Interdisciplinary Stem Cell Institute

University of Miami/Miller School of Medicine

Clinical Research Protocol

Title:	**A Phase II, Randomized, Blinded, Study of the Safety and Efficacy of Transendocardial Injection of Allogeneic Mesenchymal Stem Cells (20 Million or 100 Million Total MSCs) in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction.** **The *TR*ansendocardial Stem Cell *I*njection *D*elivery *E*ffects on *N*eomyogenesis S*T*udy (The *TRIDENT* study)**
Investigational Therapy Names:	**Allogeneic Human Mesenchymal Stem Cells (Allo-hMSCs)**
FDA IND No.:	[redacted]
Protocol No.:	**SCCT-08-003 (Version 2.3) – June 9, 2016**
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Protocol Agreement Signature:

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(Sponsor – Investigator)

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Date

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	adverse event
ALT	Alanine aminotransferase
AMBMC	autologous mononuclear bone marrow cells
AMI	acute myocardial infarction
BMC	bone marrow cell
BNP	Brain Natriuretic Peptide
BSC	biologic safety cabinet
BUN	Blood Urea Nitrogen
C of A	Certificate of Analysis
CABG	coronary artery bypass graft
CAD	coronary artery disease
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU-F	colony forming units – fibroblasts
CK-MB	creatine kinase – mb
CMV	Cytomegalovirus antibody
CRO	contract research organization
CRP	C-reactive Protein
CT	computed tomography
CXCR4	CXC Chemokine Receptor 4
DAPI	4'-6-Diamidino-2-phenylindole
DCC	Data coordinating center
DMSO	dimethyl sulfoxide
DSMB	Data and Safety Monitoring Board
EF	ejection fraction
ECG	Electrocardiogram
EPC	endothelial progenitor cells
ESR	expedited safety report
ESV	End systolic volume
FBS	fetal bovine serum
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
F/U	Follow-up
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor

HARP	Harmonic Phase
HBcAb	Hepatitis B Core antibody
hBMC	human bone marrow cell
HBsAg	Hepatitis B surface antigen
HCVAb	Hepatitis C Virus antibody
HLA	Human Leukocyte antigen
hMSC	human mesenchymal stem cell
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HSA	human serum albumin
HSC	hematopoietic stem cell
HTLV	human T-cell lymphotropic virus
ICAM	intracellular adhesion molecule
ICD	implantable cardioverter-defibrillator
ICF	informed consent form
ICH	International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use
IDM	Infectious Disease Markers
IEC	institutional ethics committee
IFN-y	Interferon-y
IIEF	International Index for Erectile Dysfunction (Male)
IM	Intramyocardial
IND	Investigational New Drug application
IRB	Institutional Review Board
I.V.	Intravenous
KDR	VEGF receptor-2
LAD	left anterior descending artery
LV	left ventricular
LVAD	left ventricular assist device
MACE	major adverse cardiac events
MEM	minimum essential medium
MHC	major histocompatibility complex
MI	myocardial infarction
MLHF	Minnesota Living with Heart Failure
MR	magnetic resonance
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell

NMDP	National Marrow Donor Program[138]
NYHA	New York Heart Association
PBS	phosphate buffered saline
QA	quality assurance
QC	quality control
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
SAE	serious adverse event
SCF	stem cell factor
SDF-1	stromal cell derived factor 1
SOP	standard operating procedures
SQOL-F	Sexual Quality of Life Questionnaire-Female[1]
SW	Stroke Work
TE-SAE	Treatment-emergent serious adverse event
TFN-a	Tumor Necrosis Factor Alpha
TTC	triphenyltetrazolium chloride
UMMSM	University of Miami Miller School of Medicine
U.S.	United States
VEGF	vascular endothelial growth factor
VT	Ventricular tachycardia
Peak VO_2	peak oxygen consumption
WBC	white blood count
WMA	Wall motion abnormalities
WMSI	Wall motion score index

SYNOPSIS

Sponsor: Interdisciplinary Stem Cell Institute University of Miami Miller School of Medicine

Name of Study Therapy: Allogeneic Human Mesenchymal Stem Cells (Allo-hMSCs)

Title of Study: The *TR*ansendocardial Stem Cell *I*njection *D*elivery *E*ffects on *N*eomyogenesis S*T*udy (The *TRIDENT* study) A Phase II, Randomized, Blinded Study of the Safety and Efficacy of Transendocardial Injection of Allogeneic Mesenchymal Stem Cells (20 Million or 100 Million MSCs) in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction.

Study Center: Interdisciplinary Stem Cell Institute - University of Miami Miller School of Medicine	**Phase of Development:** Phase II

Objectives:

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

Secondary: To demonstrate the efficacy of allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI.

Design and Investigational Plan: This is a phase II study intended to gain additional safety and efficacy assessments among two dose levels previously studied in a phase I setting. In this study, a 20 million total hMSC dose and a 100 million total hMSC dose will be randomly allocated administered via the Biocardia Helical infusion system in a blinded manner. Randomization will occur centrally through an electronic data entry system in the following two treatment groups:

Treatment Group 1

Fifteen (15) patients to be treated with Allo-hMSCs: 4 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $0.2x\ 10^8$ (20 million) Allo-hMSCs.

Treatment Group 2

Fifteen (15) patients to be treated with Allo-hMSCs: 20 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of $1x\ 10^8$ (100 million) Allo-hMSCs.

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

The Allo-hMSCs will be supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami.

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

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

Diagnosis and Main Criteria for Inclusion/Enrollment:

Major Inclusion Criteria

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

infarction left ventricular dysfunction.

- Ejection fraction less than or equal to 50%.

Major Exclusion Criteria

- Baseline glomerular filtration rate ≤35 ml/min/1.73m^2.
- Presence of a mechanical aortic valve or heart constrictive device.
- Documented presence of aortic stenosis (aortic stenosis 1.5cm^2 or less).
- Documented presence of moderate to severe aortic insufficiency (echocardiographic assessment of aortic insufficiency graded as ≥+2).
- Evidence of a life-threatening arrhythmia in the absence of a defibrillator (nonsustained ventricular tachycardia ≥ 20 consecutive beats or complete second or third degree heart block in the absence of a functioning pacemaker) or QTc interval > 550 ms on screening ECG.
- AICD firing in the past 60 days prior to study enrollment.
- Be eligible for or require coronary artery revascularization.
- Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/µl or platelet values < 100,000/µl without another explanation.
- Have liver dysfunction, as evidenced by enzymes (ALT and AST) greater than three times the ULN.
- Have a coagulopathy condition = (INR > 1.3) not due to a reversible cause.
- Known, serious radiographic contrast allergy.
- Known allergies to penicillin or streptomycin.
- Hypersensitivity to Dimethyl Sulfoxide (DMSO).
- Organ transplant recipient.
- Have a history of organ or cell transplant rejection
- 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, melanoma in situ or cervical carcinoma.
- Non-cardiac condition that limits lifespan to < 1 year.
- On chronic therapy with immunosuppressant medication.
- Serum positive for HIV, hepatitis BsAg, or viremic hepatitis C.
- Female patient who is pregnant, nursing, or of child-bearing potential and not using effective birth control**.**

Definition of Endpoints:

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

Definition of Endpoints (continued):

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

- Treatment emergent adverse event (AE) rates.
- 24-hour ambulatory electrocardiogram (ECG) recordings.
- Hematology and clinical chemistry values and urinalysis results.
- Serial troponin I and CK-MB values (every 12 hours for first 24 hours post-cardiac catheterization and one set of cardiac enzymes at week 2 visit).
- Post-cardiac catheterization echocardiogram.

Efficacy (Secondary): During the six-month follow-up period, at the month 12 visit.

- Echocardiographic measures of regional and global left ventricular function.
- Infarct size, regional and global left ventricular function by CT
- Peak VO_2 (by treadmill determination).
- Six-minute walk test.
- NYHA functional class.
- Minnesota Living with Heart Failure (MLHF) questionnaire.
- Incidence of Major Adverse Cardiac Events (MACE), defined as the composite incidence of (1) death, (2) hospitalization for worsening HF, or (3) non-fatal recurrent MI.

Study Therapy: Allogeneic human mesenchymal stem cells (Allo-hMSCs) at 20 or 100 million total hMSCs, supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami Interdisciplinary Stem Cell Institute.

Duration of Study Follow-Up: Follow-up will be at 2 weeks, 3, 6 months and 12-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[2-4]. Ideally, graft cells should be readily available, easy to culture to ensure adequate quantities for transplantation, and able to survive in host myocardium, which is often a hostile environment of limited blood supply and immunorejection. Whether effective cellular regenerative strategies require that administered cells differentiate into adult cardiomyocytes and couple electromechanically with the surrounding myocardium is increasingly controversial and recent evidence suggests that this may not be required for effective cardiac repair. Most importantly, transplantation of graft cells should improve cardiac function and prevent adverse ventricular remodeling. To date, a number of candidate cells have been transplanted in experimental models, including fetal and neonatal cardiomyocytes[5, 6], embryonic stem cell-derived myocytes[5, 7, 8], tissue engineered contractile grafts[9], skeletal myoblasts[10, 11], several cell types derived from adult bone marrow[12-20], and cardiac precursors residing within the heart itself[21-23]. There has been substantial clinical development in the use of whole bone marrow and skeletal myoblast preparations in studies enrolling both post-infarction patients and patients with chronic ischemic left ventricular dysfunction and heart failure. The effects of bone marrow-derived mesenchymal stem cells (MSCs) have also been studied clinically[24, 25].

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

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

Cells derived from adult bone marrow

Bone marrow harbors a variety of cells that may contribute to vasculogenesis or cardiomyogenesis, either directly, or by facilitating endogenous repair mechanisms. Bone marrow cells have been prepared on the basis of being 1.) endothelial precursor cells that are $CD34^+$, 2.) MSCs purified without an antigen panning technique on the basis of their fibroblast morphology, ability to divide in culture and to differentiate into mesodermal lineages[26], and 3.) cells that express stem cell factor receptor, c-Kit[27, 28]. Endothelial progenitor cells (EPCs) express the surface markers CD34, CD133, c-kit, and the vascular endothelial growth

factor receptor-2 (VEGFR2; KDR; Flk-1)[29-34]. Hematopoietic stem cells (HSCs) exhibit self-renewal and differentiation. Their cell-surface phenotype is $CD34^+$, stem cell factor antigen $(SCA\text{-}1)^+$, c-kit^+, and Lin^- (review[35]). While there has been controversy regarding the ability of bone marrow-derived cells to transdifferentiate into cardiomyocytes[36], clinical trials of bone marrow therapies continue to suggest potential benefit in terms of improving cardiac function or reducing the burden of scarred myocardium[4].

Mesenchymal Stem Cells: MSCs are a particularly promising bone marrow-derived cell for cardiac regenerative therapy because of their availability, immunologic properties, and track record of safety and efficacy[24, 37]. Studies of MSC engraftment in rodent and swine models of myocardial infarction have shown that administration of MSCs produces: 1) functional benefit in post-myocardial infarction (MI) recovery of ventricular function, 2) evidence of neoangiogenesis at the site of the infarct, 3) decrease in collagen deposition in the region of the scar, and 4) some evidence of cells expressing contractile and sarcomeric proteins but lacking true sarcomeric functional organization[38, 39]. Moreover, MSCs are thought to be ideal candidate cells for allogeneic transplantation because they show minimal major histocompatibility complex (MHC) class II and intracellular adhesion molecule (ICAM) expression and lack B-7 costimulatory molecules necessary to cause a T-cell mediated immune response[4, 37, 40].

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[41]. Some, but not all, groups have purified MSCs expressing c-Kit directly from bone marrow that have the capacity to form cardiac myocytes[42]. This is of functional significance given the demonstration that stem cell factor stimulates cardiac repair post-MI[28].

Clinical Trials

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

Previous Human Experience with Skeletal Myoblasts

There have been several case reports and very small phase I clinical trials investigating the feasibility of autologous skeletal myoblast transplantation[43-45] 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[46, 47], increased wall thickening[48], and New York Heart Association (NYHA) functional class[43, 47]. However the lack of electromechanical coupling between engrafted skeletal myoblasts and cardiac myocytes in vivo[49, 50] has raised serious concerns over the likelihood of an

increase in ventricular tachyarrhythmia's secondary to the formation of re-entry circuits[43, 48, 51]. 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[43].

