Interdisciplinary Stem Cell Institute

University of Miami/ Miller School of Medicine

Clinical Research Protocol

Study Title: A Phase I/II, Randomized, Blinded and Placebo-controlled Trial to Evaluate the Safety and Potential Efficacy of Allogeneic Human Mesenchymal Stem Cell Infusion in Patients with Aging Frailty.

***A*llogenei*C* Human Mesenchymal *S*tem C*e*lls (hMSC) in Patients with Aging F*RA*il*T*y via Intraveno*US* Delivery (CRATUS)**

Study Product: Allogeneic Human Mesenchymal Stem Cells (hMSCs)

Indication: Aging Frailty

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

Table of Contents

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List of Abbreviations

AE	Adverse event
ahMSCs	Allogeneic human Mesenchymal Stem Cells
Allo	Allogeneic
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BM	Bone Marrow
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHAMPS	Community Healthy Activities Model Program for Seniors questionnaire
CMV	Cytomegalovirus
CPF	Cell Processing Facility
CPL	Cell Processing Laboratory
CRP	C-Reactive Protein
CT	Computed tomography
DLCO	Diffusing Capacity
DMSO	Dimethyl sulfoxide
DSMB	Data Safety Monitoring Board
ECC	Eluerian Circumferential strain
EDV	End-diastolic volume
EPCs	Endothelial progenitor cells
ESR	Expedited safety report
ESV	End-systolic volume
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FEV – 1	Forced expiratory volume in 1 second
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GFP	Green fluorescent protein
GM – CSF	Granulocyte Macrophage Colony Stimulating Factor
GVHD	Graft versus host disease
HBcAb	Anti-Hepatitis B core antibody
HCV Ab	Anti-Hepatitis C virus antibody
HIPAA	Health Insurance Portability and Accountability Act Authorization
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
Has	Human serum albumin
hMSCs	Human mesenchymal stem cell
hs-CRP	High Sensitivity C-Reactive Protein
HSCs	Hematopoietic stem cells

HTLV	Human T-lymphotropic Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IL-6	Interleukin-6
IND	Investigational new drug
INR	International Normalized Ratio
IP	Investigational Product
IRB /IEC	Institutional Review Board
IV	Intravenous Infusion
LAD	Left anterior descending
LFT	Liver Function tests
LV	Left ventricular
MFI	Multi-dimensional Fatigue Inventory
MI	Myocardial Infarction
MMSE	Mini Mental State Examination
MNC	Mononuclear Cell
MRI	Magnetic resonance imaging
MSCs	Mesenchymal Stem Cells
NAT	Nucleic Acid Testing
NIH	National Institute of Health
NMDP	National Marrow Donor Program
PBMC	Peripheral blood mononuclear cells
PFTs	Pulmonary function tests
PSURs	Periodic Safety Update Reports
QOL	Quality of life
RDW	Red blood cell distribution Width
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCA-1	Stem cell factor antigen
SF – 36	Short Form – 36
SGRQ	St. George's Respiratory Questionnaire
SPPB	Standard Physical Performance Battery
TE-SAE	Treatment-emergent serious adverse event
TNF-α	Tumor necrosis factor-alpha
TTC	Triphenyltetrazolium chloride
VEGFR2	Vascular endothelial growth factor
WBC	White blood cell
WNV	West Nile virus
6MWT	Six minute walk test

Protocol Synopsis

PRODUCT	Intravenous Allogeneic Adult Human Mesenchymal Stem Cells (MSCs)
PHASE OF DEVELOPMENT	I/II
MAIN CRITERIA FOR INCLUSION	Aging Frailty
STUDY OBJECTIVES	To demonstrate the safety of intravenous allogeneic hMSCs administered in subjects' with Frailty and to explore treatment efficacy (decrease in frailty, frequency of acute exacerbations, change in symptom related quality of life, improved cardiovascular status, decrease in inflammatory biomarkers, endothelial function and 1 year survival).
STUDY DESIGN	A Phase I/II, Randomized, Blinded and Placebo-controlled
INVESTIGATIONAL PLAN	Before initiating the full-randomized study, a Pilot Safety Phase will be performed. The randomized portion of this trial will be conducted after a full review of the safety data from the Pilot Phase by the DSMB. Following the Pilot Phase of fifteen (15) subjects, thirty (30) subjects meeting all inclusion/exclusion criteria will be evaluated at baseline and scheduled to undergo infusion. <u>Pilot Phase</u> (15 subjects) In the pilot phase, the first three (3) subjects in each treatment group will not be treated less than 5 days apart for their first infusion and will each undergo full evaluation for 5 days to demonstrate there is no evidence of treatment emergent SAE's prior to proceeding with the treatment of further subjects. <u>Group 1 (5 subjects)</u>: Five (5) subjects will be treated with a single administration of allogeneic hMSCs: 2×10^7 (20 million) cells delivered via peripheral intravenous infusion. <u>Group 2 (5 subjects)</u>: Five (5) subjects will be treated with a single administration of allogeneic hMSCs: 1×10^8 (100 million) cells delivered via peripheral intravenous infusion.

	Group 3 (5 subjects): Five (5) subjects will be treated with a single administration of allogeneic hMSCs: 2 x 10^8 (200 million) cells delivered via peripheral intravenous infusion. At completion of the one year phone call visit in the pilot phase, all fifteen (15) subjects will be provided with the option of having up to three (3) additional administrations of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion. Randomized Phase (30 subjects) In the randomized phase of allo-hMSCs or matched placebo up to 30 subjects will be randomized in a 1:1:1 ratio to one of two doses of MSCs versus placebo. Treatment Strategies following successful completion of the Pilot Phase: Group A (10 subjects) – Allogeneic hMSCs: 100 million cells/ml delivered via peripheral intravenous infusion. Group B (10 subjects) – Allogeneic hMSCs: 200 million cells/ml delivered via peripheral intravenous infusion. Group C (10 subjects) - Placebo delivered via peripheral intravenous infusion. Subjects in the randomized phase that received placebo will have the option to receive one infusion of 100 million allo-hMSCs after subjects in the randomized phase have been unblinded (refer to Addendum D). Addendum B (20 subjects) Twenty (20) subjects will be treated with a single administration of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion. Subjects that participated in the Addendum B sub-study will then have the option to receive up to three (3) additional infusions administrations, per Addendum C, of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion. The Allo-hMSCs will be supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami or from a commercial clinical grade bone marrow source. Following infusion, subjects in the pilot and randomized phases will be followed at 2 week's post-infusion, and at one,

	three, and six months to complete all safety and efficacy assessments. Subjects in the addenda will be followed at one month post-infusion and then at three and six months to complete all safety and efficacy assessments. Patients participating in Addendum B will receive penicillin/streptomycin free cells. If they are participating in Addendum C or D subjects may receive cells with antibiotic (penicillin/streptomycin). All subjects, with the exception of subjects participating in Addendum C, will also have a final twelve-month contact for assessment of vital status and occurrence of hospitalization.
ROUTE OF ADMINISTRATION	Peripheral Intravenous Infusion
DURATION OF STUDY PARTICIPATION	<u>Pilot phase (15 subjects)</u> 12 months (Follow-up will be at 2 weeks, 1, 3, 6, and 12 months) for the first infusion. Pilot subjects will have an optional additional 12-month follow-up period for a second infusion (Reference Addendum A), and up to two (2) additional infusions with a 6 to 12-month follow-up period under Addendum C. The follow-up will be at 1, 3, 6, and 12 months for Addendum A. The follow-up for Addendum C will be at 1, 3, and 6 and/or at 12 months. <u>Randomized phase (30 subjects)</u> 12 months (Follow-up will be at 2 weeks, 1, 3, 6, and 12 months.) Subjects that received placebo will have the option to receive a single infusion of allogeneic MSCs under Addendum D. The follow-up will be at 1, 3, 6, and 12 months for these subjects. <u>Addendum B sub-study (20 subjects)</u> Subjects in the penicillin/streptomycin free addendum (Reference Addendum B) will have up to a 12-month follow-up schedule at 1, 3, 6, and if applicable, 12 months. Subjects continuing with the additional infusions in Addendum C will have up to a 12-month follow-up schedule at 1, 3, 6, and if applicable, 12 months for each additional infusion. Subjects will receive one initial infusion (Addendum B) and can receive up to three (3) additional infusions under Addendum C for a total of four infusions.
SUBJECT POPULATION	Forty-Five (45) subjects with frailty will be enrolled in the main study.

	An additional 20 subjects will be included in a sub-study, (Please see Addendum B).
Definition of Endpoints	**Safety (Primary):** Incidence (at one month post-infusion) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities determined per the Investigator's judgment. **Efficacy (Secondary): During the screening, baseline, Month 3 and/or Month 6 visits.** • Difference in rate of change of frailty defined as: - Reduced Activity (assessed via CHAMPS questionnaire) - Slowing of Mobility (assessed via gait speed test and SPPB assessment) - Weight Loss - Diminished handgrip strength (assessed via dynamometer) - Exhaustion (assessed via the MFI questionnaire) - Decrease in subject quality of life assessment(s) (assessed via ICECAP, SF-36, EQ-5D Questionnaires) • Death from any cause. • Change between screening and 6 months in dobutamine stress echo induced ejection fraction • Change between screening and 6 months in the following panel of inflammatory markers: CRP, IL-6, D-dimer, fibrinogen, CBC with differential, and TNFα
Safety (Additional)	• During the 12 week follow-up period and each consecutive time-point up until the final visit
Inclusion Criteria (Applicable for Main Phase of the trial; reference each individual addendum for its inclusion criteria)	1. Provide written informed consent. 2. Subjects age ≥60 and ≤95 years at the time of signing the Informed Consent Form. 3. Show signs of frailty apart from a concomitant condition as assessed by the Investigator with a frailty score of 4 to 7 using the Canadian Clinical Frailty Scale. 4. Female subjects must have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy.
Exclusion Criteria (Applicable for Main Phase of the trial; reference each individual	1. Score of ≤24 on the Mini Mental State Examination (MMSE) 2. Be a female who is pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods. Female subjects must undergo

addendum for its exclusion criteria)	a blood or urine pregnancy test at screening and within 36 hours prior to infusion. 3. Inability to perform any of the assessments required for endpoint analysis. 4. Active listing (or expected future listing) for transplant of any organ. 5. Clinically important abnormal screening laboratory values. 6. 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. 7. Hypersensitivity to dimethyl sulfoxide (DMSO). 8. Be an organ transplant recipient. 9. 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. 10. Have a non-pulmonary condition that limits lifespan to < 1 year. 11. Have a history of drug or alcohol abuse within the past 24 months. 12. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C. 13. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial. 14. 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.

1. INTRODUCTION

1.1 Background

Frailty in an aging population is defined as a medical syndrome “with multiple causes and contributors that is characterized by diminished strength, endurance, and reduced physiologic function that increases an individual’s vulnerability for developing increased dependency and/or death”[1]. Of great importance is that frailty, while not characterized as a disability, per se, does increase the risk of death in affected individuals[2-4]. Moreover, there is a close link between a subject’s health and frailty. These subjects tend to show a greater risk to frailty when there are other conditions affecting their physical and psychological well-being, such as high blood pressure, cancer, or cognitive

impairment. In an aging population frailty is characterized by reduced physical activity, slowing of mobility, weight loss, diminished handgrip strength, and exhaustion[2, 3]. There are several well-validated models to assess frailty[1]For example, the FRAIL scoring index provides a clinical diagnosis of frailty when a subject exhibits three or more of these five characteristics[2;5]. The forward trajectory in medical advances, and a more health aware society, indicates that the population is living longer and requiring more care and services than were needed in the past. As such, the increase in frail elderly subjects has adversely increased the demand for healthcare services. Currently there is a favorable movement in researching a novel medical therapy for frailty as this remains an unmet need amongst the elderly population.[6] It is widely perceived that frailty can be favorably modified, and in this regard there is a major need for effective management tools and treatment strategies. In this protocol, we will test the impact of a safe cell-based therapeutic on the frailty syndrome.

Frailty and Cardiovascular Performance

The aging cardiovascular system has some very specific phenotypic alterations[7;8]. These include left ventricular hypertrophy and a diminution in exercise induced increase in ejection fraction[9-11]. These characteristic abnormalities are hypothesized to contribute to specific symptoms of the frailty syndrome and to increase the morbidity and mortality from cardiovascular disease in elderly individuals.

Several studies document the increased risk for mortality in frail elderly subjects with cardiovascular events such as non-ST-segment elevation myocardial infarction (NSTEMI)[4;12]. Frail individuals have increased disease burden in and therefore more prolonged recuperation versus a non-frail subject[1]. There are additional associations between frailty and other cardiovascular diagnoses including angina, myocardial infarction, congestive heart failure and stroke. Gait speed is one symptom of frailty that is associated with cardiovascular mortality and an increased risk of cardiovascular events specifically STEMI subjects [13;14]. The Women's Health Initiative utilized the "Frailty Index" by Fried and colleagues[8] which was validated in the Cardiovascular Health Study[15] and the Womens Health and Aging Study. These studies demonstrated correlations in functional decline, increased risk of institutionalization and mortality with the use of these instruments.

The strong association between frailty and cardiovascular disease and the growing data base documenting safety and potential favorable effects of cell-based therapy in cardiovascular diseases provide justification for the assessment of potential benefits of cell therapy in subjects with frailty.

Inflammation in frailty

In addition and of great importance, there is a growing database of studies that highlight a connection between frailty and inflammation. For example, certain inflammatory markers, such as C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6), red blood cell distribution (RDW) and D-dimer are more likely to be elevated in frail as compared to non-frail individuals[8]. Importantly, among frail subjects, women exhibit higher concentrations of inflammatory and coagulation factors than men. CRP is an example of one marker studied that shows that women experiencing symptoms of frailty show a higher

concentration of CRP. Differential white cell counts on the other hand exhibits an increased risk of frailty in both men and women. Dysregulated inflammation is a considerable key physiological marker in correlation with the frailty syndrome[8] . There is still insufficient data to show which markers specifically affect men or women[5;16]. It is believed that frailty can ultimately be prevented or attenuated, and the link between frailty and inflammation offers a potential therapeutic target, also addressable by cell therapy.

Frailty is assessed with several instruments. For example, the Clinical Frailty Scale[2] is a clinical scale that determines the degree of a subject's frailty based on the physician's judgment and the subject's medical information. This scale consists of 7 variables ranging from fit to complete functional dependence; If a subject displays three or more of the following symptoms: low physical activity, muscle weakness, slowed performance, fatigue, unintentional weight loss, then the subject is considered to be classified as frail.[2;4]

Stem Cells in Frailty

An individual's endogenous stem cell production decreases with age; this decrease in an aging subject likely contributes to reduced ability to regenerate and repair organs and tissues. Several investigators have proposed that a regenerative treatment strategy could ameliorate signs and symptoms of aging frailty[8;16]. Currently, the FDA does not have any specific approved treatment for frail subjects and therefore no established standard of care. In many cases frailty can be masked by other physical or psychological conditions affecting well-being and functional status. The ultimate goal is to extend the health and ability of a subject to regenerate functionality. Allogeneic Human Mesenchymal Stem Cells are known to hone to sites of injury, reduce inflammation, and assist in cellular repair. Here we propose to study Allo-hMSCs (ahMSCs) as a novel therapy for treating subjects experiencing frailty.

There are specific features of the frailty syndrome that support a potential role of ahMSCs to ameliorate or improve frailty. Notably, ahMSCs are shown to improve cardiovascular status in subjects with acute MI[17] and heart failure[18]. In addition, ahMSCs are anti-inflammatory and reduce CRP levels in a sustained manner[19]. Importantly, data from heart failure studies show that ahMSCs are safe in subjects irrespective of age[18]. For these reasons, this protocol will test the safety of intravenous infusion (IV) of ahMSCs in individuals of advanced age with frailty, and will assess cardiovascular status and inflammatory markers in this population at increased risk for morbidity and mortality.

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[20], and 3.) cells that express stem cell factor receptor, c-Kit[21;22]. Endothelial progenitor cells (EPCs) express the surface markers CD34, CD133, c-kit, and the vascular endothelial growth factor receptor-2 (VEGFR2; KDR; Flk-1)[23-28]. Hematopoietic stem cells (HSCs) exhibit self-renewal and differentiation. Their cell-

surface phenotype is $CD34^+$, stem cell factor antigen $(SCA\text{-}1)^+$, c-kit^+, and Lin^- (review[29]). While there has been controversy regarding the ability of bone marrow-derived cells to transdifferentiate into cardiomyocytes[30], clinical trials of bone marrow therapies continue to suggest potential benefit in terms of improving a subject's well-being.

1.2 Mesenchymal Stem Cells

Mesenchymal stem cells (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)[31], 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[31], contribute to pancreatic islet and renal glomerular repair in diabetes[32], attenuate sepsis[33], reverse fulminant hepatic failure[34], protect against ischemic acute renal failure[35], reverse remodeling[36-38] and improve cardiac function after myocardial infarction[17], to be a potential source of multiple cell types for use in tissue engineering[39;40], and to be capable of tissue regeneration after spinal cord trauma, stroke, and connective tissue injury[41-43].

In the lung, MSCs have been shown to contribute to tissue regeneration after elastase-induced emphysema[44], home to sites of asbestos induced lung injury[45], contribute to tissue remodeling in a rat monocrotaline model of pulmonary hypertension[46], decrease chronic airway inflammation in a murine ovalbumin model of asthma[47], and to restore alveolar fluid balance after endotoxin induced acute lung injury[48].

Tracking of radioactively labeled cells shows that when administered intravenously, MSCs localize primarily to the lung, followed by the liver, and then other organs[49]. A number of studies show that MSCs preferentially home to sites of injury in the lung and contribute to tissue regeneration and repair[50-56]. Using Y-chromosome fluorescence in-situ hybridization, Y-chromosome positive male MSCs can be found at sites of lung injury in transplanted female mice[51;52;55]. 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[51].

The ability of MSCs to differentiate towards an epithelial lineage was established by studies showing that they are capable of differentiation not only to cells of mesodermal origin, but also to cells of endodermal and ectodermal (including epithelial) origin[57;58]. MSCs cultured in airway growth media differentially express lung specific epithelial markers like Clara cell secretory protein, surfactant protein-C, and thyroid transcription factor-1[57;58]. 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[56;59]. 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[60;61].

1.3 Mesenchymal Stem Cells: 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 decrease symptoms of frailty. Below we review the impact of MSCs on the cardiovascular system following injury; this provides support for an impact of MSCs in individuals with frailty.

A porcine model of anterior myocardial infarction was used to characterize the impact of cellular cardiomyoplasty on cardiac structure and function using hemodynamic, imaging, and histological analyses. A pig model was selected because of its anatomic similarity to the human heart. The following sections describe the safety and efficacy results obtained with this model[62]. Two distinct sets of studies were conducted, representing the early treatment of acute myocardial infarction, as well as the treatment of chronic ischemic cardiomyopathy.

Allogeneic mesenchymal stem cell transplantation improves global cardiac function in a swine model of acute myocardial infarction: Previously published work demonstrated that autologous MSC transplantation in post-MI pigs improved cardiac function, with histological evidence of robust engraftment at 8 weeks, and differentiation to a myocyte-like phenotype[63]. Based on *in vitro* observations that MSCs lack the B-7 costimulatory molecule and may therefore be immune-privileged, the impact of *allogeneic* MSC transplantation in porcine MI was assessed. A 14 pig randomized, placebo-controlled study (MSCs vs. placebo) using the BioCardia Helical Infusion Catheter was performed to assess safety and efficacy of allogeneic transendocardial injections[62]. Farm pigs were chronically instrumented to measure left-ventricular pressure, dimension, and oxygen consumption, and were randomized to active treatment or placebo groups. Three days following MI, placebo (n=7) or $2X10^8$ allogeneic MSCs (n=7) labeled with Di-I and DAPI (both fluorescent dyes to aid histochemical identification) were injected percutaneously into infarcted myocardium of the left ventricular cavity using a helical injection needle catheter inserted through a steerable guide catheter (BioCardia, Inc.). All animals tolerated the catheter-based injections well. Animals were then studied on a weekly basis for 8 weeks to assess hemodynamics and to examine ventricular architecture. In treated animals, MSCs engrafted within the MI (Figure 1 a, b) and expressed several myocyte proteins, including α-actinin, phospholamban, tropomyosin, and troponin T (Figure 1 c, d, e, f). In addition, there was evidence of stem-cell differentiation or incorporation into vascular structures within the infarct area (Figure 1 g, h, i). MSCs were detected in vascular structures as they expressed VEGF and vonWillebrand Factor, suggesting that they are capable of differentiating into vascular smooth muscle and/or endothelium. That the cells did not elicit rejection, despite the absence of immuno-suppressive drug therapy, was supported by the lack of a significant inflammatory response. (Note that cells surrounding vessel in Figure 1g and 1i are of MSC origin, as indicated by DAPI positivity in Figure 1h). The number of MSCs persisting in the myocardium decreased over time. Nonetheless, MSC injection produced a wide range of benefits, including improved regional and global ventricular function, reduced myocyte apoptosis, and improved tissue perfusion.

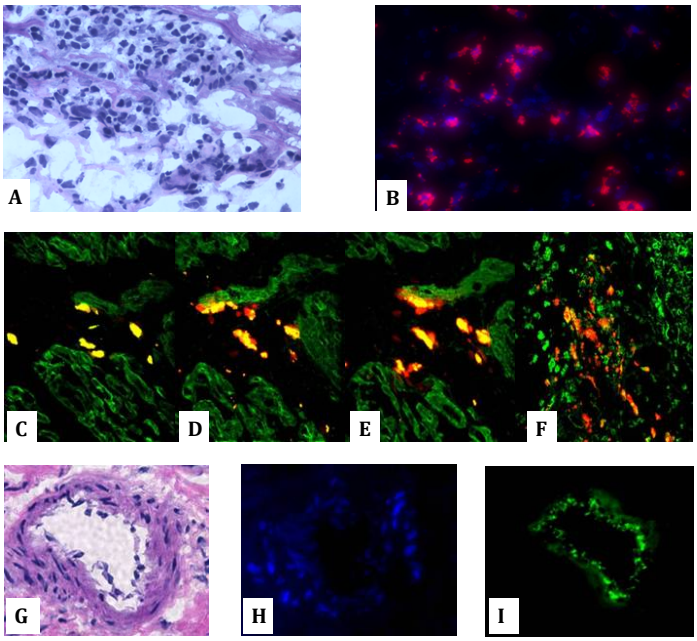


Figure 1. MSC engraftment and differentiation. MSC engraftment and muscle-specific protein-expression. DAPI and Di-I labeled MSCs (blue staining nuclei and red staining membranes, respectively) and fluorescent muscle protein-specific antibodies (green). (A) Hematoxylin and eosin (H&E)–stained section and corresponding fluorescent detection of cellular labels (B) depicts a cluster of MSCs in proximity to host myocardium. Several muscle-specific proteins are detected by immunoflouresence including α-actinin (C), phospho- lamban (D), tropomyosin (E) and troponin T (F). Yellow fluorescence indicates colocalization of immunofluorescent antibodies and DiI. (G) H&E stained sections of vascular structures at the border of the infarcted myocardium. Corresponding sections depict DAPI stained MSC nuclei (H) with immunofluoresecent detection of factor 8 (I).

In terms of functional responses, anterior MI caused dramatic deterioration of systolic and diastolic ventricular function, and impaired cardiac energy metabolism ($p<0.05$ vs. pre-MI values). Compared with injection of placebo, MSC cardiomyoplasty resulted in profound improvements in myocardial function and efficiency (Figure 2). Figure 2a depicts representative examples of pressure-dimension data from animals in either group. As shown, MSC treatment led to a pattern of LV recovery over a 2-3 month period marked by a substantial increase in stroke work (SW, the area within the loops). In the placebo-treated group, impaired cardiac function evident 3 days post infarction either persisted or worsened over 8 weeks of follow-up: indices of myocardial contraction fell and end-diastolic pressure rose (Figure 2 a,b,c,d). In marked contrast, LV end diastolic pressure increased to normal 8 weeks after MSC treatment (*$p<0.05$ vs. placebo). MSCs caused myocardial performance to recover to normal, both in systolic (Ees rose to 13.9±2.7 mmHg/mm and peak +dP/dt to 2465±575 mmHg/sec) and diastolic function (Tau fell to 37±3.8 msec).

