Interdisciplinary Stem Cell Institute

University of Miami/ Miller School of Medicine

Clinical Research Protocol

Study Title:	A Phase I/II, Randomized, Blinded, Placebo-controlled Comparative study to evaluate the Safety and Potential Efficacy of Intravenous Infusion of Umbilical Cord Tissue (UC) derived Mesenchymal Stem Cells (MSCs) Versus Bone Marrow (BM) derived MSCs to evaluate cytokine suppression in patients with chronic inflammation due to Metabolic Syndrome. The CERES Trial.
Study Product:	Allogeneic Umbilical ***C***ord Tissu***e*** de***r***ived M***e***senchymal ***S***tem Cells (UCMSCs) and Bone Marrow derived Human Mesenchymal Stem Cells (BMMSCs)
Indication:	Metabolic Syndrome
FDA IND No.:	BB-IND #17324
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Protocol Version:	**October 22, 2020 Version 3.8**
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List of Abbreviations

6MWT	6 Minute Walk Test
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BM	Bone Marrow
BMMSCs	Bone Marrow Mesenchymal Stem Cells
BNP	Brain Natriuretic Peptide
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU-F	Colony-Forming Unit in Fibroblastic
CMP	Cell Manufacturing Program
CMV	Cytomegalovirus antibody
COPD	Chronic Obstructive Pulmonary Disease
CPL	Cell Processing Laboratory
CRC	Clinical Research Center
CRF	Case Report Form
CRP	C-Reactive Protein
CRO	Contract Research Organization
CSF	Colony Stimulating Factor
CT	Computed Tomography
CVD	Cardiovascular Disease
DMARD	Disease Modifying Anti-Rheumatic Drugs
DMSO	Dimethyl Sulfoxide
DSE	Dobutamine Stress Echocardiogram
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
EDV	End Diastolic Volume
EPC	Endothelial progenitor cells
ESV	End Systolic Volume
FACS	Flow Cytometry
FDA	Food and Drug Administration
FEV-1	Forced Expiratory Volume
FMD	Flow Mediated Diameter
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GFP	Green fluorescent protein
GIT	Gastrointestinal Tract
GMP	Good Manufacturing Practices
GVHD	Graft Versus Host Disease

HARP	Harmonic Phase
HBcAb	Hepatitis B Core Antibody
HBsAg	Hepatitis B Surface Antigen
HC	Hemorrhagic Cystitis
HCVAb	Hepatitis C Virus antibody
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMWF	High Molecular Weight Fraction
HSC	Hematopoietic Stem Cells
HTLV	Human T-Lymphotropic Virus
hUC	Human Umbilical Cord Tissue
ICF	Informed Consent Form
ICH	International Conference on Harmonization (of Technical Requirements of pharmaceuticals for Human Use)
ICR	Imprinting Control Region mouse
IEC	Independent Ethics Committee
IIEF	International Index of Erectile Function Questionnaire
IND	Investigational New Drug
INR	International Normalized Ratio
IP	Investigational Product
IRB	Institutional Review Board
ISCI	Interdisciplinary Stem Cell Institute
IUD	Intrauterine Device
IV	Intravenous
LAD	Left Anterior Descending
LFTs	Liver Function Tests
LV	Left Ventricular function
MI	Myocardial Infarction
MNC	Mononuclear Cell
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cells
NMDP	National Marrow Donor Program
PBMC	Peripheral Blood Mononuclear Cells
PSURs	Periodic Safety Update Reports
QOL	Quality of Life Questionnaires
RNA	Ribonucleic Acid
RPMS	Relapsing Remitting Multiple Sclerosis
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Events
SAO_2	Oxygen Saturation
SAP	Statistical Analysis Plan
SCF	Stem Cell Factor Antigen
SF-36	Short Form – 36

SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SQOL-F	Sexual Quality of Life - Female
SW	Stroke Work
TE-SAE	Treatment Emergent Serious Adverse Events
TNFα	Tumor Necrosis Factor-alpha
TTC	Triphenyltetrazolium chloride
UC	Umbilical Cord Tissue
UCMSCs	Umbilical Cord Tissue derived Mesenchymal Stem Cells
VEGFR2	Vascular endothelial growth factor receptor-2
VO_2	Oxygen Consumption
VDRL	Venereal Disease Research Laboratory test
WBC	White Blood Count
WJ	Wharton's Jelly

Protocol Synopsis

Sponsor: Interdisciplinary Stem Cell Institute (ISCI) at the University of Miami Miller School of Medicine

Name of Study Therapy: Allogeneic Umbilical Cord Tissue derived MSCs (UCMSCs) versus Bone Marrow derived Mesenchymal Stem Cells (BMMSCs)

Title of Study: A Phase I/II, Randomized, Blinded, Placebo-controlled Comparative study to evaluate the Safety and Potential Efficacy of Intravenous Infusion of Umbilical Cord Tissue (UC) derived Mesenchymal Stem Cells (MSCs) Versus Bone Marrow (BM) derived MSCs to evaluate cytokine suppression in patients with chronic inflammation due to Metabolic Syndrome. The CERES Trial.

Study Center: ISCI at the University of Miami Miller School of Medicine	**Phase of Development:** Phase I/II

Objectives:

Primary: To compare the safety of UCMSCs and BMMSCs administered intravenously in patients with chronic inflammation due to Metabolic Syndrome.

Secondary:

- To demonstrate the efficacy of UCMSCs and BMMSCs administered intravenously in patients with chronic inflammation due to Metabolic Syndrome as assessed by (see Table 9 in Section 2.2.2):
- symptom related quality of life
- cardiovascular status
- inflammatory cytokines
- endothelial function
- lipid profile
- glucose homeostasis
- 1year survival

Design and Investigational Plan: This Study is intended as a safety assessment prior to a full comparator study. In this Study, cells administered via intravenous infusion (IV) will be tested in 42 patients in two phases (Pilot and Randomized):

Pilot Phase (12 subjects)

Group 1 (3 subjects)

Three (3) subjects will be treated with a single administration of 2×10^7 (20 million) UCMSCs delivered via peripheral intravenous infusion.

Group 2 (3 subjects)

Three (3) subjects will be treated with a single IV administration of 2×10^7 (20 million) BMMSCs delivered via peripheral intravenous infusion.

The subjects in the high dose (Groups 3 & 4) will not be treated until the subjects in the low dose (Groups 1 & 2) have completed their one-month follow-up assessments and safety measures have been reviewed to confirm that there have been no treatment emergent SAE's.

Group 3 (3 subjects)

Three (3) subjects will be treated with a single IV administration of 1 x 10^8 (100 million) UCMSCs delivered via peripheral intravenous infusion.

Group 4 (3 subjects)

Three (3) subjects will be treated with a single IV administration of 1 x 10^8 (100 million) BMMSCs delivered via peripheral intravenous infusion.

Randomized Phase (30 subjects)

Group A (10 subjects – UCMSCs)

Ten (10) subjects will be treated with a single administration of 1 x 10^8 (100 million) UCMSCs delivered via peripheral intravenous infusion.

Group B (10subjects – BMMSCs)

Ten (10) subjects will be treated with a single administration of 1 x 10^8 (100 million) BMMSC delivered via peripheral intravenous infusion.

Group C (10 subjects – Placebo)

Ten (10) subjects will be treated with a single administration of placebo delivered via peripheral intravenous infusion.

Subjects in each pilot phase group (Group 1, 2, 3 and 4) will not be treated less than 5 days apart.

The randomized portion of the study will be conducted after a full review of the safety data from the Pilot Phase by the DSMB

Patients will be randomized in a 1:1:1: ratio to one of the 3 groups in the randomized phase. For subjects randomized to B (BMMSCs); the cells will be derived from a healthy allogeneic bone marrow donor (obtained by iliac crest aspiration).

For subjects randomized to Group A (UCMSCs); the cells will be derived from a healthy allogeneic umbilical cord tissue donor.

Route of Administration	Peripheral Intravenous Infusion
Duration of Study Participation	12 months (Follow-up will be at 2 weeks, 1 Month, 3, 6, and 12 months.)
Inclusion Criteria	1. Provide written informed consent 2. Subjects age ≥ 21 and ≤ 95 years at the time of signing the Informed Consent Form. 3. Each subject must have endothelial dysfunction. Endothelial dysfunction Criteria:

	Impaired flow-mediated vasodilation (FMD <7%) 4. At the time of enrollment, each subject must meet at least 3 out of the 5 criteria under the harmonized definition of the metabolic syndrome, consisting of the following: Waist circumference - US defined: ≥ 102 cm (males) or ≥ 88 cm (females) Elevated triglycerides - ≥ 150 mg/dL (1.7 mM) Reduced HDL-C - Males: <40 mg/dL (1.0 mM) Females: <50 mg/dL (1.3 mM) Elevated blood pressure - Systolic ≥ 130 mm Hg and/or Diastolic ≥ 85 mm Hg Elevated fasting glucose - ≥ 100 mg/dL See Table 10 in section 4.1 for more details.
Exclusion Criteria	1. Be a female who is pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods. Female subjects must undergo a blood or urine pregnancy test at screening and within 36 hours prior to infusion. 2. Inability to perform any of the assessments required for endpoint analysis. 3. Active listing (or expected future listing) for transplant of any organ. 4. Clinically important abnormal screening laboratory values. (Please reference Section 4.2 for more details) 5. Serious comorbid illness or any other condition that, in the opinion of the investigator, may compromise the safety or compliance of the subject or preclude successful completion of the study. (Please reference Section 4.2 for more details) 6. Have known allergies to penicillin or streptomycin. 7. Be a solid organ transplant recipient. This does not include prior cell based therapy (>12 months prior to enrollment), bone, skin, ligament, tendon or corneal grafting. Have a history of organ or cell transplant rejection. 8. Have a clinical history of malignancy within 3 years (i.e., subjects with prior malignancy must be disease

	free for 3 years), except curatively-treated basal cell carcinoma, squamous cell carcinoma, melanoma in situ or cervical carcinoma, if recurrence occurs. 9. Have a non-pulmonary condition that limits lifespan to < 1 year. 10. History of drug abuse (illegal "street" drugs except marijuana, or prescription medications not being used appropriately for a pre-existing medical condition) or alcohol abuse (≥ 5 drinks/day for > 3 months), or documented medical, occupational, or legal problems arising from the use of alcohol or drugs within the past 24 months 11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C, and/or Syphilis – VDRL (If VDRL is reactive Confirmation with FTA-ABS is needed (Syphilis)). 12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial. 13. Patients with EF<45% (heart failure patients). 14. GFR ≤ 35 (chronic kidney disease stage 3 or higher). 15. Liver disease (elevated LFTs greater than 3x upper limit of normal). 16. Advanced pulmonary disease (requiring home oxygen and/or less than 1 year expected life span). 17. Proliferative diabetic retinopathy 18. Hemoglobin A1C greater than 7.

1. INTRODUCTION

1.1 Background

The metabolic syndrome is a group of risk factors that increases chances for developing cardiovascular disease (CVD), Type II diabetes mellitus (T2DM), and all-cause mortality by promoting a pro-inflammatory state coupled with endothelial dysfunction[1].

The metabolic syndrome is a defined condition under the WHO International Classification of Disease (ICD-9) code (277.7), and is also known as X syndrome, insulin resistance syndrome, cardiometabolic syndrome, and Reaven's syndrome. The precise definition of the metabolic syndrome has evolved over the past few decades, with definitions historically differing slightly depending upon the health organization[1,2]. To resolve this, a multi-organizational harmonized definition of the metabolic syndrome has been reached by the National Heart, Lung, and Blood Institute; the International Diabetes Federation Task Force on Epidemiology and Prevention; the American Heart Association; the World Heart Federation; the International Atherosclerosis Society; and the International Association for the Study of Obesity[3]. Specifically, the metabolic syndrome is defined as a cluster of risk factors for CVD and T2DM, with these risk factors consisting of hypertension, dyslipidemia via raised triglycerides, dyslipidemia via lowered high-density lipoprotein (HDL), fasting hyperglycemia/dysglycemia (impaired glucose tolerance; insulin resistance), and central obesity (apple-shaped adiposity) (Table 1). None of these risk factors is obligatory, but a patient having any 3 of the 5 risk factors is defined as having the metabolic syndrome. Although the first four criteria are highly agreed upon, some controversy still exist on exactly how to define abdominal obesity, and whether it should be included as an obligatory risk factor[4].

Table 1. Criteria for the Metabolic Syndrome

Measure	Categorical Cut-Off Points	Alternative Indicators
Waist circumference	US defined: ≥ 102 cm (males) or ≥ 88 cm (females)	Ethnic and country-specific definitions
Elevated triglycerides	≥ 150 mg/dL (1.7 mM)	Drug treatment for elevated triglycerides
Reduced HDL	Males: <40 mg/dL (1.0 mM) Females: <50 mg/dL (1.3 mM)	Drug treatment for reduced HDL-C
Elevated blood pressure	Systolic ≥ 130 mm Hg and/or Diastolic ≥ 85 mm Hg	Antihypertensive drug treatment for a history of hypertension
Elevated fasting glucose	≥ 100 mg/dL	Drug treatment of elevated glucose

Patients with the metabolic syndrome have double the risk of developing CVD in 5 – 10 years versus normal individuals, and a 5-fold increased risk of developing T2DM[1,3,5]. Furthermore, compared to individuals without the syndrome, patients with the metabolic syndrome are 2 to 4- times more likely to have a stroke, 3 to 4-times more likely to have a myocardial infarction (MI), and 2-times more likely to die from any such an event[5,6]. The incidence of the metabolic syndrome has reached epidemic proportions worldwide, with >40% of US adults ≥20 years of age classified as having the metabolic syndrome[3,7]. Thus, effective treatment of the metabolic syndrome is a paramount health concern.

Inflammation caused by the Metabolic Syndrome

Dysregulated inflammation is a considerable key physiological correlate of the metabolic syndrome, in which affected persons commonly manifest a proinflammatory and prothrombotic

state[8]. Inflammatory markers are predictors of cardiovascular events and progression to diabetes in healthy individuals, including individuals with metabolic syndrome. For example, those with metabolic syndrome are more likely to have elevated C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6), red blood cell distribution width (RDW) and D-dimer[8].

Excess adiposity promotes the metabolic syndrome in a least 2 major ways[8,9]. First, the adipose cells release adipose tissue-derived non-esterified fatty acids (NEFAs) that inhibit insulin signaling[10]. Second, the increased adipose cells (especially abdominal) releases adipokines, which are cytokines that promote a pro-inflammatory state[11]. These promote diminished whole-body sensitivity to insulin. Consequently, the pancreas initially increases insulin secretion to maintain normoglycemia. The result is a somewhat asymptomatic phase which can last many years, but which nevertheless leads to the development of a pro-inflammatory and pro-thrombotic state accompanied by dyslipidemia and hypertension, and eventually the co-morbidities that include T2DM and CVD.

Endothelial Dysfunction, Endothelial Progenitor Cells, and the Metabolic Syndrome

The presence of the metabolic syndrome is associated with advanced vascular damage[6] and endothelial dysfunction[12,13]. Endothelial dysfunction is characterized by a pathophysiological shift in endothelial functioning towards a state that is proinflammatory, prothrombic, and of reduced flow-mediated vasodilation (FMD)[14,15,16,17]. Endothelial dysfunction is commonly accepted to be the earliest manifestations of atherosclerosis, insulin resistance, and diabetes[15,17], and is a crucial component of the pathophysiology of numerous cardiovascular (CV) disorders, including heart failure (HF)[18,19]. These pathological changes begin with damage to the endothelium, in which production of the vasodilator, nitric oxide (NO), becomes substantially impaired, and the production of reactive oxygen species (ROS) increases[20,21]. Nitric oxide is also thought to inhibit cellular pathways involved in inflammation and thrombosis[22]. Since such pathological changes begin with damage to the endothelium, targeting endothelial dysfunction could provide a general therapeutic approach to treat the metabolic syndrome. Endothelial dysfunction and the metabolic syndrome are also characterized by reduced functioning of endothelial

progenitor cell (EPC)[23,24,25,26,27]. In fact, the metabolic syndrome is characterized by a decrease in circulating endothelial progenitor cells (EPCs), regardless of whether a patient displays T2DM or CVD[24]. EPCs are bone marrow-derived progenitor cells

Several clinical trials and research work have been done to prove that Mesenchymal Stem Cells (MSCs) derived from bone marrow or the umbilical cord tissue have anti-inflammatory properties and these cells inhibit T cells proliferation and suppress allogeneic T-cell response. The main features of the T cell response are cell proliferation and cytokines secretion. Inhibition of T cell proliferation is one of the most significant effects for MSCs. In vitro, MSCs are capable of suppressing T lymphocyte proliferation induced by mitogens, alloantigens, as well as activation of T cells by CD3 and CD28 antibodies[24,28,29,30,31,32,33]. Suppression of T cell proliferation by MSCs has no immunological restriction, similar suppressive effects being observed with cells that were autologous or allogeneic to the responder cells.

The hypothesis of this trial is that suppressing cytokines levels in subjects with metabolic syndrome will cause a decrease in the chronic inflammation and endothelial dysfunction caused by the disease[34,35,36,37,38].

