

CARDIOVASCULAR CELL THERAPY RESEARCH NETWORK

**Protocol
Study Chair:**

**Robert Simari, M.D.
Professor of Medicine
Mayo Clinic College of Medicine**

PRC/DSMB Approved Protocol 3: FOCUS Protocol

**Randomized, Controlled, Phase II, Double-Blind Trial of Intramyocardial In-
jection of Autologous Bone Marrow Mononuclear Cells under Electrome-
chanical Guidance for Patients with Chronic Ischemic Heart Disease and
Left Ventricular Dysfunction**

Supported by:

The National Heart, Lung, and Blood Institute (NHLBI)

CCTRN Authorship Roster

Emerson Perin, M.D., Ph.D. *	Texas Heart Institute
James Willerson, M.D. *	Texas Heart Institute
Barry J. Byrne, M.D., Ph.D.	University of Florida
Stephen Ellis, M.D.	Cleveland Clinic
Antonis Hatzopoulos, Ph.D.	Vanderbilt University
Timothy Henry, M.D.	Minneapolis Heart Institute Foundation
Marc Penn, M.D.	Cleveland Clinic
Carl J. Pepine, M.D.	University of Florida
Jay Traverse, M.D.	Minneapolis Heart Institute Foundation
Douglas Vaughan, M.D.	Vanderbilt University
David Zhao, M.D.	Vanderbilt University
Dejian Lai, Ph.D.	The University of Texas
Lemuel Moyé, M.D., Ph.D.	The University of Texas
Robert Simari, M.D. (Study Chair)	Mayo Clinic College of Medicine

Supported by:

The National Heart, Lung, and Blood Institute (NHLBI)
Sonia Skarlatos, Ph.D
David Gordon, M.D., Ph.D
(Grant number U01HLO87318-01)

* Principal authors

List of Abbreviations

AE- Adverse Event
BM-MNC- Bone Marrow Mononuclear Cells
BNP - B-type natriuretic peptide
CAD- Coronary Artery Disease
CBC - Complete blood count
CC- Clinical Center
CCTRN- Cardiovascular Cell Therapy Research Network
CK - Creatine kinase
DCC- Data Coordinating Center
ECG - Electrocardiography
Echo - Echocardiography
EDV-End Diastolic Volume
EMM - Electromechanical mapping
EPC- Endothelial Progenitor Cells
ESV- End Systolic Volume
FDA- Food and Drug Administration
FGF- Fibroblast Growth Factor
hsCRP - High sensitivity C-reactive protein
ICD-Internal Cardiac Defibrillator
IS- Infarct Size
IRB- Institutional Review Board
LFT- Liver Function Test
LV- Left Ventricular
LVEF- Left Ventricular Ejection Factor
MACE – Major adverse cardiac events
MI- Myocardial Infarction
MRI - magnetic resonance imaging
MVO₂ - myocardial oxygen consumption
NC – Non-contrast
NHLBI- The National Heart, Lung, and Blood Institute
NYHA – New York Heart Association
PCI- Percutaneous Coronary Intervention
PI- Principle Investigator
SAE- Serious Adverse Event
SID- Study Identification Number
SPECT - Single-photon-emission computed tomography
STEMI- ST Segment Elevation Myocardial Infarction
THI- Texas Heart Institute
TIA-Transient Ischemic Attack
TMT - Treadmill testing
VCAM - Vascular Cell Adhesion Molecule
VEGF-Vascular Endothelial Growth Factor

Table of Contents

CCTRN AUTHORSHIP TEAM.....	2
LIST OF ABBREVIATIONS.....	3
TABLE OF CONTENTS.....	4
FOCUS TRIAL Executive Summary.....	8
1.0 STUDY OBJECTIVES.....	10
1.1 Primary Objective.....	10
1.2 Hypothesis.....	10
1.3 Relevance to CCTRN.....	11
2.0 BACKGROUND.....	11
2.1 Rationale.....	12
2.2 Initial clinical studies of ABM-MNCs delivery.....	15
2.3 ABM-MNCs – The Texas Heart Institute Experience.....	16
2.3.1 Open label Study of Transendocardial, ABM-MNC Transplantation for Severe, Ischemic CHF (24).....	16
2.3.2 Conclusions.....	17
2.4 SEPAX Isolation of BM-MNCs.....	18
3.0 STUDY DESIGN.....	18
3.1 Introduction.....	18
3.2 Study Endpoints.....	18
3.2.1 Primary Endpoints.....	18
3.2.2 Secondary Endpoints.....	18
3.3 Interventions.....	19
3.3.1 Treatment Dose.....	19
3.3.2 Control Group.....	19
3.4 Sample Size Formula.....	19
3.5 Sample Size Computation.....	20
3.5.1 MVO2.....	20
3.5.2 LVESV.....	21
3.5.3 Reversible perfusion defect size.....	21
3.6 Final Sample Size Calculation.....	22
3.7 Type I Error Adjustment.....	22
4.0 SELECTION AND ENROLLMENT OF PATIENTS.....	22
4.1 Introduction.....	22
4.2 Randomization.....	23
4.3 Inclusion Criteria.....	23
4.4 Exclusion Criteria.....	24
4.5 Baseline Testing.....	25
4.6 Baseline Screening Window.....	26
5.0 INTERVENTION.....	26
5.1 Treatment Period.....	26
5.1.1 Active Therapy Group.....	27
5.1.2 Control Group.....	28
5.1.3 Randomization and Unblinding.....	28
5.1.3.1 Randomization.....	29
5.1.3.2 Unblinding.....	29
6.0 CLINICAL AND LABORATORY EVALUATIONS.....	30

6.1 "See Baseline Testing" under Section 4.3.....	30
6.2 Follow-up Evaluations.....	30
6.2.1 Laboratory Testing.....	30
6.2.2 Instructions to Patients.....	31
6.2.3 Holter Monitoring.....	31
6.2.4 Internal Cardiac Defibrillator (ICD) Interrogation.....	31
6.2.5 Echocardiographic Testing.....	31
6.2.6 Treadmill Testing.....	32
6.2.7 SPECT Imaging.....	32
6.2.8 Invasive Testing.....	32
6.2.9 Cardiac MRI.....	32
6.2.10 Adverse Event Monitoring.....	32
6.2.11 Quality of Life.....	33
6.2.12 Pregnancy Testing.....	33
6.2.13 Electrocardiograms (ECG).....	33
6.2.14 Six Minute Walk.....	33
6.2.15 Chest X-Ray (CXR).....	33
6.2.16 Biospecimens.....	33
6.3 Schedule of Tests and Procedures.....	34
6.4 Follow-up windows.....	35
7.0 EVENT REPORTING.....	36
7.1 Types of Events.....	36
7.1.1 Adverse Events (AEs).....	36
7.1.2 Serious adverse events (SAEs).....	36
7.2 Role of Abnormal Test Findings and Hospitalizations in Classifying an Event.....	36
7.2.1 Abnormal Test Findings.....	36
7.2.2 Hospitalizations.....	36
7.3 Reporting Responsibilities of the Investigator.....	37
7.3.1 Severity Assessment.....	38
7.3.2 Causality Assessment.....	38
7.3.3 Expectedness Assessment.....	38
7.4 Reporting Responsibilities of the Sponsor (DCC).....	39
7.4.1 Sponsor Reporting Requirements to the Executive Committee, NHLBI, and DSMB.....	39
7.4.2 Sponsor Reporting Requirements to FDA.....	39
7.5 Unanticipated Problems (UPs).....	39
7.6 Guidelines for Holding Product in the Event of a Catheterization Facility Event.....	40
7.7 Holding Rules.....	40
7.8 Holding Criteria.....	40
7.9 Guidance for NOGA Catheter Usage.....	41
7.10 Monitoring of Liver Function Tests (AST/ALT).....	42
8.0 ENDPOINT EVALUATION AND CLASSIFICATION.....	42
9.0 STATISTICAL PROCEDURES.....	42
9.1 Randomization.....	42
9.2 Statistical Analyses.....	43
9.3 Baseline Analyses.....	43

