Clinical Research Protocol

Title: A Phase I/II, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction.

The Percutaneous Stem Cell Injection Delivery Effects On Neomyogenesis Pilot Study (The POSEIDON-Pilot Study)

Investigational Therapy Names: Autologous Human Mesenchymal Stem Cells (Auto-hMSCs) or Allogeneic Human Mesenchymal Stem Cells (Allo-hMSCs)

FDA IND No.: BB-IND #13568

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Principal Investigator: Joshua M. Hare, MD Telephone: 305-243-1999

Protocol Agreement Signature: ___________________________ Date ___________________________

Joshua M. Hare, MD (Principal Investigator)

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<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>AMBMC</td>
<td>autologous mononuclear bone marrow cells</td>
</tr>
<tr>
<td>BMC</td>
<td>bone marrow cell</td>
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<tr>
<td>BSC</td>
<td>biologic safety cabinet</td>
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<tr>
<td>C of A</td>
<td>Certificate of Analysis</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass graft</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
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<tr>
<td>CFU-F</td>
<td>colony forming units – fibroblasts</td>
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<tr>
<td>CK-MB</td>
<td>creatine kinase – mb</td>
</tr>
<tr>
<td>CRO</td>
<td>contract research organization</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>DAPI</td>
<td>4′-6-Diamidino-2-phenylindole</td>
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<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data and Safety Monitoring Board</td>
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<tr>
<td>EF</td>
<td>ejection fraction</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EPC</td>
<td>endothelial progenitor cells</td>
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<td>ESR</td>
<td>expedited safety report</td>
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<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FEV1</td>
<td>forced expiratory volume in 1 second</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>G-CSF</td>
<td>granulocyte colony stimulating factor</td>
</tr>
<tr>
<td>HARP</td>
<td>Harmonic Phase</td>
</tr>
<tr>
<td>hBMC</td>
<td>human bone marrow cell</td>
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<tr>
<td>hMSC</td>
<td>human mesenchymal stem cell</td>
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<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
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<tr>
<td>HSA</td>
<td>human serum albumin</td>
</tr>
<tr>
<td>HSC</td>
<td>hematopoietic stem cell</td>
</tr>
<tr>
<td>HTLV</td>
<td>human T-cell lymphotropic virus</td>
</tr>
<tr>
<td>ICAM</td>
<td>intracellular adhesion molecule</td>
</tr>
<tr>
<td>ICD</td>
<td>implantable cardioverter-defibrillator</td>
</tr>
<tr>
<td>ICF</td>
<td>informed consent form</td>
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</tbody>
</table>
ICH: International Conference on Harmonisation of Technical Requirements of Pharmaceuticals for Human Use

IDM: Infectious Disease Markers

IEC: institutional ethics committee

IIEF: International Index for Erectile Dysfunction (Male)

IND: Investigational New Drug application

IRB: Institutional Review Board

I.V.: Intravenous

KDR: VEGF receptor-2

LAD: left anterior descending artery

LV: left ventricular

LVAD: left ventricular assist device

MACE: major adverse cardiac events

MEM: minimum essential medium

MHC: major histocompatibility complex

MI: myocardial infarction

MLHF: Minnesota Living with Heart Failure

MR: magnetic resonance

MRI: magnetic resonance imaging

MSC: mesenchymal stem cell

NMDP: National Marrow Donor Program

NYHA: New York Heart Association

PBS: phosphate buffered saline

QA: quality assurance

QC: quality control

SAE: serious adverse event

SCCT: Specialized Center for Cell-Based Therapy

SCF: stem cell factor

SDF-1: stromal cell derived factor 1

SOP: standard operating procedures

SQOL-F: Sexual Quality of Life Questionnaire-Female

SW: Stroke Work

TTC: triphenyltetrazolium chloride

UMMSM: University of Miami Miller School of Medicine

U.S.: United States

VEGF: vascular endothelial growth factor

Peak VO₂: peak oxygen consumption
**SYNOPSIS**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Johns Hopkins/University of Miami Miller School of Medicine SCCT</th>
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<tbody>
<tr>
<td><strong>Name of Study Therapy:</strong></td>
<td>Autologous Human Mesenchymal Stem Cells (Auto-hMSCs) or Allogeneic Human Mesenchymal Stem Cells (Allo-hMSCs)</td>
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<tr>
<td><strong>Title of Study:</strong></td>
<td>A Phase I/II, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction. - The Percutaneous Stem Cell Injection Delivery Effects On Neomyogenesis Pilot Study (The POSEIDON-Pilot Study)</td>
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<tr>
<td><strong>Study Center:</strong></td>
<td>University of Miami Miller School of Medicine - Division of Cardiology &amp; Interdisciplinary Stem Cell Institute</td>
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<td><strong>Phase of Development:</strong></td>
<td>Phase I/II</td>
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<tr>
<td><strong>Objectives:</strong></td>
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<tr>
<td><strong>Primary:</strong></td>
<td>To demonstrate the safety of allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to myocardial infarction (MI).</td>
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<tr>
<td><strong>Secondary:</strong></td>
<td></td>
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<tr>
<td>•</td>
<td>To compare the safety of allogeneic hMSCs to autologous hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI.</td>
</tr>
<tr>
<td>•</td>
<td>To demonstrate the efficacy of autologous hMSCs and allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI.</td>
</tr>
<tr>
<td><strong>Design and Investigational Plan:</strong></td>
<td>This is a Pilot Study, intended as a safety assessment prior to a full comparator study. In this Pilot Study, dose and volume escalations of cells administered via the Biocardia Helical infusion system will be tested in 30 patients in three groups:</td>
</tr>
<tr>
<td><strong>Group 1 (10 patients):</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Group 1a:</strong></td>
<td>Five (5) patients will be treated with Auto-hMSCs: 4 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 0.2 x 10⁸ (20 million) Auto-hMSCs.</td>
</tr>
<tr>
<td><strong>Group 1b:</strong></td>
<td>Five (5) patients to be treated with Allo-hMSCs: 4 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 0.2 x 10⁸ (20 million) Allo-hMSCs.</td>
</tr>
<tr>
<td>These patients will not be treated less than 5 days apart and will each undergo full evaluation for 5 days to demonstrate there is no evidence of a procedure induced myocardial infarction or myocardial perforation prior to proceeding with Group 2.</td>
<td></td>
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<tr>
<td><strong>Group 2 (10 patients):</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Group 2a:</strong></td>
<td>Five (5) patients will be treated with Auto-hMSCs: 20 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 1 x 10⁹ (100 million) Auto-hMSCs.</td>
</tr>
<tr>
<td><strong>Group 2b:</strong></td>
<td>Five (5) patients to be treated with Allo-hMSCs: 20 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 1 x 10⁹ (100 million) Allo-hMSCs.</td>
</tr>
</tbody>
</table>
Group 3 (10 patients)

Group 3a - Five (5) patients will be treated with Auto-hMSCs: 40 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of \(2 \times 10^8\) (200 million) Auto-hMSCs.

Group 3b - Five (5) patients to be treated with Allo-hMSCs: 40 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of \(2 \times 10^8\) (200 million) Allo-hMSCs.

Within each of Groups 1, 2 and 3, patients will be randomized in a 1:1 ratio to one of the two Treatment Strategies: Autologous hMSCs vs. Allogeneic hMSCs.

The Study Team will record and maintain a detailed record of injection locations.

If a patient is randomized to Groups 1a, 2a or 3a (Auto-hMSCs) and the Auto-hMSCs do not expand to the required dose (\(0.2 \times 10^8\), \(1 \times 10^8\) or \(2 \times 10^8\) cells, respectively), each injection will contain the maximum number of cells available.

The injections will be administered transendocardially during cardiac catheterization using the Biocardia Helical Infusion Catheter.

For patients randomized to Groups 1a, 2a or 3a (Auto-hMSCs); the cells will be derived from a sample of the patient’s bone marrow (obtained by iliac crest aspiration) approximately 4-6 weeks prior to cardiac catheterization. For patients randomized to Group 1b, 2b or 3b (Allo-hMSCs), the cells will be supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami.

Following cardiac catheterization and cell injections, patients will be hospitalized for a minimum of 4 days then followed at 2 weeks post-catheterization, and at monthly intervals for six months to complete all safety and efficacy assessments. Patients will also receive selected efficacy and safety assessments during a twelve month follow-up visit.

Patient Population: Thirty (30) patients with chronic ischemic left ventricular dysfunction secondary to MI scheduled to undergo cardiac catheterization will be enrolled in the study.

Diagnosis and Main Criteria for Inclusion/Enrollment:

Major Inclusion Criteria

- Diagnosis of chronic ischemic left ventricular dysfunction secondary to MI.
- Be a candidate for cardiac catheterization within 5 to 10 weeks of screening.
- Been treated with appropriate maximal medical therapy for heart failure or post-infarction left ventricular dysfunction.
- Ejection fraction below 50%.

Major Exclusion Criteria

- Baseline glomerular filtration rate < 50 ml/min/1.73m².
- Presence of a prosthetic aortic valve or heart constrictive device.
- Documented presence of aortic stenosis (aortic stenosis graded as 1.5cm² or less).
- Documented presence of moderate to severe aortic insufficiency (echocardiographic assessment of aortic insufficiency graded as ≥+2).
- Evidence of a life-threatening arrhythmia in the absence of a defibrillator (nonsustained ventricular tachycardia ≥ 20 consecutive beats or complete second or third degree heart block in the absence of a functioning pacemaker) or QTc interval > 550 ms on screening.
ECG.

- AICD firing in the past 60 days prior to study enrollment.
- Be eligible for or require coronary artery revascularization.
- Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/ul or platelet values < 100,000/ul without another explanation.
- Have liver dysfunction, as evidenced by enzymes (ALT and AST) greater than three times the ULN.
- Have a coagulopathy condition = (INR > 1.3) not due to a reversible cause.
- Known, serious radiographic contrast allergy.
- Known allergies to penicillin or streptomycin.
- Organ transplant recipient.
- Clinical history of malignancy within 5 years (i.e., patients with prior malignancy must be disease free for 5 years), except curatively-treated basal cell carcinoma, squamous cell carcinoma, or cervical carcinoma.
- Non-cardiac condition that limits lifespan to < 1 year.
- On chronic therapy with immunosuppressant medication.
- Serum positive for HIV, hepatitis BsAg, or vermic hepatitis C.
- Female patient who is pregnant, nursing, or of child-bearing potential and not using effective birth control.

**Definition of Endpoints:**

**Safety (Primary):** Incidence (at one month post-catheterization) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal MI, stroke, hospitalization for worsening heart failure, cardiac perforation, pericardial tamponade, sustained ventricular arrhythmias (characterized by ventricular arrhythmias lasting longer than 15 seconds or with hemodynamic compromise).

**Definition of Endpoints (continued):**

**Safety (Additional):** (During the six-month follow-up period, at the month 12 visit and the final month 13 visit)

- Treatment emergent adverse event (AE) rates.
- Ectopic tissue formation (as identified from CT scans of the chest, abdomen, & pelvis).
- 48-hour ambulatory electrocardiogram (ECG) recordings.
- Hematology and clinical chemistry values and urinalysis results.
- Pulmonary function – forced expiratory volume in 1 second (FEV1) results.
- Serial troponin and CK-MB values (every 12 hours for first 48 hours post-cardiac catheterization).
- Post-cardiac catheterization echocardiogram.

**Efficacy (Secondary):** (During the six-month follow-up period, at the month 12 visit and the final month 13 visit)

- X-ray computed tomography and echocardiographic measures of infarct scar size (ISS), myocardial perfusion, and left regional and global ventricular function.
- Peak VO₂ (by treadmill determination).
- Six-minute walk test.
- NYHA functional class.
- Minnesota Living with Heart Failure (MLHF) questionnaire.
- Incidence of Major Adverse Cardiac Events (MACE), defined as the composite incidence of (1) death, (2) hospitalization for worsening HF, or (3) non-fatal recurrent MI.

**Study Therapy:**  Autologous human mesenchymal stem cells (Auto-hMSCs), obtained from the patient via bone marrow aspiration, and allogeneic human mesenchymal stem cells (Allo-hMSCs), supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami.

**Duration of Study Follow-Up:** Monthly for 6 months; then a 12-month follow-up visit and a final 13-month follow-up visit.
1. INTRODUCTION

1.1 Background

The technique of transplanting progenitor cells into a region of damaged myocardium, termed cellular cardiomyoplasty\(^1\), is a potentially new therapeutic modality designed to replace or repair necrotic, scarred, or dysfunctional myocardium\(^2-4\). Ideally, graft cells should be readily available, easy to culture to ensure adequate quantities for transplantation, and able to survive in host myocardium; often a hostile environment of limited blood supply and immunorejection. Whether effective cellular regenerative strategies require that administered cells differentiate into adult cardiomyocytes and couple electromechanically with the surrounding myocardium is increasingly controversial, and recent evidence suggests that this may not be required for effective cardiac repair. Most importantly, transplantation of graft cells should improve cardiac function and prevent adverse ventricular remodeling. To date, a number of candidate cells have been transplanted in experimental models, including fetal and neonatal cardiomyocytes\(^5\), embryonic stem cell-derived myocytes\(^6, 7\), tissue engineered contractile grafts\(^8\), skeletal myoblasts\(^9\), several cell types derived from adult bone marrow\(^10-15\), and cardiac precursors residing within the heart itself\(^16\). There has been substantial clinical development in the use of whole bone marrow and skeletal myoblast preparations in studies enrolling both post-infarction patients, and patients with chronic ischemic left ventricular dysfunction and heart failure. The effects of bone-marrow derived mesenchymal stem cells (MSCs) have also been studied clinically.

Currently, bone marrow or bone marrow-derived cells represent highly promising modality for cardiac repair. The totality of evidence from trials investigating autologous whole bone marrow infusions into patients following myocardial infarction supports the safety of this approach. In terms of efficacy, increases in ejection fraction are reported in the majority of the trials.