1.2 **Rationale**

We have analyzed TNFα levels for our subjects for the CRATUS Study, allogeneic BMMSCs were tested in a range of 20M MSCs to 200M MSCs in a Phase 1/2 clinical study in 45 Aging Frailty subjects (BB-IND#15679). The study contained a run in pilot phase testing single intravenous administration of 20M, 100M or 200M MSCs, followed by a randomized, placebo-controlled double blind Phase 2 single intravenous administration of 100M MSC or 200M MSCs or placebo in 30 subjects. Results showed no significant changes in any of the treatment arms. However, the average baseline TNFα levels for the subjects in all 3 arms were less than half those in the Phase 1 study, and were already close to the low level for the reference range (1.2 – 15.3 pg/mL). No significant changes in other biomarkers were observed.

As we know that frailty is hypothesized to result from a complex interplay of biochemical and multi-systemic changes that may result in decreased physiologic reserves in older adults and biological mechanism that underlies the decline in physical function associated with aging frailty is chronic inflammation[35]. This response is initially characterized by a local release of cytokines, responsible for the amplification and regulation of the inflammatory cascade.

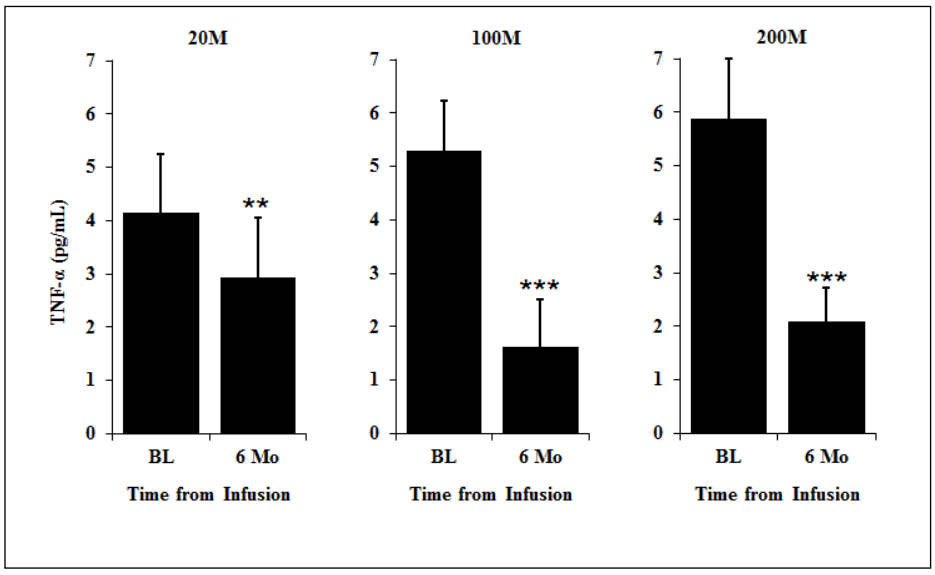
Cytokines are also involved in numerous physiological functions, such as muscle and bone tissues turnover, immunoregulation, and hematopoiesis and their circulating levels have been related to several disease processes, primarily atherosclerosis and cardiovascular disease. Chronically elevated inflammatory biomarkers have been observed in the presence of acute and chronic conditions, including atherosclerosis, Parkinson's disease, type 2 diabetes mellitus, sarcopenia, functional disability, cerebrovascular disease, coronary heart disease, CHF and arthritis. Inflammation exerts detrimental effects on muscle, bone, cardiac function, hematopoiesis and cognition[34,39,40].

Not surprisingly, data from our frailty studies indicate an association between the frailty syndrome and elevated levels of pro-inflammatory mediators including IL-6, C-reactive protein (CRP), and TNFα[41,41,42,42,43,43]. Although these various inflammatory markers are correlated with each other, the relative importance of these markers as predictors of frailty or physical decline has not been established.

While this current study will include assessment of a panel of cytokines, there is particular interest in TNFα due to evidence that I.V. infusion of allogeneic MSCs significantly decreases serum TNFα levels in aging frailty subjects.

In our frailty study, cytokine levels were measured at baseline, and at 6 months after treatment with I.V. allogeneic MSCs, the intervention proposed to be tested in the current clinical trial. Minimal effect was noted on IL-6 and CRP, but TNFα, which was elevated at baseline, decreased significantly in both the open label and randomized phases of the study. In Phase 1, mean baseline TNFα levels were 4.14 ± 2.0 pg/ml, 5.30 ± 0.94, and 5.88 ± 1.12 for the 20 million MSC, 100 million MSC and 200 million MSC frail subject groups, respectively. These levels were consistent with, if not slightly higher, than the frail (3.19 ±2.68 pg/ml) and dependent (4.58 ± 3.30 pg/ml) groups in Hubbard et al[44], and the frail group (2.11 pg/ml) in Serviddio et al[45]. Treatment with allogeneic MSCs resulted in all 3 of the Phase 1 cohorts showing a statistically significant reduction in mean TNFα (Figure 1).

Figure 1: TNFα levels decreased with allogeneic MSC treatment. Serum levels decreased

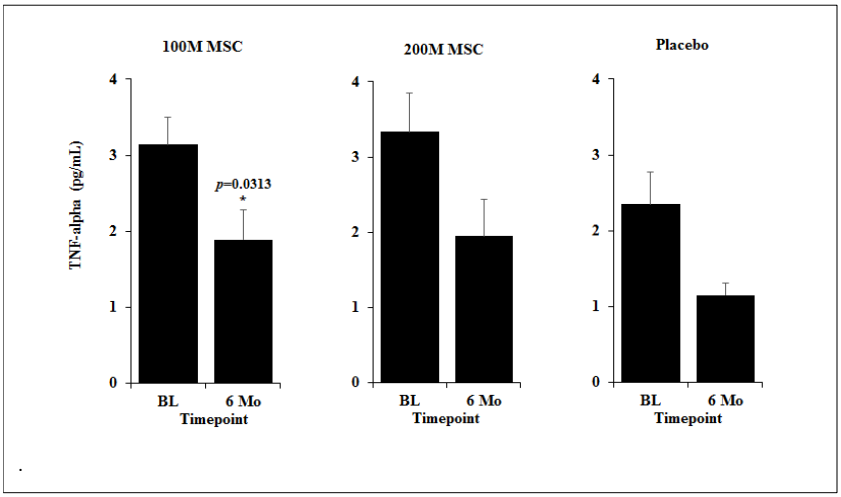


over 50% at 6-months after infusion with allogeneic MSCs. p-values are for within treatment arm versus baseline.

In Phase 2 of the CRATUS study, mean baseline TNFα was 3.14 ± 0.37 pg/ml, 3.33 ± 0.52, and 2.35 ± 0.43 for the 100 million MSC, 200 million MSC, and placebo groups, respectively. Six months following I.V. infusion with allogeneic MSCs, TNFα decreased in all cohorts, including placebo, however the 100 million MSC group showed significant reductions relative to baseline. (Figure 2)

Figure 2: Serum TNFα levels from Phase 2 randomized portion of the CRATUS study in frailty. TNFα levels were elevated at baseline (BL), and a significant reduction after i.v. infusion of 100M MSCs was observed. Plotted are averages and SEM, using only subject data for which there was both baseline and 6-month data. p-values are within group versus baseline.

Figure 2. Serum TNFα levels



Results from the CHS established a strong association between the presence of clinically manifest cardiovascular disease (CVD) and subclinical cardiovascular abnormalities, with frailty syndrome[46]. In the Health ABC Study, high levels of circulating TNFα were shown to be predictive of CVD (CHD, stroke and CHF) in older adults 70-79 years of age with no evidence of CVD at baseline[46]. The same group reported a significant association between several inflammatory markers and the syndrome of frailty *in the absence of CVD* and diabetes[44], supporting the hypothesis that age-related inflammation system changes may underlie the development of both the clinical criteria of frailty and the physiological vulnerability observed in frail older adults. However, this does not rule out the possibility of chronic inflammation due to subclinical disease such as atherosclerosis, and associated reduction in end organ functional reserve, which is thought to be part of the underlying pathophysiology that is recognized clinically as frailty[47]. While a disease-independent inflammatory mechanism for frailty is possible, subclinical versions of CVD and/or diabetes may have a greater influence on the development of frailty than previously suspected.

<u>Clinical Restoration of Endothelial Dysfunction Using MSC Therapy</u>

Endothelial dysfunction is a critical pathophysiological component of numerous cardiovascular disorders, and presents in patients with hypertension, atherosclerosis, and diabetes mellitus[18,19,48]. Furthermore, circulating EPC levels and bioactivity are diminished in patients with heart failure[49].

The pro-angiogenic properties of MSCs make them attractive as a potential therapeutic for restoring endothelial function[50]. This may be due to the capacity of MSCs to differentiate

not only into cells of mesodermal origin, but into cells of endodermal and ectodermal origin[51], as well as their ability to stimulate endogenous progenitor cells[52,53]. Given these properties of MSCs, the hypothesis was tested that MSCs could stimulate the bioactivity of circulating EPCs and improve endothelial function in patients with heart failure[54].

Specifically, the therapeutic capabilities of both autologous and allogeneic MSCs were tested for the ability to stimulate EPC function and augment vascular relaxation in patients with heart failure due to either idiopathic dilated cardiomyopathy (DCM) or ischemic cardiomyopathy (ICM)[55]. These IRB-approved studies used heart failure patients enrolled in POSEIDON-DCM (NCT01392625), "A Phase I/II, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients with Nonischemic Dilated Cardiomyopathy"[56] and in TRIDENT (NCT02013674), "The Transendocardial Stem Cell Injection Delivery Effects on Neomyogenesis Study".

The results of these studies provided insight leading to the discovery that allogeneic MSCs significantly improved endothelial dysfunction in heart failure patients. This was due to the improvement of EPC bioactivity and endothelial function, regardless of the etiology of the heart failure. Surprisingly, though, autologous MSCs did not confer similar beneficial effects.

The number of circulating endothelial progenitor cells can be assessed using an endothelial progenitor cell-colony forming unit (EPC-CFU) assay: the more EPC-CFUs that form, the greater the number of circulating EPCs. It was found that treatment of heart failure patients with allogeneic human MSCs significantly improved the number of circulating EPCs at 3 months after infusion (Figure 3). In surprising contrast, however, autologous MSCs did not provide significant improvement in the number of circulating EPCs.

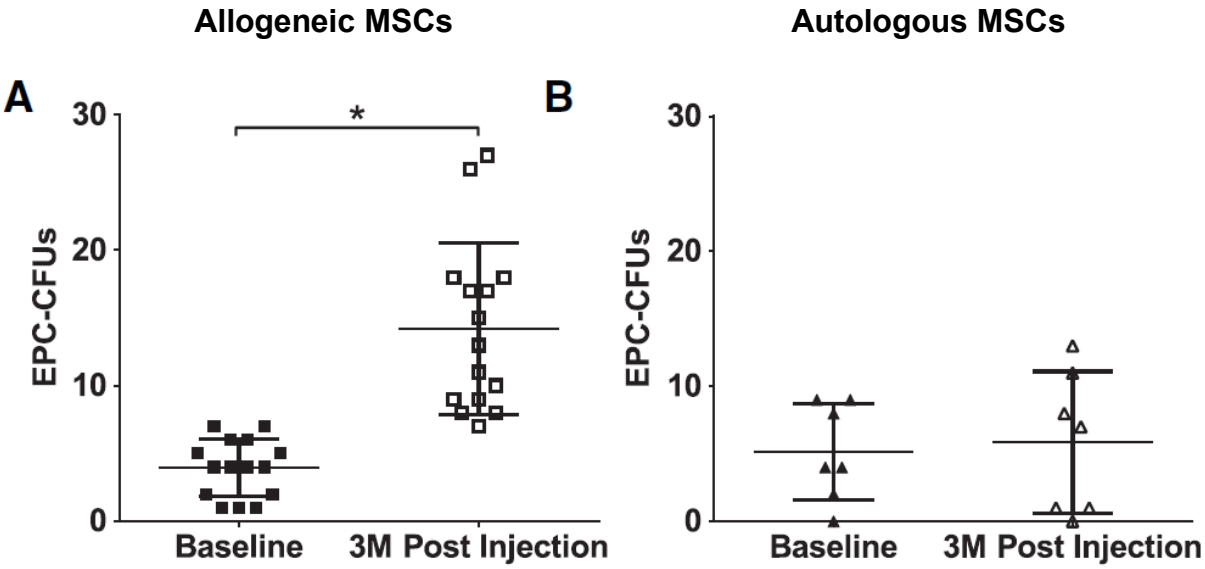


Figure 3. Allogeneic MSCs, but not autologous MSCs, improve EPC colony forming units in heart failure patients. **A**, Patients treated with allogeneic MSCs had a significant improvement in endothelial progenitor cell-colony forming units (EPC-CFUs) at 3 months post-treatment (n= 15, *P < 0. 0001, *t*-test). **B**, Patients treated with autologous MSCs had no change in EPC-CFUs post-treatment (n=7, P=NS, *t*-test).

Similarly, allogeneic human MSCs significantly increased flow-mediated vasodilation (FMD) in heart failure patients at 3 months after infusion, demonstrating improved endothelial function (Figure 4). Again, autologous MSCs did not confer significant improvement. More importantly, when assessed at an individual patient level, every subject with idiopathic dilated cardiomyopathy who received allogeneic human MSCs had improved FMD, whereas the majority of subjects receiving autologous MSCs showed either no improvement or worsening. Furthermore, the improvement in FMD directly correlated with the improvement in circulating EPC number.

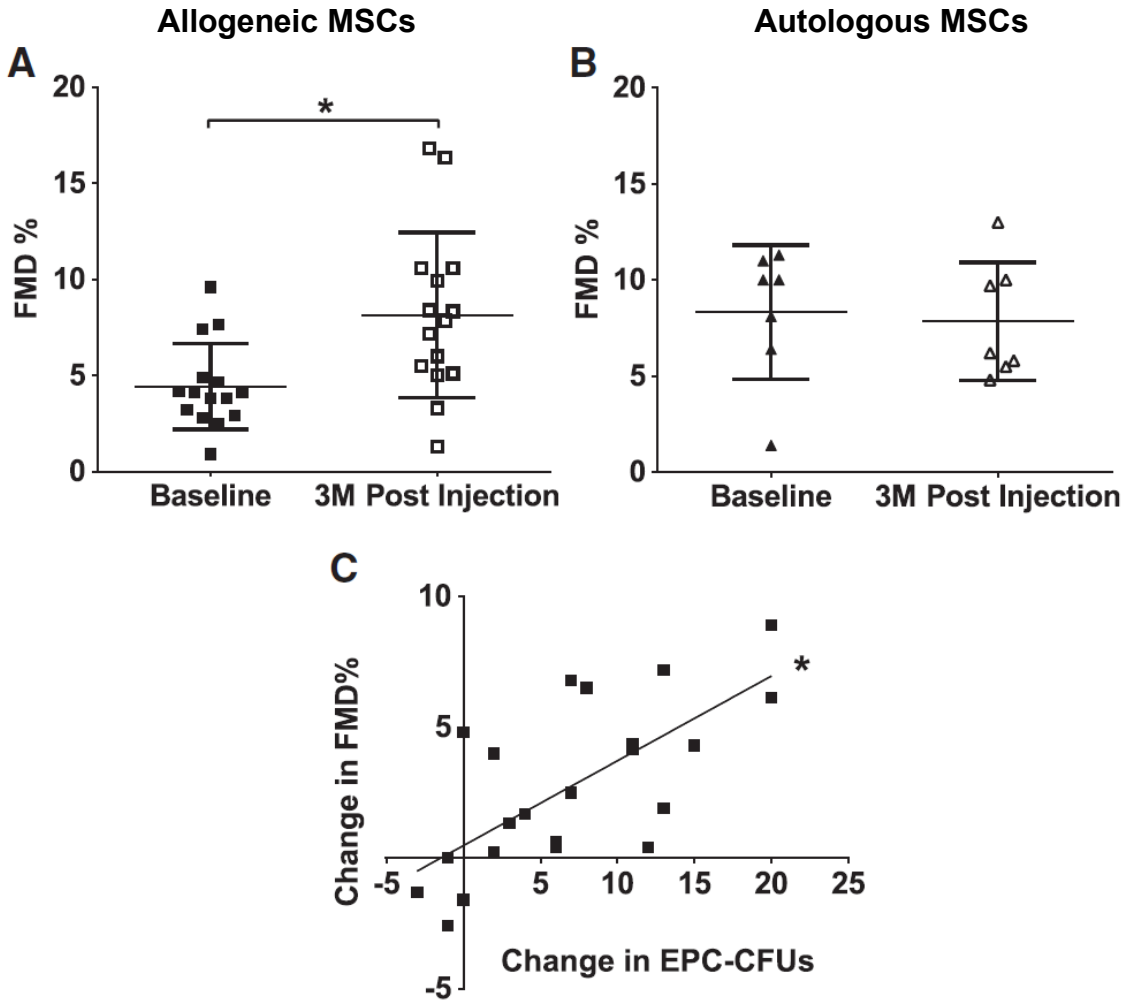


Figure 4. Allogeneic MSCs, but not autologous MSCs, improve flow-mediated vasodilation (FMD) in heart failure patients. **A**, Patients treated with allogeneic MSCs had an increase in FMD% at 3 months' post-injection (n=15, *P=0.0002, *t*-test). **B**, Patients treated with autologous MSCs had no significant difference in FMD% at 3 months' post-injection (n= 7, P=NS, *t*-test). **C**, A strong correlation existed between the absolute change in FMD% and the absolute change in EPC-CFUs from baseline to 3

At 3 months' post-infusion, subjects who received allogeneic human MSCs also had significantly suppressed serum levels of vascular endothelium growth factor (VEGF) relative to their baseline levels (Figure 5). In fact, the levels of VEGF approached those of normal, healthy (control) subjects. In contrast, autologous MSCs actually led to a significant increase VEGF levels at 3 months. Furthermore, both the absolute levels of VEGF, and the relative changes in VEGF, had an inverse correlation to the number of EPC-CFUs. It should be noted that serum level of VEGF was too low to detect in the majority of the control subjects.