9.4 Outcome Analyses.....	43
9.4.1 Primary endpoints.....	44
9.4.2 Analyses of Secondary Endpoints.....	45
9.4.3 Subgroup evaluations.....	46
9.5 Multiple Comparisons.....	46
10.0 TRIAL MANAGEMENT.....	47
10.1 Database.....	47
10.1.1 Framework.....	47
10.2 Security.....	47
10.3 Follow-up.....	48
10.4 Laboratory Data Processing Support.....	48
10.4.1 File transfers.....	48
10.5 Data Quality.....	48
10.6 Computing Infrastructure.....	49
10.7 Backup Procedure.....	49
10.8 Site Visits.....	49
11.0 HUMAN PATIENTS.....	50
11.1 Institutional Review Board (IRB) Review and Informed Consent.....	50
11.2 Subject Confidentiality.....	50
11.3 Study Modification/Discontinuation.....	50
11.4 Informed Consent.....	51
11.4.1 Human Patients Involvement and Characteristics.....	51
11.4.2 Sources of Material.....	51
11.4.3 Risks Associated with the Patient Population, Procurement, Processing, and Injection of the Study Product.....	51
11.4.4 Adequacy of Protection Against Risks.....	53
11.4.4.1 Recruitment and Informed Consent.....	54
11.4.4.2 Protection Against Risk.....	54
11.4.5 Potential Benefits of the Proposed Research to the Patients and Oth- ers.....	54
11.4.6 Importance of the Knowledge to be Gained.....	54
11.4.7 Data and Safety Monitoring Plan.....	54
11.4.8 Risk-Benefit Analysis.....	55
11.5 Recruitment Principals and Strategies.....	55
11.6 Monitoring.....	56
11.6.1 Pre-Investigation Visits.....	56
11.6.2 Scheduled monitor visits.....	56
11.6.3 Monitor Role Continued.....	57
11.6.4 Monitor Recording.....	57
11.7 Investigator responsibilities.....	57
11.7.1 Investigator Performance.....	57
11.7.2 Site Requirements.....	58
11.7.3 Institutional Review Board Approval.....	58
11.7.4 Informed Consent.....	58
11.7.5 Reporting Requirement of the Sites.....	58
11.8 Sponsor Responsibilities.....	58
11.8.1 Introduction.....	58
11.8.2 Routine Duties.....	59

11.8.3 Site Training.....	59
11.8.4 Site Monitoring.....	60
11.8.5 Reporting to the FDA.....	60
12.0 FUTURE STUDIES.....	60
13.0 DISSEMINATION.....	61
13.1 Web Site.....	61
13.2 E-network.....	61
13.3 Manuscripts and Presentations.....	62
13.4 Methodologic Developments.....	62
14.0 REFERENCES.....	63
APPENDIX 1.....	68
APPENDIX 2.....	69
APPENDIX 3.....	70
APPENDIX 4.....	74

FOCUS TRIAL
Executive Summary

Study Design: This is a randomized, Phase II, double blind, placebo controlled clinical trial that will assess the effect of autologous bone marrow mononuclear cells delivered transendocardially to patients with CAD, LV dysfunction, and limiting heart failure and/or angina. Myocardial perfusion, LV contractile performance, and maximal oxygen consumption are primary efficacy measures. Patient safety of this cell-based therapy will also be determined.

Target Population: A maximum of 95 patients (randomized 2:1 to active or placebo); male and female patients, who have no contraindications to bone marrow cell infusions and who have coronary anatomies unfavorable for coronary artery surgery or percutaneous coronary artery interventions, LV dysfunction (LVEFs $\leq 45\%$) and limiting heart failure and/or angina.

Enrollment Period: Enrollment will be continuous at all five centers until the sample size of 87 is reached (includes placebo and active groups). Consented subjects who are in screening at the time the sample size of 87 is achieved will be offered the opportunity to be randomized in the study (total study sample not to exceed 95 patients).

Rationale: Congestive heart failure (CHF) is a cardiovascular problem worldwide that continues to increase in frequency, causing major morbidity and has a 5 year mortality rate of 50% in patients with end stage disease. Existent therapies are not adequate to prevent the progressive increase in the problem. Selected bone marrow-derived mononuclear cells have the unique ability to differentiate into new blood vessels and cardiac myocytes. Through their paracrine effects to recruit and activate resident cardiac stem cells they may enhance the myocardial perfusion and contractile performance of the failing and ischemic human heart.

Primary Endpoints: The three primary endpoints are (1) change in MVO₂; (2) change in LVESV; and (3) reduction in perfusion defects.

Secondary Endpoints: The secondary endpoints of the study will compare the changes in the following measures from baseline⁺ to six month follow-up.

1. Reduction in fixed perfusion defect(s) by SPECT
 - a. Change in total defect size by SPECT
 - b. Change in fixed defect size by SPECT
 - c. Change in Sum Difference Score by SPECT
2. Progression to fixed defect by SPECT.
3. Regional wall motion by MRI (in patients who can undergo this procedure)
4. Regional blood flow improvement by MRI (in patients) who can undergo this procedure)
5. Regional wall motion by echocardiography
6. Clinical improvements at 6 months, including change in anginal score by the following measures:
 - a. Canadian Cardiovascular Society Functional Classification of Angina Pectoris (CCS)
 - b. NYHA class

- c. Decrease in antianginal medication (nitrates needed weekly)
 - d. Exercise time and level
 - e. Serum BNP levels in patients with CHF
 - f. LV diastolic dimension by contrast ECHO
 - g. Evaluation of the relationship between the degree of reversible ischemia at baseline and the effect of therapy on each of the primary endpoints and secondary endpoints 1-3.
7. MACE:
- a. New MI
 - b. Rehospitalization for PCI in coronary artery territories that were treated
 - c. Death
 - d. Rehospitalization for Acute Coronary Syndrome (ACS) and for CHF

⁺ Baseline defined as time period from day consent signed to day of treatment (not to exceed 60 days). Should patients fail screening for a reason that is likely to change over time, they may be considered for rescreening.

Subgroup Analyses: Analyses will include an examination of the effects of cell administration on prespecified subgroups:

1. Age
2. Gender
3. Patients with diabetes
4. Race
5. Serum BNP levels in patients with CHF
6. Pre-existing comorbidity (e.g. MACE)
7. Baseline LVEF
8. Functional characteristics of the cells that are used, including colony forming capability and motility in individual patients compared to subsequent influence of the cells on myocardial perfusion and contractile performance in individual patients.

Primary Hypothesis: As compared to placebo, the administration of bone marrow mononuclear cells to patients with CAD, LV dysfunction and limiting heart failure and/or angina will enhance myocardial perfusion, reduce LV end systolic volume, or enhance myocardial oxygen consumption.

Secondary Hypotheses:

1. As compared to placebo therapy, the administration of bone marrow mononuclear cells will enhance regional myocardial function.
2. As compared to placebo therapy, the administration of bone marrow mononuclear cells will diminish future MACE (new myocardial infarcts and rehospitalization for CHF).
3. As compared to placebo therapy, administration of bone marrow mononuclear cells will enhance exercise ability.

Relevance to the Goals of the CCTRN: This proposal satisfies the rationale for the Cardiovascular Cell Therapy Research Network (CCTRN), which is to investigate new cell therapy effects and examine the effects of cell therapy in cardiovascular disease. The combined expertise of experienced researchers at separate clinical centers strengthens the scientific content of this experiment. By recruiting from multiple centers, the network will accelerate the speed with which

the study is completed. The use of the network core laboratories will standardize the measures of endpoints. We believe that the regional distribution of the cell networks will amplify the dissemination of the results, improving the public health.

1.0 STUDY OBJECTIVES

1.1 Primary Objective

The objective of this randomized, Phase II, double-blinded, placebo controlled study will be to evaluate the safety and efficacy of autologous bone marrow-derived mononuclear cell injections in adult patients with coronary heart disease and ischemic left ventricular (LV) dysfunction and symptomatic angina, heart failure or both, who are not candidates for other revascularization procedures.

The efficacy of the cell delivery process will be assessed by cardiac imaging, clinical symptoms, and functional capacity. Three endpoints are proposed to reflect the potential complex effects of autologous bone marrow-derived mononuclear cell therapy.

The safety of the cell delivery process will be assessed with echocardiography prior to the procedure to establish that the targeted area(s) are appropriately thick to avoid pericardial delivery of cells. Peri-procedural surveillance for pericardial effusion, worsening of myocardial function, and arrhythmias will be provided for at least 24 hours in the hospital in a well monitored (telemetry) environment. Long-term safety, including the potential adverse effects of injected bone marrow cells, will be evaluated by clinical monitoring for signs and symptoms of inflammatory or infectious complications, surveillance for cardiac arrhythmias, and repeated evaluations of cardiac function.

1.2 Hypothesis

The hypothesis of FOCUS is that bone marrow mononuclear cells injected directly into reversibly injured areas of the heart in patients with ischemic LV dysfunction (LVEF \leq 45%), and either limiting angina (Class II to IV) or heart failure (NYHA Class II to III), will improve myocardial perfusion, regional ventricular function and clinical symptoms.

We have selected left ventricular ejection fractions (LVEFs) \leq 45% to be certain we are treating patients with LV dysfunction, but to have the widest net possible for inclusion of patients with significant CAD and limiting CHF and/or angina. We believe that bone marrow-derived mononuclear cells, and in the future, specialized stem cells that are functionally optimal and given in adequate numbers directly into reversibly injured areas of the heart in patients with coronary heart disease and LV dysfunction (LVEF \leq 45%), and either limiting angina (Class II to IV) or heart failure (NYHA Class II to III), will improve myocardial perfusion, regional ventricular function, and clinical symptoms. Stem cells' beneficial effects will be some combination of new blood flow, new myocytes, and paracrine efforts. It is

very likely that different stem cell types will promote to a greater or lesser extent either blood flow increase, myogenesis, or a balance of the two. At this time, the most dominant effect for any stem cell in altering myocardial perfusion, myogenesis, or both cannot be stated with certainty, but the bone marrow-derived mononuclear cells have clearly improved myocardial perfusion at sites where they were injected, and with that, regional LV function in our previous studies. Functionally optimal stem cells refer to those capable of motility and colony forming in culture.

