Clinical Research Protocol

Title: A Phase I/II, Randomized, Double-Blinded, Placebo-Controlled Study of the Safety and Efficacy of Intramyocardial Injection of Autologous Human Mesenchymal Stem Cells (MSCs) in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction (MI) Undergoing Cardiac Surgery for Coronary Artery Bypass Grafting (CABG).

Investigational Therapy Name: Autologous Human Mesenchymal Stem Cells (MSCs)

FDA IND No.: BB-IND #13421

Protocol No.: SCCT-07-001 (Version 2 – updated November 21, 2007)

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Protocol Agreement Signature:

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Joshua M. Hare, MD                                      Date

Principal Investigator, Project 1, SCCT

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<td>AE</td>
<td>adverse event</td>
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<tr>
<td>AMBMC</td>
<td>autologous mononuclear bone marrow cells</td>
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<td>BMC</td>
<td>bone marrow cells</td>
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<tr>
<td>BSC</td>
<td>biologic safety cabinet</td>
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<td>C of A</td>
<td>Certificate of Analysis</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass graft</td>
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<td>CAD</td>
<td>coronary artery disease</td>
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<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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<td>CK-MB</td>
<td>creatine kinase – mb</td>
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<td>CRO</td>
<td>contract research organization</td>
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<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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<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<tr>
<td>DSMB</td>
<td>Data and Safety Monitoring Board</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>EF</td>
<td>ejection fraction</td>
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<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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<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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<td>GCP</td>
<td>Good Clinical Practice</td>
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<td>G-CSF</td>
<td>granulocyte colony stimulating factor</td>
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<td>HARP</td>
<td>Harmonic Phase</td>
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<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
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<tr>
<td>MSC</td>
<td>human mesenchymal stem cell</td>
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<td>HTLV</td>
<td>human T-cell lymphotropic virus</td>
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<td>HAS</td>
<td>human serum albumin</td>
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<tr>
<td>HSC</td>
<td>hematopoietic stem cell</td>
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<tr>
<td>ICAM</td>
<td>intracellular adhesion molecule</td>
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<tr>
<td>ICD</td>
<td>implantable cardioverter-defibrillator</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>-----------</td>
<td>-------------------------------------------------------</td>
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<tr>
<td>ICF</td>
<td>informed consent form</td>
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<tr>
<td>ICH</td>
<td>International Conference on Harmonisation of Technical</td>
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<td></td>
<td>Requirements of Pharmaceuticals for Human Use</td>
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<tr>
<td>IDM</td>
<td>Infectious Disease Markers</td>
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<tr>
<td>IEC</td>
<td>institutional ethics committee</td>
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<tr>
<td>IND</td>
<td>Investigational New Drug application</td>
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<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>I.V.</td>
<td>Intravenous</td>
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<tr>
<td>KDR</td>
<td>VEGF receptor-2</td>
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<tr>
<td>LAD</td>
<td>left anterior descending artery</td>
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<td>LV</td>
<td>left ventricular</td>
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<tr>
<td>LVAD</td>
<td>left ventricular assist device</td>
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<td>MACE</td>
<td>Major adverse cardiac events</td>
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<tr>
<td>MEM</td>
<td>minimum essential medium</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>MI</td>
<td>myocardial infarction</td>
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<tr>
<td>MLHF</td>
<td>Minnesota Living with Heart Failure</td>
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<tr>
<td>MR</td>
<td>magnetic resonance</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>MSC</td>
<td>mesenchymal stem cell</td>
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<tr>
<td>NHLBI</td>
<td>National Heart, Lung and Blood Institute</td>
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<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
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<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
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<tr>
<td>QA</td>
<td>quality assurance</td>
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<td>QC</td>
<td>quality control</td>
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<tr>
<td>SAE</td>
<td>serious adverse event</td>
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<tr>
<td>SCCT</td>
<td>Specialized Center for Cell-Based Therapy</td>
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<tr>
<td>SCF</td>
<td>stem cell factor</td>
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<tr>
<td>SDF-1</td>
<td>stromal cell derived factor 1</td>
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<tr>
<td>SOP</td>
<td>standard operating procedures</td>
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<tr>
<td>SW</td>
<td>Stroke Work</td>
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<tr>
<td>TTC</td>
<td>triphenyltetrazolium chloride</td>
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<tr>
<td>U.S.</td>
<td>United States</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<tr>
<td>Peak VO\textsubscript{2}</td>
<td>peak oxygen consumption</td>
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WBC  white blood count
SYNOPSIS

**Sponsor:** Johns Hopkins / University of Miami Miller School of Medicine SCCT

**Name of Study Therapy:** Autologous Human Mesenchymal Stem Cells (MSCs)

**Title of Study:** A Phase I/II, Randomized, Double-Blinded, Placebo-Controlled Study of the Safety and Efficacy of Intramyocardial Injection of Autologous Human Mesenchymal Stem Cells (MSCs) in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction (MI) Undergoing Cardiac Surgery for Coronary Artery Bypass Grafting (CABG).

**Study Centers:** University of Miami Miller School of Medicine & Interdisciplinary Stem Cell Institute & The Johns Hopkins University School of Medicine

**Phase of Development:** Phase I/II

**Objectives:**

Primary: To demonstrate the safety of autologous MSCs administered by intramyocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to myocardial infarction (MI) undergoing cardiac surgery for CABG.

Secondary: To demonstrate the efficacy of autologous MSCs administered by intramyocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI undergoing cardiac surgery for CABG.

**Design and Investigational Plan:** Forty-five (45) patients scheduled to undergo cardiac surgery for CABG meeting all inclusion/exclusion criteria will be evaluated at baseline. Patients will be randomized to either MSCs in one of two doses: 1) 2 million cells, administered in 0.25 cc to 0.5 cc injections times 10 to 20 injections for a total of $2 \times 10^{7}$ (20 million) cells or 2.) 20 million cells, administered in 0.25 cc to 0.5 cc injections times 10 to 20 injections for a total of $2 \times 10^{8}$ (200 million) cells or to placebo (phosphate-buffered saline [PBS] and 1% human serum albumin [HSA]). The surgeon-investigators will have discretion to administer 10 to 20 separate injections; applying the volume of each 0.5 cc syringe at either 1 or 2 sites. Thus, each injection will be either 0.5 or 0.25 cc. The Study Team will record and maintain a detailed record of the locations and actual number of injections. Patients will be stratified by study site and randomized approximately 3-5 weeks prior to planned CABG surgery. Patients will be randomized using permuted blocks to one of the three arms in a 1:1:1 ratio (placebo, low dose, and high dose, respectively), providing a projected distribution of 15:15:15 patients among the groups. If the patient is randomized to a dose and the MSCs do not expand to the required dose, each syringe will contain the maximal number of cells available, but the total number of syringes will be 10, and the total overall injection volume will not exceed 5 cc. The injections will be administered epicardially using a syringe and needle following the completion of the cardiac surgical procedure (CABG). Autologous MSCs will be obtained from the patients via bone marrow aspiration approximately 3-5 weeks prior to the cardiac surgery. Following the cardiac surgical procedure and MSC (or placebo) injections, patients will be followed at monthly intervals for six months, and at 12 and 18 months to complete the safety and efficacy assessments listed below.

**Patient Population:** Forty-five patients with chronic ischemic left ventricular dysfunction secondary to MI scheduled to undergo cardiac surgery for CABG.

**Diagnosis and Main Criteria for Inclusion/Enrollment:**

**Major Inclusion Criteria**

- Diagnosis of chronic ischemic left ventricular dysfunction secondary to MI.
- Scheduled to undergo cardiac surgery for CABG.
- Ejection fraction between 20% and 50%.
• Have an akinetic or dyskinetic region by standard imaging.

**Major Exclusion Criteria**

• Baseline glomerular filtration rate < 50 ml/min/1.73m².
• Contra-indication to performance of a magnetic resonance imaging scan.
• Bone marrow dysfunction, as evidenced by evidenced by a 20% or more deviation from normal hematocrit, white blood cell count or platelet values without another explanation.
• A coagulopathy condition not due to a reversible cause (i.e., Coumadin).
• 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 B, or Hepatitis C.
• Female who is pregnant, nursing, or of child-bearing potential while not practicing effective contraceptive methods.