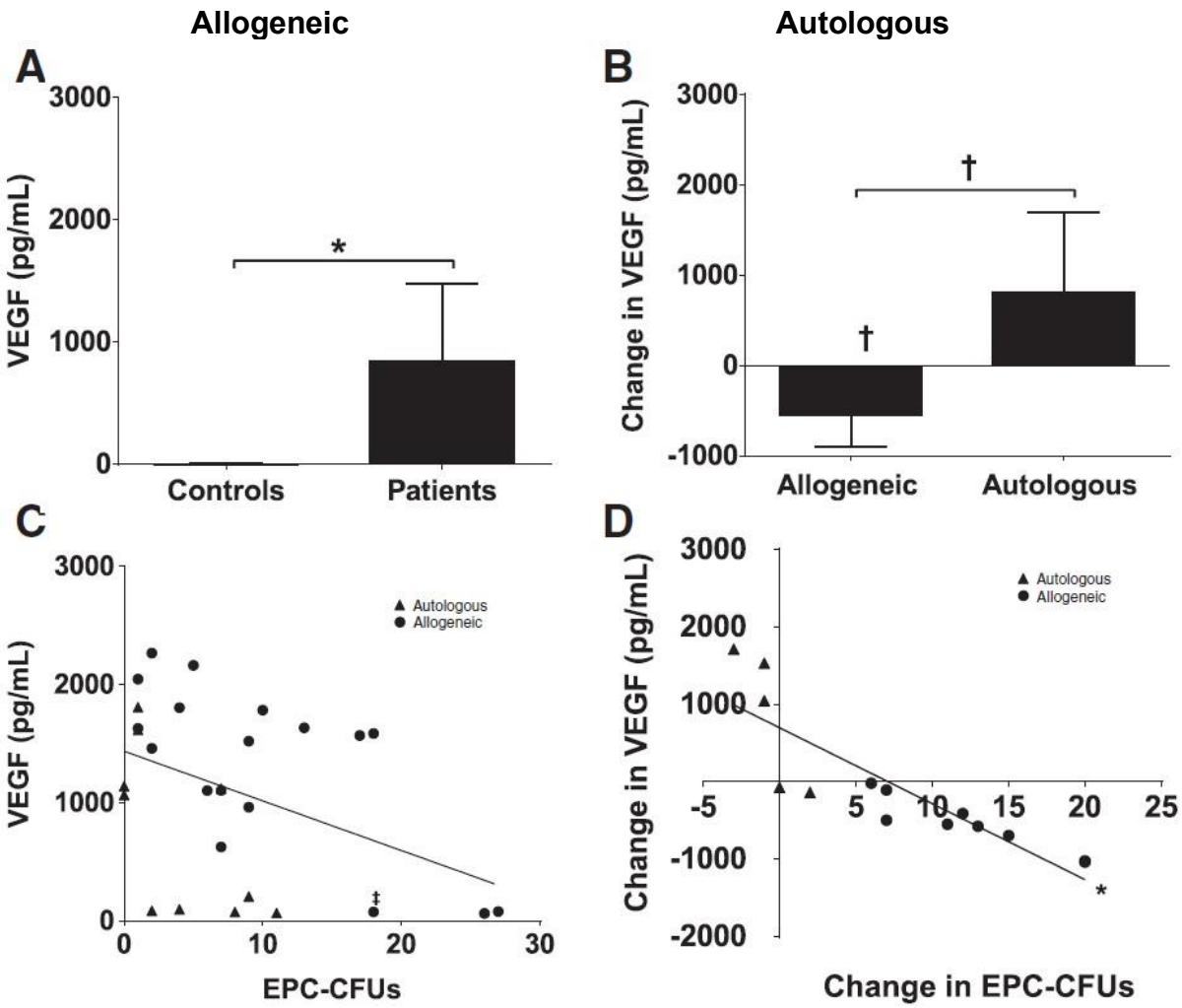


Figure 5. Allogeneic MSCs, but not autologous MSCs, improve serum VEGF levels. **A**, Patients (n=14) have a higher level of circulating VEGF compared to controls (n=9) at baseline (*P=0.0009, *t*-test; within-group, P=0.21, P<0.001, respectively, D'Agostino-Pearson omnibus normality test). **B**, Patients who received allogeneic MSCs (n=9) had a decrease in VEGF serum levels post-injection (Δ-547.5 ± 350.8 pg/mL, †P=0.0015, *t*-test; within-group P=0.96, D'Agostino-Pearson omnibus normality test), while patients who received autologous MSCs (n=5) had increased serum VEGF post-injection (Δ814.1±875.8), and there was a difference between the groups (†P=0.0012, t-test). **C**, There is an inverse correlation between endothelial progenitor cell-colony forming units (EPC-CFUs) and serum VEGF in patients at both baseline and 3months post-MSC treatment (R=−0.421, ‡P=0.026, Pearson correlation). **D**, The change in EPC-CFUs from baseline to 3 months post-treatment strongly correlated

In summary, this study demonstrated the potent and clinically relevant efficacy of allogeneic MSCs to treat endothelial dysfunction after heart failure. Specifically, allogeneic MSC therapy could restore flow-mediated vasodilation, EPC bioactivity, and VEGF levels towards normal. Furthermore, the insights gained in efficacy to treat endothelial dysfunction after heart failure implies that allogeneic MSC therapy may provide a generally useful therapy for treating all disorders associated with endothelial dysfunction.

Cells derived from adult bone marrow

Bone marrow harbors a variety of cells that may contribute to vasculogenesis or cardiomyogenesis, either directly, or by facilitating endogenous repair mechanisms. Bone marrow cells have been prepared on the basis of being 1.) endothelial precursor cells that are $CD34^+$, 2.) MSCs purified without an antigen panning technique on the basis of their fibroblast morphology, ability to divide in culture and to differentiate into mesodermal lineages[57], and 3.) cells that express stem cell factor receptor, c-Kit[58,59]. Endothelial progenitor cells (EPCs) express the surface markers CD34, CD133, c-kit, and the vascular endothelial growth factor receptor-2 (VEGFR2; KDR; Flk-1)[60,61,62,63,64,65]. Hematopoietic stem cells (HSCs) exhibit self-renewal and differentiation. Their cell-surface phenotype is $CD34^+$, stem cell factor antigen $(SCF\text{-}1)^+$, c-kit^+, and Lin^-[66]. While there has been controversy regarding the ability of bone marrow-derived cells to transdifferentiate into cardiomyocytes[67], clinical trials of bone marrow therapies continue to suggest potential benefit in terms of improving a subject's well-being and quality of life.

1.3 BMMSCs

MSCs are multipotent cells capable of differentiating into a number of different cell lines. Because of their unique combination of multipotency, migratory ability, and immunoprivileged state (MSCs do not express major histocompatibility factor-II making allogeneic transplant possible)[68], interest has abounded regarding their potential therapeutic and regenerative applications. In fact, MSCs have been shown to hold promise as a novel therapeutic agent in multiple disease processes. Treatment with MSCs has been shown to ameliorate severe graft versus host disease[68], contribute to pancreatic islet and renal glomerular repair in diabetes[69], attenuate sepsis[70], reverse fulminant hepatic failure[71], protect against ischemic acute renal failure[72], reverse remodeling[73,74,75] and improve cardiac function after myocardial infarction[76], to be a potential source of multiple cell types for use in tissue engineering[77,78], and to be capable of tissue regeneration after spinal cord trauma, stroke, and connective tissue injury[79,80,81].

In the lung, MSCs have been shown to contribute to tissue regeneration after elastase-induced emphysema[82], home to sites of asbestos induced lung injury[83], contribute to tissue remodeling in a rat monocrotaline model of pulmonary hypertension[84], decrease chronic airway inflammation in a murine ovalbumin model of asthma[85], and to restore alveolar fluid balance after endotoxin induced acute lung injury[86].

Tracking of radioactively labeled cells shows that when administered intravenously, MSCs localize primarily to the lung, followed by the liver, and then other organs[87]. A number of studies show that MSCs preferentially hone to sites of injury in the lung and contribute to tissue regeneration and repair[88,89,90,91,92,93,94]. Using Y-chromosome fluorescence in-situ hybridization, Y-chromosome positive male MSCs can be found at sites of lung injury in transplanted female mice[89,90,93]. These male MSCs appear to adopt anepithelial cell morphology, suggesting that they contribute to tissue regeneration either by fusion with resident epithelial cells or by mesenchymal to epithelial transition[89].

1.4 BMMSC Preclinical Experience

Several cell-based therapies' results propose that infusion of mesenchymal stem cells is a safe and novel approach believed to be an effective strategy to produce cytokine suppression in patients with chronic inflammation. Below we review the impact of MSCs on the cardiovascular system following injury; this provides support for the impact of MSCs on cytokine suppression.

1.4.1 MSCs in Cardiovascular Disorders

Much work has been done on animal models that demonstrate both the safety and efficacy of MSCs to treat and repair cardiovascular damage. For example, therapeutic MSCs improve cardiac function in the rat model of dilated cardiomyopathy[85], and repair the scarred myocardium and reverse remodeling after myocardial infarct in mice[47,48,86].

1.4.2 Allogeneic mesenchymal stem cell transplantation improves global cardiac function in a swine model of acute myocardial infarction:

The porcine model is an excellent model in which to study cardiac damage and repair because of the anatomical similarity to the human heart. It was initially shown that transplantation of autologous MSCs into swine after myocardial infarct (MI) improved cardiac function[87]. This was supported by histological evidence at 8 weeks post-MI, which revealed differentiation of autologous MSCs into a myocyte-like phenotype.

Because of the immune privileged nature of MSCs, we have performed a series of elegant experiments using transplantation of allogenic MSCs after porcine anterior MI, and demonstrated the safety and efficacy in repairing cardiac repair using hemodynamic analysis, imaging, and histological analyses. These experiments fell into two groups: the early treatment of acute myocardial infarction, and the treatment of chronic ischemic cardiomyopathy.

In a randomized study, swine received a single injection of either 2 x 10^8 allogenic MSCs or placebo (n = 7 for each) at 3 days following MI[88]. The allogenic MSCs were delivered via transendocardial injection using the BioCardia Helical Infusion Catheter (BioCardia, Inc.), and all the animals tolerated the catheter-based injection well. The swine were assessed on a weekly basis for 8 weeks for hemodynamics and ventricular architecture. It was found that MSC injection produced a wide range of benefits, including improved regional and global ventricular function, reduced myocyte apoptosis, and improved tissue perfusion. Over a 2-3 month period, animals that received MSC exhibited a remarkable pattern of LV recovery marked by a substantial increase in stroke work (SW) and a return to normal of LV end diastolic pressure by 8 weeks post- MI. In contrast, impaired cardiac function evident at 3 days post-MI either persisted or worsened over the same 8 week period for the placebo group. These results were further confirmed using sophisticated MRI and computed tomography (CT) to image and quantify myocardial infarcts and repair[91,92,95,96,97,98,99,100,101,102,103].

MSCs delivered during these acute myocardial infarction experiments were found to engraft within the MI, and differentiated to express myocyte proteins, including α-actinin,

phospholamban, tropomyosin, and troponin T. Furthermore, MSCs were found in vascular structures and expressed VEGF and von Willebrand Factor, which suggesting that they also differentiated into vascular endothelium and/or smooth muscle. There was also a noticeable lack of inflammatory response, supporting the observations that MSCs do not elicit immune rejection, despite the absence of immunosuppressive drug therapy. Over time, the number of MSCs persisting in the myocardium decreased.

Other experiments using intravenously-delivered MSCs at the time of coronary reperfusion homed to the myocardium, whereas MSCs injected 2 weeks post-MI preferentially engrafted into the bone marrow. Interestingly, it was found that MSCs express both SDF-1 and CXCR4, and serum levels of both of these are up-regulated immediately post-MI and remain elevated for at least 2 weeks with MSC intravenous MSC infusion[104].

Similarly, in a chronic ischemic cardiomyopathy model using Gottingen mini-swine, autologous bone marrow-derived MSCs produced reverse remodeling[105]. At 12 weeks post-MI, autologous MSCs were delivered to the infarct. Over the next 12 weeks, animals that received intramyocardial injected MSCs showed a significant reduction in scar burden via cardiac MRI (21.8+3.9% versus placebo, $p<0.05$), which could be seen as quickly as 3 days. Furthermore, there was significant improvement in regional contractility, global LV function, ejection fraction, and myocardial blood flow. The MSC therapy led to reverse remodeling, and reduced the circumferential extent of the infarct scar, suggesting the MSC therapy promoted the highly effective repair of ischemic cardiomyopathy. At a cellular level, it was found that the MSCs stimulated cardiac recovery by engrafting, promoted new blood vessels formation that increased tissue perfusion in the hypoperfused areas, formed new cardiac myocytes, and interacted with endogenous precursor cells to induce new cardiac myocyte formation.

Further experiments clearly demonstrated that allogenic MSCs restore cardiac function in chronic ischemic cardiomyopathy by trilineage differentiation[106]. This was clearly demonstrated by transplantation of MSCs from male swine donors into female recipients. Immunohistological analyses against the Y-chromosome revealed that the allogenic MSCs differentiated into cells containing markers of cardiac, endothelial, and vascular lineages. MSCs were shown to engraft in infarct and border zones, and expressed α-sarcomeric actin, GATA-4, and Nkx2.5, indicating differentiation into cardiomyocytes. Very importantly, there was a direct correlation with the number of engrafting MSCs and the improvement in cardiac function. These studies also demonstrate the effectiveness of MSCs to treat chronic damage.

These results provide strong rationale for the continued development of MSC-based cellular therapy and support ongoing human studies. Based on these pre-clinical studies, the FDA issued two INDs for clinical trials in patients with ischemic cardiomyopathy, and a third for non-ischemic cardiomyopathy.

1.4.3 MSCs in Graft-Versus-Host Disease (GVHD)

MSCs have been successfully used to prevent GVHD in mice[107]. The MSCs were required to express functional iNOS and INFα receptor to convey this immunosuppressive function, in which cytokine-induced activation of iNOS inhibited T cell proliferation.

1.4.4 MSCs in Pulmonary Disorders

MSCs have been shown to contribute to tissue regeneration after lung injury[108,109]. In mice with lipopolysaccharide-induced lung injury, bone-marrow derived progenitor cells home in on the sites of inflammation and differentiate into endothelial and epithelial cells[110]. Similarly, MSCs are involved in rodent lung repair after elastase-induced pulmonary emphysema[111], pulmonary hypertension produced by monocrotaline[112], asbestos-induced pulmonary fibrosis[113], and bacterial endotoxin-induced lung damage[114]. These and other studies have shown that MSCs contributed to pulmonary repair and regeneration by homing to sites of lung injury[115,116,117]. In particular, such homing has been elegantly shown using male murine MSCs infused into female recipients: through fluorescence in situ hybridization (FISH) against the Y-chromosome, MSCs were show to accumulate to sites of bleomycin-induced lung injury[66,118]. These allogenic MSCs appear to undergo mesenchymal to endothelial cell transition, or possibly to fuse with resident epithelial cells, since the FISH-labeled cells have an epithelial-like morphology. Likewise, allogenic human MSCs are effective at restoring normal fluid balance after endotoxin-induced acute lung injury in an ex vivo perfused human lung model [114], which may in part be due to release of MSC microvesicles [115, 116].

The allogenic and xenogeneic safety and efficacy of human MSCs has also been demonstrated in numerous animal models. For example, human MSCs are able to incorporate into murine lung epithelium [117], and are effective in repairing lung damage after endotoxin-induced acute lung injury in mice [118]. Furthermore, in the murine ovalbumin asthma model, infusion of xenogeneic human MSCs effectively decreased chronic airway inflammation, presumably due to their anti- inflammatory properties[70].

In addition, in-situ hybridization studies with co-staining for green fluorescent protein (GFP) and epithelial markers shows that GFP-labeled MSCs assume an epithelial phenotype at sites of lung injury and contribute to tissue repair [106, 119]. It is worth noting, however, that not all authors agree, with some suggesting that technical difficulties associated with immunofluorescence microscopy have led to the false conclusion that MSCs contribute to alveolar epithelium [120, 121].

1.5 BMMSC previous Experience in Humans

The use of allogeneic cellular products typically requires matching of the graft HLA to the donor in order to avoid graft rejection and graft versus host disease. However, because MSCs do not express HLA, they represent a unique immunoprivileged cell population which can be used for allogeneic cellular therapy. In addition, MSCs fail to induce proliferation of allogeneic lymphocytes *in vitro* and suppress proliferation of T cells activated by allogeneic cells or mitogens. MSCs have also been shown to exert anti-proliferative, immunomodulatory and anti-inflammatory effects. Many subjects have received allogeneic

MSCs and infusions have all been well tolerated. MSCs are currently being used at various stages in numerous clinical trials, including treatment of myocardial infarction, diabetes, graft-versus-host disease, osteogenesis imperfecta, Crohn's disease, multiple sclerosis, spinal cord injury, stroke, and traumatic brain injury [50, 57, 80, 124-138].