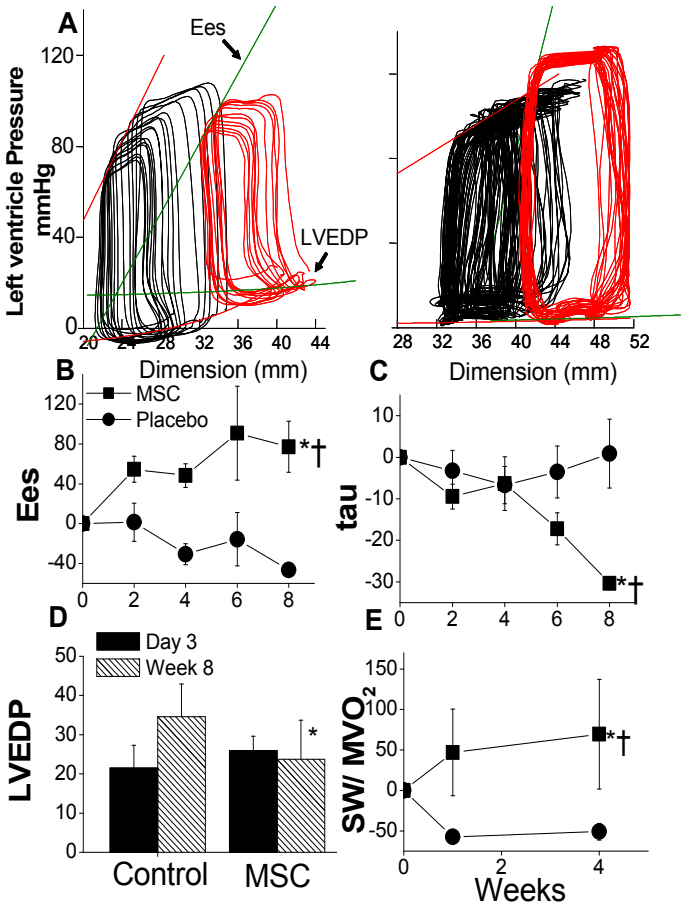


Figure 2. Physiologic impact of MSCs delivered with the BioCardia Catheter following anterior myocardial infarction (MI) in pigs. **(A)** Pressure-dimension (PD) data from placebo (left) and an MSC-treated (right) pig obtained 3 days (black loops) and 8 weeks (red loops) following MI. Placebo animals exhibit an increase in left-ventricular end-diastolic pressure (LVEDP) and dimension. Both myocardial contractility, measured by the slope of the end systolic pressure-dimension relationship (ventricular elastance, Ees), and ventricular stroke work, pressure-dimension loop area, decline in controls. In MSC-treated animals, Ees and stroke work increase to normal. **(B-E)** Average hemodynamic responses over 8 weeks showing divergent responses in cardiac function in MSC vs. placebo treated animals. **(B)** Ees declines in placebo-treated pigs but increases in the MSC group. **(C)** Isovolemic ventricular relaxation (τ), reduces to normal in MSC pigs but remains unchanged in placebo. **(D)** LVEDP increases in placebo but remains unchanged in MSC pigs. **(E)** Stroke work declines in placebo-treated animals while myocardial oxygen consumption (MVO2) increases (81±10.4%), leading to reduced SW/MVO_2. In contrast, in MSC-treated pigs, stroke work increases 89.8±15.3%, MVO_2 decreases 48.9±16.7%, resulting in augmented SW/MVO_2 and restoration of mechanoenergetic coupling toward normal. *$p<0.05$ *vs.* placebo and †$P<0.05$ vs. 3-day following MI, by ANOVA.

Heart failure and the aging cardiovascular system are characterized by mechanoenergetic uncoupling: decreased efficiency of work per unit oxygen consumption. In placebo-treated animals, SW decreased substantially during the 8-weeks following infarction, and there was a paradoxical increase in myocardial oxygen consumption, resulting in decreased ratio of SW/MVO_2. Conversely, MSC-injected animals' follow-up was marked by improving myocardial efficiency, both because of increasing SW (from 374.4±59.3 to 654.4±129.9 mmHg.mm at 8 weeks) and because of decreasing MVO_2 (from10.3±2 to 3.7±1.8 J/beat), both toward normal (Figure 2 e). Thus, MSC therapy exerts favorable effects on the damaged heart that extend to

improvements in cellular energy metabolism. The SW/MVO_2 ratio increased from 2.5±0.6 at 3 days post-MI to a normal ratio of 10±5.6 ($p<0.05$ *vs.* placebo) at 4 weeks. This improvement in mechanoenergetics was the earliest observable benefit of MSC treatment, preceding changes in global cardiac function. Improved mechanoenergetic coupling in the MSC group is consistent with several possible mechanisms, including reduced native tissue death[64], new tissue formation[42;65], or stimulation of endogenous repair mechanisms[66;67].

To further investigate the mechanisms of MSC-mediated cardiac repair, both MRI and computed tomography (CT) were used to image and quantify myocardial infarcts in MSC- and placebo-injected swine. Infarct size measurement *in vivo* by MRI and CT correlated tightly to that determined by triphenyltetrazolium chloride (TTC) staining post-mortem. Furthermore, using a 32-slice multidetector CT, the same endocardial rim of viable, non-infarcted myocardium observed in the first series of post-mortem hearts (Figure 3, Figure 4) was identified by *in vivo* imaging.

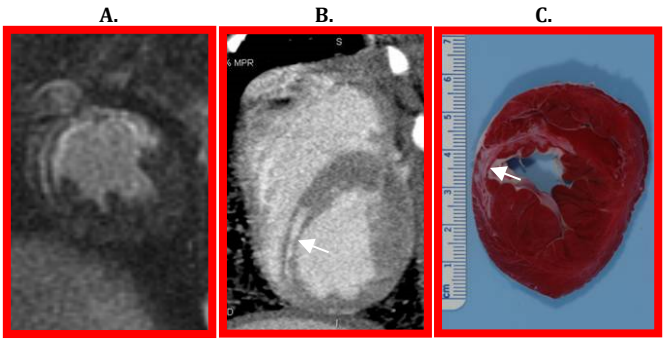


Figure 4. Comparison of infarct size using MRI (A), CT (B), and TTC (C). Images were obtained 8 weeks after closed-chest infarction in a pig and demonstrate subendocardial myocardial infarction as hyperenhancing region (~7-11 o'clock). TTC nonstaining areas (e.g., lack of brick red staining) in post-mortem slices (bottom) demonstrate concordance of infarct location and size with MRI and CT. Infarct region is notable for rim of noninfarctied myocardium along the endocardial border seen with CT and TTC staining. (arrows)

These data not only speak to the therapeutic potential of MSC cardiomyoplasty, but also establish that noninvasive imaging techniques can be used to measure the effects of cardiomyoplasty, and to study the mechanisms underlying these effects. These results in this pig model provide strong rationale for the development of MSC-based cellular cardiomyoplasty strategies and support ongoing human studies.

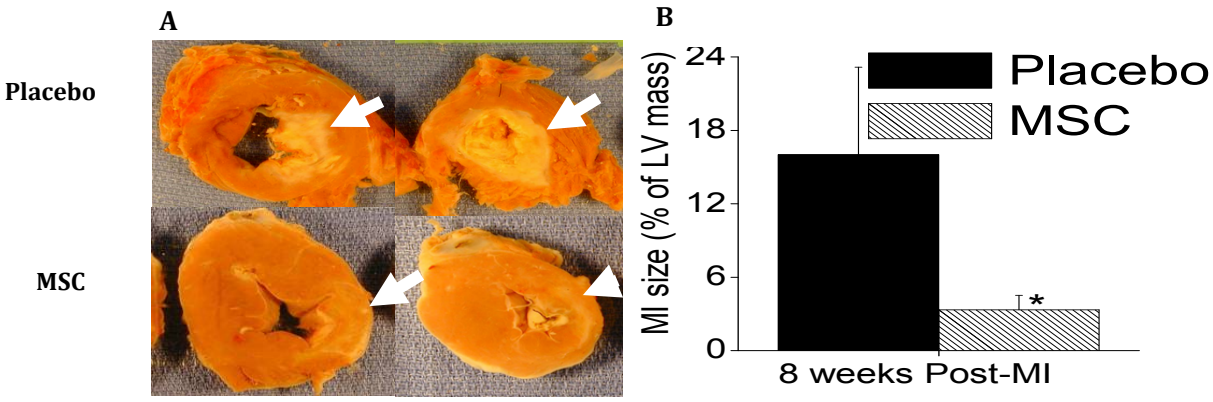


Figure 3. Myocardial infarct size 8 weeks following transient left anterior descending coronary artery occlusion. **A.** Representative example of scar formation due to myocardial infarction in placebo (top) and MSC treated animal (bottom). In placebo-treated animals the area of scar formation is transmural (arrow), while in the MSC group the scar area is barely visible and surrounded by non-scar tissue on both endo- and epicardial sides. **B.** Bar graph depicting scar formation as a percentage of LV mass. *P=0.008.

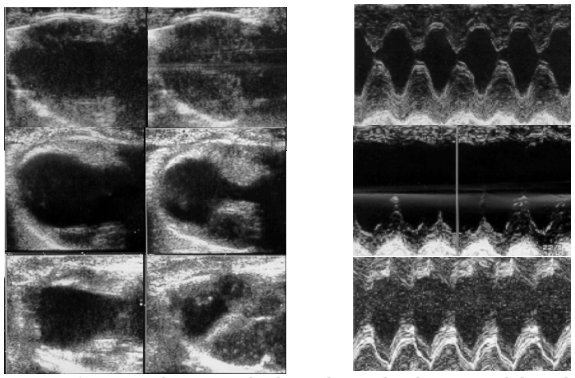


Figure 5. A: Two-dimensional echocardiography showing end diastole (left column) and end systole (right column) in a treated rat 1) before infarction 2) after infarction and prior to treatment and 3) 4 weeks after treatment with MSCs. **B:** M-Mode from same animal showing fractional shortening at the papillary level at the same time points as in A.

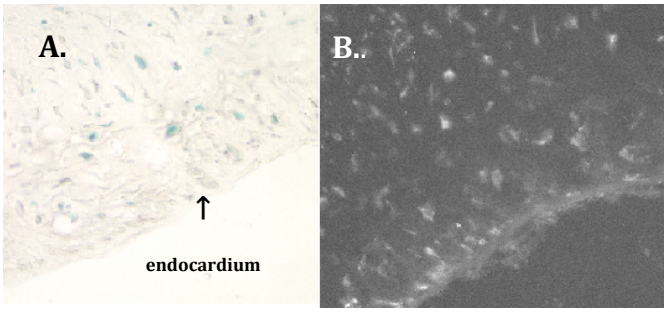


Figure 6: A: β-galactosidase positive (blue) cells are visible at 20X magnification within the infarct in young rats. These cells form a band along the endocardial surface. **B:** These cells show evidence of α-actinin expression on immunofluorescence, also at 20X magnification (bright appearing cells, B&W image). **C:** Quantification demonstrates 10-fold higher engraftment in young relative to old ($p<0.001$).

MSCs injected intravenously home to and engraft in infarcted myocardium conferring functional benefit: Preliminary studies were conducted on the efficacy of MSCs administered intravenously (*I.V.*) in a rat model of permanent left anterior descending (LAD) artery occlusion. Echocardiography was used to assess LV function at baseline, in the peri-infarct period, and four weeks after MI. MSC injection in Wistar rats led to dramatic improvement in LV function, with increased myocardial thickening and contractility in treated animals (Figure 5A and 5B). Labeled cells were identified within the infarct (Figure 6a), and were shaped like fibroblasts but expressed the cardiac protein, α-actinin, albeit at lower levels than native cardiomyocytes (Figure 6b). These labeled cells were most evident at the endocardial rim of the infarct, a finding similar to that seen in the porcine studies above.

MSCs delivered intravenously (I.V.) distributed to the heart in response to an injury signal: MSCs injected *I.V.* at the time of coronary reperfusion homed to the myocardium, while cells injected *I.V.* two weeks after reperfusion were more likely to engraft in the bone marrow (Figure 7). Determination of SDF-1 and CXCR4 levels revealed not only that both are expressed by MSCs, but also that serum levels are up regulated immediately post infarct and remain elevated for at least 2 weeks (Figure 8).

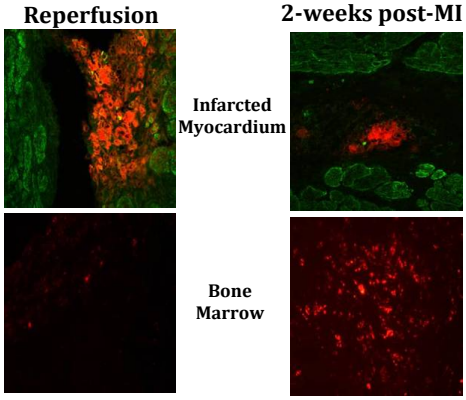


Figure 7. Confocal images of infarcted myocardium (top row) and bone marrow (bottom row) illustrating the degree of allogeneic MSC engraftment observed when 5 million MSCs were delivered via tail vein at reperfusion (left columns) or 2 weeks post-reperfusion (right column). As shown, at reperfusion cells home to the heart and not bone marrow; two weeks later, trafficking to the heart is reduced, and cells now migrate and engraft in the bone marrow. All tissues were harvested 4 weeks post-implantation. A FITC-conjugated antibody directed against desmin (green) was used to assess the myogenic differentiation. *CC

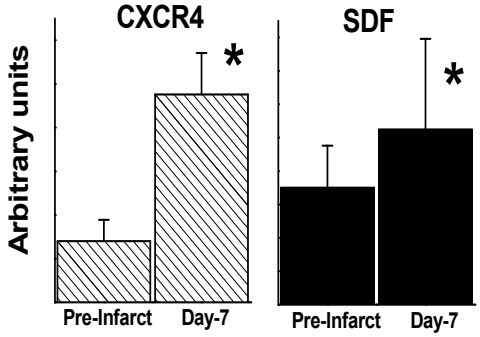


Figure 8: Both SDF-1 and CXCR4 levels are elevated following myocardial infarction. $P<0.05$ vs pre infarct

Myocardial Function can be determined *in vivo* by MRI

The clinical research team has extensive experience using tagged MRI scanning to detect and quantify alterations in regional myocardial mechanics in animal models of ischemic heart disease[68;69]. The team has also developed non-surgical, MRI-compatible animal models for studying cardiac mechanics, perfusion, and interventional procedures[70-73]. The team has recently developed new MR imaging and analysis methods that enable the rapid determination of myocardial function in infarction and ischemia[70]. This new technique, Harmonic Phase (HARP) MRI[74;75] is based on tagged MRI techniques. Computationally, the analysis of HARP MRI can be performed much more rapidly than traditional tag tracking in tagged MRI. "Real-time" HARP imaging exploits the concept that only one of the spectral peaks must be acquired for motion in one direction; this in turn accelerates the image acquisition in addition to the rapid analysis already available in HARP. An example of the use of real-time HARP to monitor the onset of ischemia in a canine model of coronary artery stenosis is depicted (Figure 9)[104]. Using this technique, we can identify abnormal regional myocardial contraction after the onset of ischemia 20 seconds earlier than can be done using conventional cine wall motion studies and one minute earlier than ECG changes. HARP MRI, similar to tagged MRI, yields quantitative motion and strain parameters on a regional basis that can be used for comparison across subjects or at serial time points after intervention. Thus, HARP MRI and analysis represents a rapid and repeatable method to assess left ventricular function serially in a quantitative manner. HARP provides fast, accurate assessment of myocardial strains in humans with and without coronary artery disease[76]. Similar techniques have been used to assess structural and functional changes after myocardial infarction in rats[77;78].

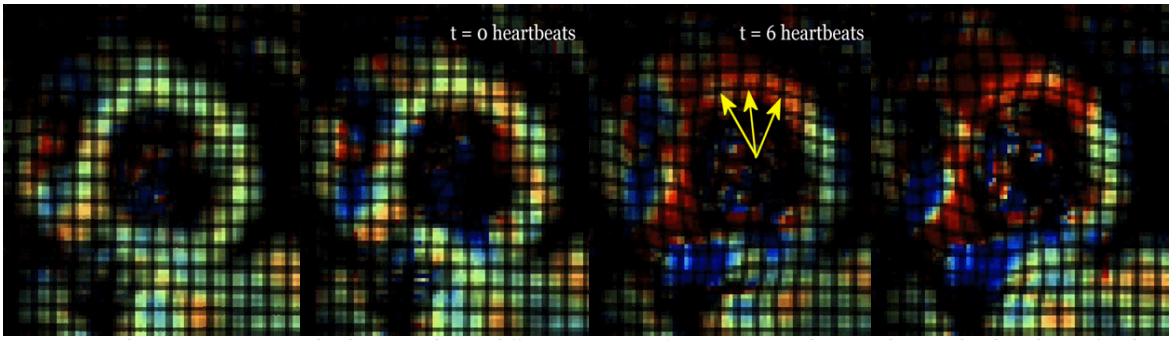


Figure 9: Real-time HARP images in the short-axis plane at different time points (10 sec prior to ischemia and 0, 6, and 20 heartbeats after the onset of ischemia from left to right) in a canine closed-chest model of acute LAD coronary artery occlusion. Overlaid on the tagged images is a pseudo-color map of circumferential shortening where green is uniform shortening, red is decreased shortening or stretching, and blue is increased shortening. At 6 heartbeats after ischemic insult, stretching of the ischemic myocardium is observed in the LAD bed whereas wall motion abnormalities by cine MRI could not be appreciated until 30 sec post-occlusion.

Noninvasive Determination of Infarct Size

Using an intravenous injection of Gd-DTPA, the study team is able to determine infarct size non-invasively with T1-weighted contrast-enhanced MRI (i.e., "Delayed Contrast-Enhanced MRI"). Short-axis image slices, which span the entire left ventricle, can be obtained using multiple breath-holds to yield images with the highest spatial resolution.

The size of the area of hyper enhancement measured on such images has been shown to be within 10% of the infarct size measured by post-mortem TTC staining (Figure 10). Alternately, using new imaging techniques, one can obtain the entire 3D left ventricular volume in a single breath hold (~16 heartbeats). It was recently shown that the 3D technique is in concordance with infarct size as measured by traditional 2D multi-slice techniques (Figure 10)[89,90]. Thus, infarct size and location can be accurately determined in less than one minute of scanning time, in order to determine the size of an infarct prior to therapy and over time.

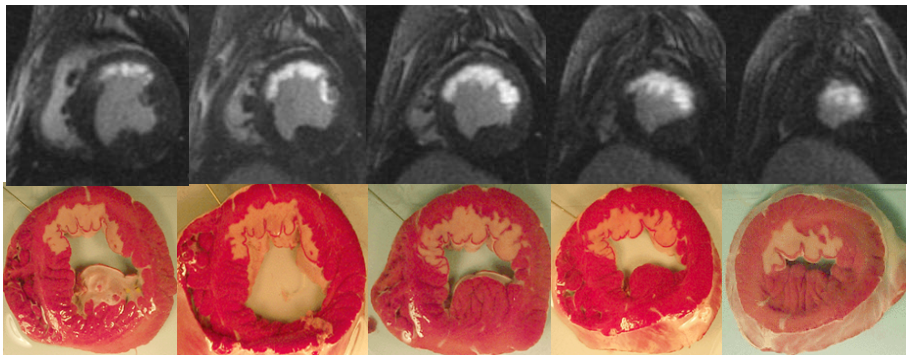


Figure 10. 2D delayed CE MRI in short axis plane (top) acquired within the first 24 hrs after closed-chest infarction demonstrating subendocardial myocardial infarction as hyperenhancing region (~11-1 o'clock) in a dog. TTC nonstaining areas (e.g., lack of brick red staining) in post-mortem slices (bottom) demonstrate concordance of infarct location and size with MRI.

Autologous Mesenchymal Stem Cells Produce Reverse Remodeling in Chronic Ischemic Cardiomyopathy: In addition to the studies outlined above using models of acute MI in the pig, we have also developed a model of chronic MI in the Gottingen mini-swine. We have used both autologous and allogeneic MSCs, with surgical and catheter delivery strategies, and have developed sufficient experience to translate the therapy from the laboratory bench to clinical trials. Together our results indicate that bone marrow derived MSCs stimulate cardiac recovery by engrafting, forming new blood vessels that increase tissue perfusion in hypoperfused areas, forming new cardiac myocytes, and importantly interacting with endogenous precursor cells to also contribute to new cardiac myocyte formation. From an immunologic perspective, MSCs may be safely used as an allogeneic graft, and have been done so extensively in clinical trials[79-81].

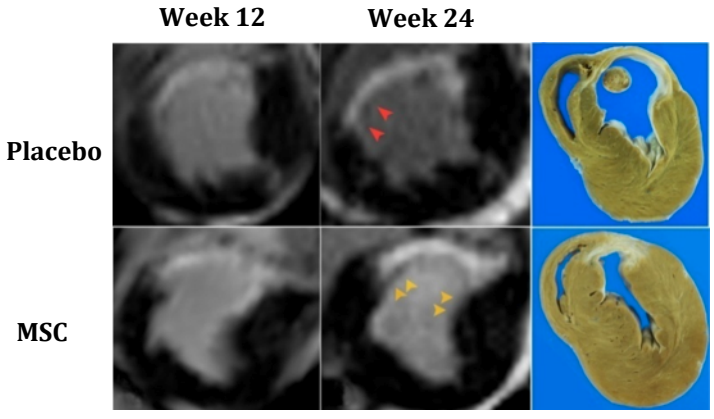


Figure 11. Myocardial regeneration in our porcine model of MI. Delayed gadolinium-enhanced MRI images depicting chronic (week 12 post MI) myocardial scar before treatment and (week 24) 12 weeks following injection of MSCs or placebo. Infarct tissue appears bright white, and healthy myocardium appears black. Comparable gross heart sections are shown adjacent to the MR images. Infarct size is reduced by MSC treatment, and cardiac performance improves.

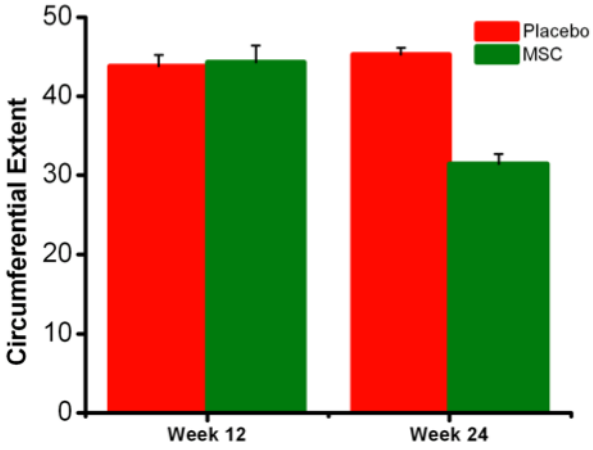


Figure 12. Infarct circumferential extent of the LV before and 12 weeks after injection in swine. Infarct size was significantly reduced by MSC treatment. $p<0.05$ MSC vs. placebo, $p<0.05$ MSC week 12 vs. week 24. [n=10]

Our experience with a 53 subject, 10-center, phase I study under the sponsorship of Osiris therapeutics, which demonstrated safety and provisional efficacy of allogeneic MSC therapy in subjects with acute infarction is outlined below[17]. In animal studies conducted in mini-swine, MSC injection via catheter into infarcted tissue reduces myocardial infarct size (Figs. 11 and 12), improves global and regional LV function, normalizes cardiac energetics, and restores tissue perfusion[63;82]. These results form the basis for our approval to conduct the CRATUS study.

