

PROTOCOL

TITLE: A phase IIa, single-blind, placebo-controlled, crossover, multi-center, randomized study to assess the safety, tolerability, and preliminary efficacy of a single intravenous dose of ischemia-tolerant allogeneic mesenchymal bone marrow cells to subjects with heart failure of non-ischemic etiology

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PROTOCOL NUMBER: STEM-104-M-CHF

INVESTIGATIONAL PRODUCT: Ischemia-Tolerant Allogeneic Mesenchymal Bone Marrow Cells, (aMBMC) (adult human)

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TABLE OF CONTENTS.....		PAGE
PROTOCOL ACCEPTANCE FORM.....		4
PROTOCOL SYNOPSIS		5
1	BACKGROUND.....	12
1.1	Non-Ischemic Heart Failure	12
1.2	Bone Marrow Mononuclear Cells (BMMNC) for N-IHF	12
2	MESENCHYMAL STEM CELLS.....	12
2.1	Mechanisms of Action of MSCs	12
2.2	Preclinical Experience with MSCs for Heart Disease	13
2.3	Allogeneic versus Autologous MSCs.....	14
2.4	Modifying MSCs for Cardiac Regeneration	14
2.5	Potential of MSCs with Hypoxic Culture	14
2.6	Potency of MSCs Cultured in a Hypoxic Environment.....	14
2.7	Clinical Experience with Stemedica itMSCs	15
2.8	Clinical Trials with MSCs in Heart Disease.....	15
2.9	Rationale for Study Design	16
3	OBJECTIVES.....	16
3.1	Primary Objective.....	16
3.2	Secondary Objective.....	16
4	STUDY DESIGN	16
4.1	Description of the Study.....	16
4.2	Endpoints.....	17
4.3	Safety Plan	18
4.4	Compliance with Laws and Regulations.....	18
5	STUDY ENROLLMENT	18
5.1	Subject Selection	18
5.2	Method of Treatment Assignment and Blinding.....	19
5.3	Subject Discontinuation and Withdrawal	19
5.4	Study Termination.....	20
6	INVESTIGATIONAL PRODUCT.....	20
6.1	Formulation, Packaging, Preparation, Randomization of aMBMC or Placebo	20
6.2	IV Administration of aMBMC or Placebo	20
6.3	Product Storage and Stability.....	20
6.4	Concomitant Medications and Rehabilitation.....	21
7	STUDY ASSESSMENTS AND SCHEDULE	21
7.1	General Assessments.....	21
7.2	Schedule of Assessments.....	22
7.3	Follow-up Assessments	24
7.4	Early Termination Visit	25
8	ASSESSMENT OF SAFETY	25
8.1	Safety Parameters	25
8.2	Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters	27
8.3	Expedited Reporting Requirements for Serious Adverse Events.....	29
8.4	Type and Duration of Follow-Up of Subjects After Adverse Events	29
8.5	Stopping Rules	29
8.6	Data Safety and Monitoring Board (DSMB).....	29
9	STATISTICAL CONSIDERATIONS.....	30
9.1	Randomization.....	30
9.2	Primary Safety Analysis	30
9.3	Efficacy Analyses.....	30
9.4	Determination of Sample Size.....	30

9.5	Interim Analysis	30
1		
10	INVESTIGATOR REQUIREMENTS	31
10.1	Study Initiation	31
10.2	Study Completion.....	31
10.3	Informed Consent Process.....	31
10.4	Institutional Review Board or Ethics Committee.....	32
10.5	Study Monitoring Requirements	32
10.6	Case Report Forms.....	32
10.7	Source Data Documentation	32
10.8	Use of Computerized Systems.....	33
10.9	Investigational Product Accountability	33
10.10	Disclosure of Data.....	33
10.11	Retention of Records (for approval enabling studies only)	33
	REFERENCES.....	35
	APPENDIX A: SCHEDULE OF ASSESSMENTS	41
	APPENDIX B: QUESTIONNAIRES	42
	APPENDIX C: FINAL FORMULATION SKIN TEST.....	46

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PROTOCOL ACCEPTANCE FORM

TITLE: A phase IIa, single-blind, placebo-controlled, crossover, multi-center, randomized study to assess the safety, tolerability, and preliminary efficacy of a single intravenous dose of ischemia-tolerant allogeneic mesenchymal bone marrow cells to subjects with heart failure of non-ischemic etiology

PROTOCOL NUMBER: STEM-104-M-CHF

INVESTIGATIONAL PRODUCT(S): Ischemia-Tolerant Allogeneic Mesenchymal Bone Marrow Cells, (aMBMC) (adult human)

I have read the protocol, including all appendices, and agree that it contains all necessary details for my staff and me to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I understand that I am not allowed to make changes to this protocol.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by CardioCell, Inc. I will discuss this material with them to ensure that they are fully informed about the investigational products and the study, and with Good Clinical Practice (GCP).

Principal Investigator's Name (print)

Principal Investigator's Signature

Date

Please fax a copy to (760) 520-0608 and retain the original for your study files.

PROTOCOL SYNOPSIS

TITLE: A phase IIa, single-blind, placebo-controlled, crossover, multi-center, randomized study to assess the safety, tolerability, and preliminary efficacy of a single intravenous dose of ischemia-tolerant allogeneic mesenchymal bone marrow cells to subjects with heart failure of non-ischemic etiology

PROTOCOL NUMBER: STEM-104-M-CHF

INVESTIGATIONAL PRODUCTS: Ischemia-Tolerant Allogeneic Mesenchymal Bone Marrow Cells (aMBMC), (adult human)

PHASE: IIa

INDICATION: Heart failure of non-ischemic etiology

IND/IDE NUMBER:

SPONSOR: CardioCell, LLC

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STUDY OBJECTIVES

Primary:

To assess the safety of human ischemia-tolerant allogeneic mesenchymal bone marrow cells (aMBMC) administered intravenously to subjects with heart failure (HF) of non-ischemic etiology (N-IHF).

Secondary:

To assess the effects of intravenously delivered human aMBMC on (a) left ventricular (LV) structure and function, and (b) clinical status of subjects with N-IHF.

STUDY DESIGN

This is a Phase IIa, single blind, placebo-controlled, crossover, multi-center, randomized study in subjects with NIHF. The study will enroll 20 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=10) or a placebo group (n=10), respectively. Subjects in the experimental group will receive 1.5 million cells per kg and subjects in the placebo group will receive 1 mL/kg Lactated Ringer's Solution. At 90 days the two groups will change arms and the placebo group will receive 1.5 million cells per kg and the initially treated group will receive a Lactated Ringer's Solution (LRS) at a volume of 1 mL/kg.

SUBJECT PARTICIPATION

To be eligible for screening, participants must be diagnosed with N-IHF with previous angiographically or cardiac CTA documented no or non-significant coronary artery disease (less than 30% left main or <50% stenosis of any other major coronary artery and no clinical history of myocardial infarction). The screening evaluation will include obtaining written informed consent, collection of a complete medical history, including current and past medications, physical examination and laboratory tests. A quantitative echocardiogram ≤ 6 months prior to evaluation is also required for assessment of LV function. If the subject's LV ejection fraction (LVEF) is $\leq 40\%$ determined by echocardiography in the past twelve months, the patient will be eligible for screening for the study.

A baseline cardiac magnetic resonance imaging (MRI) scan will be obtained with delayed contrast enhancement to measure cardiac structure and function prior to study product infusion. A T2 image will also be obtained in all subjects to provide information about tissue edema, which in turn will provide information about whether ischemia has occurred over the past several days [to determine whether ischemia is in fact present—which presumably would be on the basis small vessel disease]. A full 2D and Doppler transthoracic echocardiography with speckle tracking will be performed also prior to study product infusion.

Eligible subjects will receive either a stem cell or placebo infusion based on randomization at the two clinical sites as approved by the sponsor.

Follow-up visits will occur at days 30, 60 and 90. At 90 days the two groups will change arms and the placebo group will receive the stem cell infusion also and the initially treated group will receive a LRS at a volume of 1 mL/kg. All patients will be evaluated at 30, 60 and 90, days after this new infusion treatment. Information on adverse events, concomitant medications, vital signs, clinical laboratory tests and physical examination will be collected according to the schedule of assessments (Appendix A). All concomitant guideline based medical care will continue as prescribed by the subject's personal physician(s). Use of other investigational agents or treatments is not allowed during this study.

STUDY ENDPOINTS

PRIMARY ENDPOINT

Safety

Baseline and days 30, 60, and 90 post-initial infusion. After the crossover phase all patients will be evaluated at days 30, 60, and 90 days post the new infusion

1. Procedural complications
2. Vital signs
 - a. Temperature
 - b. Systolic blood pressure/diastolic blood pressure
 - c. Heart rate
 - d. Respiratory rate
 - e. Weight
3. Changes in heart failure medications
4. Clinical arrhythmias, defibrillator firing and anti-tachycardia pacing
5. Laboratory tests
 - a. CBC/PLT/differential
 - b. Chemistry-12, including liver function tests
 - c. Creatinine kinase
 - d. Troponin-I
6. Electrocardiogram
7. Composite of all-cause mortality all-cause admission and need for co-intervention. E.g. IV diuretics for worsening heart failure (RADIENCE trial criteria).

Baseline and day 90 post stem cell infusion

Pulmonary function test

SECONDARY ENDPOINT

Efficacy

Change in LVEF from baseline to day 90 post initial infusion. After the crossover phase all patients will be evaluated for change from the new baseline (day 90 after the initial phase) to day 90 post new infusion.

EXPLORATORY ENDPOINTS

Changes between baseline and days 90 post-initial infusion. After the crossover phase all patients will be evaluated for changes from the new baseline (day 90 after the initial phase) to day 90 post new infusion.

MRI

1. Left ventricular end systolic volume index
2. Wall motion segmental score
3. LV end-systolic and end-diastolic dimensions
4. T2 MRI imaging for tissue edema (an index of recent ischemia)

Changes between baseline and days 90 post initial infusion. After the crossover phase all patients will be evaluated for changes from the new baseline (day 90 after the initial phase) to day 90 post new infusion.