Our hypothesis stated above is supported by a clinical trial performed earlier by Drs. Willerson and Perin in Brazil (1) and is the focus of an ongoing study led by Drs. Willerson and Perin in collaboration with the other Investigators in this clinical network and NHLBI trial. The current protocol represents an extension of the former studies using higher doses of cells and refined endpoints in a multicenter environment. The results of cell delivery in the early clinical experience and previous preclinical studies suggest a benefit on ventricular function and myocardial perfusion that may result from some combination of direct and paracrine effects. The current proposal is an outgrowth of the earlier study in Brazil (2000-2003) and the recently completed similar study in Houston at the Texas Heart Institute at St. Luke's Episcopal Hospital under the leadership of Drs. Perin and Willerson (IND BB#11044). In both of these previous studies, no patient harm was identified, including no deaths, new heart attacks, perforation of the heart, or infection introduced at the time of cell injection. In the Brazilian study of 14 patients and 7 controls, there was an improvement in myocardial perfusion, MVO_2 , and LVEF in the treated patients (1).

1.3 Relevance to the CCTRN

This protocol is consistent with the scope of the Cardiovascular Cell Therapy Research Network (CCTRN) to accelerate research in the use of cell-based therapies for the management of cardiovascular diseases. This protocol is based on a growing international experience with BMC transplantation. This study will collect important mechanistic and clinical information on the efficacy and safety of direct myocardial implantation of stem cells in patients with left ventricular dysfunction. It will also provide a useful assessment of the NOGA system of cell delivery; including the investigational NOGA injection catheter.

2.0 BACKGROUND

2.1 Rationale

Coronary artery disease (CAD) is prevalent worldwide. The therapeutic armamentarium involves medical treatment and revascularization by means of either coronary artery bypass grafting (surgical approach) or coronary angioplasty and stent placement (percutaneous approach). Despite many successful technological breakthroughs, the limitations of these strategies have become clear, as the presence of myocardial scar tissue and/or an unsuitable coronary anatomy may preclude the opportunity to perform subsequent revascularization procedures. In addition, CAD is a leading cause of heart failure, posing significant morbidity and mortality risks along with increasing health costs in a rapidly enlarging patient population. Heart failure affects 4.7 million Americans, with 550,000 newly diagnosed cases per year, resulting in annual costs of 10 to 40 billion dollars. Patients with end-stage heart failure have a 5-year mortality of approximately 50%.

Because a substantial number of patients with ischemic cardiomyopathy have no further treatment options, researchers are investigating new alternative treatments, such as therapeutic angiogenesis. Therapeutic angiogenesis has been proposed for patients not eligible for revascularization procedures, and several trials have been conducted to evaluate this new therapy. Until recently, angiogenesis trials usually involved the use of two families of growth factors: fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). Strategies for applying these factors have been tested (protein, gene transfer), using different delivery routes with varying success. Recent studies demonstrated that one VEGF mechanism of action is mobilization of endothelial precursor cells originating in the bone marrow. It is important to emphasize that angiogenesis therapy has been aimed mainly at improving myocardial perfusion and anginal symptoms, not at improving left ventricular (LV) systolic dysfunction.

Preclinical studies have demonstrated myocardial regeneration and improved myocardial function with delivery of BM-derived cells in animals following MI. In a study (41) from Center for Cardiovascular Biology and Atherosclerosis, University of Texas Health Science Center at Houston; Heart Failure Research Lab, Texas Heart Institute examining murine epididymal adipose tissue resected from Rosa26 LacZ⁺ mice (Jackson Laboratory, Bar Harbor, ME), the data indicate that from the vascular-stroma of the adipose tissue, the vascular endothelial stem or progenitor cells show the ability to maintain a high-rate of self-proliferation without undergoing senescence over an extended period of culture. Moreover, examination of the feasibility of intracoronary cell transfusion reveals a new route for the adipose tissue-derived stem cell entry into the heart. The data highlights the possibility of utilizing the adipose tissue-derived stem cells for cellular therapy in heart failure. These results suggest that adipose tissue contains endothelial cell progenitors which are suitable for cardiac cellular therapy.

In a study (42) from the Departments, of Cardiology, Blood and Marrow Transplantation, and Bioimmunotherapy, The University of Texas-M.D. Andersen Cancer Center, Houston, The University of Texas Houston Health Science Center, and the Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas using female scm mice, it was observed that adult peripheral blood CD34⁺ cells can transdifferentiate into cardiomyocytes, mature endothelial cells, and smooth muscle cells in vivo. However, transdifferentiation was augmented significantly by local tissue injury. The use of peripheral blood CD34⁺ cells for cell-based therapy should greatly simplify the, procurement of cells for the regeneration of damaged myocardium.

Drs. Geng and Willerson; UT99-117 study (43) which utilized dogs that were submitted to the canine chronic ischemia protocol, revealed no abnormal growth of non-cardiac tissue detected by histopathology analysis. In the cell therapy groups when compared to the control group there was an improvement in cardiac function at rest that was more pronounced in the transendocardial group. Transendocardial delivery of MSC's was not associated with cardiac tamponade or any clinical untoward effects at immediate and up to 15 days follow-up.

In a study (44) by Perin and Willerson et al. comparing the safety and efficacy of intra-coronary versus trans-endocardial delivery of allergenic MSC cells in a canine acute ischemia model, there was no abnormal growth of non-cardiac tissue detected by histopathology analysis. In the cell therapy groups when compared to the control group there was an improvement in cardiac function at rest that was more pronounced in the transendocardial group. Transendocardial delivery of MSC's via the NOGA catheter (trans-endocardial) or intra-coronary delivery mode was not associated with cardiac tamponade or any clinical untoward effects at immediate and up to 15 days follow-up.

In the last 7 years, bone marrow-derived mononuclear cells have been intensively studied as a promising source of cells that could enhance perfusion in an injured area of the heart and help repair injured tissue (2). Bone marrow-derived stem cells are one type of "adult stem cells", being defined by their multi-potential and self-renewable cell lineages. Bone marrow-derived stems cells have been shown to differentiate into neural cells (3), hepatocytes (4), endothelium (5-8), myocytes (9), and cardiomyoctes (8,10,11). Recently accumulated preliminary evidence has demonstrated that bone marrow cells can differentiate into endothelial cells associated with angiogenesis (7,12-14) or, into cardiomyocytes (8,10,15). At least some endothelial precursor cells can be identified as CD34 positive cells, which frequently have VEGF receptors (5,16). Although the definition of endothelial progenitor cells (EPC) is controversial in nature, bone marrow contains precursors for all cell types with potential to develop an endothelial phenotype (5,16). These precursor cells include CD34⁺ cell subsets. With regards to the CD34⁺ human adult stem cells, Drs. Yeh and Willerson (17) have shown that

these cells differentiate into blood vessel cells, smooth muscle cells, and new myocytes, and that they fuse with reversibly injured murine myocytes generating new myocytes in SCID mice with experimentally-created myocardial infarcts (17-19). Losordo et al. (20) have recently completed a Phase I trial in humans with severe angina on optimal medical treatment, injecting CD34⁺ autologous stem cells transendocardially using a NOGA catheter or saline (control patients) and the 24 patients were followed for safety evaluations. No harmful effects were found in the treated or control patients.

Seeger, Zeiher, and Dimmeler have shown that cell isolation techniques for bone marrow-derived mononuclear cells are very important (21) and that isolation techniques promoting functional viability as evidenced by the ability of the cells to form colonies are very important in the subsequent impact of the transplanted cells on LVEF (21).

More recently, Yeh, Willerson, et al. have demonstrated that the α 4, β 1 and VCAM receptors mediate the fusion process in in vitro studies, and that the fusion of the human adult CD34⁺ cells with injured murine myocytes activates the cell cycle and results in the direct generation of new cardiac myocytes (18). The generation of new endothelial cells by CD34⁺ adult human stem cells in this model is not inhibited by antibodies directed against α 4, β 1 or VCAM receptors, but instead by an antibody to VEGF. Thus, the generation of new myocytes is a consequence of cell fusion, and endothelial cell generation follows a separate direct differentiation (18).

Strauer et al., Zeiher and Dimmeler et al., and Drexler et al. have all shown the benefit of using autologous human bone marrow-derived mononuclear cells in the treatment of patients with acute ST segment elevation myocardial infarction (STEMI) who are undergoing PCI in non-randomized and randomized studies (22-28). In general, these patients did not have CHF; instead, they had relatively well preserved LVEFs (low 50s typically), the cells were given after PCI directly into the infarct-related arteries between days 3 and 9 post infarct, and the cell dose was 100-200 million cells. In these studies, there was usually a 3 to 5 ejection fraction unit increase subsequently in the cell treated patients, and in Strauer's early study, there was also an increase in myocardial perfusion in the cell-treated patients (22). In more recent studies, Drexler et al. have shown that the control group improved its LVEF in the 6-18 months after PCI, becoming very similar to the cell-treated patients by 18 months following their MI (28).