1.5.1 Safety Information

Allogenic MSCs have safely been used in a number of clinical trials, in complete accord with the high safety profile repeatedly observed in animal models [139]. This stems from the immunological status of MSCs: while allogenic grafts typically require HLA matching of donor to host to prevent tissue rejection, MSCs do not detectably express HLA, conferring a unique immune privileged status to these cells[34,57,61]. Furthermore, MSCs exert anti-inflammatory and immunomodulatory effects. They also do not induce proliferation of allogeneic lymphocytes, and suppress T-cell proliferation activated by allogeneic cells or mitogens. Together, these properties make MSCs uniquely suitable for allogenic therapy, and have repeatedly been shown to be well tolerated in patients.

1.5.1.1 MSCs Safety in Cardiac Disease

The safety of allogenic MSCs has repeatedly been shown in clinical investigations of cellular cardiomyoplasty [40, 101, 135]. These studies revealed that allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions [135]. The Poseidon trial (NCT01087996) showed that MSCs are immunoprivileged and immunosuppressive, and did not lead to acute immunogenic reactions [129]. No subject had symptoms indicative of reaction to the infused cellular therapy, and only 1 of the 15 patients had mounted any detectible donor specific alloreaction, which was of low antibody titer [135]. Of particular interest in these trials was that cellular therapy using allogenic MSC appeared more effective and safer than using autologous MSCs. It has been hypothesized that autologous MSC function could be impaired due to advanced age or patient co-morbidity, which allogenic MSCs would not be subject to [140, 141]. In a follow-up Phase II study using 53 patients, no HLA-matching was performed, and MSC therapy was found to be safe and well-tolerated at any of the 3 administered doses [76]. No deaths were reported, and no SAEs were attributed to the therapy; in fact, the number of AEs decreased in the therapeutic group compared to placebo.

In the POSEIDON trial [80, 135], both allogeneic and autologous MSCs were associated with low rates of treatment-emergent serious adverse events (TE-SAEs), and no patients reported symptoms or indications of adverse reaction. The results showed that MSCs are immunoprivileged, immunosuppressive, and do not lead to acute immunogenic reactions. In an open-label pilot of the TRIDENT study (NCT02013674) to treat chronic ischemic left ventricular dysfunction secondary to myocardial infarction, subjects were infused with either allogenic or autologous MSCs [139]. SAEs were lower among the allogenic MSC-treatment group that the autologous MSCs treatment group.

The Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HFT) is an 8 patients open-label phase 1 trial to assess the safety and preliminary efficacy of MSCs and bone marrow mononuclear cells (MNCs) in patients with ischemic cardiomyopathy. All patients tolerated the treatment well, and no adverse events were observed.

1.5.1.2 MSCs Safety in Graft Versus Host Disease (GVHD)

As such, allogenic MSCs have been shown to be both safe and highly effective at treating steroid- resistant acute host-versus-graft disease (GVHD)[35]. In this study, 55 patients were given 1 – 5 infusions of either HLA-matched or mismatched MSCs. The amount of cells delivered ranged from 0.4 – 9 × 10^6 MSCs/kg body mass. There was no association between the HLA matching and improvement, and no adverse events were observed or infusion-related side-effects.

1.5.1.3 MSCs Safety in Pulmonary Disorders

The therapeutic use of allogenic MSCs have also been examined for treating COPD [142, 143]. No acute immunological reactions, infusional toxicities, deaths, or AEs related to the infusions were observed. In fact, a decrease in the inflammatory marker, CRP, was observed, suggesting that allogenic MSCs may generally decrease inflammation, particularly in older patients with co- morbidity.

1.6 Efficacy Information

Efficacy of allogenic MSCs have been shown in numerous clinical trials.

1.6.1 MSCs Efficacy in Cardiac Disease

Numerous studies have shown the efficacy of MSCs to treat cardiac disease.

Under the sponsorship of Osiris Therapeutics, an initial phase I study involving 10 centers and 53 patients demonstrated the provisional efficacy of allogeneic MSC therapy in patients with acute infarction[39]. Subsequently, a pilot study involving 8 patients with ischemic cardiomyopathy suggested that MSC therapy promotes the highly effective repair of ischemic cardiac damage [126]. The therapy produced reverse remodeling, and also reduced the circumferential extent of the infarct scar.

There is also substantial clinical evidence that intravenous delivery of bone marrow-derived mononuclear cells (MNCs) and MSCs can improve chronic left ventricular dysfunction. Under the sponsorship of Osiris Therapeutics, a multicenter phase I study suggested efficacy of intravenous allogeneic MSC therapy in patients who treated 3-10 days post-MI [144].

Additionally, a phase I randomized trial provided preliminary evidence for the potential efficacy of allogeneic versus autologous MSCs delivered via transendocardial stem cell injection (TESI) in patients with chronic ischemic cardiomyopathy [145]

In the POSEIDON trial [80, 135], therapeutic MSC favorably affected patient functional capacity, quality of life, and ventricular remodeling. In this phase 1 open-label trial, 30 patients were divided into 6 subgroups and given either autologous or allogenic MSC at a single dose or 20, 100, and 200 x 10^6 MSCs. Relative to baseline, autologous but not allogeneic MSC therapy was associated with an improvement in the 6-min walk test (6MWT) and the MLHFQ score. However, allogeneic MSCs reduced end-diastolic volume (EDV), possibly better than autologous MSCs. Both allogenic and autologous MSCs significantly reduced sphericity index and infarct size, but did not increase ejection fraction (EF).

In the Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HFT) open-label phase 1 trial, 8 patients with ischemic cardiomyopathy were administered MSCs and bone marrow mononuclear cells (MNCs) [134]. The patients fell into one of two age groups: younger than 60 years old, or 60 years or older. These preliminary studies showed that MSC therapy improved 6MWT and MLHFQ scores, and produced reverse remodeling with ~12% EDV reduction and regional function as measured by –Ecc, a cardiac MRI derived index reduced myocardial infarction size. Furthermore, older patients did not have an impaired response to MSC therapy compared to the young patients.

A multi-center, randomized, double-blind, placebo-controlled study was performed to evaluate the safety and preliminary efficacy of allogeneic MSCs administered after myocardial infarction[40]. In this study, 53 patients were treated with one of three cell-dose levels of allogeneic MSCs (0.5, 1.6 and 5.0 cells/kg body weight) or placebo administered intravenously. No HLA matching was performed in this study and administration was found to be safe and well tolerated at all dose levels (with 5.3 adverse events per patient in the MSC-treated group vs. 7.0 in the placebo group). No deaths were reported and no serious adverse events were attributed to MSC administration. Compared to those who received placebo, patients that received MSCs had improvement in the frequency of arrhythmic events and premature ventricular contractions, post-event ejection fraction for patients with major anterior wall infarctions, overall clinical status, and notably post-infusion pulmonary function as measured by FEV1 percent predicted (increased 17% in the MSC- treated group vs. 6% for placebo group, $p < 0.05$).

Cumulatively, these data support the hypothesis that cellular cardiomyoplasty using allogeneic MSC therapy is a safe and effective. These results formed the basis for the approved CRATUS study on aging frailty (NCT02065245).

1.6.2 MSCs Efficacy in Graft Versus Host Disease (GVHD)

In a phase II trial for treating acute GVHD, 55 patients received between 1 or more intravenous infusions of 1.4 x 106 allogenic MSCs/kg body mass (median dose)[35]. Twenty-seven (27) patients received one dose, 22 received two doses, and 6 received 3-5 doses. The MSCs were obtained from HLA-identical sibling donors (n=5), haploidentical donors (n=18), or HLA-mismatched donors (n=69). Thirty (30) of the patients had a complete response, and improvement was seen in a further 9 patients. The complete responders had significantly higher 1-year and 2-year survival rate. Furthermore, the response rates were not associated with HLA-matching.

1.6.3 MSCs Efficacy in Pulmonary Disorders

The therapeutic use of allogenic MSCs have also been examined for treating COPD [143, 146]. Patients received 4 monthly infusions of 1 x 10^8 MSCs. While the preclinical results indicated the promise of MSCs to treat COPD, preliminary efficacy results suggest that, while not detrimental to the patients, MSCs may not be effective at treating this condition. However, the sample size for these preliminary trials was small, so further investigation is required.

1.6.4 MSCs Efficacy in Endothelial Disorders and Metabolic Syndrome

Mesenchymal stem cells (MSCs) have the potential for clinical benefit in the metabolic syndrome by virtue of their anti-inflammatory, antifibrotic, and pro-angiogenic properties[54,55], and their ability to stimulate endogenous progenitor cells [57, 147]. Thus, it was recently examined whether MSCs are capable of stimulating the bioactivity of circulating EPCs and improve endothelial function in failing circulation[77]. The results showed that allogeneic, but not autologous, MSCs improve EPC bioactivity and endothelial function in heart failure patients, regardless of the etiology. These findings suggest a novel clinical beneficial effect of allogeneic MSCs transplantation as a potential general treatment for disorders associated with endothelial dysfunction.

1.6.5 Tabulated Previous Human Experience

Table 2. Previous Human Experience

Trial Name	Start Date	PI	Disease	N	Cell Doses	Safety Result	Efficacy Results
Stem Cell Therapy for Vasculogenesis in Patients With Severe Myocardial Ischemia	2005-Dec	Kastrup, J	CAD	31	3 - 72	Safe	3M and 6M post ↑ Exercise ↓ Angina attacks ↑ Cardiac function 3 years post ↑ Exercise ↓ Angina Rates
Combined CABG and Stem-	2006-Oct	Harjula, A Patila,	CAD	39	5.2 - 13.5	Safe	↓Local scar No Δ LVEF
Cell Transplantation for Heart Failure							
TAC-HFT	2008-Aug	Heldman, A	CAD	59	100, 200 placebo	Safe	↑ MLHFQ ↑ 6 minute walk test ↓ Infarct size

Poseidon	2010-Mar	Hare, J	CAD	30	20, 100, 200	Safe	↓ Mean EED ↓ SI Autologous MSCs ↑ 6 minute walk test ↑MLHFQ, Allogeneic MSCs ↓ LVED
OPTIPEC	2005-Jan	Emmerich, J	PAD/ CLI	20		Safe	Active neoangiogenesis
Study on Induced Wound Healing Through Application of Expanded Autologous Bone Marrow Stem Cells in Diabetic Patients With Ischemia-induced Chronic Tissue Ulcers Affecting the Lower Limbs	2005-Aug	Tschoepe, D	PAD/ CLI	22	~50	Safe	↑Microcirculation
A Phase I Study of Human Cord Blood-Derived Mesenchymal Stem Cell Therapy in Patients with Peripheral Arterial Occlusive Disease	2007-May	Kim, D	PAD/ CLI	8	10	Safe	NoΔ ABI ↑ Mean value pain-free walking distance Complete ulceration healing in 3 patients No amputations, ↑Angiography scores
Phase II Combination Stem Cell Therapy for the Treatment of Severe Coronary Ischemia (CI)	2008-Nov	Lasala, G	PAD/ CLI	26	9	Safe	↑Walking time ↑ABI ↑Perfusion via 99mTc-TF

A Randomized, Double Blind, Multicentric, Placebo Controlled, Single Dose, Phase - i/ii Study Assessing the Safety and Efficacy of Intramuscular ex Vivo Cultured Adult Allogeneic Mesenchymal Stem Cells in Patients With Critical Limb Ischemia (Cli)	2009-Apr	Suresh, K S	PAD/ CLI	20	2/kg or placebo	Safe	↑ABPI ↑TcPO2
Mesenchymal Stem Cells in Multiple Sclerosis (MSCIMS)	2008-Oct	Connick	MS	18	Autologous 1-2/kg	Safe	No efficacy data
AllogeneiC Human Mesenchymal Stem Cells (hMSC) in Patients With Aging FRAilTy Via IntravenoUS Delivery. (CRATUS)	2014-Feb	Hare, J	Frailty	45	Allogeneic 20, 100 or 200 MSCs per infusion	Safe	↑MMSE scores ↓Inflammatory markers

1.7 Safety and Efficacy of Multiple Doses of MSCs

Active clinical trials and ongoing preclinical work have increasingly demonstrated the therapeutic efficacy and safety of allogeneic mesenchymal stem cells (MSCs). Mesenchymal stem cells are both immunoprivileged and immunosuppressive, thereby enabling their allogenic allograftic use [148]. For example, the POSEIDON study addressed the major issue of the use of allogeneic MSCs as a cell-based therapeutic [135].

Accumulating preclinical evidence now also supports the concept that repeated doses and/or co- administration of MSCs could further enhance the therapeutic outcomes. This is an extremely important area for research, since repeat dosing could potentially have an additive effect and/or reverse disease pathology, depending on the disorder.

Several clinical studies have demonstrated that multiple injections of MSCs are well tolerated with minimal side-effects; are safe; and exhibit no detriment to efficacy over single doses. For example, results from Franco Locatelli's group clearly demonstrate the safety and efficacy of multiple MSC infusions in children with steroid-refractory acute graft versus host disease (aGvHD) [127]. Doses in these studies were 1-2x10^6 MSCs/kg recipient body mass, and each child received on average 2 doses (range was 1-13 doses) separated

on average by 15 dy (range 3- 43 dy). Their results indicated increased effectiveness when the therapy was commenced early in the disease. Furthermore, their results indicated the therapeutic benefits of repeat doses to patients who did not achieve complete remission after a single dose.

In another very interesting study, a single patient with aggressive relapsing-remitting multiple sclerosis received MSC infusions for 4 years, starting after the second year of disease onset [128]. Eight sets of injections were given every 3-9 months apart, using either autologous bone marrow-derived MSCs, or allogenic umbilical-derived MSCs, ranging from 1.3 x107 - 3.27 x 10^8 cells per intravenous injection. Throughout the treatment period, the patient showed no disease progression clinically or radiologically. And in fact, the patient showed significant improvement (EDSS score improved from 3.5 to 2.0), oligoclonal bands were no longer detected, and many lesions that were observed prior to commencement of the therapy had resolved. All of which occurred without significant adverse effects.

In another study by Kurtz's group, compassionate use of adipose-derived MSCs were used to successfully treat a variety of autoimmune diseases in which other treatment options had been exhausted [149]. These included autoimmune inner ear disorder, multiple sclerosis, polymyositis, atopic dermatitis, and rheumatoid arthritis. The patients received 1-6 intravenous infusions of 2 x 10^8 MSCs within 1 month, and showed considerable improvement over the following months. Although the sample size was small, precluding statistical analyses, the preliminary results warrant further studies. In short, their results demonstrated the safety of MSCs, and suggest a therapeutic advantage to multiple MSC injections.

A Phase II study on the 3-year efficacy of allogenic MSCs in the treatment of system lupus erythrematosus (SLE) was reported by Lingyun Sun's group [150]. Their results demonstrated/confirmed the therapeutic efficacy of MSCs in treating SLE. However, no advantage was found by administering a second or higher dose of MSCs. Despite this lack of additional efficacy over a single dose, the results of this study confirmed the safety of multiple infusions of MSCs. It is also worth noting that the doses (1x10^6 MSCs/kg body mass) were administered only 1 week apart, and a potential benefit of a longer therapeutic dosing interval, e.g., monthly, has yet to be assessed. Along these lines, a recent report on the use of MSCs to treat chronic obstructive pulmonary disease (COPD) showed minimal therapeutic efficacy after 2 years [142]. While these preliminary results were somewhat disappointing, they nevertheless again demonstrated the safety of repeated MSC infusions, which were given 4 times at 30-day intervals (1x108 MSCs/infusion).

Taken together, these various clinical studies provide a solid rationale for repeat MSC infusions. In no case did the administration of multiple doses lead to a significant increase in the frequency of adverse effects, or to a decrease in therapeutic efficacy over a single dose. In many cases, repeated infusions indeed improved clinical outcomes, demonstrating an additive effect of MSC therapy. Given these promising results, investigations are now warranted to determine optimal dosing frequency and total doses of MSCs to administer.

1.8 Pharmacology and Toxicology Studies of BMSC

Preclinical data suggest that MSCs may safely be used in the treatment patients with chronic inflammation to produce cytokine suppression. Using the murine bleomycin model, several groups have shown that the administration of stem cells ameliorates bleomycin induced lung injury with no significant adverse effects.

Lee et al intravenously administered $1x10^6$ bone marrow derived MSCs to rats treated with bleomycin and found a decrease in bleomycin induced lung edema, neutrophil infiltration, collagen deposition, and overall mortality with no adverse effects reported[93]. Similarly, Ortiz et al intravenously administered 5×10^5 bone marrow derived MSCs to mice with no adverse effects reported. They found that after bleomycin exposure, MSCs home to sites of lung injury, lead to decreased fibrosis and extracellular matrix collagen deposition, and contribute to tissue repair[89]. Rojas et al intravenously administered 5×10^5 allogeneic bone marrow derived MSCs to mice treated with bleomycin and observed that lung injury attracts bone marrow derived MSCs via the production of soluble factors like G-CSF and GM-CSF which lead to MSC proliferation and migration and ultimately improved survival with no reported adverse effects[94].