Echocardiogram

1. Changes in LV mechanics by speckle-tracking echocardiography (longitudinal and circumferential strain and strain rate)
2. Estimated systolic pulmonary artery pressures
3. Changes in MR severity (most patients have mild to moderate MR)

Changes between baseline and days 30, 60, and 90 post-initial infusion. After the crossover phase all patients will be evaluated for changes from the new baseline (day 90 after the initial phase) to day 30 and 90 post new infusion

Exercise capacity

6 minute walk distance

Health status

- a. New York Heart Association Class (NYHA) class
- b. Kansas City Cardiomyopathy Questionnaire

Heart failure status

Clinical Congestion Score

Biomarkers

1. Brain natriuretic peptide, troponin
2. Growth factors (FGF, VEGF)

SUBJECT SELECTION

Subjects will be selected using the following inclusion and exclusion criteria:

INCLUSION CRITERIA

1. Males and females ≥ 18 years of age
2. LVEF $\leq 35\%$ on echocardiogram within 6 months of randomization to undergo MRI
3. Screening cardiac MRI at baseline with
 - a. Ejection fraction $\leq 40\%$
 - b. No evidence of hyper-enhancement
4. Absent or non-obstructive coronary artery disease on x-ray angiography or coronary computed tomography within the past 3 years
5. On standard therapy medical therapy (at the discretion of the investigator) including ACE-inhibitors, beta-blockers, and mineralocorticoid receptor antagonists, as tolerated, for at least 3 months
6. NYHA class II-III symptoms
7. Ability to understand and provide signed informed consent
8. Reasonable expectation that patient will receive standard post-treatment care and attend all scheduled safety follow-up visits

KEY EXCLUSION CRITERIA

1. Pregnant or nursing women or those of childbearing age and not using an effective method of contraception
2. History of stroke within 3 months
3. Cardiac surgery within 3 months prior to randomization or the likelihood of a requirement for such procedures during the study period
4. Current ICD or CRT or implantation planned within 6 months of infusion
5. Presence of clinically significant, uncorrected valvular heart disease, hypertrophic or restrictive cardiomyopathy, active myocarditis, or uncontrolled hypertension
6. History of cardiac arrest or life-threatening arrhythmias within 3 months
7. Treatment with parenteral inotropic agents within 1 month of randomization
8. Anticipated cardiac transplantation within 1 year
9. Illness other than heart failure with life expectancy less than 1 year
10. Received an experimental drug or device within 30 days of randomization
11. Left ventricular assist device or implantation planned in the next 6 months
12. Patients with complex congenital heart disease
13. Uncontrolled seizure disorder
14. Presence of immune deficiency
15. Clinically significant hematologic, hepatic, or renal impairment as determined by screening clinical laboratory tests
16. Presence of any other clinically-significant medical condition, psychiatric condition, or laboratory abnormality, that in the judgment of the investigator or sponsor for which participation in the study would pose a safety risk to the subject
17. Inability to comply with the conditions of the protocol

18. Malignancy within the previous five years, except basal cell carcinoma, provided that it is neither infiltrating nor sclerosing, and carcinoma in situ of the cervix
19. Active myocarditis or early postpartum cardiomyopathy (within six months).
20. Systemic corticosteroids, cytostatics, immunosuppressive drug therapy (cyclophosphamide, methotrexate, cyclosporine, azathioprine, etc.), and DNA depleting or cytotoxic drugs taken within four weeks prior to study treatment
21. Porphyria
22. Allergy to sodium citrate or any "caine" type of local anesthetic
23. Any contraindication for gadolinium use for MRI
24. Patient scheduled for hospice care
25. Clinically relevant abnormal findings in the clinical history, physical examination, ECG, or laboratory tests at the screening assessment that would interfere with the objectives of the study or would preclude safe completion of the study. Abnormal findings could include: known HIV infection or other immunodeficiency state, chronic active viral infection (such as hepatitis B or C), acute systemic infections (defined as patients undergoing treatment with antibiotics), gastrointestinal tract bleeding, or any severe or acute concomitant illness or injury
26. Any other medical, social, or geographical factor that would make it unlikely that the patient could comply with study procedures (e.g., alcohol abuse, lack of permanent residence, severe depression, disorientation, distant location, or noncompliance)

STUDY TREATMENT

Study subjects will receive infusion at two study sites under the supervision of the site Principal Investigator or qualified co-investigator. Study product administration will be performed 7 ± 3 days after randomization.

Administration of Human Ischemia-Tolerant Allogeneic Mesenchymal Bone Marrow Cells (aMBMC)

The study will enroll a minimum of 20 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=10) or a placebo group (n=10). Subjects in the experimental group will receive 1.5 million cells per kg and subjects in the placebo group will receive 1 mL/kg LRS. At 90 days the placebo group will receive 1.5 million cells per kg and the initially treated group will receive 1 mL/kg LRS.

Once the subject is randomized into one of the two cohorts, the final formulation of aMBMC or placebo will be prepared. Within 8 hours of infusion, the appropriate number of cells will be thawed at the study site pharmacy and re-suspended in LRS at a concentration of 1×10^6 cells/mL. Subjects in placebo group will receive LRS at a volume of 1 mL/kg. The study solution (cells or placebo) will be labeled to indicate that it is an investigational agent and will contain subject's information, date, and time of formulation.

On the day of infusion, prior to study product administration, a skin test will be administered using 0.1 mL aliquot of the Final Formulation (Appendix C).

An intravenous line will be placed into an appropriate vein on the upper extremity and 0.9% sodium chloride solution will be run to keep the vein open. The study product will be drawn using an 18-gauge needle into a 60 mL syringe with an eccentric (offset) tip. The needle will be removed and infusion tubing will be attached to the syringe. The syringe with infusion tubing will be placed in a metered dose syringe pump positioned horizontally with the syringe rotated such that the syringe tip is at the lowest position. The product will be infused intravenously into the subject's arm at a constant rate of approximately 2 mL/min. The applicable volume for the target dose will be delivered within ± 2 mL. Depending on subject's weight, up to three 60 mL syringes of product may be infused. After the entire product volume is delivered, 25 mL of LRS (without cells) will be infused at 2 mL/min to deliver cells from the infusion line for more accurate dosing. Subjects in the placebo group will receive intravenous LRS. Their syringes will be labeled as above to preserve blinding and followed by 25 mL of LRS infusion as above.

If the subject has an adverse reaction during the infusion, the infusion may be interrupted or slowed according to the severity of the reaction.

All appropriate treatment will be given to reduce any discomfort and ensure the subject's safety. The infusion may be restarted, if interrupted, if the subject is not considered to be at risk by the Investigator and the subject consents.

All subjects will receive the follow-up tests and evaluations as per Appendix A.

CONCOMITANT THERAPY AND CLINICAL PRACTICE

Subjects will continue all of their regular medications unless contraindicated. Subjects may be administered beta-blockers, ACE inhibitors or angiotensin receptors blockers, mineralocorticoid receptor antagonists, statin agents, or other medications, as tolerated.

STATISTICAL METHODS

All randomized subjects who receive at least part of a dose of aMBMC or LRS will be included in the safety analysis. Subject safety data will be analyzed by actual dose level (i.e., number of cells/kg). Adverse events will be coded according to the MedDRA adverse event dictionary. The results will be tabulated to examine their frequency, affected organ systems, severity and relationship to study stem cell administration. The results of laboratory tests will be similarly evaluated. The efficacy analyses will include subjects that received the full dose of aMBMC or LRS and have the appropriate follow-up completed at the scheduled assessments (i.e., efficacy evaluable population). Safety measures include the composite of all-cause mortality, admission for worsening HF and all-cause admissions. Measurement of left ventricle end diastolic volume, left ventricle end systolic volume, left ventricular ejection fraction (LVEF), longitudinal strain and strain rate, global and segmental strain and strain rate, exercise capacity by 6 minute walk distance test, biomarkers, Kansas City Cardiomyopathy Questionnaire (KCCQ) and the New York Heart Association (NYHA) Classification will be completed in the entire efficacy evaluable population. A formal statistical analysis plan will be completed before the end of the study and used for the final analysis of study data. This study will be conducted in accordance with the guidelines of Good Clinical Practice (GCP), including the archiving of essential documents.

ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
AHA	American Heart Association
ALT	Alanine amino transferase
AST	Aspartate amino transferase
BMMNC	Bone marrow mononuclear cells
BUN	Blood urea nitrogen
CBC	Complete blood count
CK	Creatine kinase
CRF	Case report form
CRP	C-reactive protein
CVD	Cardiovascular disease
ECG	Electrocardiogram
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
GCP	Good Clinical Practice
HF	Heart failure
ICF	Informed consent form
ICH	International Conference on Harmonization
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IND	Investigational New Drug
IRB	Institutional Review Board
KCCQ	Kansas City Cardiomyopathy Questionnaire
LFT	Liver function test
LVEF	Left ventricular ejection fraction
MACE	Major adverse cardiac events
aMBMC	Allogeneic mesenchymal bone marrow cells
MedDRA	Medical Directory for Regulatory Activities
N-IHF	Non-ischemic heart failure
NT-proBNP	N-Terminal Prohormone of Brain Natriotic Peptide
NYHA	New York Heart Association Classification
SAE	Serious adverse event
SDF-1	Stromal derived factor 1
STEMI	ST segment elevation myocardial infarction
VEGF	Vascular Endothelial Growth Factor

BACKGROUND

1.1 Non-Ischemic Heart Failure

Heart failure (HF) is a major cause of morbidity and mortality worldwide, affecting in the United States more than 5.1 million people.¹ Projections show that by 2030, the prevalence of HF will increase 25% from 2013 estimates.² The lifetime risk of developing HF is 20% for Americans ≥ 40 years of age.³ In the United States, HF incidence has largely remained stable over the past several decades, with $>650,000$ new HF cases diagnosed annually.^{1,4} HF incidence increases with age, rising from approximately 20 per 1,000 individuals 65 to 69 years of age to >80 per 1,000 individuals among those >85 years of age.⁴ In 2009, HF as an underline cause of mortality was 56,410.¹

Non-ischemic cardiomyopathy is defined as disease of the myocardium associated with mechanical or electrical dysfunction exhibiting inappropriate ventricular hypertrophy or dilatation. There are numerous causes leading to non-ischemic cardiomyopathy. Non-ischemic cardiomyopathy may be either primary or secondary to systemic diseases. In this study we will evaluate patients with dilated cardiomyopathy, which is a primary mixed disease, who have heart failure symptoms. Prevalence of non-ischemic HF (N-IHF),⁵⁻⁷ in large-scale therapeutic trials the proportion of patients with N-IHF ranged from 18% to 53%.⁸⁻¹⁴ There is a relation between sex, age and etiology of HF; non-ischemic cardiomyopathy is more frequent in women and in younger individuals. The prognosis for HF is poor, with an overall mortality of 50% within 4 years, and a 1-year mortality of 50% in patients with severe HF.¹ Although the prognosis of N-IHF was shown to be better than that in IHF, mortality is high in N-IHF reported from 23% in 1 year, 48% in 2 years and 60% in 5 years.^{7,15} Although there has been some progress in the past decades in the treatment of HF by using pharmacological therapy, surgery and device-based therapy,¹⁶ current interventional and pharmacological therapies for HF have limits and the need for a more efficient regenerative therapeutic strategy is urgent. This treatment could delay disease progression and reduce mortality and morbidity by helping injured myocardium recovery and/or facilitating cardiac repairing and regeneration.