The Perin and Willerson study in Brazil, as described below, was the first study in patients with chronic and multivessel CAD and severe CHF in which patients were treated with autologous bone marrow-derived stem cells with direct transendocardial injections into reversibly injured myocardium using a NOGA catheter (1). This study was done in the same time period (2000-2002) as Strauer's

and Zeiher's and Dimmeler's studies in Germany in patients with acute MIs. In Zeiher's and Dimmeler's most recent studies in patients with MIs, they described that the maximal improvement in LVEF occurred in patients with the lowest LVEFs and the highest serum BNP (biomarkers of the severity of CHF) when the autologous bone marrow-derived mononuclear cells that were used had the greatest colony forming capability (23). Zeiher and Dimmeler et al. have also found that patients treated with functionally viable bone marrow-derived mononuclear cells who derive functional benefit in terms of increased LVEFs, also have a reduction in the clinical composite end point risk of new MI, need of repeat PCI, and death in the one-year follow-up period (21,23).

Perin and Willerson have very recently completed a randomized trial in patients with severe CHF and multivessel CAD using a similar protocol to that used in Brazil with transendocardial injections of the cells using a NOGA catheter (30). It is a 30 patient study with 20 patients receiving cells and 10 serving as controls. It is the first such FDA approved trial in patients with severe CHF in the U.S. The follow-up period is 6 months following cell treatment. Neither Drs. Perin, Willerson, nor the German physicians, physician/scientists have seen any harm from the autologous stem cell injections in patients with acute MIs and those with chronic ischemic CAD and CHF (1,23-30).

2.2 Initial clinical studies of ABM-MNCs delivery

The first study to use autologous bone marrow mononuclear cells (ABM-MNCs) for angiogenesis in patients with critical limb ischemia involved intramuscular implants of ABM-MNCs and showed an improvement in perfusion. Importantly, angiogenesis was demonstrated by the visualization of new blood vessels with arteriography (31).

In other clinical studies, Assmus et al. (24) studied 20 patients with preserved left ventricular ejection fractions (LVEF) (mean, 52%). Nine patients received intracoronary ABM-MNCs, and 11 pts received peripheral endothelial precursor cells expanded in vitro 4.3±1.5 days post myocardial infarction. The patients were compared to non-randomized matched control patients (n=10). At 4-months follow-up, there was a significant increase in the LVEF, improved wall motion in the infarct zone, and a reduced LVESV. In addition, coronary flow reserve had increased in the infarct-related artery, and a quantitative FDG-18 positron emission tomography (PET) scan revealed a significant increase in myocardial viability in the infarct zone. No inflammatory response or malignant arrhythmias were observed.

Stamm et al. (32) utilized a surgical approach for cell delivery. In six post myocardial-infarction patients undergoing surgical revascularization, ABM-MNCs were injected at infarct borders of non-revascularizable territories. At 3- to 10-

months follow-up examination, the LVEFs (n=4) and myocardial perfusion (n=5) were improved.

Tse et al. (33) utilized a NOGA Myostar catheter for transendocardial delivery of ABM-MNCs in eight patients with stable angina refractory to maximal medical therapy (mean LVEF, 57.6%). At 3-month follow-up evaluations, the researchers noted a decrease in mean anginal episodes and nitroglycerin use per week from 26.5 to 10.1 ($p < 0.0001$) and from 23.9 to 6.8 ($p = 0.002$), respectively. Cardiac magnetic resonance imaging (MRI) revealed improvement in target wall thickening and target wall motion (11.6% and 5.5%, respectively), but no improvement in global LVEF. There was a 3.9% reduction in the mass of the hypoperfused myocardium. More importantly, the procedure was demonstrated to be safe, as no pericardial effusions, arrhythmias, or other acute complications were recorded.

2.3 ABM-MNCs – The Texas Heart Institute Experience

The application of bone marrow-derived stem cells in humans for the treatment of CAD by inducing vasculogenesis and possibly cardiomyogenesis has been under investigation for the past 7 years by Drs. Perin and Willerson and their colleagues in Brazil and the U.S. This effort has included a nonrandomized open-label study in Brazil, and a randomized two center study in the US with Texas Heart Institute in Houston and the Minnesota Heart Institute who are both CCTRN sites (1,30). The results of the latter study that delivered 30×10^6 cells were described in section 1.3

2.3.1 Open label Study of Transendocardial, ABM-MNC Transplantation for Severe, Ischemic CHF (24)

In Dr. Perin's and Dr. Willerson's previous experience (1) with ABM-MNCs, they evaluated the hypothesis that transendocardial injections of autologous mononuclear bone-marrow cells in patients with end-stage ischemic heart disease and severe heart failure is safe, can promote neovascularization, and improve myocardial perfusion and myocardial contractility. In alliance with Procardiaco Hospital – Rio de Janeiro, Brazil, Drs. Perin and Willerson at Texas Heart Institute (THI) completed one of the first protocols for bone marrow-derived stem cell intramyocardial implantation in humans. Perin, et al. utilized the LV endocardial mapping (NOGA mapping) system as the platform for catheter-based intramyocardial implantation of autologous bone marrow derived mononuclear cells, injected into areas of ischemic hibernating myocardium (1). (Dr. Perin possesses the world's most extensive experience with endocardial mapping, having performed more than 500 procedures with an excellent safety profile) (34). The NOGA system reconstructs electromechanical maps of the left ventricle, therefore permitting online diagnosis of myocardial viability by measuring endocardial elec-

trical activity (34). It has been widely utilized as the delivery system for endothelial growth factors and has an excellent safety profile (20,34,35).

Twenty-one patients were enrolled into this prospective, non-randomized, open-label, controlled study (treatment group, first 14 patients; control group, last 7 patients) (1). Baseline evaluations included complete clinical and laboratory tests, exercise stress (ramp treadmill) studies, 2-D Doppler echocardiography, SPECT perfusion scanning, and 24-hour Holter monitoring. Bone marrow-derived mononuclear cells (BM-MNCs) were harvested, isolated, washed, and resuspended in saline for injection by the NOGA catheter (15 injections of 0.2 cc, totaling 30 million cells per patient). Electromechanical mapping (EMM) was used to identify viable myocardium (unipolar voltage ≥ 6.9 mV) for treatment. All patients underwent noninvasive follow-up tests at 2 months, and the treatment group also underwent invasive studies at 4 months, using standard protocols and the same procedures as at baseline.

The demographic and exercise test variables did not differ significantly between the treatment and control groups. The injection procedure was safe, and there were no procedural complications. At 2 months, there was a significant reduction in the total reversible perfusion defect in the treatment group and between the treatment and control groups ($p=0.02$) on quantitative SPECT analysis (1). At 4 months, the LVEF was improved from a baseline of 20% to 29% ($p=0.003$), and the LVESV was reduced ($p=0.03$) in the treated patients. EMM revealed significant mechanical improvement of the injected segments ($p<0.0005$). Perin and Willerson et al. concluded that the transendocardial application of BM-MNCs is safe and that further investigation of this therapy was warranted to further evaluate efficacy endpoints (1).

2.3.2 Conclusions

To further confirm those initial results, the CCTRN will pursue a randomized study of autologous bone marrow mononuclear cell transplantation utilizing the same endocardial delivery platform and to evaluate a larger number of bone marrow-derived mononuclear cells in a similar patient population. In the proposed study, we will evaluate the effects of a larger number of cells (the initial number of cells given was very conservative because of safety issues), and we will utilize a more sophisticated study design to better evaluate efficacy endpoints and safety. Additionally, a highly translatable cell isolation system is proposed for this CCTRN study (Section 2.4).

The CCTRN is a network of clinical centers committed to the study of stem cells in humans with cardiovascular disease and to identify optimal methods for delivery of these cells and the identification of the most efficacious stem cell(s) for repair of injured hearts and blood vessels without harming the patient. This net-

work should allow for more rapid recruitment of appropriate patients and more efficient evaluation of different methods for administration of the cells as well as more rapid progress toward the identification of the most optimal and safest stem cell type(s).

2.4 SEPAX isolation of BM-MNCS

See Appendix 3.

3.0 STUDY DESIGN

3.1 Introduction

To evaluate the safety and efficacy of autologous bone marrow mononuclear cell injections in patients with ischemic LV dysfunction and symptomatic angina, heart failure or both, the CCTRN proposes a randomized, Phase II, double-blind clinical trial. This trial will randomize a maximum of 95 patients.

3.2 Study Endpoints

3.2.1 Primary Endpoints

The Investigators propose three co-primary endpoints of LV function:

- a. Change in maximal oxygen consumption (MVO_2);
- b. Change in left ventricular end systolic volume (LVESV) as assessed by echocardiography with contrast;
- c. Change in reversible defect size as assessed by SPECT.

3.2.2 Secondary Endpoints

The secondary endpoints of the study will compare the changes in the following measures from baseline⁺ to six month follow-up.