Chronic ischemic left ventricular dysfunction resulting from heart disease is a common and problematic condition; definitive therapy in the form of heart transplantation is available to only a tiny minority of eligible patients. Cellular cardiomyoplasty for chronic heart failure has been studied less than for acute MI, but represents a potentially important alternative for this disease.

**Cells derived from adult bone marrow**

Bone marrow harbors a variety of cells that may contribute to vasculogenesis or cardiomyogenesis, either directly, or by facilitating endogenous repair mechanisms. Bone marrow cells have been prepared on the basis of being 1.) endothelial precursor cells that are CD34\(^+\), 2.) MSCs purified without an antigen panning technique on the basis of their fibroblast morphology, ability to divide in culture and to differentiate into mesodermal lineages\(^17\), and 3.) cells that express stem cell factor receptor, c-Kit\(^18\). Endothelial progenitor cells (EPCs) express the surface markers CD34, CD133, c-kit, and the vascular endothelial growth factor receptor-2 (VEGFR2; KDR; Flk-1)\(^19-24\). Hematopoietic stem cells (HSCs) exhibit
self-renewal and differentiation. Their cell-surface phenotype is CD34⁺, stem cell factor antigen (SCA-1)+, c-kit+, and Lin⁻ (review²⁵). While there has been controversy regarding the ability of bone marrow-derived cells to transdifferentiate into cardiomyocytes²⁶, clinical trials of whole bone marrow therapies continue to suggest potential benefit in terms of improving cardiac function or reducing the burden of scarred myocardium.

**Mesenchymal Stem Cells:** MSCs are a particularly promising bone marrow-derived cell for cardiac regenerative therapy because of their availability, immunologic properties, and track-record of safety and efficacy²⁷. Studies of MSC engraftment in rodent and swine models of myocardial infarction have shown that administration of MSCs produces: 1) functional benefit in post-myocardial infarction (MI) recovery of ventricular function 2) evidence of neoangiogenesis at the site of the infarct 3) decrease in collagen deposition in the region of the scar 4) some evidence of cells expressing contractile and sarcomeric proteins but lacking true sarcomeric functional organization²⁸, ²⁹. Moreover, MSCs are thought to be ideal candidate cells for allogeneic transplantation because they show minimal major histocompatibility complex (MHC) class II and intracellular adhesion molecule (ICAM) expression and lack B-7 costimulatory molecules necessary to cause a T-cell mediated immune response³⁰, ³¹.

Although there is no agreed upon cell surface marker that characterizes MSCs, they appear related to c-Kit+ cells discussed next as they pass through a stage of cardiac differentiation in which they express this cell surface marker. C-Kit is the 145 KD tyrosine kinase receptor for stem cell factor³². Some, but not all, groups have purified MSCs expressing c-Kit directly from bone marrow that have the capacity to form cardiac myocytes³³. This is of functional significance given the demonstration that stem cell factor stimulates cardiac repair post-MI³⁴.

**Clinical Trials**

Several cell-based therapies have entered early studies. As described below, the results continue to suggest that cellular cardiomyoplasty is a safe and effective strategy to improve cardiac function in patients with acute MI or chronic heart failure.

**Previous Human Experience with Skeletal Myoblasts**

There have been several case reports and very small phase I clinical trials investigating the feasibility of autologous skeletal myoblast transplantation³⁵-³⁷ for ischemic cardiomyopathy, as well as the ability of transplanted cells to survive and differentiate in human myocardium. Though limited by extremely small numbers of patients (typically fewer than 10 to 15) as well as a lack of blinding, control groups and randomization, these studies suggest potential improvements in left ventricular ejection fraction³⁸, ³⁹, increased wall thickening⁴⁰, and New York Heart Association (NYHA) functional class⁴¹, ⁴². However the lack of electromechanical coupling between engrafted skeletal myoblasts and cardiac myocytes in vivo⁴³, ⁴⁴ has raised serious concerns over the likelihood of an increase in ventricular tachyarhythmias secondary to the formation of re-entry
Indeed because of reports of increased arrhythmias in these patients, ongoing trials have mandated the use of implantable cardioverter defibrillator (ICD) placement for enrolled patients.\footnote{48}

Recently, the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, a large multicenter Phase II study comparing two doses of autologous skeletal myoblasts to placebo in patients undergoing CABG, was terminated early by the DSMB on a futility basis, with virtually no chance that either the high-dose group or the low-dose group would demonstrate an improvement in the primary endpoint (survival).\footnote{49} However, although neither group randomized to skeletal myoblast therapy demonstrated improvement in survival, the high-dose group did show statistically significant reductions in both end diastolic volume (EDV) and end systolic volume (ESV); effects that were not observed in the low-dose group.

**Previous Human Experience with Autologous Mononuclear Bone Marrow Cells (AMBMCs)**

Clinical studies using autologous mononuclear bone marrow cells have been performed for a variety of indications, including peripheral vascular and cardiac diseases. The Therapeutic Angiogenesis using Cell Transplantation Study investigators\footnote{50} injected bone marrow mononuclear cells into the gastrocnemius muscles of patients with lower extremity ischemia and demonstrated significant improvement in ankle-brachial pressure index, rest pain, and pain free walking time. The authors concluded that the efficacy related to these implanted cells is due to the supply of endothelial progenitor cells.

**AMBMCs in Acute MI:** As with skeletal myoblasts, there have been several small studies evaluating the safety and feasibility of AMBMC cardiomyoplasty in patients in the peri-infarct period. Although these studies are also limited by similarly small numbers of patients, lack of blinding, control groups, and randomization, they do offer promising insights into the potential of MSC transplantation. In an early study, Strauer et al. randomized 20 patients following transmural MI to standard therapy plus intracoronary AMBMC injection 12 hours after acute MI, or to standard medical therapy alone. Intracoronary AMBMC decreased infarct size from 30±13% to 12±7% and the size of perfusion defects, as assessed by \(^{201}\) thallium scintigraphy, by 26% (174±99 cm\(^2\) to 128±71 cm\(^2\)) compared to baseline values.\footnote{51} Subsequently, Stamm et al. demonstrated similar improvements in perfusion, left ventricle dimensions, and ejection fraction (EF) in an uncontrolled, non-blinded phase I study of 12 patients with transmural MI and left ventricular (LV) dysfunction (EF of 39.7±9%). These patients had infarct areas not amenable to surgical or interventional revascularization; they received intraoperative AMBMC injections during elective coronary artery bypass to non-infarct-related arteries in the first 3 months post-MI.\footnote{52, 53}

In the TOPCARE-AMI\footnote{54} trial, post-MI patients were randomized to receive either AMBMC (n=9) or peripheral blood derived progenitor cells (n=11) infused into the infarct artery approximately four days after reperfusion with coronary stenting. Over 90% of the cells derived from peripheral blood exhibited endothelial cell
characteristics including KDR, von Willebrand factor, CD31, and VE-Cadherin; while those derived from bone marrow cells exhibiting CD34 and CD133. The results demonstrated a ~9% absolute increase in LVEF (from 51.6±9.6% at baseline to 60.1±8.6% after 4 months), as well as improvement in wall motion abnormalities in the infarct area and a reduction in end-systolic LV size. Furthermore there was complete normalization of coronary flow reserve in the infarct artery, and a significant increase in myocardial viability within the infarcted segments. Interestingly, these improvements did not differ between patients receiving bone marrow or peripheral blood derived progenitor cells. Though this was a pilot trial, limited by the lack of a control group and only four months of follow-up, the results were quite promising; supporting the conduct of larger, controlled clinical trials.

In the randomized controlled BOOST clinical trial, patients received both standard post-infarct medical therapy and intracoronary transfer of AMBMC (n=30), or standard post-infarct therapy alone, 4 to 8 days after percutaneous coronary intervention for their first acute ST segment elevation MI. There was a 6.7±9.5% absolute improvement in global LVEF in the cell-treated group (46.3±10.6% at baseline to 53.0±15.5% at 6 months), compared to 1.1±11.8% increase in the control group (47.8±9.7% at baseline to 48.9±15.2%; p=0.0026). Furthermore cell transplantation was associated with increased systolic wall motion in the MI border zone. Importantly, infarct size as measured by late enhancement magnetic resonance imaging (MRI) was not reduced compared to placebo in the BOOST trial. Recent reports from the BOOST investigators suggest that the relative improvement in EF between placebo and AMBMC treated patients may wane over time, but this was due to increases in EF in the placebo patients, not deterioration in the AMBMC-treated patients.

In the REPAIR AMI study, the largest trial of bone marrow-derived cellular therapy to date, Schächinger et al. randomized 204 patients to intracoronary infusion of bone-marrow cells or placebo 3 to 7 days after successful reperfusion therapy. At the four month follow-up period, LVEF improved by 5.5% with the bone marrow cells versus 3% with placebo infusion (p=0.014). Interestingly, the benefit was greatest in patients with the worst ejection fractions at baseline. Other studies suggest relatively less benefit in EF than that reported above, although AMBMCs appeared to reduce infarct size.

**AMBMCs In Chronic Ischemia:** There are several small studies investigating the safety and feasibility of autologous bone marrow cell transplantation for ischemic heart disease.

Hamano and colleagues performed a non-randomized study of direct injection of AMBMC into ungraftable or peri-infarct myocardial segments during CABG in five patients and reported improved perfusion to the treated areas up to one year after surgery. Ozbaran and colleagues injected peripheral blood stem cells mobilized with granulocyte colony stimulating factor (G-CSF) in the myocardium of 6 patients with severe ischemic cardiomyopathy (EF <25%); they found improvements in NYHA functional class and quality of life. However, it is
important to note the difficulty in determining how much of the improved perfusion is secondary to the stem cells compared to surgical revascularization.

In a randomized, crossover trial known as TOPCARE-CHD, Assmus et al. compared bone marrow-derived progenitor cells, and progenitor cells derived from circulating blood, to no cellular therapy in 75 patients with chronic left ventricular dysfunction. Results showed a modest benefit at three months in the group receiving the bone marrow-derived cells: EF in the patients treated with these had an absolute increase of 2.9% versus (1) a decrease of 0.4% in the patients who received injections of progenitor cells derived from circulating blood (P=0.003), and (2) a decrease of 1.4% in the patients who received no infusion (P<0.001).67

Several smaller (n<20) non-randomized studies have performed endocardial catheter injections of AMBMC into chronically ischemic myocardium and demonstrated improved myocardial function and perfusion, as well as reduced symptoms.68-70 Perin and colleagues performed a nonrandomized, open-label study comparing AMBMC injection (n=14) to standard therapy (n=7) in 21 patients with severe ischemic heart failure. They used a NOGA™ endocardial mapping catheter to inject AMBMC into hibernating myocardial segments of patients with severe ischemic heart failure. They reported a 73% reduction in the total reversible perfusion defect, improved mechanical function of injected myocardial segments as determined by electromechanical mapping, improved global EF (9%), and improved NYHA functional class and Canadian Cardiovascular Society Angina score.70 Together these results support ongoing research in AMBMC transplant for patients with chronic ischemic cardiomyopathy.

**Previous Human Experience with Autologous and Allogeneic Human Mesenchymal Stem Cells (MSCs)**

Administration of autologous or allogeneic human MSCs to cardiovascular patients was performed in three clinical studies to date, all in the post-myocardial infarction (MI) setting. Two studies administered MSCs via the intracoronary route (IC), and one via peripheral intravenous (IV) injection.

In a clinical study reported by Chen et al.,71 69 patients were randomly assigned to receive IC infusions of autologous MSCs (average cell dose: 5.4 x 10^10) or placebo (saline) 18 days after the onset of acute MI symptoms. At the three-month follow-up visit, LVEF was significantly improved in the MSC-treated group (from 49% ± 9% at baseline to 67% ± 11%) compared to the placebo group (from 48% ± 10% at baseline to 53% ± 18%; P < 0.01 for the between-group comparison). This improved EF was sustained at six months post-infusion. In addition, significant reductions in perfusion defect, left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were reported in the MSC-treated group. No adverse events were reported in this study. Although it is unclear if the cell preparation used was purified MSCs or whole bone marrow, even in the latter case, the likely range of MSC cells infused was 5 x 10^7 – 5 x 10^8 cells, since the MSC fraction is generally considered to be 0.1 – 1.0 % of a whole bone
marrow aspirate.
Katritsis et al.\textsuperscript{72} investigated the effects of IC infusions of autologous MSCs and endothelial progenitor cells (average cell dose: $1.5 \times 10^6$) in 11 patients approximately 8.6 months post-MI compared to 11 age- and sex-matched patients used as controls. Statistically significant improvements in both wall motion score and myocardial contractility on stress echocardiography, as well as restoration of uptake of Tc\textsuperscript{99m} sestamibi in previously nonviable myocardial scars, were observed at four months post-infusion. No arrhythmias were detected on ambulatory ECG monitoring throughout the four-month follow-up period. Moreover, no ventricular arrhythmias were detected in three patients treated with an implantable cardioverter-defibrillator due to clinical and inducible ventricular tachycardia or fibrillation during the follow-up period.