**Definition of Endpoints:**

- **Safety (Primary):** Incidence of serious adverse events (SAEs) defined as the six-month post-CABG surgery serious adverse event (SAE) proportion of patients experiencing sustained ventricular arrhythmias, characterized by ventricular arrhythmias lasting longer than 15 seconds or with hemodynamic compromise, ectopic tissue formation, or sudden unexpected death.

- **Safety (Additional):**
  (During the six-month follow-up period and final month 18 visit)
  • Treatment emergent adverse event (AE) rates.
  • 48-hour ambulatory electrocardiogram (ECG) recordings.
  • Hematology, clinical chemistry, and urinalysis values.
  • Pulmonary function – forced expiratory volume in 1 second (FEV1) results.
  • Serial troponin and CK-MB values (first 48 hours post CABG surgery).
  • Post-CABG surgery echocardiogram (day 2 post-op).

- **Efficacy (Secondary):**
  (During the six-month follow-up period and final month 18 visit)
  • Magnetic resonance imaging (MRI) and echocardiographic measures of Infarct Scar Size (ISS), and left regional and global ventricular function.
  • Cardiac CT measures of ISS, LVEF, and end diastolic and end systolic volumes.
  • Peak VO₂ (by exercycle 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
<table>
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<th>composite incidence of (1) death, (2) hospitalization for heart failure, or (3) non-fatal recurrent MI.</th>
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**Study Therapy:** Autologous human MSCs, obtained from each patient approximately three to five weeks prior to the cardiac surgical procedure.

**Duration of Study Follow-Up:** Monthly visits for the first 6 months, and at 12 and 18 months.
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\(^2\text{-}^4\). 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 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\(^6\text{-}^7\), tissue engineered contractile grafts\(^8\), skeletal myoblasts\(^9\), several cell types derived from adult bone marrow\(^10\text{-}^15\), and cardiac precursors resident within the heart itself\(^16\). There has been substantial clinical development of the use of whole bone marrow and skeletal myoblast preparations in trials enrolling both post-infarction patients and patients with chronic ischemic left ventricular dysfunction. The effects of bone-marrow derived mesenchymal stem cells (MSCs) have also been studied in clinical trials.

**Cells derived from adult bone marrow**

Bone marrow harbors a variety of cells that potentially contribute to vasculogenesis and 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 and ability to divide in culture and to differentiate into mesodermal lineages\(^17\), 3.) cells that express stem cell factor receptor, c-Kit\(^18\). Endothelial progenitor cells (EPCs) express the surface markers CD34, CD133, ckit, and the vascular endothelial growth factor receptor-2 (VEGFR2; KDR; Flk-1)\(^19\text{-}24\). 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\(^-)\) (review \(^25\)). While there has been controversy regarding the ability of bone-marrow derived cells to transdifferentiate into cardiomyocytes\(^26\), clinical trials of whole bone marrow therapies continue to suggest potential benefit in terms of improving cardiac function and 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 record of safety and efficacy\(^27\). Studies of MSC engraftment in rodent and swine models of myocardial infarction demonstrate: 1)
functional benefit in post-myocardial infarction (MI) recovery with administration 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 organization(28;29).

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 factor(30;31). Some, but not all, groups have purified MSCs expressing c-Kit directly from bone marrow that have the capacity to form cardiac myocytes. This is of functional significance given the demonstration that stem cell factor stimulates cardiac repair post-MI(32).

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 left ventricular dysfunction.

Previous Human Experience with Skeletal Myoblasts

There are several case reports and very small phase I clinical trials investigating the feasibility of autologous skeletal myoblast transplantation(33-35) 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 fraction(36;37), increased wall thickening(38), and New York Heart Association (NYHA) functional class(39;40). However the lack of electromechanical coupling between engrafted skeletal myoblasts and cardiac myocytes in vivo(41;42) has raised serious concerns over the likelihood of an increase in ventricular tachyarrhythmias secondary to the formation of re-entry circuits(43-45). 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(46).

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)(47). 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 (AMBMC)
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(48) injected bone marrow mononuclear cells into the gastrocnemius of patients with lower extremity ischemia; the study demonstrated significant improvement in ankle-brachial pressure index, rest pain, and pain free walking time, and the investigators concluded that the efficacy of implantation of these 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 also decreased the size of perfusion defects as assessed with 201 thallium scintigraphy by 26% (174±99cm² to 128±71 cm²) compared to baseline values(49). Subsequently, Stamm et al. demonstrated similar improvements in perfusion, left ventricular (LV) dimensions, and ejection fraction (EF) in an uncontrolled, non-blinded phase I study in 12 patients with transmural MI and LV dysfunction (EF of 39.7±9%). These patients had infarct areas not amenable to surgical or interventional revascularization; they received intraoperative AMBMC injection during elective CABG performed to bypass occlusions of coronary arteries other than the infarct vessel in the first 3 months post-MI(50,51). In the TOPCARE-AMI(52) trial, post-MI patients were randomized to receive either AMBMC (n=9) or peripheral blood derived progenitor cells (n=11) 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 included 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(53). 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 either 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 group (46.3±10.6% at baseline to 53.0±15.5% at 6 months) as compared to only a 1.1±11.8 % increase in the control group (47.8±9.7% at baseline to 48.9±15.2%; p=0.0026). Furthermore stem cell transplantation was also associated with increased systolic wall motion in the MI border zone\(^{(54)}\). Importantly, infarct size as measured by late enhancement magnetic resonance imaging (MRI) was not reduced in the cell therapy, as compared to placebo in 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\(^{(55)}\).

In the REPAIR AMI study, the largest trial of bone marrow-derived cellular therapy to date, Schächinger \textit{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\(^{(56)}\). Other studies suggest relatively less benefit in EF than that reported above, although AMBMCs appeared to reduce infarct size\(^{(57)}\).

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\(^{(58)}\). The combined results of these studies support the clinical safety of administering both BMC and MSC preparations for cardiac repair.

\textbf{AMBMCs In Chronic Ischemia:} There are several small studies investigating the safety and feasibility of autologous bone marrow cell transplantation for patients with ischemic heart disease\(^{(59-64)}\).

Hamano and colleagues performed a non-randomized study of direct injection of AMBMC into the ungraftable or peri-infarct myocardium during CABG in five patients and reported improved perfusion of the treated areas up to one year after surgery\(^{(65)}\). Ozbaran and colleagues injected peripheral blood stem cells mobilized with granulocyte colony stimulating factor (G-CSF) into the myocardium of 6 patients with severe ischemic cardiomyopathy (EF <25%) demonstrating significant improvements in NYHA functional class and quality of life \(^{(66)}\). However, it is important to note that it is difficult to determine how much of the improved perfusion is secondary to the stem cells as opposed to the surgical revascularization.

In a randomized, crossover trial known as TOPCARE-CHD, Assmus \textit{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 small non-randomized studies using sample sizes of fewer than 20 patients have performed catheter based injections of AMBMC into chronically ischemic myocardium and demonstrated improved myocardial function and perfusion as well as symptoms\(^{68-70}\). Perin and colleagues performed a nonrandomized, open-labeled study comparing AMBMC injection (n=14) to standard therapy (n=7) in 21 patients with ischemic heart failure. They used a NOGA\(^{\text{TM}}\) catheter to percutaneously inject AMBMC into the hibernating myocardium of patients with severe ischemic left ventricular dysfunction and reported a 73% reduction in the total reversible perfusion defect, improved mechanical function of injected segments as determined by electromechanical mapping, improved EF (9%) and global LV function, as well as improved NYHA functional class and Canadian Cardiovascular Society Angina score\(^{71}\). Together these results support ongoing research into AMBMC transplant for patients with chronic ischemic cardiomyopathy\(^{72}\).