1. Reduction in fixed perfusion defect(s) by SPECT.
 - a. Change in total defect size by SPECT
 - b. Change in fixed defect size by SPECT
 - c. Change in Sum Difference Score by SPECT
2. Progression to fixed defect by SPECT.
3. Regional wall motion by MRI (in eligible patients).
4. Regional blood flow improvement by MRI (in eligible patients).
5. Regional wall motion by echocardiography.
6. Clinical improvements at 6 months, including change in anginal score by the following measures:
 - a. Canadian Cardiovascular Society Functional Classification of Angina Pectoris (CCS) (Appendix 1).

- b. NYHA Class (Appendix 2).
 - c. Decrease in antianginal medication (nitrates needed weekly).
 - d. Exercise time and level.
 - e. Serum BNP levels in patients with CHF.
 - f. LV diastolic dimension by contrast ECHO.
 - g. Evaluation of the relationship between the degree of reversible ischemia at baseline and the effect of therapy on each of the primary endpoints and secondary endpoints 1-3.
7. MACE:
- a. New MI.
 - b. Rehospitalization for PCI in coronary artery territories that were treated.
 - c. Death.
 - d. Rehospitalization for Acute Coronary Syndrome (ACS) and for CHF.

+ Baseline defined as time period from day consent signed to day of treatment (not to exceed 60 days).

3.3 Interventions

This clinical trial will have two treatment arms (active and placebo).

3.3.1 Treatment Dose

The treatment group will have intramyocardial electromechanical-guided injection of approximately 100×10^6 BM-MNCs, administered in 15 different injection sites.

3.3.2 Control Group

The control group will have intramyocardial electromechanical-guided needle insertions and injection of 5% human serum albumin/saline in 15 different injection sites.

3.4 Sample Size Formula

The evaluation for each of these three primary endpoints, (MVO₂, LVESV, and reversible defect size) will compare the change (follow-up minus baseline) of the measure in the control group to the change in the measure of the cell delivery group. Let $\mu_{\Delta x}$ be the expected change in the control group, and $\mu_{\Delta y}$ be the expected change in the cell delivery group. Then, the statistical hypothesis to be examined for each of the three co-primary endpoints is

$$H_0 : \mu_{\Delta x} = \mu_{\Delta y} \quad \text{versus} \quad H_1 : \mu_{\Delta x} - \mu_{\Delta y} = \Delta \neq 0$$

All subsequent sample sizes are based on the following assumptions: Type I error is apportioned at the 0.05 level (reduced from 0.05 as a correction

for alpha allocation should the Data Safety Monitoring Board choose to carry out interim monitoring for efficacy) to be carried out at 80% power. All testing is two-sided. There will be twice as many patients in the active group as in the control group.

In estimating the sample sizes for the primary analysis, we used sample size and power analysis based on the normal approximation to the distribution of continuous variables:

$$N = \frac{(k+1) \left(\sigma_{\Delta x}^2 + \frac{\sigma_{\Delta y}^2}{k} \right) (Z_{1-\alpha/2} - Z_{\beta})^2}{(1-f)\Delta^2}$$

where

N = total sample size of the study;

$\sigma_{\Delta x}^2$ = variance of the difference in the outcome variable in the control group;

$\sigma_{\Delta y}^2$ = variance of the difference in the outcome variable in the active group;

α = Type I error;

β = Type II error;

Z_c = the c^{th} percentile from the standard normal probability distribution;

Δ = effect size, i.e., the expected difference between the change in the control group and the change in the cell delivery group over time;

k = ratio of number of active group to control group patients, $k = 2$;

f = expected proportion of patients anticipated to be lost to follow-up, $f = 10\%$.

3.5 Sample Size Computation

For each of the three co-primary endpoints, a sample size was computed based on estimates of the effect size and standard deviation of the difference. To ensure adequate power for each of the three endpoints, the sample size was computed for each of them, and the maximum sample size was selected. The data for effect size and standard deviation of the difference for each of MVO₂, LVESV, and reversible defect size were obtained from Perin, et al. (1). Correlations for LVESV and reversible defect size were obtained from an evaluation of the data published in the INSPIRE study, Mahmarian et al. (38).

3.5.1 MVO₂

From the data in Perin et al. (1), the mean MVO₂ before therapy was 17.96. The standard deviation before therapy was 8.78 and 8.31 at two months. Under the null hypothesis, it is assumed no change will occur in this mean MVO₂ over the six-month duration of the study. In the cell therapy group, it is anticipated the change over time will be 5.42, conservatively reduced to 5. The correlation be-

tween MVO₂ at baseline and at six months was estimated to be 0.65. These data produce $\sigma_{\Delta x} = \sigma_{\Delta y} = 7$. This produces a total sample size (control group + cell therapy group) of 77. The variability of the sample size for MVO₂ as a function of the standard deviation of the difference and the effect size is available (Table 1).

Table 1. Sample size (control + cell therapy group) as a function of the effect size and standard deviation of the change over time for MVO₂.

		Effect size						
		4	4.5	5	5.5	6	6.5	7
σ_{Δ}	5.5	74	59	48	39	33	28	24
	6	89	70	57	47	39	34	29
	6.5	104	82	67	55	46	39	34
	7	121	95	77	64	54	46	39
	7.5	138	109	89	73	62	52	45
	8	157	124	101	83	70	60	51

3.5.2 LVESV

From the data in Perin et al. (1), the mean LVESV before therapy was 146.78 cc. Under the null hypothesis, it is assumed no change will occur in this mean LVESV over the six month duration of the study. In the cell therapy group, it is anticipated the change over time will be 22 ccs. The standard deviation before therapy was 53.46 and 47.88 after therapy. The correlation between baseline and follow-up LVESV was 0.80. These data generate $\sigma_{\Delta x} = \sigma_{\Delta y} = 32.50$. This produces a total sample size (control group + cell therapy group) of 86. The variability of the sample size for LVESV as a function of the standard deviation of the difference and the effect size is available (Table 2).

Table 2. Sample size (control + cell therapy group) as a function of the effect size and standard deviation of the change over time for LVESV.

		Effect size						
		17	18	20	21	22	23	24
σ_{Δ}	30	123	109	89	80	73	67	62
	32.5	144	128	104	94	86	79	72
	35	167	149	121	109	100	91	84
	40	218	194	157	143	130	119	109
	42.5	246	219	178	161	147	134	123
	40	218	194	157	143	130	119	109

3.5.3 Reversible perfusion defect size

From the data in Perin et al. (1), the mean reversible perfusion defect size before therapy was 15.15 (measured as a percent). Under the null hypothesis, it is as-

sumed a 5% (measured in absolute percentage points) decrease in mean reversible perfusion defect size will occur over the six-month duration of the study. A 15% decrease is anticipated in the cell therapy group. The standard deviation before therapy was 14.99 before therapy and 10.61 after therapy. The correlation between baseline and six-month reversible defect size was 0.60. These results generate a standard deviation of the difference for each group $\sigma_{\Delta x} = \sigma_{\Delta y} = 10.70$, conservatively increased to 11. This produces a total sample size (control group + cell therapy group) of 48. The variability of the sample size for LVESV as a function of the standard deviation of the difference and the effect size is available (Table 3).

Table 3. Sample size (control + cell therapy group) as a function of the effect size and standard deviation of the change over time for reversible defect size.

		Effect size						
		9	9.5	10	10.5	11	11.5	12
σ_{Δ}	9	39	35	32	29	26	24	22
	10	49	44	39	36	33	30	27
	11	59	53	48	43	39	36	33
	12	70	63	57	51	47	43	39
	13	82	74	67	60	55	50	46
	14	95	85	77	70	64	58	54

3.6 Final Sample Size Calculation

A sample size of 86 patients, administratively increased to 87 patients (29 in the control group, 58 in the active group), provides adequate power to examine the effect of cell therapy for MVO_2 , LVESV, and reversible defect size. All randomized patients (up to 95 patients) will be evaluated in the analyses.

3.7 Type I Error Adjustment

No type I error adjustment was incorporated.

4.0 SELECTION AND ENROLLMENT OF PATIENTS

A maximum of 95 patients who meet the following inclusion/exclusion criteria will be enrolled.

4.1 Introduction

According to the inclusion criteria, the patient cohort will have both LV dysfunction and myocardial ischemia. Compared to patients with CAD and preserved LV function, those with LV dysfunction have a higher morbidity and mortality and can potentially benefit the most from alleviation of ischemia. Patients with LV dysfunction secondary to CAD may have predominant symptoms of heart failure or angina despite maximal medical therapy. Some patients may have “silent ischemia”, making the absolute presence or absence of anginal symptoms unreliable as a marker of underlying ischemic cardiomyopathy. Therefore, we have also considered heart failure symptoms as a cardinal manifestation of CAD in these patients. Once the patient has met all inclusion criteria, and they have no exclusions, informed consent will be obtained.

4.2 Randomization

Randomization will occur at the Data Coordinating Center (DCC) incorporating a scheme devised by a biostatistician that will randomized patients into two groups (control and treatment).The patients and research staff will be blinded to the treatment group. Further details of the randomization (using a variable block scheme) are provided in sections 5.1.3.2 and 9.