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)[52]. However, although neither group randomized to skeletal myoblast therapy demonstrated improvement in survival, the high-dose group did show statistically significant reductions in both end diastolic volume (EDV) and end systolic volume (ESV); effects that were not observed in the low-dose group.

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

Clinical studies using autologous mononuclear bone marrow cells have been performed for a variety of indications, including peripheral vascular and cardiac diseases. The Therapeutic Angiogenesis using Cell Transplantation Study investigators[53] injected bone marrow mononuclear cells into the gastrocnemius muscles of patients with lower extremity ischemia and demonstrated significant improvement in ankle-brachial pressure index, rest pain, and pain free walking time. The authors concluded that the efficacy related to these implanted cells is due to the supply of endothelial progenitor cells.

AMBMCs in Acute MI: As with skeletal myoblasts, there have been several small studies evaluating the safety and feasibility of AMBMC cardiomyoplasty in patients in the peri-infarct period. Although these studies are also limited by similarly small numbers of patients and lack of blinding, control groups, and randomization, they do offer promising insights into the potential of MSC transplantation. In an early study, Strauer *et al*. randomized 20 patients following transmural MI to standard therapy plus intracoronary AMBMC injection 12 hours after acute MI, or to standard medical therapy alone. Intracoronary AMBMC decreased infarct size from 30±13% to 12±7% and the size of perfusion defects, as assessed by ^{201}thallium scintigraphy, by 26% (174±99cm^2 to 128±71 cm^2) compared to baseline values[54]. Subsequently, Stamm *et al*. demonstrated similar improvements in perfusion, left ventricle dimensions, and ejection fraction (EF) in an uncontrolled, non-blinded phase I study of 12 patients with transmural MI and left ventricular (LV) dysfunction (EF of 39.7±9%). These patients had infarct areas not amenable to surgical or interventional revascularization; they received intraoperative AMBMC injections during elective coronary artery bypass to non-infarct-related arteries in the first 3 months post-MI[55, 56].

In the TOPCARE-AMI[57] trial, post-MI patients were randomized to receive either AMBMC (n=9) or peripheral blood-derived progenitor cells (n=11) infused into the infarct artery approximately four days after reperfusion with coronary stenting.

Over 90% of the cells derived from peripheral blood exhibited endothelial cell characteristics including KDR, von Willebrand factor, CD31, and VE-Cadherin; while those derived from bone marrow cells exhibited 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. There was also complete normalization of coronary flow reserve in the infarct artery, and a significant increase in myocardial viability within the infarcted segments. Furthermore, a sustained improvement in LV function, reduction in end-systolic volume, and prevention of remodeling was observed at 1-year. These findings were observed in both the AMBMC and peripheral blood-derived progenitor cell treatment groups. The recently completed 5-year follow up showed that the improvement in LVEF in the treated groups was sustained[58]. These results are quite promising; supporting the conduct of larger, controlled clinical trials.

In the randomized controlled BOOST clinical trial, patients received both standard post-infarct medical therapy and intracoronary transfer of AMBMC (n=30), or standard post-infarct therapy alone, 4 to 8 days after percutaneous coronary intervention for their first acute ST segment elevation MI. There was a 6.7±9.5% absolute improvement in global LVEF in the cell-treated group (46.3±10.6% at baseline to 53.0±15.5% at 6 months), compared to 1.1±11.8% increase in the control group (47.8±9.7% at baseline to 48.9±15.2%; p=0.0026). Furthermore cell transplantation was associated with increased systolic wall motion in the MI border zone[59]. Importantly, infarct size as measured by late enhancement magnetic resonance imaging (MRI) was not reduced compared to placebo in the BOOST trial. Reports from the BOOST investigators suggest that the relative improvement in LVEF 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[60]. The recently completed 5-year follow-up study showed that the 6-month improvement in LVEF in the treated group was not sustained[61], emphasizing the importance of examining in future trials the long-term sustainability of cell-based therapy.

In the REPAIR AMI study, the largest trial of bone marrow-derived cellular therapy to date, Schächinger *et al.* randomized 204 patients to intracoronary infusion of bone-marrow cells or placebo 3 to 7 days after successful reperfusion therapy. At the four-month follow-up period, LVEF improved by 5.5% with the bone marrow cells versus 3% with placebo infusion (p=0.014). Interestingly, the benefit was greatest in patients with the worst ejection fractions at baseline[62]. Other studies suggest relatively less benefit in EF than that reported above, although AMBMCs appeared to reduce infarct size[63].

AMBMCs In Chronic Ischemia: There are several small studies investigating the safety and feasibility of autologous bone marrow cell transplantation for ischemic heart disease[17, 64-68].

Hamano and colleagues performed a non-randomized study of direct injection of AMBMC into ungraftable or peri-infarct myocardial segments during CABG in five

patients and reported improved perfusion to the treated areas up to one year after surgery[64]. Ozbaran and colleagues injected peripheral blood stem cells mobilized with granulocyte colony stimulating factor (G-CSF) in the myocardium of 6 patients with severe ischemic cardiomyopathy (EF <25%); they found improvements in NYHA functional class and quality of life[69]. However, it is important to note the difficultly in determining how much of the improved perfusion is secondary to the stem cells compared to surgical revascularization.

In a randomized, crossover trial known as TOPCARE-CHD, Assmus *et al.* compared bone marrow-derived progenitor cells and progenitor cells derived from circulating blood to no cellular therapy in 75 patients with chronic left ventricular dysfunction. Results showed a modest benefit at three months in the group receiving the bone marrow-derived cells: EF in the patients treated with these had an absolute increase of 2.9% versus (1) a decrease of 0.4% in the patients who received injections of progenitor cells derived from circulating blood (P=0.003), and (2) a decrease of 1.4% in the patients who received no infusion (P<0.001)[70].

Several smaller (n<20) non-randomized studies have performed endocardial catheter injections of AMBMC into chronically ischemic myocardium and demonstrated improved myocardial function and perfusion, as well as reduced symptoms[66-68]. Perin and colleagues performed a nonrandomized, open-label study comparing AMBMC injection (n=14) to standard therapy (n=7) in 21 patients with severe ischemic heart failure. They used a NOGA™ endocardial-mapping catheter to inject AMBMC into hibernating myocardial segments of patients with severe ischemic heart failure. They reported a 73% reduction in the total reversible perfusion defect, improved mechanical function of injected myocardial segments as determined by electromechanical mapping, improved global EF (9%), and improved NYHA functional class and Canadian Cardiovascular Society Angina score[66].

Recently, the First Mononuclear Cells injected in the United States conducted by the Cardiovascular Cell Therapy Research Network (FOCUS-CCTRN) phase 2 trial evaluated whether transendocardial delivery of AMBMCs improves LV performance and perfusion at 6 months in patients with chronic ischemic cardiomyopathy[71]. Although there was no improvement compared to placebo in LV end-systolic volume, maximal oxygen consumption, or myocardial perfusion, exploratory analyses showed significant improvement in LVEF and stroke volume. Interestingly, the improvement in EF was associated with higher bone marrow $CD34^+$ and $CD133^+$ progenitor cell counts. These results suggest that the cellular composition of the bone marrow may determine clinical end points, with certain cell populations providing a greater regenerative benefit. Together these results support ongoing research in AMBMC transplant for patients with chronic ischemic cardiomyopathy.

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

Administration of autologous or allogeneic human MSCs to cardiovascular patients has been performed in several clinical studies to date. In the post-myocardial infarction (MI) setting, previous studies have administered MSCs via the intracoronary route (IC) and via peripheral intravenous (IV) injection.

In a clinical study reported by Chen *et al.*[72], 69 patients were randomly assigned to receive IC infusions of autologous MSCs (average cell dose: 5.4 x 10^{10}) or placebo (saline) 18 days after the onset of acute MI symptoms. At the three-month follow-up visit, LVEF was significantly improved in the MSC-treated group (from 49% ± 9% at baseline to 67% ± 11%) compared to the placebo group (from 48% ± 10% at baseline to 53% ± 18%; $P < 0.01$ for the between-group comparison). This improved EF was sustained at six months post-infusion. In addition, significant reductions in perfusion defect, left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were reported in the MSC-treated group. No adverse events were reported in this study. Although it is unclear if the cell preparation used was purified MSCs or whole bone marrow, even in the latter case, the likely range of MSC cells infused was 5 x 10^7 – 5 x 10^8 cells, since the MSC fraction is generally considered to be 0.1 – 1.0 % of a whole bone marrow aspirate.

Katritsis *et al.*[73] investigated the effects of IC infusions of autologous MSCs and endothelial progenitor cells (average cell dose: 1.5 x 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, multi-center, randomized, double-blinded, placebo-controlled study was performed in 53 patients who were treated 3-10 days post-MI[74]. Patients received one of three cell-dose levels of allogeneic MSCs (0.5, 1.6 and 5.0 M 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 administered via peripheral IV injection, and followed for six months. There were no deaths reported in the study; no toxicity was observed with the administration of the allogeneic MSCs (which were found to be well-tolerated at all dose levels administered, with 5.3 adverse events per patient in the MSC-treated group vs. 7.0 in the placebo group); and no serious adverse events were attributed to MSC administration. In fact, several signals from the trial indicate that the allogeneic MSCs were very safe, and also provided preliminary evidence of the following clinical benefits:

- Patients in the MSC-treated group were four times less likely to experience an arrhythmic event compared to those receiving placebo (9% vs. 37%, p=0.025).

- Fewer patients experienced clinically significant premature ventricular contractions (PVCs) after receiving MSCs as compared to placebo across all time points (11% vs. 24%, $p < 0.001$).
- The MSC-treated patients with major anterior wall myocardial infarctions had a statistically significant 7.0 point absolute improvement (24%) in EF at three months and a 7.3 point absolute improvement (25%) at six months over baseline ($p<0.05$), while similar patients receiving placebo did not have significant improvement.
- Patients in the MSC-treated group had significantly improved pulmonary function as measured by improvement in FEV_1 (% predicted), which increased 17% in the MSC-treated group vs. 6% in the placebo, $p < 0.05$.

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

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

There is substantial clinical experience with the intramyocardial delivery of autologous bone marrow-derived mononuclear cells (BMCs) in the clinical setting of chronic left ventricular dysfunction. Table 1 lists the 11 studies in which intramyocardial delivery of BMCs was performed. Cell delivery has been performed via either (1) direct intramyocardial (IM) injection during coronary artery bypass graft (CABG) surgery, or (2) catheter-based intramyocardial injection (including transendocardial, or TEC, delivery)[24, 55, 56, 65-68, 75-80]. These results clearly support the clinical safety of the intramyocardial injection delivery method.

BMC cell doses as high as $292 \pm 232 \times 10^6$ have been injected via the IM route without untoward effects, and have produced global improvements in ventricular function[80]. In addition, a recent clinical study (the TABMMI trial) using the BioCardia Helical Infusion system (the delivery device to be used for the clinical study in this protocol) has been reported. In this study, transplantation of autologous BMCs ($86 \pm 3 \times 10^6$ cells) into the peri-infarct region of patients with chronic ischemic heart failure[79] was performed using the Biocardial Helical Infusion catheter to improve ease, efficiency, and safety of delivery. The study demonstrated statistically significant functional improvements in transthoracic echocardiographic measurements at both 6 and 12 months of follow-up, with no adverse events associated with the catheter.

In addition, a recent meta-analysis of all clinical trials of adult, bone marrow derived cell therapy (either BMCs or MSCs) for cardiac repair has been published[81]. The combined results of these studies support the clinical safety of administering both BMC and MSC preparations for cardiac repair.

Previous Human Experience Using the Biocardia Helical Infusion Catheter

The initial clinical study performed using the BioCardia Helical Infusion system

for intramyocardial delivery in patients with coronary artery disease undergoing PCI or diagnostic heart catheterization provided support that the procedure and device are safe and well-tolerated[82].