Our work in large animal models with fully healed scars after MI showed that MSC administration can significantly improve left ventricular structural and functional indices, indicating meaningful repair. Using sophisticated imaging techniques, we tracked phenotypic improvements triggered by implantation of MSCs in a porcine model of chronic ischemic cardiomyopathy and quantified these changes morphometrically. MI was created in swine; after 12 weeks, the infarct segment had thinned, leaving a transmural scar (Figure 11). Autologous MSCs were expanded from each animal[83], and these cells or placebo were delivered to the infarct and surrounding border zone at this time. During a further 12-week follow up period, cardiac MRI revealed that intramyocardial injections of MSCs not only reduced the scar burden (as a percentage of LV mass) by 21.8$\pm$3.9% ($p<0.05$ vs. placebo and week 12 vs. week 24) (Figure 11, 12), but also significantly improved regional contractility, global LV function, ejection fraction, and myocardial blood flow. Importantly, the therapy produced reverse remodeling and reduced the circumferential extent of the infarct scar (Figure 12). This constellation of effects suggests highly effective repair of ischemic cardiomyopathy. We subsequently confirmed reverse remodeling in a pilot study of 8 subjects with ischemic cardiomyopathy (Figure 21)[43].

Allogeneic Mesenchymal Stem Cells Restore Cardiac Function in Chronic Ischemic Cardiomyopathy Via Trilineage Differentiating Capacity:

We tested the hypothesis that MSC based cardiac repair regenerates the heart via mechanisms comprising long-term engraftment and by differentiation into both myocardial and vascular elements. We generated allogeneic MSCs from a male swine donor, and administered sex mismatched cells by transendocardial injection into female swine 12 weeks post-MI. Animals were followed with serial MRI, and 12 weeks later the hearts were collected for immunohistological evaluation. The fate of the male donor cells was determined by co-localization of Y-chromosome (Y^{pos}) cells with markers of cardiac, vascular, and endothelial lineages. MSCs engrafted in infarct and border zones and differentiated into cardiomyocytes (Figure 13) as ascertained by co-localization with GATA-4, Nkx2.5, and α-sarcomeric actin markers. In addition, Y^{pos}MSCs exhibited vascular smooth muscle and endothelial cell differentiation, contributing to large and small vessel formation. The number of cells engrafting correlated with the functional changes that occurred (Figure 13F). Thus, MSCs could engraft and repair hearts in chronic ischemic cardiomyopathy[84].

Ventricular remodeling is a progressive disease causing the myocardium to reorganize itself from an elliptical to a spherical shape. During its reorganization the heart will become enlarged and ventricular dimensions increase, assisting in reshaping a subject's heart. In swine models, MSCs show that the reduction in scar size supports reverse remodeling, which show a reduction in scar size as quickly as 3 days after injection. MSCs have shown as a promising cell-based therapy where previously there was limited means of improving a subject's heart function and survival[85].

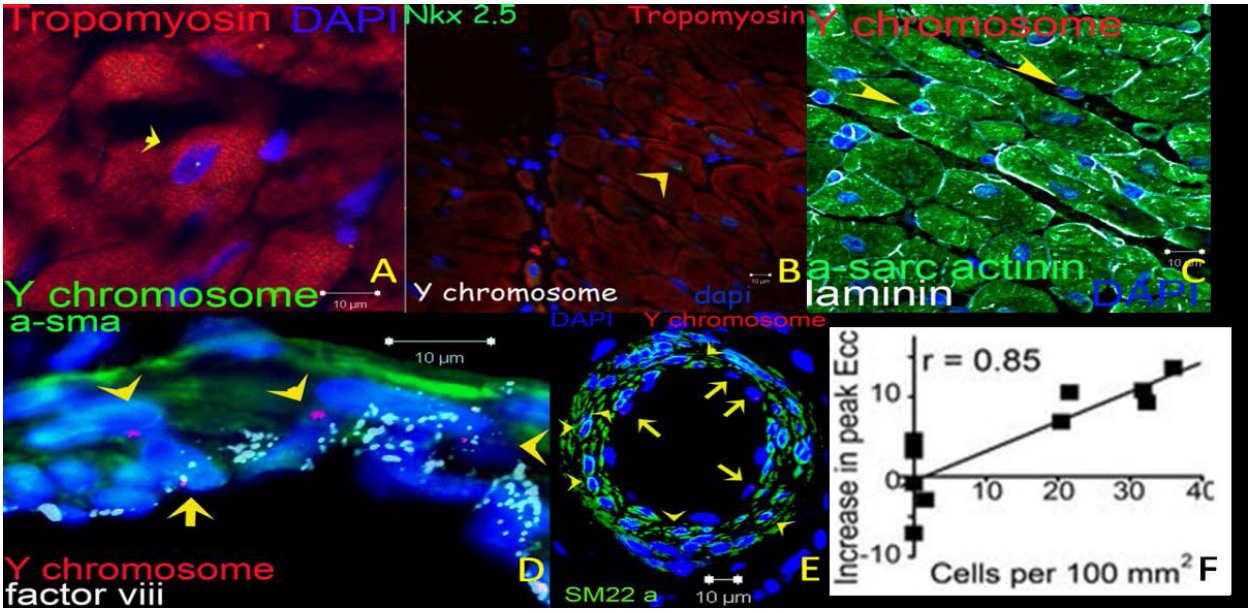


Figure 13. Engraftment of MSCs in chronic myocardial infarction. (**A**) Colocalization Y chromosome and troponin, indicative of an MSC differentiated into a cardiomyocyte. (**B**) Evidence of cardiac commitment in the transplanted cardiomyocyte by the colocalization of cardiac transcription factor Nkx2.5 (green, arrow). (**C**) Ypos cells also reside in the interstitial compartment (arrows) of border myocardium in a nondifferentiated stage. (**D**) Ypos cells that colocalize with smooth muscle actinin (arrowheads) and factor viiirelated antigen (white, arrows) demonstrating vascular smooth muscle and endothelial commitment, respectively. (**E**) Confirmation of Ypos cells commitment into vascular structures as depicted by colocalization with SM22-alpha (**F**) The functional outcomes (i.e. infarct size reduction, increase in contractility and increase in tissue perfusion) showed related interaction with the magnitude of transplanted cells detected.

Figure 14. Merging CMR angiography and electroanatomical mapping to guide transendocardial mesenchymal stem cell injection.

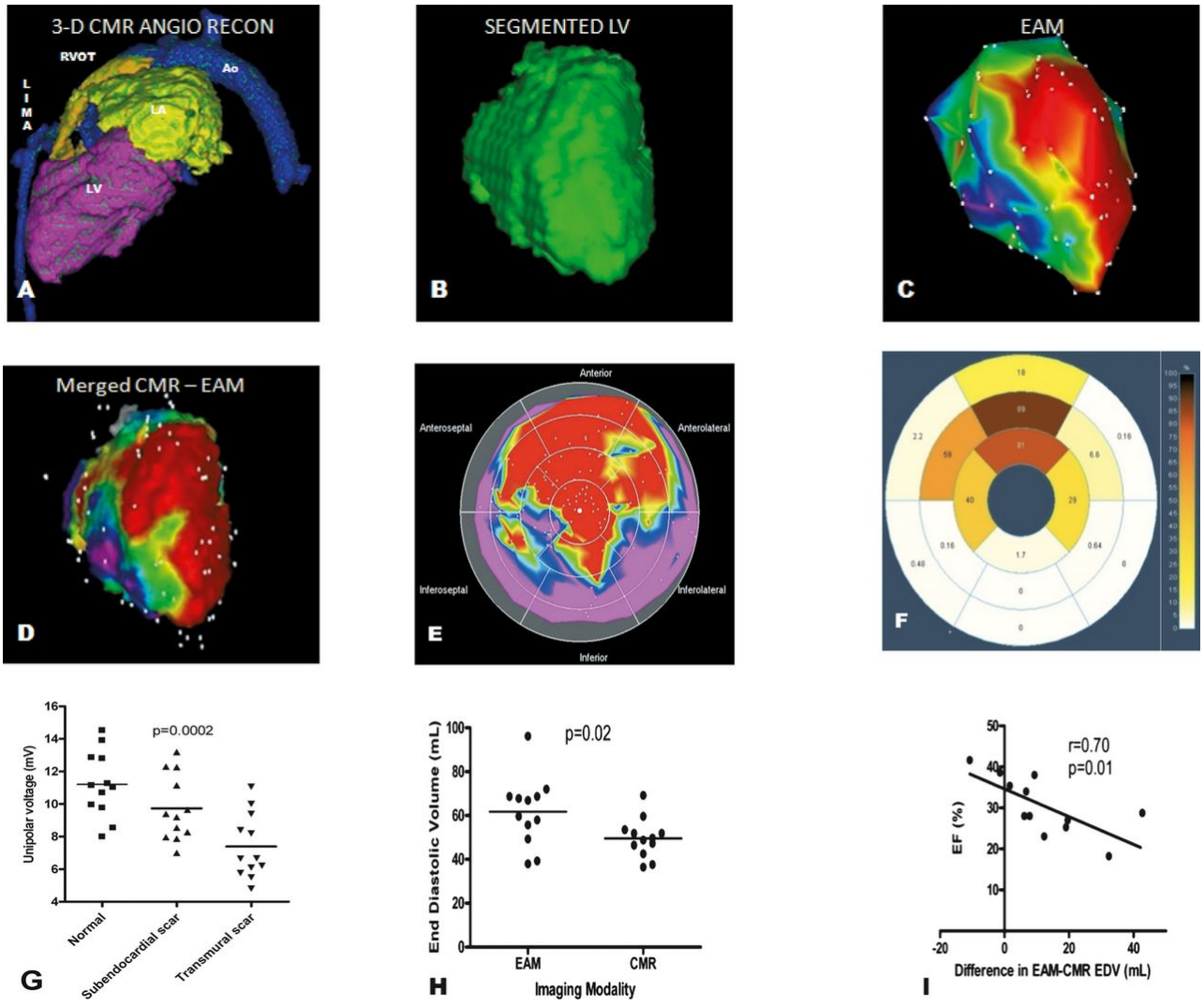


Figure 15: Durable and progressive scar size reduction due to intramyocardial injection of allogeneic bone marrow MSCs. A, Delayed-enhancement CMR scar size images show durable reduction in scar size with MSC therapy.

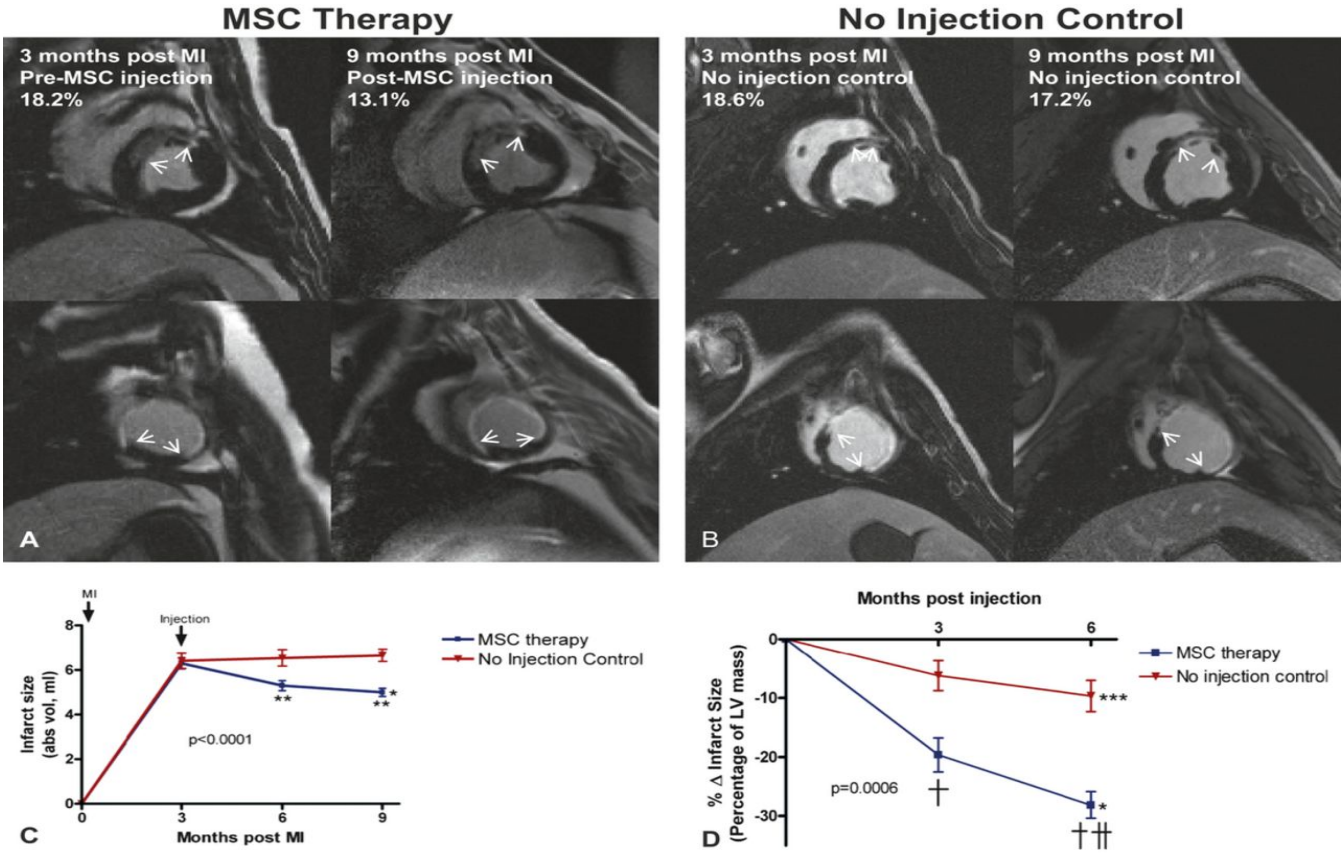
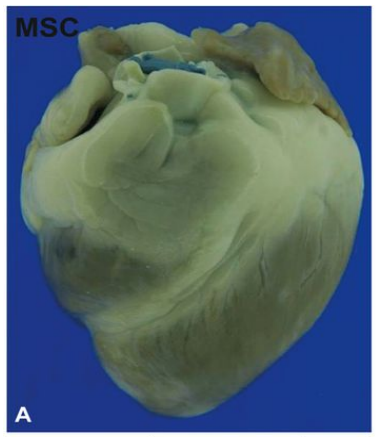
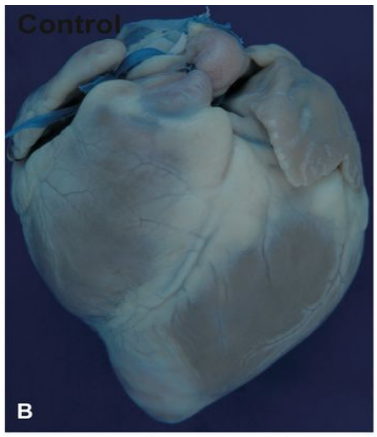


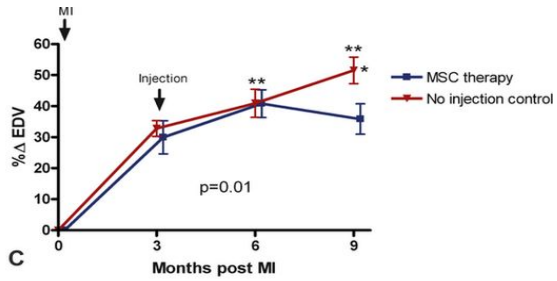
Figure 16: Allogeneic MSC therapy reverses remodeling in ischemic cardiomyopathy.



	3 mo	9 mo
EDV (mL)	37.6	37.7
ESV (mL)	24.3	19.3
Infarct size (% LV mass)	21.1	14.5



	3 mo	9mo
EDV (mL)	38.6	52.9
ESV (mL)	26.3	39.3
Infarct size (% LV mass)	20.3	18.2



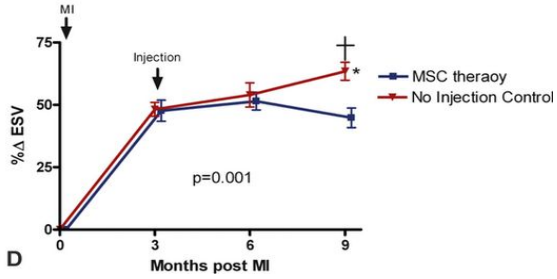
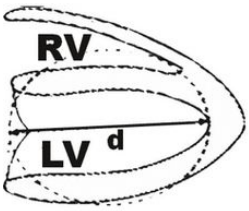
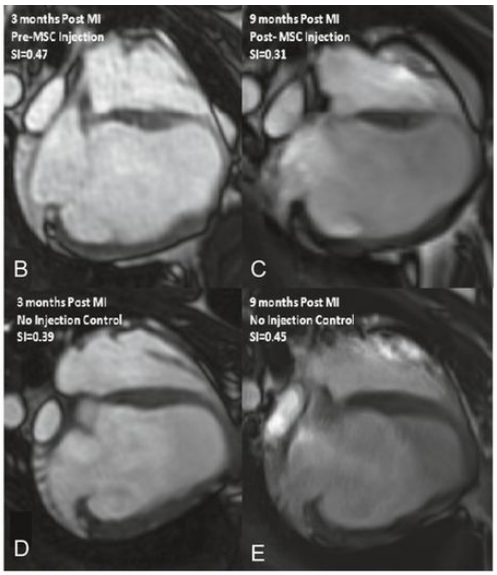


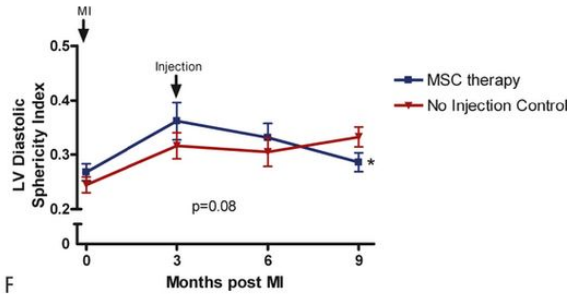
Figure 17: Allogeneic MSC therapy improves LV sphericity index.

LV Diastolic SI=
LV EDV
[4/3 x π x (d/2)³]

LV Systolic SI=
LV ESV
[4/3 x π x (d/2)³]







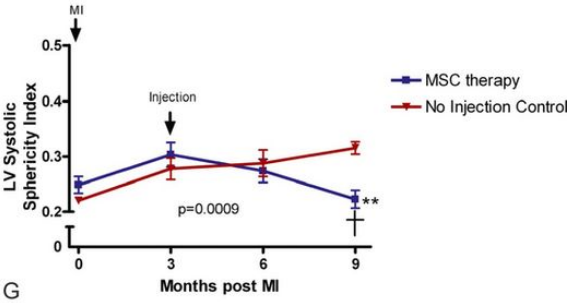
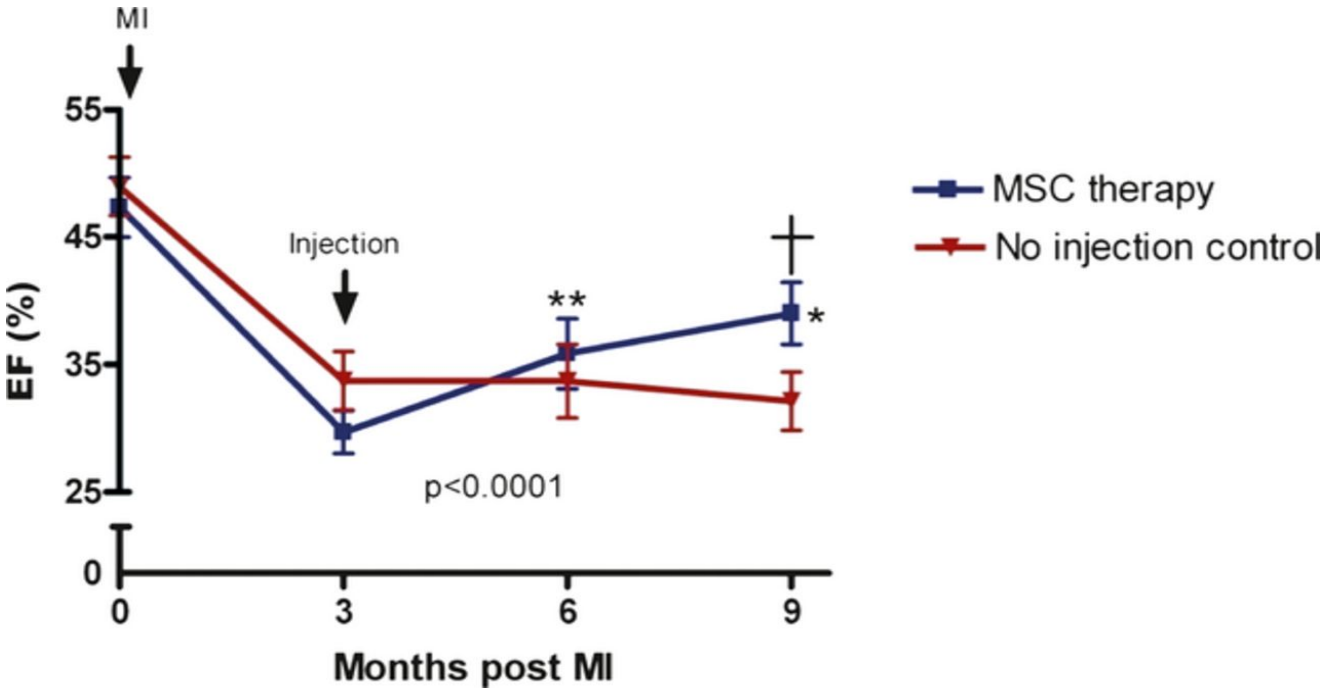


Figure 18. Progressive improvement in LV function with allogeneic MSC therapy.



1.4 Allogeneic Mesenchymal Stem Cells: 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.

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[17]. 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. In this study, 55 subjects received 1-5 intravenous infusions of 1.4 x 10^6 cells/kg body weight from HLA matched and mis-matched donors. A complete response was seen in 30 subjects and improvement was seen in 9 subjects. 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[86]. 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.

The Poseidon trial (IND #13568; NCT01087996), has provided additional validity that MSCs reduce infarct size and reverse remodeling of the myocardium[85]. Study data also reveal that MSCs are immunoprivileged and immunosuppressive, and do not cause acute immunogenic reactions. In this regard, no subject reported symptoms or indications of a reaction. Only one of 15 subjects receiving ahMSCs mounted a donor specific alloreaction, of low antibody titer[18]. Furthermore, subjects treated with allogeneic MSCs suffered fewer AE's versus autologous MSCs subjects. It is also hypothesized that the function of autologous MSCs could be impaired in subjects with co-mobility or advanced age[87;88]. Reverse cardiac remodeling was a prominent factor subjects showed as assessed by the study CT scan results[18]. Allogeneic MSCs were shown in this trial to be a safer and more effective form of cell-therapy than autologous MSCs. Figures 19 and 20 depict functional and quality of life improvements in subjects received both allogeneic and autologous MSCs.

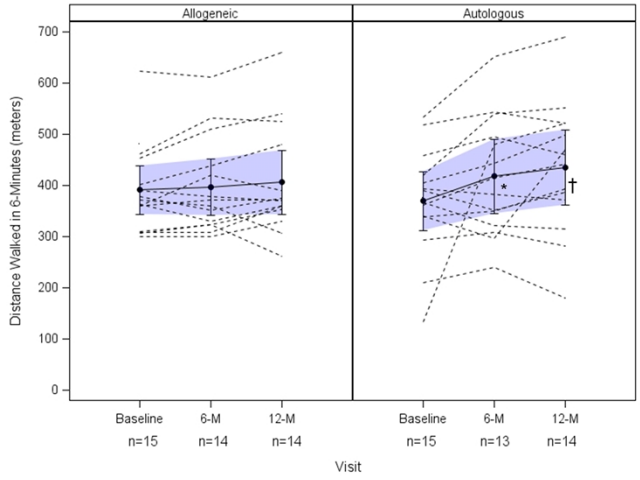
We randomized 30 subjects in an open-label study of either allogeneic or autologous hMSCs at 3 doses; 20, 100, or 200 million total hMSCs. The study was designed as a pilot study to compare the safety and efficacy of allogeneic versus autologous hMSCs in subjects with chronic ischemic left ventricular dysfunction secondary to myocardial infarction. Autologous MSCs were derived from a sample of the subject's bone marrow approximately 4-6 weeks prior to cardiac catheterization. Allogeneic MSCs were supplied from a human MSC source manufactured by the University of Miami. All 30 subjects tolerated the procedure well and have been followed-up with serial cardiac CT at 13 months post injection. The serious adverse event rate (SAE) was lower among subjects treated with allogeneic MSCs as compared to autologous MSCs (6-months: 20%-allogeneic MSCs and 40%-autologous MSCs). The injection of MSCs into the hearts of these subjects produced reverse remodeling, with reductions in EDV of ~23 ml and reduction in spherecity index of 0.8.