1.2 Bone Marrow Mononuclear Cells (BMMNC) for N-IHF

To date, limited number of randomized controlled trials has investigated intracoronary or intracardiac infusion of BMMNCs in patients with N-IHF.¹⁷⁻¹⁹ The First-in-Man ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) Trial was a prospective, randomized, open, and parallel 2-arm study in a stable HF population with a history of dilated cardiomyopathy.¹⁷ In this study autologous bone marrow-derived stem cells were used which were administered intracoronary. This study showed a significant improvement in the left ventricular ejection fraction (LVEF) and New York Heart Association (NYHA) Functional Classification.¹⁷ The Transplantation of Progenitor Cells and Functional Regeneration Enhancement Pilot Trial in Patients with Nonischemic Dilated Cardiomyopathy (TOPCARE-DCM) was a prospective, open-label study testing the efficacy of selective intracoronary infusion of BMMNC on LV function.¹⁸ This study showed that intracoronary administration of bone marrow-derived progenitor cells seems to be associated with improvements in cardiac contractile and microvascular function.¹⁸ The results of the first randomized, double blind, placebo-controlled trial worldwide investigating the role of G-CSF and BMMNC for cardiac function improvement are expected.¹⁹

2 MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs), also known as marrow stromal cells, represent 0.001%–0.01% of all nucleated bone marrow cells.²⁰⁻²¹ They are self-renewing, multipotent progenitor cells with the capacity to differentiate into several distinct mesenchymal lineages including osteoblasts, chondrocytes, adipocytes, endothelial cells, vascular smooth muscle cells, and cardiomyocytes.^{20,22,23} Although MSCs were initially identified in the bone marrow, they have been isolated from a number of tissues including muscle, adipose and connective tissues.²⁴⁻²⁷ Human MSCs have been defined as cells that (1) are plastic-adherent, (2) express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules, and (3) differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*.^{28-29,30} Even after expansion *in vitro*, mesenchymal differentiation potential of MSCs is retained.^{31,32}

2.1 Mechanisms of Action of MSCs

Although there is *in vitro* and preclinical evidence that MSCs differentiate into cardiomyocyte-like cells with sarcomeric organization when injected into the adult myocardium, it appears that a low percentage of cells

engraft, independently of cell source, dose, and route of delivery. In ischemic settings and on appropriate growth factor and/or cytokine stimulation, MSCs readily acquire an endothelial cell phenotype *in vitro*, form capillary-like networks in extracellular matrix gels, express and secrete proangiogenic growth factors, and increase blood vessel growth *in vivo*.⁴⁴⁻⁴⁹ MSCs also participate in the formation of the vessel media by differentiating into smooth muscle cells and fibroblasts, and the vascular tree is a predominant reservoir for tissue-specific MSCs that express CD146, NG2, and PDGFRB (platelet-derived growth factor receptor, β polypeptide) but no hematopoietic, endothelial, or myogenic markers.³³⁻³⁵ MSCs delay scar formation due to connective tissue remodeling indicating that they may directly interact with extracellular matrix components.^{36, 37}

MSCs secrete a wide spectrum of growth and other paracrine factors including vascular endothelial growth factor (VEGF), HGF, insulin-like growth factor (IGF)-1, TGF- β , basic fibroblast growth factor (bFGF), leukemia inhibitory factor (LIF), stromal-derived factor (SDF)-1, angiopoietin, interleukins, numerous chemoattractants, and “survival factors” that exert antiapoptotic effects.⁵⁵⁻⁵⁸ Their secretion profile is importantly influenced by the inflammatory milieu, hypoxia, and mechanical stress.³⁸⁻⁴⁰ These paracrine actions may be sufficient to enhance repair and regeneration even in remote tissues.^{41, 42} Evidence suggests that secreted phospholipid particles (exosomes) rather than or in addition to cytokine proteins mediate MSC cardioprotection.⁴³ In the *in vivo* setting, reduced numbers of apoptotic cells are found in the vicinity of transplanted MSCs that may explain the reduction in infarct size despite absence of formation of new cardiomyocytes.⁴⁴

MSCs also reduce myocardial fibrosis and thereby attenuate LV dilatation by modulating the expression of extracellular matrix proteins and matrix metalloproteinases.⁴⁵ They secrete antiapoptotic and antifibrotic factors including hepatocyte growth factor and adrenomedullin. Indeed, MSC-conditioned medium decreases type I and III collagen expression, attenuates proliferation of fibroblasts, and upregulates expression of elastin, resulting in reduction of myocardial fibrosis and favorable changes in remodeling.^{46, 47} MSCs increased proliferation of endogenous CSCs, enhanced lineage commitment of the CSCs, and reconstitution of niche-like structures. Importantly, cardiac MRI documented a reduction of infarct size as early as 4 days after cell injection, which was progressive over 8 weeks.⁴⁷

2.2 Preclinical Experience with MSCs for Heart Disease

MSC transplantation using either autologous or allogeneic cells in animal models leads to reduction of ischemia, reduced apoptosis, and increased angio- and arteriogenesis in ischemic tissue, leading to improved tissue vascularization and function. Successful delivery strategies include intravenous, intramyocardial, and intracoronary administration^{22, 28, 48-52} not only in ischemic but models of non-ischemic cardiomyopathy.^{50, 53}

Intravenous injection of 3 and 10 million allogeneic porcine MSCs/kg at the onset of reperfusion in a closed-chest pig model of acute MI increased vascular density, hyperemic infarct region blood flow, and LV contractile function evaluated by pressure–volume indices after 12 weeks compared to the controls.²⁸ The functional and vascular density data suggest that the 1 million MSCs/kg dose may represent the lowest effective dose for protection. In another study, apical infarction in swine was created and bone marrow derived MSCs injected intravenously 30 min post-reperfusion. At 3 months, treated animals had higher LVEF and less wall thickening of non-infarcted myocardium compared to controls.⁵⁴ Similarly, Kocher et al. reported an increase in microvasculature in the rat infarct model with intravenous injection of human endothelial progenitors.⁵⁵ In a porcine model of occlusion-reperfusion, LV function measured by MRI 8 weeks after MI was better in hearts receiving MSCs by intramyocardial injection 3 days after infarction.^{48, 56} Others reported improved LV function in the absence of observable MSCs one month after transplantation.⁴⁹

In contrast to administration of stem cells by direct myocardial injection or intracoronary administration, intravenous delivery results in entrapment of cells mainly in the lungs during initial distribution reducing recruitment to the site of infarction. Intravenous infusion resulted in a lower efficiency of cell uptake compared to direct intramyocardial injection.⁵⁷ However, a recent comparative study with intracoronary or intravenous injection of BMMNCs found accumulation of cells in the infarcted myocardium although more cells accumulate after intracoronary than after intravenous injection.⁵⁸ It is therefore likely that paracrine release by MSCs of growth factors and cytokines is largely responsible for their therapeutic effect after intravenous administration.

2.3 Allogeneic versus Autologous MSCs

Bone marrow-derived MSCs are to some extent immunoprivileged, and allogeneic MSCs may escape elimination by the host immune system because they do not express MHC class II receptors and only low levels of MHC class I receptors.^{48,59} As with endothelial progenitor cells, MSCs are subject to age- and disease-related changes, decreasing in number in the bone marrow with age.^{60,61} Although terminal differentiation is preserved in older MSCs,⁶² it is lower when compared to younger cells.⁸⁶⁻⁸⁸ The gene expression profile of aging MSCs shows increased abundance of differentiation- and growth arrest-related transcripts and downregulation of transcripts involved in RNA processing.⁶³ Moreover, these MSCs did not improve cardiac function.⁶⁴ *These findings support the concept that allogeneic MSCs from young donors may have greater regenerative capacity than autologous cells from older subjects with CAD.*

2.4 Modifying MSCs for Cardiac Regeneration

Overexpression of antiapoptotic proteins and growth factors, and inhibition of MSC differentiation by blocking Wnt and BMP signaling pathways, have proven powerful strategies to enhance the cardiac regeneration capacity of MSCs, but their clinical application remains problematic at present.^{44,65-68} Experimentally, several modifications in MSCs have resulted in their enhanced functionality. Among these strategies are MSCs enriched for Stro-1 expression, treatment with heat shock proteins, preconditioning by oxidative stress, diazoxide-mediated NF- κ B activation, and nitric oxide donors, hyperbaric oxygenation of the recipient heart, adjunct pharmacologic therapy with statins and estradiol, sildenafil, and oxytocin.⁹⁴⁻⁶⁹ Mechanical pre-stimulation by microbubbles and embedding MSCs in extracellular matrix components or artificial polymer scaffolds may facilitate cell retention.^{53,103-107} *One strategy that is ready for clinical translation is use of enhanced MSCs grown in hypoxic culture conditions.*⁷⁰

2.5 Potentiation of MSCs with Hypoxic Culture

It is well known that stem cells reside in defined microenvironmental niches and that oxygen conditions critically regulate cellular responses. For example, oxygen tension in human bone marrow is ~3.0%. Hypoxic culture conditions can maintain MSCs in an undifferentiated state that favors stem cell self-renewal. It enhances expression of genes involved in developing mesodermal and non-mesodermal cell lines, increases multipotency and transdifferentiation, increases the half-life of VEGF mRNA significantly (6-8h in low oxygen conditions compared to 30-40 min. in normoxia), and decreases transversions from oxidative damage.¹⁰⁹⁻¹¹⁰

2.6 Potency of MSCs Cultured in a Hypoxic Environment

Stemmedica has obtained a patent for culture of human donor MSCs in hypoxic conditions (ischemia-tolerant MSCs, itMSCs). The biological potency of these cells has been studied in vitro experiments compared to MSCs cultured in normoxic conditions. itMSCs formed more colonies in culture assays, had a greater proliferation rate, produced greater quantities of VEGF, SDF-1, HIF-1 α , and angiopoietin 1. itMSCs also demonstrated greater migratory capacity in response to chemokines and cytokines, and formed more gap junctions than normoxic MSCs. In summary, itMSCs compared to those grown in normoxic conditions have (1) a higher proliferation rate, (2) higher clonogenicity as determined by colony forming unit assay, (3) increased production of cytokines participating in homing of host stem cells to the site of injury (SDF-1) and in neovascularization (VEGF), (4) higher resistance to factors adversely affecting performance of MSC at the site of injury such as tumor necrosis factor, (5) higher expression of connexins, proteins involved in cell communication via gap junctions, (6) accelerated and enhanced differentiation into certain types of tissues such as cartilage, and (7) increased migration towards the factors produced at the ischemic site such as Hepatocyte Growth Factor. *These properties of itMSCs likely increase their ability to migrate to ischemic tissues, enhance angiogenesis and arteriogenesis through paracrine effects, inhibit apoptosis, and mobilize host progenitors to promote tissue regeneration.*

In vivo bio-distribution studies after intravenous injection in a rat model of ischemic stroke demonstrate a greater retention of human itMSC cells in stroke animals as compared to controls. With radiolabelled cells, it was clear that most of the itMSCs reside in lungs after 1 hour of intravenous injection, but a greater number are present in stroke animals compared to controls. After 24 hrs, the majority of itMSCs were found in the kidneys and liver, but were also greater in stroke animals compared to controls. After 72 hours, very little

activity was detected in control animals, while there was significant activity detected in the liver and spleen of stroke animals. Very little activity in either group was noted after 5 days.

2.7 Clinical Experience with Stemedica itMSCs

Two clinical studies are currently ongoing using cells prepared at Stemedica's cGMP manufacturing facility. The first study targets ischemic stroke and is being conducted at the University of California, San Diego (UCSD), and at Mercy Gilbert Medical Center in Gilbert, Arizona using Stemedica's specially formulated ischemic adult allogeneic mesenchymal stem cells under US IND 14328. Stemedica completed the Phase I enrollment and treatment of 15 patients. All safety data were provided to DSMB for review. Only one mild event was possibly related to the study product. There were no clinically significant abnormalities reported on any post-administration scans or on post-administration physical examinations. In the second study "Intravenous Administration of Ischemic Tolerant Allogeneic Mesenchymal Stem Cells for Acute Coronary Syndrome Patients," Stemedica Cell Technologies, Inc. provided cGMP allogeneic itMSCs to the National Scientific Medical Center (NSMC) in Astana, Kazakhstan to conduct a clinical study in patients with STEMI, Principal Investigators: Professors A.U. Djolbasbekova, MD, PhD and Daniyar Jumaniyazov, MD, PhD. itMSCs were provided under 21 CFR §312.110 (4)(b) regulations and the study was conducted in compliance with the ICH-E6 (Good Clinical Practice) guidelines and all subjects provided signed informed consent. Nineteen subjects in the itMSC group received intravenously. There were no deaths and no SAEs reported to be related to the study drug. In 19 itMSC treated and 15 open control patients, i.v. injection of 45-50 million cells 7 days after stenting for STEMI was administered without any SAEs reported. LVEF improved significantly from 42.1% to 52.3% and 54.7% after 3 and 6 months, respectively. In contrast there was no improvement in LVEF in the control group; LVEF 42.3%, 42.8% and 46.1%, at baseline, 3 and 6 months respectively. Similar changes in end-systolic and end-diastolic volumes were noted. There was also an increase reported in circulating levels of growth factors (VEGF and FGF) in the itMSC treated group, and CRP and BNP levels were lower in the treated group compared to controls during follow-up.