A recent clinical study (the TABMMI trial) using the BioCardia Helical Infusion system has been reported. In this study, transplantation of autologous BMCs into the peri-infarct region of patients with chronic ischemic heart failure[79] was performed using the Biocardial Helical Infusion catheter (the delivery device to be used for the clinical study in this protocol) to improve ease, efficiency, and safety of delivery. The study demonstrated statistically significant functional improvements in transthoracic echocardiographic measurements at both 6 and 12 months of follow-up, with no adverse events associated with the catheter.

In conclusion, the experience utilizing the BioCardia Helical Infusion system in these two clinical studies and in our recently published TAC-HFT clinical study[24], described below, supports the clinical safety of the technique and provides preliminary evidence of patient benefit.

TABLE 1

CLINICAL STUDIES: AUTOLOGOUS BONE MARROW-DERIVED MONONUCLEAR CELLS (BMCs) ADMINISTERED VIA INTRAMYOCARDIAL (IM) INJECTION IN PATIENTS WITH CHRONIC LEFT VENTRICULAR DYSFUNCTION

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

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

Potential mechanisms for MSC mediated improvements in cardiac function

As noted above, prior studies have shown that a variety of cellular sources are capable of differentiating into phenotypes that strongly resemble the three principal cell types of myocardium; cardiomyocytes, smooth muscle and vascular endothelium. Our preliminary data and reports from other labs cited above suggest that MSCs, the cells employed in our model of cellular cardiomyoplasty, have the potential to form all three cell types within infarcted myocardium *in vivo*[16]. Nevertheless, it is important to consider that MSCs may exert other favorable effects on cardiac repair above and beyond differentiation[4, 37]. For example, these cells may also participate in the recruitment and/or stimulation of other cells to differentiate into a cardiac phenotype[83].

There is a wealth of evidence suggesting that stem cell homing to injured myocardium is directed by injury signal(s) emanating from the area within or surrounding the infarct. For example, stromal-cell-derived factor 1 (SDF-1), a chemokine that is a natural ligand for the CXCR4 receptor, is crucial for bone marrow retention of hematopoietic stem cells[84, 85], cardiogenesis[86], recruitment of endothelial progenitor cells to sites of ischemic tissue[87] and, potentially, migration of tissue-committed stem/progenitor cells[88]. Interestingly, it was recently shown that the CXCR4 receptor is strongly expressed by a proportion of MSCs and it plays an important role in MSC mobilization[89]. Expression of SDF-1 dramatically increased over the first week following infarction, and exogenous expression of SDF-1 increased the numbers of mobilized bone marrow cells (BMCs) homing to the heart at time periods remote from infarction[90]. 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[91], and intramyocardial administration of MSCs is associated with increases in vascular endothelium growth factor (VEGF) levels[19]. Furthermore, it has been shown that MSCs participate in angiogenesis and arteriogenesis, differentiating into endothelium and vascular smooth muscle in a VEGF-dependent manner[92].

Once cells successfully home and engraft in the heart, they must survive in a hostile environment if they are to effect successful cardiac repair. It is thought that apoptosis within the infarct region is responsible for the fact that only a fraction of cells injected directly into the heart will engraft and survive, and that such cell death reduces the efficacy of cellular cardiomyoplasty. In a dramatic proof of principle study, Mangi *et al.* genetically engineered rat MSCs using *ex vivo* retroviral transduction to overexpress the anti-apoptotic protein Akt1, a serine-threonine kinase[39]. 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 USA). At present, several types of adult stem cells (possibly enhanced by concomitant strategies aimed at enhancing trafficking or survival) hold great promise to improve recovery following MI. This clinical study will utilize allogeneic bone marrow-derived hMSCs as a therapy for chronic ischemic left ventricular dysfunction and heart failure. Allogeneic MSCs have been chosen because they have shown effectiveness in small and large animal models as well as safety and effectiveness in clinical trials[4, 74], and offer the substantial advantage of already having approval from the FDA for use in humans.

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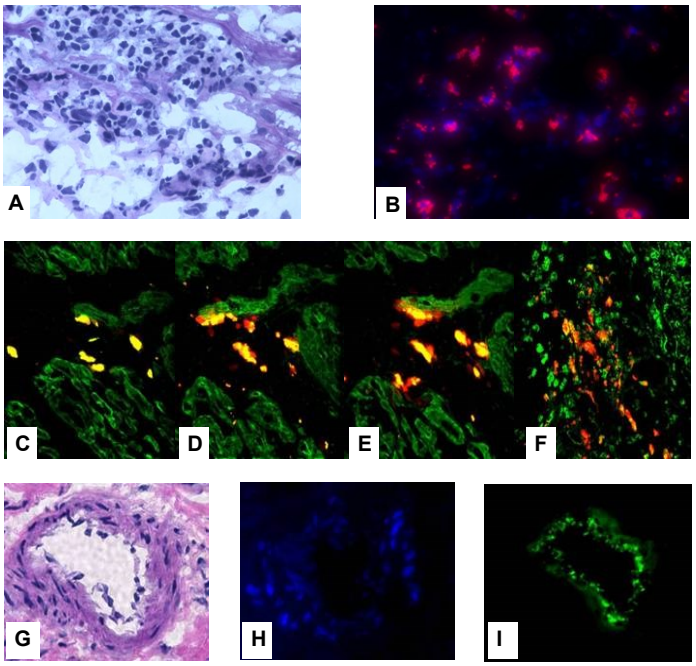

Figure 1. MSC engraftment and differentiation. MSC engraftment and muscle-specific protein-expression. DAPI and Di-I labeled MSCs (blue staining nuclei and red staining membranes, respectively) and fluorescent muscle protein-specific antibodies (green). (A) Hematoxylin and eosin (H&E)–stained section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

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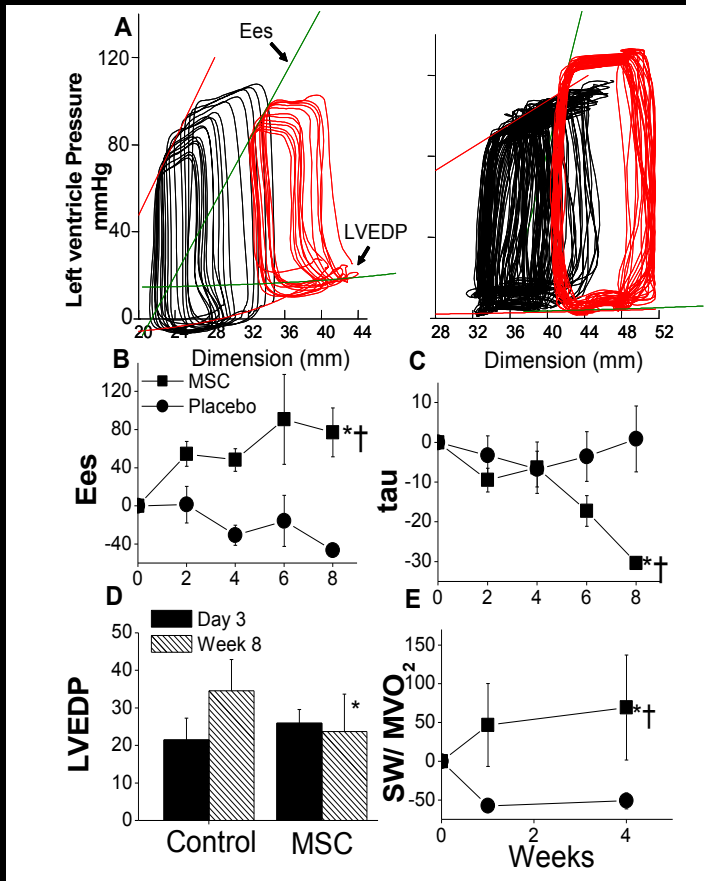

Figure 2. Physiologic impact of MSCs delivered with the BioCardia Catheter following anterior myocardial infarction (MI) in pigs. **(A)** Pressure-dimension (PD) data from placebo (left) and an MSC-treated (right) pig obtained 3 days (black loops) and 8 weeks (red loops) following MI. Placebo animals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

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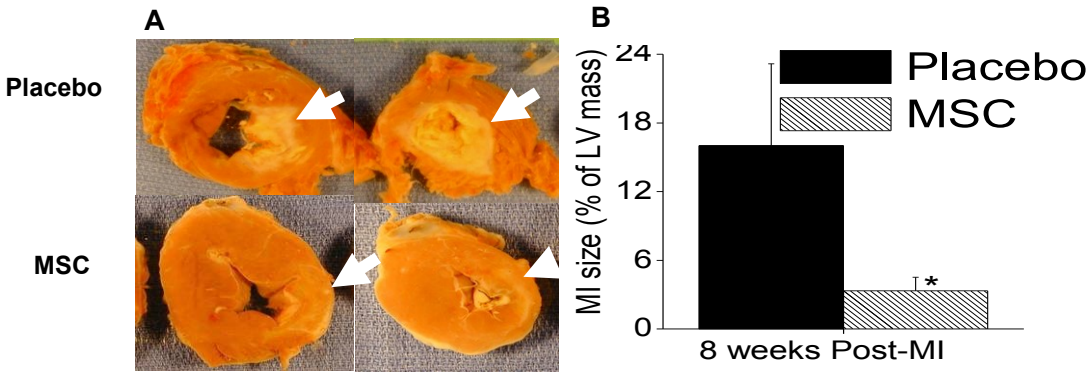
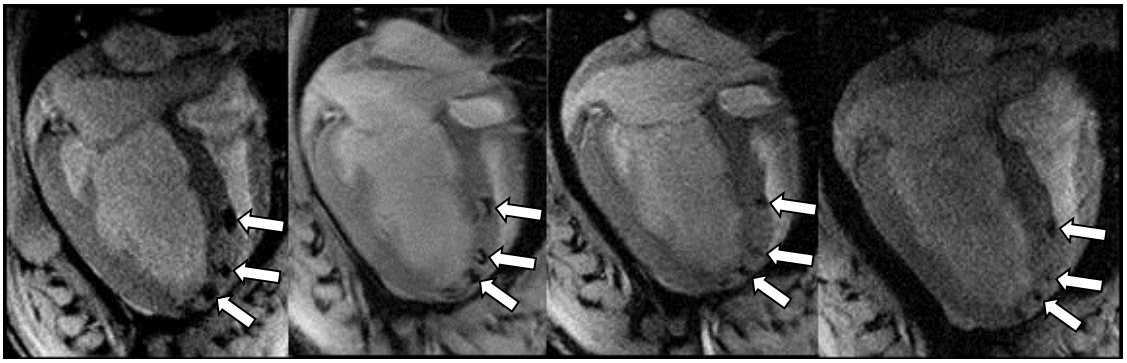


Figure 3. Myocardial infarct size 8 weeks following transient left anterior descending coronary artery occlusion. **A.** Representative example of scar formation due to myocardial infarction in placebo (top) and MSC treated animal (bottom). In placebo-treated animals the area of scar formation is transmural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

Figure 4. MRI image of swine myocardium obtained after myocardial infarction and injection of Feridex labeled mesenchymal stem cells. Feridex labeled cells can be seen as dark hypoenhancing regions in the epicardium (arrows) using an ECG-gated, fast gradient echo (fgre) pulse sequence. As shown, Feridex labeling remains evident for up to eight weeks after stem cell injection.