Together, these preliminary data support our hypothesis that allogeneic MSC administration is a safe and successful option for cellular cardiomyoplasty. We have

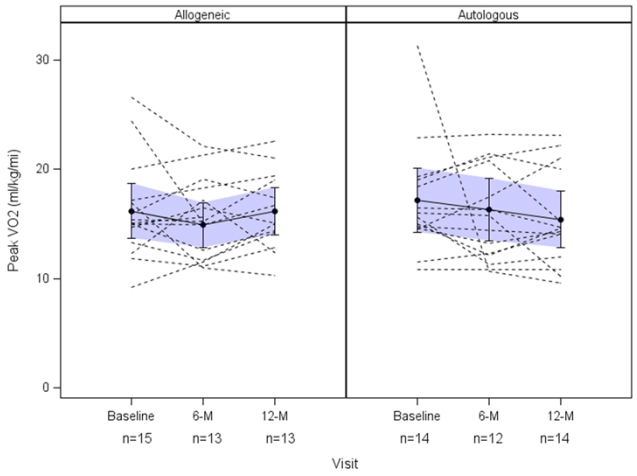
demonstrated long-term MSC survival, engraftment, and differentiation into myocardial, vascular, and endothelial lineages following transplantation into chronically scarred porcine myocardium. These cells' capacity for cardiomyogenesis and vasculogenesis both likely contribute to their ability to repair chronically scarred myocardium.

Figure 19: Functional Outcomes

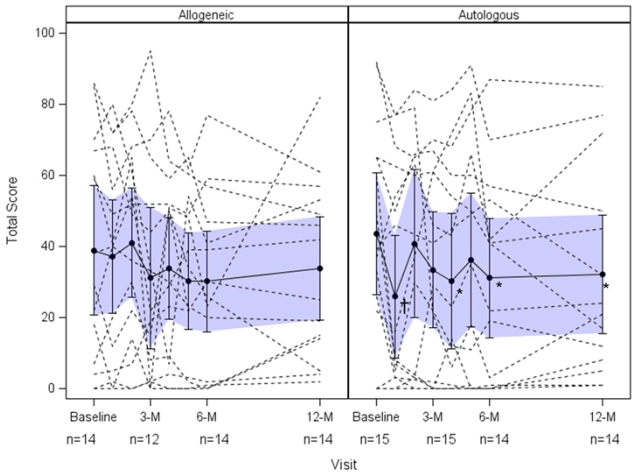
A) Six-Minute Walk Test (meters)



B) Peak VO_2 (ml/kg/min)



C) Minnesota Living with Heart Failure



D) New York Heart Association Class

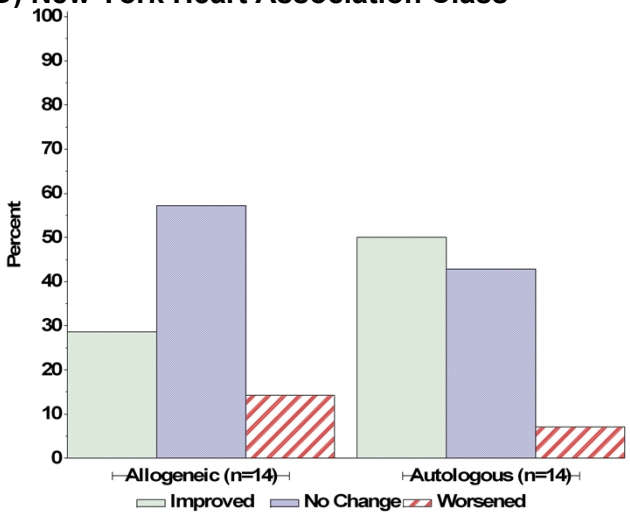
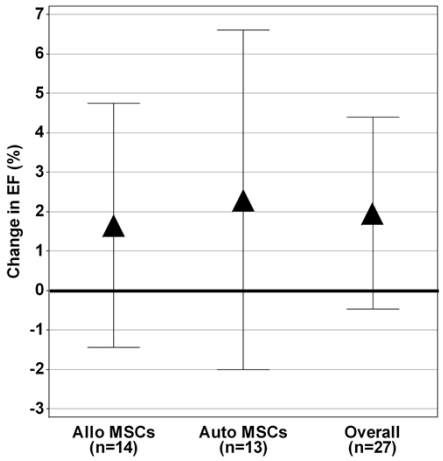
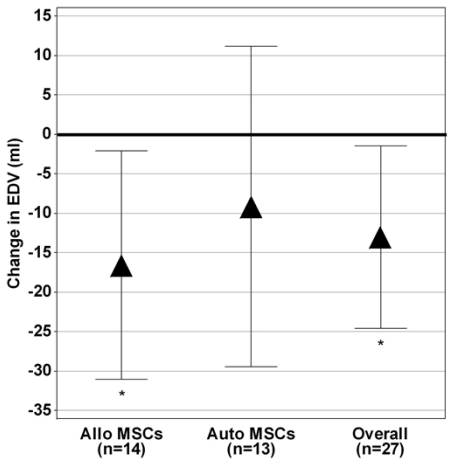


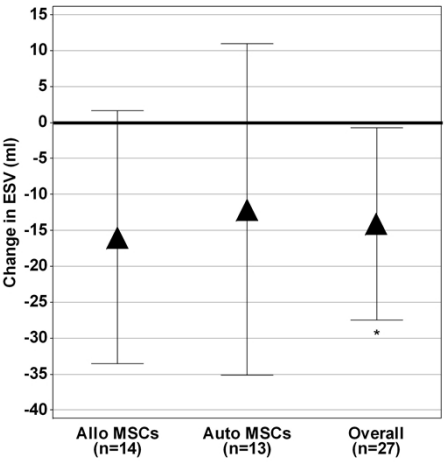
Figure 20: CT Parameters Change from Baseline

A) Left Ventricular Ejection Fraction (%) **B) End Diastolic Volume (ml)**

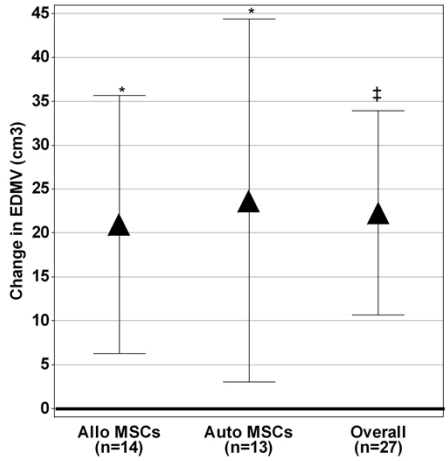




C) End Systolic Volume (ml)

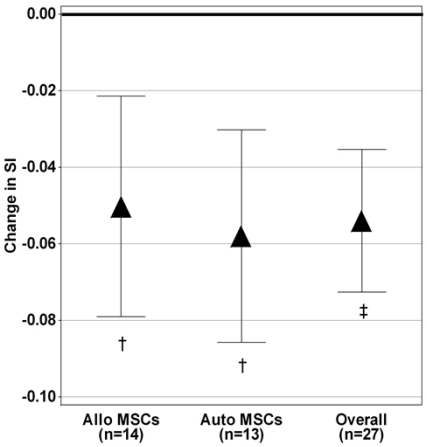


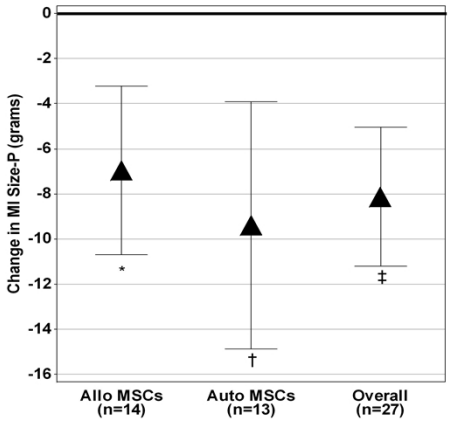
D) End Diastolic Myocardial Volume (cm^3)



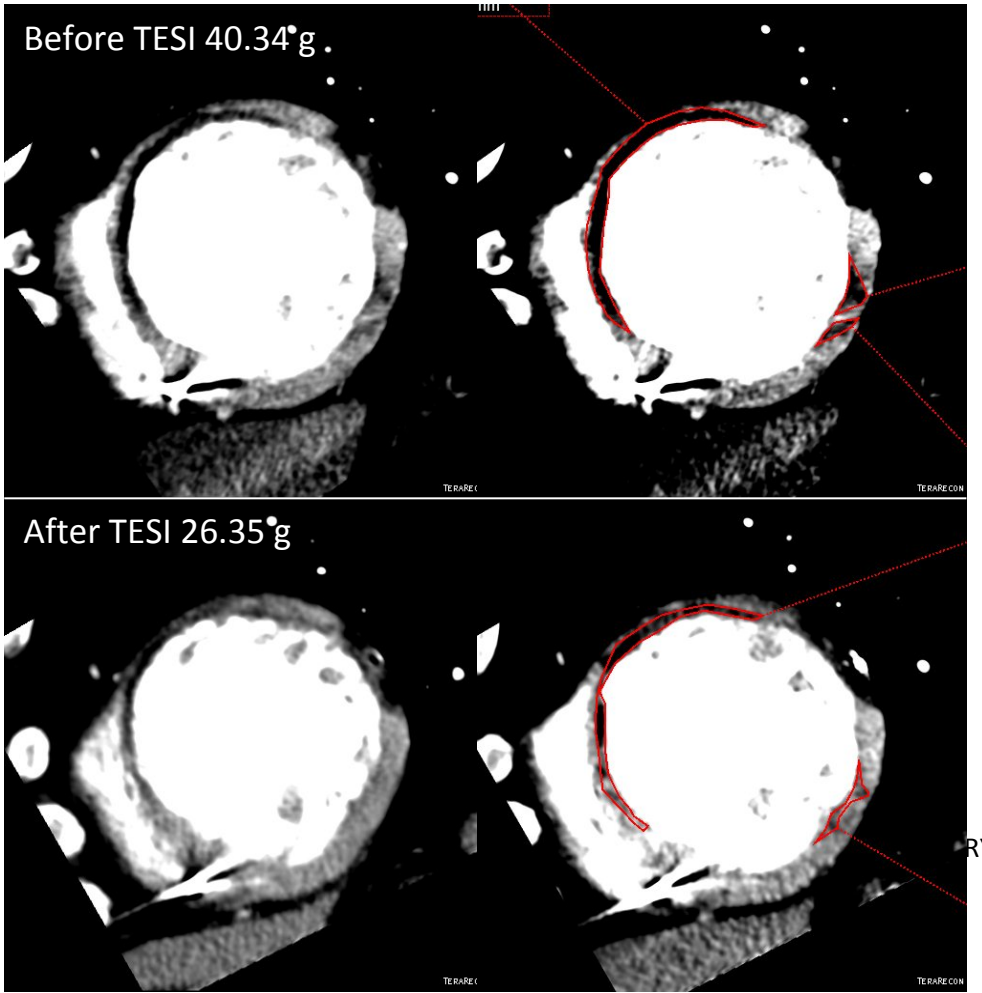
E) Sphericity Index

F) MI Size (Early enhancement defect; grams)


0.00
-0.02
-0.04
-0.06
-0.08
-0.10
Change in SI
†
†
‡
Allo MSCs (n=14)
Auto MSCs (n=13)
Overall (n=27)


0
-2
-4
-6
-8
-10
-12
-14
-16
Change in MI Size-P (grams)
*
†
‡
Allo MSCs (n=14)
Auto MSCs (n=13)
Overall (n=27)

G)

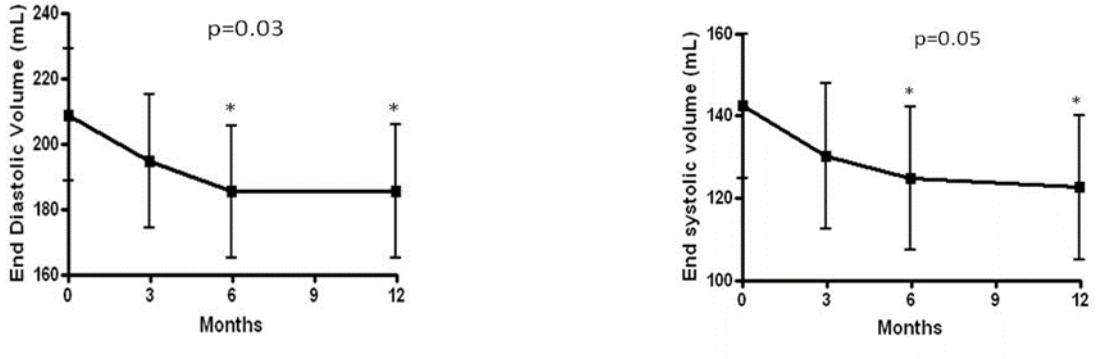

Before TESI 40.34 g
After TESI 26.35 g

RY***

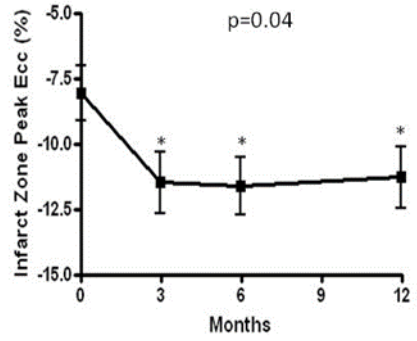
The study by Weiss et al, which investigated ahMSC therapy for COPD, reflected similar trial results indicating as the Poseidon trial that allogeneic MSCs did not cause acute immuogeneic reactions in subjects participating in the trial. Importantly, ahMSCs produced a decrease in the inflammatory marker CRP. Systemic administration of MSCs appears to be safe and decrease inflammation in an older and co-morbid population of subjects with compromised lung function due to moderate COPD[19].

Preliminary Results from the Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HFT): We enrolled 8 subjects in an open-label run-in phase of the TAC-HFT trial to assess the safety and preliminary efficacy of bone marrow mononuclear cells (MNCs) and MSCs in subjects with ischemic cardiomyopathy[24, 25]. At baseline each subject underwent a bone marrow aspiration and transendocardial injection (Helix Infusion Catheter; Biocardia, Inc., CA) of bone marrow derived MNCs or MSCs to the infarct and border zone using biplane fluoroscopic guidance. All 8 subjects tolerated the procedure well and have been followed-up with serial cardiac MRI at 3, 6, and 12 months post injection (Figure 21). The injection of bone marrow derived cells into the hearts of these subjects produced reverse remodeling, with reductions in EDV of ~12% and improved regional function as measured by –Ecc, a cardiac MRI derived index (Figure 21). As described above, we have also treated subjects with ahMSCs in the POSEIDON trial and have demonstrated an outstanding safety profile.

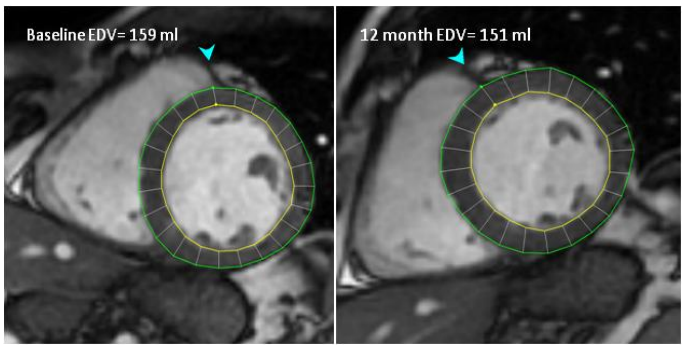
A. **B.**



C.



D. **E.**



F. **G.**

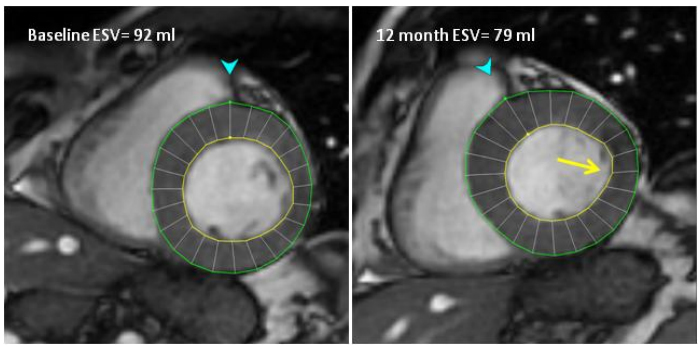


Figure 21. Cardiac MRI follow-up data of (A) end-diastolic volume, (B) end-systolic volume, and (C) peak Eulerian circumferential strain (Ecc) of the infarct zone from tagged imaging. Example cardiac MRI images of change in EDV from (D) baseline to (E) 1 year, and change in ESV from (F) baseline to (G) 1 year after stem cell injection. As depicted, EDV and ESV are reduced by 6 months following injection, and do not return towards baseline by 12 months. Peak Ecc is dramatically reduced by 3 months following injection (the more negative Ecc corresponds to *improving* regional LV function). *p<0.05 in post-hoc analysis compared to baseline. Yellow Arrow: Note improved systolic thickening in lateral wall, the site of cell therapy in this example.

1.5. Pharmacology and Toxicology Studies of Mesenchymal Stem Cells

Preclinical data suggest that MSCs may safely be used in the treatment of Frailty. 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[55]. Similarly, Ortiz et al intravenously administered 5 x 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[51]. Rojas et al intravenously administered 5 x 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[56].

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)[50]. 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[52].

Table 1. 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 BM-MSCs**	**Intravenous, 1 x 10^6 cells**	**No adverse effects reported.**	**Reduced edema, neutrophil infiltratration, collagen deposition. Improved survival.**
Ortiz et al, 2003	**Mouse**	**Allogeneic BM-MSCs**	**Intravenous, 5 x 10^5 cells**	**No adverse effects reported.**	**Reduced inflammation and collagen deposition.**
Rojas et al, 2005	**Mouse**	**Allogeneic BM-MSCs**	**Intravenous, 5 x 10^5 cells**	**No adverse effects reported.**	**Reduced pro-inflammatory 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 subjects 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[17]. 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[31]. 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 subjects. 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[86]. 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.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

- To demonstrate the safety of intravenous allogeneic hMSCs administered in subjects with Frailty and to explore treatment efficacy (decrease in frailty, frequency of acute exacerbations, change in symptom related quality of life, improved cardiovascular status, decrease in inflammatory biomarkers, endothelial function and 1 year survival).

2.1.2 Secondary Objectives

- To explore effects of allo-hMSCs on symptom related quality of life, cardiovascular performance, endothelial function and inflammation.

2.2 Study Endpoints

2.2.1 Primary Endpoints (Safety)

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

    - Serum chemistry: chloride, sodium, Carbon Dioxide, BUN, creatinine, glucose, calcium, AST/SGOT, ALT/SGPT, total bilirubin (fractionate if total >1.5 times normal), alkaline phosphatase, albumin,
    - Hematology (CBC): hemoglobin, hematocrit, platelets, WBC, WBC differential

2.2.2. Secondary Endpoints (Efficacy)

The following efficacy endpoints will be evaluated in this trial (During the screening, baseline, Month 3 and/or Month 6 visits):

1. Difference in rate of decline of Frailty defined as:
    a. Reduced Activity (assessed via CHAMPS questionnaire)

b. Slowing of Mobility (assessed via a 4 meter gait speed test and SPPB assessment)
c. Weight Loss
d. Diminished handgrip strength (assessed via dynamometer)
e. Exhaustion (assessed via the MFI questionnaire)
f. Decrease in subject quality of life assessment(s) (assessed via ICECAP, SF-36, EQ-5D Questionnaires)

2. Death from any cause.

3. Change between screening and 6 months in dobutamine stress echo induced ejection fraction.

4. Change between screening and 6 months for the following panel of inflammatory markers: CRP, IL-6, D-dimer, fibrinogen, CBC with differential, and TNFα

3. STUDY DESIGN

3.1 Description of the Study

A Pilot Phase will be performed to test the safety of dose and volume escalation of cells administered via peripheral intravenous infusion. The randomized portion of the study will be conducted after a full review of the safety data from the Pilot Phase by the DSMB.

In the pilot phase, the first three (3) subjects in each treatment group will not be treated less than 5 days apart for their first infusion and will each undergo full evaluation for 5 days to demonstrate there is no evidence of 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.

The fifteen (15) subjects that participated In the pilot phase will be able to receive up to an additional three (3) infusions of the study product for a total of four (4) possible infusions. See Addendum A and Addendum C for rationale and description.

Following the pilot study, thirty (30) subjects meeting all inclusion/exclusion criteria will be evaluated at baseline and 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 allo-hMSCs in subjects with Frailty and to explore potential efficacy at 3 and 6 months.

Approximately fifteen (15) subjects will be enrolled in the pilot phase and thirty (30) subjects with Frailty will be enrolled in the randomized phase for a total of up to forty-five (45) subjects. In the randomized phase, subjects will then be enrolled and randomized

1:1:1 to an active arm or placebo. Additional subjects may be enrolled if deemed appropriate.

Eligible subjects must have a diagnosis or symptoms of frailty as defined by the Canadian Study on Health & Aging[2]. Following informed consent before or at the screening visit, the diagnosis of frailty will be confirmed by investigator review of medical history.

In the randomized phase of the trial, electronic randomization will be performed using the Advantage EDC system and communicated to cellular laboratory personnel who have no contact with the investigators or subjects. 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 allo-hMSCs (100 million cells), 2 x 10^8 allo-hMSCs (200 million cells), 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 (Gait speed, handgrip strength, and QOL questionnaires) will be assessed following Day 0 until Month 6. Clinical laboratory tests to assess safety will be performed at every clinic visit, excluding the baseline visit and month 12 visit.

In the randomized phase of the trial, subjects who received placebo will have the option to receive 1 x 10^8 (100 million) cells of allogeneic hMSCs, if all study endpoints are met. If all endpoints are met then the subject will be administered the study drug and follow the study schedule listed in Section D.14 (Table D1) of Addendum D after subjects in the randomized phase have been unblinded.

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 ≥60 and ≤95 years at the time of signing the Informed Consent Form.
3. Show signs of frailty apart from a concomitant condition as assessed by the Investigator with a frailty score of 4 to 7 using the Canadian Clinical Frailty Scale
4. Female subjects must have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy.

4.2 Exclusion Criteria

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

1. Score of ≤24 on the Mini Mental State Examination (MMSE)

2. Inability to perform any of the assessments required for endpoint analysis (report safety or tolerability concerns, perform PFTs, undergo blood draws, read and respond to questionnaires.

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, total bilirubin > 1.5 mg/dl.5.

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. 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.

7. Be an organ transplant recipient.

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

11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C.

12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial.

13. 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.

14. Hypersensitivity to dimethyl sulfoxide (DMSO).

4.3 Concomitant Treatments, Procedures, and Nondrug Therapies

All concomitant medications (prescription or over-the counter) as well as procedures or nondrug therapies (e.g. continuous positive airway pressure, pulmonary rehabilitation) will be recorded at the initial screening visit and updated at each subsequent visit. Except for other experimental treatments or medications with putative disease modifying effects

in FRAILTY, subjects will continue all prior concomitant medications for comorbid diseases to ensure optimal general medical care.

4.4 Withdrawal Criteria

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

1. Subject request.
2. Subject is unable or unwilling to comply with the protocol.
3. 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 hMSCs (allo-hMSC) offers the potential for an "off the shelf" product for subjects. Significant data has been generated to demonstrate that the allogeneic hMSCs are immunoprivileged 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 and RPR. Potential donors testing positive for any of these infectious diseases with the exception of CMV, will be ineligible. Bone marrow (BM) aspirates will be obtained from normal individuals and allo-hMSCs will be isolated and expanded.

5.1 Bone Marrow Aspiration for Generation of MSCs

A total of approximately 60mL to 120mL of BM will be obtained from each normal volunteer. 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 antibiotic free (ie. No 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, first passage (P0) cells will be harvested by trypsin treatment and expanded to passage 1, 2, and 3. At passage 3, cells are harvested and cryopreserved.