2.8 Clinical Trials with MSCs in Heart Disease

A few studies using either allogeneic or autologous MSCs employing different delivery strategies have been performed in subjects with a variety of cardiac diseases including HF, STEMI, and chronic ischemia.

2.8.1 STEMI Trials with MSCs

In 3 studies where autologous bone marrow-derived MSCs were injected i.c. into the infarct artery of patients with STEMI, there were no serious adverse events reported, and regional and global LV function improved. Intracoronary infusion of bone-marrow-derived MSCs in 69 patients with STEMI showed improvement in perfusion defects at 3 months after the therapy with improvement of LVEF and LV chamber size.⁷¹ In 11 patients with STEMI treated with a combination of autologous bone marrow MSCs and endothelial progenitor cells, LV function and perfusion were better in MSC-treated patients and there was a reduction in malignant arrhythmia that indicated a reduced arrhythmogenic potential.^{72, 73}

An allogeneic MSC product (Prochymal by Osiris Therapeutics, Columbia, MD) was administered intravenously in 53 patients with STEMI using a double-blind, placebo-controlled, dose-ranging (0.5, 1.6, and 5 million cells/kg) design.⁷⁴ This demonstrated that intravenous infusion of hMSCs was safe, ventricular tachycardia episodes were lowered, and pulmonary function improved (forced expiratory volume) compared to placebo. Global symptom score in all patients and LVEF in the subset of anterior MI patients were both significantly better in hMSCs versus placebo subjects. In the cardiac MRI substudy, hMSC treatment increased LVEF and led to reverse remodeling.⁷⁴

2.8.2 MSC Trials in Heart Failure and Chronic Ischemia

In a randomized trial in patients with severe chronic ischemic cardiomyopathy, MSC-treated patients had significantly improved LVEF, exercise capacity, and change of New York Heart Association (NYHA) class.⁷⁵ The POSEIDON trial studied patients with ischemic cardiomyopathy and compared autologous and allogeneic MSCs administered transendocardially via injection catheters.⁷⁶ Thirty patients were randomly assigned to 1 of 6 permutations of cell origin (self or donor) and dosage (n=5 patients each), and the safety and efficacy of self-

derived MSCs with allogeneic MSCs was studied. Twenty million, 100 million, or 200 million cells were delivered by transcatheter injection into 10 LV sites. Relative to baseline, autologous but not allogeneic MSC therapy was associated with an improvement in the 6-minute walk test and the MLHFQ score, but neither improved exercise VO₂ max. Low-dose concentration MSCs (20 million cells) produced greatest reductions in LV volumes and increased EF. Allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions.⁷⁶

2.9 Rationale for Study Design

Allogeneic MSCs are excellent for consideration for cell therapy in patients with IHF for the following reasons:

- (a) Human MSCs are accessible and their isolation and expansion to clinical scale in a relatively short period of time is feasible^{32, 77}
- (b) Patients are spared the discomfort of harvesting procedures
- (c) Early therapy is feasible without the delay imposed by autologous cell cultures
- (d) MSCs can be biopreserved with minimal loss of potency and stored for point-of-care delivery^{78, 79}
- (e) Human trials of MSCs thus far have shown no adverse reactions to allogeneic versus autologous MSC transplants, enabling creation of an inventory of third-party donor MSCs to widen the number of patients treated by a single isolation⁸⁰⁻⁸²
- (f) MSC transplantation is considered safe and has been tested in Phase I clinical trials of cardiovascular, neurological, and immunological disease with encouraging results¹²³⁻¹²⁸
- (g) Preliminary Phase I studies with Stemmeda iMSCs administered in patients with ischemic stroke and STEMI has proven to be safe and associated with some improvement in clinical outcomes.

3 OBJECTIVES

3.1 Primary Objective

To assess the safety of human allogeneic mesenchymal bone marrow cells (aMBMC) administered intravenously to subjects with N-IHF.

3.2 Secondary Objective

To assess the effects of intravenously delivered human aMBMC on (a) left ventricular (LV) structure and function, and (b) clinical status of subjects with N-IHF.

4 STUDY DESIGN

4.1 Description of the Study

This is a Phase IIa, single-blind, placebo-controlled, crossover, multi-center, randomized study in subjects with N-IHF. Within 3 ±2 days all study participants who meet the eligibility criteria will receive the treatment.

The study will enroll a minimum of 20 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=10), or a placebo group (n=10). Subjects in the experimental group will receive 1.5 million cells per kg and subjects in the placebo group will receive 1 mL/kg LRS. At 90 days the two groups will change arms and the placebo group will receive 1.5 million cells per kg and the initially treated group will receive a Lactated Ringer's Solution (LRS) at a volume of 1 mL/kg .

After discharge, subjects will have safety evaluations per Appendix A. Adverse events, concomitant medications, and assessments will be recorded at each follow-up visit. Complete examinations will be used to assess disability and functional status at baseline (prior to treatment), and at the visits scheduled at 30, 60, and 90, after the initial administration. The same process will be followed at visits scheduled at 30, 60, and 90 days after the new administration (crossover phase). A baseline magnetic resonance imaging (MRI) with delayed contrast enhancement and T2 imaging, as well as a full 2D and Doppler transthoracic echocardiography with speckle-tracking will be performed at baseline and at specific time points during follow-up. All performed MRIs will be assessed in a blinded core lab.

Subjects will continue all of their regular medications unless contraindicated. Subjects may be administered beta-blockers, ACE inhibitors or angiotensin receptors blockers, mineralocorticoid receptor antagonists, statin agents, or other medications, as tolerated.

4.2 Endpoints

PRIMARY ENDPOINT

Safety

Baseline and days 30, 60, and 90 post-initial infusion. After the crossover phase all patients will be evaluated at days 30, 60, and 90 days post the new infusion

8. Procedural complications
9. Vital signs
 - a. Temperature
 - b. Systolic blood pressure/diastolic blood pressure
 - c. Heart rate
 - d. Respiratory rate
 - e. Weight
10. Changes in heart failure medications
11. Clinical arrhythmias, defibrillator firing and anti-tachycardia pacing
12. Laboratory tests
 - a. CBC/PLT/differential
 - b. Chemistry-12, including liver function tests
 - c. Creatinine kinase
 - d. Troponin-I
13. Electrocardiogram
14. Composite of all-cause mortality all-cause admission and need for co-intervention. E.g. IV diuretics for worsening heart failure (RADIENCE trial criteria).

Baseline and day-90 post infusion

Pulmonary function test

SECONDARY ENDPOINT

Efficacy

Change in LVEF from baseline to day 90 post-initial infusion. After the crossover phase all patients will be evaluated for change from the new baseline (day 90 after the initial phase) to day 90 post new infusion

EXPLORATORY ENDPOINTS

Changes between baseline and days 90 post-initial infusion. After the crossover phase all patients will be evaluated for changes from the new baseline (day 90 after the initial phase) to day 90 post new infusion.

MRI

5. Left ventricular end systolic volume index
6. Wall motion segmental score
7. LV end-systolic and end-diastolic dimensions
8. T2 MRI imaging

Changes between baseline and days 90 post-initial infusion. After the crossover phase all patients will be evaluated for changes from the new baseline (day 90 after the initial phase) to day 90 post new infusion.

Echocardiogram

4. Changes in LV mechanics by speckle-tracking echocardiography (longitudinal and circumferential strain and strain rate)
5. Estimated systolic pulmonary artery pressures
6. Changes in MR severity (most patients have mild to moderate MR).

Changes between baseline and days 30, 60, and 90 post-initial infusion. After the crossover phase all patients will be evaluated for changes from the new baseline (day 90 after the initial phase) to day 30 and 90 post new infusion.

Exercise capacity

6 minute walk distance

Health status

- a. New York Heart Association Class (NYHA) class
- b. Kansas City Cardiomyopathy Questionnaire

Heart failure status

Clinical Congestion Score

Biomarkers

3. Brain natriuretic peptide, troponin
4. Growth factors (FGF, VEGF)

4.3 Safety Plan

The safety of subjects will be carefully monitored and reviewed by an independent Data and Safety Monitoring Board (DSMB).

4.4 Compliance with Laws and Regulations

This study will be conducted in accordance with the International Conference on Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP), the Declaration of Helsinki (October 1996) and applicable local, state, and federal laws, as well as other applicable country laws.

5 STUDY ENROLLMENT

Subjects will participate in this study for 180 days as follows: up to 3±2 days after screening, followed by aMBMC or placebo (LRS) administration, follow-up, crossover phase and follow-up.

5.1 Subject Selection

The study will enroll a minimum of 20 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=10) or a placebo group (n=10). Subjects in the experimental group will receive 1.5 million cells per kg and subjects in the placebo group will receive 1 mL/kg Lactated Ringer's Solution. At 90 days the two groups will change arms and the placebo group will receive the stem cell infusion also and the initially treated group will receive a LRS at a volume of 1 mL/kg.

Eligibility will be determined by the inclusion and exclusion criteria listed below.

INCLUSION CRITERIA

1. Males and females ≥18 years of age
2. LVEF ≤35% on echocardiogram within 6 months of randomization to undergo MRI
3. Screening cardiac MRI at baseline with
 - a. Ejection fraction ≤40%
 - b. No evidence of hyper-enhancement
4. Absent or non-obstructive coronary artery disease on x-ray angiography or coronary computed tomography within the past 3 years
5. On standard therapy medical therapy (at the discretion of the investigator) including ACE-inhibitors, beta-blockers, and mineralocorticoid receptor antagonists, as tolerated, for at least 3 months
- 6.
7. NYHA class II-III symptoms
8. Ability to understand and provide signed informed consent
9. Reasonable expectation that patient will receive standard post-treatment care and attend all scheduled safety follow-up visits

KEY EXCLUSION CRITERIA

1. Pregnant or nursing women or those of childbearing age and not using an effective method of contraception.
2. Acute myocardial infarction, acute coronary syndrome, or stroke within 3 months
3. Cardiac surgery within 3 months prior to randomization or the likelihood of a requirement for such procedures during the study period
4. Current ICD or CRT or implantation planned with 6 months of infusion