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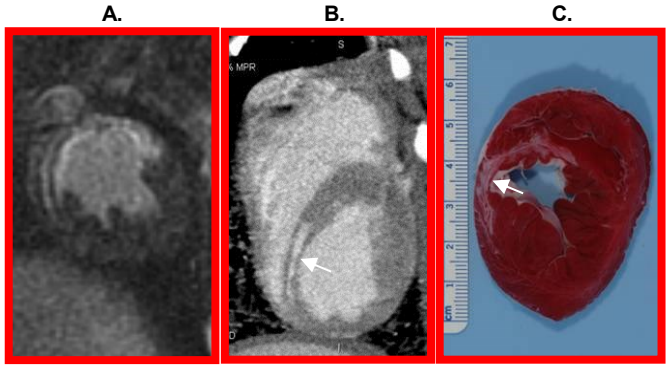

Figure 5. Comparison of infarct size using MRI (A), CT (B), and TTC (C). Images were obtained 8 weeks after closed-chest infarction in a pig and demonstrate subendocardial myocardial infarction as hyperenhancing region (~7-11 o'clock). TTC nonstaining areas (e.g., lack of brick red staining) in post-mortem slices (bottom) demonstrate concordance of infarct location and size with MRI and CT. Infarct region is notable for rim of noninfarcted myocardium along the endocardial border seen with CT and TTC staining. (arrows)

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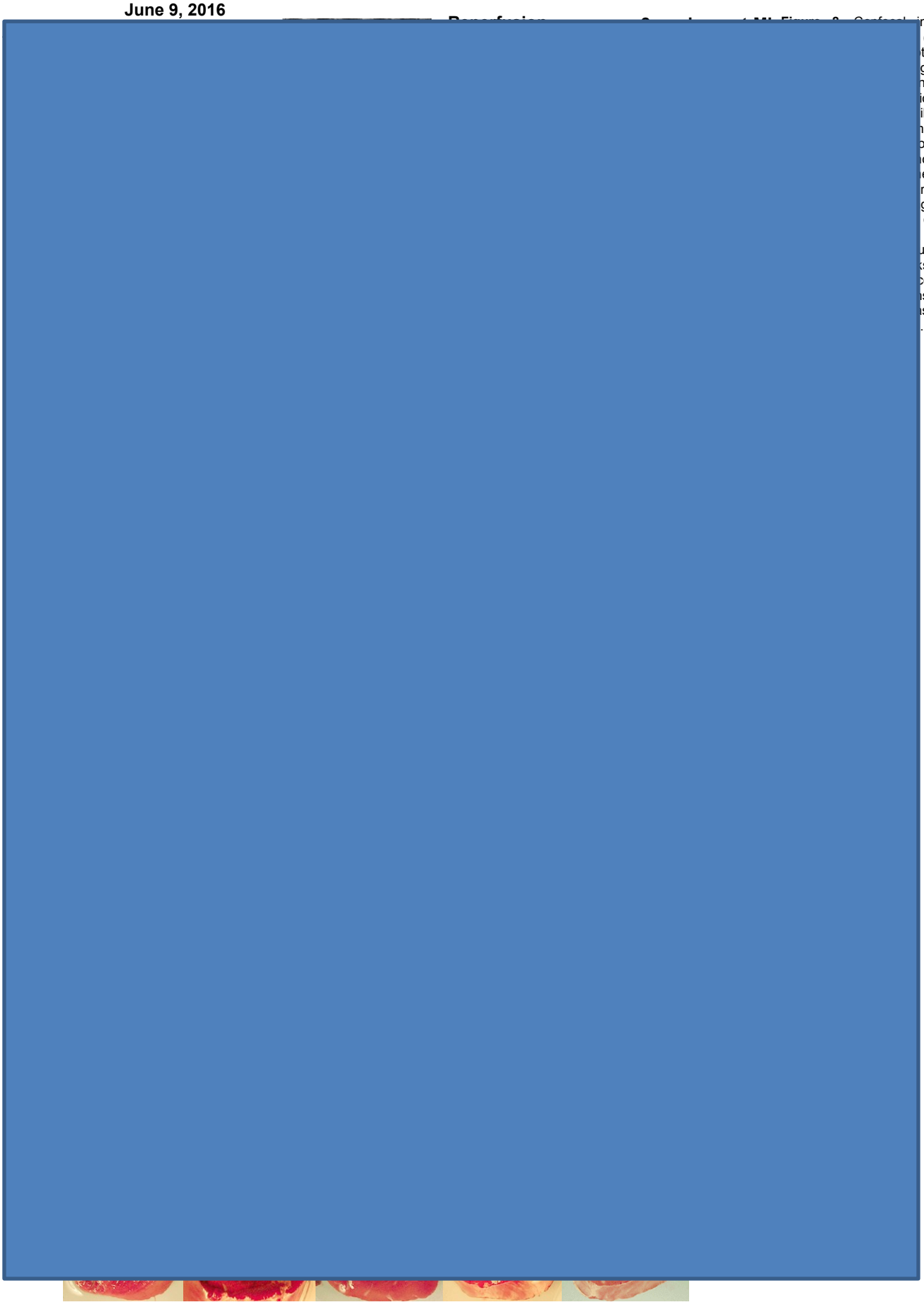

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Autologous Mesenchymal Stem Cells Produce Reverse Remodeling in Chronic Ischemic Cardiomyopathy: In addition to the studies outlined above using models of acute MI in the pig, we have also developed a model of chronic MI in the Gottingen mini-swine. We have used both autologous and allogeneic MSCs, with surgical and catheter delivery strategies, and have developed sufficient experience to translate the therapy from the laboratory bench to clinical trials. Together our results indicate that bone marrow derived MSCs stimulate cardiac recovery by engrafting, forming new blood vessels that increase tissue perfusion in hypoperfused areas, forming new cardiac myocytes, and importantly interacting with endogenous precursor cells to also contribute to new cardiac myocyte formation. From an immunologic perspective, MSCs may be safely used as an allogeneic graft, and have been done so extensively in clinical trials[112-114]. Our experience with a 53 patient, 10-center, phase I study under the sponsorship of Osiris therapeutics, which demonstrated safety and provisional efficacy of allogeneic MSC therapy in patients with acute infarction is outlined below[74]. In animal studies conducted in mini-swine, MSC injection via catheter into infarcted tissue reduces myocardial infarct size (Figs. 12 and 13), improves global and regional LV function, normalizes cardiac energetics, and restores tissue perfusion[93, 115]. These results form the basis for our approval to conduct the TRIDENT study.

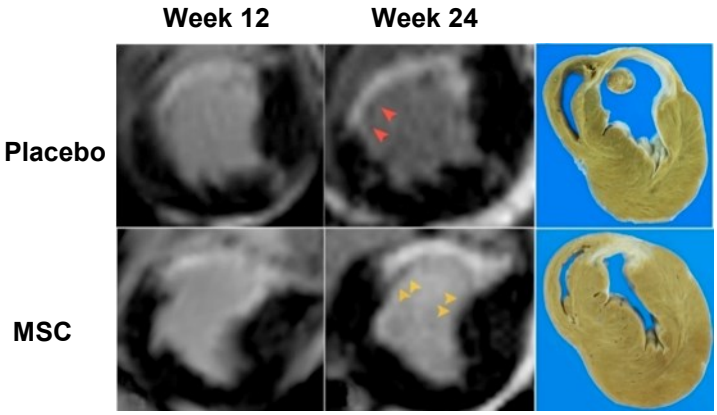

Figure 12. Myocardial regeneration in our porcine model of MI. Delayed gadolinium-enhanced MRI images depicting chronic (week 12 post MI) myocardial scar before treatment and (week 24) 12 weeks following injection of MSCs or placebo. Infarct tissue appears bright white, and healthy myocardium appears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

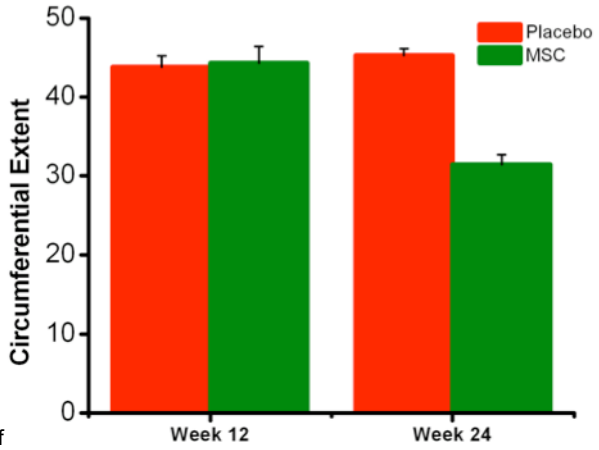

Figure 13. Infarct circumferential extent of the LV before and 12 weeks after injection in swine. Infarct size was significantly reduced by MSC treatment. $p<0.05$ MSC vs. placebo, $p<0.05$ MSC week 12 vs. week 24. [n=10]

Our work in large animal models with fully healed scars after MI showed that MSC administration can significantly improve left ventricular structure and functional indices, indicating meaningful repair. Through exploitation of our experience with imaging techniques we were able to track phenotypic improvements triggered by implantation of MSCs in our porcine model of chronic ischemic cardiomyopathy, and to quantify these changes morphometrically. MI was created in swine; after 12 weeks, the infarct segment had thinned, leaving a transmural scar (Figure 12). Autologous MSCs were expanded from each

animal[116], and these cells or placebo were delivered to the infarct and surrounding border zone at this time. During a further 12-week follow up period, cardiac MRI revealed that intramyocardial injections of MSCs not only reduced the scar burden (as a percentage of LV mass) by 21.8±3.9% ($p<0.05$ vs. placebo and week 12 vs. week 24) (Figure 12, 13), but also significantly improved regional contractility, global LV function, ejection fraction, and myocardial blood flow. Importantly, the therapy produced reverse remodeling and reduced the circumferential extent of the infarct scar (Figure 13). This constellation of effects suggests highly effective repair of ischemic cardiomyopathy. We subsequently confirmed reverse remodeling in a pilot study of 8 patients with ischemic cardiomyopathy (Figure 15)[24].

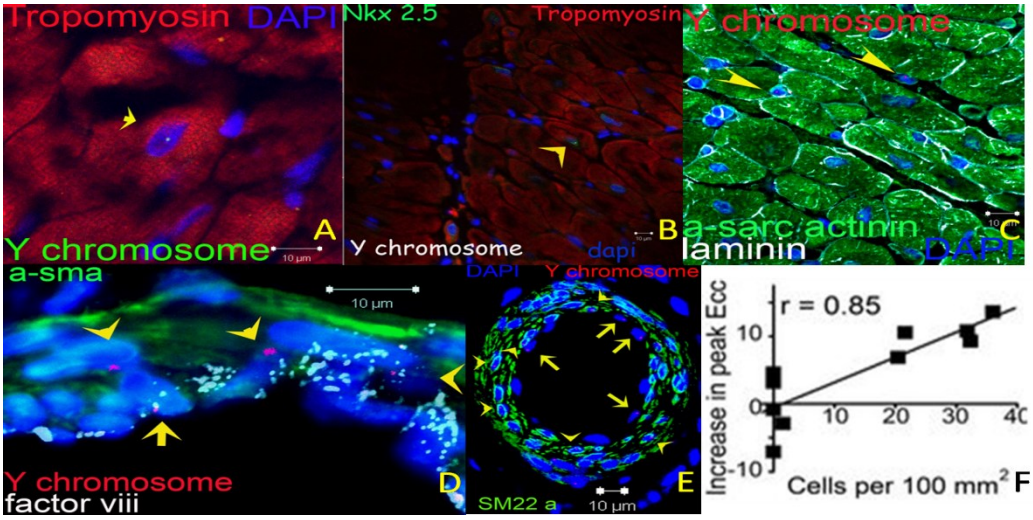

Figure 14. Engraftment of MSCs in chronic myocardial infarction. (**A**) Colocalization Y chromosome and troponin, indicative of an MSC differentiated into a cardiomyocyte. (**B**) Evidence of cardiac commitment in the transplanted cardiomyocyte by the colocalization of cardiac transcription factor Nkx2.5 (green, arrow). (**C**) Ypos cells also reside in the interstitial compartment (arrows) of border myocardium in a non-differentiated stage. (**D**) Ypos cells that colocalize with smooth muscle actinin (arrowheads) and factor viii-related antigen (white, arrows) demonstrating vascular smooth muscle and endothelial commitment, respectively. (**E**) Confirmation of Ypos cells commitment into vascular structures as depicted by co-localization with SM22-alpha (**F**) The functional outcomes (i.e. infarct size reduction, increase in contractility and increase in tissue perfusion) showed related interaction with the magnitude of transplanted cells detected.