These findings appear to extend beyond bone marrow derived MSCs. Cargnoni et al administered fetal membrane derived cells (both allogeneic murine and xenogeneic human) to bleomycin treated mice and found that placental derived stem cells, like MSCs, localize to the lung and reduce tissue damage associated with bleomycin exposure regardless of source or route of administration (intravenous, intraperitoneal, or intratracheal)[88]. They did find that intraperitoneal and intratracheal administration of cells led to mild to moderate lung inflammation but no fibrosis in the absence of bleomycin injury. Importantly, this was not seen with intravenous administration of cells. In another murine model, xenogeneic human umbilical cord derived MSCs were also shown to home to sites of bleomycin induced lung injury, inhibit the production of pro-inflammatory cytokines, and reduce lung injury and collagen deposition with no adverse effects reported[90].

Table 3. Results of preclinical animal studies of mesenchymal stem cell therapy for IPF.

Study	Model	Cell Type	Cell Delivery and Dose	Safety Results	Efficacy Results
Lee at al, 2006	**Rat**	**Allogeneic BMMSCs**	**Intravenous, 1 x 10^6 cells**	**No adverse effects reported.**	**Reduced edema, neutrophil infiltratration, collagen deposition. Improved survival.**
Ortiz et al, 2003	**Mouse**	**Allogeneic BMMSCs**	**Intravenous, 5 x 10^5 cells**	**No adverse effects reported.**	**Reduced inflammation and collagen deposition.**
Rojas et	**Mouse**	**Allogeneic**	**Intravenous,**	**No adverse effects**	**Reduced pro-inflammatory**

al, 2005		**BMMSCs**	**5 x 10^5 cells**	**reported.**	**cytokines.** **Improved survival.**
Cargnoni et al, 2009	**Mouse**	**Fetal membrane derived** **(Allogeneic murine and xenogeneic human)**	**Intra-peritoneal,** **4 x 10^6 cells** **Intratracheal, 1 x 10^6 cells** **Intravenous, 1 x 10^6 cells**	**Mild to moderate lung inflammation but no fibrosis induced by intraperitoneal or intratracheal administration.** **No adverse effects reported with I.V. administration.**	**Decreased neutrophil infiltration and fibrosis regardless of route of administration or source of cells.**
Moodley et al, 2009	**Mouse**	**Xenogeneic human umbilical cord derived MSCs**	**Intravenous, 1 x 10^6 cells**	**No adverse effects reported.**	**Reduced inflammation, pro-inflammatory cytokine production, and collagen deposition.**

In addition to safety data from preclinical animal studies, many patients have received allogeneic MSCs in clinical trials and infusions have all been well tolerated.

A multi-center, randomized, double-blind, placebo-controlled study was performed to evaluate the safety and preliminary efficacy of allogeneic MSCs administered after myocardial infarction[76]. In this study, 53 subjects were treated with one of three cell-dose levels of allogeneic MSCs (0.5, 1.6 and 5.0 cells/kg body weight) or placebo administered intravenously. No HLA matching was performed in this study and administration was found to be safe and well tolerated at all dose levels (with 5.3 adverse events per subject in the MSC-treated group vs. 7.0 in the placebo group). No deaths were reported and no serious adverse events were attributed to MSC administration. Improvements were seen in subjects receiving MSCs as compared with those receiving placebo in the frequency of arrhythmic events and premature ventricular contractions, post-event ejection fraction for subjects with major anterior wall infarctions, overall clinical status, and notably post-infusion pulmonary function as measured by FEV1 percent predicted (increased 17% in the MSC-treated group vs. 6% for placebo group, $p < 0.05$).

Allogeneic MSC infusion has also been studied in a phase II trial of MSCs for the treatment of severe acute graft versus host disease[68]. In this study, 55 subjects received 1-5 intravenous infusions of 1.4 x 10^6 cells/kg body weight from HLA matched and mismatched donors. A complete response was seen in 30 subjects and improvement was seen in 9

patients. Of note, response rates were not associated with HLA-matching. No infusion related side effects were noted and no long term adverse events were observed.

The safety and efficacy of allogeneic MSCs for the treatment of refractory lupus has also been explored[119]. Fifteen subjects received a single intravenous infusion of 1 x 10^6 cells/kg body weight. MSCs were derived from family members but were not HLA-matched. At 12 months, all subjects had improvement in disease activity as measured by 24-hour proteinuria (decreased from 2505.0±1323.9 to 858.0±800.7 mg/24hr, $p<0.05$) and SLE Disease Activity Index scores (decreased from 12.2±3.3 to 3.2±2.8, $p<0.05$). No serious adverse events were noted in any of the subjects.

1.9 UCMSCs

The alternative sources of MSC is of significant value. It has been reported that MSC could be isolated from various tissues, including periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle, deciduous teeth, fetal pancreas, lung, liver, amniotic fluid, cord blood and umbilical cord tissues[98,120,121]. Among those, cord blood and UC may be ideal sources due to their accessibility, painless procedures to donors, promising sources for autologous cell therapy and lower risk of viral contamination. However, the data on the isolation of cord blood-derived MSC are controversial[120,122]. In addition, the process of isolation of MSC is at the expense of losing hematopoietic stem cells in cord blood. Thus, UC should be focused on as an alternative source of MSC.

MSC's can be isolated from many different tissues like, bone marrow, adipose tissue, dental pulp etc. Umbilical cords are readily accessible, fewer limitation, (a usual medical waste) and are more similar to early embryonic sketch of human cell biology. Cells in the Umbilical cord are divided into different groups based on the regions like, Wharton's Jelly, Cord lining, and perivascular area, or cell type, epithelial, stromal, and smooth muscles; respectively, of umbilical cord. However, majority of research and investigation on Umbilical cord in vivo are based on use of accumulated fraction of whole umbilical cord tissue or Wharton's Jelly/perivascular area. MSC's derived from Wharton's Jelly matrix, located close to the vasculature of cord tissue, are more primitive, proliferative and immunosuppressive than their counterparts. MSC cultured from individual cord digested enzymatically, demonstrated plastic adherence, flow cytometric profile and immune-profile showed no significant difference than its adult counterpart.

The healing potential of UC derived stem cells and other MSC's in regenerative medicine serves as direct repairing, tissue remodeling, paracrine effects and influence on microenvironment and immunomodulation. Researchers have reported that gradual exposure to MSCs at a lesion site resulted in accumulation of various therapeutic proteins that were secreted and reacted with the microenvironment. Therefore, many different proteins secreted by MSCs influenced paracrine actions for recovery of the lesion site. The therapeutic proteins secreted by MSCs include growth factors, cytokines, extracellular matrix proteins, and antioxidants. When the paracrine effect of MSCs was assessed in various disease models, there were similar therapeutic effects through the actions of the proteins mentioned above. Although the general characteristics of MSCs include stemness,

tropism, differentiation, motivation, and a therapeutic paracrine effect, it is possible that the origin of the donor cells may influence their therapeutic effects.

UCMSCs shared most of the characteristics with BMMSCs, including fibroblastic morphology, typical immunophenotypic markers, cell cycle status, adipogeneic and osteogenic differentiation capacity, cytokine spectrum as well as hematopoiesis-supportive function. However, several differences have been observed.

The CFU-F frequency was significantly higher in UC-derived nucleated cells than in BM-derived nucleated cells. Secondly, the proliferation analysis revealed that UCMSC have a faster population doubling time[123].

With regard to their multipotency[124,125,126,127] UCMSC can be differentiated into bone, cartilage, neural and muscle cells as well as cardiomyocyte-like cells, as they express cardiac troponin-I and *N*-cadherin[108,124,125,126,127,128].

Our group and other groups, successfully managed to grow UCMSC under GMP-compliant culture conditions, while retaining their phenotypic and functional properties[112]..

The Superiority of UCMSCs in Clinical Application

BM is considered as a traditional source of MSCs, and most of the knowledge concerning MSCs comes from BM studies. However, several limitations restrict the clinical application of BMMSCs. Harvesting BMMSCs involves an invasive and painful procedure, which can cause infection, bleeding, and chronic pain. On the contrary, UCMSCs are obtained after delivery of a baby from a sample that would be discarded inevitably. The process is noninvasive, painless, and harmless for the mother and the baby. UC contains a significant amount of MSCs which can be easily collected and cultured[98,113]. In vitro, UCMSCs have greater expansion capability and faster growth rate[98,123,129,130], indicating the advantage for rapid expansion and consequent downstream application. UCMSCs express a lower level of HLA-class I than BMMSCs[98]. UCMSCs appear to have greater immunosuppressive effects[130], indicating their better role in the management of GVHD. Therefore, UC represents a good alternative source of MSCs and should not be discarded as medical waste[131].

1.10 UCMSCs: Preclinical Experience

The use of adult Bone Marrow is considered an extensive and renowned source of MSCs. MSCs can also be obtained from umbilical cord, which was contemplated as clinical waste (Zhimai et al, 2012).

In a study for the treatment of Spinal cord injuries in dogs (Hak-Hyun et al, 2012), the study concentrated on the assimilation and survival of allogeneic MSCs (Adipose derived MSC, Bone Marrow MSC, Umbilical Cord Blood MSC, and Wharton's WJ derived MSC) in the injured spinal cord. Overall, 6.0×10^6 cells were injected into the parts of the injured spinal cord. The results showed macrophage infiltration into the lesion epicenter in the MSCs group and reduced levels reactive astrogliosis but most notably there was a decrease of

COX-2 protein in Umbilical Cord stem cell compared to the other MSC groups (Hak-Hyun et al, 2012). The Levels of GFAP were decreased, while the expression of Tuj1, NeuN, and NF160 were increased compared to the other MSCs groups. As a result, there was less inflammation to the site and more nerve regeneration, as well as neuroprotection (Hak-Hyun et al, 2012). This study concluded that allogeneic MSCs can survive in injured spinal cords where they can develop into host tissue without the need of immunosuppressive agents and improve hind-limb function (Hak-Hyun et al, 2012).

Another study (Mei-Juan et al, 2011) researched the effects of UC MSCs on ataxic mice by cytosine beta-D- arabinofuranoside (Ara-C). 2×10^6 HU-MSCs were delivered intravenously once a week for three consecutive weeks. The use of open field test and rotarod test, measured the Neurological function score of the mice on a weekly basis. The results showed that ataxic mice (n = 6) remained on the machine much shorter than normal ICR-mice (n = 6) (68.11 ± 9.59 s vs. 330.11 ± 51.87 s, $p < 0.01$). On week 7 there was an improvement in behavior performance in terms of relief of behavior impairment and morphologic atrophy of ataxic mice. By the 9^{th} week a significant improvement was achieved following UC-MSCs treatment (UC-MSCs vs. control: 90.56 ± 13.75 seconds vs. 58.00 ± 9.43 s, $p < 0.05$) until the 12th week (Mei-Juan et al, 2011). With the Open field test, there was also a difference between the control group (n = 6) and UC-MSCs group (n = 6) at week 6 post-transplant and a statistical significance at the 8th week (74.33 ± 10.05 vs. 128.67 ± 16.98, $p < 0.05$) (Mei-Juan et al, 2011). These results indicated 8 weeks after the application UC-MSCs implantation considerably improved the motor skills of ataxic mice. Cerebellar atrophy was also relieved as well as a decrease in the number of apoptotic cells in the therapeutic group using UC-MSCs (Mei-Juan et al, 2011). Vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) had a heighted expression in UC-MSC treated mice.

Table 4:

Study	Model	Cell Type	Cell Delivery and Dose	Safety Results	Efficacy Results
Mei-Juan et al, 2011	**Rat**	**Allogeneic UCMSCs**	**Intravenous 2×10^6 HU-MSCs**	**No adverse effects reported.**	**Relief of behavior impairment and morphologic atrophy**

Hak-Hyun et al, 2012	**Dog**	**Allogeneic UCMSCs**	**Cranial, epicenter, and caudal lesions of the spinal cord parenchyma; 20 µl/site 6.0 × 10^6 cells**	**No adverse effects reported.**	**Improve hind-limb function**
		UCMSCs	**10^6 MSC/200 µL/PBS**		
		UCMSCs	**6 × 10^5 hUC-MSCs**		
		UCMSCs	**5 × 10^5 hUC-MSCs**		

1.11 UCMSCs: Previous Experience in Humans

Due to its noninvasive procedure and its lesser possibility for genetic alterations, Human UC tissue is viewed as a greater MSC source for bone regeneration. It has also shown great therapeutic effects in many diseases in animal studies due to their ability for differentiation and self- renewal[132]. Native MSCs ($CD90^+$, $CD45^-$, and $CD235a^-$ cells) and native ECs ($CD31^+$, $CD235a^-$, and $CD45^-$) from numerous experiments showed that from a single UC donation, the number of cells obtained was higher on an average than a 20-ml specimen of BM aspirate (average 90- and 11-fold, respectively)[132]. In this procedure, the UC cells were set apart from 19 cesarean section patients that were on their full term. About 0.2 g of UC tissue was mechanically cut up into miniature pieces. 7.5 × 10^7 nucleated cells/per gram of tissue was considered the average released UC isolates[132].

Sun et al, 2010[118] did a study to understand the safety and efficacy of allogeneic UC MSCT in patients with severe and treatment-refractory systemic lupus Erythematosus (SLE). A single-arm trial was conducted using 16 SLE patients who either had life-threatening visceral involvement or whose disease wasn't applicable to standard treatment. From 2007 to 2009, the results concluded with a significant improvement in the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score, anti-dsDNA antibody, renal function, levels of serum ANA, complement C3, and serum albumin. There was also a balance between TH1- and Th2 related cytokynes. This resulted in a reduction of disease activity in patients. For each patient who had lupus nephritis, there was a 24-hour proteinuria measurement that had impaired renal function at baseline. At the 3-month visit, 15 patients had a decreased effect in proteinuria. Eight patients had greater reduction in proteinuria after 6 months. Two patients stayed negative of preoteinuria after 2 years[118]. There was higher than normal elevated serum creatinine levels at baseline for six patients. This improved after 3 months and even greater at 6 months[118]. Two out of16 patients had

seizures and after the UC MSCT, none demonstrated recurrences. In some patients that had severe hypertension, there symptoms were controlled after the UC MSCT[118].

LU et al, 2006[98] created a study using 36 UC and six BM samples. Fibroblastic-like morphology, adipogenic and osteogenic differentiation potentials immunophenotype, hemotapoiesis supportive function and cell cycle status are some of the mostly shared characteristics that UC-MSC and BM-MSC share, yet there are still other differences that have set them apart. For example, CFU-F, which represent mesenchymal progenitor cell, frequency tested lower in BM nucleated cells compared to UC nucleated cells. Another comparison with UC- MSC, BM-MSC have higher levels of expression CD106 and HLA-ABC and a lower proliferation capacity (LU et al, 2006). In contrast, proliferation analysis has shown that UC-MSC as opposed to BM-MSC have a faster population doubling time and it maintained that way even after 30 passages, while BM-MSC decreased in population doubling time after P6[98].

Table 5

Study	**Model**	**Cell Type**	**Cell Delivery and Dose**	**Safety Results**	**Efficacy Results**
Kouroupis et al, 2013[132]	Human	Allogeneic **UCMSCs**	7.5×10^7 nucle-ated cells/per gram of tissue	No adverse effects reported.	Higher count of MSC and EC in a UC donation compared to 20 mL of BM aspirate
Sun et al, 2010[118]	Human	Allogeneic **UCMSCs**	1×10^6 per kg of body weight	No adverse effects reported.	Reduced proteinuria, decreased (SLE)
Lu et al, 2006[98]	Human	Allogeneic **UCMSCs**	1×10^6/cm mean number of nucleated cells	No adverse effects reported.	HigherCFU-F frequency, faster population doubling time
Hu et al, 2013	Human	Allogeneic	Intravenous $1.5 – 3.2 \times 10^7$	No adverse effects reported.	Reduced dosage of insulin

		UCMSCs /WJMSCs	cells		supplementation
Wang et al, 2015[128]	Human	Allogeneic **UCMSCs / WJMSCs**	Central line, 0.8-1.6 x 10^6/kg cells	No adverse effects reported.	Decrease in hematuria

Hu et al, 2013 administered two doses of 1.5–3.2 × 10^7 of WJ-MSC to 15 patients in a double-blind study for an onset of type 1 diabetes. It was done at a 4-week interval by intravenous delivery. For 3 out of 15 the use of insulin supplementation was terminated and 3 out of 15 the daily dosage was decreased from 15-50%. 8 out of 15 patients, the dosage was decreased by more than 50%, all within a period of 24 months. There was just 1 patient that did no effect from the MSC treatment (Hu et al, 2013). There were no adverse effects during the treatment.

A study done by Wang et al, presented that HC (Late-Onset Hemorrhagic Cystitis) though a common complication after allogeneic hematopoietic stem cell transplantation, can be detrimental to the patient by causing hemorrhagic inflammation of the bladder. There is a continuous association with Graft-versus- host disease (GVHD) and late onset of HC. 1-3 doses of 0.8–1.6 × 10^6 /kg of WJ-MSC were administered through a central line to seven patients that contained this disease. Due to this stem cell treatment, gross hematuria was relieved within 2-12 days as opposed to patients that did not have this treatment, which took longer to clear up, thus showing a significant difference between the two groups. Multiple results have confirmed that WJ-MSC are a helpful treatment for late onset hemorrhagic cystitis[128].