A third, recently-reported multi-center, randomized, double-blinded, placebo-controlled study was performed in 53 patients who were treated 3-10 days post-MI\textsuperscript{73}. Patients were administered with one of three cell-dose levels of allogeneic MSCs (0.5, 1.6 and 5.0 cells/kg; corresponding to $3.5 \times 10^7$, $1.1 \times 10^8$, and $3.5 \times 10^8$ cells per patient for a 70 kg body weight patient), or placebo administered via peripheral IV injection, and followed for six months. There were no deaths reported in the study; no toxicity was observed with the administration of the allogeneic MSCs (which were found to be well-tolerated at all dose levels administered, with 5.3 adverse events per patient in the MSC-treated group vs. 7.0 in the placebo group); and no serious adverse events were attributed to MSC administration. In fact, several signals from the trial indicate that the allogeneic MSCs were very safe, and also provided preliminary evidence of the following clinical benefits:

- Patients in the MSC-treated group were four times less likely to experience an arrhythmic event compared to those receiving placebo (9% vs. 37%, $p=0.025$).
- Fewer patients experienced clinically significant premature ventricular contractions (PVCs) after receiving MSCs as compared to placebo across all time points (11% vs. 24%, $p < 0.001$).
- The MSC-treated patients with major anterior wall myocardial infarctions had a statistically significant 7.0 point absolute improvement (24%) in EF at three months and a 7.3 point absolute improvement (25%) at six months over baseline ($p<0.05$), while similar patients receiving placebo did not have significant improvement.
- Patients in the MSC-treated group had significantly improved pulmonary function as measured by improvement in FEV\textsubscript{1} (% predicted), which increased 17% in the MSC-treated group vs. 6% in the placebo, $p < 0.05$.
- Significantly more patients who received the MSCs experienced improvement in their overall clinical status at six months as compared to those receiving placebo (42% vs. 11%, $p=0.027$).
Immunological Properties of MSC and HLA-Matching

The use of allogeneic cellular products typically requires matching of the graft HLA to the donor. Mismatched grafts can result in graft rejection and can induce graft versus host disease (GVHD). However, MSC represent a unique cell population for allogeneic cellular therapy. MSCs have been shown to exert anti-proliferative, immunomodulatory and anti-inflammatory effects. Human MSC fail to induce proliferation of allogeneic lymphocytes in vitro. Also MSC suppress proliferation of T cells activated by allogeneic cells or mitogens. The suppression appears to be mediated, at least in part, by soluble factors and effects several types of immune cells.

In humans, several hundred patients have received MSC, in most cases, the MSC were derived from an allogeneic donor. Infusion of the MSC was well tolerated and no side-effects were noted. Several patients have been treated with MSC to treat severe graft versus host disease (GVHD). In a 9-year old boy who received a matched unrelated donor hematopoietic stem cell transplant for leukemia, severe acute steroid-resistant GVHD of the gut and liver was reversed by infusion of haplo-identical MSC derived from the patient’s mother. These data suggest that human MSC may be a unique cell population for regenerative medicine with minimal immune reactivity decreasing the potential of graft rejection and/or GVHD.

As described in the previous section, the use of allogeneic MSC has been explored in cardiac patients by Osiris Therapeutics. In March 2007, Osiris announced the results of a study of MSC (Provacel) in heart attack patients. The study consisted of 53-patient to evaluate the safety and preliminary efficacy of the intravenous administration of PROVACEL. No matching of the grafts to the donor HLA was performed in this study. Administration of PROVACEL was found to be safe and well tolerated at all dose levels. The efficacy described above suggest the MSC engrafted in the cardiac tissue and were not rejected due to any HLA mismatching.

Previous Human Experience with Autologous Human Bone Marrow-Derived Mononuclear Cells

There is substantial clinical experience with the intramyocardial delivery of autologous bone marrow-derived mononuclear cells (BMCs) in the clinical setting of chronic left ventricular dysfunction. Table 1 lists the 11 studies in which intramyocardial delivery of BMCs was performed. Cell delivery has been performed via either (1) direct intramyocardial (IM) injection during coronary artery bypass graft (CABG) surgery, or (2) catheter-based intramyocardial injection (including transendocardial, or TEC, delivery). These results clearly support the clinical safety of the intramyocardial injection delivery method. BMC cell doses as high as 292 ± 232 x 10^6 have been injected via the IM route without untoward effects, and have produced global improvements in ventricular function. In addition, a recent clinical study (the TABMMI trial) using the
BioCardia Helical Infusion system (the delivery device to be used for the clinical study in this IND application) has been reported. In this study, transplantation of autologous BMCs (86 ± 3 x 10^6 cells) into the peri-infarct region of patients with chronic ischemic heart failure was performed using the Biocardial Helical Infusion catheter to improve ease, efficiency, and safety of delivery. The study demonstrated statistically significant functional improvements in transthoracic echocardiographic measurements at both 6 and 12 months of follow-up, with no adverse events associated with the catheter.

In addition, a recent meta-analysis of all clinical trials of adult, bone-marrow derived cell therapy (either BMCs or MSCs) for cardiac repair has been published. The combined results of these studies support the clinical safety of administering both BMC and MSC preparations for cardiac repair.

**Previous Human Experience Using the Biocardia Helical Infusion Catheter**

The initial clinical study performed using the BioCardia Helical Infusion system for intramyocardial delivery in patients with coronary artery disease undergoing PCI or diagnostic heart catheterization provided support that the procedure and device are safe and well-tolerated.

A recent clinical study (the TABMMI trial) using the BioCardia Helical Infusion system has been reported. In this study, transplantation of autologous BMCs into the peri-infarct region of patients with chronic ischemic heart failure was performed using the Biocardial Helical Infusion catheter (the delivery device to be used for the clinical study in this IND application) to improve ease, efficiency, and safety of delivery. The study demonstrated statistically significant functional improvements in transthoracic echocardiographic measurements at both 6 and 12 months of follow-up, with no adverse events associated with the catheter.

In conclusion, the experience utilizing the BioCardia Helical Infusion system in these two clinical studies supports the clinical safety of the technique and provides preliminary evidence of patient benefit.
### TABLE 1
CLINICAL STUDIES: AUTOLOGOUS BONE MARROW-DERIVED MONONUCLEAR CELLS (BMCs) ADMINISTERED VIA INTRAMYOCARDIAL (IM) INJECTION IN PATIENTS WITH CHRONIC LEFT VENTRICULAR DYSFUNCTION

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Cell Delivery (Injection) Method</th>
<th>Cell Source &amp; Type</th>
<th>Cell Dose (x 10^6)</th>
<th>Safety Results</th>
<th>Efficacy Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stamm</td>
<td>6</td>
<td>Direct IM during CABG surgery</td>
<td>Autologous, AC133⁺ BMCs</td>
<td>1.2 - 3.4</td>
<td>No arrhythmias; no neoplasia</td>
<td>↑ global contractility (EF)</td>
</tr>
<tr>
<td>Tse</td>
<td>8</td>
<td>Catheter-based IM</td>
<td>Autologous BMCs</td>
<td>2.6 - 21.2</td>
<td>No arrhythmias</td>
<td>↑ wall motion &amp; thickening</td>
</tr>
<tr>
<td>Fuchs</td>
<td>10</td>
<td>Catheter-based IM</td>
<td>Autologous BMCs</td>
<td>32.6 ± 27.5</td>
<td>No arrhythmias or other SAEs</td>
<td>↓ angina score; ↓ ischemia</td>
</tr>
<tr>
<td>Perin</td>
<td>14</td>
<td>Catheter-based IM</td>
<td>Autologous CD34⁺ BMCs</td>
<td>25.5 ± 6.3</td>
<td>No arrhythmias at 6-mo. F/U</td>
<td>↑ global contractility (EF); ↓ ESV</td>
</tr>
<tr>
<td>Beeres</td>
<td>25</td>
<td>Catheter-based IM</td>
<td>Autologous BMCs</td>
<td>84.1 ± 28.7</td>
<td>No arrhythmias or pericardial effusion</td>
<td>↑ global contractility (EF); ↓ ESV</td>
</tr>
<tr>
<td>Briguori</td>
<td>10</td>
<td>Catheter-based IM</td>
<td>Autologous CD34⁻ BMCs</td>
<td>4.6 ± 1.5</td>
<td>No arrhythmias or AMI</td>
<td>↑ quality of life; ↑ perfusion</td>
</tr>
<tr>
<td>de La Fuente</td>
<td>10</td>
<td>Catheter-based IM</td>
<td>Autologous CD34⁺ BMCs</td>
<td>86 ± 3</td>
<td>No arrhythmias at 12-mo. F/U</td>
<td>↑ global contractility (EF)</td>
</tr>
<tr>
<td>Mocini</td>
<td>36</td>
<td>Direct IM during CABG surgery</td>
<td>Autologous CD34⁺ BMCs</td>
<td>292 ± 232</td>
<td>No SAEs</td>
<td>↑ global contractility (EF)</td>
</tr>
<tr>
<td>Hendrixx</td>
<td>20</td>
<td>Direct IM during CABG surgery</td>
<td>Autologous BMCs</td>
<td>60.1 ± 31.1</td>
<td>Possible inducible VT</td>
<td>↑ global contractility (EF)</td>
</tr>
<tr>
<td>Stamm</td>
<td>55</td>
<td>Direct IM during CABG surgery</td>
<td>Autologous, CD133⁺ BMCs</td>
<td>3.85 - 103.0</td>
<td>No arrhythmias</td>
<td>↑ global contractility (EF)</td>
</tr>
<tr>
<td>Li</td>
<td>6</td>
<td>Direct IM during CABG surgery</td>
<td>Autologous BMCs</td>
<td>50 – 100</td>
<td>No arrhythmias; no neoplasia</td>
<td>Not assessed</td>
</tr>
</tbody>
</table>

AMI: acute myocardial infarction; IM: intramyocardial; CABG: coronary artery bypass graft; BMC: bone marrow-derived mononuclear cells; EF: Ejection Fraction; ESV: end systolic volume; F/U: follow-up; SAE: serious adverse event; VT: ventricular tachycardia.
Potential mechanisms for MSC mediated improvements in cardiac function

As noted above, prior studies have shown that a variety of cellular sources are capable of differentiating into phenotypes that strongly resemble the three principle cell types of myocardium; cardiomyocytes, smooth muscle and vascular endothelium. Our preliminary data and reports from other labs cited above suggest that MSCs, the cells employed in our model of cellular cardiomyoplasty, have the potential to form all three cell types within infarcted myocardium in vivo. Nevertheless, it is important to consider that MSCs may exert other favorable effects on cardiac repair above and beyond differentiation. For example, these cells may also participate in the recruitment and/or stimulation of other cells to differentiate into a cardiac phenotype.

There is a wealth of evidence suggesting that stem cell homing to damaged myocardium is directed by injury signal(s) emanating from the area within or surrounding the infarct. For example, stromal-cell-derived factor 1 (SDF-1), a chemokine that is a natural ligand for the CXCR4 receptor, is crucial for bone marrow retention of hematopoietic stem cells, cardiogenesis, recruitment of endothelial progenitor cells to sites of ischemic tissue and, potentially, migration of tissue-committed stem/progenitor cells. Interestingly, it was recently shown that the CXCR4 receptor is strongly expressed by a proportion of MSCs and it plays an important role in MSC mobilization. Expression of SDF-1 dramatically increased over the first week following infarction, and exogenous expression of SDF-1 increases the numbers of mobilized bone marrow cells (BMCs) homing to the heart at time periods remote from infarction. These findings suggest that MSCs participate in the complex autocatalytic cascade of cytokines and growth factors that is activated following MI. Indeed, human MSCs are capable of secreting several cytokines, including stem cell factor (SCF) and G-CSF, and intramyocardial administration of MSCs is associated with increases in vascular endothelium growth factor (VEGF) levels. Furthermore, it has been shown that MSCs participate in angiogenesis and arteriogenesis; differentiating into endothelium and vascular smooth muscle in a VEGF-dependent manner.

Once cells successfully home and engraft in the heart, they must survive in a hostile environment if they are to effect successful cardiac repair. It is thought that apoptosis within the infarct region is responsible for the fact that only a fraction of cells injected directly into the heart will engraft and survive, and that such cell death reduces the efficacy of cellular cardiomyoplasty. In a dramatic proof of principle study, Mangi et al. genetically engineered rat MSCs using ex vivo retroviral transduction to overexpress the anti-apoptotic protein Akt1, a serine-threonine kinase. Transplantation of 5x10^6 cells overexpressing Akt into the ischemic rat myocardium led to dramatic improvements in structure and function that far exceeded those seen with injection of control MSCs transduced with Lac-Z. MSCs reduced inflammation, collagen deposition and cardiomyocyte hypertrophy, regenerated 80-90% of lost myocardium, and completely normalized systolic and diastolic cardiac function in a dose-dependent fashion.
1.2 Study Rationale

Introduction

The field of stem cell mediated myocardial repair has advanced rapidly over the past few years, and early studies have been performed in humans (including new studies in the US). At present, several types of adult stem cells (possibly enhanced by concomitant strategies aimed at enhancing trafficking or survival) hold great promise to improve recovery following MI. This clinical study will utilize allogeneic bone marrow-derived hMSCs or autologous bone marrow-derived hMSCs as a therapy for chronic ischemic left ventricular dysfunction and heart failure. MSCs have been chosen because they have shown effectiveness in small and large animal models, and offer the substantial advantage of already having approval from the FDA for use in humans.

Preliminary Studies

A porcine model of anterior myocardial infarction was used to characterize the impact of cellular cardiomyoplasty on cardiac structure and function using hemodynamic, imaging, and histological analyses. A pig model was selected because of its anatomic similarity to the human heart. The following sections describe the safety and efficacy results obtained with this model101.. Two distinct sets of studies were conducted, representing the early treatment of acute myocardial infarction, as well as the treatment of chronic ischemic cardiomyopathy.

Allogeneic mesenchymal stem cell transplantation improves global cardiac function in a swine model of acute myocardial infarction: Previously published work demonstrated that autologous MSC transplantation in post-MI pigs improved cardiac function, with histological evidence of robust engraftment at 8 weeks, and differentiation to a myocyte-like phenotype28. Based on in vitro observations that MSCs lack the B-7 costimulatory molecule and may therefore be immune-privileged, the impact of allogeneic MSC transplantation in porcine MI was assessed. A 14 pig randomized, placebo-controlled study (MSCs vs. placebo)
using the BioCardia Helical Infusion Catheter was performed to assess safety and efficacy of allogeneic transendocardial injections. Farm pigs were chronically instrumented to measure left-ventricular pressure, dimension, and oxygen consumption, and were randomized to active treatment or placebo groups. Three days following MI, placebo (n=7) or 2X10^8 allogeneic MSCs (n=7) labeled with Di-I and DAPI (both fluorescent dyes to aid histochemical identification) were injected percutaneously into infarcted myocardium of the left ventricular cavity using a helical injection needle catheter inserted through a steerable guide catheter (BioCardia, Inc). All animals tolerated the catheter-based injections well. Animals were then studied on a weekly basis for 8 weeks to assess hemodynamics and to examine ventricular architecture. In treated animals, MSCs engrafted within the MI (Figure 1 a, b) and expressed several myocyte proteins, including α-actinin, phospholamban, tropomyosin, and troponin T (Figure 1 c, d, e, f). In addition, there was evidence of stem-cell differentiation or incorporation into vascular structures within the infarct area (Figure 1 g, h, i). MSCs were detected in vascular structures as they expressed VEGF and von Willebrand Factor, suggesting that they are capable of differentiating into vascular smooth muscle and/or endothelium. That the cells did not elicit rejection, despite the absence of immuno-suppressive drug therapy, was supported by the lack of a significant inflammatory response. (Note that cells surrounding vessel in Figure 1g and 1i are of MSC origin, as indicated by DAPI positivity in Figure 1h). The number of MSCs persisting in the myocardium decreased over

![Figure 2](image-url)
time. Nonetheless, MSC injection produced a wide range of benefits, including improved regional and global ventricular function, reduced myocyte apoptosis, and improved tissue perfusion.