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

We tested the hypothesis that MSC based cardiac repair regenerates the heart via mechanisms comprising long-term engraftment and by differentiation into both myocardial and vascular elements. We generated allogeneic MSCs from a male swine donor, and administered sex mismatched cells by transendocardial injection into female swine 12 weeks post-MI. Animals were followed with serial MRI, and 12 weeks later the hearts were collected for immunohistological evaluation. The fate of the male donor cells was determined by co-localization of Y-chromosome (Y^{pos}) cells with markers of cardiac, vascular, and endothelial lineages. MSCs engrafted in infarct and border zones and differentiated into

cardiomyocytes (Figure 14) as ascertained by co-localization with GATA-4, Nkx2.5, and α-sarcomeric actin markers. In addition, Y^{pos} MSCs exhibited vascular smooth muscle and endothelial cell differentiation, contributing to large and small vessel formation. The number of cells engrafting correlated with the functional changes that occurred (Figure 14F). Thus, MSCs could engraft and repair hearts in chronic ischemic cardiomyopathy[16].

Preliminary Results from the Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HFT): We enrolled 8 patients in an open-label run-in phase of the TAC-HFT trial to assess the safety and preliminary efficacy of bone marrow mononuclear cells (MNCs) and MSCs in patients with ischemic cardiomyopathy[24, 25]. At baseline each patient underwent a bone marrow aspiration and transendocardial injection (Helix Infusion Catheter; Biocardia, Inc., CA) of bone marrow derived MNCs or MSCs to the infarct and border zone using biplane fluoroscopic guidance. All 8 patients tolerated the procedure well and have been followed-up with serial cardiac MRI at 3, 6, and 12 months post injection (Figure 15). The injection of bone marrow derived cells into the hearts of these patients produced reverse remodeling, with reductions in EDV of ~12% and improved regional function as measured by –Ecc, a cardiac MRI derived index (Figure 15). We have also treated patients with Allogeneic MSCs in the POSEIDON trial and have demonstrated an acceptable safety profile, as per the interim analysis of the NIH DSMB.

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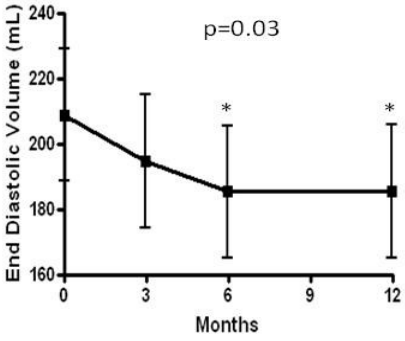

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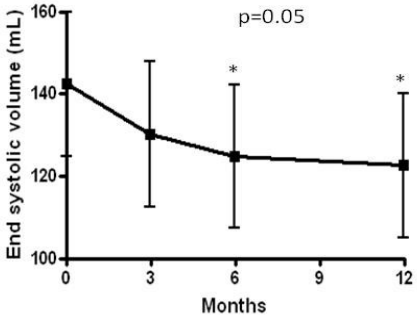

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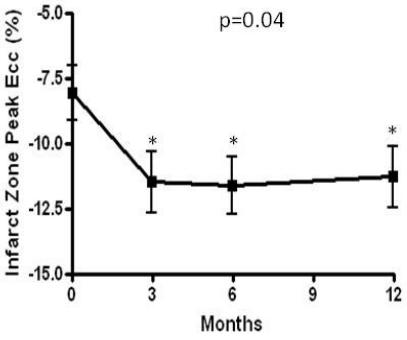

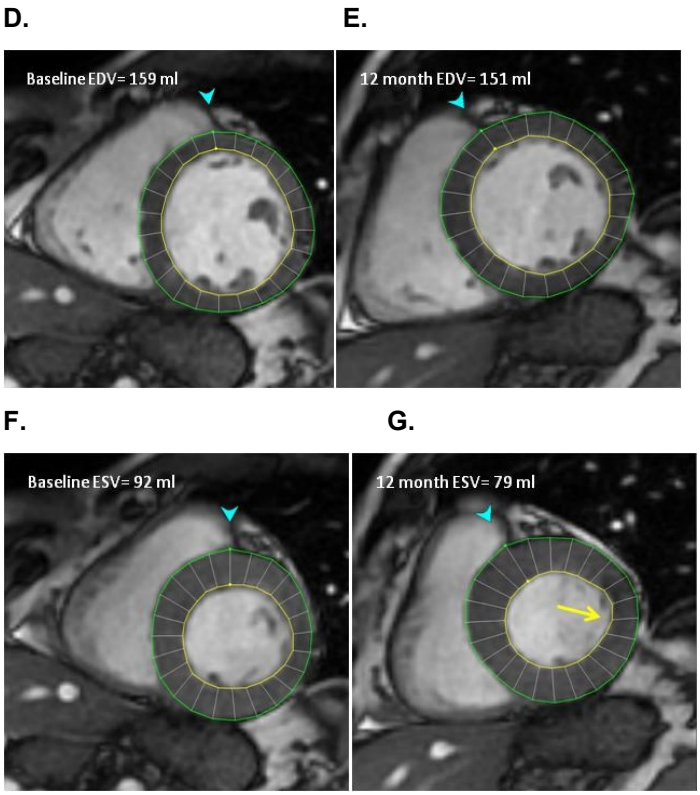

Figure 15. Cardiac MRI follow-up data of (A) end-diastolic volume, (B) end-systolic volume, and (C) peak Eulerian circumferential strain (Ecc) of the infarct zone from tagged imaging. Example cardiac MRI images of change in EDV from (D) baseline to (E) 1 year, and change in ESV from (F) baseline to (G) 1 year after stem cell injection. As depicted, EDV and ESV are reduced by 6 months following injection, and do not return towards baseline by 12 months. Peak Ecc is dramatically reduced by 3 months following injection (the more negative Ecc corresponds to *improving* regional LV function). *p<0.05 in post-hoc analysis compared to baseline. Yellow Arrow: Note improved systolic thickening in lateral wall, the site of cell therapy in this example.

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

Together, these preliminary data support our hypothesis that allogeneic MSC administration is a safe and successful option for cellular cardiomyoplasty. We have demonstrated long-term MSC survival, engraftment, and differentiation into myocardial, vascular, and endothelial lineages following transplantation into chronically scarred porcine myocardium. These cells' capacity for cardiomyogenesis and vasculogenesis both likely contribute to their ability to repair chronically scarred myocardium.

Animal Pharmacology and Toxicology Studies of MSCs Delivered Via Intramyocardial Injection

Seven preclinical studies (Table 2) and 2 clinical studies (described above) have been performed using autologous or allogeneic MSCs delivered to ischemic myocardium via intramyocardial injection. The cell doses administered in these studies cover the proposed dose (1.0×10^8 MSCs) for the clinical study in this IND application.

Shake *et al.*[38] investigated the engraftment and functional effects of transplanted autologous MSCs (cell dose = 60×10^6, administered via direct intramyocardial injection with a 30-gauge needle) 14 days after MI in a porcine animal model. No ectopic tissue formation was observed. Furthermore, there was no evidence of MSC differentiation to tissues other than cardiac muscle, and no significant inflammatory infiltrates at the MSC implantation sites. Microscopic analysis showed robust engraftment of MSCs in all treated animals. Expression of muscle-specific proteins was seen as early as 2 weeks and could be identified in all animals at sacrifice. The degree of contractile dysfunction was significantly attenuated at 4 weeks in animals transplanted with MSCs (+5.4% ± 2.2% versus -3.37% ± 2.7% in control). In addition, the extent of wall thinning after MI[117] was markedly reduced in treated animals.

In a porcine animal model, Cattaneo *et al.*[96] transplanted allogeneic MSCs (cell dose = 200×10^6, administered via direct intramyocardial injection with a 27-gauge needle) shortly (1 day) after MI. No ectopic tissue formation, significant inflammatory responses, or other adverse events were observed. Robust engraftment of allogeneic MSCs was seen in all treated animals. Furthermore, engrafted MSCs were found to express numerous muscle specific proteins and exhibited morphological changes consistent with cardiomyogenesis. A marked improvement in both ejection fraction and global wall motion score was observed in treated animals at 10 weeks post-MSC implantation. Systolic wall thickening and diastolic wall thickness were also augmented in MSC-treated animals. Since no significant differences in infarct size or cardiac loading were noted between groups (MSC-treated of placebo), improvements in cardiac function were likely attributable to MSC implantation.

The two porcine animal model studies by Amado *et al.*[93, 118] investigated the intramyocardial injection of allogeneic porcine MSCs (cell dose = 200×10^6) via transendocardial catheter delivery 3 days after MI. One of the studies, a

randomized, placebo-controlled study of 14 pigs using the BioCardia Helical Infusion Catheter found[93]:

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

Additionally, several important findings emerged from both Amado and investigators' transendocardial porcine animal studies[93, 118], including:

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

Schuleri et al[115] used a porcine animal model and injected autologous MSCs (low cell dose= 20 x 10^6 and high cell dose= 200 x 10^6, administered via direct intramyocardial injection using a 1.0 ml syringe with a 29-guage needle) 12 weeks (90 days) after MI. Between groups there was no difference in mortality, systemic inflammatory responses, or post-injection arrhythmias. No ectopic tissue formation was observed in the treatment groups. A trend towards reduction was noted in the low dose group, whereas the high dose MSC treatment group saw a significant reduction in infarct size. Additionally this therapy produced new tissue that is contractile and perfused, and an overall increase in LVEF following a single administration of MSCs.

A study by Quevedo, et al[16] sought to test the ability of allogeneic MSCs to engraft and differentiate in chronically infarcted myocardium. Female porcine animals were injected transendocardially with allogeneic MSCs using a catheter (cell dose= 200 x 10^6) 12 weeks after MI. The study saw the presence of viable MSCs in infarct and border zones 12 weeks after transplantation into a chronic ischemic scar. The MSCs differentiated into cardiomyocytes and blood vessel elements that integrated into host myocardium, formed gap junctions, and contributed to the restoration of cardiac function and tissue perfusion. The decrease in infarct size together with increased contractility and perfusion resulted in improved global LV function. Also a significant increase in ejection fraction was seen in the MSC-treated group. This study was able to document MSC engraftment and differentiation into cardiac cell constituents in an infarcted heart.

Hatzistergos et al.[83] investigated the ability of bone marrow derived MSCs to stimulate the proliferation and differentiation of endogenous cardiac stem cells (CSCs). The study had a 2 phase porcine model. In phase 1, eight pigs were transendocardially injected with 75 x 10^6 GFP labeled MSCs, eight more pigs received placebo, and three pigs were part of a no injection comparison group. The injections occurred at 3-days post MI. The animals were euthanized at varying points after transplantation. The second phase of the study contained 12 pigs, which were equally randomized to one of two groups. Six pigs were treated with 100 x 10^6 male GFP-labeled MSCs and six pigs were treated with their 10x concentrated conditioned medium (CCM). The animals from phase 2 were followed by MRI analyses at several time-points post-injection to assess the amount of functional recovery; the animals were euthanized at varying points.

The absolute size of the myocardial infarct was reduced in the animals treated with MSCs as early as 4 days post-procedure while no change was seen in those treated with CCM. By two weeks, the MSC group also had a significant improvement in ejection fraction compared to post-MI, and this result persisted through the 8-week follow-up period. Two weeks after injection MSC-treated hearts exhibited chimeric clusters containing both immature MSCs of exogenous origin and endogenous CSCs. The bone marrow derived MSCs injected post-MI facilitated engraftment, differentiation, and substantial cardiac recovery involving host cell-based repair.

The results of these preclinical studies, and the ongoing clinical trials TAC-HFT and POSEIDON, support the safety and potential efficacy of the dose (1.0 X 10^8 MSCs) for the proposed clinical study in this IND application.