**Previous Human Experience With Autologous or Allogeneic Human Mesenchymal Stem Cells (MSCs)**

MSCs have also been studied clinically. 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.*\(^{73}\), 69 patients were randomly assigned to receive IC infusions of autologous MSCs (average cell dose: \(5.4 \times 10^{10}\)) 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 \(5 \times 10^7 - 5 \times 10^8\) cells, since the MSC fraction is generally considered to be 0.1 – 1.0 % of a whole bone marrow aspirate.

Katritsis *et al.*\(^{74}\) 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\(^{99m}\) 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\(^{(75)}\) was performed in 53 patients who were treated 3-10 days post-MI. Patients were administered one of three cell-dose levels of allogeneic MSCs (0.5, 1.6, and 5.0 x 10^6 cells/kg; corresponding to 3.5 x 10^7, 1.1 x 10^8, and 3.5 x 10^8 cells per patient for a 70 kg body weight patient), or placebo via peripheral IV injection, and followed for six months. There were no deaths in the study population, no toxicity 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 attributed to MSC administration. In fact, several signals from the trial indicated that the allogeneic MSCs were not only very safe, but 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 compared to placebo across all time points (11% vs. 24%, \(p < 0.001\)).

- The MSC-treated patients with 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 EF in similar patients receiving placebo did not significantly improve.

- Patients in the MSC-treated group had significantly improved pulmonary function as measured by FEV1 (% predicted), which increased 17% in the MSC-treated group vs. 6% in the placebo-treated group, \(p < 0.05\).

- Significantly more patients who received the MSCs experienced improvement in their overall clinical status at six months compared to those receiving placebo (42% vs. 11%, \(p=0.027\)).

**Previous Human Experience with Intramyocardial Injections**

Although no clinical study has been performed using intramyocardial injection of autologous MSCs in chronic left ventricular dysfunction, there is a substantial clinical experience with the intramyocardial delivery of autologous bone marrow-derived mononuclear cells (BMCs) in this clinical setting\(^{(76-86)}\). Cell delivery has been performed via either (1) direct intramyocardial injection during coronary artery bypass graft (CABG) surgery, or (2) catheter-based intramyocardial injection. These results clearly support the clinical safety of the intramyocardial injection delivery method.

**Thus, the experience with administration of MSCs in the three clinical cardiovascular studies noted above** (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 to be used in this clinical study, two of the three studies utilized cell doses that were over 50% greater\(^74\), and as much as two hundred-fold greater\(^87\), than the cell doses proposed in this clinical study.

**Potential mechanisms for MSC mediated improvements in cardiac function**

As noted above, several prior studies have shown that a variety of cellular sources are capable of differentiating into phenotypes that strongly resemble the three principal cell types of myocardium; cardiomyocytes, smooth muscle and vascular endothelium. Most importantly, 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 \textit{in vivo}. Nevertheless, it is important to consider that MSCs may exert favorable effects on cardiac repair above and beyond differentiation \(^88\). 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 to suggest that stem cell homing 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\(^89;90\), cardiogenesis\(^91\), recruitment of endothelial progenitor cells to sites of ischemic tissue\(^92\) and, perhaps, migration of tissue-committed stem/progenitor cells\(^93\). Interestingly, it was recently shown that the CXCR4 receptor is strongly expressed by a proportion of MSCs and that it plays an important role in MSC mobilization\(^94\). 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\(^95\). 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\(^96\) and intramyocardial administration of MSCs is associated with increases in vascular endothelial growth factor (VEGF) levels\(^97\). Furthermore, it has been shown that MSCs participate in angiogenesis and arteriogenesis, differentiating into endothelium and vascular smooth muscle in a VEGF-dependent manner\(^98\).

Once cells have 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 this principle, Mangi et al. genetically engineered rat MSCs using \textit{ex vivo} retroviral
transduction to overexpress the anti-apoptotic protein Akt1, a serine-threonine kinase\(^9\). 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 human autologous MSCs as a therapy for chronic ischemic LV dysfunction. MSCs were chosen because they are effective in small and large animal models, and offer the advantage of already having FDA approval 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 histologic analyses. The pig model was selected due to its anatomic similarity to the human heart. The following sections describe the results obtained with this model\(^{100}\).

Allogeneic mesenchymal stem cell transplantation improves global cardiac function in a swine model of myocardial infarction: In previously published work, it was demonstrated that autologous MSC transplantation in post-MI pigs improved cardiac function, with histologic evidence of robust engraftment.
at 8 weeks, and differentiated to a myocyte-like phenotype. Based on *in vitro* observations that MSCs lack the B-7 costimulatory molecule and may therefore be immunoprivileged, the impact of *allogeneic* MSC transplantation in porcine MI was assessed. 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 $2 \times 10^8$ allogeneic MSCs (n=7) labeled with Di-I and DAPI (both fluorescent dyes to aid histochemical identification) were injected percutaneously into infarcted myocardium using a helical injection needle catheter inserted through a steerable guide catheter (BioCardia, Inc). All animals tolerated the catheter-based injections well. Conscious animals were then studied on a weekly basis for 8 weeks to assess hemodynamics and ventricular architecture. In treated animals, MSCs engrafted within the MI (Figure 1 a, b) and expressed several myocyte proteins including a-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. The fact that the cells were not rejected was further 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 positively in Figure 1h).
In terms of functional responses, anterior MI caused a dramatic deterioration in systolic and diastolic ventricular function and impaired cardiac energy metabolism \((p<0.05\) vs. pre-MI values). MSC cardiomyoplasty produced profound improvements in myocardial function and efficiency (Figure 2). Figure 2a depicts representative examples of pressure-dimension data from animals in both groups. As shown, MSC treatment led to a pattern of LV function over a 2-3 month period marked by a substantial increase in stroke work (SW, the area demarcated by the loops). In the placebo-treated group, impaired cardiac function evident at 3 days post infarction showed either no sign of recovery or a tendency to worsen 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, though LV end diastolic pressure increased in the placebo group, it remained unchanged at 8 weeks in the MSC group \((^p<0.05\) vs. placebo). Furthermore, animals receiving MSCs exhibited recovery to essentially normal levels of both systolic (Ees rose to 13.9±2.7 mmHg/mm and peak +dP/dt to 2465±575 mmHg/sec) and diastolic function (Tau fell to 37±3.8 msec).

In placebo-treated animals, SW decreased substantially during the 8-week period accompanied by a paradoxical gain in oxygen consumption, both factors together depressing the SW/MVO\(_2\) ratio. Conversely, MSC therapy significantly improved myocardial efficiency, increasing SW (from 374.4±59.3 to 654.4±129.9 mmHg.mm at 8 weeks) while simultaneously decreasing MVO\(_2\) (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. SW/MVO\(_2\) ratio increased from 2.5±0.6 at 3 days post- MI to a normal level 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 the changes in global cardiac function. Improved mechanoenergetic coupling in the MSC group is consistent with several mechanisms, including reduced native tissue death \(^{102}\), new tissue formation \(^{103;104}\), and stimulation of endogenous repair mechanisms \(^{105}\).

Interestingly, despite near-identical hemodynamic impairment immediately post-MI, infarct size at autopsy was substantially reduced with MSC therapy \((16±7.2%\) and 3.3±1.2% of the LV, respectively for the placebo and MSC groups; \(p<0.05\) (Figure 3). Importantly, this was due to reduced scar within ventricles of similar mass, due likely to the engraftment and persistence of the MSCs within the area of infarction. Interestingly, placebo-treated animals exhibited evidence of transmural myocardial infarction, while those injected with MSCs had mid-myocardial infarcts with non-scar tissue surrounding the infarct on both the endo- and epicardial sides. To identify the mechanisms responsible for these findings, we used noninvasive imaging techniques to track the fate of MSCs in the swine myocardium. As is described in detail below, magnetically labeled MSCs can be detected \textit{in vivo} using MRI. Using this technique, it has been shown that Feridex labeling can identify sites of stem cell injection for up to 8 weeks (Figure 4). The Feridex signal fades with time,
suggesting either death of the MSCs or loss of Feridex from their cytoplasm, possibly during differentiation. Nevertheless, clear evidence of restoration of contractile function in areas of MSC injection was observed, supporting the notion that MSCs persist in the myocardium and differentiate into contractile cells. The profound decrease in infarct size and improved hemodynamic function suggests the possibility of other concomitant repair mechanisms, possibly the recruitment and stimulation of endogenous cardiac progenitor cells.