5. Presence of clinically significant, uncorrected valvular heart disease, hypertrophic or restrictive cardiomyopathy, active myocarditis, or uncontrolled hypertension
6. History of cardiac arrest or life-threatening arrhythmias within 3 months
7. Treatment with parenteral inotropic agents within 1 month of randomization
8. Anticipated cardiac transplantation within 1 year
9. Illness other than heart failure with life expectancy less than 1 year
10. Received an experimental drug or device within 30 days of randomization
11. Left ventricular assist device or implantation planned in the next 6 months
12. Patients with complex congenital heart disease
13. Uncontrolled seizure disorder.
14. Presence of immune deficiency.
15. Clinically significant hematologic, hepatic, or renal impairment as determined by screening clinical laboratory tests
16. Presence of any other clinically-significant medical condition, psychiatric condition, or laboratory abnormality, that in the judgment of the investigator or sponsor for which participation in the study would pose a safety risk to the subject
17. Inability to comply with the conditions of the protocol.
18. Malignancy within the previous five years, except basal cell carcinoma, provided that it is neither infiltrating nor sclerosing, and carcinoma in situ of the cervix.
19. Active myocarditis or early postpartum cardiomyopathy (within six months).
20. Systemic corticosteroids, cytostatics, immunosuppressive drug therapy (cyclophosphamide, methotrexate, cyclosporine, azathioprine, etc.), and DNA depleting or cytotoxic drugs taken within four weeks prior to study treatment.
21. Porphyria.
22. Allergy to sodium citrate or any "caine" type of local anesthetic.
23. Any contraindication for gadolinium use for MRI
24. Patient scheduled for hospice care.
25. Clinically relevant abnormal findings in the clinical history, physical examination, ECG, or laboratory tests at the screening assessment that would interfere with the objectives of the study or would preclude safe completion of the study. Abnormal findings could include: known HIV infection or other immunodeficiency state, chronic active viral infection (such as hepatitis B or C), acute systemic infections (defined as patients undergoing treatment with antibiotics), gastrointestinal tract bleeding, or any severe or acute concomitant illness or injury.
26. Any other medical, social, or geographical factor that would make it unlikely that the patient could comply with study procedures (e.g., alcohol abuse, lack of permanent residence, severe depression, disorientation, distant location, or noncompliance)

5.2 Method of Treatment Assignment and Blinding

This study is a Phase IIa, single-blind, placebo-controlled, crossover, two-site, randomized trial with blinded endpoints comprised of 20 subjects with an experimental groups (n=10 in each group) and a placebo group (n=15). At 90 days the two groups will change arms and the placebo group will receive the stem cell infusion also and the initially treated group will receive a LRS at a volume of 1 mL/kg.

5.3 Subject Discontinuation and Withdrawal

Subjects may voluntarily withdraw from the study at any time. In the event of early withdrawal, the reason for withdrawal will be recorded on the appropriate CRF. Attempts to complete early termination exam as defined in 7.4 will be made. The Investigator has the right to discontinue a subject from the study for any medical reason that the investigator determines may jeopardize the subject's safety if he or she continues in the study, or for reasons of noncompliance (e.g., missed visits, use of other investigational drugs, etc.). Each site Investigator may enroll up to 3 additional subjects to each group to offset subjects that are withdrawn or terminated.

5.4 Study Termination

The Sponsor has the right to terminate the study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to subjects.
- Subject enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.

6 INVESTIGATIONAL PRODUCT

6.1 Formulation, Packaging, Preparation, Randomization of aMBMC or Placebo

The study will enroll a minimum of 20 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=15) or a placebo group (n=15). Subjects in the experimental group will receive 1.5 million cells per kg and subjects in the placebo group will receive 1 mL/kg Lactated Ringer's Solution.

Once the subject is randomized into one of the two cohorts, the cells or placebo will be prepared. Within 8 hours of infusion, the appropriate number of cells will be thawed at the study site pharmacy and re-suspended in Lactated Ringer's Solution (LRS) at a concentration of 1×10^6 cells/mL. Subjects in placebo group will receive LRS at a volume of 1 mL/kg. The study solution (cells or placebo) will be labeled to indicate that it is an investigational agent and will contain a subject's information, date, and time of formulation.

After discharge, subjects will have safety and efficacy evaluations as per Appendix A.

6.2 IV Administration of aMBMC or Placebo

On the day of infusion, prior to study product administration, a skin test will be administered using 0.1 mL aliquot of the Final Formulation (Appendix C).

An intravenous line will be placed into an appropriate vein on the upper extremity or hand with 0.9% sodium chloride solution running to keep the vein open. The study solution described above will be taken up in an 18-gauge needle into a 60 mL syringe with an eccentric (offset) tip. The needle will be removed and infusion tubing will be attached to the syringe. The syringe with infusion tubing will be placed in a metered dose syringe pump positioned horizontally with the syringe rotated such that the syringe tip is at the lowest position. The product will be infused intravenously into the subject's arm at a constant rate of approximately 2 mL/min. The applicable volume for the target dose will be delivered within ± 2 mL. Depending on subject's weight, up to three 60 mL syringes of product may be infused. After the entire product volume is delivered, 25 mL of LRS (without cells) will be infused at 2 mL/min to deliver cells from the infusion line for more accurate dosing. Subjects in the placebo group will receive intravenous LRS. Their syringes will be labeled as above to preserve blinding and followed by 25 mL of LRS infusion as above.

If the subject has an adverse reaction during the infusion, the infusion may be interrupted or slowed according to the severity of the reaction. All appropriate treatment will be given to reduce any discomfort and ensure the subject's safety. The infusion may be restarted, if interrupted, if the subject is not considered to be at risk by the Investigator and the subject consents.

All subjects will receive the follow-up tests and evaluations as per Appendix A.

6.3 Product Storage and Stability

Once the cell suspension is prepared for administration to a subject as described above, the dose of allogeneic mesenchymal bone marrow cells will be stored at +4°C until the time of administration. All cells will be administered to the subject within 8 hours of preparation. Cells that are not used within this time period will be discarded.

6.4 Concomitant Medications and Rehabilitation

Concomitant medications (any prescription and/or over-the-counter preparations) and therapies (nondrug or procedures) used by a subject while participating in this clinical trial will be optimized per AHA Guidelines and recorded at screening and until the end of the study or the end of a subject's participation in the study. Subjects may be administered beta-blockers, ACE inhibitors or angiotensin receptors blockers, mineralocorticoid receptor antagonists, statin agents, or other medications, as tolerated. All other prescription and over-the-counter preparations approved by the patients' physician will be permitted.

7 STUDY ASSESSMENTS AND SCHEDULE

7.1 General Assessments

Clinical Assessments

- Complete medical history, including medication use and lifestyle history
- Physical and cardiac examination, including vital signs (temperature, systolic blood pressure/diastolic blood pressure, weight, heart rate, respiratory rate)
- Cardiac Examination
- Composite of all-cause mortality, admission for worsening HF and all-cause admissions.
- Exercise capacity: 6 minute walk distance
- Kansas City Cardiomyopathy Questionnaire (KCCQ)(Appendix B)
- New York Heart Association (NYHA) Classification (Appendix B)

Clinical Laboratory Assessments

- Hematology (CBC): hemoglobin, hematocrit, platelet count, red blood cell count, white blood cell count, automated differential
- Serum Chemistry: includes sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, glucose, calcium, phosphorous, total bilirubin, albumin and total protein, and the following liver function tests (LFT's): alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST)
- Immunoglobulin A (IgA), immunoglobulin E (IgE), immunoglobulin G (IgG), immunoglobulin M (IgM), and Lymphocyte Proliferation Panel
- Serum Pregnancy test: only for women with child-bearing potential
- Vascular Endothelial Growth Factor (VEGF)
- Fibroblast Growth Factor (FGF)
- NT-pro Brain Natriuretic Peptide (NT-proBNP) and cardiac troponin

ECG

A 12-lead ECG will be performed at all study visits. Standard interval assessments, including QT and QTc, will be calculated from a single lead (typically lead II). QTc will be calculated on the baseline ECG in order to determine if the subject meets enrollment criteria. QTc must be <550ms in order for the subject to be randomized.

Echocardiography

- A full 2D and Doppler echocardiogram will be performed at screening, at day 90, after initial infusion. After the crossover phase a full 2D and Doppler echocardiogram will be performed at day 90 after the new infusion.
- Images will be acquired with the patient positioned in the recumbent lateral position. Left ventricular volumes will be determined at end-diastole and end-systole by quantitative biplane assessment. Endocardial borders will be manually traced from apical four-chamber and two-chamber views. Left ventricular volumes will be used to calculate ejection fraction using the biplane modified Simpson's summation-of-disks method recommended by the American Society of Echocardiography.
- High frame-rate images of the LV in the three apical views (4-chamber, 2-chamber, and apical long axis) will be acquired for speckle tracking echocardiography. Images will be analyzed offline on a dedicated workstation using EchoPAC BTO 12 (GE Healthcare) software for calculation of segmental and global

longitudinal strain and strain rate. Global longitudinal strain and strain rate will be calculated from all 18 segments of the LV. Segmental strain and strain rate will be calculated for each segment separately. To assess effect of therapy on impaired regions, we will average strain and strain rate of the dysfunctional segments at baseline and compare the average of these segments over time.

Cardiac MRI

- Cardiac MRI with gadolinium contrast will be performed at baseline and after 90 days after initial infusion. After the crossover phase an MRI with gadolinium contrast will be performed at new baseline (day 90 after the initial phase) and after 90 days. MRI sequences will include balanced steady state free precession sequence (b_SSFP) and a delayed contrast enhanced imaging sequence using IV gadolinium contrast, and T2 imaging. Image analysis will be performed by an experienced central laboratory blinded to the subjects' treatment. Efficacy endpoints evaluated by MRI will include: LV end diastolic volume EDV, LV end systolic volume, ESV, and global Left Ventricular Ejection Fraction, and assessment of T2 images for assessing the tissue status
- End-diastolic and end-systolic endocardial traces from contiguous short axis slices will be used to determine end-diastolic and end-systolic left ventricular volumes and ejection fraction. Subjects with contraindications (e.g. implanted devices, surgical prosthesis, renal impairment or claustrophobia) will not undergo MRI.

Holter monitoring

Those patients who do not have dual chamber defibrillators implanted will undergo twenty four hour Holter monitoring at baseline, day of infusion, at day 30, 60, and 90, after initial infusion and at 30, 60, and 90 days post the new infusion after the crossover phase. For the purposes of this study, life-threatening arrhythmias are defined as ventricular tachycardia (defined as 3 or more consecutive beats arising below the atrioventricular node with a rate >120 beats/min, and complete heart block).

Pulmonary Function Tests

Spirometry will be used to assess lung function at screening and at 90 days for the initial phase and at 90 days after the crossover phase. Spirometry tests should be conducted in accordance with the American Association for Respiratory Care (AARC) Spirometry Clinical Practice Guidelines

(www.rcjournal.com/cpgs/spirupdatepg.html). Tests will obtain information about FEV1 (L) and Percent Predicted FEV1. Prior to lung function testing, subjects will be advised to avoid the following activities: 1) Smoking within at least 2 hours of testing, 2) consuming alcohol within 4 hours of testing, 3) vigorous exercise within 4 hours of testing, 4) eating a large meal within 2 hours of testing, 5) consuming caffeine such as coffee, tea, cola drinks, or chocolate on the day of testing.

Diffusing capacity of the lungs for carbon monoxide (DLCO) will also be used to assess lung function at screening and at 90 days. DLCO will be conducted in accordance with the American Thoracic Society and European Respiratory Society guidelines for standardization of the test.⁸³

7.2 Schedule of Assessments

7.2.1 Screening (Day 1-2)

All screening evaluations must be within 1-2 days after identification of possible eligible patients.