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

Heart failure is characterized by mechanoenenergetic uncoupling: decreased efficiency of work per unit oxygen consumption. In placebo-treated animals, SW decreased substantially during the 8-weeks following infarction, and there was a paradoxical increase in myocardial oxygen consumption, resulting in decreased ratio of SW/MVO₂. Conversely, MSC-injected animals’ follow-up was marked by improving myocardial efficiency, both because of increasing SW (from 374.4±59.3 to 654.4±129.9 mmHg.mm at 8 weeks) and because of decreasing MVO₂ (from 10.3±2 to 3.7±1.8 J/beat), both toward normal (Figure 2 e). Thus, MSC therapy exerts favorable effects on the damaged heart that extend to improvements in cellular energy metabolism. The SW/MVO₂ ratio increased from 2.5±0.6 at 3 days post- MI to a normal ratio of 10±5.6 (p<0.05 vs. placebo) at 4 weeks. This improvement in mechanoenergetics was the earliest observable benefit of MSC treatment, preceding changes in global cardiac function. Improved mechanoenergetic coupling in the MSC group is consistent with several possible mechanisms, including reduced native tissue death⁹⁸, new tissue formation²⁹,¹⁰², or stimulation of endogenous repair mechanisms¹⁰⁰.

Besides these metabolic effects, MSC treatment also reduced infarct size. At 8 weeks following treatment, the percentage of LV mass made up by infarcted myocardium was 16±7.2% in placebo-treated and 3.3±1.2% of MSC-treated hearts ; p<0.05) (Figure 3). This finding resulted from reduced scarring within ventricles of similar mass. Placebo-treated animals had transmural myocardial infarctions, while those injected with MSCs had mid-myocardial infarcts; with viable non-scar myocardium surrounding the infarct on both the endo- and epicardial sides. In order to identify the mechanism behind these findings, we tracked the fate of MSCs in the swine myocardium using noninvasive imaging techniques.
As described in detail below, magnetically labeled MSCs can be detected in vivo using MRI. Using this technique, the site of injection of Feridex-labeled stem cells can be identified for up to 8 weeks (Figure 4). The Feridex signal fades over time, suggesting either death of the MSCs or loss of Feridex from their cytoplasm, possibly during differentiation. Restoration of contractile function was observed in areas of MSC injection; supporting the notion that MSCs persist in the myocardium and differentiate into contractile cells. Other potential mechanisms are not excluded by this finding, and the profound decreases in infarct size and improvements in cardiac function suggest the possibility of other concomitant repair mechanisms, for example recruitment and stimulation of endogenous cardiac progenitor cells.

To further investigate the mechanisms of MSC-mediated cardiac repair, both MRI

Figure 4. MRI image of swine myocardium obtained after myocardial infarction and injection of Feridex labeled mesenchymal stem cells. Feridex labeled cells can be seen as dark hypoenhancing regions in the epicardium (arrows) using an ECG-gated, fast gradient echo (fgre) pulse sequence. As shown, Feridex labeling remains evident for up to eight weeks after stem cell injection.
Infarcts in MSC- and placebo-injected swine. Infarct size measurement in vivo by MRI and CT correlated tightly to that determined by triphenyl tetrazolium chloride (TTC) staining post-mortem. Furthermore, using a 32 slice multidetector CT, the same endocardial rim of viable, non-infarcted myocardium observed in the first series of post-mortem hearts (Figure 3, Figure 5) was identified by in vivo imaging. These data not only speak to the therapeutic potential of MSC cardiomyoplasty, but also establish that noninvasive imaging techniques can be used to measure the effects of cardiomyoplasty, and to study the mechanisms underlying these effects. These results in this pig model provide strong rationale for the development of MSC-based cellular cardiomyoplasty strategies and suggest that human studies are warranted.

**MSCs injected I.V. home to and engraft in infarcted myocardium conferring functional benefit:** Preliminary studies were conducted on the efficacy of MSCs administered intravenously in a rat model of permanent left anterior descending (LAD) artery occlusion. Echocardiography was used to assess LV function at baseline, in the peri-infarct period and four weeks after MI. MSC injection in Wistar rats led to dramatic improvement in LV function, with increased myocardial thickening and contractility, and motion in treated animals (Figure 6A and 6B). Labeled cells were identified within the infarct (Figure 7a), and were shaped like fibroblasts but expressed the cardiac protein, α-actinin, albeit at lower levels than native cardiomyocytes (Figure 7b). These labeled cells were most evident at the endocardial rim of the infarct, a finding similar to that seen in the porcine studies above.

**MSCs delivered intravenously (I.V.) distributed to the heart in response to an injury signal:** MSCs injected I.V. at the time of coronary reperfusion homed to the myocardium, while cells injected I.V. two weeks after reperfusion were more likely to engraft in the bone marrow (Figure 8). Determination of SDF-1 and CXCR4 levels revealed not only that both are expressed by MSCs, but that serum levels are up-regulated immediately post infarct and remain elevated for at least two weeks (Figure 9).

**Myocardial Function, Perfusion, and Infarct Size Can be Determined in vivo by CT**

The clinical research team has extensive experience using X-ray computed tomography for the evaluation of myocardial function, perfusion, and infarct size. Our team and others have shown that CT compares well with MRI and echocardiography for the evaluation of global, regional, and segmental wall function.
motion and thickening. Our group and others also have extensive experience in myocardial perfusion imaging. Studies have shown that CT perfusion imaging performed at rest is an accurate method of identifying acute and chronic myocardial infarction. Furthermore, we have shown that adenosine stress CT perfusion imaging is capable of qualitative and absolute quantification of myocardial blood flow in preclinical models of myocardial ischemia. Translation of these protocols have established that rest and stress CT perfusion imaging is an accurate method for the evaluation of myocardial ischemia. Similar to MRI, delayed enhanced CT is also capable of identifying chronic myocardial scar. CT, when compared with MRI is an accurate method for quantifying infarct scar size and this can be done at relatively low radiation doses.

Animal Pharmacology and Toxicology Studies of MSCs Delivered Via Intramyocardial Injection

Five preclinical studies have been performed using autologous or allogeneic MSCs delivered to ischemic myocardium via intramyocardial injection (Table 2). The cell doses administered in these studies covers the proposed dose (2.0 x 10^8 MSCs) for the clinical study in this IND application.

Shake et al. investigated the engraftment and functional effects of transplanted autologous MSCs (cell dose = 60 x 10^6, administered via direct intramyocardial injection with a 30-gauge needle) 14 days after myocardial infarction in a porcine animal model. No ectopic tissue formation was observed. Furthermore, there was no evidence of MSC differentiation to tissues other than cardiac muscle, and no significant inflammatory infiltrates at the MSC implantation sites. Microscopic analysis showed robust engraftment of MSCs in all treated animals. Expression of muscle-specific proteins was seen as early as 2 weeks and could be identified in all animals at sacrifice. The degree of contractile dysfunction was significantly attenuated at 4 weeks in animals implanted with MSCs (+5.4% ± 2.2% versus -3.37% ± 2.7% in control). In addition, the extent of wall thinning after myocardial infarction was markedly reduced in treated animals.

In a porcine animal model, Cattaneo et al. transplanted allogeneic MSCs (cell dose = 200 x 10^6, administered via direct intramyocardial injection with a 27-gauge needle) shortly (1 day) after myocardial infarction. No ectopic tissue formation, significant inflammatory responses or other adverse events were observed. Robust engraftment of allogeneic MSCs was seen in all treated animals. Furthermore, engrafted MSCs were found to express numerous muscle specific proteins and exhibited morphological changes consistent with cardiomyogenesis. A marked improvement in both ejection fraction and global wall motion score was observed in treated animals at 10 weeks post-MSC implantation. Systolic wall thickening and diastolic wall thickness were also augmented in MSC-treated animals. Since no significant differences in infarct size or cardiac loading were noted between groups (MSC-treated of placebo), improvements in cardiac function were likely attributable to MSC implantation.
A study by Hare et al. is particularly informative for establishing the safety of the dose ($2.0 \times 10^8$) of MSC in the proposed clinical study. Autologous porcine MSCs (cell doses of $20 \times 10^6$, $200 \times 10^6$, or placebo) were transplanted into the myocardium of infarcted pigs via direct intramyocardial injection using a 1.0 ml syringe with a 29-gauge needle. A total of 15-25 injections (0.25 ml each) were used to administer the specified cell dose (or placebo). No ectopic tissue formation, change in body weight, or clinical laboratory abnormalities were observed; providing evidence for the safety of the administered doses. MRI assessments at 3 months showed a decrease in infarct scar size in the high-dose group ($200 \times 10^6$ MSCs) that was not evident in the lower dose or placebo groups, providing support of a potential treatment effect at the higher cell dose level. All animals were monitored for cardiac arrhythmias after injection of the MSCs. Although a limited number of heart blocks were seen, a common event after open heart procedures, no ventricular arrhythmias or sudden deaths occurred. Additionally, electrophysiology studies on six animals were completed at 12 weeks before injection and before sacrifice (12 weeks after injection): No animals were inducible for arrhythmias. Animals in this study had whole body autopsy exams to assess any pathology in tissues adjacent to the injection sites,
the heart itself, and remote solid organs. No abnormal pathological lesions were noted in any of these sites.

The two porcine animal model studies by Amado et al.\textsuperscript{101,124} investigated the intramyocardial injection of allogeneic porcine MSCs (cell dose = $200 \times 10^6$) via transendocardial catheter delivery 3 days after myocardial infarction. One of the studies, a randomized, placebo-controlled study of 14 pigs using the BioCardia Helical Infusion Catheter found\textsuperscript{101}:

1. MSC and placebo transendocardial injections were safe and well-tolerated.
2. At the 8 week follow-up assessment, MSC injections resulted in profound improvements in LV end diastolic pressure (LVEDP) and dimension.
3. MSC-treated pigs showed improved LV recovery, as demonstrated by a substantial increase in stroke work.
4. MSC treatment resulted in myocardial performance recovery to normal, both in systolic and diastolic function.

Additionally, several important findings emerged from both Amado and investigators\textsuperscript{97,121} transendocardial porcine animal studies, including:

1. MSCs were safely injected via the transendocardial catheter delivery route using two different catheter-needle systems.
2. Cellular transplantation of MSCs resulted in long-term engraftment and profound reduction in scar formation.
3. Transplanted MSCs were prepared from an allogeneic donor and were not rejected; a major practical advance for the potential widespread application of this therapy.

The results of these five preclinical studies support the safety and potential efficacy of the dose (2.0 x $10^8$ MSCs) for the proposed clinical study in this IND application.
### TABLE 2
PRECLINICAL STUDIES: AUTOLOGOUS AND ALLOGENEIC MESENCHYMAL STEM CELLS (MSCs) ADMINISTERED VIA INTRAMYOCARDIAL INJECTION

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>N</th>
<th>Cell Delivery</th>
<th>Cell Source &amp; Type</th>
<th>Cell Doses (x 10^6)</th>
<th>Safety Results</th>
<th>Efficacy Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shake (28)</td>
<td>14-Day</td>
<td>14</td>
<td>Surgical (needle) IM injection</td>
<td>Autologous, porcine MSCs</td>
<td>60.0</td>
<td>- No ectopic tissue formation</td>
<td>- MSC engraftment</td>
</tr>
<tr>
<td></td>
<td>Post-MI Pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- No MSC differentiation to non-cardiac tissue</td>
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<td></td>
<td></td>
<td>- No significant inflammatory infiltrates at site of MSC implantation</td>
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<td></td>
<td></td>
<td></td>
<td>- No significant inflammatory infiltrates at site of MSC implantation</td>
<td></td>
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<tr>
<td>Cattaneo (122)</td>
<td>1-Day</td>
<td>13</td>
<td>Surgical (needle) IM injection</td>
<td>Allogeneic, porcine MSCs</td>
<td>200.0</td>
<td>- No ectopic tissue formation</td>
<td>- MSC engraftment</td>
</tr>
<tr>
<td></td>
<td>Post-MI Pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- No significant inflammatory response</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>- MSC engraftment</td>
<td></td>
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<tr>
<td>Hare (123)</td>
<td>90-Day</td>
<td>9</td>
<td>Surgical (needle) IM injection</td>
<td>Autologous, porcine MSCs</td>
<td>20.0 (Low) 200.0 (High)</td>
<td>- No ectopic tissue formation</td>
<td>- MSC engraftment</td>
</tr>
<tr>
<td></td>
<td>Post-MI Pig</td>
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<td></td>
<td></td>
<td></td>
<td>- No ectopic tissue formation</td>
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<td></td>
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<td></td>
<td></td>
<td>- No change in body weight</td>
<td></td>
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<td></td>
<td></td>
<td>- No clinically relevant laboratory abnormalities</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>- No arrhythmias or inducible VT</td>
<td></td>
</tr>
<tr>
<td>Amado (101)</td>
<td>3-Day</td>
<td>14</td>
<td>PIM (catheter) injection</td>
<td>Allogeneic, porcine MSCs</td>
<td>200.0</td>
<td>- No deaths; no malignant arrhythmias</td>
<td>- MSC engraftment</td>
</tr>
<tr>
<td></td>
<td>Post-MI Pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- No death deaths; no malignant arrhythmias</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>- No evidence of cardiac perforation during injection</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Decrease in infarct scar</td>
<td></td>
</tr>
<tr>
<td>Amado (124)</td>
<td>3-Day</td>
<td>22</td>
<td>PIM (catheter) injection</td>
<td>Allogeneic, porcine MSCs</td>
<td>200.0</td>
<td>- No difference in deaths between treated/placebo</td>
<td>- MSC engraftment</td>
</tr>
<tr>
<td></td>
<td>Post-MI Pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- No difference in deaths between treated/placebo</td>
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<td></td>
<td></td>
<td>- Viable myocardium</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- ↓Infarct scar</td>
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</tbody>
</table>

EF: Ejection Fraction; IM: intramyocardial; MSC: Mesenchymal Stem Cell; PIM: percutaneous intramyocardial; VT: ventricular tachycardia
Animal Pharmacology and Toxicology Studies of BMCs Delivered Via Intramyocardial Injection

The inconsistent efficacy results reported in the controlled trials of bone marrow-derived cells (BMCs) administered via intracoronary (IC) or intravenous (IV) delivery are not seen in a smaller trial of BMCs administered via intramyocardial (or more specifically, transendocardial, TEC) delivery. There is growing recognition that there is limited homing and retention of bone-marrow derived cells after intracoronary cell delivery. Furthermore, microvascular obstruction caused by infusion of cells into the coronary arteries has been shown to impair coronary flow in canine and swine studies.