To further investigate this notion of MSC mediated cardiac repair, both MRI and computed tomography (CT) were used to image myocardial infarcts in the MSC injected swine. It was found that infarct size, as determined by MRI and CT, correlated to that determined by triphenyl tetrazolium chloride (TTC) staining. Furthermore, using a 32 slice multidetector CT, the same endocardial rim of non-infarcted tissue observed in the first series (Figure 3, Figure 5) was identified, once again suggesting that MSC injection is associated with clear-cut cardiac repair. These data not only suggest the enormous potential of MSC cardiomyoplasty, but also establish that noninvasive imaging techniques can be used as experimental tools to both investigate the hemodynamic effects of cardiomyoplasty and the mechanisms responsible for 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. Using echocardiography to assess LV function at baseline, in the peri-infarct period, and four weeks after MI, it was found that MSC injection in Wistar rats leads to dramatic improvement in LV function. There is clear evidence of myocardial wall thickening and increased contractility in treated animals at four weeks (Figure 6A) as well as increased anterior wall motion at the level of the papillary muscle (Figure 6B). Histology shows the presence of a band of β-gal
positive cells within the infarct region (Figure 7a) shaped like fibroblasts yet expressing the cardiac protein, α-actinin, albeit at lower levels than native myocytes (Figure 7b). This band is greatest at the endocardial rim of the infarct, correlating with the localization of the rim of non-infarcted tissue described in the porcine studies above.

Interestingly, the degree of engraftment of MSCs at the infarct site seen with systemic I.V. injection is comparable to that seen with direct intramyocardial injection. Overall, MSCs injected I.V. at reperfusion showed increased homing to the myocardium, whereas those 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 Determined by MRI**

This SCCT clinical research team has extensive experience using tagged MRI to demonstrate alterations in regional myocardial mechanics in animal models of ischemic heart disease (106,107). The team has also developed closed-chest, MRI-compatible animal models for studying cardiac mechanics, perfusion, and interventional procedures (108-111), and has recently developed new MR imaging and analysis methods.
that enable the rapid determination of myocardial function in infarction and ischemia \((112;113)\). This new technique, Harmonic Phase (HARP) MRI \((114;115)\), developed by collaborators on this SCCT team, is based on tagged MRI techniques. Computationally, the analysis of HARP MRI can be performed much more rapidly than traditional tag tracking in tagged MRI. “Real-time” HARP imaging exploits the concept that only one of the spectral peaks must be acquired for motion in one direction; this in turn accelerates the image acquisition in addition to the rapid analysis already available in HARP. Our SCCT team demonstrated the power of real-time HARP to monitor the onset of ischemia in a canine model of coronary artery stenosis (Figure 10) \((113)\). Using this technique, one can identify abnormal regional myocardial contraction after the onset of ischemia on average 20 seconds earlier than conventional cine wall motion studies and ~1 minute earlier than echocardiographic changes. HARP MRI, similar to tagged MRI, yields quantitative motion and strain parameters on a regional basis that can be used for comparison across patients or at serial time points after intervention. Thus, HARP MRI and analysis represents a rapid and repeatable method to assess left ventricular function serially in a quantitative manner. HARP provides fast, accurate assessment of myocardial strains in humans with and without coronary artery disease \((116)\). Similar techniques have been used to assess structural and functional changes after myocardial infarction in rats \((117;118)\).

**Noninvasive Determination of Infarct Size**

Using an intravenous injection of Gd-DTPA, our SCCT team is able to non-invasively determine the infarct size with T1-weighted contrast-enhanced MRI (i.e., “Delayed Contrast-Enhanced MRI”). Short-axis image slices, which span the entire left ventricle, can be obtained using multiple breath-holds to yield images with the highest spatial resolution. The area of hyperenhancement on the delayed images has been shown to be within 10% of the infarct size measured post-mortem by TTC staining (Figure 11). Alternately, using new imaging techniques, one can obtain the entire 3D left ventricular volume in a single breath (~16 heartbeats). It was recently shown that the 3D technique is in concordance with infarct size as measured by traditional 2D multi-slice techniques (Figure 11) \((119;120)\). Thus, infarct size and location can be accurately determined in less than one minute of scanning time, in order to determine the size of an infarct prior to therapy and/or whether magnetically labeled MSCs home to the infarct.
Figure 6. A: Two-dimensional echocardiography showing end diastole (left column) and end systole (right column) in a treated rat 1) before infarction 2) after infarction and prior to treatment and 3) 4 weeks after treatment with MSCs. B: M-Mode from same animal showing fractional shortening at the papillary level at the same time points as in A.

Figure 7: A: β-galactosidase positive (blue) cells are visible at 20X magnification within the infarct in young rats. These cells form a band along the endocardial surface. B: These cells show evidence of α-actinin expression on immunofluorescence, also at 20X magnification (bright appearing cells, B&W image). C: Quantification demonstrates 10-fold higher engraftment in young relative to old (p<0.001).

Figure 8: Confocal images of infarcted myocardium (top row) and bone marrow (bottom row) illustrating the degree of allogeneic MSC engraftment observed when 5 million MSCs were delivered via tail vein at reperfusion (left columns) or 2 weeks post-reperfusion (right column). As shown, at reperfusion cells home to the heart and not bone marrow; two weeks later, trafficking to the heart is reduced, and cells now migrate and engraft in the bone marrow. All tissues were harvested 4 weeks post-implantation. A FITC-conjugated antibody directed against desmin (green) was used to assess the myogenic differentiation.

Figure 9: Both SDF-1 and CXCR4 levels are elevated following myocardial infarction. P<0.05 vs pre infarct

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

Figure 11. 2D delayed CE MRI in short axis plane (top) acquired within the first 24 hrs after closed-chest infarction demonstrating subendocardial myocardial infarction as hyperenhancing region (~11 o’clock) in a dog. TTC nonstaining areas (e.g., lack of brick red staining) in post-mortem slices (bottom) demonstrate concordance of infarct location and size with MRI.
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 1A). The cell doses administered in these studies covered the doses for this clinical study (2.0 x 10^7 and 2.0 x 10^8 MSCs).

Shake et al.(121) 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 four weeks in animals implanted with MSCs. Mean ejection fraction increased by 5.4 ± 2.2% in the treated group and decreased by 3.4 ± 2.7% in the control group. In addition, the extent of wall thinning after myocardial infarction was markedly reduced in treated animals.

In a porcine animal model, Cattaneo et al.(122) transplanted allogeneic MSCs (cell dose = 200 x 10^6, administered via direct intramyocardial injection with a 27-gauge needle) one 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. Marked improvement in both ejection fraction and global wall motion score were 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 or placebo), improvements in cardiac function were likely attributable to MSC implantation.

The study by Hare et al.(123) is particularly informative for establishing the safety of the doses in this clinical study (2.0 x 10^7 and 2.0 x 10^8 MSCs). Autologous porcine MSCs (cell doses of 20 x 10^6, 200 x 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 x 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 one pig exhibited transient heart block after open heart surgery, a
common event, no ventricular arrhythmias or sudden death occurred throughout the
study period. In addition, no arrhythmias were induced on electrophysiology testing
performed in six animals 12 weeks after injection and prior to sacrifice. Animals in
this study also 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\(^{100,124}\) investigated the
intramyocardial injection of allogeneic porcine MSCs (cell dose = \(200 \times 10^6\)) via
transendocardial catheter delivery 3 days after myocardial infarction. Several
important findings emerged from these studies:

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, profound
   reduction in scar formation, time-dependent recovery of active contractility
   paralleling new tissue appearance, and near-normalization of cardiac
   function.