5.2 Normal Donor Eligibility

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

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

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

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

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

- Complete blood count with differential

- Complete metabolic panel, magnesium, calcium, and uric acid
- Urinalysis
- Pregnancy test (Female only)

Eligibility Criteria for Normal Donors will include:

- Male and female gender
- No history of malignancy
- No active coagulopathy and/or hypocoagulable state
- No history of cardio/pulmonary conditions
- Negative tests for Hepatitis B, Hepatitis C, RPR, Chagas, HIV ½, HTLV I/II and NAT for HCV, HIV, Zika Virus, and WNV.
- Hemoglobin ≥ 13.0 g/dL if male; and if female donor hemoglobin ≥ 11.0 g/dL
- Platelet count 140,000 to 440,000/ul
- WBC 3.0 to 11.0 K/ul
- No anomalies on the CBC and differential suggestive of a hematopoietic disorder
- Creatinine ≤1.5 mg/dL
- ALT≤ 112 IU/L
- Bilirubin < 1.5 mg/dL
- No diabetes
- Systolic blood pressure ≤ 170
- Diastolic blood pressure ≤ 90
- No history of autoimmune disorders
- Negative serum or urine pregnancy test for female donors
- Body Mass Index (BMI) ≤ 30

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

5.3 Donor Consent

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

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

5.4 Follow-up Schedule for Donors

After discharge from the hospital, the bone marrow donor will be contacted by the study team with a follow-up telephone call 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 Donors

A separate 7 mL blood sample for gene expression profiling will be obtained at the donation visit. All samples will be identified so that they can be linked to individual subjects. These samples may be stored indefinitely. Individual results will not be returned to the subject or the study physician. The samples will be linked to subjects, but there will be no recontact. Data presented in publications will not contain individual subjects' clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

6. TREATMENT OF SUBJECTS

6.1 Study Investigational Product

The investigational product (IP) consists of hMSCs obtained from a healthy bone marrow donor. 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. BM will be obtained from normal volunteers with approximately 60 ml to 120 ml aspirated from the posterior iliac crest. The BM will be aspirated into heparinized syringes. The MNC fraction will be isolated using a density gradient with Lymphocyte Separation Media (specific gravity 1.077). The low-density cells will be collected and washed with Plasma-LyteA containing 1% HSA. The MNCs will be prepared antibiotic free (ie. No penicillin or streptomycin). The washed cells will be samples and viable cell numbers determined. The BM MNC will be seeded into 175 cm^2 tissue culture flasks in alpha MEM containing 20% FBS. After approximately 14 days of culture, passage zero (P0) cells will be harvested by trypsin treatment and expanded P1, P2 and P3. Cells are harvested using the Trypin treatment and each passage lasts approximately 7 to 10 days. At P3, cells are harvested and cryopreserved. This process may or may not contain penicillin and/or streptomycin. Any subject that test positive for a penicillin or streptomycin allergy will be provided with an antibiotic free MSC product.

6.2 Dosing

During the pilot phase of the study 15 subjects will receive a single infusion of hMSCs

Group 1 will consist of Five (5) subjects and will be treated with a single administration of allogeneic hMSCs: 2 x 10^7 (20 million) cells delivered via peripheral intravenous infusion.

Group 2 will consist of Five (5) subjects and will be treated with a single administration of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion.

Group 3 will consist of Five (5) subjects and will be treated with a single administration of allogeneic hMSCs: 2 x 10^8 (200 million) cells delivered via peripheral intravenous infusion.

After subjects complete their Month 12 follow-up phone call visit in the pilot phase, all 15 subjects will then have the option of receiving up to three (3) additional infusions of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion. The option will be provided if subjects in the pilot phase continue to meet all inclusion / exclusion criteria. See Addendum A and Addendum C for rationale and description for initial 15 subjects.

In the randomized phase of allo-hMSCs or matched placebo 30 subjects will be randomized in a 1:1:1 ratio to one of two Treatment Strategies or placebo following successful completion of the Pilot Phase.

Group A will consist of 10 subjects who will receive 100 million Allogeneic hMSCs delivered via peripheral intravenous infusion.

Group B will consist of 10 subjects who will receive 200 million Allogeneic hMSCs delivered via peripheral intravenous infusion.

Group C will consist of 10 subjects who will receive placebo via peripheral intravenous infusion.

The Allo-hMSCs will be derived from donors meeting criteria for allogeneic unrelated human bone marrow stem cell source manufactured by the University of Miami or from a commercial clinical grade bone marrow source.

6.3 Dosage Rationale

A safety profile for IV infusion of hMSCs was based on results from previous completed toxicology results[17]. 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 10^6hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6hMSC/kg per infusion).

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

Therefore, results from previous trials support the rationale on the safety and potential efficacy of the selected maximum dose of 200 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 20x 10^6, and 100×10^6hMSC/infusion, and 200x10^6 reconstituted with the 2.5 million hMSC/ml, in the following total volume

- 25 ml for 20 million dose (5 million hMSC/min)
- 40ml for 100 million dose (5 million hMSC/min)
- 80ml for 200 million dose (5 million hMSC/min)

For the randomized double blinded phase we will prepare the following doses of 100 million, 200 million and Placebo in an 80ml bag.

Cell will be delivered at a rate of 2ml/min, and delivered at a maximum rate of 16×10^6 hMSC/minute and will last approximately:

- 12.5 minutes for 25ml for 20 million dose
- 40 minutes for 40ml for 100 million dose
- 40 minutes for 80ml for 200 million dose.

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.

During the randomized phase each dose will take place over approximately 40 minutes in order to maintain the blind.

6.4.1. Infusion Monitoring

Subjects will be monitored in the ICU for two hours prior to infusion to establish baseline vital signs (oxygen saturation, heart rate, respiratory rate, blood pressure, and temperature) every 15 minutes. Monitoring will also continue throughout the infusion.

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

6.5 Concomitant Therapy

6.5.1 Permitted therapy

Concomitant medications will be recorded on the case report form (CRF), which includes all FDA-approved medications, 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, 2002) all applicable national and local regulations. Monitoring will be conducted by a qualified outside source at the study site.

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

6.6 Blinding and Unblinding

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- MSC product. CMV status of the recipient and donor of the Allo-MSC product will be matched. CMV positive Allo-MSC product will only be infused to a CMV positive recipient. All CMV negative recipients will receive CMV negative Allo-MSC product[91].

The designated cell-processing technicians will prepare both the allogeneic hMSCs 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 unblinding is thought to be necessary, the Investigator may identify the treatment assignment by obtaining the randomization assignment by contacting the Director of the Cell Manufacturing Program at ISCI who is responsible for maintaining randomization records for all subjects.

6.7 Study Investigational Therapy Management

6.7.1 Investigational Product Labeling and Storage

The product label contains the elements required by the CFR and other national and local authorities for investigational products. ISCI GMP Cell Processing Facility (CPF) will

directly store and deliver the designated cell processing technologist in the CPL/CPF, and will be kept cryopreserved in liquid nitrogen vapor phase until shortly before administration must be stored in a securely locked enclosure. Access is strictly limited to unblinded CPL/CPF personnel prior to preparation for infusion. After preparation for infusion, the Investigator and his or her designees are permitted to administer the Investigational Product to subjects participating in this protocol.

6.7.2 Investigational Product Accountability Procedures

In accordance with all applicable regulatory requirements, the Cell Processing Laboratory/Cell Processing Facility will maintain a record of the investigational products hMSCs received, dispensed, administered, destroyed, or returned. The final disposition of all unused, empty, and partially used Cryocyte™ bags will be handled in accordance with the drug preparation manual. An independent unblinded clinical research associate (monitor) or auditor will perform compliance monitoring during the study.

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 2

Schedule of Assessments

Table 2: Time and Events Table for Pilot and Randomized Phases

VISIT	Screening Day -56 ± 28 days	Baseline (-4 weeks)	Day 1	Week 2 (Day 14) +/- 2 days	Month 1 (Day 30) (±/-2 weeks)	Month 3 (Day 90) (±/-2 weeks)	Month 6 (Day 180) (±/-2 weeks)	Month 12 (Day 365) (±/-2 weeks) *Phone Call Follow-up
Informed Consent	x							
Full Medical History	x							
Physical Exam	x	x	x	x	x	x	x	
12-lead (ECG)	x	x	x	x	x	x	x	
Concomitant Medications	x	x	x	x	x	x	x	x
Mini Mental State Examination (MMSE)	x						x	
Randomization		x						
Infusion Treatment (IP)			x					
Dobutamine Stress Echo Test (DSE)	x						x	
Bone Density Scan (DEXA)[8]		x					x	
FEV-1		x				x	x	
6 Minute Walk Test		x				x	x	
4 Meter Gait Speed Test[7]		x				x	x	
SPPB Assessment		x				x	x	
Dynamometer (handgrip)[10]		x				x	x	
Smell Identification Test (UPSIT)		x				x	x	
IIEF, SQOL-F Questionnaires		x			x	x	x	
QOL Questionnaires (ICECAP, EQ-5D, SF-36,CHAMPS, MFI)		x			x	x	x	
Questionnaires (Subject and Physician Global Assessment[9])							x	
Urinalysis	x				x	x	x	
Hemat., Chem., CBC, LFTs, INR, and other labs[1]	x		x	x	x	x	x	
HIV 1, HIV 2, Hep. B & C, and CMV	x							
Serum or Urine Pregnancy Test[2]	x		x					
Review Adverse Events			x	x	x	x	x	x
Immune Monitoring[4]			x	x	x	x	x	
Biomarker Assessment[3]			x				x	
Optional: Brachial Ultrasound [5]		x				x		
Optional: Endothelial blood samples[6]		x				x		

Time and Events Table Key:

1 – The minimal laboratory requirements for hematological, liver function and renal function include:

Hematology Tests: white blood cell count, platelet count, hemoglobin and hematocrit.

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase, asparate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin(fractionate if total >1.5 times normal).

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

Serum Uric Acid, Pro-BNP, and C-reactive protein (CRP), IL6, fibrinogen, D-Dimer, DNA, **TNFα, testosterone (males only) and estrogen (females only).**

2 – A serum or urine pregnancy test will be completed within 36 hours prior to infusion for females of childbearing potential.

3 – The following biomarkers will be analyzed:

- **Cell-surface markers**: CD19, CD27, IgD, and CD5 (for Switched Memory, Naïve, Late/Exhausted, IgM memory B cells and regulatory B cells); CD19 and intracellular TNF-α (to assess intracellular TNF-α); CD3, CD4, CD8, CCR7 and CD45RA (for Central Memory, Naïve, Effector Memory, TEMRA T cells and CD4 to CD8 ratio); CD3, CD25 and FOXP3 (to assess regulatory T cells)
- **Transcriptomic/Proteome:** RNA, miRNA, protein samples, and telomerase, akt
- **Growth factors**: Sdf-1, notch,
- **Functional Assays:** cell growth rate, VEGF, and CFU assay

4 – Immune monitoring for graft rejection. Calculated panel reactive antibodies will be performed from the serum of the subjects on day 1 and 6 months post treatment. In addition, the following cell surface markers will be used to assess for activated T-cells based upon a $CD3^+CD25^+$ (late/chronic T cell activation) or $CD3^+CD69^+$ phenotype (early T cell activation).

5 – Optional brachial ultrasound to assess endothelial function.

6 – Optional: An additional 5 lavender top tubes (EDTA) will be drawn to assess the ability of endothelial progenitor cells to form colonies.

7 – 4 meter gait speed test will be performed twice per visit and the average of the exams will be taken.

8 – DEXA scan will be performed at baseline and Month 6. The scans of the hip and spine for bone density, and total body composition will be assessed at both visits.

9 – Subject and Physician Global Assessments are not applicable to pilot subjects in the initial infusion of the trial.

10 – Dynamometer will be performed at least three (3) times during each applicable visit for each hand. The three values collected will be averaged for each hand. (Please reference the instruction manual on how to administer.)

7.2 Study Phases and Visits

7.2.1 Screening Visit

See Table 2 for the procedures and assessment to be performed during the screening visit of the study. Screening visit test and procedures will begin upon the subject 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 will take place over several days and will need to be completed prior to the subject starting the baseline visit.

Physical exam

A complete physical examination will include general appearance, skin, head and neck, Lymph nodes, musculoskeletal/extremities, cardiovascular, chest/lungs, abdomen, and neurological assessment. At screening information about the physical examination and any significant findings must be recorded in the source documentation at the study site. Weight should be measured at each physical exam on a calibrated scale with the subject wearing only light clothing and no shoes. Height (in bare feet or wearing only thin socks) will be measured at the Screening visit.

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 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.

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 when under stress to better evaluate ejection fraction in frail subjects. Guidelines for completing the (DSE) are outlined in Appendix 2 of this protocol.

7.2.2 Baseline Visit

See Table 2 for the procedures and assessment to be performed during the baseline visit of the study. Once all screening exams are completed and it has been determined that the subject remains eligible for the study, subjects will be enrolled into the study. The baseline visit will take place within four weeks from treatment. The listed procedures should all be performed as soon as practicable.

Bone density Dexa scan will be performed at the baseline visit (if not done within three months prior to enrollment).

The Smell Identification Test (UPSIT) test will be performed once at baseline and once at the Month 3 and Month 6 follow-up visits. Age-related olfactory dysfunction is felt to be due to cumulative inflammatory damage affecting the olfactory mucosa. The Smell Identification Test (UPSIT) is a self-administered 40-item olfactory test. It provides an absolute indication of smell loss (anosmia, mild, moderate, or severe microsomia) as well as an index to detect malingering.

Endothelial function (Optional Assessments) will occur upon the subject signing the optional section of the informed consent form (ICF). No endothelial function tests will take place until the subject is fully informed of the research and signs the optional portion of the consent form.

- Brachial ultrasound testing and blood collection will be performed to assess endothelial function in the aging frailty 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.

- Flow Mediated Diameter percent change (FMD%): All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C. Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the 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. In each examination, recording of vessel images will be followed by inflation of a cuff to supra-systolic pressure (40 to 50 mmHg above systolic pressure) for 5 minutes. Then the cuff will be deflated and the brachial artery diameter will be imaged and recorded for 3 minutes. FMD% more than 10% is considered a normal response. Lower than 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.

- Blood drawn from fasting subjects will be separated and the serum will be frozen until processed as one batch towards the end of the study. Blood will be processed twice – in the beginning of the study and after 3 months.

- Biochemical analysis: soluble pro inflammatory cytokines (interleukin-1, interleukin-6, interleukin-10, VEGFR2, TNF-a).

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

7.2.3 Day 1 Visit

See Table 2 for the procedures and assessment to be performed during the Day 1 visit of the study. The Day 1 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 2 for the procedures and assessment to be performed for week 2 through month 6 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 – Month 6 Visits

See Table 2 for the procedures and assessment to be performed for week 2 through month 6 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 weeks for the Month 1 through month 6 study visits.

7.2.6 Month 12 Visit

See Table 2 for the procedures and assessments to be performed for Month 12 visit. This visit will be conducted via a phone interview with the subject. A phone script will be

provided to the study personnel to use when interviewing the subject. There will be a +/- window of 2 weeks for this visit. All fifteen (15) pilot phase subjects will be informed with the option of having additional administrations of allogeneic hMSCs at this visit.

7.2.7 Biomarker Assessment

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.

7.2.8 Immune Monitoring for Graft Rejection

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

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

As part of the CRATUS protocol we will obtain peripheral blood samples from all subjects to evaluate the panel reactive antibodies (PRA) as well as 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: at day one prior to infusion of MSC and visits through month 6. 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 α). We will monitor the activation of T cells by flow cytometric analysis of CD3+CD25+CD69+ cells in thawed PBMC. CD69 is an immediate/early marker of CD3+ T cell activation while CD25 expression increases within 1-2 days of activation and remains sustained over the intermediate-long term during chronic immune activation. Given the differences in the kinetics of CD69 and CD25 up regulation, assessment of both activation phenotypes (CD3+CD69+ and CD3+CD25+) will maximize the sensitivity of detection of T cell activation following autologous or allogeneic MSC infusion.

8. SAFETY

8.1 Safety Variables

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

8.2 Laboratory Evaluations

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

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

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

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

8.2.1 Pulse Oximetry

Pulse oximetry will be used to observe oxygen saturation when measuring vital signs at screening. Pulse oximetry will also be used throughout infusions and 2 hours following infusions. 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.

8.2.2 Pregnancy

There is no information allogeneic hMSCs and its effects or potential risks to a fetus or unborn child. The Principal Investigator and DSMB must be notified within twenty-four hours of investigator's awareness of the pregnancy via facsimile if a study subject becomes pregnant during the study. Males and females of childbearing potential $\geq$ 60 to $\leq$ 95 years of age at the time of signing the Informed Consent Form with documented FRAILTY must practice any one of the enumerated forms of contraception. Items will be acceptable for meeting the studies contraceptive requirements as listed in section 8.2.2. Females will be defined as non-childbearing potential if surgically sterilized (i.e. bilateral tubal litigation, bilateral oophorectomy, or complete hysterectomy) or post-menopausal (defined as 12 months no menses with an alternative medical cause and with a follicle stimulating hormone (FSH $\geq$ 25.8 mIU/mL). 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.

8.2.3 Determination of Infusional Toxicity

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

8.2.4 Subject Stopping Guidelines

This guideline is to be used to indicate boundaries requiring discussion by the Data and Safety Monitoring Board (DSMB) and is designed to assist the independent DSMB in overseeing the study. The DSMB may also request additional interim analyses and develop other criteria including provision for monitoring of potential late effects to determine when to intervene in the enrollment or treatment of subjects in the study. The first more conservative stopping guideline is to monitor subjects for unexpected SAEs where there is a reasonable possibility that the study product or administration procedure caused the event within 30 days of administration including subject death, grade 3 myocardial infarction, or grade 3 hemodynamically unstable ventricular tachycardia. 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:

1. Any subject who develops persistent (that is, still existing more than 3 hours after the end of IP infusion) cardiorespiratory signs or symptoms (for example, shortness of breath, tachypnea, tachycardia, hypotension, or palpitations) will continue with all scheduled follow-up if such follow-up is considered safe in the opinion of the Investigator. A subject may still be able to receive additional infusions, based on the investigator's judgement.

2. Any subject whose infusion is stopped due to cardiorespiratory distress will not receive further IP infusions until it is considered safe in the opinion of the Investigator, but subjects will continue with all scheduled follow-up if such follow-up is considered safe. A subject may still be able to receive additional infusions, based on the investigator's judgement.

3. 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.

4. Monitoring of key safety endpoints will be conducted (Please reference section 2.2.1). If rates significantly exceed the pre-set threshold, then the DSMB will be advised.

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.

8.2.5 Subject observation and discontinuation after IP administration

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

8.3 Definition of an Adverse Event

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

Examples of an AE include:

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

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

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

4. 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:

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

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

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

4. 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.

8.4 Definition of Adverse Reaction

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

8.5 Definition of Suspected Adverse Reaction

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

8.6 Definition of Serious

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

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

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

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

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

8.7 Definition of Unexpected

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

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

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

8.9 Recording of Adverse Events and Serious Adverse Events

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

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

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

Pregnancies

Subject pregnancy must be reported to the Principal Investigator within 1 working day of knowledge of the event. Any subject that becomes pregnant during the study must be

promptly withdrawn from the study. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

8.10 Intensity of Adverse Events and Serious Adverse Events

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

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

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

8.11 Causality of Adverse Events and Serious Adverse Events

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

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

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

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

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

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

8.12 Follow-Up of Adverse Events and Serious Adverse Events

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

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

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

8.13 Timeframes for Submitting SAE Reports

Once an Investigator becomes aware that an SAE has occurred in a study subject, he/she will record the information in the electronic data record within 48 hours. Any fatal or life-threatening event must be reported within 24 hours. If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before recording the event in the data system and completing as much information known at the time of the submission. The reporting timeframes for any SAE occurring during the study are summarized in Table 3.

TABLE 3
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

8.14 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 subject s will be randomized according to a fixed allocation permuted block randomization schema. The allocation ratio will be 1:1:1. 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 $\alpha=0.05$ 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), which occur at approximately quarterly intervals, but can be postponed at the DSMB's discretion. The DSMB Chair will be notified each time an SAE occurs. After all subjects in phase I have received their first infusion and 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 1:1:1 or to recommend a dose modification for the randomized placebo study.

These should not be considered formal interim analyses as no hypothesis testing will be done.

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 and Safety Monitoring Board (DSMB)

9.4.1. ROLE OF THE DSMB

This study is designed to test the safety of hMSCs in subjects with frailty.

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

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 FRAILTY, a fatal disease. Given the importance of mortality in FRAILTY, a stopping boundary based on the all-cause mortality rate will be implemented to guide the DSMB. This stopping boundary will not be applied until after all subjects have enrolled in the study.

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
2. Review protocol amendments, as deemed necessary by the DSMB Chair.
3. Review the statistical analysis plan with particular attention to the portions describing the data to be provided to the DSMB.
4. Evaluate on an ongoing basis, the accumulating unblinded safety summaries including AEs, SAEs, discontinuations and post-baseline laboratory results, to ensure the ongoing safety of study 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.
5. Review efficacy and mortality data (although the DSMB shall not recommend stopping the study for efficacy because the primary objective of this study does not include efficacy).
6. Evaluate the conduct of the study including enrollment rates, the selection and retention of subjects, protocol deviations, treatment adherence and quality and completeness of the data.
7. Review all documents provided in the DSMB data review packets as soon as possible upon receipt.
8. Protect the confidentiality of the DSMB discussions and the trial data, with particular attention to safeguarding the confidentiality of unblinded treatment information to minimize the potential for premature conclusions regarding the study results and the potential for introducing bias into the study.
9. Determine any safety issues, which may suggest risk to the subjects currently enrolled in the study or to future subjects to be enrolled
10. Ensure that the reports of the DSMB are based upon an unbiased and comprehensive evaluation of the data.
11. Make recommendations to modify or terminate the trial if there is a safety concern following review of data.
12. 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).

13. Assure confidentiality and the scientific integrity of the study, the members will not disclose any data prior to the completion of the study and release of final results to investigators.

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 FRAILTY subjects.

9.4.2.6 DSMB Chair Responsibilities

The responsibilities of the DSMB Chair will include the following:

- Serve as a voting member
- Convene and facilitate the meetings, assist in the development of the agenda, and ensure that the meeting minutes and recommendation(s) are appropriately documented
- Ensure that the Board is duly constituted
- DSMB Chair (or designee) serves as the primary contact person for the DSMB
- Review and approves the Charter
- Review protocol amendments
- Ensure that those involved in the day-to-day management of the study are excluded from DSMB voting procedures
- Will be responsible for deciding whether consultancies of financial interests of the members materially impact their objectivity

- DSMB Chair (or designee) will be responsible for presenting requests to University of Miami/ISCI for unscheduled (not planned) meetings
- DSMB Chair (or designee) will be responsible for discussing DSMB recommendations with University of Miami/ISCI and appropriate members of the project team via teleconference. These discussions will be conducted in a manner that preserves the study blind.
- Will have the authority to ask for the resignation of a member of the DSMB who exhibits poor attendance, inadequate demonstration of effort, unprofessional conduct, or failure to act consistently with the DSMB objectives
- Will be responsible for selecting an appropriate replacement for any DSMB member that withdraws from the committee

9.4.2.7 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 Safety /SAE Reports

As part of ongoing safety review and obligation to regulatory agencies, cumulative safety and SAE reports will be furnished for each DSMB meeting. 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.

9.4.3.3 Data Review Meetings

After all subjects 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 subjects are enrolled in the randomized phase. Meetings may be postponed due to accrual rates or to coincide with study milestones (e.g. after all subject s' Day 30 data are entered) 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.

Other safety data obtained at protocol-specified follow-up visits, including but not limited to data from the assessments listed below, will also be evaluated by the DSMB as appropriate:

- 12-lead ECGs
- Hematology, clinical chemistry, and urinalysis data
- Serum or Urine Pregnancy test
- Medical history
- Physical Exam
- Vital Signs
- FEV-1
- 6 – Minute Walk Test
- QOL Questionnaire
- Adverse Events

The DSMB will evaluate all safety data available for each subject as appropriate. In addition to those assessments listed above, data for DEXA scan, Dobutamine stress echocardiogram test, dynamometer test, and specific biomarkers may be available.