Recent cell distribution and retention studies suggest that intramyocardial delivery of cells has advantages in efficiency of delivery and retention in the heart when compared to intracoronary delivery of cells, but that there is room for improvement to reduce variation in trans-epicardial delivery with a syringe. There may also be advantages to preventing the loss of delivered cells (presumably through potentially embolic back-leak into the ventricular chamber) from straight needle transendocardial delivery catheters. A study in a porcine model demonstrated that TEC delivery of bone-marrow-derived cells (in this case, mesenchymal stem cells, MSCs) into the myocardium was not only safe and well tolerated, but was associated with decreased remote organ engraftment when compared to IC or IV delivery. Furthermore, IC delivery was associated with a higher incidence of decreased coronary blood flow.

Percutaneous delivery in an interventional setting enables minimally invasive introduction of cells into the myocardium during Percutaneous Coronary Intervention (PCI) or a diagnostic catheterization. BioCardia has developed an investigational catheter (Helical Infusion Catheter; United States Food and Drug Administration Device Master File MAF-1229) that is tipped with a small helical needle that enables fixation to, and infusion of, cells within the myocardium. The design of the BioCardia Helical Infusion Catheter evolved from the design of active fixation pacing leads, which have long history of safe anchoring in myocardial tissue. The Helical Infusion Catheter is advanced into the chambers of the heart through a deflectable guide catheter that is introduced into the left ventricle over a guide wire. The Helical Infusion Catheter can be directed to different locations within the heart using the steerable guiding catheter (the 8 French Universal Deflectable Guide Catheter, which has received marketing clearance from the United States Food and Drug Administration).

Animal studies using the BioCardia Helical Infusion Catheter to transplant cells into the heart have demonstrated that the procedure and devices are safe and effective, while facilitating practical application of cell therapy technologies in the treatment of myocardial infarction. Using this system, stem cells were successfully and safely administered by endocardial injection into a myocardial infarct produced by occlusion of the left anterior descending artery in domestic swine. Cell engraftment was observed in all treated animals, with transmural migration of implanted cells from endocardium to epicardium. Fibroblast cells
injected through the Helical Infusion System were estimated to have an efficacy of delivery of 17.3 ± 24.3% of cells identified after 21 days, with >96% of the cells within 5 mm of the infarct zone without complication. The feasibility of using the catheter to deliver mammalian cells was initially determined in vitro by determining the viability of BMCs which were injected through the anchoring infusion system. Subsequently, intramyocardial cell transplantation was performed using porcine skeletal bone marrow cells through the Helical Infusion Catheter system. The histological distribution of the transplanted cells was determined both acutely and chronically (after 3 weeks), with cells remaining viable and incorporated within the myocardial architecture, with some inflammatory reaction around the injection sites.

Rationale for Proposed Mesenchymal Stem Cell (MSC) Dose

The clinical experience with the administration of MSCs in the three clinical studies performed to date (two utilizing the IC route, and one via peripheral IV injection) provide substantial evidence of clinical safety at the cell doses administered as well as preliminary support of clinical efficacy. Although none of the studies employed the delivery method planned in this IND application’s proposed clinical study, two of the three studies utilized cell doses that were over 50% greater and as much as two hundred-fold greater than the highest MSC cell dose proposed herein (2.0 x 10^8).

Summary

Preliminary data strongly support the hypothesis that MSCs may improve heart function and prevent the development of heart failure following myocardial infarction. The study team has extensive experience using both rat and swine models of myocardial infarction and assessing left ventricular function in vivo with imaging techniques in large and small animals.

Given the experience and encouraging preliminary results with stem cell therapies and tracking, the University of Miami Miller School of Medicine (UMMSM) team is conducting this randomized, pilot clinical study. The approach includes the following rationales:

- Recent randomized trials of cellular cardiomyoplasty with endothelial progenitor cells or hematopoetic stem cells have proven to be more rigorous assessments than earlier, uncontrolled studies.
- If allogeneic h-MSC strategies prove to be as safe and effective as autologous h-MSC strategies, the former will be preferred due to avoidance of the need for bone marrow aspiration in the patient, as well as earlier time-to-treatment.
- The specific delivery method, using the BioCardia Helical Infusion System for the transcatheter delivery of the hMSCs, may result in improved cell delivery to the target myocardial tissue.
An ancillary study will determine biomarkers that may be predictive of response to cell therapy, and the variability in effective cell growth and resultant cell quantity are likely determinants. These factors will be measured in each patient and correlated with baseline clinical variables and co-morbidities.

If this Pilot Study supports the clinical safety of the hMSC delivery at the cell concentrations, injection volumes and injection numbers used, a subsequent study will investigate the comparative efficacy and safety of allogeneic vs. autologous hMSC strategies in a more comprehensive manner.
2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective
To demonstrate the safety of allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to myocardial infarction (MI).

2.1.2 Secondary Objectives
- To compare the safety of allogeneic hMSCs to autologous hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI.
- To demonstrate the efficacy of autologous hMSCs and allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI.

2.2 Study Endpoints

2.2.1 Primary Endpoint (Safety)
Incidence (at one month post-catheterization) of any treatment-emergent serious adverse events (TE-SAEs), defined as the composite of: death, non-fatal MI, stroke, hospitalization for worsening heart failure, cardiac perforation, pericardial tamponade, sustained ventricular arrhythmias (characterized by ventricular arrhythmias lasting longer than 15 seconds or with hemodynamic compromise) or any other potential late effects detected and corroborated by clinical presentation, laboratory investigations, image analysis and when necessary with biopsy from suspected target sites in the body.

2.2.2 Secondary Endpoints (Efficacy)
The following efficacy endpoints will be evaluated in this trial (during the six-month follow-up period, at the month 12 visit and at the final month 13 visit):
- CT and Echocardiographic-derived measures of left ventricular function:
  1. Difference between the baseline and 13-month infarct scar size (ISS) as determined by delayed contrast-enhanced CT
  2. Difference between the baseline and 13-month regional left ventricular function (at the site of autologous cell injections) as determined by CT.
  3. Difference between the baseline and 13-month regional left ventricular wall thickening as determined by CT.
4. Difference between the baseline, 6-month (echocardiogram only), and 13-month left ventricular end diastolic wall thickness as determined by CT and echocardiogram.

5. Difference between the baseline, 6-month (echocardiogram only), and 13-month left ventricular ejection fraction, end diastolic and end systolic volumes, as determined by CT and echocardiogram.

6. Difference between the baseline and 13-month left ventricular regional myocardial perfusion as determined by CT.

Note: If the 6-month observations for echocardiogram are not available, the 3-month observations will be used.

- Tissue perfusion measured by CT.
- Peak VO₂ (by treadmill determination).
- Six-minute walk test.
- NYHA functional class.
- Minnesota Living with Heart Failure (MLHF) Questionnaire.
- Incidence of the Major Adverse Cardiac Events (MACE) endpoint, defined as the composite incidence of (1) death, (2) hospitalization for worsening heart failure, or (3) non-fatal recurrent MI.

2.2.3 **Secondary Endpoints (Safety)**

The following safety endpoints will be evaluated in this trial (during the six-month follow-up period, at the month 12 visit and at the final month 13 visit):

- Treatment emergent adverse event (AE) rates.
- Ectopic tissue formation (as identified from CT scans of the chest, abdomen, and pelvis).
- 48-hour ambulatory electrocardiogram (ECG) recordings.
- Hematology, clinical chemistry and urinalysis values.
- Pulmonary function (measured by the forced expiratory volume in 1 second [FEV₁]).
- Serial troponin and CK-MB values (every 12 hours for the first 48 hours post cardiac catheterization).
- Post-cardiac catheterization echocardiogram (day 1 post-catheterization).

### 3. STUDY DESIGN

This is a Pilot Phase I/II Study, intended as a safety assessment prior to a full comparator study. In this study, dose and volume escalations of cells
administered via the Biocardia Helical infusion system will be tested in 30 patients in three groups:

**Group 1** (10 patients)

**Group 1a** - Five (5) patients will be treated with Auto-hMSCs: 4 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $0.2 \times 10^8$ (20 million) Auto-hMSCs.

**Group 1b** - Five (5) patients to be treated with Allo-hMSCs: 4 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $0.2 \times 10^8$ (20 million) Allo-hMSCs.

These patients will not be treated less than 5 days apart and will each undergo full evaluation for 5 days to demonstrate there is no evidence of a procedure induced myocardial infarction or myocardial perforation prior to proceeding with Group 2.

**Group 2** (10 patients)

**Group 2a** - Five (5) patients will be treated with Auto-hMSCs: 20 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $1 \times 10^8$ (100 million) Auto-hMSCs.

**Group 2b** - Five (5) patients to be treated with Allo-hMSCs: 20 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $1 \times 10^8$ (100 million) Allo-hMSCs.

**Group 3** (10 patients)

**Group 3a** - Five (5) patients will be treated with Auto-hMSCs: 40 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $2 \times 10^8$ (200 million) Auto-hMSCs.

**Group 3b** - Five (5) patients to be treated with Allo-hMSCs: 40 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $2 \times 10^8$ (200 million) Allo-hMSCs.

Within each of Groups 1, 2 and 3, patients will be randomized in a 1:1 ratio to one of the two Treatment Strategies: Autologous hMSCs vs. Allogeneic hMSCs. The Study Team will record and maintain a detailed record of injection locations.

If a patient is randomized to Groups 1a, 2a or 3a (Auto-hMSCs) and the Auto-hMSCs do not expand to the required dose ($0.2 \times 10^8$, $1 \times 10^8$ or $2 \times 10^8$ cells, respectively), each injection will contain the maximum number of cells available.

The injections will be administered transendocardially during cardiac catheterization using the Biocardia Helical Infusion Catheter.

For patients randomized to Groups 1a, 2a or 3a (Auto-hMSCs); the cells will be derived from a sample of the patient’s bone marrow (obtained by iliac crest aspiration) approximately 4-6 weeks prior to cardiac catheterization. For patients
randomized to Group 1b, 2b or 3b Allo- hMSCs), the cells will be supplied from an allogeneic human mesenchymal stem cell source isolated from bone marrow cells from normal human donors and manufactured by the University of Miami.

Following cardiac catheterization and cell injections, patients will be hospitalized for a minimum of 4 days then followed at 2 weeks post-catheterization, and at monthly intervals for six months to complete all safety and efficacy assessments. Patients will also receive selected efficacy and safety assessments during a 12 month follow-up visit and a 13 month follow-up visit.

* Definition of Myocardial Infarction:

Myocardial infarction (MI) will be defined by an adaptation of the diagnostic criteria for myocardial infarction with coronary bypass graft (CABG) surgery, as outlined in the recent consensus document from the ACC/AHA/ESC/WHF that has become the authoritative standard for the definition of MI (Thygesen K, Alpert JS, White HD et al, J Am Coll Cardiol, 2007; 50:2173-2195). This definition for post-CABG MI is most applicable because it recognizes that cardiopulmonary bypass is associated with myocyte death, even when successful, and that at low biomarker levels, patient prognosis is not adversely affected.

The administration of cells in this Pilot Phase requires a transendocardial injection, thus an elevation of cardiac biomarkers is expected concurrent with successful cell delivery (please see documentation of 7-fold elevation in the clinical study reported in Section 3, Attachment 1 of the IND). Accordingly the above MI criteria require modification of the troponin elevation threshold. Although an average 7.7-fold elevation in troponin I is documented as associated with this procedure, we will use troponin I values more than 5 times the 99th percentile of the normal reference range as our guideline to define a procedure-related MI, as used in the consensus document. Thus, the definition for procedure-related MI will be more sensitive than specific.

In summary, a procedure-related MI will be defined within the first 48 hours after drug delivery if at least 2 of the following 3 criteria are met:

1. There is typical ischemic cardiac pain lasting at least 30 minutes.
2. There are troponin I values more than 5 times the 99th percentile of the normal reference range or CPK-MB levels more than 5 times the 99th percentile of the normal reference range during the first 48 h following intramyocardial cell delivery.
3. There are new pathological Q-waves or new LBBB in conjunction with echocardiographic evidence of new loss of viable myocardium.

** Definition of myocardial perforation:

Myocardial perforation will be considered to have occurred if a) a new pericardial effusion >1cm thick is detected by transthoracic echocardiography immediately post, 4-6 hours post, or on day 2 post catheter injection; or b) a new ventricular...
septal defect is detected by Doppler echocardiography immediately post, 4-6 hours post, or on day 2 post catheter injection; or c) the tip or any part of the catheter system is observed by the operator under fluoroscopy to exit the left ventricular cavity across its myocardium, even if neither pericardial effusion nor ventricular septal defect results from the catheter exit.