TABLE 2
PRECLINICAL STUDIES: AUTOLOGOUS AND ALLOGENEIC MESENCHYMAL STEM CELLS (MSCs) ADMINISTERED VIA INTRAMYOCARDIAL INJECTION

Study	Model	N	Cell Delivery	Cell Source & Type	Cell Doses (x 10^6)	Safety Results	Efficacy Results
Shake	14-Day Post-MI Pig	14	Surgical (needle) IM injection	Autologous, porcine MSCs	60.0	- No ectopic tissue formation - No MSC differentiation to non-cardiac tissue - No significant inflammatory infiltrates at site of MSC implantation	- MSC engraftment - ↑regional contractile function
Cattaneo	1-Day Post-MI Pig	13	Surgical (needle) IM injection	Allogeneic, porcine MSCs	200.0	- No ectopic tissue formation - No significant inflammatory response	- MSC engraftment - ↑EF and global wall motion score
Amado	3-Day Post-MI Pig	14	PIM (catheter) injection	Allogeneic, porcine MSCs	200.0	- No deaths; no malignant arrhythmias - No evidence of cardiac perforation during injection	- MSC engraftment - ↓infarct scar - Improved systolic and diastolic function
Amado	3-Day Post-MI Pig	22	PIM (catheter) injection	Allogeneic, porcine MSCs	200.0	- No difference in deaths between treated/placebo	- MSC engraftment - ↑Viable myocardium - ↓infarct scar
Schuleri	90-Day Post-MI Pig	9	Surgical (needle) IM injection	Autologous porcine MSCs	20.0 (Low) 200.0 (High)	-No difference in deaths between groups -No evidence of post-injection arrhythmias -No ectopic tissue formation	- ↓infarct scar at High Dose -↑EF
Quevedo	90-Day Post-MI Pig	6	PIM (catheter injection)	Allogeneic, porcine MSCs	200.00		-MSC engraftment & differentiation -↓infarct scar -↑global LV function and EF
Hatzisterg os	3-Day Post-MI Pig	12	PIM (catheter injection)	Allogeneic porcine MSCs & Concentrated Condition Medium	75 100 & 10 x concentrated condition medium		-MSC engraftment & differentiation -↑EF

EF: Ejection Fraction; IM: intramyocardial; MSC: Mesenchymal Stem Cell; PIM: percutaneous intramyocardial; VT: ventricular tachycardia

Rationale for Proposed Mesenchymal Stem Cell (MSC) Dose

The clinical experience with the administration of MSCs in the three clinical studies performed to date (two utilizing the IC route, and one via peripheral IV injection) as well as our experience on the POSEIDON study provide substantial evidence of clinical safety at the cell doses administered as well as preliminary support of clinical efficacy. Although none of the studies employed the delivery method planned in this IND application's proposed clinical study, two of the three studies utilized cell doses that were over 50% greater[72] and as much as two hundred-fold greater[70] than the two MSC cell doses proposed herein (0.2×10^8 and 1.0×10^8).

Summary

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

Given the experience and encouraging preliminary results with stem cell therapies and tracking, the University of Miami Miller School of Medicine (UMMSM) team is conducting this clinical study.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

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

2.1.2 Secondary Objectives

To evaluate the safety of allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI. To demonstrate the efficacy of allogeneic hMSCs administered by transendocardial injection in patients with chronic ischemic left ventricular dysfunction secondary to MI.

2.2 Study Endpoints

2.2.1 Primary Endpoint (Safety)

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

2.2.2 Secondary Endpoints (Efficacy)

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

- CT and Echocardiographic-derived measures of left ventricular function:
    1. Difference between the baseline and 12-month infarct scar size (ISS) as determined by delayed contrast-enhanced CT
    2. Difference between the baseline and 12-month regional left ventricular function (at the site of allogeneic cell injections) as determined by CT.
    3. Difference between the baseline and 12-month regional left ventricular wall thickening as determined by CT.

4. Difference between the baseline, 6-month (echocardiogram only), and 12-month left ventricular end diastolic wall thickness as determined by CT and echocardiogram.
5. Difference between the baseline, 6-month (echocardiogram only), and 12-month left ventricular ejection fraction, end diastolic and end systolic volumes, as determined by CT and echocardiogram.
6. Difference between the baseline and 12-month left ventricular regional myocardial perfusion as determined by CT.

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

- Tissue perfusion measured by CT.
- Peak VO_2 (by treadmill determination).
- Six-minute walk test.
- NYHA functional class.
- Minnesota Living with Heart Failure (MLHF) Questionnaire.
- Incidence of the Major Adverse Cardiac Events (MACE) endpoint, defined as the composite incidence of (1) death, (2) hospitalization for worsening heart failure, or (3) non-fatal recurrent MI.

2.2.3 Secondary Endpoints (Safety)

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

- Treatment emergent adverse event (AE) rates.
- 24-hour ambulatory electrocardiogram (ECG) recordings.
- Hematology and clinical chemistry values and urinalysis results.
- Serial Troponin I and CK-MB values (every 12 hours for the first 24 hours post-cardiac catheterization).
- Post-cardiac catheterization echocardiogram.

3. STUDY DESIGN

This is a Phase II, randomized, blinded study, intended to further evaluate the 20 million and 100 million dose levels of allogeneic MSCs. A total of 30 patients will be randomized and receive the study injection via the Biocardia Helical infusion system:

Treatment Group 1 (15 patients)

Fifteen (15) patients will be treated with Allo hMSCs: 4 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 0.2 x 10^8 (20 million) Allo-hMSCs.

Treatment Group 2 (15 patients)

Fifteen (15) patients will be treated with Allo hMSCs: 20 million cells/ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 1 x 10^8 (100 million) Allo-hMSCs.

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

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

The Allo-hMSCs will be supplied from an allogeneic human mesenchymal stem cell source isolated from bone marrow cells from normal human donors and manufactured by the University of Miami.

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

* Definition of Myocardial Infarction:

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

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

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

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

** Definition of myocardial perforation:

Myocardial perforation will be considered to have occurred if a) a new pericardial effusion >1cm thick is detected by transthoracic echocardiography immediately post, 4-6 hours post, or on day 2 post catheter injection; or b) a new ventricular septal defect is detected by Doppler echocardiography immediately post, 4-6 hours post, or on day 2 post catheter injection.

3.1 Inclusion Criteria

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

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

3.2 Exclusion Criteria

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

1. Have a baseline glomerular filtration rate ≤ 35 ml/min/1.73m^2.
2. Have a known, serious radiographic contrast allergy.
3. Have a Mechanical aortic valve or heart constrictive device.

4. Have a documented presence of aortic stenosis (aortic stenosis graded as 1.5cm^2 or less).
5. Have a documented presence of moderate to severe aortic insufficiency (echocardiographic assessment of aortic insufficiency graded as ≥+2).
6. Require coronary artery revascularization. Patients who require or undergo revascularization procedures should undergo these procedures a minimum of 3 months in advance of treatment in this study. In addition, patients who develop a need for revascularization following enrollment will be submitted for this therapy without delay.
7. Have evidence of a life-threatening arrhythmia in the absence of a defibrillator (non-sustained ventricular tachycardia ≥ 20 consecutive beats or complete second or third degree heart block in the absence of a functioning pacemaker) or QTc interval > 550 ms on screening ECG
8. AICD firing in the past 60 days prior to enrollment.
9. Have a hematologic abnormality as evidenced by hematocrit < 25%, white blood cell < 2,500/µl or platelet values < 100,000/µl without another explanation.
10. Have liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the ULN.
11. Have a coagulopathy = (INR > 1.3) not due to a reversible cause (i.e., Coumadin). Patients on Coumadin will be withdrawn 5 days before the procedure and confirmed to have an INR < 1.3. Patients who cannot be withdrawn from Coumadin will be excluded from enrollment
12. Have known allergies to penicillin or streptomycin.
13. Hypersensitivity to Dimethyl Sulfoxide (DMSO).
14. Be an organ transplant recipient.
15. Have a history of organ or cell transplant rejection
16. 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, melanoma in situ or cervical carcinoma.
17. Have a non-cardiac condition that limits lifespan to < 1 year.
18. Have a history of drug or alcohol abuse within the past 24 months.
19. Be on chronic therapy with immunosuppressant medication, such as corticosteroids or TNFα antagonists.
20. Be serum positive for HIV, hepatitis BsAg or viremic hepatitis C.
21. Be currently participating (or participated within the previous 30 days) in an investigational therapeutic or device trial.

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

4. TREATMENT OF PATIENTS

4.1 Study Therapy and Dosages

4.1.1 Study Investigational Therapy

Harvesting for Allo-hMSCs

Bone marrow (BM) will be harvested from normal healthy donors with 30 to 120 ml aspirated from the posterior iliac crest, which is the thickened superior margin of the ilium terminating in the iliac spine. The patient will lay on his/her side to enable the physician to have optimal access to the posterior iliac crest of the hip area. This area will be cleaned with a germ-killing cleanser followed by the application of local anesthetic. The BM aspiration will be done with a special needle attached to the heparinized syringes. The mononuclear fraction (MNC) will be isolated using a density gradient with Lymphocyte Separation Medium (LSM; specific gravity 1.077). The low density cells will be collected from the gradient and washed with Plasma-Lyte A containing 1% human serum albumin (HSA). The washed cells will be sampled and viable cell counts performed to determine the total number of viable cells.

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

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

Allo-hMSC Preparation for Administration

The required dose of Allo-hMSC will be generated using standard human MSC culture conditions. Preclinical validation studies have demonstrated the reproducible generation of more than 200 million hMSCs in 21 days of culture. The bone marrow mononuclear cells are seeded into 225 cm^2 tissue culture flasks in alpha MEM containing 20% FBS. After 14 days of culture passage, zero

(P0) cells are harvested by trypsin treatment and expanded into 30 flasks. These flasks are incubated for a further 7 days and the MSCs are harvested by trypsin treatment (P1 cells). The P1 cells are washed and total viable cell counts determined.

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

Upon request, the frozen bags will be thawed in a 37^0C water bath. In a BSC, the cell suspension will be transferred to conical tubes and slowly diluted with a PBS buffer supplemented with 1% HSA or Plasma-Lyte A supplemented with 1% HSA. The suspension will be centrifuged and the cell pellet re-suspended in the dilution buffer. The cells will be counted to determine the total viable cells. After release, the cells will be placed in a labeled conical tube and delivered to the appropriate medical staff.

Technique for Administration

Please see **Addendum A**, which specifies the technique for percutaneous endocardial administration of Allo-hMSCs. **Addendum B** provides a detailed training program for the Biocardia Helical Catheter System.

4.1.2 Dose Rationale

In preclinical studies in a porcine model[16, 93, 115], MSC therapy was safely administered via intramyocardial injection at doses of up to 2 x 10^8 cells. Results of the POSEIDON study support the use of 0.2 x 10^8 Allo-MSCs and 1.0 x 10^8 Allo-MSCs in this clinical study.

4.1.3 Dosages and Dosing

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

Fifteen (15) patients will be treated with Allo hMSCs: 4 million cells / ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 0.2 x 10^8 (20 million) Allo-hMSCs and fifteen (15) patients will be treated with Allo hMSCs: 20 million cells / ml delivered in a dose of 0.5 ml per injection x 10 injections for a total of 1 x 10^8 (100 million) Allo-hMSCs.

The injections will be administered transendocardially during cardiac catheterization using a Biocardia Helical Infusion Catheter. Intramyocardial injections will be discontinued during the procedure if one or more of the following occur:

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

The cells will be supplied from an allogeneic human mesenchymal stem cell source manufactured by the University of Miami. Cardiac catheterization will be scheduled following successful enrollment.

4.2 Blinding and Unblinding

The administration of either dose of allo-MSCs will be performed in a double-blind manner; the study investigators and patients will not be made aware of the assigned treatment regimen. If for important medical reasons unblinding is thought to be necessary, the Investigator may identify the treatment assignment by obtaining the randomization assignment by contacting the Director of Experimental and Clinical Cell Based Therapies who is responsible for maintaining randomization records for all patients. Standard Operating Procedures (SOPs) will be in place at the center outlining the emergency unblinding procedures.

4.2.1 Storage and Handling of Study Investigational Therapy

Study therapy (Allo-hMSCs) should only be dispensed once a patient has (1) provided written informed consent, (2) met all eligibility criteria for entry into the study, and (3) completed all baseline evaluations.

Before dispensing the investigational product, Cell Therapy Lab staff will confirm the CMV status of eligible recipient. This information will be used to select Allo-MSC product. CMV status of the recipient and donor of the Allo-MSC product will be matched. CMV positive Allo-MSC product will only be infused to a CMV positive recipient. All CMV negative recipients will receive CMV negative Allo-MSC product[125].