The EMMES Corporation will coordinate the scheduling of the teleconferences to review safety data during the randomized phase.

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

During the closed session, the DSMB chairperson will issue one of the following recommendations as determined by the DSMB and will be reported in the meeting minutes:

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 support team, then reviewed and issued by the DSMB chairperson. The DSMB support team will distribute the finalized minutes. 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

Written recommendations will be forwarded to University of Miami/ISCI in the finalized open session meeting minutes within two weeks of the DSMB meeting.

All recommendations of the DSMB, such as a change in the study protocol or early termination of the study, must be documented in the DSMB meeting minutes. If the DSMB recommends modification to, or termination of, the study, the CRO will contact the investigators within 24 hours of the DSMB 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.

In cases where a new ICF is issued between the Month 6 (Office Visit) and Month 12 (Telephone visit) the subject will be contacted and informed of the changes. The subject will be asked if they are available to come to the site. If the subject is unable to come to the site the subject may be reconsented via phone.

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.

Subject s 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 and Donors

Subjects will be reimbursed $25 at the end of each follow-up visit (Month 1 – Month 6) for a total remuneration of $75. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

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
10. Return the blinded IP infusion bag to the cell-processing technician.

Procedures post-infusion:

1. Vital signs will be monitored at 15 minutes, 30 minutes, 1 hour, and 2 hours post IP infusion
2. The subject will be monitored for a minimum of 2 hours post IP infusion with continuous pulse oximetry
3. If the O_2 saturation decreases to < 90% over a continual period of 3 – 5 minutes then supplemental oxygen may be added or increased during the two-three 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, if no complaints are experienced, such as shortness of breath or other objective signs of cardiorespiratory compromise.
6. Subjects not meeting criteria for discharge will be assessed by the Investigator during the observation period to further determine hospitalization otherwise not specified in the protocol.

<u>Subject Stopping Guidelines:</u>

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 Echocardiogram (DSE) Guidelines

A Dobutamine Stress Echocardiogram (DSE) 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:

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

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

Addendum A: Pilot Subjects Optional Follow-on Phase

A.1 Rationale and Description of second infusion for first 15 subjects on pilot phase

This addendum protocol is designed to test the safety of a second infusion of allogeneic mesenchymal stem cells 12 to 18 months following a first infusion.

Subjects in the pilot phase that received one (1) infusion of allogeneic MSCs are eligible to participate in this optional follow-on phase for a second dose. Because several studies, reviewed below, have now shown safety of repeat doses of MSCs in a variety of medical conditions, additional safety testing in the subject population of aging frailty enrolled in the CRATUS trial is required[92]. All study specific processes and procedures included in the pilot phase of the protocol apply in this addendum unless otherwise noted.

A.2 Demonstrated Safety and Increased Efficacy of Multiple Infusions of Allogeneic Mesenchymal Stem Cells

Active clinical trials and ongoing preclinical work provide an accumulating data set supporting the safety and, in some cases, therapeutic efficacy of allogeneic mesenchymal stem cells (MSCs). Allogeneic mesenchymal stem cells are both immunoprivileged and immunosuppressive, thereby enabling their use as an allograft[93]. Cellular therapy with allogeneic MSCs has abundant support from both animal studies and human clinical trials for a wide range of disorders (Table 1). For example, the POSEIDON study addressed the major issue of the use of allogeneic MSCs as a cell-based therapeutic[18]. In this study, subjects with cardiac failure were randomized to receive either autologous MSCs or identically prepared allogeneic MSCs from healthy donors.
Accumulating evidence also supports the concept that repeated doses and/or co-administration of allogeneic MSCs could further enhance therapeutic outcomes. This is an extremely important area for medical investigation, since repeat dosing could potentially have an additive effect and/or reverse disease pathology, depending on the disorder.

Recently, several clinical studies have demonstrated that multiple infusions of allogeneic MSCs are well tolerated with minimal side-effects. Importantly, repeat dosing has no increased level of side effects compared with single doses. For example, results from Franco Locatelli's group[92] clearly demonstrate the safety and efficacy of multiple allogeneic MSC infusions in children with steroid-refractory acute graft versus host disease (aGvHD). 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 days (range 3-43 days). The results of this study indicated increased effectiveness when the therapy was commenced early in the disease. Furthermore, the study indicated the therapeutic benefits of repeat doses to subjects who did not achieve complete remission after a single dose.

Koc et al[94]; further investigated Hurler syndrome (mucopolysaccharidosis type-IH) and metachromatic leukodystrophy (MLD) and studied allogeneic MSCs based on their potential to differentiate into cells of bone, cartilage, tendon, muscle and other adventitial tissues and offer potential for corrective cellular therapy[95]. Their results demonstrated no infusion-related toxicity, improvement in nerve conduction, and bone mineral density was either maintained or improved in all subjects.

A Phase II study on the 3-year efficacy of allogeneic MSCs in the treatment of system lupus erythrematosus (SLE) was reported by Lingyun Sun's group[96]. The results of this study demonstrated the therapeutic efficacy of allogeneic MSCs in treating SLE. However, no advantage was found by administering a second or higher dose of allogeneic MSCs. Despite this lack of additional efficacy over a single dose, the results of this study confirmed the safety of multiple infusions of allogeneic MSCs. The doses ($1x10^6$ MSCs/kg body mass) were administered 1 week apart. Another study reported on the use of repeated dosing of allogeneic MSCs in subjects with chronic obstructive pulmonary disease. The study showed minimal therapeutic efficacy after 2 years[19]. Importantly, in this subject population repeated allogeneic MSC infusions, which were given 4 times at 30 day intervals ($1x10^8$ MSCs/infusion) were safe.

Taken together, these various clinical studies provide a solid rationale for repeat allogeneic 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 allogeneic MSC therapy. Given these promising results, investigations are now warranted to determine optimal dosing frequency and total doses of allogeneic MSCs to administer.

It is particularly important to establish safety of repeat doses of allogeneic MSCs in the subject population enrolled in the CRATUS study. These individuals are of older age and are not represented in the previous studies described. In this follow-on study subject s in an earlier open label pilot study will be asked to participate in this repeat dosing protocol.

A.3 STUDY OBJECTIVES AND ENDPOINTS

A.3.1 Study Objectives

A.3.1.1 Primary Objective

1. Demonstrate the safety and tolerability of a second intravenous infusion of allo-hMSCs in subjects with aging frailty who had previously received an infusion of allogeneic MSCs as part of the pilot phase of the protocol.

A.3.1.2 Secondary Objectives

1. To explore treatment efficacy (decrease in frailty, frequency of acute exacerbations, change in symptom related quality of life, improved cardiovascular status, decrease in inflammatory biomarkers, endothelial function and 1 year survival).

A.3.2 Study Endpoints

A.3.2.1 Primary Endpoints (Safety)

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

   Laboratory tests included in the primary endpoint include the following:

   - Serum chemistry: chloride, sodium, Carbon Dioxide, BUN, creatinine, glucose, calcium, AST/SGOT, ALT/SGPT, total bilirubin (fractionate if total >1.5 times normal), alkaline phosphatase, albumin,
   - Hematology (CBC): hemoglobin, hematocrit, platelets, WBC, WBC differential

A.3.2.2 Secondary Endpoints (Efficacy)

The following efficacy endpoints will be evaluated in this trial (During the screening, baseline, Month 3 and/or Month 6 visits):

1. Difference in rate of decline of Frailty defined as:
   - Reduced Activity (assessed via CHAMPS questionnaire)
   - Slowing of Mobility (assessed via a 4 meter gait speed test and SPPB assessment)
   - Weight Loss
   - Diminished handgrip strength (assessed via dynamometer)
   - Exhaustion (assessed via the MFI questionnaire)
   - Decrease in subject quality of life assessment(s) (assessed via ICECAP, SF-36, EQ-5D Questionnaires)
2. Death from any cause.
3. Change between screening and 6 months in dobutamine stress echo induced ejection fraction.
4. Change between screening and 6 months for the following panel of inflammatory markers: CRP, IL-6, D-dimer, fibrinogen, CBC with differential, and TNFα.

5. The incidence of each component of the primary endpoint including non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities

6. Change in Smell Identification Test (UPSIT)

A.4 Inclusion and Exclusion Criteria

A.4.1 Inclusion Criteria for Follow-on Phase

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

1. Provide written informed consent.
2. Subjects age ≥60 and ≤95 years at the time of signing the Informed Consent Form for the second infusion.
3. Have previously participated in the pilot phase of this trial
4. Female subjects must have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy.

A.4.2 Exclusion Criteria for Follow-on Phase

In order to participate in this study, a subject MUST NOT have any of the following:

1. Score of ≤24 on the Mini Mental State Examination (MMSE)
2. Inability to perform any of the assessments required for endpoint analysis (report safety or tolerability concerns, perform PFTs, undergo blood draws, read and respond to questionnaires.
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, total bilirubin > 1.5 mg/dl.

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. 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.
7. Be an organ transplant recipient.
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. Have a history of drug or alcohol abuse within the past 24 months.
11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C.

12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial. This does not exclude subjects that participated in the pilot phase.

13. 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.
14. Have hypersensitivity to dimethyl sulfoxide (DMSO)

A.5 Dosing

After subjects complete their Month 12 follow-up phone call visit in the pilot phase (first infusion), all 15 subjects will then have the option of receiving additional infusions of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion.

The Allo-hMSCs will be derived from donors meeting criteria for allogeneic unrelated human bone marrow stem cell source manufactured by the University of Miami.

A.6 Dosage Rationale

A safety profile for IV infusion of hMSCs was based on results from previous completed toxicology results (Hare et al. 2277-86). 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 10^6hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6hMSC/kg per infusion).

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

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

A.7 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 addendum, the cell dose to be delivered is 1 x 10^8 (100 million), in the following total volume:

- 40ml for 100 million dose (5 million hMSC/min)

Cells are placed in an 80ml bag and will be delivered at a rate of 2ml/min, and delivered at a maximum rate of 16×10^6 hMSC/minute and will last approximately:

- 40 minutes for delivery of 40ml of 100 million dose in an 80 ml bag

A.8 Data and Safety Monitoring Board (DSMB)

DSMB will continue to provide the same oversight as noted in Section 9.4 of the Main protocol.

A.9 Concomitant Treatments, Procedures, and Nondrug Therapies

Refer to section 4.3 of the main study protocol

A.10 Infusion Monitoring Guidelines

Refer to section 6.4.1 and Appendix 1 of the main protocol.

A.11 Adverse Events and Serious Adverse Events

Refer to section 8.9 through 8.15 of the main study protocol.

A.12 Stopping Guidelines

Refer to section 8.2.4 of the main study protocol for further stopping guidelines.

A.13 Payments to Subjects and Donors in the Follow-on Phase

Subjects will be reimbursed $25 at the end of each follow-up visit (Month 1 – Month 6 for a total remuneration of $75. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

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.

A.14 Study Procedures for Pilot Phase Optional Second Infusion

Table A1: Time and Events Table for Optional Pilot Phase Second Infusion Schedule

Visit Schedule for Second Infusion	Screening ± 6 Months	Baseline (-4 weeks)	Day 1	Month 1 (Day 30) (+/- 2 weeks)	Month 3 (Day 90) (+/- 30 days)	Month 6 (Day 180) (+/-30 days)	Month 12 (Day 365) (+/-2 weeks) *Phone Call Follow-up
Informed Consent	x						
Full Medical History	x						
Physical Exam	x	x	x	x	x	x	
12-lead (ECG)	x	x	x	x	x	x	
Concomitant Medications	x	x	x	x	x	x	x
Mini Mental State Examination (MMSE)	x					x	
Infusion Treatment (IP)			x				
Dobutamine Stress Echo Test (DSE)	x					x	
Bone Density Scan (DEXA)[8]		x				x	
FEV-1		x				x	
6 Minute Walk Test		x				x	
4 Meter Gait Speed Test[7]		x				x	
SPPB Assessment		x				x	
Dynamometer (handgrip)[9]		x				x	
Smell Identification Test (UPSIT)		x				x	
IIEF, SQOL-F Questionnaires		x		x	x	x	
QOL Questionnaires (ICECAP, EQ-5D, SF-36,CHAMPS, MFI)		x		x	x	x	
Urinalysis	x			x	x	x	
Hemat., Chem., CBC, LFTs, INR, and other labs[1]	x		x	x	x	x	
HIV 1, HIV 2, Hep. B & C, and CMV	x						
Serum or Urine Pregnancy Test[2]	x		x				
Review Adverse Events			x	x	x	x	x
Immune Monitoring[4]			x	x	x	x	
Biomarker Assessment[3]			x			x	
Optional: Brachial Ultrasound [5]		x			x		
Optional: Endothelial blood samples[6]		x			x		

Time and Events Table Key:

1 – The minimal laboratory requirements for hematological, liver function and renal function include:

Hematology Tests: white blood cell count, platelet count, hemoglobin and hematocrit, red blood cell count, CBC with Differential: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase, asparate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin(fractionate if total >1.5 times normal).

Renal Function Tests: creatinine, creatinine clearance, blood urea nitrogen (BUN), glomerular filtration rate, sodium, potassium, chloride, calcium, carbon dioxide, total protein, glomerular filtration rate (GFR), and glucose, (HbA1c) will be collected at every follow-up visit only for subjects diagnosed with diabetes mellitus (DM).

Serum Uric Acid, Pro-BNP, and high sensitivity C-reactive protein (hs-CRP), IL6, fibrinogen, D-Dimer, DNA, TNFα, testosterone (males only) and estrogen (females only).**

- Laboratory work may be completed at a diagnostic center or home health agency should the subject be unable to come to the site.

*** Deoxyribonucleic acid (DNA) will only be collected once during the subject's participation. There is no specific point DNA needs to be collected as it can occur at any time point.*

2 – A serum or urine pregnancy test will be completed within 36 hours prior to infusion for females of childbearing potential.

3 – The following biomarkers will be analyzed (Approximately 10mL blood samples will be collected):

- **Cell-surface markers**: CD19, CD27, IgD, and CD5 (for Switched Memory, Naïve, Late/Exhausted, IgM memory B cells and regulatory B cells); CD19 and intracellular TNF-α (to assess intracellular TNF-α); CD3, CD4, CD8, CCR7 and CD45RA (for Central Memory, Naïve, Effector Memory, TEMRA T cells and CD4 to CD8 ratio); CD3, CD25 and FOXP3 (to assess regulatory T cells)
- **Transcriptomic/Proteome:** RNA, miRNA, protein samples, and telomerase, akt
- **Growth factors**: Sdf-1, notch,
- **Functional Assays:** cell growth rate, VEGF, and CFU assay

4 – Immune monitoring for graft rejection. Calculated panel reactive antibodies (cPRA) will be performed on day 1 and 6 months post treatment to assess for donor specific antibodies. In addition, the following markers will be used for analysis to assess for activated T-cells based upon a $CD3^{+}CD25^{+}$ (late/chronic T cell activation) or $CD3^{+}CD69^{+}$ phenotype (early T cell activation).

5 – Optional brachial ultrasound to assess endothelial function.

6 – Optional: An additional five (5) lavender top tubes (EDTA) will be drawn of approximately 8ml per tube.

7 – 4 meter gait speed test will be performed twice per visit and the average of the exams will be taken.

8 – DEXA scan will be performed at baseline and Month 6. The scans of the hip and spine for bone density, and total body composition will be assessed at both visits.

9 – Dynamometer will be performed at least three (3) times during each applicable visit for each hand. The three values collected will be averaged for each hand. (Please reference the instruction manual on how to administer.)

10– Infectious disease tests – HIV, HIV 2, Hep B (HBsAG, Anti-HBs, Anti-HBc), and Hep C (Anti-HCV), VDRL, Syphilis, West Nile Virus, and CMV. (Conducted within 30 days of infusion, if test expire prior infusion, they must be performed again)

A.15 Study Visits

A.15.1 Screening Visit for second infusion

See Table A1 for the procedures and assessments to be performed during the screening visit of the study for the 15 pilot subjects that consent to participate in a second infusion. 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 the start of the baseline visit. There will be up to a 6 month window from the subjects Month 12 follow-up visit to the subject's additional infusion.

A.15.2 Baseline Visit for second infusion

See Table A1 for the procedures and assessment to be performed during the baseline visit of the study. Once all screening exams are completed and it has been determined that the subject remains eligible for the study, subjects will be enrolled into the study. The baseline visit will take place within four weeks of the infusion. The listed procedures should all be performed as soon as practicable.

Endothelial function (Optional Assessments) will occur upon the subject signing the optional section of the informed consent form (ICF). No endothelial function tests will take

place until the subject is fully informed of the research and signs the optional portion of the consent form.

- Brachial ultrasound testing and blood collection will be performed to assess endothelial function in the aging frailty 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.

- Flow Mediated Diameter percent change (FMD%): All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C. Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the 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. In each examination, recording of vessel images will be followed by inflation of a cuff to supra-systolic pressure (40 to 50 mmHg above systolic pressure) for 5 minutes. Then the cuff will be deflated and the brachial artery diameter will be imaged and recorded for 3 minutes. FMD% more than 10% is considered a normal response. Lower than 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.

- Blood drawn from fasting subjects will be separated and the serum will be frozen until processed as one batch towards the end of the study. Blood will be processed twice – in the beginning of the study and after 3 months.

- Biochemical analysis: soluble pro inflammatory cytokines (interleukin-1, interleukin-6, interleukin-10, VEGFR2, TNF-a).

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

A.15.3 Day 1 Visit for second infusion

See Table A1 for the procedures and assessment to be performed during the Day 1 visit of the study. The Day 1 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. The subject will be monitored for 2-3 hours, following administration of the investigational product, and will be sent home the same day.

A.15.4 Month 1 Visit for second infusion

See Table A1 for the procedures and assessment to be performed for the Month 1 visit of the study. Subject visits should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 2 weeks for the month 1 study visit.

A.15.5 Month 3 and Month 6 Visit for second infusion

See Table A1 for the procedures and assessment to be performed for month 3 and 6 visit of the study. Subject visit should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 30 days for the month 3 and 6 study visit.

A.15.6 Month 12 Visit

See Table A1 for the procedures and assessments to be performed for Month 12 visit. This visit will be conducted via a phone interview with the subject. A phone script will be provided to the study personnel to use when interviewing the subject. There will be a +/- window of 2 weeks for this visit.

A.16 Statistical Considerations

All subjects will be offered the re-infusion and formal statistical considerations regarding sample size and power are not provided. Based on prior studies, a second infusion of stem cells are safe but strict safety monitoring will be employed to continue the safety assessment of allogeneic infusion of MSCs. Detailed analyses will be described in the separate statistical analysis plan.

A.17 Safety Monitoring of 30-Day Rate of TE-SAEs

Monitoring of the rate of TE-SAEs by 30-days post-second infusion among subjects who received the infusion of allogeneic MSCs will be employed to assist the DSMB in prospective monitoring of this study. The guideline is to be used to indicate boundaries requiring discussion by the 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. Monitoring of key safety endpoints will be conducted. If rates significantly exceed the pre-set threshold, then the DSMB will be notified.

A Bayesian motivated safety stopping guideline for monitoring the 30-day TE-SAE rate will be used for this trial. The expected underlying rate of TE-SAE at 30 days post-second infusion is assumed to be 13.3% based on the assumption that 2 out of 15 subjects experience a TE-SAE. It would then be assumed that a rate of greater than 40.0% is unacceptable.

A Beta distribution can be used as the prior distribution of θ; where θ is the proportion of subjects who experience an TE-SAE by 30-days post-second infusion. The stopping rule is based on the beta-binomial methodology and assumes a prior expected failure rate. This leads to prior Beta parameters where a=0.8 and b=5.2. The Beta distribution will have a prior mean of 0.13 and a prior probability of <0.05 of exceeding 0.40. The guideline is derived such that there is strong evidence (posterior probability >0.95) that the probability of the event is greater than 40%, the trial will be stopped. The resulting boundaries tabulated in Table A2 were rounded to be conservative with the stopping guideline and is considered after 5 subjects are enrolled on the study.

TABLE A2
Bayesian Stopping Guideline for Event Rate of 13% *

# Events	# Subjects in Study
3	5
4	6-9
5	10-14
6	15

* 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.

A simulation study was conducted to evaluate the operating characteristics of this stopping rule. Data were generated from the binomial distribution with varying probabilities of failure (θ) and assuming a sample size of 15 subjects. Table A3 shows the probability of stopping the trial early and the average sample size (N), conditional on stopping early, at which the boundary is crossed for each value of θ. The unconditional average sample size of the trials for each value of θ is displayed.

Table A3
Operating Characteristics for Bayesian Motivated Stopping Guideline

Mean of Prior Distribution	θ	Probability of stopping	Conditional Average Sample Size (N)	Unconditional Average Sample Size of Trials (N)
0.13	0.13	0.05	8.4	14.7
	0.18	0.12	8.8	14.2
	0.23	0.25	8.9	13.5
	0.28	0.40	8.8	12.5
	0.33	0.56	8.5	11.4
	0.40	0.75	8.0	9.8

Although the motivation for the boundary is Bayesian, the operating characteristics can be evaluated from a frequentist perspective of Type I error and power. The stopping rule for a 13% event rate has a 5% chance ("Type I error") of suggesting early termination when the true rate is 0.13, and a 75% chance ("power") when the true rate is 0.40.

Limitations

A major limitation to the second infusion approach is the small sample size. While it is assumed that all 15 subjects will be eligible for a second infusion, there is a chance that subjects will not want to undergo a second infusion and thus would further limit the amount of information received. However, the second infusion strategy is being implemented to obtain information on the safety of a second infusion of allogeneic MSCs to help guide future studies.

Addendum B: Penicillin/streptomycin free cell safety study

B.1 Rationale and Description for penicillin/streptomycin free cell safety study

This addendum to the protocol is designed to gain additional safety of penicillin/streptomycin free intravenous infusion of allo-hMSCs in subjects with aging frailty. Up to twenty subjects will be recruited to participate in a single dose of allogeneic MSCs in this follow-on phase. All study specific processes and procedures included in the protocol apply in this addendum unless otherwise noted. This population will not be included in the main phase of the trial, which included 15 pilot and 30 randomized subjects. Additionally, subjects receiving a dose in the penicillin/streptomycin free study are eligible to receive up to three (3) additional infusions.

B.2 STUDY OBJECTIVES AND ENDPOINTS

B.2.1 Study Objectives

B.2.1.1 Primary Objective

1. Demonstrate the safety and tolerability of penicillin/streptomycin free intravenous infusion of allo-hMSCs in subjects with aging frailty.

B.2.1.2 Secondary Objectives

6. To explore treatment efficacy (decrease in frailty, frequency of acute exacerbations, change in symptom related quality of life, improved cardiovascular status, decrease in inflammatory biomarkers, endothelial function and 1 year survival).

B.2.2 Study Endpoints

B.2.2.1 Primary Endpoints (Safety)

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

   - Laboratory tests included in the primary endpoint include the following:

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

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

B.2.2.2 Secondary Endpoints (Efficacy)

The following efficacy endpoints will be evaluated in this trial (During the screening, baseline, Month 3 and/or Month 6 visits):

1. Difference in rate of decline of Frailty defined as:
    - Reduced Activity (assessed via CHAMPS questionnaire)
    - Slowing of Mobility (assessed via a 4 meter gait speed test and SPPB assessment)
    - Weight Loss
    - Diminished handgrip strength (assessed via dynamometer)
    - Exhaustion (assessed via the MFI questionnaire)
    - Decrease in subject quality of life assessment(s) (assessed via ICECAP, SF-36, EQ-5D Questionnaires)
2. Death from any cause.
3. Change between screening and 6 months in dobutamine stress echo induced ejection fraction.
4. Change between screening and 6 months for the following panel of inflammatory markers: CRP, IL-6, D-dimer, fibrinogen, CBC with differential, and TNFα
5. The incidence of each component of the primary endpoint including non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities
6. Change in Smell Identification Test (UPSIT)

B.3 Inclusion and Exclusion Criteria

B.3.1 Inclusion Criteria for penicillin/streptomycin free cell safety study

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

1. Provide written informed consent.
2. Subjects age ≥ 60 and ≤95 years at the time of signing the Informed Consent Form.
3. Show signs of frailty apart from a concomitant condition as assessed

by the Investigator with a frailty score of 4 to 7 using the Canadian Clinical Frailty Scale

4. Female subjects must have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy.

B.3.2 Exclusion Criteria for penicillin/streptomycin free cell safety study

In order to participate in this study, a subject MUST NOT have any of the following:

1. Score of ≤24 on the Mini Mental State Examination (MMSE)
2. Inability to perform any of the assessments required for endpoint analysis (report safety or tolerability concerns, perform PFTs, undergo blood draws, read and respond to questionnaires.
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, total bilirubin > 1.5 mg/dl.
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. 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.
7. Be an organ transplant recipient.
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. Have a history of drug or alcohol abuse within the past 24 months.
11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C.
12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial.
13. 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.