3.1 Inclusion Criteria

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

1. Be ≥ 21 and < 90 years of age.
2. Provide written informed consent.
3. Have a diagnosis of chronic ischemic left ventricular dysfunction secondary to myocardial infarction (MI) as defined by previous myocardial infarction documented by an imaging study demonstrating coronary artery disease with corresponding areas of akinesis, dyskinesis, or severe hypokinesis.
4. Been treated with appropriate maximal medical therapy for heart failure or post-infarction left ventricular dysfunction. For beta-blockade, the patient must have been on a stable dose of a clinically appropriate beta-blocker for 3 months. For angiotensin-converting enzyme inhibition, the patient must have been on a stable dose of a clinically appropriate agent for 1 month.
5. Be a candidate for cardiac catheterization within 5 to 10 weeks of screening as determined by doctors.
6. Have an ejection fraction less than 50% by gated blood pool scan, two-dimensional echocardiogram, cardiac MRI, or left ventriculogram within the prior six months and not in the setting of a recent ischemic event.

3.2 Exclusion Criteria

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

1. Have a baseline glomerular filtration rate < 50 ml/min1.73m2.
2. Have a known, serious radiographic contrast allergy.
3. Have a prosthetic aortic valve or heart constrictive device.
4. Have a documented presence of aortic stenosis (aortic stenosis graded as 1.5cm² or less).
5. Have a documented presence of moderate to severe aortic insufficiency (echocardiographic assessment of aortic insufficiency graded as ≥+2).
6. Require coronary artery revascularization. Patients who require or undergo revascularization procedures should undergo these procedures a minimum of 3 months in advance of treatment in this study. In addition, patients who develop a need for revascularization following enrollment will be submitted for this therapy without delay.
7. Have evidence of a life-threatening arrhythmia in the absence of a defibrillator (nonsustained ventricular tachycardia ≥ 20 consecutive beats or complete second or third degree heart block in the absence of a functioning pacemaker) or QTc interval > 550 ms on screening ECG.

8. AICD firing in the past 60 days prior to enrollment.

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

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

11. Have a coagulopathy = (INR > 1.3) not due to a reversible cause (i.e., Coumadin). Patients on Coumadin will be withdrawn 5 days before the procedure and confirmed to have an INR < 1.3. Patients who cannot be withdrawn from Coumadin will be excluded from enrollment.

12. Have known allergies to penicillin or streptomycin.

13. Be an organ transplant recipient.

14. Have a history of organ or cell transplant rejection.

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

16. Have a non-cardiac condition that limits lifespan to < 1 year.

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

18. Be on chronic therapy with immunosuppressant medication, such as corticosteroids or TNFα antagonists.

19. Be serum positive for HIV, hepatitis BsAg or vermic hepatitis C.

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

21. Be a female who is pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods. Female patients must undergo a blood or urine pregnancy test at screening and within 36 hours prior to injection.

4. TREATMENT OF PATIENTS

4.1 Study Therapy and Dosages

4.1.1 Study Investigational Therapy
Harvesting for Auto-hMSCs

Bone marrow (BM) will be harvested from all patients with 30 to 60 ml aspirated from the posterior iliac crest, which is the thickened superior margin of the ilium terminating in the iliac spine. The patient will lie on his/her side to enable the physician to have optimal access to the posterior iliac crest of the hip area. This area will be cleaned with a germ-killing cleanser followed by the application of local anesthetic. The BM aspiration will be done with a special needle attached to the heparinized syringes. The mononuclear fraction (MNC) will be isolated using a density gradient with Lymphocyte Separation Medium (LSM; specific gravity 1.077). The low density cells will be collected from the gradient and washed with Plasma-Lyte A containing 1% human serum albumin (HSA). The washed cells will be sampled and viable cell counts performed to determine the total number of viable cells.

The required dose of Auto-hMSC (for Groups 1a, 2a and 3a) will be generated using standard hMSC culture conditions. Preclinical validation studies have demonstrated the reproducible generation of more than 200 million hMSCs in 21 days of culture. The BM MNC are seeded into 225 cm² tissue culture flasks in alpha MEM containing 20% FBS. After 14 days of culture passage, zero (P0) cells are harvested by trypsin treatment and expanded into 30 flasks. These flasks are incubated for a further 7 days and the hMSCs are harvested by trypsin treatment (P1 cells). The P1 cells are washed and total viable cell counts determined.

The Auto-hMSCs will be resuspended in cryoprotectant consisting of Pentaspan (10% pentastarch in 0.9% sodium chloride) supplemented with 2% HSA and 5% DMSO. The cells are transferred to cryo-bags and frozen using a control rate freezer. The frozen cells will be stored in a liquid nitrogen freezer until issue.

Auto-hMSC Preparation for Administration

Upon request, the frozen bags will be thawed in a 37°C water bath. In a BSC, the cell suspension will be transferred to conical tubes and slowly diluted with a PBS buffer supplemented with 1% HSA or Plasma-Lyte A supplemented with 1% HSA. The suspension will be centrifuged and the cell pellet re-suspended in the dilution buffer. The cells will be counted to determine the total viable cells. After release, the cells will be placed in a labeled conical tube and delivered to the appropriate medical staff.

Technique for Administration

Please see Addendum A, which specifies the technique for percutaneous endocardial administration of Auto-hMSCs and Allo-hMSCs. Addendum B provides a detailed training program for the Biocardia Helical Catheter System.
4.1.2 Dose Rationale
In two preclinical studies in a porcine model, MSC therapy was safely administered via intramyocardial injection at doses of up to $2 \times 10^8$ cells, thus supporting the use this dose level for the hMSC preparation in this clinical study. The hMSC doses were also chosen based on practical considerations and the ability to grow this quantity of cells for most patients within approximately 28 days.

4.1.3 Dosages and Dosing
The Study Team will record and maintain a detailed record of the locations and actual number of injections in the study.

If a patient is randomized to Groups 1a, 2a or 3a and the Auto-hMSCs do not expand to the required dose ($0.2 \times 10^8$, $1 \times 10^8$ or $2 \times 10^8$ cells, respectively), each injection will contain the maximum number of cells available. The injections will be administered transendocardially during cardiac catheterization using a Biocardia Helical Infusion Catheter. Intramyocardial injections will be discontinued during the procedure if one or more of the following occur:

a.) Sustained drop in blood pressure exceeding 20mm/Hg not responsive to fluid administration.
b.) Clinical signs and symptoms indicating acute coronary syndrome
c.) Clinical signs and symptoms indicating a cerebrovascular accident
d.) Two episodes of sustained ventricular tachycardia / ventricular fibrillation requiring cardioversion

If a patient is randomized to Groups 1a, 2a or 3a (Auto-hMSCs), the cells will be obtained from the patients via bone marrow aspiration approximately four – six weeks prior to the cardiac catheterization. Patients whose bone marrow MNC preparation fails to generate Auto-hMSCs will be removed from study without limit. The randomization sequence will be maintained such that subsequent patient enrollment will not be impacted.

If a patient is randomized to Groups 1b, 2b or 3b (Allo-hMSCs), the cells will be supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami. Cardiac catheterization will be scheduled following randomization.

4.2 Blinding and Unblinding
The study will not be blinded, since the need to perform a bone marrow aspiration for the patients randomized to Groups 1a, 2a and 3a (Auto-hMSCs) effectively prevents such.
4.2.1 Storage and Handling of Study Investigational Therapy

Study therapy (Auto-hMSCs and Allo-hMSCs) should only be dispensed once a patient has (1) provided written informed consent, (2) met all eligibility criteria for entry into the study, and (3) completed all baseline evaluations. If a patient’s Auto-hMSCs cells are not used within 5 years of entry into the study, the cells will be destroyed.

4.2.2 Study Investigational Therapy Accountability Procedures

The Investigator is responsible for study investigational therapy accountability, reconciliation, and record maintenance. In accordance with all applicable regulatory requirements, the Investigator or designated study center personnel must maintain accountability records throughout the study.

5. STUDY PROCEDURES

5.1 Time and Events Schedule

The Time and Events Schedule for the conduct of this study is shown in Table 3.
# TABLE 3: Overall Schedule of Time and Events

| Study Procedure | Screening (Weeks -10 to -5) | Baseline Visit 1 # (Weeks -6 to -2) | Baseline Visit 2 * (Weeks -6 to -2) | Day 1 (Week 1) | Day 2 (Week 1) | Day 3 (Week 1) | Day 4 (Week 1) | Day 14±3 (Week 2) | Month 1 (Week 4 ±10 days) | Month 2 (Week 8 ±10 days) | Month 3 (Week 12 ±10 days) | Month 4 (Week 16 ±10 days) | Month 5 (Week 20 ±10 days) | Month 6 (Week 24 ±10 days) | Month 12 (Week 52 ±21 days) | Month 13 (Week 56 ±21 days) |
|-----------------|-----------------------------|----------------------------------|----------------------------------|---------------|---------------|---------------|---------------|----------------|----------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|--------------------------|--------------------------|
| Informed Consent | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| History and Physical | X                           | X                                | X                                | X             | X             | X             | X             | X              |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Vital Signs     | X                           | X                                | X                                | X             | X             | X             | X             | X              |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| 12-lead ECG     | X ##                        | X                                | X                                | X             | X             | X             | X             | X              |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Concomitant Medications | X                           | X                                | X                                | X             | X             | X             | X             | X              |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Randomization   | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Bone Marrow Aspiration | X                           | (Groups 1a, 2a & 3a)            |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Catheterization | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Investigational agent | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Standard Post-Procedural Care | X                           | X                                | X                                | X             | X             | X             | X             |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| CT Assessment of Heart | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Echocardiogram $ | X                           | X E                              | $                                |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Treadmill Determin. of Peak VO$_2$ | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Six-Minute Walk Test | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| NYHA Functional Class | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Questionnaire’s MLHF, IIEF (male), SQOL-F(female) | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| CT Scans (chest, abd., pelvis) | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Pulmonary function (FEV1) | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| 48 Hour Ambulatory ECG | X ##                        |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Serum Troponin & CK-MB $**$ | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Hematology & Clinical Chem. % BNP, Uric acid, and CRP | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Urinalysis      | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Serum or urine pregnancy test | X                           | X $$$                          |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| HIV 1, HIV 2 & Hepatitis B & C, | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Donor Screening Tests | X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Biomarkers Assessment %| X                           |                                  |                                  |               |               |               |               |                |                      |                     |                      |                      |                      |                      |                      |                          |                          |
| Adverse Events  | X                           | X                                | X                                | X             | X             | X             | X             | X              |                      |                     |                      |                      |                      |                      |                      |                          |                          |
Time and Events Table Key:

# - All Baseline Visit tests will occur within 28 days of the final Screening Visit.

## - If there is a sustained or short run of ventricular tachycardia on the 12-lead ECG or 48 hour Ambulatory ECG obtained in the Screening phase testing, the patient will be removed from the study.

** - Serial troponin and CK-MB laboratory assays will be performed every 12 hours for the first 48 hours post-cardiac catheterization.

*** - Unless the patient is not capable, then 48 hours prior to discharge.

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

- **Hematology Tests:** white blood cell count, platelet count, hemoglobin and hematocrit.
- **Liver Function Tests:** Albumin, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin.
- **Renal Function Tests:** creatinine, blood urea nitrogen (BUN), creatinine clearance, glomerular filtration rate, sodium, potassium, chloride, bicarbonate, and glucose.

**Serum Uric Acid, BNP, and C-reactive protein (CRP)**

%% - The following biomarkers will be analyzed:

- **Cell-surface markers:** CXCR4, C-Kit, & Connexin 43
- **Transcriptomic/Proteome:** RNA, miRNA, protein samples, and telomerase, akt
- **Growth factors:** Sdf-1, notch,
- **Functional Assays:** cell growth rate, and CFU assay

$ - All subjects will undergo transthoracic echocardiographic assessment of overall and regional LV systolic function at baseline, day 2, and months 3, 6, and 12.

₤ - All subjects will undergo a limited transthoracic echocardiographic assessment immediately following the catheterization procedure, and 4-6 hours later.

$$ - A serum or urine pregnancy test will be completed within 36 hours prior to injection.

XX - Immune monitoring for graft rejection. The following markers will be used for analysis to assess for activated T-cells based upon a CD3⁺CD25⁺ or CD3⁺CD69⁺ phenotype:

- CD3, CD25, CD69
5.2 Study Phases and Visits

5.2.1 Screening Phase
See Table 3 for the procedures and assessments to be performed during this phase of the study. Any patient who has a sustained or short run of ventricular tachycardia on ECG or 48 Ambulatory ECG performed during the screening phase will be removed from the study. The donor screening blood work is specific to each cell manufacturing facility and may include hepatitis A, West Nile Virus, human T-cell lymphotropic virus (HTLV) I/II, syphilis, and. All screening visit tests and procedures will occur within five weeks of the signed informed consent (IC) and 5 to 10 weeks prior to cardiac catheterization. No screening exams will take place until the patient is fully informed of the research and signs the consent form.

5.2.2 Baseline Phase
See Table 3 for the procedures and assessments to be performed during this phase of the study. After all screening exams, patients will be enrolled into the study and randomized to either Groups 1a, 2a and 3a (to receive autologous hMSCs) or Groups 1b, 2b and 3b (to receive allogeneic hMSCs).

The Baseline Phase will take place over a four week period, and is split into two visits. The Baseline Visit #1 will occur once the screening tests are completed and it has been determined that the patient remains eligible for the study. Patients in Group 1a, 2a and 3a will return for their bone marrow aspiration procedure, which will occur 4 to 6 weeks prior to catheterization. Successful bone marrow aspiration will trigger scheduling of the patient’s catheterization. Patients whose bone marrow MNC preparation fails to generate Auto-hMSCs, will be removed from study. Baseline Visit #2 will take place within four weeks of the final screening exam at which time all patients will undergo additional tests to gather baseline data. Patients in Groups 1b, 2b and 3b will undergo their baseline tests and be scheduled for catheterization as soon as possible after randomization.