4.2.2 Study Investigational Therapy Accountability Procedures

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

5. STUDY PROCEDURES

5.1 Time and Events Schedule

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

Study Procedure	Screening (Weeks -10 to -5)	Baseline # (Weeks -10 to -2)	Day 1	Day 2	Day 14 ±3 days (Week 2)	Month 3 ±10 days (Week 12)	Month 6 ±10 days (Week 24)	Month 12 ±21 days (Week 52)
Informed Consent	X							
History and Physical	X		X	X	X	X	X	X
Vital Signs	X		X	X	X	X	X	X
12-lead ECG	X		X	X	X	X	X	X
Concomitant Medications	X		X	X	X	X	X	X
Eligibility for Enrollment		X						
Catheterization			X					
Investigational agent			X					
Standard Post-Procedural Care			X	X				
CT Assessment of Heart /complete chest, abdomen and pelvis CT ‡	X							X
Echocardiogram $	X		X £	X		X	X	X
Treadmill Determination. of Peak VO_2		X					X	X
Six-Minute Walk Test		X				X	X	X
NYHA Functional Class	X					X	X	X
Questionnaires MLHF, IIEF (male), SQOL-F(female)	X					X	X	X
Pulmonary function (FEV1)		X		X***	X		X	X
24 Hour Ambulatory ECG*	X		X *		X	X	X	X
Serum Troponin I & CK-MB **			X		X			
Hematology & Clinical Chem. % Pro-BNP, Uric acid, and CRP **Immune monitoring (XX)**	X		X, XX	X,XX		X	X, XX	X
Urinalysis	X		X	X		X	X	X
Serum or urine pregnancy test	X		X $$			X	X	
HIV 1, HIV 2, Hepatitis B & C, and CMV	X							
Donor Screening Tests	X							
Biomarkers Assessment %%			X				X	
Adverse Events			X	X	X	X	X	X

Time and Events Table Key:

- All Baseline Visit tests will occur 2 to 10 weeks prior to the cardiac catheterization.

‡ - Cardiac CT and whole body scan (CT of chest, abdomen and pelvis) will occur at screening and final month 12 visit

* - 24 hour Holter will capture day one and day two of hospitalization post injection.

** - Serial Troponin I and CK-MB laboratory assays will be performed every 12 hours for the first 24 hours post-cardiac catheterization and one time assay at week 2 visit.

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

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

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

Liver Function Tests: Albumin, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, prothrombin time / activated partial thromboplastin time, and bilirubin.

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

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

%% - The following biomarkers will be analyzed:

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

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

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

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

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

- CD3, CD25, CD69

5.2 Study Phases and Visits

5.2.1 Screening Phase

See Table 3 for the procedures and assessments to be performed during this phase of the study. Any patient who has a life-threatening arrhythmia in the absence of a defibrillator (sustained or short run ≥ 20 consecutive beats of ventricular tachycardia or complete second or third degree heart block in the absence of a functioning pacemaker) on ECG or 24-hour Ambulatory ECG performed during the screening phase will be removed from the study. The donor screening blood work is specific to each cell manufacturing facility and may include hepatitis A, West Nile Virus, human T-cell lymphotropic virus (HTLV) I/II, CMV and syphilis. All screening visit tests and procedures will occur within five weeks of the signed informed consent (IC) and 5 to 10 weeks prior to cardiac catheterization. No screening exams will take place until the patient is fully informed of the research and signs the consent form.

5.2.2 Baseline Phase

See Table 3 for the procedures and assessments to be performed during this phase of the study. After all screening exams, patients will be enrolled into the study to receive allogeneic hMSCs.

The Baseline Phase will take place 2 to 10 weeks prior to the cardiac catheterization. The Baseline Visit will occur once the screening tests are completed and it has been determined that the patient remains eligible for the study. Upon successful enrollment of the patients, they will be scheduled for catheterization as soon as possible.

5.2.3 Day 1 – Day 2 Post-Catheterization & Week 2 Evaluations

See Table 3 for the procedures and assessments to be performed during these evaluations. The listed procedures (other than catheterization and Investigational Agent administration) should all be performed as soon as practicable after the catheterization procedure. All patients will have Troponin I and CK-MB laboratory work completed prior to catheterization, and every 12 hours post catheterization for the first 24 hours, as well as an echocardiogram prior to catheterization, immediately following the procedure, 4-6 hours later and 24 hours post-cardiac catheterization. Additionally, an FEV1 assessment will be completed during day 2, unless the patient is unable, then 48 hours prior to discharge.

During Week 2 (Day 14 ± 3), patients will return to the clinic for a 12-lead ECG, 24-hour ambulatory ECG, one time assay of Troponin I and CK-MB and an FEV1 assessment.

5.2.4 Month 3 – Month 12 Visits

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

5.2.4 Biomarkers Assessment

A separate 7 mL blood sample for gene expression profiling of WBC RNA will be obtained at day 1 and at 6 months if the patient consents. All samples will be identified so that they can be linked to individual patients. These samples may be stored indefinitely. Individual results will not be returned to the patient or the study physician. Data presented in publications will not contain individual patient's gene expression or clinical characteristics or outcomes; only aggregate data from the entire study will be disclosed.

5.2.5 Immune Monitoring for Graft Rejection

The studies planned in the TRIDENT protocol will utilize allogeneic mesenchymal stem cells (MSC) in patients with heart disease. The use of an allogeneic graft raises the potential of graft rejection through immune cells resulting in failure of the therapy. MSCs are ideal candidates for allogeneic transplantation because they show minimal MHC class II and ICAM expression and lack B-7 co-stimulatory molecules necessary for T-cell mediated immune responses[119, 120]. Indeed MSCs do not stimulate a proliferative response from alloreactive T-cells even when the MSCs have differentiated into other lineages or are exposed to proinflammatory cytokines. Previous studies have demonstrated that MSCs have significant immunomodulatory effects, inhibiting T-cell proliferation, prolonging skin allograft survival, and decreasing graft-versus-host disease (GVHD). Recently human MSCs were shown to alter the cytokine secretion profile of dendritic cells, T cells, and natural killer cells *in vitro*, inhibiting secretion of proinflammatory cytokines (e.g. TNF-a, IFN-γ) and increasing expression of suppressive cytokines (e.g.IL-10), possibly via a prostaglandin E2 mediated pathway.

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

our demonstration of efficacy of these cells for cardiac repair, solidify the notion of using MSCs as an allograft for successful tissue regeneration.

As part of the Trident protocol we will obtain peripheral blood samples from all patients to evaluate the presence of activated T cells. Two heparinized (green top) vacutainer tubes (approx. 15 cc total blood) will be collected at different time points during the study: at day one prior to infusion of MSC, within 24 hours (day 2) of injection (post infusion of MSC), and at 6 months. Peripheral blood mononuclear cells (PBMC) will be isolated from heparinized blood by ficoll sedimentation and will be viably cryopreserved for planned assessments of T cell activation.

5.2.7 Enrollment Contingency Plans

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

1. Any patient whose catheterization is delayed will be allowed to remain in the study and receive the study product for up to 6 months from the date of the signed informed consent; as long as the patient remains eligible based upon a successful repeat screening exam.
2. Enrolled patients who unexpectedly die prior to catheterization, or otherwise withdraw from the study after the screening period but before surgery, may be replaced without limit.
3. Patients who are in the middle of the cardiac catheterization and additional therapy is given may be withdrawn from the study and replaced by newly enrolled subjects without limit.

5.3 Computed Tomography (CT) Protocol

CT Protocol

Patients will undergo contrast-enhanced CT at screening and at the 12 month follow-up visit. In addition to screening the chest, abdomen, and pelvis for ectopic tissue formation, cardiac CT will be used to measure global left ventricular function, regional wall motion, and volumetric parameters; rest perfusion; and infarct scar size.

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

Cardiac CT Function and Rest Perfusion Imaging

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

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

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

Chest / Abdomen / Pelvic CT

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

Delayed Enhanced CT Infarct Scar Imaging

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

5.4 Echocardiogram Protocols

Echocardiogram Protocol

All subjects will undergo transthoracic echocardiographic assessment of overall and regional LV systolic function at screening. A limited 2D echocardiogram

assessment will be performed prior to catheterization, immediately following the catheterization procedure, and 4-6 hours later on Day 1. On day 2, months 3, 6, and 12 a complete 3D echo will be performed.

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.

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

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

6. ADVERSE EVENT MANAGEMENT

6.1 Definition of an Adverse Event

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

(new or exacerbated) temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Examples of an AE include:

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

An AE does **not** include:

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

6.2 Definition of Adverse Reaction

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

6.3 Definition of Suspected Adverse Reaction

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

6.4 Definition of Serious

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

1. results in death
2. is life-threatening (at risk of death at the time of the event)

3. requires in inpatient hospitalization or prolongation of existing hospitalization

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

4. results in disability/incapacity

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

5. is a congenital anomaly/birth defect.

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

6.5 Definition of Unexpected

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

6.6 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 screening and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the Investigator as more severe than expected for the 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.

6.7 Recording of Adverse Events and Serious Adverse Events

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

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

Pregnancies

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

6.8 Intensity of Adverse Events and Serious Adverse Events

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

Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.

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

Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is described as 'serious' when it meets one of the pre-defined outcomes as described in Section 6.4, "Definition of Serious."

6.9 Causality of Adverse Events and Serious Adverse Events

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

Brochure and/or Product Information, for marketed products, in the determination of his/her assessment.

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

Is there a reasonable possibility that the study therapy caused the event? Reasonable possibility implies that there is evidence that the vent was caused by the study product.

An affirmative answer designates the event as a suspected adverse reaction.

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

6.10 Follow-Up of Adverse Events and Serious Adverse Events

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

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

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

6.11 Timeframes for Submitting SAE Reports

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

TABLE 4

Serious Adverse Event Reporting Requirements

	Initial Reports		**Follow-Up Reports**
Type of SAE	Fatal or Life-Threatening	Other SAEs	Any SAE
Reporting Timeframes	24 hours	48 hours	48 hours
Documents Required	24 hours: Complete as much information in the electronic data system that is known. 48 hours: Fully complete all AE forms	Fully completed AE forms	Updated AE Forms

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6.12 Post-Study Adverse Events and Serious Adverse Events

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

6.13 Regulatory Aspects of Adverse Event Reporting

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

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

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

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

6.14 Monitoring of Adverse Events

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

- All serious suspected adverse reactions will be reviewed by the Medical Monitor at the CRO within 2 business days of the site entering the information into the EDC.
- If the Medical Monitor requires additional information to make his/her assessment, the clinical centers will have 2 business days to respond to the request for additional information.
- The CRO is responsible for initial notification of the DSMB immediately of all unexpected, serious suspected adverse reactions, regardless of attribution, and of any concerns regarding the frequency or type of SAE(s) on a study or treatment arm according to the DSMB charter.
- The attribution, as assessed by the clinical center and the CRO Medical Monitor, will be provided to the DSMB within 2 business days of receiving the clinical center report and available data supporting the attribution if the event is both unexpected and a serious suspected adverse reaction.

The CRO will prepare semi-annual summary reports of all AEs/SAEs for the DSMB. Semi-annual reports will be made available on a secure website; the DSMB Chair will be notified by e-mail when the materials are posted.

7. DATA COLLECTION AND STATISTICAL ISSUES

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

7.1 Enrollment

Patients will be registered using the following procedures:

1. An authorized user completes the initial screening by entering patient demographics and inclusion/exclusion criteria on the Enrollment Form. The enrollment screen includes a question confirming that the patient signed the informed consent form.
2. If the patient is eligible as determined by the electronic data entry system, a unique patient study number is generated as well as a blinded study product number. The blinded study product number will allow the cell therapy lab to know what dose to prepare. Only the unblinded statistician and cell lab will be aware of the treatment assignment.
3. The study coordinator informs the Cell Therapy Laboratory of the blinded study number and will prepare the study product based on an unblinded treatment assignment table.
4. The study coordinator will inform the Cell Therapy Laboratory of the blinded study number and prepare the study product based on an unblinded treatment assignment table.