- Study participants must meet eligibility criteria (inclusion/exclusion criteria review).
- Signed the written informed consent
- Full 2D and Doppler transthoracic echocardiography with speckle-tracking with LVEF $\leq 40\%$
If the patient is eligible based on the evaluation above, then
- Complete medical history, including medications history
- Complete physical and cardiac examination, including vital signs (oral temperature, blood pressure, and pulse rate), weight, and height
- 12-lead ECG
- Cardiac MRI, with delayed gadolinium contrast enhancement – Subjects with contraindications to MRI because of cardiac devices (pacemakers and defibrillators) will undergo an MRI scan after deactivation

of their device by an electrophysiologist. Other contraindicated patients, e.g. surgical prosthesis, renal impairment or claustrophobia, will not undergo MRI.

- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- Spirometry/DLCO
- Clinical labs: serum chemistry including BUN & creatinine, CBC with diff, LFT's, total protein and albumin,
- Cardiac Biomarkers (CK, CK-MB fraction)
- Pregnancy test, when appropriate
- Serum chemistry
- Serum/plasma stored for VEGF, FGF, NT-proBNP, troponin
- HIV test
- Pulse oximetry
- 24 Hour Holter ECG recording or ICD assessment
- Randomization into experimental or control group
- 6 minute walk test

7.2.2 Assessments and Treatment

Day 7 ± 3 Days (day of infusion)

Pre-infusion:

- Inclusion/exclusion criteria review.
- Review and record any changes in medical history, including concomitant medications from screening.
- Confirm Informed Consent Form (ICF) is complete with signature of subject, or legal guardian & witness.
- Measure and record weight and vital signs (oral temperature, blood pressure, pulse rate) after subjects rest quietly in a supine or semi-recumbent position.
- Final Formulation Skin Test. Performed prior to infusion (Appendix C)
- Physical and Cardiac Examination
- Draw and store blood samples for IgA, IgE, IgG, IgM and for Lymphocyte Proliferation Panel
- Place an IV line in the upper extremity or hand with 0.9% sodium chloride running to keep the vein open

During infusion:

- IV infusion of the study product or placebo
- Monitoring for any signs of adverse reaction, record adverse event/s and concomitant medications
- Record vital signs every 2 hours, and as clinically indicated
- Continuous ECG monitoring during infusion of study product
- Pulse oximetry continuously during and for 2 hours post infusion

Post- infusion:

- 12-lead ECG
- Monitoring for any signs of adverse reaction, record adverse event/s and concomitant medications
- Record vital signs every 2 hours till discharge
- Continuous ECG monitoring for 2 hours
- Pulse oximetry continuously for 2 hours
- Cardiac biomarkers (CK and CK-MB fraction, troponin I).
- Serum/plasma stored for VEGF, FGF, NT-proBNP, troponin
- Subjects will be observed for a minimum of 6 hours ± 30 minutes post infusion or until deemed stable in the opinion of the investigator
- Subject is free to ambulate according to investigators discretion
- Physical exam, prior to discharge if clinically stable
- Provide subject or responsible individual accompanying the subject with the phone number for contacting the study nurse and/or investigator for any questions, concerns or changes in health status.

7.3 Follow-up Assessments

Day 30 (± 7) days post-infusion

- Measure and record vital signs
- 12-lead ECG
- Pulse Oximetry
- 24 hour Holter ECG recording or ICD assessment
- Record the composite of all-cause mortality, admission for worsening HF and all-cause admissions, and concomitant medication use
- Physical and cardiac examination
- Clinical labs: serum chemistry including BUN & creatinine, CBC with diff, LFT's, total protein and albumin
- Cardiac biomarkers (CK and CK-MB fraction, troponin I)
- Serum/plasma stored for VEGF, FGF, NT-proBNP, troponin
- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- 6 minute walk test

Day 60 (± 7) days post infusion)

- Measure and record vital signs
- 12-lead ECG
- Pulse Oximetry
- 24 hour Holter ECG monitoring or ACD assessment
- Record the composite of all-cause mortality, admission for worsening HF and all-cause admissions, and concomitant medication use
- Physical and cardiac examination
- Clinical labs: serum chemistry including BUN & creatinine, CBC with diff, LFT's, total protein and albumin
- Cardiac biomarkers (CK and CK-MB fraction, troponin I)
- Serum/plasma stored for VEGF, FGF, NT-proBNP, troponin
- Draw and store blood samples for IgA, IgE, IgG, IgM and for Lymphocyte Proliferation Panel
- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- Spirometry/DLCO
- 6 minute walk test

Day 90 (± 7) days post initial infusion, day of crossover infusion)

- Measure and record vital signs
- Final Formulation Skin Test. Performed prior to infusion (Appendix C)
- 12-lead ECG
- Pulse Oximetry
- 24 hour Holter ECG monitoring or ACD assessment
- Record the composite of all-cause mortality, admission for worsening HF and all-cause admissions, and concomitant medication use
- Physical and cardiac examination
- Clinical labs: serum chemistry including BUN & creatinine, CBC with diff, LFT's, total protein and albumin,
- Cardiac biomarkers (CK and CK-MB fraction, troponin I)
- Serum/plasma stored for VEGF, FGF, NT-proBNP, troponin
- Draw and store blood samples for IgA, IgE, IgG, IgM and for Lymphocyte Proliferation Panel

- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- Spirometry/DLCO
- 6 minute walk test
- Cardiac MRI, with delayed contrast enhancement
- Full 2D and Doppler transthoracic echocardiography with speckle-tracking
- Pregnancy test for women of childbearing potential

7.4 Early Termination Visit

- Measure and record weight and vital signs
- 12-lead ECG
- Pulse Oximetry
- 24 hour Holter ECG monitoring or ACD assessment
- Record the composite of all-cause mortality admission for worsening HF and all-cause admissions, and concomitant medication use
- Physical and cardiac examination
- Clinical labs: serum chemistry including BUN & creatinine, CBC with diff, LFT's, total protein and albumin
- Serum/plasma stored for VEGF, FGF, NT-proBNP, troponin
- Blood sample for IgA, IgE, IgG, IgM and for Lymphocyte Proliferation Panel
- 6 minute walk test
- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- Cardiac MRI, with delayed contrast enhancement
- Full 2D and Doppler transthoracic echocardiography with speckle-tracking

The same follow-up visits will be performed after the crossover phase.

8 ASSESSMENT OF SAFETY

8.1 Safety Parameters

Safety assessments will consist of monitoring and recording adverse treatment events. Changes in clinical laboratory, clinical status and physical examination values will be used for evaluation.

8.1.1 Adverse Event (AE)

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study stem cell administration. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis) which would include MD, DO, PA or NP and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

Severity of Event: All AEs will be assessed by the clinician using the following grading system of AE intensity.

- **Mild:** events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate:** events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe:** events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Life threatening:** any adverse drug experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician's assessment of an AE's relationship to investigational product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to investigational product assessed. In a clinical trial, the investigational product must always be suspect. To help assess, the following guidelines are used.

- **Related** – There is a plausible temporal relationship between the onset of the AE and administration of the investigational product, and the AE cannot be readily explained by the subject's pre-existing clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the investigational product; and/or the AE abates or resolves upon discontinuation of the investigational product and, if applicable, reappears upon re-challenge.
- **Not Related** – There is good evidence that the AE has an etiology other than the investigational product (e.g., pre-existing medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to administration of the investigational product (e.g., cancer diagnosed 2 days after treatment).

8.1.2 Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes:

- Death (i.e., the AE actually causes or leads to death)
- Life threatening event (i.e., the AE, in the view of the Investigator, places the subject at immediate risk of death)
- Requires or prolongs inpatient hospitalization
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- A congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product(s)

Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of relatively minor medical significance (such as severe headache). "Serious" is a regulatory definition and is based on subject or event outcome or action criteria

usually associated with events that pose a threat to a subject's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness will be independently assessed when recording AEs and SAEs on the CRF.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The Investigator is responsible for ensuring that all study-emergent AEs and SAEs (as defined in Section 7.1) are recorded on the CRF and reported to the sponsor in accordance with protocol instructions. For each SAE observed, the investigator will obtain all of the information available about the event, including (but not limited to): hospital discharge diagnoses, hospital discharge note, death certificate, appropriate laboratory findings (including autopsies and biopsy results), and clinical examinations (including radiological examinations and clinical consultations).

8.2.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after initiation of study treatment and ends at study completion or study discontinuation/termination, whichever is earlier. Any condition present between consenting and time of treatment will be considered as baseline and not recorded as an AE. After this period, investigators should report only SAEs that are attributed to study treatment.

All AEs that are observed or reported prior to initiation of study treatment will be recorded as unrelated to treatment and those occurring after initiation of treatment will be recorded as treatment-emergent.

8.2.2 Assessment of Adverse Events

Investigators will seek information on AEs and SAEs at each subject contact by specific questioning and, as appropriate, by examination. All AEs and SAEs, whether spontaneously reported by the subject or noted by authorized study personnel, will be recorded in the subject's medical record and on the appropriate AE or SAE CRF page.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, regulatory seriousness criteria if applicable, suspected relationship to the investigational product, and actions taken.

Note: The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the "Yes" or "No" causality assessment for individual AE reports, the sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities. Attribution of SAEs will be reviewed on an ongoing basis, and may be changed as additional clinical data emerge (e.g., reversibility of AE, new clinical findings in subject with AE, effects of retreatment, AEs in other subjects).

8.2.3 Recording Adverse Events on the CRF

Investigators will use correct medical terminology/concepts when recording AEs or SAEs on the CRF. Avoid colloquialisms and abbreviations.

AEs will be recorded either on an AE CRF page (if no serious criteria are met) or SAE CRF page, but not both. For each SAE observed, the investigator must report the triggering event for that particular episode of illness as the primary event.

Only one medical concept will be recorded in the event field on the AE or SAE CRF page.

a. Diagnosis versus Signs and Symptoms

If known, a diagnosis will be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event will be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it will be reported as follow-up information.

b. Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) will be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, medically significant AEs occurring secondary to an initiating event that are separated in time will be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events will be recorded separately on the CRF.

c. Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution between subject evaluation time points. Such events will only be recorded once in the CRF unless their severity increases. If a persistent AE becomes more severe, it will be recorded again on an AE or SAE CRF page.

A recurrent AE is one that occurs and resolves between subject evaluation time points and subsequently recurs. All recurrent AEs will be recorded on an AE or SAE CRF page.

d. Abnormal Laboratory Values

Laboratory data collection will be limited to those laboratory parameters that are relevant to safety, study outcome measures, and/or clinical outcome. Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require more frequent follow-up assessments, further diagnostic investigation, etc.) If the Investigator is uncertain about the appropriateness of reporting particular laboratory data, he/she will contact the Medical Monitor for the study.

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin five times the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the AE or SAE CRF page.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself will be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term will be recorded as the AE or SAE. For example, an elevated serum potassium level of 7.0mEq/L will be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit will not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

e. Deaths

For any deaths that occur during the study, regardless of attribution, the investigator will report the death immediately to the Medical Monitor for the study at the telephone number provided on the cover of this protocol and record the death on the appropriate SAE CRF page.

When recording a death, the event or condition that caused or contributed to the fatal outcome will be recorded as the single medical concept on the SAE CRF page. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" on the SAE CRF page.

f. Pre-existing Medical Conditions

A pre-existing medical condition is one that is present at the start of the study. This includes any abnormal physical examination finding noted during screening. Such conditions will be recorded on the Medical and Surgical History CRF page.