5.2.3 Day 1 – Day 4 Post-Catheterization & Week 2 Evaluations
See Table 3 for the procedures and assessments to be performed during these evaluations. The listed procedures (other than catheterization and Investigational Agent administration) should all be performed as soon as practicable after the catheterization procedure. All patients will have troponin and CK-MB laboratory work completed every 12 hours for the first 48 hours, as well as an echocardiogram immediately following the procedure, 4-6 hours later and 24 hours post-cardiac catheterization. Additionally, an FEV1 assessment will be completed during day 4, unless the patient is unable, then 48 hours prior to discharge.
During Week 2 (Day 14 ± 3), patients will return to the clinic for a 12-lead ECG, 48-hour ambulatory ECG, and an FEV1 assessment.

5.2.4 **Month 1 – Month 13 Visits**

See Table 3 for the procedures and assessments to be performed during these follow-up visits. Outpatient visits should be completed as close to the scheduled visit dates as possible. For visits months 1-6, the visit window is ± 10 days from the intended date of the visit. For visits during month 12 & 13, the visit window is ± 21 days. If required, outpatient visit procedures may take place over more than one day. If procedures are performed on more than one day, the date of the history and physical will be considered the visit day. Every attempt will be made to have the visit close to the target date.

5.2.5 **Biomarkers Assessment**

A separate 7 mL blood sample for gene expression profiling of WBC RNA will be obtained at baseline and at 6 months if the donor consents. In addition, for Group A, a 5-7 mL sample of each patient’s bone marrow aspirate will be obtained at baseline for the same purpose if the donor consents. All samples will be identified so that they can be linked to individual patients. These samples may be stored indefinitely. Individual results will not be returned to the patient or the study physician. Data presented in publications will not contain individual patients’ gene expression or clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

5.2.6 **Immune Monitoring for Graft Rejection**

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

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

As part of the Poseidon protocol we will obtain peripheral blood samples from all patients to evaluate the presence of activated T cells. Two heparinized (green top) vacutainer tubes (approx 15 cc total blood) will be collected at different time points during the study: at baseline prior to infusion of MSC (at screening), within 24 hours of injection, 1 month and 6 months. Peripheral blood mononuclear cells (PBMC) will be isolated from heparinized blood by ficoll sedimentation and will be viably cryopreserved for planned assessments of T cell activation.

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

### 5.2.7 Enrollment Contingency Plans

The following list will be followed to take into account certain unexpected events that can disrupt the planned study schedule:

1. Any patient whose catheterization is delayed will be allowed to remain in the study and receive investigational cells for up to 6 months from the date of the signed informed consent; as long as the patient remains eligible based upon a successful repeat screening exam.

2. Enrolled patients who unexpectedly die prior to catheterization, or otherwise withdraw from the study after the screening period but before surgery, may be replaced without limit.

3. Patients who are in the middle of the cardiac catheterization and additional therapy is given may be withdrawn from the study and replaced by newly enrolled subjects without limit.

### 5.3 Computed Tomography (CT) and Echocardiogram Protocols

**CT Protocol**
All patients will undergo contrast-enhanced CT at screening and at the 13 month follow-up visit. In addition to screening the chest, abdomen, and pelvis for ectopic tissue formation, cardiac CT will be used to measure global left ventricular function, regional wall motion, and volumetric parameters; rest perfusion; and infarct scar size.

Imaging will be performed using a 128 slice CT scanning system (Siemens AS+, Siemens Medical Solutions). Patients will lie supine on the scanner table and be attached to the scanner’s electrocardiographic monitor. Baseline heart rate will be recorded. Beta-blockers will not be given, except for the patient’s normally prescribed dose (if applicable). Scout images will be obtained for determining scan range.

**Cardiac CT Function and Rest Perfusion Imaging**

Cardiac CT function and rest perfusion imaging will be performed with a temporal resolution of 100-150 msec. Rest perfusion imaging will be acquired at peak tube current and an optimal diastolic phase of the R-R interval. Dose modulation and low tube voltage (100 kV) will be used to minimize the radiation dose. Contrast dose will be 140 ml. The estimated radiation dose for function and rest perfusion imaging is estimated to not exceed 10 mSv.

Functional images will be reconstructed using segmental reconstruction every 5% of the R-R interval. Global left ventricular systolic function, regional wall motion and wall thickening, end-systolic and end-diastolic volumes will be measured.

Rest perfusion images will be reconstructed in mid-diastole using myoperfusion convolution kernels with beam hardening correction. Custom myocardial perfusion software will be used to measure segmental and regional perfusion.

**Chest / Abdomen / Pelvic CT**

The chest, abdominal, and pelvic CT will be performed according to standard clinical protocol, immediately following the function and rest perfusion scan. Oral gastrografin will be used for bowel opacification. No additional intravenous contrast will be administered. The estimated radiation dose will be 17 mSv.

**Delayed Enhanced CT Infarct Scar Imaging**

Five minutes following contrast injection, delayed enhanced viability imaging will be performed using a prospective ECG-gated protocol. No additional contrast will be administered. Prospective ECG-gating will limit X-ray exposure to 70-90% of the R-R interval. Tube voltage will remain low (100 kV) to minimize radiation dose. The estimated radiation dose for delayed enhanced imaging is estimated to not exceed 5 mSv.

**Echocardiogram Protocol**

All subjects will undergo transthoracic echocardiographic assessment of overall and regional LV systolic function at baseline, immediately following the catheterization procedure, 4-6 hours later, day 2, and months 3, 6, and 12.
The echocardiograms will be performed using commercially available ultrasound machines. Images will be recorded for off-line analysis. Multiple views will be recorded, including the parasternal long and short axis views, the apical two and four chamber views and the subcostal views. The parasternal short-axis views will be recorded at the basal (mitral valve level), mid (papillary muscle level), and apical positions. Subject angulation and transducer position will be recorded for serial examinations.

End-diastolic wall thickness will be measured from the parasternal long- and short-axis views. Wall motion analysis will be performed using the 16-segment model proposed by the American Society of Echocardiography. Each wall segment will be scored using a visual grading system (1=normal, 2=hypokinetic, 3=akineti c, 4=dyskinetic, 5=aneurysmal). A wall motion score index (WMSI) will be defined as the average wall motion score for all segments divided by the number of segments analyzable. A percentage of wall motion abnormalities (%WMA) will be obtained by dividing the number of akinetic, dyskinetic, and aneurysmal segments by the total number of segments evaluated.

Left ventricular volumes will be determined at end-diastole and end-systole. Endocardial borders will be manually traced from apical four-chamber and two-chamber views and the volumes obtained will be used to calculate ejection fraction using the biplane summation-of-disks method recommended by the American Society of Echocardiography.

In addition, we will perform 3D echocardiography which can simultaneously integrate the effects of radial, circumferential and longitudinal contraction of all 17 myocardial segments on cardiac dyssynchrony. A semi automated detection process, through endocardial traces of 2D subsections of the full volume data, can be used to generate a mathematically based "cast" of the LV cavity that provides time volume data for the entire cardiac cycle. These time volume data are subsequently divided into time volume estimates for each of the 17 standard segments. The echocardiogram procedures are described in detail in Appendix A (Transthoracic Echocardiogram SOP).

6. ADVERSE EVENT MANAGEMENT

6.1 Definition of an Adverse Event

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

Examples of an AE include:
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency or intensity of the condition.
- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- A new condition detected or diagnosed after study therapy administration even though it may have been present prior to the start of the study.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (e.g., invasive protocol-defined procedures, modification of a patient’s previous treatment regimen).

An AE does not include:

- Medical or surgical procedures (e.g., colonoscopy, biopsy). The medical condition that leads to the procedure is an AE.
- Social or convenience hospital admissions where an untoward medical occurrence did not occur.
- Day to day fluctuations of pre-existing disease or conditions present or detected at the start of the study that do not worsen.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied unless more severe than expected for the patient’s condition.

6.2 Definition of a Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse experience occurring at any dose that:

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

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

4. results in disability/incapacity

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

5. is a congenital anomaly/birth defect.

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

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

Abnormal laboratory findings (e.g. clinical chemistry, hematology, urinalysis) or other abnormal assessments (e.g., ECGs, vital signs) that are judged by the Investigator as clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE as defined in Section 6.1 (“Definition of an Adverse Event”) or SAE, as defined in Section 6.2 (“Definition of a Serious Adverse Event”). Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the Investigator as more severe than expected for the patient’s condition, or that are present or detected at the start of the study but do not worsen, will not be reported as AEs or SAEs.

The Investigator will exercise medical judgment in deciding whether abnormal laboratory values are clinically significant.

6.4 Recording of Adverse Events and Serious Adverse Events

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

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

Pregnancies

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

6.5 Intensity of Adverse Events and Serious Adverse Events

The Investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the Investigator’s clinical judgment. The intensity of each AE and SAE should be assigned to one of the following categories:
Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is described as ‘serious’ when it meets one of the pre-defined outcomes as described in Section 6.2, “Definition of an SAE.”

6.6 Relationship of Adverse Events and Serious Adverse Events to Study Therapy

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

All AE/SAE that occur during the course of the clinical study will be evaluated and a determination of relatedness to the investigational product will be defined according to one of the following categories:

- **Definite** - The AE/SAE is clearly related to the investigational product.
- **Probable** - The AE/SAE is likely related to the investigational product.
- **Possible** - The AE/SAE may be related to the investigational product.
- **Unlikely** - The AE/SAE is doubtfully related to the investigational product.
- **Unrelated** - The AE/SAE is clearly NOT related to the investigational product.

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

6.7 Follow-Up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each patient and provide further information on the patient’s condition. All AEs and SAEs documented at a previous visit/contact that are designated as ongoing will be reviewed at subsequent visits/contacts.
Adverse events and SAEs will be followed until resolution, until no further changes in the event are expected (i.e. the point at which a patient experiencing a critical adverse event is treated successfully and stabilized even though they may continue to experience lingering sequelae that may never resolve), until the patient is lost to follow-up, or until it is agreed that further follow-up of the event is not warranted (e.g. non-serious, study therapy unrelated, mild or moderate adverse events ongoing at a patient’s final study visit). If a patient dies during participation in the study or during a recognized follow-up period, the Investigator will provide a copy of any post-mortem findings, including histopathology.

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

### 6.8 Timeframes for Submitting SAE Reports

Once an Investigator becomes aware that an SAE has occurred in a study patient, he/she will record the information in the electronic data record within 48 hours. Any fatal or life-threatening event must be reported within 24 hours. If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before recording the event in the data system and completing as much information known at the time of the submission. The reporting timeframes for any SAE occurring during the study are summarized in Table 4.

<table>
<thead>
<tr>
<th>Type of SAE</th>
<th>Reporting Timeframes</th>
<th>Documents Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatal or Life-Threatening</td>
<td>24 hours</td>
<td>24 hours: Complete as much information in the electronic data system that is known. 48 hours: Fully complete all AE forms</td>
</tr>
<tr>
<td>Other SAEs</td>
<td>48 hours</td>
<td>Fully completed AE forms</td>
</tr>
<tr>
<td>Any SAE</td>
<td>48 hours</td>
<td>Updated AE Forms</td>
</tr>
</tbody>
</table>

### 6.9 Post-Study Adverse Events and Serious Adverse Events

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

6.10 Regulatory Aspects of Adverse Event Reporting

The Investigator will promptly report all SAEs within the timeframes specified in Section 6.8. Prompt notification of SAEs by the Investigator is essential so that UMMSM can meet legal obligations and fulfill ethical responsibilities towards the safety of all patients participating in UMMSM-sponsored investigational trials.

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

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

The ESRs are prepared according to UMMSM policy and are forwarded to the Investigator as necessary. The purpose of the ESR is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements regarding the product under investigation.

6.11 Monitoring of Adverse Events

The following list summarizes the Contract Research Organization’s (CRO’s) role in monitoring AE/SAEs:

- All SAEs will be reviewed by the Medical Monitor at the CRO within 1 business day of receiving the adverse event form (or MedWatch form) from the clinical center.
- If the Medical Monitor requires additional information to make his/her assessment, the clinical centers will have 2 business days to respond to the request for additional information.
- The CRO is responsible for notifying the NHLBI Project Officer immediately of all SAEs, regardless of attribution, and of any concerns regarding the frequency or type of SAE(s) on a study or treatment arm.
- The attribution, as assessed by the clinical center and the CRO Medical Monitor, will be provided to the NHLBI Project Officer within 2 business days of receiving the report.
- The NHLBI Project Officer will forward all SAEs, at least possibly related, to the DSMB Executive Secretary for distribution to the DSMB chair.

The NHLBI Project Officer (or designee) is responsible for reviewing the SAE materials to determine if the documents are complete. If there are any concerns regarding the type or frequency of the event, the NHLBI Project Officer will request that the DSMB Executive Secretary notify the DSMB chair. The DSMB chair will review the SAE materials, determine if additional DSMB review is required and make recommendations concerning continuation of the study.
The DCC will prepare semi-annual summary reports of all AEs/SAEs for the NHLBI Project Officer and DSMB Chairman. Semi-annual reports will be made available on a secure website; the DSMB Chair will be notified by e-mail when the materials are posted.

**7. DATA COLLECTION AND STATISTICAL ISSUES**

This section describes methods for randomization, data collection, sample size determination, analysis populations, and planned analyses for safety and efficacy endpoints.

**7.1 Enrollment and Randomization**

Patients will be registered using the following procedures:

1. An authorized user completes the initial screening by entering patient demographics and inclusion/exclusion criteria of the Eligibility Form approximately 2-10 weeks prior to the cardiac catheterization.
2. If the patient is eligible, a study number and random treatment assignment is generated.

**7.2 Randomization**

Patients will be randomized to treatment strategy (Auto-MSCs: Groups 1a, 2a and 3a; or Allo-MSCs: Groups 1b, 2b and 3b) in a 1:1 ratio. Randomization will be stratified by site. The treatment assignment will be sent via email to the Cell Therapy Laboratory, which will have the table that identifies the treatment associated with the treatment number. A visit schedule based on treatment start date is prepared and displayed for printing.

**7.3 Data Collection**

A description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide. The Investigator or designee must record all required patient data, and an explanation must be documented for any missing data.