4.3 Inclusion Criteria

To be considered eligible to participate in this study, the patient must have the following conditions:

1. Patients ≥ 18 years of age with significant coronary heart disease not amenable to revascularization.
2. LVEF $\leq 45\%$ (by echocardiogram) and limiting angina (Class II to IV) and /or CHF (NYHA class II - III).
3. Patients should be on maximal medical therapy. Maximal medical therapy for anginal symptoms is defined as a medical regimen that includes the maximal tolerated dose of at least two anti-angina medications, such as beta-blockers, nitrates, or calcium-channel blockers. One anticipates using a beta blocker or calcium channel blocker to reduce heart rate to 50-60 beats per minute and systolic blood pressure to 100-115 mm Hg in patients with angina or as tolerated clinically in order to evaluate them for limiting angina or angina that interferes with the life style the patient wishes to lead. Maximal medical therapy for heart failure symptoms includes beta-blockers (either a beta 1 blocker, such as metoprolol or non-specific beta blocker, such as carvedilol), ACE-1 or ARB (if creatinine ≤ 2.5) + diuretics. Patients with LVEF's less than or equal to 35%, sinus rhythm, and NYHA functional class III or ambulatory class IV symptoms despite rec-

ommended, optimal medical therapy and who have cardiac dyssynchrony, which is currently defined as QRS duration greater than 0.12 ms, should receive cardiac resynchronization therapy unless contraindicated (ACC AHA CHF guidelines 2005).

4. Presence of a defect as identified by SPECT (isotope protocol) and/or viability as identified by NOGA.
5. Coronary artery disease not well suited to any other type of revascularization procedure (percutaneous or surgical) in the target region of the ventricle. A cardiovascular surgeon and an interventional cardiologist (who are not Investigators in the trial) will assess the subject's eligibility by chart review and recent diagnostic arteriogram (within 12 months) to determine percutaneous or surgical revascularization options. Patients should not be considered for revascularization procedures, if they have an unsuitable coronary anatomy, including total occlusion, poor targets for bypass grafts, small vessels, or diffuse disease affecting the distal vessel and making proximal revascularization ineffective. Patients could also have significant co-morbidities that would pose an unacceptable risk for surgical revascularization
6. Hemodynamic stability as defined by systolic BP \geq 80 mmHg without IV pressors or support devices.
7. Females of childbearing potential must be willing to use two forms of birth control for the duration of the study.
8. A signed consent form approved by the institutional review board.

4.4 Exclusion Criteria

Patients will be excluded from the study if they have any of the following conditions:

1. Atrial fibrillation or flutter without a pace maker that guarantees a stable heart rate
2. Unstable angina.
3. LV thrombus, as documented by echocardiography or LV angiography.
4. A vascular anatomy that precludes cardiac catheterization.
5. Severe valvular disease or mechanical aortic valve that would preclude safe entry of the catheter into the left ventricle.
6. Pregnant or lactating status. Pregnancy as determined by a positive pregnancy test at baseline.
7. Platelet count $<$ 100 K/mm³. †
8. WBC $<$ 2 K/mm³. †
9. Revascularization within 30 days of consent
10. TIA or stroke within 60 days of study consent.
11. ICD shock within 30 days of baseline consent, and within 30 days of randomization.

12. Presence of sustained ventricular tachycardia (30 or more seconds) on 24 hour Holter monitor or ECG performed during baseline screening period.
13. A bleeding diathesis defined as an INR \geq 2.0 in the absence of warfarin therapy.
14. A history of malignancy in the last 5 years excluding basal cell carcinoma that has been surgically removed with proof of surgical clean margins.
15. Has a known history of HIV, or has active Hepatitis B or active Hepatitis C
16. Any condition requiring immunosuppressive medication.
17. A high-risk acute coronary syndrome (ACS) or a myocardial infarction in the month prior to consent (ACS is defined as the presence of chest pain characteristic for angina, dynamic electrocardiography changes of ST segment depression or elevation and/or serum elevation of troponin I or T > 3X ULN (according to local laboratory).
18. A left ventricular wall thickness of <8 mm (by echocardiogram) of the infero-lateral wall at the target site for cell injection.
19. Inability to walk on a treadmill except for class IV angina patients who will be evaluated separately. (If only reason patient is unable to walk on treadmill is class IV angina, then patient will be included.)
20. Potential patients enrolled in an investigational device or drug study within the previous 30 days.
21. Hepatic dysfunction, as defined as aspartate aminotransferase (AST) and /or alanine aminotranferase (ALT) > 1.5 times the upper limit of normal range (x ULN) prior to study entry.
22. Chronic renal insufficiency as defined as a serum creatinine > 2.5 mg/dL or requires dialysis.
23. Any other condition that in the judgment of the Investigator would be a contraindication to enrollment or follow-up.

† To minimize risk of bleeding and infection.

If at any time prior to the study procedure the randomized patient meets any of the above exclusion criterion, treatment will be postponed or if the condition is not resolvable, the patient will be excluded from participation.

4.5 Baseline Testing

The following evaluations will be carried out at baseline. (Baseline is defined as day informed consent signed to day of treatment (not to exceed 60 days)).

1. Laboratory examinations:
 - a. Complete blood count (CBC) with differential and platelet count

- b. Chemistry-8 panel (Na, K, BUN, Creat, glucose, calcium, chloride, CO₂)
 - c. Liver function tests (LFTs)
 - d. Partial thromboplastin time (PTT) and prothrombin time / international normalized ratio (PT/INR)
 - e. High sensitivity C-reactive protein (hsCRP)
 - f. B-type natriuretic peptide (BNP)
 - g. Creatine kinase (CK), and CK-MB levels
 - h. Pregnancy for females of childbearing potential
2. Infectious Disease Labs: HIV, hepatitis B (HBV), hepatitis C virus (HCV), human T-lymphotropic virus (HTLV) I/II antibody, rapid plasma reagin (RPR), cytomegalovirus (CMV), ABO group, Rh status, West Nile virus and serum protein electrophoresis. * (Virology testing as required by GMP Cell labs)
 3. Cardiac Markers: CK, CK-MB, Troponin T or Troponin I
 4. 24-hour Holter monitoring
 5. Treadmill testing with myocardial oxygen consumption (MVO₂) determination (MVO₂ Core Lab Procedure Manual)
 6. SPECT Imaging (SPECT Core Lab Procedure Manual)
 7. Physical examination, including a neurological evaluation (NIH stroke scale) to establish the baseline neurological status
 8. Medical history
 9. Chest x-ray
 10. Contrast 2D Echocardiography with strain rate imaging (Echo Core Lab Procedure Manual)
 11. Six minute walk
 12. ICD interrogation
 13. Quality of Life Questionnaires assessed with the 36-Item Short-Form Health Survey Questionnaire (SF36) and the Minnesota Living with Heart Failure Questionnaire
 14. MRI (in those patients without contraindication to cardiac MRI) (MRI Core Lab Procedure Manual)

4.6 Baseline Screening Window

Baseline screening period will not exceed 60 days.

5.0 INTERVENTION

5.1 Treatment Period

After baseline testing is complete, patients will be randomized via a computer-generated randomization sequence to the active cell therapy group or the control group.

5.1.1 Active Therapy Group

After undergoing hospital admission and clinical evaluation, patients will undergo bone marrow aspiration by trained physician with substantial experience in carrying out bone marrow harvesting procedures. The details of the aspiration procedure are located in Appendix 4. Approximately 80-90ml (± 10 ml) of bone-marrow aspirate will be obtained and transferred in a sterile manner to a local stem cell laboratory for processing. Patients on aspirin and Plavix (clopidogrel) at the time of consent should remain on aspirin and Plavix (clopidogrel) for the bone marrow aspiration procedure. Continuance or discontinuance of other medications at the time of bone marrow aspiration, (e.g. Coumadin) are left to the discretion of the Study Physician. The Sepax system (investigational) will be used to select bone marrow mononuclear cells from the aspirate. Data to be recorded in the cell lab includes the population of stem cell types, including CD34⁺ and CD133⁺ fractions. Access to subject identities will be limited to the Investigators and research staff. Approximately 4 hours (but no more than 12) after the bone marrow procedure, patients will be admitted to the cardiac catheterization lab for the injection procedure. Coronary and LV angiography and LV EMM will be performed to locate the target area to be injected. The infero-lateral wall must be at least 8mm thick as measured by echocardiograph.

All cell injections will be performed in areas of a SPECT defect (fixed or reversible) associated with viability, as per NOGA criteria. The target myocardial area must display points that show electrical viability, defined as a unipolar voltage of ≥ 6.9 mV, thus representing viable myocardial tissue(1). In the case of divergent results between the perfusion imaging test and EMM regarding the target ischemic area, the coronary anatomy will be taken into account. EMM will be relied on for point source viability for the injections.