14. Have hypersensitivity to dimethyl sulfoxide (DMSO)

B.4 Dosing

After subjects complete have completed their screening and continue to meet inclusion/exclusion criteria subjects will receive a single infusion of allogeneic penicillin/streptomycin free hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion.

The penicillin/streptomycin free Allo-hMSCs will be derived from donors meeting criteria for allogeneic unrelated human bone marrow stem cell source manufactured by the University of Miami or from a commercial clinical grade bone marrow source.

B.5 Dosage Rationale

A safety profile for IV infusion of hMSCs was based on results from previous completed toxicology results(Hare et al. 2277-86). The results from previous studies demonstrate that the product can be administered intravenously without toxic events at up to 65 x 10^6 hMSC/kg dose delivered in one bolus infusion or at 100 x 10^6 hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6 hMSC/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, the weight range for this study.

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

B.6 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 addendum, the cell dose to be delivered is 1 x 10^8 (100 million), in the following total volume

- 40ml for 100 million dose (5 million hMSC/min)

Cells are placed in an 80ml bag and will be delivered at a rate of 2ml/min, and delivered at a maximum rate of 16×10^6 hMSC/minute and will last approximately:

- 40 minutes for delivery of 40ml of 100 million dose in an 80ml bag.

B.7 Data and Safety Monitoring Board (DSMB)

DSMB will continue to provide the same oversight as noted in Section 9.4 of the Main protocol.

B.8 Concomitant Treatments, Procedures, and Nondrug Therapies

Refer to section 4.3 of the main study protocol

B.9 Infusion Monitoring Guidelines

Refer to section 6.4.1 and Appendix 1 of the main protocol.

B.10 Adverse Events and Serious Adverse Events

Refer to section 8.9 through 8.15 of the main study protocol.

B.11 Stopping Guidelines

Refer to section 8.2.4 of the main study protocol for further stopping guidelines.

B.12 Payments to Subjects and Donors in the Follow-on Phase

Subjects will be reimbursed $25 at the end of each follow-up visit (Month 1 – Month 6) for a total remuneration of $75. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

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.

B.13 Study Procedures for Penicillin/Streptomycin Free hMSCs Infusion

Table B1: Time and Events Table for Penicillin/Streptomycin Free Dose Schedule

Visit Schedule	Screening - 45 days From baseline	Baseline (0 to -4 weeks)	Day 1	Month 1 (Day 30) (+/- 2 weeks)	Month 3 (Day 90) (+/- 30 days)	Month 6 (Day 180) (+/-30 days)	Month 12 (Day 365) (+/-2 weeks) *Phone Call Follow-up [12]
Informed Consent	x						
Full Medical History	x						
Physical Exam	x	x	x	x	x	x	
12-lead (ECG)	x	x	x	x	x	x	
Concomitant Medications	x	x	x	x	x	x	x
Mini Mental State Examination (MMSE)	x					x	
Infusion Treatment (IP)			x				
Dobutamine Stress Echo Test (DSE)[11]	x					x	
Bone Density Scan (DEXA)[8]		x				x	
FEV-1		x				x	
6 Minute Walk Test		x				x	
4 Meter Gait Speed Test[7]		x				x	
SPPB Assessment		x				x	
Dynamometer (handgrip)		x				x	
Smell Identification Test (UPSIT)		x				x	
IIEF, SQOL-F Questionnaires		x		x	x	x	
QOL Questionnaires (ICECAP, EQ-5D, SF-36,CHAMPS, MFI)		x		x	x	x	
Urinalysis	x			x	x	x	
Hemat., Chem., CBC, LFTs, INR, and other labs[1]	x		x	x	x	x	
HIV 1, HIV 2, Hep. B & C, and CMV[10]	x						
Serum or Urine Pregnancy Test[2]	x		x				
Review Adverse Events			x	x	x	x	x
Immune Monitoring[4]			x	x	x	x	
Biomarker Assessment[3]			x			x	
Optional: Brachial Ultrasound [5]		x			x		
Optional: Endothelial blood samples[6]		x			x		

Time and Events Table Key:

1 – The minimal laboratory requirements for hematological, liver function and renal function include:

Hematology Tests: white blood cell count, platelet count, hemoglobin and hematocrit, red blood cell count, CBC with Differential: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase, asparate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin(fractionate if total >1.5 times normal).

Renal Function Tests: creatinine, creatinine clearance, blood urea nitrogen (BUN), glomerular filtration rate, sodium, potassium, chloride, calcium, carbon dioxide, total protein, glomerular filtration rate (GFR), and glucose, (HbA1c) will be collected at every follow-up visit only for subjects diagnosed with diabetes mellitus (DM).

Serum Uric Acid, Pro-BNP, and high sensitivity C-reactive protein (hs-CRP), IL6, fibrinogen, D-Dimer, DNA, TNFα**, **testosterone (males only), estrogen (females only), and FSH***.**

- Laboratory work may be completed at a diagnostic center or home health agency should the subject be unable to come to the site.

*** Deoxyribonucleic acid (DNA) will only be collected once during the subject's participation. There is no specific point DNA needs to be collected as it can occur at any time point.*

****FSH will need to be collected at screening to verify that female subjects have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy for inclusion in the trial.*

2 – A serum or urine pregnancy test will be completed within 36 hours prior to infusion for females of childbearing potential.

3 – The following biomarkers will be analyzed:

- **Cell-surface markers**: CD19, CD27, IgD, and CD5 (for Switched Memory, Naïve, Late/Exhausted, IgM memory B cells and regulatory B cells); CD19 and intracellular TNF-α (to assess intracellular TNF-α); CD3, CD4, CD8, CCR7 and CD45RA (for Central Memory, Naïve, Effector Memory, TEMRA T cells and CD4 to CD8 ratio); CD3, CD25 and FOXP3 (to assess regulatory T cells)
- **Transcriptomic/Proteome:** RNA, miRNA, protein samples, and telomerase, akt
- **Growth factors**: Sdf-1, notch,

- **Functional Assays:** cell growth rate, VEGF, and CFU assay

4 – Immune monitoring for graft rejection. Calculated panel reactive antibodies (cPRA) will be performed from the serum of the subjects on day 1 and 6 months post infusion to check for donor specific antibodies. In addition, the following markers will be used for analysis to assess for activated T-cells based upon a $CD3^{+}CD25^{+}$ (late/chronic T cell activation) or $CD3^{+}CD69^{+}$ phenotype (early T cell activation).

5 – Optional brachial ultrasound to assess endothelial function.

6 – Optional: An additional five (5) lavender top tubes (EDTA) will be drawn of approximately 8ml per tube.

7 – 4 meter gait speed test will be performed twice per visit and the average of the exams will be taken.

8 – DEXA scan will be performed twice at each visit. The first scan will be of the hip and spine for bone density and the second will be to assess the total body composition.

9 – Dynamometer will be performed at least three (3) times during each applicable visit for each hand. The three values collected will be averaged for each hand. (Please reference the instruction manual on how to administer)**.**

10– Infectious disease tests – HIV, HIV 2, Hep B (HBsAG, Anti-HBs, Anti-HBc), and Hep C (Anti-HCV), VDRL, Syphilis, West Nile Virus, and CMV. (Conducted within 30 days of infusion, if test expire prior infusion, they must be performed again)

11– Guidelines for completing the (DSE) are outlined in Appendix 2 of this protocol.

12–Month 12 follow-up phone call may be waived if the subject has a screening visit for an additional infusion prior to this visit.

B.14 Study Visits

B.14.1 Screening Visit

See Table B1 for the procedures and assessments to be performed during the screening visit. 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 the start of 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. There will be up to a +/-2 week window from the subjects Month 12 phone call from the subject's previous infusion to begin screening procedures. Month 12 follow-up phone call may be waived if the subject has a screening visit for an additional infusion prior to this visit.

If a dobutamine stress echo was performed within 6 months, the report may be used in place of screening DSE for additional infusions.

B.14.2 Baseline Visit

See Table B1 for the procedures and assessment to be performed during the baseline visit of the study. Once all screening exams are completed and it has been determined that the subject remains eligible for the study, subjects will be enrolled into the study. The baseline visit will take place within four weeks from treatment. The listed procedures should all be performed as soon as practicable. If DEXA was performed within 6 months, the report may be used in place of baseline DEXA for additional infusions.

Endothelial function (Optional Assessments) will occur upon the subject signing the optional section of the informed consent form (ICF). No endothelial function tests will take place until the subject is fully informed of the research and signs the optional portion of the consent form.

- Brachial ultrasound testing and blood collection will be performed to assess endothelial function in the aging frailty 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.

- Flow Mediated Diameter percent change (FMD%): All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C. Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the 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. In each examination, recording of vessel images will be followed by inflation of a cuff to supra-systolic pressure (40 to 50 mmHg above systolic pressure) for 5 minutes. Then the cuff will be deflated and the brachial artery diameter will be imaged and recorded for 3 minutes. FMD% more than 10% is considered a normal response. Lower than 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.

  Blood drawn from fasting subjects will be separated and the serum will be frozen until processed as one batch towards the end of the study. Blood will be processed twice – in the beginning of the study and after 3 months.
- Biochemical analysis: soluble pro inflammatory cytokines (interleukin-1, interleukin-6, interleukin-10, VEGFR2, TNF-a).

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

B.14.3 Day 1 Visit

See Table B1 for the procedures and assessment to be performed during the Day 1 visit of the study. The Day 1 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. The subject will be monitored for 2 – 3 hours, following administration of the investigational product, and will be sent home the same day.

B.14.4 Month 1 Visit

See Table B1 for the procedures and assessment to be performed for the Month 1 visit of the study. Subject visits should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 2 weeks for the month 1 study visit.

B.14.5 Month 3 and Month 6 Visit

See Table B1 for the procedures and assessment to be performed for month 3 and 6 visit of the study. Subject visit should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 30 days for the month 3 and 6 study visit.

B.14.6 Month 12 Visit

See Table B1 for the procedures and assessments to be performed for Month 12 visit. This visit will be conducted via a phone interview with the subject. A phone script will be provided to the study personnel to use when interviewing the subject. There will be a +/- window of 2 weeks for this visit.

The Month 12 visit will be waived if screening visits for subsequent infusions take place prior to this visit.

B.15 Statistical Considerations

All subjects will be offered the penicillin/streptomycin free hMSCs and formal statistical considerations regarding sample size and power are not provided. Based on prior studies, stem cells are safe but strict safety monitoring will be employed to continue the safety assessment of penicillin/streptomycin free allogeneic infusion of MSCs. No detailed analyses will be described in the statistical analysis plan.

B.16 Safety Monitoring of 30-Day Rate of TE-SAEs

Monitoring of the rate of TE-SAEs by 30-days post infusion among subjects who received the infusion of penicillin/streptomycin free allogeneic MSCs will be employed to assist the DSMB in prospective monitoring of this study. The guideline is to be used to indicate boundaries requiring discussion by the 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. Monitoring of key safety endpoints will be conducted. If rates significantly exceed the pre-set threshold, then the DSMB will be notified.

A Bayesian motivated safety stopping guideline for monitoring the 30-day TE-SAE rate will be used for this trial. The expected underlying rate of TE-SAE at 30 days post-infusion is assumed to be 10% based on the assumption that 2 out of 20 subjects experience a TE-SAE. It would then be assumed that a rate of greater than 40.0% is unacceptable.

A Beta distribution can be used as the prior distribution of θ; where θ is the proportion of subjects who experience an TE-SAE by 30-days post infusion. The stopping rule is based on the beta-binomial methodology and assumes a prior expected failure rate. This leads to prior Beta parameters where a=0.8 and b=5.2. The Beta distribution will have a prior mean of 0.13 and a prior probability of <0.05 of exceeding 0.40. The guideline is derived such that there is strong evidence (posterior probability >0.95) that the probability of the event is greater than 40%, the trial will be stopped. The resulting boundaries tabulated in table B2 were rounded to be conservative with the stopping guideline and is considered after 5 subjects are enrolled on the study.

TABLE B2

Bayesian Stopping Guideline for Event Rate of 10% *

# Events	# Subjects in Study
3	5
4	6-9
5	10-14
6	15

* 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.

A simulation study was conducted to evaluate the operating characteristics of this stopping rule. Data were generated from the binomial distribution with varying probabilities of failure (θ) and assuming a sample size of 15 subjects. Table B3 shows the probability of stopping the trial early and the average sample size (N), conditional on stopping early, at which the boundary is crossed for each value of θ. The unconditional average sample size of the trials for each value of θ is displayed.

Table B3
Operating Characteristics for Bayesian Motivated Stopping Guideline

Mean of Prior Distribution	θ	Probability of stopping	Conditional Average Sample Size (N)	Unconditional Average Sample Size of Trials (N)
0.13	0.13	0.05	8.4	14.7
	0.18	0.12	8.8	14.2
	0.23	0.25	8.9	13.5
	0.28	0.40	8.8	12.5
	0.33	0.56	8.5	11.4
	0.40	0.75	8.0	9.8

Although the motivation for the boundary is Bayesian, the operating characteristics can be evaluated from a frequentist perspective of Type I error and power. The stopping rule for a 13% event rate has a 5% chance ("Type I error") of suggesting early termination when the true rate is 0.13, and a 75% chance ("power") when the true rate is 0.40.

Limitations

A major limitation to this approach is the small sample size. However, the penicillin streptomycin free infusion strategy is being implemented to obtain information on the safety of a penicillin/streptomycin free allogeneic MSCs to help guide future studies.

Addendum C: Pilot and Penicillin/Streptomycin Free Subjects Optional Follow-on Phase

C.1 Rationale and Description of additional infusions for subjects who have received one or two infusions in the phase I open-label phases of this protocol.

This addendum protocol is designed to test the safety of sequential infusions of allogeneic mesenchymal stem cells 6 to 18 months following previous infusion. Subjects in this protocol have previously participated in protocols receiving open label infusions (phase I subjects). There is an important need to assess the safety and immunologic tolerability of additional doses of intravenous allogeneic MSCs in the target population. Previously a group of 12 subjects received two doses delivered 12-18 month apart with excellent safety and no adverse effects attributed to the study agent. A second group of 20 subjects received a single dose of MSCs, in the penicillin/streptomycin free cohort. Subjects receiving either one or two sequential doses have not mounted significant immunologic reactions to the study agent.

Subjects in the pilot phase would have received two (2) of the possible four (4) infusions of allogeneic MSCs and are eligible to participate in this optional follow-on phase. Additionally, subjects receiving one dose (100 Million cells) in the penicillin/streptomycin free protocol are eligible to receive up to three (3) additional infusions. Because several studies, reviewed below, have now shown safety of repeat doses of MSCs in a variety of medical conditions, additional safety testing in the subject population of aging frailty enrolled in the CRATUS trial is required[92]. All study specific processes and procedures included in the pilot phase and penicillin free follow-on phase of the protocol apply in this addendum unless otherwise noted.

Pilot subjects participating in this addendum are eligible to receive up to two (2) additional infusions and subjects participating from Penicillin Free cohort are eligible to receive up to three additional infusions of MSCs (100 Million cells) administered 6 -18 months apart. The MSCs provided to study subjects in this addendum may be penicillin/streptomycin free. Subjects from the Pilot phase returning for their additional infusions may receive cells with penicillin/streptomycin. Altogether, subjects will have received four (4) infusions over a time span ranging approximately 3 to 6 years. Subjects will have ongoing safety monitoring that will include detailed immunologic assessments.

Subjects that do not want an additional infusion will not be eligible to continue in the study and/or receive additional infusions under this addendum.

C.2 Demonstrated Safety and Increased Efficacy of Multiple Infusions of Allogeneic Mesenchymal Stem Cells

Active clinical trials and ongoing preclinical work provide an accumulating data set supporting the safety and, in some cases, therapeutic efficacy of allogeneic

mesenchymal stem cells (MSCs). Allogeneic mesenchymal stem cells are both immunoprivileged and immunosuppressive, thereby enabling their use as an allograft[93]. Cellular therapy with allogeneic MSCs has abundant support from both animal studies and human clinical trials for a wide range of disorders (Table 1). For example, the POSEIDON study addressed the major issue of the use of allogeneic MSCs as a cell-based therapeutic[18]. In this study, subjects with cardiac failure were randomized to receive either autologous MSCs or identically prepared allogeneic MSCs from healthy donors.

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

Recently, several clinical studies have demonstrated that multiple infusions of allogeneic MSCs are well tolerated with minimal side-effects. Importantly, repeat dosing has no increased level of side effects compared with single doses. For example, results from Franco Locatelli's group[92] clearly demonstrate the safety and efficacy of multiple allogeneic MSC infusions in children with steroid-refractory acute graft versus host disease (aGvHD). 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 days (range 3-43 days). The results of this study indicated increased effectiveness when the therapy was commenced early in the disease. Furthermore, the study indicated the therapeutic benefits of repeat doses to subjects who did not achieve complete remission after a single dose.

Koc et al[94]; further investigated Hurler syndrome (mucopolysaccharidosis type-IH) and metachromatic leukodystrophy (MLD) and studied allogeneic MSCs based on their potential to differentiate into cells of bone, cartilage, tendon, muscle and other adventitial tissues and offer potential for corrective cellular therapy[95]. Their results demonstrated no infusion-related toxicity, improvement in nerve conduction, and bone mineral density was either maintained or improved in all subjects.

A Phase II study on the 3-year efficacy of allogeneic MSCs in the treatment of system lupus erythrematosus (SLE) was reported by Lingyun Sun's group[96]. The results of this study demonstrated the therapeutic efficacy of allogeneic MSCs in treating SLE. However, no advantage was found by administering a second or higher dose of allogeneic MSCs. Despite this lack of additional efficacy over a single dose, the results of this study confirmed the safety of multiple infusions of allogeneic MSCs. The doses (1x10^6 MSCs/kg body mass) were administered 1 week apart. Another study reported on the use of repeated dosing of allogeneic MSCs in subjects with chronic obstructive pulmonary disease. The study showed minimal therapeutic efficacy after 2 years[19]. Importantly, in this subject population repeated allogeneic MSC infusions, which were given 4 times at 30 day intervals (1x10^8 MSCs/infusion) were safe.

In the pilot phase of this trial, subjects (N=12) have been administered two doses of MSCs 12 to 18 months apart with no adverse effects.

Taken together, these various clinical studies provide a solid rationale for repeat allogeneic 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 allogeneic MSC therapy. Given these promising results, investigations are now warranted to determine optimal dosing frequency and total doses of allogeneic MSCs to administer.

It is particularly important to establish safety of repeat doses of allogeneic MSCs in the subject population enrolled in the CRATUS study. These individuals are of older age and are not represented in the previous studies described. In this follow-on study subject s in earlier open label pilot study and penicillin/streptomycin free phase will be asked to participate in this repeat infusion protocol. Subjects in the pilot-phase or penicillin/streptomycin free phase, which received either one or two infusions previously will be eligible, and all subjects will get a total of four infusions. Each infusion will be 6 to 18 months apart.

C.3 STUDY OBJECTIVES AND ENDPOINTS

C.3.1 Study Objectives

C.3.1.1 Primary Objective

1. Demonstrate the safety and tolerability of up to four intravenous infusions of allo-hMSCs in subjects with aging frailty who had previously received an infusion of allogeneic MSCs as part of an open label phase (phase I) of the protocol or the Penicillin/streptomycin free addendum (Addendum B).

2. To determine immunologic safety of sequential infusions of allo-hMSCs in subjects with aging frailty.

C.3.1.2 Secondary Objectives

1. To explore treatment efficacy (decrease in frailty, frequency of acute exacerbations, change in symptom related quality of life, improved cardiovascular status, decrease in inflammatory biomarkers, endothelial function and 1 year survival).

C.3.2 Study Endpoints

C.3.2.1 Primary Endpoints (Safety)

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

Laboratory tests included in the primary endpoint include the following:

- Serum chemistry: chloride, sodium, Carbon Dioxide, BUN, creatinine, glucose, calcium, AST/SGOT, ALT/SGPT, total bilirubin (fractionate if total >1.5 times normal), alkaline phosphatase, albumin,
- Hematology (CBC): hemoglobin, hematocrit, platelets, WBC, WBC differential

C.3.2.2 Secondary Endpoints (Efficacy)

The following efficacy endpoints will be evaluated in this trial (During the screening, baseline, Month 3 and/or Month 6 visits):

1. Difference in rate of decline of Frailty defined as:
    - Reduced Activity (assessed via CHAMPS questionnaire)
    - Slowing of Mobility (assessed via a 4 meter gait speed test and SPPB assessment)
    - Weight Loss
    - Diminished handgrip strength (assessed via dynamometer)
    - Exhaustion (assessed via the MFI questionnaire)
    - Decrease in subject quality of life assessment(s) (assessed via ICECAP, SF-36, EQ-5D Questionnaires)
2. Death from any cause.
3. Change between screening and 6 months in dobutamine stress echo induced ejection fraction.
4. Change between screening and 6 months for the following panel of inflammatory markers: CRP, IL-6, D-dimer, fibrinogen, CBC with differential, and TNFα
5. The incidence of each component of the primary endpoint including non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities
6. Change in Smell Identification Test (UPSIT)

C.4 Inclusion and Exclusion Criteria

C.4.1 Inclusion Criteria for Follow-on Phase

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

1. Provide written informed consent.
2. Subjects age ≥60 and ≤95 years at the time of signing the Informed Consent Form for this follow-on phase.
3. Have previously participated in the pilot phase of the trial, or have previously participated in the penicillin free (open label) phase of the trial.
4. Female subjects must have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy.

C.4.2 Exclusion Criteria for Follow-on Phase

In order to participate in this study, a subject MUST NOT have any of the following:

1. Score of ≤24 on the Mini Mental State Examination (MMSE)
2. Inability to perform any of the assessments required for endpoint analysis (report safety or tolerability concerns, perform PFTs, undergo blood draws, read and respond to questionnaires.
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, total bilirubin > 1.5 mg/dl.
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. 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.
7. Be an organ transplant recipient.
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. Have a history of drug or alcohol abuse within the past 24 months.

11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C.
12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial. This does not exclude subjects that participated in addendum A or B.
13. 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.
14. Have hypersensitivity to dimethyl sulfoxide (DMSO)

C.5 Dosing

Subjects participating in this protocol will have received either one or two infusions of study agent. All subjects who have received their first two infusions, will then have the option of receiving an additional infusion of allogeneic hMSCs: 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion. Subjects will be eligible to receive a total of four (n=4) infusions, 6 to 18 months apart.

The Allo-hMSCs will be derived from donors meeting criteria for allogeneic unrelated human bone marrow stem cell source manufactured by the University of Miami or from a commercial clinical grade bone marrow source. Subjects from the Pilot phase returning for their additional infusions may receive cells with penicillin/streptomycin.

C.6 Dosage Rationale

A safety profile for IV infusion of hMSCs was based on results from previous completed toxicology results(Hare et al. 2277-86). 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 10^6hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6hMSC/kg per infusion).

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

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

C.7 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 addendum, the cell dose to be delivered is 1 x 10^8 (100 million), in the following total volume

- 40ml for 100 million dose (5 million hMSC/min)

Cell s are placed in an 80ml bag and will be delivered at a rate of 2ml/min, and delivered at a maximum rate of 16×10^6 hMSC/minute and will last approximately:

- 40 minutes for delivery of 40ml of 100 million dose in an 80 ml bag

C.8 Data and Safety Monitoring Board (DSMB)

DSMB will continue to provide the same oversight as noted in Section 9.4 of the Main protocol.