**Follow-up Assessments**: The timing of follow-up visits is based on the date of the cardiac catheterization procedure. Following catheterization, a Patient Visit Schedule listing target dates for assessments will be prepared.

**7.4 Study Design and Sample Size Considerations**

The primary focus of this study is to assess the 30-day post-cardiac catheterization SAE proportion as defined in Section 2. Thirty patients (15 in each arm; Groups A and B) will be enrolled over a 12-18 month accrual period. The first dose level of 10 patients will be randomized to receive either $0.2 \times 10^8$ (20 million) Auto-hMSCs (Group 1a) or $0.2 \times 10^8$ (20 million) Allo-hMSCs (Group...
1b) followed by a second dose level of 10 patient randomized to receive either $1.0 \times 10^8$ (100 million) Auto-hMSCs (Group 2a) or $1.0 \times 10^8$ (100 million) Allo-hMSCs (Group 2b) ending with a third dose level of 10 patients randomized to receive either $2.0 \times 10^8$ (200 million) Auto-hMSCs (Group 3a) or $2.0 \times 10^8$ (200 million) Allo-hMSCs (Group 3b). The DSMB will evaluate the SAE proportion after the tenth patient of each dose level is followed for 30 days. The DSMB will make the decision to continue to the next dose level.

The total sample size is 30 patients for the trial equally distributed between Auto-hMSC and Allo-hMSC in a 1:1 ratio. As a result, the sample size and power considerations will be based on the 15 subjects infused with the respective cell doses. Exact binomial ninety-five percent confidence intervals were calculated for varying number of events based on the sample size. Table 5 provides confidence intervals for a variety of true underlying number of events. Of particular interest, is the anticipated 30 day proportion of treatment-emergent serious adverse events (TE-SAE) as defined in 2.2.1. The underlying rate of 30-day TE-SAE is 25% which translates into between 3 and 4 patients with events out of 15 patients. For the setting of 3 events, the confidence interval is 0.04-0.48. The probabilities above and below 3 represent other plausible scenarios.

Exact power computations based on the binomial distribution were conducted using the SAS system version 9.1. The precision of the estimates could be viewed as a lower bound on the number of events reported. The probability to rule out the number of patients experiencing a TE-SAE of a certain size can be interpreted as “power.” Table 4 provides the probability (or power) that the lower bound of a 95% two-sided confidence interval for the primary endpoint will be greater than thresholds of 1, 2, 3, 4, 5, 6, and 7 events. When the true number of events is 3, there is 65% power to rule out a number of patients experiencing the primary endpoint of 8 or higher.

<table>
<thead>
<tr>
<th>Patients randomized to the first and second dose group</th>
<th>Number of patients experiencing the primary endpoint</th>
<th>Exact 95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1</td>
<td>0.002, 0.32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.02, 0.40</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.04, 0.48</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.08, 0.55</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.12, 0.62</td>
</tr>
</tbody>
</table>
Table 6. Probability of Ruling Out a Threshold of Size T or larger for Various Sample Sizes and True Underlying SAE Proportion of Patients Experiencing the Primary Endpoint

<table>
<thead>
<tr>
<th>N</th>
<th>True Number of Events</th>
<th>Probability of Ruling Out Events of Size T or Larger</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td></td>
<td>1  2   3   4   5   6   7   8</td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.01     &lt;0.01     0.35   0.73   0.73   0.93   0.99</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.12      &lt;0.01     0.12   0.40   0.40   0.69   0.88</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.35      0.06      0.04   0.17   0.17   0.40   0.65</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.60      0.19      0.08   0.06   0.06   0.19   0.40</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.78      0.37      0.20   0.03   0.02   0.08   0.22</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.91      0.60      0.39   0.10   0.04   0.03   0.09</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.97      0.79      0.61   0.23   0.10   0.04   0.03</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.99      0.90      0.77   0.39   0.21   0.09   0.03</td>
<td></td>
</tr>
</tbody>
</table>

7.5 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, ejection fraction, Six-minute walk test performance, peak VO₂, etc.

7.6 Analysis of the Primary Endpoint

Descriptive analyses of the primary endpoint will be performed. Point estimates and confidence intervals of TE-SAE proportion by treatment arm and dose-level will be computed. Event rates will be compared across treatment arms and against historical data, recognizing that in this limited sample size only very large differences can be detected. The frequency, severity, timing and the potential relationship to the intervention will be assessed in order to characterize the safety of the intervention.

7.7 Stopping Guidelines

The proportion of patients experiencing TE-SAE as defined in 2.2.1 will be monitored within 30 days of infusion. This guideline is to be used to indicate boundaries requiring discussion by the NHLBI-appointed Data and Safety Monitoring Board (DSMB) and is designed to assist the independent DSMB in overseeing the study. The DSMB may also request additional interim analyses and develop other criteria including provision for monitoring of potential late
effects to determine when to intervene in the enrollment or treatment of patients in the study.

Monitoring of key safety endpoints will be conducted. If rates significantly exceed the pre-set threshold, then the NHLBI will be notified in order that the DSMB can be advised.

A Bayesian motivated safety stopping guideline will be used for this trial. The expected underlying TE-SAE at 30 days post-catheterization probability is assumed to be 25% and that a probability of greater than 45% is unacceptable. The difference in event rates of 25% and 45% is a 80% increase.

A Beta distribution can be used as the prior distribution of \( \theta \); where \( \theta \) is the proportion of patients who experience a TE-SAE. The stopping rule is based on the beta-binomial methodology and assumes a prior expected failure rate. This leads to prior Beta parameters where \( a=3.6 \) and \( b=10.8 \). The Beta distribution will have a prior mean of 0.25 and a prior probability of <0.05 of exceeding 0.45. The guideline is derived such that there is strong evidence (posterior probability >0.95) that the probability of the event is greater than 45%, the trial will be stopped. The resulting boundaries tabulated in table 7 were rounded to be conservative with the stopping guideline and is considered after 5 patients are enrolled on the study.

### TABLE 7

**Bayesian Stopping Guideline for Event Rate of 25%**

<table>
<thead>
<tr>
<th># Events</th>
<th># Patients in Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4-6</td>
</tr>
<tr>
<td>5</td>
<td>7-9</td>
</tr>
<tr>
<td>6</td>
<td>10-12</td>
</tr>
<tr>
<td>7</td>
<td>13-15</td>
</tr>
</tbody>
</table>

* The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

A simulation study was conducted to evaluate the operating characteristics of this stopping rule. Data were generated from the binomial distribution with varying probabilities of failure (\( \theta \)) and assuming a sample size of 25 patients. Table 8 shows the probability of stopping the trial early and the average sample size (N), conditional on stopping early, at which the boundary is crossed for each value of
θ. The unconditional average sample size of the trials for each value of θ is displayed.

### Table 8

**Operating Characteristics for Bayesian Motivated Stopping Guideline**

<table>
<thead>
<tr>
<th>Mean of Prior Distribution</th>
<th>θ</th>
<th>Probability of stopping</th>
<th>Conditional Average Sample Size (N)</th>
<th>Unconditional Average Sample Size of Trials (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>0.10</td>
<td>9.1</td>
<td>14.4</td>
</tr>
<tr>
<td>0.30</td>
<td>0.19</td>
<td>9.1</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>0.35</td>
<td>0.32</td>
<td>9.2</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>0.47</td>
<td>9.0</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>0.45</td>
<td>0.62</td>
<td>8.8</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>0.75</td>
<td>8.4</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>0.55</td>
<td>0.85</td>
<td>7.9</td>
<td>9.0</td>
<td></td>
</tr>
</tbody>
</table>

Although the motivation for the boundary is Bayesian, the operating characteristics can be evaluated from a frequentist perspective of Type I error and power. The stopping rule for a 25% event rate has a 10% chance (“Type I error”) of suggesting early termination when the true rate is 0.25, and a 62% chance (“power”) when the true rate is 0.45, rising to 85% when the true rate is 0.55.

### 7.8 Analysis of Secondary Endpoints

Descriptive analyses of all secondary endpoints will be performed using point and confidence interval estimation. Treatment effects will be assessed using appropriate methods for continuous, dichotomous, or ordinal categorical data, such as the Wilcoxon, Fisher's exact, and linear rank tests. Graphical methods will be used to describe the time course of repeated measurements.

### 7.9 Data and Safety Monitoring Board (DSMB)

Interim analyses will be conducted at times coincident with regularly scheduled meetings of the Data and Safety Monitoring Board (DSMB) at approximately six-month intervals. The DSMB Chair will be notified each time an SAE occurs. The DSMB will evaluate unblinded AE data (including SAEs) in each Group (A or B) at the following pre-specified intervals: (1) when 5 patients are enrolled in each group and completed 1 month of follow-up. Other safety data, such as 48-hour
ambulatory ECGs and laboratory data will also be evaluated by the DSMB as appropriate. Monitoring of key safety endpoints will be conducted as described above, and if rates significantly exceed pre-set thresholds, the DSMB Chair will be notified and information will be supplied to the DSMB. Policies of the DSMB will be described in the DSMB Charter, which will be prepared by the DSMB prior to study initiation. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

8. NORMAL DONORS FOR GENERATION OF ALLOGENEIC MSC

The availability of allogeneic MSC (allo-MSC) offers the potential for an “off the shelf” product for patients. Significant data has been generated (see Background Section 2) to demonstrate that the allo-MSC are immuno suppressive. In addition, allo-MSC are immuno privilege and can be infused without immune rejection despite disparate HLA phenotypes. BM aspirates will be obtained from maximum of 15 normal individuals and MSC isolated and expanded.

8.1 Bone Marrow Aspiration for Generation of Allo-MSC

BM will be obtained from normal volunteers with 60 ml aspirated from the posterior iliac crest. The BM will be aspirated into heparinized syringes. The MNC fraction will be isolated using a density gradient with Lymphocyte Separation Media (specific gravity 1.077). The low-density cells will be collected and washed with Plasma-Lyte A containing 1% HSA. The washed cells will be samples and viable cell numbers determined. The BM MNC will be seeded into 225 cm² tissue culture flasks in alpha MEM containing 20% FBS. After 14 days of culture, passage zero (P0) cells will be harvested by trypsin treatment and expanded into 60 flasks. These flasks are incubated for a further 7 to 10 days and the MSC harvested by trypsin treatment (P1 cells).

8.2 Normal Donor Eligibility

Male donors between the ages of 20 to 35 will be screened as potential BM donors (maximum of 15) and shall be evaluated by history and physical examination, as well as, completion of the National Marrow Donor Program (NMDP) questionnaire. The history shall include the following:

- History of malignancy
- Bleeding abnormalities
- Deep venous thrombosis
- Cardio/pulmonary conditions
- Blood transfusions
- Vaccinations
- Questions to identify persons at risks of infectious disease transmission
- Questions to identify persons at risk of transmitting hematological or immunological disease
The physical examination shall be completed and include evaluation for potential risk of the BM aspiration procedure.

Prospective donors shall have infectious Disease Testing including:

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

Prospective donors shall have the following blood tests:

- CBC, differential, platelet count
- Creatinine, ALT, bilirubin, alkaline phosphatase, glucose, BNP and uric acid
- Na, K, Cl, Mg, calcium

**Eligibility Criteria for Normal Donors**

- No history of malignancy
- No active coagulopathy and/or hypocoagulable state
- No history of cardio/pulmonary conditions
- Negative tests for Hepatitis B, Hepatitis C, RPR, Chagas, HIV ½, HTLV I/II and NAT for HCV, HIV, and WNV
- Hemoglobin ≥ 13.0 g/dL
- Platelet count 140,000 to 440,000/ul
- WBC 3.0 to 11.0 K/ul
- No anomalies on the CBC and differential suggestive of a hematopoietic disorder
- Creatinine ≤ 1.5 mg/dL
- ALT ≤ 112 IU/L
- Bilirubin < 1.5 mg/dL
- No diabetes
- Systolic blood pressure ≤ 170
- Diastolic blood pressure ≤ 90
- No history of autoimmune disorders

**8.3 Donor Consent**
Informed consent shall be obtained and documented. The procedure shall be explained in terms the donor can understand, and shall include information about the significant risks of the procedure. The donor shall have the right to review the results of tests.

The donor shall have the opportunity to ask questions and the right to refuse to donate.

8.4 Follow-up Schedule for Donors

After discharge from the hospital, the bone marrow donor will be contacted by the study team with periodical follow-up telephone calls over a period of one year to determine the well-being and health status of the donor. The donor will be provided with contact telephone numbers in the consent form for any questions or comments.

9. STUDY ADMINISTRATION

9.1 Regulatory and Ethical Considerations

9.1.1 Regulatory Authority Approval

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

9.1.2 Ethics Approval

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

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

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

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

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

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

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

9.2. Confidentiality of Information

Patients' names will remain confidential and will not be included in the database. Only patient number, patient initials, and birth date will be recorded in the data system. If the patient name appears on any other document collected (e.g., hospital discharge summary), the name must be obliterated before the document is transmitted. All study findings will be stored in electronic databases. The patients will give explicit permission for representatives of regulatory authorities and the IRB/IEC to inspect their medical records to verify the information collected.

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

All participants in the United States will provide written authorization to disclose private health information either as a part of the written ICF or as a separate authorization form. The authorization will contain all required elements specified by 45 CFR 164, and will contain a waiver of patient access to study-related private health information until the conclusion of the clinical study. The authorization will remain valid and in full force and effect until the first to occur of (1) the expiration of 2 years after the study therapy is approved for the indication being studied, or (2) the expiration of 2 years after the research program is
discontinued. Individual patient medical information obtained during this study is confidential and its disclosure to third parties (other than those mentioned in this Section 9.7) is strictly prohibited. In addition, medical information obtained during this study may be provided to the patient's personal physician or to other appropriate medical personnel when required in connection with the patient's continued health and welfare.

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

### 9.3 Payments to Patients

Patients will be reimbursed $25 at the end of each follow-up visit (months 1-6, month 12 and month 13) for a total remuneration of $200. These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

Normal donors for generation of allo-MSC will be reimbursed $350 at the end of BM aspiration. This payment will compensate donors for lost time, parking and travel expenses.

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