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 cell doses in this clinical study \((2.0 \times 10^7 \text{ and } 2.0 \times 10^8 \text{ MSCs})\)._
## TABLE 1A
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 10⁶)</th>
<th>Safety Results</th>
<th>Efficacy Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shake (28)</td>
<td>14-Day Post-MI Pig</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>
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<td></td>
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<td></td>
<td></td>
<td>- No MSC differentiation to non-cardiac tissue</td>
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<td>- No significant inflammatory infiltrates at site of MSC implantation</td>
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<td>- MSC engraftment</td>
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<td></td>
<td></td>
<td></td>
<td>- Regional contractile function</td>
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<tr>
<td>Cattaneo (114)</td>
<td>1-Day Post-MI Pig</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>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td>- No significant inflammatory response</td>
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<tr>
<td>Hare (115)</td>
<td>90-Day Post-MI Pig</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>
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<td>- No change in body weight</td>
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<td></td>
<td>- No arrhythmias or inducible VT</td>
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<td></td>
<td></td>
<td></td>
<td>- Decrease in infarct size at High Dose</td>
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</tr>
<tr>
<td>Amado (95)</td>
<td>3-Day Post-MI Pig</td>
<td>14</td>
<td>PIM (catheter) injection</td>
<td>Allogeneic, porcine MSCs</td>
<td>200.00</td>
<td>- No deaths; no malignant arrhythmias</td>
<td>- MSC engraftment</td>
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<td></td>
<td>- No evidence of cardiac perforation during injection</td>
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<td>- Improved systolic and diastolic function</td>
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<td>Amado (116)</td>
<td>3-Day Post-MI Pig</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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Viable myocardium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Infarct scar</td>
<td></td>
</tr>
</tbody>
</table>

EF: Ejection Fraction; IM: intramyocardial; MSC: Mesenchymal Stem Cell; PIM: percutaneous intramyocardial; VT: ventricular tachycardia
Summary
Preliminary data strongly support the hypothesis that MSCs are a safe and effective therapy following myocardial infarction. Moreover, this SCCT team has extensive experience working with both rat and swine models of myocardial infarction and assessing left ventricular function in large and small animals. Given the experience and encouraging preliminary results with stem cell therapies and tracking, our SCCT team is proposing to conduct this randomized, placebo-controlled clinical study. The choice of a randomized, blinded, placebo-controlled trial design is justified on the following:

- Recent randomized, placebo-controlled trials of cardiac cell therapy have been conducted with endothelial progenitor cells or hematopoietic stem cells have provided more rigorous assessments than earlier, uncontrolled studies.
- Obtaining bone marrow aspirates and preparing MSCs on all patients (including those eventually randomized to the placebo group) will insure that the trial is double-blind, reducing potential bias.
- 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.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective
To demonstrate the safety of autologous MSCs administered by intramyocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI undergoing cardiac surgery for CABG.

2.1.2 Secondary Objectives
To demonstrate the efficacy of autologous MSCs administered by intramyocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI undergoing cardiac surgery for CABG.

2.2 Study Endpoints

2.2.1 Primary Endpoint (Safety)
The primary safety endpoint is the six-month post-CABG surgery serious adverse event (SAE) proportion of patients experiencing sustained ventricular arrhythmias, defined as ventricular arrhythmias lasting longer than 15 seconds or with hemodynamic compromise,
or sudden unexpected death at six months, or ectopic tissue formation at 12 months by chest/abdomen/pelvis CT exam.

2.2.2 Secondary Endpoints (Efficacy)

The following additional efficacy endpoints will be evaluated in this trial (during the six-month follow-up period and a final month 18 visit):

- MRI and echocardiographic-derived measures of left ventricular function:
  1. Difference between the baseline, 6-month, and 18-month infarct scar size (ISS) as determined by delayed contrast-enhanced MRI
  2. Difference between the baseline, 6-month, and 18-month regional left ventricular function (region of MSC injection) as determined by MRI.
  3. Difference between the baseline, 6-month, and 18-month regional left ventricular wall thickening as determined by MRI.
  4. Difference between the baseline, 6-month, and 18-month left ventricular end diastolic wall thickness as determined by MRI and echocardiogram.
  5. Difference between the baseline, 6-month, and 18-month left ventricular ejection fraction, and end diastolic and end systolic volumes, as determined by MRI and echocardiogram.
  6. Difference between the baseline, 6-month, and 18-month left ventricular regional myocardial perfusion as determined by MRI.

- Peak VO$_2$ (by exercycle 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 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 and a final month 18 visit):

- Treatment emergent adverse event (AE) rates.
- 48-hour ambulatory 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 CABG).
• Post-CABG surgery echocardiogram (day 2 post-op).

3. STUDY DESIGN

Forty-five patients scheduled to undergo cardiac surgery for CABG meeting all inclusion and no exclusion criteria will be evaluated at baseline. Following informed consent and bone marrow aspiration, patients will be randomized to either MSCs in one of two doses: 1) 2 million cells, administered in 0.25 cc to 0.5 cc injections times 10 to 20 injections for a total of $2 \times 10^7$ cells (20 million MSCs) or 2.) 20 million cells, administered in 0.25 cc to 0.5 cc injections times 10 to 20 injections for a total of $2 \times 10^8$ cells (200 million MSCs) or to placebo (phosphate buffered saline [PBS] and 1% human serum albumin [HSA]). The surgeon-investigators will have discretion to administer 10 to 20 separate injections; applying the volume of each 0.5 cc syringe at either 1 or 2 sites. Thus, each injection will be either 0.5 or 0.25 cc. The injections will be administered epicardially under direct visualization using a syringe and needle following the completion of the CABG procedure, and a detailed record of the locations and actual number of injections will be recorded. The cells will be injected into and surrounding an akinetic or severely hypokinetic territory not undergoing revascularization. The region of injection will be marked with surgical suture material visible by MRI. Autologous MSCs will be obtained from each patient via bone marrow aspiration three-five weeks prior to the cardiac surgery. If the patient is randomized to a dose and the MSCs do not expand to the required dose, each syringe will contain the maximal number of cells available, but the total number of syringes will be 10 and the total overall injection volume will not exceed 5 cc.

Factors which will be considered in determining the areas of the myocardium suitable for revascularization, and those areas not suitable for intervention, during the CABG surgical procedure include (1) the size of the vessel at the site where insertion could technically be performed, (2) the location of that site, (3) the extent of disease distal to that site, (4) the viability of the territory being supplied by that vessel, (5) the availability of appropriate conduits, and (6) an assessment of the operative time the patient could tolerate.

Please refer to APPENDIX A: Procedures for Isolation, Preparation and Administration of Autologous Human Mesenchymal Stem Cells (MSCs) for additional details.

Following the cardiac surgical procedure and the MSC injections, patients will be followed at monthly intervals for six months, and at 12 and 18 months to obtain the safety and efficacy assessments.

3.1 Inclusion Criteria

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

1. Be 21-80 years of age.
2. Provide written informed consent.
3. Have a diagnosis of chronic ischemic left ventricular dysfunction secondary to myocardial infarction (MI).
4. Be scheduled to undergo cardiac surgery for CABG.

5. Have an ejection fraction between 20% and 50% by gated blood pool scan, two-dimension echocardiogram, cardiac MRI, or left ventriculogram within the prior six months and not in the setting of a recent ischemic event.

6. Have an akinetic or dyskinetic region by standard imaging.

3.2 Exclusion Criteria

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

1. Have a baseline glomerular filtration rate < 50 ml/min/1.73m².

2. Have a known, serious radiographic contrast allergy.

3. Have bone marrow dysfunction, as evidenced by a 20% or more deviation from normal hematocrit, white blood cell or platelet values without another explanation.

4. Have a coagulopathy condition not due to a reversible cause (i.e., Coumadin).

5. Have known allergies to penicillin or streptomycin.

6. Have a contra-indication to performance of an MRI scan.

7. Be an organ transplant recipient.

8. 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.

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

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

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


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

14. Be a female patient who is pregnant, nursing, or of childbearing potential while not practicing effective contraceptive methods.

4. TREATMENT OF PATIENTS

4.1 Study Therapy and Dosages

4.1.1 Study Investigational Therapy

Please refer to APPENDIX A: Procedures for Isolation, Preparation and Administration of Autologous Human Mesenchymal Stem Cells (MSCs) for additional details.
4.1.2 **Dose Rationale**

The study doses are selected to provide a ten-fold difference in cell concentration between the two cell therapy-treated groups, in order to assess a cell concentration versus response effect. The doses were also chosen based on practical considerations and the ability to grow this quantity of cells for most patients within 28 days.