Table 6

Study	Phase	Model	Cell Type	Cell Delivery and Dose	Identifier
Clinical Study of Umbilical Cord Tissue Mesenchymal Stem Cells (UC-MSC) for Treatment of Osteoarthritis Translational Biosciences Panama City, Panama Ruben Berrocal, MD	I/II	Human	Allogeneic UC-MSCs Intra-arterial	Arm 1: will receive one intra-articular injection of UC-MSC into the Knee and Arm 2: will receive IV UC-MSC once per day for 3 consecutive days.	NCT02237846

An Open-label, Non-randomized, Phase I/II Study of Allogeneic Human Umbilical Cord Tissue-Derived Mesenchymal Stem Cells (UC-MSC) and Liberation Therapy (When Associated With Chronic Cerebrovascular Venous Insufficiency) in Patients With Relapsing Remitting Multiple Sclerosis (RRMS) Novo Cellular Medicine Institute San Fernando, Trinidad, Trinidad and Tobago PI: Dr. Bill Brashier, M.D.	I/II	Human	Allogeneic UC-MSCs IV-infusion & Intrathecal	Intravenous administration of 50 million Allogeneic UC-MSCs and intrathecal administration of UC-MSCs in dose of 100 million along with liberation therapy (when associated with CCSVI)	NCT02587715
Feasibility Study of Umbilical Cord Tissue Derived Mesenchymal Stem Cells (UC-MSC) in Disease Modifying Anti-Rheumatic Drugs (DMARD) Resistant Rheumatoid Arthritis Translational Biosciences Panama City, Panama PI: Jorge Paz-Rodriguez, MD	I/II	Human	Allogeneic UC-MSCs	Not available	NCT01985464

1.12 Pharmacology and Toxicology Studies of Mesenchymal Stem Cells

Preclinical data suggest that UC MSCs may safely be used in the treatment of patients with chronic inflammation to produce cytokine suppression.

New studies are developing for the future and prevention of health incidents. Radiation injuries can occur as a result of nuclear accidents and radio-therapy. One of the most radiosensitive intraabdominal organ is the small intestine. The function and integrity of the small intestine can be affected by abdominal irradiation, which can cause acute and/or chronic gastrointestinal tract (GIT) disorders (Zhimai et al, 2012). After the injection of high molecular weight fraction (HMWF) from hypoxic-conditioned media of UC MSC on radiation-induced intestinal injury of 10 Gy (60Coγ-ray), there was an increased survival rate in mice, improved small intestinal structural integrity and a decrease of diarrhea occurrences (Zhimai et al, 2012). Zhimai concluded that the infusion of an MSC-secreted HMWF has a key role in accelerating the recovery of radiation-induced intestine structural damage and averting radiation-induced intestinal injury in an abdominal irradiation mice mode. Thus, presenting a therapeutic approach for treatment of radiation induced injury (Zhimai et al, 2012).

(Koh et al, 2008) implanted hUC-MSCs into the damaged hemisphere of immunosuppressed ischemic stroke rats. Results showed a reduction in infarct volume and an improvement of neurobehavioral function relative to control rats. Three weeks after implantation, a great amount of the hUC-MSCs that was implanted were existent in the damaged hemisphere, even some of these cells showed levels of neuron-specific markers. There was also an indication of increased Nestin expression in the hippocampus in the hUC-MSC-implanted group as appose to the control group. Signs of neuroprotection on neurological diseases (i.e Parkinson diseases) in animal were exhibited in transplanted HU-MSCs[133]. The study concludes that hUC-MSCs like most MSCs could possibly have advantages for the induction of neuronal differentiation[133].

The loss of neurons and glia, degeneration of axons, and demyelination around the lesion site are common effects of mammalian spinal cord injury. Post lesion scar barriers and myelin- associated inhibitors can obstruct axonal regeneration. Even intrinsic cell renewal after application of mitogenic agents, does not have the capability to permit the recovery of a spinal cord injury (Chang-Ching et al, 2008). HUMSCs can differentiate into chondrogenic, adipogenic, myogenic, and myogenic osteogenic cells in vitro. In this study, using a complete transection model in rats, (Chang-Ching et al, 2008) assessed the effect of transplantation of UCMSCs on axon regeneration in the injured spinal cord. Within the first three weeks after transplantation, signs of recovery in locomotor function were detected and showed significant difference between the control group, which had no locomotor function throughout the 16-week trial, and the transplanted groups. By the end of the study, (Chang-Ching et al, 2008) uncovered that the transplanted rats were able to synchronize movement between the forelimbs and the hind-limbs to achieve a walking motion. This study shows proof that transplantation of HUC-MSCs can help with the locomotor recovery, depression in the activities of microglia and reactive astrocytes, reduction of astroglial scarring in the lesion, and restoration of corticospinal fibers after spinal cord transection in rat (Chang-Ching et al, 2008).

Table 7

Study	Model	Cell Type	Cell Delivery and Dose	Safety Results	Efficacy Results
Zhimai et al, 2012	Mice	Allogeneic	Intravenously by the tail	No adverse effects reported.	Accelerates the recovery of radiation-induced intestine structural damage
		UCMSCs	10^6 MSC/200 µL/PBS		
Koh et al, 2008	Rat	Allogeneic	Implanted into the damaged hemisphere	No adverse effects reported.	Exhibit neuroprotection on neurological diseases in animal models i.e Parkinson disease
		UCMSCs	6 × 10^5 hUC-MSCs		
Chang-Ching et al, 2008	Rat	Allogeneic	transplanted into the lesion site	No adverse effects reported.	Improvements in locomotion
		UCMSCs	5 × 10^5 hUC-MSCs		

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

- To compare the safety of UCMSCs and BMMSCs administered intravenously in patients with chronic inflammation due to metabolic syndrome.

2.1.2 Secondary Objectives

- To demonstrate the efficacy of UCMSCs and BMMSCs administered intravenously in patients with chronic inflammation due to Metabolic Syndrome as assessed by (see Table 9 in Section 2.2.2):
    - symptom related quality of life
    - cardiovascular status
    - inflammatory cytokines
    - endothelial function
    - lipid profile
    - glucose homeostasis
    - 1 year survival

2.2 Study Endpoints

2.2.1 Primary Endpoints (Safety)

Safety will be assessed as the incidence within one month after an infusion of any treatment emergent serious adverse events (TE-SAEs), defined as one or more of the following events that: is life-threatening event (e.g., stroke or non-fatal pulmonary embolism); requires inpatient hospitalization or prolongation of existing hospitalization (e.g., for worsening dyspnea); results in persistent or significant disability/incapacity; results in death; or leads to clinically significant untoward laboratory test result(s) or medical condition(s), determined per Investigator' judgments.

2.2.2. Secondary Endpoints (Efficacy)

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

3. STUDY DESIGN

3.1 Description of the Study

A Pilot Phase will be performed to test the safety of dose and volume of cells administered via peripheral intravenous infusion. The first three (3) subjects in-group 1 (UCMSCs) will be treated first and safety will be fully assessed. Only if there is no evidence of treatment emergent SAEs in Group 1, treatment of subjects in Group 3 be undertaken.

The subjects in the high dose (Groups 3 & 4) will not be treated until the subjects in the low dose (Groups 1 & 2) have completed their one-month follow-up assessments and safety measures have been reviewed to confirm that there have been no treatment emergent SAE's. Treatment emergent SAE's, defined as the composite of: death, non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities, prior to proceeding with the treatment of further subjects.

Subjects in each pilot phase group (Group 1, 2, 3 and 4) will not be treated less than 5 days apart.

Subjects in the pilot phase will receive a telephone call 5 days post infusion (+/- 1 day) to review adverse events.

The randomized portion of the study will be conducted after a full review of the safety data from the Pilot Phase by the DSMB.

Following the pilot study (12 subjects), thirty (30) subjects that meet inclusion criteria will be scheduled to undergo peripheral intravenous infusion.

3.2. RANDOMIZATION STUDY

This Phase I/II, randomized, blinded, placebo-controlled study is designed to evaluate the safety and tolerability of UCMSCs versus BMMSCs in subjects with chronic inflammation due to the metabolic syndrome and to explore potential efficacy at baseline, 3 and 6 months.

In the randomized phase of the trial, product will be prepared by ISCI GMP laboratory personnel. The selected personnel will not have contact with the investigators or subjects for this trial. At the time of administration, opaque tubing will be used to maintain double blinding. Treatments will be administered once and will consist of 1 x 10^8 (100 million) UCMSCs, 1 x 10^8 (100 million) BMMSCs or placebo. After each infusion, subjects will be monitored for immediate complications.

Continued safety and tolerability with review of adverse events (AEs) will be monitored at each visit. Efficacy parameters (6MWT and QOL questionnaires) will be assessed at various study visits. Clinical laboratory tests to assess safety will be performed at every clinic visit, excluding the baseline visit.

4. SUBJECT SELECTION

4.1 Inclusion Criteria

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

1. Provide written informed consent
2. Subjects age ≥ 21 and ≤ 95 years at the time of signing the Informed Consent Form.
3. Each subject must have endothelial dysfunction. Endothelial dysfunction criteria: impaired flow-mediated vasodilation (FMD < 7%).
4. At the time of enrollment, each subject must meet at least 3 out of the 5 criteria under the harmonized definition of the metabolic syndrome, consisting of the following:

Table 10. Criteria for the Metabolic Syndrome

Measure	Categorical Cut-Off Points	Alternative Indicators
Waist circumference	US defined: ≥ 102 cm (males) or ≥ 88 cm (females)	Ethnic and country-specific definitions
Elevated triglycerides	≥ 150 mg/dL (1.7 mM)	Drug treatment for elevated triglycerides
Reduced HDL	Males: <40 mg/dL (1.0 mM)	Drug treatment for reduced

	Females: <50 mg/dL (1.3 mM)	HDL-C
Elevated blood pressure	Systolic ≥ 130 mm Hg and/or Diastolic ≥ 85 mm Hg	Antihypertensive drug treatment for a history of hypertension
Elevated fasting glucose	≥ 100 mg/dL	Drug treatment of elevated glucose

4.2 Exclusion Criteria

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

1. Be a female who is pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods. Female subjects must undergo a blood or urine pregnancy test at screening and within 36 hours prior to infusion.
2. Inability to perform any of the assessments required for endpoint analysis.
3. Active listing (or expected future listing) for transplant of any organ.
4. Clinically important abnormal screening laboratory values, including but not limited to: hemoglobin <8 g/dl, white blood cell count <3000/mm^3, platelets <80,000/mm^3, INR > 1.5 not due to a reversible cause (i.e. Coumadin), aspartate transaminase, alanine transaminase, or alkaline phosphatase > 3 times upper limit of normal.
5. Serious comorbid illness that, in the opinion of the investigator, may compromise the safety or compliance of the subject or preclude successful completion of the study. Including, but not limited to: HIV, advanced liver or renal failure, class III/IV congestive heart failure, myocardial infarction, unstable angina, or cardiac revascularization within the last six months, or severe obstructive ventilatory defect.
6. Have known allergies to penicillin or streptomycin.
7. Be a solid organ transplant recipient. This does not include prior cell-based therapy (>12 months prior enrollment) bone, skin, ligament, tendon or corneal grafting. Have a history of organ or cell transplant rejection.
8. Have a clinical history of malignancy within 3 years (i.e., subjects with prior malignancy must be disease free for 3 years), except curatively-treated basal cell carcinoma, squamous cell carcinoma, melanoma in situ or cervical carcinoma, if recurrence occurs.
9. Have a non-pulmonary condition that limits lifespan to < 1 year .
10. History of drug abuse (illegal "street" drugs except marijuana, or prescription medications not being used appropriately for a pre-existing medical condition) or alcohol abuse (≥ 5 drinks/day for > 3 months), or documented medical, occupational, or legal problems arising from the use of alcohol or drugs within the past 24 months
11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C, and/or Syphilis – VDRL (If VDRL is reactive Confirmation with FTA-ABS is needed (Syphilis)).
12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial.
13. Patients with EF<45% (heart failure patients).

14. GFR ≤ 35 (chronic kidney disease stage 3 or higher).
15. Liver disease (elevated LFTs greater than 3x upper limit of normal).
16. Advanced pulmonary disease (requiring home oxygen and/or less than 1 expected life span).
17. Proliferative diabetic retinopathy if history of diabetes
18. Hemoglobin A1C greater than 7.

4.3 Concomitant Treatments, Procedures, and Nondrug Therapies

Subjects will receive standard-of-care medical management of metabolic syndrome, as recommended by the ATP III, American Heart Association (AHA) and the National Institutes of Health (NIH), and the Endocrine Society. All concomitant medications (prescription or over-the counter) as well as procedures or nondrug therapies (e.g. continuous positive airway pressure, pulmonary rehabilitation) will be recorded at the initial screening visit and updated at each subsequent visit.

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 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. MESENCHYMAL STEM CELL DONORS

The availability of allogeneic BMMSCs offers the potential for an "off the shelf" product for subjects. Significant data has been generated to demonstrate that the allogeneic BMMSCs are immune-privileged and can be infused without immune rejection despite disparate HLA phenotypes.

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.

Allogeneic donor testing will include anti-HIV-1/2, anti-HTLV I/II, anti-HCV, HIV-1 nucleic acid testing, HCV nucleic acid testing, HBsAg, anti-HBc(IgG and IgM), CMV, West Nile Virus nucleic acid, *T. cruzi* ELISA (Chagas), Zika virus, Syphilis – VDRL (Confirmation with FTA-ABS if needed (Syphilis)) and RPR. Potential donors testing positive for any of these infectious diseases with the exception of CMV, will be ineligible. Bone marrow aspirates will be obtained from normal individuals and BMMSCs will be isolated and expanded.

5.1 Bone Marrow Aspiration, cord tissue harvest, MSC culture for generation of MSCs and Placebo formulation.

A total of approximately 60ml to 120ml of BM will be obtained from a healthy donor's bone marrow. BM will be aspirated from the posterior iliac crest into heparinized syringes. The mononuclear cell 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 washed cells will be sampled and viable cell numbers determined. The MNCs will be prepared with antibiotic (ie. penicillin or streptomycin). The BM mononuclear cells 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. 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 consenting and collecting the umbilical cord tissue, the maternal blood will be screened for infectious disease.

Collection of Umbilical Cord Tissue: The cord will be clamped then cut and 6 to 15 cm of the cord will be transferred into a labeled container containing the collection media supplemented with antibiotics. A specialized validated temperature controlled container (2–15°C) will be used. The collection kit with the cord will be transported to the processing facility within 36 hrs. Upon arrival at the processing facility, the sample will be assigned a unique ID that enables the unit to be tracked throughout the process.

The umbilical cord tissue will be enzymatically digested. The digested cells will be washed and cultured. An expansion process of 3 passages will take place followed by cryopreservation.

Placebo will be formulated as following:

The placebo group will receive infusion of PlasmaLyte-A supplemented with 1% HSA. The placebo product will undergo QC testing before injection for gram stain and endotoxin. The sterility (aerobic, anaerobic and fungal) samples will be taken from final product and cultured for 14 days. Product will only be released if testing meets following criteria:
Gram Stain – Negative
Endotoxin - < 5EU/kg/hr.

5.2 Donor Eligibility

5.2.1 Bone Marrow Donors Screening and Testing

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

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

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

- Hepatitis B surface antigen (HBsAg)
- Hepatitis B core antibody (HBcAb)
- Hepatitis C virus antibody (HCV Ab)
- Human Immunodeficiency Virus (HIV) antibody (HIV 1/2)
- Cytomegalovirus antibody (CMV)
- HCV/HIV Nucleic Acid test
- West Nile Virus Nucleic Acid test
- Rapid Plasma Reagin (RPR) or VDRL (if VDRL reactive confirmation with FTA-ABS is needed (Syphilis))
- Human T-lymphotropic Virus I/II (HTLV I/II)
- *T. cruzi* ELISA test (Chagas disease)
- Zika Virus testing (RNA qualitative Real Time RT-PCR, Serum/Urine or Zika Virus Antibody (IgM), MAC-ELISA)

If a donor tests positive for CMV the donor may still be able to participate.

b. Prospective donors will also have the following tests:

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

c. Eligibility Criteria for Normal Donors will include:

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

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

5.2.2 Umbilical Cord Tissue Donor Screening and Testing

Upon consent the maternal blood will be screened for infectious disease prior to collecting the umbilical cord tissue. This will prevent the introduction, transmission, and spread of communicable disease agents and diseases.

All Umbilical cord tissue donors between the ages of 18 to 35 must be tested and screened for the following:

a. Donor screening and reviewing of relevant medical history records and social history for risk factors will be evaluated by history and physical examination. The history will include:
   - History of malignancy

- Bleeding abnormalities
- Prior deep venous thrombosis
- Known cardiac or pulmonary conditions
- Prior blood transfusions
- Vaccinations
- Questions to identify persons at risks of infectious disease transmission
- Questions to identify persons at risk of transmitting hematological or immunological disease

b. Umbilical donors are tested for transmissible infectious diseases as follow (results from any viral or infections agent testing must be negative):
   - Hepatitis B surface antigen (HBsAg)
   - Hepatitis B core antibody (HBcAb)
   - Hepatitis C virus antibody (HCV Ab)
   - Human Immunodeficiency Virus (HIV) antibody (HIV 1,2)
   - Cytomegalovirus antibody (CMV) Immune Screen (total immunoglobulin, including IgG and IgM). Positive samples are tested for CMV DNA.
   - HCV/HIV Nucleic Acid test
   - West Nile Virus Nucleic Acid test
   - Rapid Plasma Reagin (RPR)
   - Human T-lymphotropic Virus I/II (HTLV I/II)
   - *T. cruzi* ELISA test (Chagas disease)
   - Zika Virus Testing
   - VDRL (confirmation with FTA-ABS if needed (Syphilis))

   Results from any viral or infectious agent testing must be negative.

c. Eligibility Criteria for Umbilical Donors will also include:
   - Female gender
   - No history of malignancy
   - Cancer
   - Bleeding abnormalities
   - Neurological diseases
   - Blood and bleeding disorders, including sickle cell disease
   - History of autoimmune disorders
   - No family history (biologic mother, father or sibling) of genetic disorder that may affect the recipient.
   - Negative tests for Hepatitis B, Hepatitis C, RPR, VDRL, Chagas, HIV ½, HTLV I/II and NAT for HCV, HIV, Zika Virus and West Nile Virus.
   - No diabetes (gestational diabetes is not an exclusion criteria)

5.3 Bone Marrow Donor Consent

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

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

5.4 Follow-up Schedule for Bone Marrow Donors

After discharge from the hospital, the bone marrow donor will be contacted by the study team with a follow-up telephone call to determine the well-being, health status of the donor and/or if any adverse events have occurred. Attempts should be made to follow-up via phone within a week of the BMA procedure. The donor will be provided with contact telephone numbers in the consent form for any questions or comments.