The NOGA injection catheter (investigational) will be prepared by adjusting the needle extension at 0° and 90° flex and by placing 0.1 cc of ABM-MNCs to fill the needle dead space. All study product will be retained to complete the 15 required injections. Following priming, the interventionalist will retract the drop that is hanging from the tip of the needle back into the catheter. To ensure safety and limit the potential for extracardiac administration of the injectate, the needle extension/wall thickness ratio will be ≤ 0.5 at all times. The maximum needle extension permitted will be of 6mm. Following insertion through an 8F sheath placed in the femoral artery, the injection catheter will be advanced to the aortic valve. In a retrograde fashion, the catheter will cross the aortic valve into the left ventricle and the catheter tip will be placed against the endocardium at the target area. Each injection site will be carefully evaluated prior to cell injection, as follows, to

enhance safety and ensure intramyocardial delivery of the cellular product. The following criteria will have to be met: (1) perpendicular position of the catheter to the LV wall; (2) excellent loop stability (4 mm); (3) underlying voltage ≥ 6.9 mV; and (4) presence of a premature ventricular contraction on extension of the needle into the myocardium. Each of the 15 injections will contain 0.2 ml of cells (6.5 to 7 million cells per injection site) for the 100×10^6 arm. For the final injection, 0.1 cc of saline should be placed in the catheter and injected. This will allow the 0.1 already in the catheter (from priming) to be administered into the myocardium, constituting the 15th injection.

After the injection procedure, patients will be monitored overnight in the cardiac telemetry unit (simple telemetry). A transthoracic 2-D echocardiogram without contrast will be performed immediately after procedure and on the day after injection (prior to discharge) to detect possible pericardial effusion. Serial myocardial necrosis markers (CK and CK-MB) and troponin I or T will be evaluated 8, 16, and 24 hours after the procedure.

5.1.2 Control Group

Patients randomized to the control group will undergo the same baseline testing and bone-marrow aspiration procedure. Approximately 4 hours (but no more than 12 hours) later the patient will be taken to the cardiac catheterization laboratory where he or she will undergo coronary and LV angiography, as well as an EMM procedure with needle injection of 5 % HSA/Saline at each of 15 different sites without the presence of cells. The ABM-MNC's will undergo cryopreservation.

Regardless of group assignment, patients will remain hospitalized for a minimum of 24 hours post injection procedure.

5.1.3 Randomization and Unblinding

Blinding of the interventionalist giving the injections will be eased by the fact that all patients, placebo or treatment will undergo bone marrow harvest as well as an injection after mapping. As the study product cannot affectively be masked, the process of blinding resides with endpoint and adverse event determination. Each of the three primary endpoints of the study will be determined by a core laboratory whose personnel are blinded to therapy assignment. In addition, each of the clinical centers will take steps to ensure that adverse event assessments are carried out in a blinded fashion

The bone marrow aspiration and automated cell processing will take place in a blinded fashion as described below.

Randomization and unblinding are each necessary procedures for clinical trials in CCTRN. Randomization, or the random allocation of therapy, is a well-accepted

mechanism for reducing potential bias in evaluating treatment effects. Unblinding is the process by which knowledge of a patient's therapy assignment is provided to specific, predetermined individuals. Of necessity, these two important procedures must occur at different time points. The sequence of steps is as follows:

5.1.3.1 Randomization

After the Clinical Center research team has determined that a patient satisfies the inclusion and exclusion criteria of the study and the patient has read and signed the informed consent, the Research Coordinator completes a secure form on the CCTRN web application (described further in section 10). Completing this form validates that the patient has met the inclusion/exclusion criteria and acknowledges the informed consent has been completed. The computerized randomization algorithm now assigns a study ID number to the patient.

5.1.3.2 Unblinding

Subsequently, the patient undergoes a bone marrow aspiration of 80-90ml (± 10 ml), the aspirate is processed through the investigational Sepax system, and samples are drawn for rapid release and other testing. Should there be less than 30 million cells, the subject will not be randomized to therapy condition and no cells will be provided. At this point, the designated cell processing technician is unblinded. The unblinding proceeds in the following manner:

- 1) Laboratory staff log on the CCTRN website;
- 2) The logged-on staff member confirms that cell processing is complete, inputting date and time of aspiration, arrival of aspirate at the laboratory, and cell processing;
- 3) The logged-on staff member informs laboratory staff of randomization so final product packaging can proceed.

The web server responds with the patient's therapy assignment, producing a printable, written report. This process guards against knowledge of treatment assignment affecting cell processing. All product testing will be conducted by blinded laboratory personnel to the extent possible. Staff must input date and time of release on the CCTRN website.

If the cell product passes rapid release testing and the patient is in the active group, then the cell therapy product is prepared for injection. If a control group patient's product passes rapid release testing, then a placebo infusate is prepared, and the patient's cells are cryopreserved and sent to the biorepository, assuming the patient has consented to have their cells donated to the repository.

If the cell product fails the viability rapid release testing, then the patient is removed from the study regardless of therapy assignment.

If the cell product passes the release criteria, then it is delivered to the Investigator who will be providing the product to the patient. Thus the transporter and the Investigator who injects the product into the patient are unblinded.

Should the 14-day sterility culture testing produce a positive culture after the cell processing product has been administered to the patient, then regardless of therapy assignment the following steps will take place:

- a) A laboratory investigation will take place. Reporting requirements of an “unanticipated problem” will proceed for the NIH, DSMB, FDA, and IRB’s
- b) The patient’s doctor will be notified at once by the cell processing laboratory that the specimen was positive.
- c) The patient will remain in the study and be monitored for clinical signs of infection. Any resultant adverse events will be evaluated and reported.
- d) Antibiotic prophylaxis will be considered.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 See “Baseline Testing” under section 4.3.

6.2 Follow-up Evaluations

6.2.1 Laboratory Testing

After the injection procedure, all patients will have the following blood drawn: troponin I or T, CK, CK-MB at 8, 16 and 24 hours after the procedure. If the subject is discharged prior to 24 hours after the procedure, the cardiac markers will be evaluated at discharge if more than 2 hours have elapsed since the previous evaluation. The day following injection (with the 16 hours enzymes if possible to save subject discomfort), a CBC with differential and platelet count, blood urea nitrogen (BUN), creatinine, hsCRP, BNP levels and LFTs will be drawn. Patients will be discharged the day after the procedure, if no adverse events have occurred that require prolongation of the hospitalization. The same labs as on the day following the injection will be drawn at week 1, week 4, and months 3 and 6. A BNP only will be drawn at month 12 (see section 6.3, Table 1).

Five 10 mL venous blood (purple top) tubes and one 10 mL venous blood (green top heparin) tube will be drawn, on the day of study product injection, for the biorepository, FACS analysis and plasma cryostorage, respectively. Similar blood draws will take place on the day after study product injection, and 4 weeks, 3 months, and 6 months post study product injection.

6.2.2 Instructions to Patients

Prior to discharge, patients and their care providers will be given detailed instructions regarding early signs and symptoms of infectious syndromes (such as fever, malaise, and disorientation). They will be instructed to take and record their body temperatures twice daily for the first 2 weeks and to report to the Investigators immediately if their temperature is $\geq 99.5^{\circ}$ F. Clinical visits will occur at 1 and 4 weeks post-procedure, then at months 3 and 6, and 12, and an annual telephone call at years 2-5.

6.2.3 Holter Monitoring

To help detect life-threatening arrhythmias and/or conduction abnormalities, Holter monitoring will be performed at 1 and 4 weeks and at 3 and 6 months.

6.2.4 Internal Cardiac Defibrillator (ICD) Interrogation

An ICD interrogation is a standard non-invasive assessment of the function of the implantable cardiac defibrillator (ICD). This assessment (interrogation) identifies the occurrence of any significant ventricular arrhythmias over a certain time period and identifies any potential therapeutic interventions (such as shocks or anti-tachycardia pacing) that were used by the ICD to treat any ventricular arrhythmia. ICD interrogation will occur at baseline and at 3 and 6 months.

6.2.5 Echocardiographic Testing (Echo Core Lab Procedure Manual)

The methods used for performing 2D Transthoracic Echocardiograms (TTE) are described in detail in the Core Lab for Echocardiography evaluations. Standard 2D transthoracic (TTEs) will be obtained by experienced personnel using a parasternal view with both long and short axis studies. Doppler evaluations will also be obtained. Apical, subcostal, and suprasternal views will also be obtained. We anticipate from previous studies done to evaluate reproducibility of LV end systolic and end diastolic dimensions and LVEF, a range from 3% to 9% from different Investigators (36).

To determine the potential effects of the product on myocardial contractility and integrity and to detect pericardial effusion, evaluation of global and regional left ventricular function and imaging of the pericardium with contrast echocardiography will be obtained. This ECHO testing will be performed at baseline; on the day after procedure (prior to discharge), 1 week, and at 3 and 6 months after study product administration (with baseline and 6 month Echoes sent to the Echo Core Lab). A routine transthoracic ECHO will be completed immediately after product

delivery while in the coronary angiography recovery area to determine the presence of a pericardial effusion. An additional routine ECHO will also be done on the day after the procedure (prior to discharge).

6.2.6 Treadmill Testing (MVO₂ Core Lab Procedure Manual)

Treadmill testing with MVO₂ assessment will be performed at baseline and at 6 months after the administration of the cells and in the control patients. Methods are described in the MVO₂ Core Lab Procedure Manual.