4.1.3 **Dosages and Dosing**

Based upon the randomization assignment, patients will receive one of the following three regimens:

- 20 million MSCs (2 million cells per 0.25 cc to 0.5 cc injection times 10 to 20 injections for a total of $2 \times 10^7$ cells)
- 200 million MSCs (20 million cells per 0.25 cc to 0.5 cc injection times 10 to 20 injections for a total of $2 \times 10^8$ cells)
- Placebo (PBS and 1% HSA).

The surgeon-investigators will have discretion to administer 10 to 20 separate injections; applying the volume of each 0.5 cc syringe at either 1 or 2 sites. Thus, each injection will be either 0.5 cc or 0.25 cc. The injections will be administered epicardially using a syringe and needle following the completion of the CABG procedure, and a detailed record of the locations and actual number of injections will be recorded. If the patient is randomized to a dose and the MSCs do not expand to the required dose, each syringe will contain the maximal number of cells available, but the total number of syringes will be 10, and the total overall injection volume will not exceed 5 cc. Please refer to **APPENDIX A: Procedures for Isolation, Preparation and Administration of Autologous Human Mesenchymal Stem Cells (MSCs)** for additional details concerning the administration and delivery of the MSCs.

4.2 **Blinding and Unblinding**

Administration of both MSCs and placebo will be performed in a double-blind manner; i.e., the study investigators and patients will not be aware of the assigned treatment regimen. If for medical reasons unblinding is thought to be necessary, the Investigator may identify the treatment assignment by opening a sealed envelope that will be retained with the individual patient’s study record.

4.3 **Study Investigational Therapy Management**

4.3.1 **Storage and Handling of Study Investigational Therapy**

Study therapy (MSCs or placebo) will 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 cells are not used within 2 years of entry into the study, the cells will be destroyed.
4.3.2 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. Please refer to APPENDIX A: Procedures for Isolation, Preparation and Administration of Autologous Human Mesenchymal Stem Cells (MSCs) for additional details of accountability procedures.

5. STUDY PROCEDURES

5.1 Time and Events Schedule

The Time and Events Schedule for the conduct of this study is shown in Table 1.

Patients will be screened approximately 5-7 weeks prior to CABG surgery. During the screening period (weeks -7 to -5), and after written informed consent is obtained, patients will undergo a battery of tests to ensure they meet all inclusion and no exclusion criteria.

Patients will return to the hospital for two baseline visits after their screening exams have been completed and prior to surgery. The first visit will consist of baseline tests and evaluations (Baseline Visit #1). Subsequently, during Baseline Visit #2, patients will return for their bone marrow aspiration procedure. Performing the bone marrow aspiration approximately 3-5 weeks before surgery will provide sufficient time for harvesting and preparation of the MSCs. Additionally study patients will be randomized at this time to one of three treatment arms in a 1:1:1 ratio, and randomization will be stratified by Study Center. Further details regarding these visits are discussed after the Table of Time and Events.

The study team, with the assistance of the hospital health care team, will also educate the patient about the CABG surgery and what to expect during recovery.
### Schedule of Time and Events

| Study Procedure                  | Screening | Baseline Visit #1 | Baseline Visit 2 | Day 1     | Day 2     | Day 3     | Day 4     | Day 14+3 | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 | Month 7 | Month 8 | Month 9 | Month 10 | Month 11 | Month 12 | Month 13 | Month 14 | Month 15 | Month 16 | Month 17 | Month 18 |
|---------------------------------|-----------|------------------|------------------|-----------|-----------|-----------|-----------|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Informed Consent                |           |                  |                  | X         |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| History and Physical            | X         | X                | X                | X         | X         | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| Vital Signs                     | X         | X                | X                | X         | X         | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| 12-lead ECG                     |           |                  |                  | X         |           |           |           |           |          |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Concomitant Medications         | X         | X                | X                | X         | X         | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| Randomization                   |           |                  |                  |           | X         |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Bone Marrow Aspiration          |           |                  |                  |           |           | X         |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| CABG/Investigational Agent      |           |                  |                  |           |           |           | X         |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Standard Post-op Care           |           |                  |                  |           |           |           |           |         X | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| Cardiac MRI                     |           |                  |                  |           |           |           |           | X         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Determin. of Peak VO2           |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Six-Minute Walk Test            |           |                  |                  |           |           |           |           |           |         X |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| NYHA Functional Class           |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| MLHF Questionnaire              |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| CT Scan of chest, adb & pelvis  |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Echocardiogram                  |           |                  |                  |           |           |           |           | X         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Pulmonary function (FEV1)       |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| 48 Hour Ambulatory ECG          | X         | X                | X                | X         | X         | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| Serum Troponin & CK-MB **       |           |                  |                  |           |           |           |           |           |         |     X   |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Hematolgy & Chemistry           | X         | X                | X                | X         | X         | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| Urinalysis                      |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Serum 8-HCG                     |           |                  |                  |           |           | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |
| HIV & Hepatitis Screens         | X         |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Coagulation Studies             |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Biomarkers Assessment           |           |                  |                  |           |           |           |           |           |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Adverse Events                  | X         | X                | X                | X         | X         | X         | X         | X         | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       | X       |

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Time and Events Table Key:

# All Baseline Visit #1 tests will occur within 10 days of the Screening Visit.

* Baseline Visit #2 will occur within 7 days of Baseline Visit #1. Also, completion of Baseline Visit #2 (bone marrow aspiration) will trigger scheduling of the CABG surgery and randomization.

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

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

5.2 Study Phases and Visits

5.2.1 Screening Phase

See Table 1 for the procedures and assessments to be performed during this phase of 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, and/or syphilis. All screening visit tests and procedures will occur within 5 days of the signed informed consent (IC) and 5 to 7 weeks prior to CABG surgery. No screening exams will take place until the patient is fully informed of the research and signs the informed consent.

5.2.2 Baseline Phase

See Table 1 for the procedures and assessments to be performed during this phase of the study. The Baseline Phase will take place over a two week period, and is split into two visits. During Baseline Visit #1, which will occur within 10 days of the final screening exam, and 4 to 6 weeks prior to CABG, patients will undergo additional tests to assess continued eligibility for the study and to gather baseline data. This visit may take place over more than one day as long as all studies are completed within 10 days of the final screening exam.

Baseline Visit #2 will take place within 7 days of the final Baseline Visit #1 test, and 3 to 5 weeks prior to CABG; at which time patients will return for their bone marrow aspiration procedure. Successful bone marrow aspiration will trigger scheduling of the patient’s CABG surgery and randomization.

5.2.3 Day 1 – Day 4 Post-Surgery Evaluations

See Table 1 for the procedures and assessments to be performed during these evaluations. All patients will have troponin and CK-MB laboratory work completed every 12 hours for the first 48 hours post-CABG, as well as an echocardiogram on
the second day after surgery. Additionally, an FEV1 assessment will be completed during day 4, unless the patient is unable, then 48 hours prior to discharge.

5.2.4  **Week 2 Visit**

During Week 2 (Day 14 ± 3), patients will return to the clinic for a 48-hour ambulatory ECG exam and FEV1 assessment.

5.2.5  **Month 1 – Month 6 and Month 18 Visits**

See Table 1 for the safety and efficacy tests, procedures, and assessments to be performed during these visits. Outpatient visits should be completed as close to the scheduled visit dates as possible. The visit window is ± 5 days from the intended date of the visit. If needed, outpatient visit procedures may be completed over more than one day and, if so, the date of the history and physical exam will be considered the visit date.

5.2.6  **Biomarkers Assessment**

A separate 7 mL blood sample for gene expression profiling of WBC RNA will be obtained at baseline and at 6 months. In addition, a 57 mL sample of each patient’s bone marrow aspirate will be obtained for the same purpose. All samples will be de-identified (anonymized) so that they cannot be linked to individual patients. These samples may be stored indefinitely. Because of the anonymized manner in which these data will be analyzed, 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.