5.5 Biomarker Assessment for Bone Marrow Donors

A separate blood sample of about 10mL will be obtained at the donation visit for gene expression profiling. 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' gene expression or clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

6. TREATMENT OF SUBJECTS

6.1 Study Investigational Product

The investigational product (IP) consists of BMMSCs obtained from donor bone marrow. 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.

The investigational product (IP) consists of UCMSCs obtained from donor cord tissue. 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. Cord tissue will be obtained from normal donors.

6.2 Dosing

The allogeneic BMMSCs or UCMSCs will be derived from normal donors meeting criteria for allogeneic unrelated human bone marrow stem cell source or human cord tissue manufactured by the ISCI Clinical Research Cell Manufacturing facility at the University of Miami.

6.3 Dosage Rationale

A safety profile for IV infusion of BMMSCs and UCMSCs is based on results from previous completed toxicology results[76]. The results from previous studies demonstrate that the product can be administered intravenously without toxic events at up to 65 x 10^6hMSC/kg dose delivered in one bolus infusion or at 100 x 10^6 hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6hMSC/kg per infusion).

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

Therefore, results from previous trials support the rationale on the safety and potential efficacy of the selected maximum dose of 100 x10^6 allo-hMSCs.

6.4 Administration Rate

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

In the proposed study, the cell dose to be delivered is 20 or 100 million cells in 80 mL Cells will be delivered at a rate of 2mL/min, and delivered at a maximum rate of 2.5×10_6 hMSC/minute and will last approximately 40 minutes for both doses.

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

6.5 Concomitant Therapy

6.5.1 Permitted therapy

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

6.5.2 Excluded therapy

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

6.5.3. Subject monitoring

All aspects of the study will be conducted in accordance with Good Clinical Practice (GCP) as described in the ICH Guideline (CFR ICH Selected Regulations and Guidance for Drug

Studies, CFR Title 21 Food and Drugs Revised as of April 1, 2016) all applicable national and local regulations. Monitoring will be conducted by a qualified outside source at the study site.

Monitoring of key safety endpoints (refer to Table 8 in section 2.2.1) will be conducted.

6.6 Blinding and Un-blinding

Subjects will be randomized into active groups. Only designated technicians in the ISCI Cell Processing Laboratory will be unblinded to treatment. The investigator, study staff, subject and anyone involved in the care of the subject will not be made aware of the assigned treatment regimen. Before dispensing the investigational product, Cell Therapy Lab staff will confirm the CMV status of eligible recipient. This information will be used to select Allo-BM and UC MSC product. CMV status of the recipient and donor of the Allo-BM and UC MSC product will be matched. CMV positive Allo-BM or UC MSC product will only be infused to a CMV positive recipient. All CMV negative recipients will receive CMV negative Allo-BM or UC MSC product[134].

The designated cell-processing technicians will prepare both (BM and UC) the allogeneic MSCs infusions. The investigational agent infusions will be prepared in identical infusion bags and labeled with the identical investigational drug labels. A brown plastic slip cover will be placed over the infusion lines as well as the bags to maintain the blind. The designated technicians in the ISCI Cell Processing Laboratory (or designee) will be responsible for maintaining the investigational product records including randomized treatment assignments by subject identification.

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

6.7 Study Investigational Therapy Management

6.7.1 Investigational Product Labeling and Storage

The product label contains the elements required by the CFR and other national and local authorities for investigational products. ISCI CMP will directly store and deliver the designated cell processing technologist in the CPL, and will be kept cryopreserved in a liquid nitrogen vapor phase until shortly before administration and 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 BMMSCs or UCMSCs 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.

Only unblinded personnel may access accountability records until the study blind has been broken.

7. STUDY PROCEDURES

7.1. Time and Events Schedule

The Time and Events Schedule for the conduct of this study is shown in Table 11

Schedule of Assessments
Table 11: Time and Events Table

VISIT	Screening + 45 days	Baseline (0 to -4 weeks prior to Day 0)	Day 0	Week 2 (Day 14) ± 2 days	Month 1 (Day 30) ±1 weeks	Month 3 (Day 90) ±4 weeks	Month 6 (Day 180) ±6 weeks	Month 12 (Day 365) ±2 weeks
Informed Consent	X							
Full Medical History	X							
Physical Exam	X	X	X	X	X	X	X	X
Vital Signs[1]	X	X	X	X	X	X	X	X
12-lead (ECG)	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X
Randomization		X						
Infusion Treatment (IP)			X					
6 Minute Walk Test		X				X	X	
4 Meter Gait Speed Test		X				X	X	
QOL Questionnaires (EQ-5D, SF-36, IIEF (Male), SQOL-F(Female))		X			X	X	X	
Laboratory Testing[2]								
Review Adverse Events		X	X	X	X	X	X	X
Brachial Ultrasound [3]	X	X				X		
Dobutamine Stress Echo (DSE)[4]	X						X	

Subjects in the pilot phase will receive a telephone call 5 days post infusion (+/- 1 day) to review adverse events

1- Weight, Height (will only be collected at screening)
2- See table 12 for specific tests done at each time point
3- Brachial ultrasound to assess endothelial function should be completed in the morning prior to 12p.m. Instruction for completing this assessment are outline in Appendix 3 of this protocol
4- Dobutamine Stress Echocardiogram (DSE) will be performed, at screening and at the Month 6 follow-up visit. Instructions for completing the (DSE) are outlined in Appendix 2 of this protocol. If the stress test needs to be stopped due to unforeseeable side effects and cannot be completed on a subject, this will not prevent them from being able to take part in the study.

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Schedule of Laboratory Testing
Table 12: Time and Events Table

VISIT		SCR	BSL	DAY 0	W2	M 1	M 3	M 6	M 12
Standard Labs	CBC with Differential [1]	X		X	X	X	X	X	
	Comprehensive Metabolic Panel [2]	X		X	X	X	X	X	
	PT, INR, PTT	X							
	Uric Acid, Pro BNP, hsCRP, IL6, Fib, D-dimer, TNFa,	X		X	X	X	X	X	
	Lipid Profile [3]	X		X	X	X	X	X	
	Urinalysis	X		X		X	X	X	
	HbA1c	X				X	X	X	
	Pregnancy (childbearing women)[4]	X		X					
Biomarkers / Immune	SST / PRA[5]			X				X	
	MNCs[6]			X	X	X	X	X	
EPCs	Endothelial Blood Samples[7]		X				X		
Gene Expression	DNA[8]			X					
	RNA			X				X	
Viral Serology	Infectious Disease Tests[9]	X							

1. Complete Blood Count with Differential - WBC, RBC, hemoglobin, hematocrit, MCV, and platelets; Diff: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.
2. Complete Metabolic Panel – glucose, sodium, potassium, chloride, biocarbonate, 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.
3. Lipid Profile: Triglyceride levels, Total cholesterol, LDL levels, and HDL levels
4. Pregnancy Serum β HCG: will be completed within 36 hours prior to infusion
5. SST: (2 Gold or Tiger top tube) Transcriptomic/proteome (RNA, mRNA, protein samples, and telomerase, akt); growth factors (sdf-1, notch); functional assays (cell-growth rate, VEGF, and CFU assays) and more.
6. MNCs: (2 Green top tube) CD3*CD25* or CD3*CD69* and more
7. Endothelial Blood Samples: (5 Lavender top tubes) Endothelial Progenitor Cell- Colony Forming Units (EPC-CFU). Must be performed on the same day as the day as the brachial artery ultrasound.
8. Genetic Testing: Deoxyribonucleic acid (DNA) to be collected at one time point only.
9. Infectious disease tests – HIV, HIV 2, Hep B (HBsAg, HBs Ab, HBc Ab), Hep C (Anti-HCV), VDRL/Syphilis (reflex FTA Ab), and CMV.

7.1.1 Remote Visits

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

- Physical Examination – Can be completed via telemedicine using a virtual platform.
- Vital Signs and 12-lead EKG – If subject has completed an EKG or vital signs within the protocol specified time window with their PCP or any other treating physician, a copy can be requested. Alternatively, subjects may record and report their own vital signs or EKG using home testing equipment (such as thermometer, automated blood pressure monitor, pulse oximeter, wearable health devices, or other method for recording vital signs.
- Questionnaires, concomitant medications and review of adverse events – may be completed by study team via email, phone contact, virtual visit or alternative methods.
- Laboratory assessments – A copy of SOC labs (urinalysis, hematology, chemistry, Hg A1C , lipid profile as well as any labs required by the protocol for particular time point) may be obtained from PCP or treating physician if done within the protocol specified time window. If participant has not completed any laboratory assessment they may complete them in any local laboratory facility.
- Brachial Artery Ultrasound, 6 minute walk test, 4 meter gait speed test, Dobutamine Stress Echo, Immune Monitoring, EPC-CFUs and Biomarkers Assessment – if participant is willing to come to site to complete these assessments, all necessary safety precautions will be taken. Study team will provide the subject with a mask and gloves upon arrival to the site. If subject prefers to complete this assessment at another time, the study team must document if the assessment will be completed out of the protocol specified time window and report to the regulatory authorities accordingly.

7.2 Study Phases and Visits

7.2.1 Screening Visit

See Table 11 for the procedures and assessment to be performed during the screening visit of the study. 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. The tests may take place over several days and will need to be completed prior to performing the baseline visit. There will be up to a 45-day window from the time the subject signs the informed consent form to the baseline visit to complete screening procedures.

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.

7.2.2 Baseline Visit

See Table 1 for the procedures and assessment to be performed during the baseline visit of the study. This visit will occur after all screening tests are completed and it has been determined that the subject meets eligibility criteria. This visit should occur within 4 weeks prior to Day 0 (the infusion visit).

7.2.3 Day 0 Visit

See Table 11 for the procedures and assessment to be performed during the Day 0 visit of the study. The Day 0 visit will occur after all baseline tests are completed and it has been determined that the subject remains eligible. Once the subject is deemed eligible to continue in the study the subject will be administered the investigational product.

7.2.4 Week 2 Visit

See Table 11 for the procedures and assessment to be performed for the week 2 visit of the study. Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of +/-2 days for the week 2 study visits.

7.2.5 Month 1 Visit

See Table 11 for the procedures and assessment to be performed for the Month 1 study visit. Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of +/-1 weeks for the Month 1 study visits.

7.2.6 Month 3

See Table 11 for the procedures and assessment to be performed for month 3 study visit. . Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of +/-4 weeks for the Month 3 study visit.

7.2.7 Month 6 - Month 12 Visit (Final Study Visit)

See Table 11 for the procedures and assessments to be performed for Month 6 through month 12 visit of the study. Outpatient visits should be completed as close to the scheduled visit dates as possible. There will be a window of ±6 weeks for the Month 6, and ±2 weeks for the Month 12 study visits.

7.3 Assessment Details

7.3.1 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. Similar physical exams will be conducted at each additional clinic visit during the study including vital signs, weight, review of AEs, and concomitant medications.

7.3.2 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. Respiratory rate, heart rate, and blood pressure should be measured in a sitting position after 5 minutes of rest. Height will only be collected at the screening visit.

7.3.3 Dobutamine Stress Echocardiography (DSE)

A Dobutamine Stress Echocardiography will be performed twice during the study, once at screening and at the Month 6 follow-up visit. This exam will assist in mimicking the effect of exercise on subjects to assess the heart muscle and ejection fraction when under stress. (refer to appendix 2)

7.3.4 Flow Mediated Diameter percent change (FMD%)

A Flow Mediated Diameter percent change will be performed three times during the study, once at screening, baseline, and at the Month 6 follow-up visit. This exam will assess vascular 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)

7.3.5 Laboratory Testing

- **Gene Expression**
  A separate blood sample of approximately 17mL for gene expression (DNA) profiling of WBC (at Day 0 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 12. A separate genetic consent form is completed by the study participants.

  All samples will be identified so that they can be linked to individual subjects. These samples may be stored indefinitely. Individual results will not be returned to the subject or the study physician. The samples will be linked to subjects, but there will be no recontact. Data presented in publications will not contain individual subjects' clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

- **Biomarkers/Immune**
  The studies planned in this protocol will utilize allogeneic hBMMSCs or hUCMSCs in subjects with chronic inflammation due to the metabolic syndrome. The use of an allogeneic graft raises the potential of graft rejection through immune cells resulting in failure of the therapy. hBMMSCs or hUCMSCs are ideal candidates for allogeneic transplantation because they show minimal MHC class II and ICAM expression and lack B-7 co-stimulatory molecules necessary for T-cell mediated immune responses[57, 58]. Indeed 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 CERES 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 12 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.

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

Additionally, in female subjects who receive allogeneic hBMMSCs or hUCMSCs, the stored baseline serum will 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 CERES 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 metabolic syndrome.

- **EPCs**
  Blood collection will be performed to assess endothelial function in the metabolic syndrome population at baseline and 3 months post stem cell infusion. This will

help provide cumulative data in assessing whether or not stem cell infusion improves endothelial function.

Assay of colony forming units: Fresh blood will be processed for cell culture assays for endothelial progenitor stem cells colonies counting (a 5 days' protocol). Fifty milliliter of blood will be processed; peripheral-blood mononuclear cells will be isolated by Ficoll density-gradient centrifugation, will be washed twice in phosphate buffered saline with 5% fetal bovine serum and re-suspended in media (EndoCult basal media with supplements; StemCell Technologies, Vancouver, British Columbia, Canada) for EPC colony-forming assay. Cells will be planted on human fibronectin-coated plates (BIOCOAT; Becton Dickenson Labware, Bedford, Massachusetts) at a density of 5X106 cells/well and incubated at 370C in humidified 5% CO2. After 48 hours, the non-adherent cells will be re-plated onto fibronectin-coated 24 well plates at a density of 1X106 cells/well. After 5 days, colony forming units (defined as a central core of rounded cells surrounded by elongated and spindle-shaped cells) will be counted manually in 8 wells out of a 24-well plate.

8. SAFETY

8.1 Safety Variables

1. Vital signs
2. Physical examination
3. Clinical laboratory tests
4. Adverse events

8.1.1 Laboratory Evaluations

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

Serum chemistry: sodium, potassium, chloride, BUN, creatinine, glucose, calcium, AST/SGOT, ALT/SGPT, carbon dioxide, total bilirubin (fractionate if total >1.5 times normal), alkaline phosphatase, albumin, fibrinogen, IL6, D-Dimer, Coagulation studies

Hematology: hemoglobin, hematocrit, platelets, WBC, RBC,

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.

8.1.2 Pulse Oximetry

Pulse oximetry will be used to observe oxygen saturation when measuring vital signs. Pulse oximetry will also be used throughout infusions and 2 hours following infusions. Subjects should have a resting oxygen saturation of ≥93% in order to be randomly assigned. 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.

8.1.3 Pregnancy

There is no information regarding allogeneic BMMSCs or UCMSCs and their 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 one of the enumerated contraceptive items will be acceptable for meeting the studies contraceptive requirements as listed in this section. Females will be defined as non-childbearing potential if surgically sterilized (i.e. bilateral tubal litigation, bilateral oophorectomy, or complete hysterectomy) or post-menopausal (defined as 12 months no menses with an alternative medical cause and with a follicle stimulating hormone (FSH $\geq$ 25.8 IU/L). Non-sterilized males who are sexually active with a female partner of childbearing potential must use any one of the enumerated contraceptive items as listed in section 8.2.2 throughout the study.

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.

8.1.4 Determination of Infusional Toxicity

Infusional toxicity will be evaluated by continuously monitoring the subject's vital signs and O_2 saturation by pulse oximetry from the time of allogeneic BMMSCs or UCMSCs

administration until two hours after infusion is complete. Since there is no specific or antidotal therapy for AEs arising from allogeneic BMMSCs or UCMSCs, 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.

8.1.5 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 subject death, grade 3 myocardial infarction, grade 3 hemodynamically unstable ventricular tachycardia or grade 3 LV perforation. Study accrual and further treatment of subjects will be put on hold 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:

5. 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, Bradycardia, hypotension, or palpitations) will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator.

6. 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.

7. 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.

8. 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.

9. The proportion of subjects experiencing TE-SAE as defined in Section 2.2.1 will be monitored within 30 days of infusion. 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.

10. Monitoring of key safety endpoints (refer to Table 8 in section 2.2.1) will be conducted.

11. 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.