A pre-existing medical condition will be re-assessed throughout the trial and recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an AE or SAE CRF page, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

h. Hospitalization, Prolonged Hospitalization or Surgery

Any AE that results in hospitalization or prolonged hospitalization will be documented and reported as an SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an AE if the condition meets the definition of an AE. Surgical procedures are not to be recorded as SAEs or AEs.

i. Pregnancy

If a female subject becomes pregnant during the study, a Pregnancy Report CRF page will be completed and expeditiously submitted to the sponsor to facilitate outcome follow-up. Pregnancy is not an SAE.

Abortion, whether accidental, therapeutic, or spontaneous, will always be classified as serious, recorded on an SAE CRF page, and expeditiously reported to the sponsor. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the investigational product will be recorded and reported as an SAE.

8.3 Expedited Reporting Requirements for Serious Adverse Events

Any life-threatening (i.e., imminent risk of death) or fatal AE that is attributed by the investigator to the investigational product will be **telephoned to the Medical Monitor (or alternate) immediately**.

Medical Monitor: Nikolai Tankovich, MD, PhD
Office Telephone No.: 858-658-0910, ext. 7214
Mobile Phone: 858-610-2588

For initial SAE reports, investigators will record all case details that can be gathered within 24 hours on an SAE CRF page. The completed SAE CRF page will be faxed immediately upon completion to the attention of xxx

Relevant follow-up information will be submitted as soon as it becomes available and/or upon request.

8.4 Type and Duration of Follow-Up of Subjects After Adverse Events

The Investigator will follow all unresolved study-emergent AEs and SAEs until the events are resolved, stabilized, the subject is lost to follow-up, or until it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) will be documented on the appropriate AE or SAE CRF page and in the subject's medical record to facilitate source data verification. For some SAEs, the sponsor or its designee may follow-up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

8.5 Stopping Rules

The following findings will trigger a temporary halt in enrollment and administration of the product to new subject pending a safety review:

- Allergic reaction after product administration which resulted in severe anaphylactic reaction
- Occurrence of a severe cardiovascular event or pulmonary embolism in a minimum of two patients as evidenced by clinical and CT findings

8.6 Data Safety and Monitoring Board (DSMB)

An independent DSMB appointed by the Sponsor will review the protocol and will thereafter provide medical and ethical guidance related to the conduct of this trial. During the study, the board will conduct ongoing review of the nature, frequency and severity of safety data. The DSMB Chairperson has the option to recommend suspension of enrollment in the study if, upon initial deliberation by the DSMB, urgent safety issues or alarming trends in summary safety data are identified that require further investigation.

The study sponsor will report all serious adverse events to the study DSMB. The DSMB will determine whether enrollment will be continued, suspended, or terminated. In the event that the DSMB determines that the study will be suspended or terminated, the study sponsor will notify the Institutional Review Boards and the appropriate regulatory agencies.

9 STATISTICAL CONSIDERATIONS

Statistical analyses will be descriptive. Summary statistics will consist of sample size (N), means, standard deviations, medians, and minimum and maximum values for continuous variables, and counts and percentages for categorical variables. Tables will summarize all safety and efficacy outcome measures by actual treatment received. Summary tables will indicate the number of subjects with complete data for each measurement, event or outcome. No substitutions will be made for missing data. All analyses will be based on available data.

A detailed Statistical Analysis Plan will be written prior to data analysis. Significant deviations from the original statistical analysis plan will be detailed in the final study report.

9.1 Randomization

Once eligibility and consent are confirmed, participants will be randomly assigned to the experimental or placebo group in a 1:1 allocation ratio, stratified by site. At 90 days the two groups will change arms and the placebo group will receive the stem cell infusion and the initially treated group will receive a LRS at a volume of 1 mL/kg.

9.2 Primary Safety Analysis

All safety outcomes will be summarized in tables and listings presented by experimental group. Continuous measures will be summarized with descriptive statistics (N, mean, SD, median, min, max). Categorical measures will be summarized using counts and percentages. The incidence of adverse events will be presented by toxicity grade, MedDRA system organ class and preferred term, and by relationship of the adverse events to the study treatment and compared using a Fisher's Exact Test. Laboratory and vital signs data will be presented using shift tables and summaries of change from baseline to the maximum post-administration value and compared using analysis of covariance (ANCOVA) models.

9.3 Efficacy Analyses

The actual score and calculated change from baseline for each outcome measure (LV end diastolic volume, LVEF, Ventricular Arrhythmias, Kansas City Cardiomyopathy Questionnaire and the New York Heart Association Classification) will be tabulated for each scheduled assessment visit. Summary statistics will consist of sample size (N), means, standard deviations, medians, and minimum and maximum values will be used to summarize the data. Subjects who receive complete dose of investigational product or LRS and have post-administration assessments will be used as the efficacy evaluable population for statistical analysis.

The primary efficacy endpoint will be evaluated and is the change from baseline to Day 90, and 180 on the above measures. For each of the efficacy endpoints, a mixed-effect repeated measures (MMRM) analysis will be conducted to determine if the change from baseline to six months in the experimental group is different than that for the placebo group. The model will include the change in endpoint score from baseline to each post-baseline visit as the outcome variable, the baseline score, time, study site, treatment group and time-by-treatment interaction as fixed effects, and participant as a random effect. An unstructured covariance matrix will be used to model the within-subject variance-covariance errors. Time (0, 30d, 90d, and 180d) will be treated as a categorical variable in the analysis. The mixed model seamlessly accommodates different times of measurement as well as missed measurement and data from participants who are lost to follow up.

9.4 Determination of Sample Size

This Study is exploratory and its sample size is not determined by statistical power considerations, but is considered appropriate for an early phase safety trial.

9.5 Interim Analysis

No formal interim analysis for efficacy is planned, as this is a study with careful monitoring of subject safety in an ongoing manner by the principal investigator, Sponsor and independent Data Safety Monitoring Board.

9.6 Data Quality Assurance

The following steps will be taken to ensure the accuracy, consistency, completeness, and reliability of the data:

- Investigator meeting
- Routine study site monitoring
- CRF review against source documents
- Data management quality control checks
- Sponsor medical review

The sponsor or designee (e.g., contract research organization (CRO)) will be responsible for the data management of this clinical trial. The responsible party will design and distribute the case report forms (CRFs), and also conduct monitoring of the CRFs. The responsible party will generate queries in the event of incomplete or inconsistent data to be reconciled by the study site.

10 INVESTIGATOR REQUIREMENTS

10.1 Study Initiation

Before the release of investigational products to the study site, the following documents must be on file with CardioCell LLC or its designee:

- U.S. FDA Form 1572 signed by the Principal Investigator or equivalent for non-U.S. sites if applicable.
 - The names of any sub-investigators must appear on this form. Investigators must also complete all regulatory documentation as required by local and national regulations.
- Current curricula vitae (CV) of the Principal Investigator and all subinvestigators
- subinvestigators
- Complete financial disclosure forms for the Principal Investigator and all subinvestigators
- Written documentation of Institutional Review Board (IRB)/Ethics Committee (EC) approval of the protocol (identified by protocol number or title and date of approval) and Informed Consent Form (identified by protocol number or title and date of approval)
- A copy of the IRB/EC-approved Informed Consent Form
- IRB/EC composition or assurance letter as applicable
- Current laboratory certification of the clinical laboratory performing sample analysis, as well as current references ranges for all laboratory tests (*if applicable*)
- A Clinical Trial Agreement signed and dated by the study site
- Investigator Brochure receipt signed and dated by the Principal Investigator (*if applicable*)
- A Protocol Acceptance Form signed and dated by the Principal Investigator

10.2 Study Completion

The following data and materials are required before a study can be considered complete or terminated:

- All essential documents (e.g., curriculum vitae for the Principal Investigator and subinvestigator, U.S. FDA Form 1572 or equivalent (non-U.S.))
- Copies of protocol amendments, IRB/EC approval/notification, and signed and dated Protocol Amendment Acceptance Form(s) (*if applicable*)
- All data collection and query resolution complete Original, final SAE reports and all supporting documentation (i.e., discharge summaries, laboratory results)
- Original, final Pregnancy Information forms and all supporting documentation (*if applicable*)
- Complete and accurate investigational product accountability records and inventory log
- Laboratory results, clinical data, and all special test results from screening through the end of the study
- A summary of the study prepared by the principal investigator (IRB/EC summary close out letter is acceptable)

10.3 Informed Consent Process

CardioCell, LLC Informed Consent Form (ICF) will be provided to the hospital site. CardioCell, LLC or its designee must review and approve any proposed deviations from the ICF or any alternate consent forms proposed by the site before IRB/EC submission. Subjects must be re-consented to the most current version of

the ICF during their participation in the study. The final IRB/EC-approved ICF must be provided to CardioCell, LLC or its designee for regulatory purposes.

The ICF must be signed by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document the informed consent process and that written informed consent was obtained prior to participation in the study. A copy of each signed ICF must be provided to the subject or the subject's legally authorized representative. It will be provided in a certified translation of the local language(s).

All signed and dated ICF must remain in each subject's study file and must be available for verification by study monitors at any time.

The ICF will be revised whenever there are changes to procedures outlined in the informed consent or when new information becomes available that may affect the willingness of the subject to participate.

10.4 Institutional Review Board or Ethics Committee

This protocol, the ICF, any information to be given to the subject and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator for review and approval before the study is initiated as required by the country regulatory authority. In addition, any subject recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the regulatory requirements and policies and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol changes or amendments and of any unanticipated problems involving risk to human subjects or others. In addition to the requirements to report protocol-defined AEs to the Sponsor, Investigators may be required to promptly report to their respective IRB/EC all unanticipated problems involving risk to human subjects. Some IRB/ECs may want prompt notification of all SAEs, whereas others require notification only about events that are serious, assessed to be related to study stem cell administration, and are unexpected. Investigators may receive written Investigational New Drug (IND) safety reports or other safety-related communications from CardioCell, LLC. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with regulatory requirements and with the policies and procedures established by their IRB and archived in the site's Study File.

10.5 Study Monitoring Requirements

Site visits will be conducted by an authorized CardioCell, LLC representative to inspect study data, subjects' medical records, and CRFs. The Principal Investigator will permit CardioCell, LLC monitors/representatives and collaborators, the U.S. FDA or other regulatory agencies, Ethics Committees and the respective national or local health authorities to inspect facilities and records relevant to this study, in accordance with GCP and the ICH E6 guidance. If the investigator is notified of an audit pertaining to this study by the U.S. FDA or other applicable regulatory authorities, the Investigator must notify CardioCell, LLC immediately.

The Investigator must provide sufficient space and allocate sufficient time for the monitor to inspect subject source records, CRFs, investigational product accountability records, and regulatory documents.

10.6 Case Report Forms

CRFs will be supplied by CardioCell, LLC or its designee and will be handled in accordance with the instructions. All CRFs will be filled out completely by designated personnel.

10.7 Source Data Documentation

Study monitors will perform ongoing source data verification to confirm that critical protocol data (i.e., source data) transcribed on the CRFs by authorized site personnel are accurate, complete, and verifiable from source documents. Source documents are where subject data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated

instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, laboratories, and medico-technical departments involved in a clinical trial. Source documents that are required to verify the validity and completeness of data transcribed on the CRFs must never be obliterated or destroyed. To facilitate source data verification, the investigator(s) and institution(s) must provide the sponsor direct access to applicable source documents and reports for trial-related monitoring, sponsor audits, and IRB/EC review. The investigational site must also allow inspection by applicable regulatory authorities.