5.2.7  **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 surgery is delayed will be allowed to remain in the study and receive investigational cells or placebo 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 surgery, or otherwise withdraw from the study after the screening period but before surgery, may be replaced without limit.

3. Patients who are in the midst of the surgical procedure, but whose investigational cells can no longer be used, may be withdrawn from the study and replaced by newly enrolled subjects. This replacement process is limited to 3 patients.
5.3 **Magnetic Resonance Imaging (MRI) and Echocardiogram Protocols**

**MRI Protocol**

All patients will undergo contrast-enhanced MRI at baseline and months 3, 6 and 18. Prior to the MRI examination, patients will be informed about the peculiarities of the MRI environment and coached on performing adequate breath-holds. Electrocardiographic leads and a blood pressure cuff will be positioned. The cardiac phased-array coil will be wrapped around the patient’s chest and correctly positioned over the precordium. Participants will lie supine on the magnet table and enter feet-first into the center of the scanner. All cardiac images will be obtained during a 12-15 heartbeat breathhold at end-expiration, averaging 10-15 seconds with adequate rest periods between breathholds (about 10-15 seconds). The imaging protocol will first include sagittal, axial and oblique scout images to localize the heart. It is anticipated that the duration each MRI session will be 45-60 minutes.

**Tissue tagging protocol for ejection fraction determination:** This will be performed prior to contrast administration in order to minimize tag line fading post-gadolinium. The protocol is based upon an ECG triggered fast gradient echo pulse sequence. The tagging pulse consists of nonselective radiofrequency pulses separated by spatial modulation of magnetization encoding gradients to achieve a tag separation of 6 mm. Tagging pulses are applied immediately before the imaging pulse and triggered by the upslope of the electrocardiographic QRS complex. After scout images, 6 contiguous stacks of short-axis images with stripes in each of two directions are prescribed to cover the entire heart from base to apex. Thus, two sets of short axis series will be obtained with stripes oriented at 0° and 90°. Six long-axis views will also be prescribed in a radial fashion every 30°. Imaging parameters are: 36-40 cm field of view, 8 mm slice thickness, matrix size 256x128, TR=5.1 msec, TE=1.4 msec, flip angle=12°, and 8 phase-encoded views per frame.

**Delayed contrast-enhancement protocol:** This is the most accurate assessment of infarct scar size. Following MRI tissue tagging, patients will receive a bolus intravenous injection of 0.2 mmol/kg of gadolinium-DTPA. High-resolution delayed enhancement images will be obtained using an inversion-recovery prepared gated fast gradient echo pulse sequence. Eight to ten short-axis cross sections of the left ventricle to ensure entire cardiac coverage will then be acquired. Beginning with the peripheral bolus of gadolinium, the entire heart will be imaged in short-axis every 4-5 minutes, visually adjusting the inversion time (TI) for optimal suppression of the normal myocardium. This will continue for 25-30 minutes post-contrast bolus. Pulse sequence parameters are as follows: TR 5.1 msec, TE 2 msec, flip angle 25°, field of view 36-40 cm, matrix size 256 x 192, bandwidth 15.6
kHz, inversion pulse 180°, 8 mm slice thickness with 0 gap, and inversion time (TI) 150-250 msec.

**Tissue characterization protocol:** Standard echo planar imaging sequence will be adapted to run the Look-Locker sequence. Approximately 20 minutes following contrast-bolus, 6 breath-held short-axis images to cover the entire ventricle will be acquired. Pulse sequence parameters are as follows: TR 8.9msec, TE 4.8msec, flip angle 10°, EPI factor 5, spatial resolution 2.7x2.7x8mm³, temporal resolution 34msec.

**Echocardiogram Protocol**

All subjects will undergo transthoracic echocardiographic assessment of overall and regional LV systolic function at baseline, day 2, and months 3, 6, and 18.

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=akinetic, 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.

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 will 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. However, there may be instances when the medical monitor (an independent physician who is not participating in the clinical study), requests copies of medical records for certain cases. In this instance, all patient identifiers will be blinded on the copies of the medical records prior to submission to the medical monitor.
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.

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 will 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 will 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 pharmacological action of the study therapy was responsible for the 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 to the medical monitor 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 medical monitor will be provided with 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 to the SCCT

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 2.

The medical monitor will review the information and contact the Investigator for additional information, as necessary. The principal investigator, or his designee, is responsible for notifying the NHLBI DSMB of all SAEs.
### TABLE 2

**Serious Adverse Event Reporting Requirements**

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

6.9 **SCCT Post-Study Adverse Events and Serious Adverse Events**

The Investigator should notify the medical monitor of 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 this SCCT 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 SCCT can meet legal obligations and fulfill ethical responsibilities towards the safety of all patients participating in SCCT-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 is being filed under an Investigational New Drug (IND) application with the FDA. A given SAE may qualify for 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 the ESR.

The ESRs are prepared according to SCCT 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 SCCT Monitoring of Adverse Events

The following list summarizes the SCCT’s role in monitoring AE/SAEs:

- All SAEs will be reviewed by the Medical Monitor at the Data Coordinating Center, 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 DCC 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 DCC Medical Monitor, will be provided to the NHLBI Project Officer within 2 business days of receiving the report.
- All SAEs, at least possibly related, will also be sent 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 the information is complete, determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study.

The DCC will prepare semi-annual summary reports of all AEs/SAEs for the NHLBI Project Officer and DSMB Chair. Semi-annual reports will be made available on a secure website and the NHLBI Project Officer and 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

Patients will be registered using the SCCT Advantage Electronic Data Capture (EDC). The following procedures shall be followed:

1. An authorized user at the clinical center completes the initial screening by entering patient demographics and Segment A information (inclusion/exclusion
criteria) of the Eligibility Form approximately 5-7 weeks prior to planned CABG surgery.

2. If the patient is eligible and undergoes bone marrow aspiration, a study number and random treatment assignment is generated (3-5 weeks prior to CABG surgery).

If a connection is interrupted during a randomization session, the process is completely canceled and logged. A backup manual registration and randomization system will also be available to provide for short-term system failure or unavailability.

### 7.2 Randomization

Patients will be randomized approximately 3-5 weeks prior to planned CABG surgery. Patients will be randomized using permuted blocks to one of the three arms in a 1:1:1 ratio (placebo, low dose, high dose, respectively), providing a projected distribution of 15:15:15 patients among the groups. Randomization will be stratified by treatment center. The treatment assignment is sent via email to the SCCT Cell Therapy Laboratory, which will have the table to identify the treatment associated with the treatment number. A visit schedule based on treatment start date is displayed for printing.

### 7.3 Data Collection

Data will be entered using the SCCT Advantage EDC. A detailed 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 study therapy administration, during the CABG surgery. Following surgery, a Patient Visit Schedule listing target dates for assessments can be printed from the EDC system.

**Criteria for Forms Submission:** Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User’s Guide. Forms that are not received at the Data Coordinating Center (DCC) within the specified time will be considered delinquent. Past due forms can be viewed via the Web-based data entry system. A missing form will continue to appear until the form is entered into the DCC’s master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook and User’s Guide.
7.4 Statistical Considerations

7.4.1 Study Design
The study is a Phase I/II, randomized, double-blinded, placebo-controlled, multi-center trial. It is designed to assess the safety and efficacy of intramyocardial injection of autologous human mesenchymal stem cells (MSCs) in patients with chronic ischemic left ventricular dysfunction secondary to myocardial infarction undergoing cardiac surgery for coronary artery bypass grafting (CABG). The sample size is 45 patients for this trial.

7.4.2 Accrual
It is estimated that two years of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, gender, and age.

7.4.3 Study Duration
Patients will be followed for 18 months (monthly visits for the first 6 months plus a final month 18 visit).

7.4.4 Randomization
The study is designed with three arms: placebo, low dose and high dose. Patients are stratified by Study Center and randomized in 1:1:1 ratio, with 15 in each of the three arms for a total sample size of 45 patients.