12. Study accrual and further treatment of subjects will be stopped if multiple (i.e. more than 1) subjects develop cardiorespiratory signs and symptoms that are persistent or lead to discontinuation of infusion.

8.1.6 Subject observation and discontinuation after IP administration

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

8.2 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.

8.3 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.

8.4 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.

8.5 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.

8.6 Definition of Unexpected

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

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

Abnormal laboratory findings (e.g. clinical chemistry, hematology) or other abnormal assessments (e.g., vital signs) that are judged by the Investigator as clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE as defined in Section 8.3 ("Definition of an Adverse Event") or SAE, as defined in Section 8.6 ("Definition of a Serious Adverse Event"). 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 exercise medical judgment in deciding whether abnormal laboratory values are clinically significant.

8.8 Recording of Adverse Events and Serious Adverse Events

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

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

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

Pregnancies

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

8.9 Intensity of Adverse Events and Serious Adverse Events

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

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

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

8.10 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 make 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.

8.11 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

8.12 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 12.

TABLE 12
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

8.13 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.

8.14 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.

9. STATISTICAL ANALYSIS

9.1 Determination of Sample Size and Analysis Population

No formal statistical justification was performed to determine sample size in the Phase I study. Cohort size was determined based on expected requirements for safety analyses and projected enrollment rates. Study participants will be randomized according to a fixed allocation permuted block randomization schema. The allocation ratio will be 1:1:1: and the block size will be 4. All enrolled subject who received at least one treatment dose will be included in summaries of baseline characteristics, safety, and efficacy. Reasons for study discontinuation will be tabulated.

9.2 General Statistical Methods

All statistical tests will be performed at an $\mu=0.05$ level of significance, using two-sided tests. Because this is a Phase I 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.

9.3 Interim Analyses

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

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.

9.4 Data Safety Monitoring Board (DSMB)

9.4.1. ROLE OF THE DSMB

This study is designed to test the safety of the study investigational product in subjects with chronic inflammation due to metabolic syndrome.

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

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.

9.4.2. Purpose of the DSMB

The primary function of the 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 chronic inflammation due to metabolic syndrome. Given the importance of mortality in patients with metabolic syndrome who are at high risk for cardiovascular disease, a stopping boundary based on the all-cause mortality rate will be implemented to guide the DSMB.

9.4.2.1 DSMB MEMBERSHIP

The DSMB is an independent, multidisciplinary group consisting of four members (inclusive of the DSMB chair). The members include a clinical trialist, a biostatistician, an expert gerontologist and an expert cardiologist.

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.

9.4.2.2 Financial Disclosure and Conflict of Interest

DSMB membership is restricted to individuals without significant potential or perceived conflicts of interest. The source of these conflicts may be financial, scientific, or regulatory in nature.

Members must disclose to the DSMB chairperson their consultancies (direct or indirect) in excess of $5,000 or financial interests in any pharmaceutical companies, biotechnology companies, or CROs, if these relationships could lead to any conflict of interest or these companies' products involve hMSCs.

The DSMB chairperson will be responsible for deciding whether consultancies or financial interests of the members materially impact their objectivity. This decision is to be based on the reasonable belief that their objectivity is not in doubt. DSMB members will be responsible for advising the DSMB chairperson of any changes in financial interests in pharmaceutical companies, biotechnology companies, including consultancies, during the course of their membership. Members of the DSMB who develop significant potential or perceived conflicts of interest that may materially impact their objectivity will be asked to resign from the DSMB.

9.4.2.3 DSMB Responsibilities

The DSMB has the responsibility to:

1. Review the protocol and all amendments. The DSMB will also be provided the Investigator's Brochure, as it is updated, that includes preclinical and clinical efficacy and safety data.

2. Review the statistical analysis plan with particular attention to the portions describing the data to be provided to the DSMB.

3. Review Periodic Safety Update Reports (PSURs).

4. Evaluate the conduct of the study including the selection and retention of subjects, extent of protocol deviations, treatment adherence, and the quality of the data. The DSMB shall make recommendations regarding study conduct as necessary to protect the scientific

integrity of the study. The DSMB shall not make recommendations regarding the efficacy outcomes or associated analyses.

5. Review unblinded periodic safety summaries including adverse events, serious adverse events, discontinuations, and post-baseline laboratory results. In addition, efficacy and mortality data will be reviewed, although the DSMB shall not recommend stopping the study for efficacy.

6. Evaluate the conduct of the study including enrollment rates, the selection and retention of patients, protocol deviations, treatment adherence and quality and completeness of the data

7. The DSMB will make recommendations regarding modification or termination of the study in the event that significant safety concerns arise during study conduct.

9.4.2.4 Confidentiality

The DSMB will be unblinded in its assessment of safety and efficacy data to ensure that the DSMB is fully informed in its primary mission of safeguarding the interest of participating subjects. The DSMB will have sole access to comparative results of safety data aggregated by treatment arm. The DSMB will take all necessary and appropriate steps to safeguard the confidentiality of unblinded treatment information it receives to minimize the potential for premature conclusions regarding the study results as well as the potential for introducing bias into the study.

9.4.2.5 Study Conduct and Termination

The DSMB will provide recommendations following review and assessment of the quality of study conduct. More specifically, the DSMB will review enrollment rates, consistency in complying with eligibility requirements, compliance with the study protocol as well as the completeness of the data. In their review of the data, the DSMB will be responsible for protecting the safety of the enrolled subjects. If any potential question of safety arises, the DSMB will use the efficacy data to assess the possible safety risk in the context of the benefit-to-risk profile of study treatment. Based on this information, the DSMB may make recommendations to terminate the study if members believe that an undue risk (relative to benefit) would be incurred by allowing the study to continue to completion. Otherwise the study will be completed to allow investigators to complete the protocol-mandated assessments to evaluate the safety of hMSCs in patients with chronic inflammation due to metabolic syndrome subjects.

9.4.2.6 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.

9.4.3 COMMITTEE MEETINGS

9.4.3.1 Organizational Meeting

At an organizational meeting the DSMB will discuss the operational aspects of the committee. This meeting will include DSMB members and the clinical monitor. The documents to be provided before this meeting are:

1. Study protocol
2. Preliminary DSMB Statistical Analysis Plan (SAP)
3. Preliminary list of tables and listings to be provided for interim assessments
4. Investigator's Brochure
5. Food and Drug Administration Guidance: *Establishment and Operation of Clinical Trial Data Monitoring Committees.*

For all DSMB meetings, a quorum is defined as at least two members of the DSMB in addition to the DSMB chairperson.

9.4.3.2 Review of Periodic Safety Update Reports

As part of ongoing safety review and obligation to regulatory agencies, safety reports will be reported every month. These reports will be available to the DSMB, who shall review them in the context of providing additional information to assist the committee's consideration of subject safety. The DSMB members may choose to discuss the PSURs during an ad hoc teleconference.

9.4.3.3 Data Review Meetings

After all patients enrolled in the Pilot Phase have received the study therapy infusion and been followed for 30 days, the DSMB will conduct a full review of all cumulative safety data before the trial proceeds to the Randomized Phase. As part of the cumulative safety data review meeting for the pilot phase, the DSMB will recommend that the trial proceed to the protocol-specified randomized phase or recommend a dose modification for the randomized placebo study.

Ongoing Monitoring During Randomized Phase

Formal data review meetings that include the entire DSMB will be conducted via teleconference approximately every three months. The timeline for the quarterly DSMB data review meetings will begin after approximately 25% of patients are enrolled in the randomized phase. Meetings may be postponed due to accrual rates at the DSMB's discretion. The purpose of the data review is for safety evaluation, and the study may be stopped because of significant safety concerns. SAEs which are related to stopping rules will be continuously evaluated and the full DSMB will be informed of any extra risk. The DSMB will evaluate all safety data available for each patient as appropriate.

Open session

Blinded data will be provided to the DSMB approximately 1 week before each data review meeting. The report will contain:

1. Protocol status including any protocol changes
2. Data sources and cutoff dates
3. Analysis methods applied specifically to the open session report
4. Subject enrollment by month
5. Protocol deviations
6. Early treatment discontinuations and study withdrawals
7. Demographic and baseline characteristics
8. Duration of follow-up at time of data cutoff

Closed Session

Only the DSMB members will participate in closed sessions. Unblinded data will be presented to the DSMB in closed session and discussed by the DSMB. "Unblinded" means that the name of actual treatment arm is associated with individual subject data listings and summaries of data. At the chairperson's discretion, the DSMB may discuss or vote on potential study conduct recommendations at closed session.

The closed session report will contain data separated and identified by treatment group. This report (hard copy) will be provided to the DSMB approximately 1 week before each data review meeting and will include:

1. Data sources and cutoff dates
2. Analysis methods applied specifically to the closed session report
3. Subject enrollment
4. Protocol deviations
5. Early treatment discontinuations and study withdrawals
6. Demographic and baseline characteristics
7. Primary and secondary efficacy outcome measures
8. Prohibited concomitant medications
9. Adverse events
10. Serious adverse events

Following the closed session, the DSMB chairperson will issue, within 24 hours, one of the following recommendations as determined by the DSMB:

1. Continue the study, with or without modifications.
2. Terminate the study for safety concerns.

Separate meeting minutes for the open and closed sessions will be prepared by the DSMB project manager, then reviewed and issued by the DSMB chairperson. The DSMB support team will maintain copies of the meeting minutes for both the open and closed meetings. To preserve the integrity of the study, the detailed rationale and discussion of comparative unblinded data will be included only in the closed meeting minutes

Follow-Up Open Session

Immediately following the closed session, the DSMB will meet with the investigators to discuss any study conduct concerns. This follow-up open session may be attended by DSMB Members, clinical monitor, and study staff. Potential recommendations from the DSMB regarding study discontinuation or continuation, with or without modification, will not be communicated in this open session.

Closed Executive Session

At the discretion of the DSMB chairperson, a closed executive session may be held. Closed executive sessions will include only DSMB members. Discussion of unblinded study data and potential DSMB recommendations and voting may take place in closed executive session. The DSMB may choose whether to write minutes of the closed executive sessions.

9.4.4 DATA FLOW

9.4.4.1 Communications and Reports

For each DSMB meeting, the open session and closed session reports will be prepared by the DSMB support team. Open session reports will be circulated to all attendees of the open session. The closed session reports will be circulated to DSMB members only. The closed session reports will be retrieved from the DSMB members by the DSMB support team and destroyed after the closed session. The DSMB support team will maintain copies of all reports from the open and closed sessions.

9.4.4.2 Review of Unblinding Requests

Except as required by regulatory authorities for safety reporting, individual subjects' treatment assignments will not be unblinded during the conduct of the study, unless a subject safety issue arises in which unblinding is necessary to ensure optimal subject management. It is not anticipated that unblinding will be necessary, given the hMSCs can be safely discontinued at any time a safety concern arises. The DSMB will be informed in a timely manner of any case for which unblinding was requested and performed.

9.4.4.3 DSMB Additional Analysis Requests

The DSMB may request additional analyses from the statistician if deemed necessary to fulfill the mission of the DSMB. If based on the additional data the DSMB feels there is a need for an unscheduled formal meeting, the DSMB chairperson will arrange.

9.4.4.4 Confidentiality

All documents will be held in strict confidence by the DSMB, and all documents provided to the DSMB will be collected and destroyed at the end of all DSMB meetings by the DSMB support team.

9.4.5 COMMUNICATION

9.4.5.1 DSMB Minutes

The DSMB chairperson is responsible for issuing minutes of the open and closed sessions. Minutes of the open session will be distributed to all open meeting attendees within two weeks of the meeting. Minutes of the closed session will be distributed to the members of the DSMB within two weeks of the meeting. At the conclusion of the study, the DSMB support team will send a complete set of the open and closed reports, minutes of the open and closed sessions with the tables and listings, all presentations and copies of the recommendation forms to the investigators.

9.4.5.2 DSMB Recommendations

If the DSMB recommends modification to, or termination of, the study, the chairperson of the DSMB will contact the investigators within 24 hours of making the decision.

Recommendations for modifications other than termination should be accompanied by the rationale for the recommendation and the minimum amount of data required to make a decision. The investigator will be responsible for promptly reviewing the DSMB recommendations and determining whether amendments to the protocol or changes regarding the study conduct are required and if reporting to regulatory authorities is warranted (FDA, 2006).

Should there be disagreement between the DSMB and the investigators around the decision to stop or modify the study a separate compliance committee will be appointed. This committee is comprised of individuals who have extensive experience in the pharmaceutical industry and a deep appreciation of the ethical issues surrounding the conduct of clinical studies and are responsible for the investigator's code of ethics. The Compliance Committee is charged with independently evaluating differing opinions that may arise between the investigator and DSMB and applying the highest ethical standards with respect to determining the best interests of subjects enrolled in the study.

10. STUDY ADMINISTRATION

10.1 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.

10.2 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.

10.3 Subject Informed Consent

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

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

The investigator or designee will review the study with each subject. The review will include the nature, scope, procedures, and possible consequences of the subject's participation in the study. The ICF and review must be in a form understandable to the subject. The Investigator or designee and the subject must both sign and date the ICF after review and before the subject can participate in the study. The subject will receive a copy of the signed and dated form, and the original will be retained in the site study files. The

Investigator or his/her designee must emphasize to the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

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

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

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

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

10.4 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.

10.5 Payments to Subjects

Subjects will be reimbursed $25 at the end of each follow-up visit (Week 2 – Month 12) for a total remuneration of $125. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

10.6 Payments to Donors

Normal donors for generation of allo-MSC will be reimbursed $500 at the end of BM aspiration. This payment will compensate donors for lost time, parking, and travel expenses.

APPENDIX 1: Infusion Guidelines

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

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

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

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

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

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

1. Monitor the subject continuously with pulse oximetry
2. Hang the blinded infusion bag. Investigational product (IP) should not be "piggybacked" through another line
3. Intravenously administer the IP at a rate of 2ml/min.
   Note: *Study personnel administering the IP must be present throughout the infusion process. The Investigator must be available at the site during the infusion process in case an emergency should arise.*
4. Record the start time of the infusion bag

5. Gently squeeze the infusion bag several times every 15 minutes to assure uniform dispersion of contents
6. Vital signs and O_2 saturation will be measured every 15 minutes until the end of IP infusion
7. Record the total volume infused from the IP bag
8. At the end of the IP infusion, close the line and flush 25ml of 0.9% normal saline into the luer lock connector on the bottom of the IP bag, reopen line and allow to infuse at a rate of 2mL/min until completion.
9. Discard IV tubing according to established guidelines

Procedures post-infusion:

1. Vital signs will be monitored at 15 minutes, 30 minutes, 1 hour, and 2 hours post IP infusion
2. The subject will be monitored for a minimum of 2 hours post IP infusion with continuous pulse oximetry
3. If the O_2 saturation decreases to < 90% 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 90% then the subject will be provided additional oxygen to maintain a saturation of >90% at room air up to 4 hours post infusion.
5. After the minimum two-hour observation period, the subject will be continuously monitored and discharged the following day, 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:

1. 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.

2. 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.

3. 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.

4. 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: Dobutamine Stress Echo (DSE) Instructions

A Dobutamine Stress Echocardiogram will be performed at screening and at the Month 6 follow-up visit. Dobutamine is a chemical, which can assist in mimicking the effect of exercise on subjects in order to assess the heart muscle and ejection fraction when under stress.

Procedure Details:

- Obtain baseline heart rate, blood pressure, Electrocardiogram (ECG), physical exam, and echocardiographic images on the study participant.
- Place an Intravenous (IV) line to administer Dobutamine.
- Administer progressively increasing doses of Dobutamine (5, 10, 20, and 30 mcg/kg/min) in 3-minute intervals
- Obtain Heart rate, Blood Pressure, ECG, and echocardiographic images at each dosage.
- After completing measurements at 30mcg/kg/min dosage, discontinue Dobutamine and allow heart rate to recover to baseline and obtain repeat echocardiographic images.

Required Data Points:

1. Right Ventricular end-diastolic dimension (RVDd)
2. Interventricular Septal end-diastolic dimension (IVSd)
3. Left Ventricular Internal end-diastolic dimension (LVIDd)
4. Left Ventricular Internal end-systolic dimension (LVIDs)
5. Left Ventricular Posterior Wall end-diastolic dimension (LVPWd)
6. End-Diastolic Volume (EDV)
7. End-Systolic Volume (ESV)
8. Left Atrial (LA) dimension
9. Peak infusion rate
10. Peak heart rate
11. Peak blood pressure (systolic/diastolic)
12. Resting biplane ejection fraction
13. Ejection fraction at 5 mcg/kg/min
14. Ejection fraction at 10 mcg/kg/min
15. Ejection fraction at 20 mcg/kg/min
16. Ejection fraction at 30 mcg/kg/min
17. Evidence of Mitral Valve Regurgitation (MR)
18. Evidence of Pericardial Effusion (PE)

APPENDIX 3: Flow Mediated Diameter percent change (FMD%) Instructions

A Flow Mediated Diameter percent change will be performed three times during the study, once at screening, baseline, and at the Month 3 follow-up visit. This exam will assess vascular function.

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 subjects 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 10% is considered a normal response. Lower than 10% FMD% reflects endothelial dysfunction, which means a high likelihood to develop cardiovascular event in the future. Subjects with negative FMD% results (the artery is constricted after stress and not dilated as was expected) have the worst prognosis.

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