10.8 Use of Computerized Systems

When clinical observations are entered directly into an investigational site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with ICH requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system (for clinical research purposes) would be one that (1) allows data entry only by authorized individuals; (2) prevents the deletion or alteration of previously entered data and provides an audit trail for such data changes (e.g., modification of file); (3) protects the database from tampering; and (4) ensures data preservation. If a site's computerized medical record system is not adequately validated for the purposes of clinical research (as opposed to general clinical practice), applicable hardcopy source documents must be maintained to ensure that critical protocol data transcribed on the CRFs can be verified.

10.9 Investigational Product Accountability

All investigational products required for completion of this study will be provided by CardioCell, LLC. The recipient will acknowledge receipt of the drug by returning the appropriate documentation form indicating shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study drug received at, dispensed from, returned to and disposed of by the study site will be recorded.

Investigational product will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to CardioCell, LLC with the appropriate documentation, as determined by the study site. If the study site chooses to destroy investigational product, the method of destruction must be documented.

CardioCell, LLC must evaluate and approve the study site's drug destruction standard operating procedure prior to the initiation of drug destruction by the study site.

10.10 Disclosure of Data

Subject medical information obtained by this study is confidential, and may only be disclosed to third parties as permitted by the ICF and Authorization Form (or separate authorization to use and disclose personal health information) signed by the subject or unless permitted or required by law.

Medical information may be given to the subject's personal physician or other appropriate medical personnel responsible for the subject's welfare for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the applicable regulatory agencies, national and local health authorities, CardioCell, LLC monitors/representatives and collaborators, and the IRB/EC for each study site, if appropriate.

10.11 Retention of Records (for approval enabling studies only)

United States FDA regulations (21 CFR §312.62[c]) and the ICH Guideline for GCP (E6) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after the last marketing application approval in an ICH region or after at least

2 years have elapsed since formal discontinuation of clinical development of the investigational product. All state and local laws for retention of records also apply.

No records will be disposed of without written approval from CardioCell, LLC. Written notification will be provided to CardioCell, LLC for transfer of any records to another party or moving them to another location.

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APPENDIX A: SCHEDULE OF ASSESSMENTS

Visit	1	3	4	5	6	7	8	9	Early Termination
Day	Days 1 to 2	Day 7±3	Day 30±7	Day 60±7	Day 90±7	Day 120±10	Day 150 ±7	Day 180±10	
Informed Consent Form	X								
IV Infusion		X			X				
Final Formulation Skin Test ^x		X			X				
Pregnancy Test [^]	X				X			X	X
Troponin, HIV	X								
Randomization into experimental or control group	X								
Cardiac MRI	X				X			X	X
Medical History	X								
Spirometry/DLCO	X				X			X	
Full 2D Doppler transthoracic echocardiography with speckle-tracking †	X				X			X	X
SF-36 Health Assessment, Canadian Cardiovascular Society (CCS) angina classification, KCCQ, NYHA	X		X	X	X	X	X	X	X
Concomitant medications	X		X	X	X	X	X	X	X
12-lead ECG	X	X	X	X	X	X	X	X	X
24 hour Holter ECG recording*	X		X	X	X	X	X	X	X
Pulse Oximetry**	X	X	X	X	X	X	X	X	X
Vital Signs**	X	X	X	X	X	X	X	X	X
6 minute walk test	X		X	X	X	X	X	X	X
Physical Examination	X	X	X	X	X	X	X	X	X
Cardiac Examination	X	X	X	X	X	X	X	X	X
CBC with Diff	X		X	X	X	X	X	X	X
Serum Chemistry with BUN/Creatinine, LFT	X	X	X	X	X	X	X	X	X
VEGF, FGF, NT-proBNP	X	X	X	X	X	X	X	X	X
IgA, IgE, IgG, IgM and Lymphocyte Proliferation Panel [✓]		X		X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X
Cardiac Biomarkers (CK and CK-MB fraction, troponin I)	X	X	X	X	X	X	X	X	X
Composite of all-cause mortality, myocardial infarction, coronary revascularization, and admission for worsening HF and all-cause admissions			X	X	X	X	X	X	X

† Ejection fraction must be ≤ 40%.

‡ A coronary angiogram performed during the index MI may be used to determine eligibility.

*Skin Test (Appendix C) to be performed prior to IV infusion

*24 hour Holter ECG recording must be completed prior to IV infusion.

✓Collect 5mL of blood into tube for lymphocyte proliferation panel prior to IV infusion. Provide 2mL of serum in another tube of IgA, IgE, IgG and IgM test. Serum is taken from blood in 5mL tube, which is centrifuged for 15 minutes at 3500 RPM.

**Vital signs taken every 2 hours after infusion and pulse oximetry taken continuously for 2 hours after IV infusion. Vital signs include temperature, blood pressure, heart rate respiratory rate and weight

†† A test will be taken prior to IV infusion to establish baseline. Another test will be taken 6 hours ± 30 minutes after infusion. If levels are significantly elevated, two more tests will be taken every 6 hours for the next 12 hours.

Need continuous ECG monitoring during infusion

Patient to go home with Holter monitor to assure no arrhythmias immediately post

[^]Pregnancy test for women of childbearing potential only.

Protocol:

STEM-104-M-CHF

APPENDIX B: QUESTIONNAIRES

THE KANSAS CITY CARDIOMYOPATHY QUESTIONNAIRE (KCCQ)

The following questions refer to your **heart failure** and how it may affect your life. Please read and complete the following questions. There are no right or wrong answers. Please mark the answer that best applies to you.

1. **Heart Failure** affects different people in different ways. Some feel shortness of breath while others feel fatigue. Please indicate how much you are limited by **heart failure** (shortness of breath or fatigue) in your ability to do the following activities over the past 2 weeks.

Place an X in one box on each line

Activity for other reasons not do the activity	Extremely limited	Quite a bit limited	Moderately limited	Slightly limited	Not at all limited	Limited or did
Dressing yourself	†	†	†	†	†	†
Showering/Bathing	†		†	†	†	†
Walking 1 block on level ground	†	†	†	†	†	†
Doing yardwork, housework or carrying groceries	†	†	†	†	†	†
Climbing a flight of stairs without stopping	†	†	†	†		†
Hurrying or jogging (as if to catch a bus)	†	†	†	†	†	†

2. Compared with 2 weeks ago, have your symptoms of **heart failure** (shortness of breath, fatigue or ankle swelling) changed? My symptoms of **heart failure** have become...

Much worse	Slightly worse	Not changed	Slightly better	Much better	I've had no symptoms over the last 2 weeks
†	†	†	†	†	†

3. Over the past 2 weeks, how many times did you have **swelling** in your feet, ankles or legs when you woke up in the morning?

Every morning	3 or more times a week, but not every day	1-2 times a week	Less than once a week	Never over the past 2 weeks
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†	†	†	†	†
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4. Over the past 2 weeks, how much has **swelling** in your feet, ankles or legs bothered you? It has been...

Extremely bothersome	Quite a bit bothersome	Moderately bothersome	Slightly bothersome	Not at all bothersome	I've had no swelling
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Protocol:

5. Over the past 2 weeks, on average, how many times has **fatigue** limited your ability to do what you want?

↑	↑	↑	↑	↑	↑
All of the time once a the past 2 weeks	Several times Never over per day	At least once a day	3 or more times per week but not every day	1-2 times per week	Less than week

6. Over the past 2 weeks, how much has your **fatigue** bothered you?
It has been...

Extremely bothersome	Quite a bit bothersome	Moderately bothersome	Slightly bothersome	Not at all bothersome	I've had no fatigue
↑	↑	↑	↑	↑	↑

7. Over the past 2 weeks, on average, how many times has **shortness of breath** limited your ability to do what you wanted?

↑	↑	↑	↑	↑	↑	↑
All of the time once a the past 2 weeks	Several times Never over per day	At least once a day	3 or more times per week but not every day	1-2 times per week	Less than week	

8. Over the past 2 weeks, how much has your shortness of breath bothered you?

Extremely bothersome	Quite a bit bothersome	Moderately bothersome	Slightly bothersome	Not at all bothersome	I've had no shortness of breath
↑	↑	↑	↑	↑	↑

9. Over the past 2 weeks, on average, how many times have you been forced to sleep sitting up in a chair or with at least 3 pillows to prop you up because of **shortness of breath**?

↑	↑	↑	
Every night a week, but not every day	3 or more times a week, but not every day	1-2 times a week	Less than once a week
			Never over the past 2 weeks

10. **Heart Failure** symptoms can worsen for a number of reasons. How sure are you that you know what to do, or whom to call, if your **heart failure** gets worse?

Not at all sure	Not very sure	Somewhat sure	Mostly sure	Completely sure
↑	↑	↑	↑	↑

11. How well do you understand what things you are able to do to keep your **heart failure** symptoms from getting worse? (for example, weighing yourself, eating a low salt diet, etc.)

Do not understand at all	Do not understand very well	Somewhat understand	Mostly understand	Completely understand
↑	↑	↑	↑	↑

12. Over the past 2 weeks, how much has your **heart failure** limited your enjoyment of life?

It has extremely limited my enjoyment of life	It has limited my enjoyment of life quite a bit	It has moderately limited my enjoyment of life	It has slightly limited my enjoyment of life	It has not limited my enjoyment of life at all
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13. If you had to spend the rest of your life with your **heart failure** the way it is right now, how would you feel about this?

Not at all satisfied	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
↑	↑	↑	↑	↑

14. Over the past 2 weeks, how often have you felt discouraged or down in the dumps because of your **heart failure**?

I felt that way all of the time	I felt that way most of the time	I felt that way occasionally	I felt that way rarely	I felt that way never
↑	↑	↑	↑	↑

15. How much does your **heart failure** affect your lifestyle? Please indicate how your **heart failure** may have limited your

participation in the following activities over the past 2 weeks?

Please place an X in one box on each line

Activity	Severely limited	Limited quite a bit	Moderately limited	Slightly limited	Did not limit at all	Does not do for other reasons
Hobbies, recreational activities	↑	↑	↑	↑	↑	
Working or doing household chores	↑	↑	↑	↑	↑	
Visiting family or friends out of your home	↑	↑	↑	↑	↑	
Intimate relationships with loved ones	↑	↑	↑	↑	↑	

Developed by John Spertus et al., Mid America Heart Institute, Saint Luke's Hospital, Kansas City, MO.

NEW YORK HEART ASSOCIATION (NYHA)

Protocol Number	Site Number □ □ □	Subject Number □ □ □ □	Subject Initials □ □ □	Visit Date (dd-MMM-yyyy) - - - - -
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Examination	Not Done	Result		If abnormal findings, describe
		Yes	No	
Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Class III: patients marked with limitation of activity; they are comfortable only at rest	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

APPENDIX C: FINAL FORMULATION SKIN TEST

Testing Materials:

0.1 ml Aliquot of the Solution from Final Formulation Preparation (30 gauge needle syringe)

Procedure to be performed prior to infusion:

Testing is usually done on the volar surface of the forearm or lateral surface of the upper part of the arm.

1. Intradermal test: raise a bleb by intradermal injection using the 0.1 mL of aliquot. Read the test results at 10-15 minutes. A positive result disqualifies the subject.

Interpretation:

A positive reaction includes a wheal of at least 4mm accompanied by erythema. Questionable results will be repeated.