7.4.5 Primary Objective
The primary objective is to demonstrate the safety of autologous MSCs administered by intramyocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI undergoing CABG surgery. The primary endpoint is the proportion of patients who experience ventricular arrhythmias requiring therapy, sudden unexpected death, or ectopic tissue formation in the MSC- and placebo-treated patients. The six month serious adverse event rates in the two groups will also be a primary focus.

7.4.6 Sample Size and Power Calculations
The sample size is 45 patients for this trial. Ninety-five percent confidence intervals were calculated for varying probabilities based on the sample size. Table 3 provides confidence intervals for a variety of true underlying proportions. Of particular interest is the anticipated six-month SAE probability, which is 40%. For this setting, the confidence interval length is 28.6%. The probabilities above and below 40% represent other plausible SAE scenarios.
The precision of the estimates alternatively could be viewed as a lower bound on the rate of SAE. The probability to rule out SAE percentages 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 SAE probability will be greater than thresholds of 30%, 50%, 60%, 65% and 70%. When the true percentage is 40%, there is 91% power to rule out a SAE percentage of 65% or larger.

**TABLE 3**

Confidence Interval Lengths and a Possible Confidence Interval for Various Sample Sizes and Underlying SAE Proportions

<table>
<thead>
<tr>
<th>N</th>
<th>SAE Proportion %</th>
<th>Length of 95% Confidence Interval</th>
<th>Possible Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>30</td>
<td>26.8</td>
<td>16.6, 43.4</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>27.9</td>
<td>21.1, 48.9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>28.6</td>
<td>25.7, 54.3</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>29.1</td>
<td>30.5, 59.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>29.2</td>
<td>35.4, 64.6</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>32.8</td>
<td>13.6, 46.4</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>34.1</td>
<td>17.9, 52.1</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>35.1</td>
<td>22.5, 57.5</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>35.6</td>
<td>27.2, 62.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>35.8</td>
<td>32.1, 67.9</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>46.4</td>
<td>6.8, 53.2</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>48.3</td>
<td>10.9, 59.1</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>49.6</td>
<td>15.2, 64.8</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>50.4</td>
<td>19.8, 70.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50.6</td>
<td>24.7, 75.3</td>
</tr>
</tbody>
</table>
### TABLE 4

**Probability of Ruling Out a Threshold of Size T or larger for Various Sample Sizes and True Underlying SAE Proportion**

<table>
<thead>
<tr>
<th>N</th>
<th>True SAE Proportion %</th>
<th>Probability of Ruling Out SAE Percentages of Size T or Larger</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T=30%</td>
</tr>
<tr>
<td>45</td>
<td>30</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.72</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.57</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.30</td>
</tr>
</tbody>
</table>

### 7.4.7 Stopping Guidelines

Two statistical stopping guidelines are employed in this study: the proportion of SAEs by 30 days post-CABG surgery and the 30 day probability of SAEs. These guidelines are designed to assist an independent NHLBI-appointed Data and Safety Monitoring Board (DSMB) in overseeing the study. The DSMB may also request additional interim analyses and develop other criteria for determining when to intervene in the enrollment or treatment of patients in the study.
Monitoring of key safety endpoints will be conducted and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised.

The proportion of SAEs by 30 days post-CABG surgery will be monitored using a stopping guideline that compares the rate of adverse events in all pooled arms to an external standard for safety in patients undergoing CABG surgery.

A Bayesian motivated safety stopping guideline will be used for this trial (125; 126). The expected underlying SAE at 30 days post-CABG probability is assumed to be 25% (127; 128) and that a probability of greater than 45% is unacceptable.

A Beta distribution can be used as the prior distribution of \( \theta \); \( \theta \) is the proportion of patients who experience a SAE. The Beta distribution will have prior mean of 0.25 and a prior probability <0.05 of exceeding 0.45. The guideline is derived such that if there is strong evidence (posterior probability \( \geq 0.95 \)) that the probability of the event is greater than 25%, the trial will be stopped. The resulting boundaries are tabulated in Table 5.

**TABLE 5**

Bayesian Stopping Guideline for Event Rate of 25% *

<table>
<thead>
<tr>
<th># Events</th>
<th># Patients in Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>6-8</td>
</tr>
<tr>
<td>7</td>
<td>9-11</td>
</tr>
<tr>
<td>8</td>
<td>12-14</td>
</tr>
<tr>
<td>9</td>
<td>15-18</td>
</tr>
<tr>
<td>10</td>
<td>19-21</td>
</tr>
<tr>
<td>11</td>
<td>22-24</td>
</tr>
<tr>
<td>12</td>
<td>25-27</td>
</tr>
<tr>
<td>13</td>
<td>28-31</td>
</tr>
<tr>
<td>14</td>
<td>32-34</td>
</tr>
<tr>
<td>15</td>
<td>35-37</td>
</tr>
<tr>
<td>16</td>
<td>38-41</td>
</tr>
<tr>
<td>17</td>
<td>42-44</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 (\(\pi\)) and assuming a sample size of 45 patients. Table 6 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 \(\pi\).

<table>
<thead>
<tr>
<th>Mean of Prior Distribution</th>
<th>(\theta)</th>
<th>Probability of stopping</th>
<th>Average Sample Size (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>0.07</td>
<td>25</td>
</tr>
<tr>
<td>0.35</td>
<td>0.46</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>0.45</td>
<td>0.88</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>0.55</td>
<td>0.99</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>1.00</td>
<td>13</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 7% chance (“Type I error”) of suggesting early termination when the true rate is 0.25, and an 88% chance (“power”) when the true rate is 0.45, rising to 99% when the true rate is 0.55.

A second stopping guideline will also be used which will compare the 30 day probability of SAEs, \(\pi_T\), in the pooled treatment arms to the 30 day probability of SAEs, \(\pi_P\), in the placebo group. This rule will be applied once, after 10 patients on the placebo have been followed for the 30 day outcome. A one-sided, \(a=0.1\), Barnard’s test for superiority will be used to test if the event rates are higher in the treated group as compared to the placebo group. Table 7 shows the power of the test given various underlying probabilities of 30 day SAEs. Of note if the true 30 day SAE probability is 0.25, there is 91% power to detect an increase in the
probability to 0.75. Note that due to the small sample size there is only sufficient power to detect large disparities in event rates.

![Table 7](image)

**Table 7**

Operating Characteristics for Barnard’s Stopping Guideline

<table>
<thead>
<tr>
<th>$\theta_T$</th>
<th>$\theta_p$</th>
<th>Probability of stopping</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>0.09</td>
</tr>
<tr>
<td>0.50</td>
<td>0.25</td>
<td>0.47</td>
</tr>
<tr>
<td>0.75</td>
<td>0.25</td>
<td>0.91</td>
</tr>
<tr>
<td>0.10</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>0.20</td>
<td>0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>0.30</td>
<td>0.10</td>
<td>0.47</td>
</tr>
<tr>
<td>0.40</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>0.80</td>
<td>0.40</td>
<td>0.78</td>
</tr>
</tbody>
</table>

7.4.8 **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 VO$_2$, etc.

7.4.9 **Analysis of the Primary Endpoint**

Descriptive analyses of the primary endpoint will be performed. Point estimates and confidence intervals of SAE proportion by treatment arm 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.
7.4.10 **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.

7.4.11 **Data and Safety Monitoring Board (DSMB)**

Interim analyses will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed 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) when 12 and 24 CABG patients have completed 1 month of follow-up (Visit 1). 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, NHLBI will be notified and information will be supplied to the DSMB. Policies of the DSMB are described in the SCCT Manual of Procedures. 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. **STUDY ADMINISTRATION**

8.1 **Regulatory and Ethical Considerations**

8.1.1 **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.

8.1.2 **Ethics Approval**

It is the Investigators’ 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.

8.1.3 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 (HIPAA) authorization 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 will 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 will 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.

8.2 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 will 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 the Sponsor, 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 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.

8.3 Payments to Patients

Patients will be reimbursed $50 for all post surgical monthly visits except for month 6 and 18, when $100 will be provided. Patients will also be remunerated a total of $100 for the baseline period (Baseline Visits 1 and 2). These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.
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