C.9 Concomitant Treatments, Procedures, and Nondrug Therapies

Refer to section 4.3 of the main study protocol

C.10 Infusion Monitoring Guidelines

Refer to section 6.4.1 and Appendix 1 of the main protocol.

C.11 Adverse Events and Serious Adverse Events

Refer to section 8.9 through 8.15 of the main study protocol.

C.12 Stopping Guidelines

Refer to section 8.2.4 of the main study protocol for further stopping guidelines.

C.13 Payments to Subjects and Donors

Subjects will be reimbursed $25 at the end of each follow-up visit (Month 1 – Month 6 for a total remuneration of $75. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

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.

C.14 Study Procedures for Optional Additional Infusions for Pilot and Penicillin/Streptomycin Free Subjects (See Table C1)

Table C1: Time and Events Table for Optional Pilot Phase and PCN/Streptomycin Free Additional Infusion(s) Schedule

Visit Schedule for Additional Infusions (repeating for up to 4 doses every 6 -18 months)	Screening - 45 days from baseline	Baseline (-4 weeks)	Day 1	Month 1 (Day 30) (+/- 2 weeks)	Month 3 (Day 90) (+/- 30 days)	Month 6 (Day 180) (+/-30 days)	Month 12 (Day 365) (+/- 2 weeks) *Phone Call Follow-up[11]
Informed Consent	x						
Full Medical History	x						
Physical Exam	x	x	x	x	x	x	
12-lead (ECG)	x	x	x	x	x	x	
Concomitant Medications	x	x	x	x	x	x	x
Mini Mental State Examination (MMSE)	x					x	
Infusion Treatment (IP)			x				
Dobutamine Stress Echo Test (DSE)[12]	x					x	
Bone Density Scan (DEXA)[8]		x				x	
FEV-1		x				x	
6 Minute Walk Test		x				x	
4 Meter Gait Speed Test[7]		x				x	
SPPB Assessment		x				x	
Dynamometer (handgrip)[9]		x				x	
Smell Identification Test (UPSIT)		x				x	
IIEF, SQOL-F Questionnaires		x		x	x	x	
QOL Questionnaires (ICECAP, EQ-5D, SF-36,CHAMPS, MFI)		x		x	x	x	
Urinalysis	x			x	x	x	
Hemat., Chem., CBC, LFTs, INR, and other labs[1]	x		x	x	x	x	
HIV 1, HIV 2, Hep. B & C, and CMV	x						
Serum or Urine Pregnancy Test[2]	x		x				
Review Adverse Events			x	x	x	x	x
Immune Monitoring[4]			x	x	x	x	
Biomarker Assessment[3]			x			x	
Optional: Brachial Ultrasound [5]		x			x		
Optional: Endothelial blood samples[6]		x			x		

Time and Events Table Key:

1 - The minimal laboratory requirements for hematological, liver function and renal function include:

Hematology Tests: white blood cell count, platelet count, hemoglobin and Hematocrit, red blood cell count, CBC with Differential: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase, asparate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin (fractionate if total >1.5 times normal).

Renal Function Tests: creatinine, creatinine clearance, blood urea nitrogen (BUN), glomerular filtration rate, sodium, potassium, chloride, calcium, carbon dioxide, total protein, glomerular filtration rate (GFR) and glucose, (HbA1c) will be collected at every follow-up visit only for subjects diagnosed with diabetes mellitus (DM).

Serum Uric Acid, Pro-BNP, and high sensitivity C-reactive protein (hs-CRP), IL6, fibrinogen, D-Dimer, TNFα, DNA, testosterone (males only), estrogen (females only) and FSH***.**

- Laboratory work may be completed at a diagnostic center or home health agency should the subject be unable to come to the site.

*** Deoxyribonucleic acid (DNA) will only be collected once during the subject's participation. There is no specific point DNA needs to be collected as it can occur at any time point.*

****FSH will need to be collected at screening to verify that female subjects have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy for inclusion in the trial.*

2 - A serum or urine pregnancy test will be completed within 36 hours prior to infusion for females of childbearing potential.

3 - The following biomarkers will be analyzed (Approximately 10mL blood samples will be collected):

- **Cell-surface markers**: CD19, CD27, IgD, and CD5 (for Switched Memory, Naïve, Late/Exhausted, IgM memory B cells and regulatory B cells); CD19 and intracellular TNF-α (to assess intracellular TNF-α); CD3, CD4, CD8, CCR7 and CD45RA (for Central Memory, Naïve, Effector Memory, TEMRA T cells and CD4 to CD8 ratio); CD3, CD25 and FOXP3 (to assess regulatory T cells)
- Transcriptomic/Proteome: RNA, miRNA, protein samples, and telomerase, akt

- Growth factors: Sdf-1, notch,
- Functional Assays: cell growth rate, VEGF, and CFU assay

4 - Immune monitoring for graft rejection. Calculated panel reactive antibodies (cPRA) will be performed at day 1, Month 1, 3 and 6 months post infusion to check for donor specific antibodies. In addition, the following markers will be used to assess for activated T-cells based upon a $CD3^{+}CD25^{+}$ (late/chronic T cell activation) or $CD3^{+}CD69^{+}$ phenotype(early T cell activation).

5 - Optional brachial ultrasound to assess endothelial function.

6 - Optional: An additional five (5) lavender top tubes (EDTA) will be drawn of approximately 8ml per tube.

7 – 4 meter gait speed test will be performed twice per visit and the average of the exams will be taken.

8 – DEXA scan will be performed at baseline and Month 6. The scans of the hip and spine for bone density, and total body composition will be assessed at both visits.

9 – Dynamometer will be performed at least three (3) times during each applicable visit for each hand. The three values collected will be averaged for each hand. (Please reference the instruction manual on how to administer.)

10– Infectious disease tests – HIV, HIV 2, Hep B (HBsAG, Anti-HBs, Anti-HBc), and Hep C (Anti-HCV), VDRL, Syphilis, West Nile Virus, and CMV. (Conducted within 30 days of infusion, if test expire prior infusion, they must be performed again)

11–Month 12 follow-up phone call may be waived if the subject has a screening visit for an additional infusion prior to this visit.

12 - Guidelines for completing the (DSE) are outlined in Appendix 2 of this protocol.

C.15 Study Visits

C.15.1 Screening Visit for additional infusion

See Table C1 for the procedures and assessments to be performed during the screening visit of the study for the subjects that consent to participate in the additional infusions. Once the subject signs the informed consent form (ICF), the subject will then have a 45-day window from the time the subject signs the ICF to the start of the baseline visit to complete all screening procedures. The tests may take place over several days to complete. No screening exams will take place until the subject is fully informed of the research and signs the consent form.

There will be up to a +/-2 week window from the subjects Month 12 phone call from the subject's previous infusion to begin screening procedures, at which point the month 12 phone call visit from the original infusion will be waived, if screening visits for subsequent infusions take placed prior to this visit.

If a dobutamine stress echo was performed within 6 months, that report can be used in place of screening DSE for additional infusions

C.15.2 Baseline Visit for additional infusion

See Table C1 for the procedures and assessment to be performed during the baseline visit of the study. Once all screening exams are completed and it has been determined that the subject remains eligible for the study, subjects will be enrolled into the study. The baseline visit will take place within four weeks of the infusion. The listed procedures should all be performed as soon as practicable. If DEXA was performed within 6 months, that report can be used in place of baseline DEXA for additional infusions.

Endothelial function (Optional Assessments) will occur upon the subject signing the optional section of the informed consent form (ICF). No endothelial function tests will take place until the subject is fully informed of the research and signs the optional portion of the consent form.

- Brachial ultrasound testing and blood collection will be performed to assess endothelial function in the aging frailty 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.

- Flow Mediated Diameter percent change (FMD%): All measurements of the brachial artery diameter and FMD will be performed in the morning, in a quiet and dark room and at controlled ambient temperatures between 20°C and 26°C. Studies will be conducted after an overnight fast of at least 10 hours (water is permitted), with the 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. In each examination, recording of vessel images will be followed by inflation of a cuff to supra-systolic pressure (40 to 50 mmHg

above systolic pressure) for 5 minutes. Then the cuff will be deflated and the brachial artery diameter will be imaged and recorded for 3 minutes. FMD% more than 10% is considered a normal response. Lower than 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.

- Blood drawn from fasting subjects will be separated and the serum will be frozen until processed as one batch towards the end of the study. Blood will be processed twice – in the beginning of the study and after 3 months.
- Biochemical analysis: soluble pro inflammatory cytokines (interleukin-1, interleukin-6, interleukin-10, VEGFR2, TNF-a).
- Assay of colony forming units: Fresh blood will be processed for cell culture assays for endothelial progenitor stem cells colonies counting (a 5 days' protocol). Fifty milliliter of blood will be processed; peripheral-blood mononuclear cells will be isolated by Ficoll density-gradient centrifugation, will be washed twice in phosphate buffered saline with 5% fetal bovine serum and re-suspended in media (EndoCult basal media with supplements; StemCell Technologies, Vancouver, British Columbia, Canada) for EPC colony-forming assay. Cells will be planted on human fibronectin-coated plates (BIOCOAT; Becton Dickenson Labware, Bedford, Massachusetts) at a density of $5X10^6$ cells/well and incubated at 37^0C in humidified 5% CO_2. After 48 hours, the non-adherent cells will be re-plated onto fibronectin-coated 24 well plates at a density of $1X10^6$ cells/well. After 5 days, colony forming units (defined as a central core of rounded cells surrounded by elongated and spindle-shaped cells) will be counted manually in 8 wells out of a 24-well plate.

C.15.3 Day 1 Visit for additional infusion

See Table C1 for the procedures and assessment to be performed during the Day 1 visit of the study. The Day 1 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. The subject will be monitored for 2-3 hours, following administration of the investigational product, and will be sent home the same day.

C.15.4 Month 1 Visit for additional infusion

See Table C1 for the procedures and assessment to be performed for the Month 1 visit of the study. Subject visits should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 2 weeks for the month 1 study visit.

C.15.5 Month 3 and Month 6 Visit for additional infusion

See Table C1 for the procedures and assessment to be performed for month 3 and 6 visit of the study. Subject visit should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 30 days for the month 3 and 6 study visit.

C.15.6 Month 12 Visit

See Table C1 for the procedures and assessments to be performed for Month 12 visit. This visit will be conducted via a phone interview with the subject. A phone script will be provided to the study personnel to use when interviewing the subject. There will be a +/- window of 2 weeks for this visit. The Month 12 visit will be waived, if screening visits for subsequent infusions take place prior to this visit.

C.16 Statistical Considerations

All subjects will be offered the re-infusion and formal statistical considerations regarding sample size and power are not provided. Based on prior studies, additional infusions of stem cells are safe but strict safety monitoring will be employed to continue the safety assessment of allogeneic infusion of MSCs. Detailed analyses will be described in the separate statistical analysis plan.

Addendum D: Placebo Group Optional Follow-on Phase

D.1 Rationale and Description of infusion for 10 subjects randomized to placebo group

This addendum protocol is for subjects that have previously participated in the randomized portion of the study and received placebo. Ten (10) subjects are eligible for a single infusion of 100 million allogeneic human mesenchymal stem cells (Reference section 3.2).

Subjects from the randomized phase who previously received placebo infusions and are participating in this addendum will receive one (1) infusion of 100 Million allogeneic hMSCs. The hMSCs provided to study subjects in this addendum may have penicillin/streptomycin. Altogether, subjects in this addendum will have the option to receive one infusion of MSCs for a total of two (2) infusions (1 placebo infusion from the main phase and one infusion of 100 Million hMSCs) once all eligibility criteria have been met.

Subjects will have ongoing safety monitoring that will include detailed immunologic assessments.

D.2 STUDY OBJECTIVES AND ENDPOINTS:

D 2.1 - Study Objectives

D.2.1.1 Primary Objective:

- To demonstrate the safety of intravenous allogeneic hMSCs administered in subjects with Frailty and to explore treatment efficacy (decrease in frailty, frequency of acute exacerbations, change in symptom related quality of life, improved cardiovascular status, decrease in inflammatory biomarkers, endothelial function and 1 year survival).

D.2.1.2 Secondary Objective:

- To explore effects of allo-hMSCs on symptom related quality of life, cardiovascular performance, endothelial function and inflammation.

D.2.2 Study Endpoints

D.2.2.1 Primary Endpoints (Safety)

• Safety (Primary): Incidence (at one month post infusion) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-

fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities, determined per the Investigator's judgment.

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

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

D.2.2.2 Secondary Endpoints (Efficacy)

The following efficacy endpoints will be evaluated in this trial (During the screening, baseline, Month 3 and/or Month 6 visits):

1. Difference in rate of decline of Frailty defined as:

    - Reduced Activity (assessed via CHAMPS questionnaire)
    - Slowing of Mobility (assessed via a 4 meter gait speed test and SPPB assessment)
    - Weight Loss
    - Diminished handgrip strength (assessed via dynamometer)
    - Exhaustion (assessed via the MFI questionnaire)
    - Decrease in subject quality of life assessment(s) (assessed via ICECAP, SF-36, EQ-5D Questionnaires)

2. Death from any cause.
3. Change between screening and 6 months in dobutamine stress echo induced ejection fraction.
4. Change between screening and 6 months for the following panel of inflammatory markers: CRP, IL-6, D-dimer, fibrinogen,CBC with differential, and TNFα
5. The incidence of each component of the primary endpoint including non-fatal pulmonary embolism, stroke, hospitalization for worsening dyspnea and clinically significant laboratory test abnormalities
6. Change in Smell Identification Test (UPSIT)

D.3 Study Design

Subjects who received placebo in the randomized phase of the trial will have the option to receive 1 x 10^8 (100 million) allogeneic hMSCs, if all study endpoints are met from the randomized phase. Up to ten (10) subjects may be enrolled in this addendum and

subjects will follow the study schedule listed in Section D14 (Table D1) of this addendum.

D.4 Inclusion and Exclusion Criteria

D.4.1 Inclusion Criteria

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

1. Provide written informed consent.

2. Subjects age ≥60 and ≤95 years at the time of signing the Informed Consent Form for this follow on phase.

3. Have previously participated in the randomized phase of the trial and received placebo.

4. Female subjects must have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy.

D.4.2 Exclusion Criteria

In order to participate in this study, a subject MUST NOT have any of the following:

1. Score of ≤24 on the Mini Mental State Examination (MMSE)

2. Inability to perform any of the assessments required for endpoint analysis (report safety or tolerability concerns, perform PFTs, undergo blood draws, read and respond to questionnaires.

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, total bilirubin > 1.5 mg/dl.

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. 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.

7. Be an organ transplant recipient.
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. Have a history of drug or alcohol abuse within the past 24 months.
11. Be serum positive for HIV, hepatitis BsAg or Viremic hepatitis C.
12. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial. This does not exclude subjects that participated in the randomized phase of this trial.
13. 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.
14. Have hypersensitivity to dimethyl sulfoxide (DMSO)

D.5 Dosing

Subjects participating in this protocol will receive one infusion of 1 x 10^8 (100 million) cells delivered via peripheral intravenous infusion.

The Allo-hMSCs cells may have penicillin/streptomycin and will be derived from donors meeting criteria for allogeneic unrelated human bone marrow stem cell source manufactured by the University of Miami or from a commercial clinical grade bone marrow source.

D.6 Dosage Rationale

A safety profile for IV infusion of hMSCs was based on results from previous completed toxicology results(Hare et al. 2277-86). 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 10^6hMSC/kg cumulative dose delivered by 5 infusions (20 x 10^6hMSC/kg per infusion).

The evidence supports the conclusion that it is feasible to dose subjects in this study based on a standard dose of hMSCs rather than per kilogram of body weight. The total

cell number corresponds to a range of 1.3 - 4.4 x 10^6 hMSCs per kg per infusion for subjects with 45 to 150kg body weight, the weight range for this study.

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

D.7 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 addendum, the cell dose to be delivered is 1 x 10^8 (100 million), in the following total volume

- 40ml for 100 million dose (5 million hMSC/min)

Cell s are placed in an 80ml bag and will be delivered at a rate of 2ml/min, and delivered at a maximum rate of 16×10^6 hMSC/minute and will last approximately:

- 40 minutes for delivery of 40ml of 100 million dose in an 80 ml bag

D.8 Data and Safety Monitoring Board (DSMB)

DSMB will continue to provide the same oversight as noted in Section 9.4 of the Main protocol.

D.9 Concomitant Treatments, Procedures, and Nondrug Therapies

Refer to section 4.3 of the main study protocol

D.10 Infusion Monitoring Guidelines

Refer to section 6.4.1 and Appendix 1 of the main protocol.

D.11 Adverse Events and Serious Adverse Events

Refer to section 8.9 through 8.15 of the main study protocol.

D.12 Stopping Guidelines

Refer to section 8.2.4 of the main study protocol for further stopping guidelines.

D.13 Payments to Subjects and Donors

Subjects will be reimbursed $25 at the end of each follow-up visit (Month 1 – Month 6 for a total remuneration of $75. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

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.

D.14 Study Procedures for Optional Infusion for Placebo Subjects from the Randomized phase.

Table D1: Time and Events Table for Optional Infusion for Placebo Subjects from the Randomized Phase

VISIT Schedule (Table D1)	Screening - 45 days	Baseline (-4 weeks)	Day 1	Month 1 (Day 30) (+/-2 weeks)	Month 3 (Day 90) (+/-30 days)	Month 6 (Day 180) (+/-30 days)	Month 12 (Day 365) (+/-2 weeks) *Phone Call Follow-up
Informed Consent	x						
Full Medical History	x						
Physical Exam	x	x	x	x	x	x	
12-lead (ECG)	x	x	x	x	x	x	
Concomitant Medications	x	x	x	x	x	x	x
Mini Mental State Examination (MMSE)	x					x	
Randomization		x					
Infusion Treatment (IP)			x				
Dobutamine Stress Echo Test (DSE)[11]	x					x	
Bone Density Scan (DEXA)[8]		x				x	
FEV-1		x				x	
6 Minute Walk Test		x				x	
4 Meter Gait Speed Test[7]		x				x	
SPPB Assessment		x				x	
Dynamometer (handgrip)[9]		x				x	
Smell Identification Test (UPSIT)		x				x	
IIEF, SQOL-F Questionnaires		x		x	x	x	
QOL Questionnaires (ICECAP, EQ-5D, SF-36,CHAMPS, MFI)		x		x	x	x	
Urinalysis	x			x	x	x	
Hemat., Chem., CBC, LFTs, INR, and other labs[1]	x		x	x	x	x	
HIV 1, HIV 2, Hep. B & C, and CMV	x						
Serum or Urine Pregnancy Test[2]	x		x				
Review Adverse Events			x	x	x	x	x
Immune Monitoring[4]			x	x	x	x	
Biomarker Assessment[3]			x			x	
Optional: Brachial Ultrasound [5]		x			x		
Optional: Endothelial blood samples[6]		x			x		

Time and Events Table Key:

1 – The minimal laboratory requirements for hematological, liver function and renal function include:

Hematology Tests: white blood cell count, platelet count, hemoglobin and hematocrit, red blood cell count, CBC with Differential: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase, asparate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin(fractionate if total >1.5 times normal).

Renal Function Tests: creatinine, creatinine clearance, blood urea nitrogen (BUN), glomerular filtration rate, sodium, potassium, chloride, calcium, carbon dioxide, total protein, glomerular filtration rate (GFR), and glucose, (HbA1c) will be collected at every follow-up visit only for subjects diagnosed with diabetes mellitus (DM).

Serum Uric Acid, Brain Natriuetic Peptide Pro-BNP, and high sensitivity C-reactive protein (hs-CRP), IL6, fibrinogen, D-Dimer, DNA, Serum TNFα, testosterone (males only), estrogen (females only) and FSH***.**

- Laboratory work may be completed at a diagnostic center or home health agency should the subject be unable to come to the site.

*** Deoxyribonucleic acid (DNA) will only be collected once during the subject's participation. There is no specific point DNA needs to be collected as it can occur at any time point.*

****FSH will need to be collected at screening to verify that female subjects have an FSH ≥ 25.8 mIU/mL, if not currently on hormone replacement therapy for inclusion in the trial.*

2 – A serum or urine pregnancy test will be completed within 36 hours prior to infusion for females of childbearing potential.

3 – The following biomarkers will be analyzed (Approximately 10mL blood samples will be collected):

- **Cell-surface markers**: CD19, CD27, IgD, and CD5 (for Switched Memory, Naïve, Late/Exhausted, IgM memory B cells and regulatory B cells); CD19 and intracellular TNF-α (to assess intracellular TNF-α); CD3, CD4, CD8, CCR7 and CD45RA (for Central Memory, Naïve, Effector Memory, TEMRA T cells and CD4 to CD8 ratio); CD3, CD25 and FOXP3 (to assess regulatory T cells)
- **Transcriptomic/Proteome:** RNA, miRNA, protein samples, and telomerase, akt
- **Growth factors**: Sdf-1, notch,

- **Functional Assays:** cell growth rate, VEGF, and CFU assay

4 – Immune monitoring for graft rejection. Calculated panel reactive antibodies will be performed from the serum of the subjects on day 1 and 6 months post treatment. In addition, the following markers will be used to assess for activated T-cells based upon a $CD3^+CD25^+$ (late/chronic T cell activation) or $CD3^+CD69^+$ phenotype (early T cell activation).

5 – Optional brachial ultrasound to assess endothelial function.

6 – Optional: An additional five (5) lavender top tubes (EDTA) will be drawn of approximately 8ml per tube.

7 – 4 meter gait speed test will be performed twice per visit and the average of the exams will be taken.

8 – DEXA scan will be performed twice at baseline and Month 6. The scans of the hip and spine for bone density, and total body composition will be assessed at both visits.

9 – Dynamometer will be performed at least three (3) times during each applicable visit. for each hand. The three values collected will be averaged for each hand. (Please reference the instruction manual on how to administer.)

10 – Infectious disease tests – HIV, HIV 2, Hep B (HBsAG, Anti-HBs, Anti-HBc), and Hep C (Anti-HCV), VDRL, Syphilis, West Nile Virus, and CMV.(Conducted within 30 days of infusion, if test expire prior infusion, they must be performed again)

11 – Guidelines for completing the (DSE) are outlined in Appendix 2 of this protocol.

D.15 Study Visits

D.15.1 Screening Visit

See Table D1 for the procedures and assessments to be performed during the screening visit of the study for the subjects that consent to participate in the additional infusion. Once the subject signs the consent form the subject will then have a 45-day window from the time the subject signs prior to the start of any baseline visit procedures to complete all screening procedures. The tests may take place over several days to complete. No screening exams will take place until the subject is fully informed of the research and signs the consent form.

There will be up to a + 1 year window for the subject to consent to begin screening procedures, from the subject's receipt of the un-blinding letter for the randomized phase.

D.15.2 Baseline Visit

See Table D1 for the procedures and assessment to be performed during the baseline visit of the study. Once all screening exams are completed and it has been determined

that the subject remains eligible for the study, subjects will be enrolled into the study. The baseline visit will take place within four weeks of the infusion. The listed procedures should all be performed as soon as practicable.

D.15.3 Day 1 Visit

See Table D1 for the procedures and assessment to be performed during the Day 1 visit of the study. The Day 1 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. The subject will be monitored for 2-3 hours, following administration of the investigational product, and will be sent home the same day.

D.15.4 Month 1 Visit

See Table D1 for the procedures and assessment to be performed for the Month 1 visit of the study. Subject visits should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 2 weeks for the month 1 study visit.

D.15.5 Month 3 and Month 6 Visit

See Table D1 for the procedures and assessment to be performed for month 3 and 6 visit of the study. Subject visit should be completed as close to the scheduled visit dates as possible. There will be a +/- window of 30 days for the month 3 and 6 study visit.

D.15.6 Month 12 Visit

See Table D1 for the procedures and assessments to be performed for Month 12 visit. This visit will be conducted via a phone interview with the subject. A phone script will be provided to the study personnel to use when interviewing the subject. There will be a +/- window of 2 weeks for this visit.

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