5. Cell Therapy Lab will also be provided with the CMV test results of eligible recipient.
6. This information will be used to select Allo- MSC product for eligible recipient.
7. CMV status of the recipient and donor of the Allo-MSC product will be matched.
8. CMV positive Allo-MSC product will only be infused to a CMV positive recipient.
9. All CMV negative recipients will receive CMV negative Allo-MSC product.
10. Upon the user saving the injection date on the Injection Form in Advantage EDC, a visit schedule based on the study product injection date is available for printing and is referred to as “Segment A Follow-up.”

7.2 Data Collection

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

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

7.3 Study Design and Sample Size Considerations

The primary focus of this study is to assess the 30-day post-cardiac catheterization SAE proportion as defined in Section 2. Thirty patients will be enrolled over a 12-18 month accrual period. Fifteen patients will receive allo-hMSCs at each dose level in a blinded fashion.

The total sample size is 30 patients for the trial equally distributed between the 20 million and 100 million dose levels. As a result, the sample size and power considerations will be based on the 15 subjects who received the injection in each treatment group. Exact binomial ninety-five percent confidence intervals were calculated for varying number of events based on the sample size. Table 5 provides confidence intervals for a variety of true underlying number of events. Of particular interest, is the anticipated 30 day proportion of treatment-emergent serious adverse events (TE-SAE) as defined in 2.2.1. The underlying rate of 30-day TE-SAE is 20%, which translates into 3 patients with events out of 15 patients. For the setting of 3 events, the confidence interval is 0.04-0.48. The probabilities above and below 3 represent other plausible scenarios.

Exact power computations based on the binomial distribution were conducted using the SAS system version 9.1. The precision of the estimates could be viewed as a lower bound on the number of events reported. The probability to rule out the number of patients experiencing a TE-SAE of a certain size can be interpreted as “power.” Table 4 provides the probability (or power) that the lower

bound of a 95% two-sided confidence interval for the primary endpoint will be greater than thresholds of 1, 2, 3, 4, 5, 6, and 7 events. When the true number of events is 3, there is 65% power to rule out a number of patients experiencing the primary endpoint of 8 or higher.

Table 5. Exact Binomial 95% Confidence Intervals for Various Sample Sizes and Proportion of Patients Experiencing the Primary Endpoint

Patients randomized to each treatment group	Number of patients experiencing the primary endpoint	Exact 95% Confidence Intervals	
15	1	0.002,	0.32
	2	0.02,	0.40
	3	0.04,	0.48
	4	0.08,	0.55
	5	0.12,	0.62
	6	0.16,	0.68
	7	0.21,	0.73

Table 6. Probability of Ruling Out a Threshold of Size T or larger for Various Sample Sizes and True Underlying SAE Proportion of Patients Experiencing the Primary Endpoint

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

7.4 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.

7.5 Analysis of the Primary Endpoint

Descriptive analyses of the primary endpoint will be performed. Point estimates and confidence intervals of TE-SAE proportion will be computed. Fisher Exact Test will be used to compare the two dose levels.

7.6 Stopping Guidelines

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

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

A Bayesian motivated safety stopping guideline will be used for this trial[123, 124]. The expected underlying TE-SAE at 30 days post-catheterization probability is assumed to be 20% and that a probability of greater than 40% is unacceptable.

A Beta distribution can be used as the prior distribution of θ; where θ is the proportion of patients who experience a TE-SAE. The stopping rule is based on the beta-binomial methodology and assumes a prior expected failure rate. This leads to prior Beta parameters where a=2.5 and b=10.0. The Beta distribution will have a prior mean of 0.20 and a prior probability of <0.05 of exceeding 0.40. The guideline is derived such that there is strong evidence (posterior probability >0.95) that the probability of the event is greater than 40%, the trial will be stopped. The resulting boundaries tabulated in table 7 were rounded to be conservative with the stopping guideline and is considered after 5 patients are enrolled on the study.

TABLE 7

Bayesian Stopping Guideline for Event Rate of 20% *

# Events	# Patients in Study
4	5-8
5	9-12
6	13-15

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

A simulation study was conducted to evaluate the operating characteristics of this stopping rule. Data were generated from the binomial distribution with varying probabilities of failure (θ) and assuming a sample size of 15 patients. Table 8 shows the probability of stopping the trial early and the average sample size (N), conditional on stopping early, at which the boundary is crossed for each value of θ. The unconditional average sample size of the trials for each value of θ is displayed.

Table 8

Operating Characteristics for Bayesian Motivated Stopping Guideline

Mean of Prior Distribution	θ	Probability of stopping	Conditional Average Sample Size (N)	Unconditional Average Sample Size of Trials (N)
0.20	0.20	0.11	9.5	14.4
	0.25	0.22	9.4	13.8
	0.30	0.36	9.4	13.0
	0.35	0.52	9.1	11.9
	0.40	0.67	8.8	10.9
	0.45	0.79	8.4	9.8
	0.50	0.88	7.9	8.8
	0.55	0.94	7.4	7.8

Although the motivation for the boundary is Bayesian, the operating characteristics can be evaluated from a frequentist perspective of Type I error and power. The stopping rule for a 20% event rate has a 11% chance ("Type I error") of suggesting early termination when the true rate is 0.20, and a 67% chance ("power") when the true rate is 0.40, rising to 88% when the true rate is 50%.

7.7 Analysis of Secondary Endpoints

A number of secondary endpoints as noted in chapter 2 will be examined to compare the safety and efficacy of each of the allo-MSC dose levels. The analysis of the secondary endpoints that are planned is included below.

- Adverse Events: Fisher exact tests will be used to compare groups on the incidence of adverse events (including percentages of patients with $\geq$ 1 adverse events, possibly-related MSC adverse events, possibly-related to the catheter adverse events, and serious adverse events. All adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). MedDRA coded events will be displayed by system organ class and preferred term. The time periods would be within 30-days, 6 and 12-months post-injection.
- MACE: Fisher exact tests will be used to compare groups on the incidence of major adverse cardiac events (MACE) as defined in section 2.2.3. The time periods would be within 30-days, 6 and 12-months post-injection.
- TE-SAE: Fisher exact tests will be used to compare groups on the incidence of treatment emergent adverse events (TE-SAE) as defined in section 2.2.2. The time periods would be within 30-days, 6 and 12-months post-injection.
- Ectopic Tissue Formation: The incidence of ectopic tissue formation will be described between groups and compared using Fishers exact test. The time periods would be within 30-days, 6 and 12-months post-injection.
- Cardiac Enzymes: CK-MB and serum troponin will be monitored at baseline and every 12-hours for the first 48 hours post-injection. Generalized linear models will be employed to model the effects of treatment on cardiac enzymes.
- Hospitalizations will be described based on adjudicated readmissions.
    - The rate of any hospitalization and hospitalization for worsening heart failure will be compared using Fishers Exact test.
    - The probability of hospitalization-free survival will be estimated using a binomial proportion at 1-year as well as using the Kaplan-Meier method if the number of events permits.
    - The time to hospitalization will be estimated using the cumulative incidence method treating death as a competing risk and censoring

at the last date of follow-up. The time periods would be 6 and 12-months post-injection

- Deaths will be described by treatment arm and evaluated using the Kaplan-Meier method if the number of events permits.
- Changes from baseline will be evaluated for the 6-minute walk test, peak Vo2, forced expiratory volume in 1-second (FEV1), echocardiographic measures, and CT parameters. General linear models will be used to model the effects of treatment on the change from baseline.
- New York Heart Association class will be analyzed as either improvement, no change or worsening of class at 6 and 12-months post-injection as compared to pre-injection and will be analyzed using a chi-square test.
- Quality of Life
    - The Minnesota Living with Heart Failure Questionnaire will be administered at 6 and 12-months post-injection. The total score, emotional score and physical dimension score will be calculated and compared using general linear models.
    - Sexual Quality of Life Questionnaire (Female) will be administered at 3, 6 and 12-months post-injection. The score will be calculated and compared using general linear models.
    - International Index for Erectile Dysfunction (Male) will be administered at 3, 6 and 12-months post-injection. The score will be calculated and compared using general linear models.
- Composite Score will be computed based on death, hospitalization, Minnesota Living with Heart Failure Total Score, change in 6-minute walk test, ejection fraction and change in NYHA class. Groups will be compared at 3, 6, and 12-months using general linear models.

7.8 Criteria for Dose Selection

The criteria for moving to the next phase of the study will be based on the totality of evidence including the safety and efficacy endpoints previously described.

7.9 Data and Safety Monitoring Board (DSMB)

Interim analyses will be conducted at times coincident with regularly scheduled meetings of the Data and Safety Monitoring Board (DSMB) at approximately six-month intervals. The DSMB Chair will be notified each time an unexpected, serious suspected adverse reaction occurs. The DSMB will evaluate AE data (including SAEs) at the following pre-specified intervals: (1) when 5 patients are enrolled and completed 1 month of follow-up. Other safety data, such as 24-hour ambulatory ECGs and laboratory data will also be evaluated by the DSMB as appropriate. Monitoring of key safety endpoints will be conducted as described above, and if rates significantly exceed pre-set thresholds, the DSMB Chair will be notified and information will be supplied to the DSMB. Policies of the DSMB

will be described in the DSMB Charter, which will be prepared by the DSMB prior to study initiation. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal "stopping rules" that would mandate automatic closure of study enrollment.

8. NORMAL DONORS FOR GENERATION OF ALLOGENEIC MSC

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

The recipient 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, CMV and syphilis."

8.1 Bone Marrow Aspiration for Generation of Allo-MSC

[redacted]

8.2 Normal Donor Eligibility

Donors (male or female) between the ages of 20 to 45 will be screened as potential BM donors (maximum of 15) and shall be evaluated by history and physical examination, as well as, completion of the National Marrow Donor Program (NMDP) questionnaire[138]. The history shall include the following:

- History of malignancy
- Bleeding abnormalities
- Deep venous thrombosis
- Cardio/pulmonary conditions
- Blood transfusions
- Vaccinations
- Questions to identify persons at risks of infectious disease transmission

- Questions to identify persons at risk of transmitting hematological or immunological disease

The physical examination shall be completed and include evaluation for potential risk of the BM aspiration procedure.

Prospective donors shall have infectious Disease Testing including:

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

Prospective donors shall have the following blood tests:

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

Eligibility Criteria for Normal Donors

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

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

8.3 Donor Consent

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

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

8.4 Follow-up Schedule for Donors

After discharge from the hospital, the bone marrow donor will be contacted by the study team with 24 hours post BMA follow-up telephone calls to determine the well-being and health status of the donor. The donor will be provided with contact telephone numbers in the consent form for any questions or comments.

9. STUDY ADMINISTRATION

9.1 Regulatory and Ethical Considerations

9.1.1 Regulatory Authority Approval

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

9.1.2 Ethics Approval

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

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

If it is necessary to amend the protocol or the ICF during the study, the Investigator will be responsible for ensuring that the IRB/IEC reviews and approves these amended documents. An IRB/IEC approval of the amended protocol and/or ICF must be obtained in writing before implementation of the

amended procedures and before new patients are consented to participate in the study using the amended version of the ICF.

9.1.3 Patient Informed Consent

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

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

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

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

9.2 Confidentiality of Information

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

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

All participants in the United States will provide written authorization to disclose private health information either as a part of the written ICF or as a separate authorization form. The authorization will contain all required elements specified by 45 CFR 164, and will contain a waiver of patient access to study-related private health information until the conclusion of the clinical study. The

authorization will remain valid and in full force and effect until the first to occur of (1) the expiration of 2 years after the study therapy is approved for the indication being studied, or (2) the expiration of 2 years after the research program is discontinued. Individual patient medical information obtained during this study is confidential and its disclosure to third parties (other than those mentioned in this Section 9.7) is strictly prohibited. In addition, medical information obtained during this study may be provided to the patient's personal physician or to other appropriate medical personnel when required in connection with the patient's continued health and welfare.

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

9.3 Payments to Patients

Patients will be reimbursed $50 at the end of each follow-up visit (months 3, 6, and 12) for a total remuneration of $150). These disbursements are meant to cover the time required to complete these study visits and all necessary travel and parking expenses.

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

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