6.2.7 SPECT Imaging (SPECT Imaging Core Lab Procedure Manual)

Baseline single-photon emission computed tomography (SPECT) imaging with adenosine infusion over 4 minutes (or if contraindicated, regadenoson administered as a bolus) will be performed at rest and following pharmacologic stress using standard clinical protocols. In order to enhance the detection of viability on resting images, sublingual nitroglycerin (NTG) will be administered 15 minutes prior to injecting technitium for the resting image. Should technitium be unavailable due to shortages of the isotope, a protocol using thallium may be used. Details for the use of either isotope are included in the SPECT Core Lab Procedure Manual. The SPECT procedure will be repeated at 6 months using the same isotope used to collect the baseline SPECT (i.e. technitium or thallium). Images will be displayed as raw data, multiplanar tomographic slices, and 3 dimensional sets for both rest and stress. Quantitative data analysis will be performed using standard clinical software. The studies will be interpreted at the Core Lab by an experienced nuclear medicine physician who is blinded to the patient's specific clinical situation and methods of treatment. The SPECT Imaging Core Lab Procedure Manual provides details of the SPECT imaging protocol. Repeated studies of SPECT imaging in patients with CAD in a 3 to 9 day period in 20 patients by Garcia, King, and their colleagues at Emory, showed that the studies were reproducible in 15 patients in whom the ECG/exercise tests were reproducible (94%). Interobserver agreement was 95% (37).

6.2.8 Invasive Testing

Invasive follow-up testing will not be conducted.

6.2.9 Cardiac MRI (MRI Core Lab Procedure Manual)

Cardiac MRI will be repeated in those patients without a contraindication to MRI at 6 months. Methods to be used are described in the MRI Core Lab Procedure Manual.

6.2.10 Adverse Event Monitoring

Patients will be monitored for major adverse cardiac events (death, nonfatal myocardial infarction, need for coronary artery revascularization, and hospitalization for angina and/or congestive heart failure). This is discussed in detail in section 7.

6.2.11 Quality of life

The patients' quality of life will be assessed with the 36-Item Short-Form Health Survey Questionnaire (SF36) and the Minnesota Living With Heart Failure Questionnaire at 3, 6, and 12 months following the procedure.

6.2.12 Pregnancy Testing

Pregnancy testing will be performed with women of childbearing potential at each of the following clinic visits; Week 4, Month 3, Month 6, and Month 12.

6.2.13 Electrocardiograms (ECG)

A 12 Lead ECG will be performed at baseline, Days 1 and 2, Weeks 1 and 4, and Months 3, 6 and 12.

6.2.14 Six -Minute Walk

Patient will perform a 6 minute walk at baseline and at 6 months.

6.2.15 Chest X-Ray (CXR)

Patient will have a CXR performed at baseline and at 6 months.

6.2.16 Biospecimens

Creation of a CCTRN Biorepository for patient blood, bone marrow, and progenitor cell samples.

Recently, a loss in the number of circulating endothelial progenitor cells (EPCs) and a defect in their ability to migrate were shown in patients at increased risk of coronary artery disease, including acute MI. However, these observations are in contrast to a recent study that showed an increased number of EPCs in circulation following AMI. This disparity reflects how little is known about circulating progenitor cells and their impact on cardiovascular disease.

The goal of this biorepository is three-fold: 1) to provide **storage of critical biomaterials** derived from patients enrolled in clinical protocols within the Cardiovascular Cell Therapy Research Network 2) to provide **long-term integrity** (up to 10 years) of these specimens and samples, and 3) to provide progenitor cell

profiles and cytokine analyses of samples obtained during the clinical protocols undertaken by the CCTRN with an aim toward gaining insight into diagnostics of disease progression and prognostics of successful intervention. A central CCTRN Biorepository will be established at the Center for Cardiovascular Repair at the University of Minnesota and maintained by Dr. Doris Taylor and her associates. Specifically, Dr. Taylor's group will store these cells in cryovials, up to 10 years, in the University of Minnesota Masonic Cancer Center Liquid Nitrogen Storage Facility. In addition, the CCTRN biorepository will carry out a collection of prospectively described analyses as discussed in Appendix 3.

These stem cells will be used for research purposes only (not for profit), will be stored without personal identifying information, and will be shared with approved researchers who will conduct studies to improve the understanding of the effects of cell therapies. Cell samples will be destroyed after 10 years.

6.3 Schedule of Tests and Procedures

Table I. Schedule of Tests and Procedures

	Base line	Day 1	Day 2	wk 1	wk4	mo 3	mo 6	mo 12	Mo 24, 36, 48, 60
Compl. Med. hx.	X								
F/U Med hx				X	X	X	X	X	
Physical Exam/Neuro	X	X	X	X	X	X	X	X	
Informed consent	X								
ECG	X	X	X	X	X	X	X	X	
Infectious Disease Labs	X								
Laboratory evaluations	X		X ¹	X ¹	X ¹	X ¹	X ¹	X ²	
Biorepository specimens		X	X		X	X	X		
Pregnancy Testing	X				X	X	X	X	
Cardiac markers	X	X ³	X ³	X	X	X	X		
SPECT Imaging	X						X		
TMT with MVO ₂	X						X		
Bone Marrow Aspiration		X							
Angiography		X							
EMM		X							

Echocardiogram ⁶	X	X		X		X	X		
Clinic visit	X			X	X	X	X	X	
Adverse event eval.	X	X	X	X	X	X	X	X	
Study Product Injection		X							
24 Hr. Holter Monitoring	X	X ⁴		X	X	X	X		
In-Hospital telemetry		X	X						
Six Minute Walk test	X						X		
ICD Interrogation	X					X	X		
Qual. of life assmts. ⁵	X					X	X	X	
Phone call F/U									X
Chest X-Ray	X						X		
Cardiac MRI	X						X		

1. CBC with platelet count, BUN, CR, LFT's, CRP, BNP
2. BNP only
3. Collected at 8,16 and 24 hrs
4. Applied after the procedure
5. Quality of Life Questionnaires assessed with the 36-Item Short-Form Health Survey Questionnaire (SF36) and the Minnesota Living with Heart Failure Questionnaire.
6. Routine transthoracic ECHO will be completed immediately after product delivery while in the coronary angiography recovery area and the day after procedure (prior to discharge) to rule out pericardial effusion.

6.4 Follow-up windows

The timeline for follow up will initiate with the day of injection (day 1). The time windows for each of the subsequent follow up visits will be as follows:

1. The 1-week visit will be 7 \pm 2 days (from day of injection).
2. The 4-week visit will be at 30 \pm 5 days.
3. The 3-month visit will be at 90 \pm 7 days.
4. The 6-month visit will be at 180 \pm 30 days.
5. The 12 month visit will be at 360 \pm 30 days.
6. Yearly phone call will be annually \pm 30 days (from date of injection).

7.0 EVENT REPORTING

7.1 Types of Events

7.1.1 Adverse Events (AEs)

An adverse event is any untoward medical occurrence in a clinical investigation subject which has been consented, administered a product or medical device. The event need not necessarily have a causal relationship with the treatment or usage.

Examples of adverse events include but are not limited to: abnormal test findings, clinically significant symptoms and signs, changes in physical examination findings, and hypersensitivity. Additionally, they may include the signs or symptoms resulting from drug misuse and drug interactions.

7.1.2 Serious Adverse Events (SAEs)

A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that (1) Results in death; (2) is life-threatening (immediate risk of death); (3) requires inpatient hospitalization or prolongation of existing hospitalization; (4) results in persistent or significant disability/incapacity; or (5) results in congenital anomaly/birth defect.

Examples of serious adverse events include but are not limited to: acute coronary syndrome, pulmonary embolus, and serious ventricular arrhythmias.

7.2 Role of Abnormal Test Findings and Hospitalizations in Classifying an Event

7.2.1 Abnormal Test Findings

If a test result is associated with accompanying symptoms, and/or the test result requires additional diagnostic testing or medical/surgical intervention, and/or the test result is considered to be an adverse event by the investigator or DCC it should be reported as an adverse event.

NOTE: Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an adverse event. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

7.2.2 Hospitalizations

Adverse events reported from studies associated with hospitalization or prolongations of hospitalization are considered serious. Admission also includes trans-

fer within the hospital to an acute/intensive care unit (e.g., from the cardiac wing to the medical floor for an infection, or from the medical division to the neurologic unit for a stroke).

Hospitalization does not include rehabilitation facilities, hospice facilities, respite care (i.e., caregiver relief), skilled nursing facilities or homes, routine emergency room admissions, same day surgeries (as outpatient/same day/ambulatory procedures)

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event is not in itself a serious adverse event.

7.3 Reporting Responsibilities of the Investigator

For all events (adverse events and serious adverse events), monitoring and reporting to the DCC begins at the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, i.e., prior to undergoing any study related procedure and/or receiving investigational product, through and including 30 calendar days after the subject completes the study. Adverse events (serious and non-serious) should be recorded on the eCRFs (AE form and SAE form). **Do not delay the initial reporting of a serious adverse event in order to obtain resolution or follow-up information.**

For all adverse events, the investigator must pursue and obtain adequate information both to determine the severity and causality of the event. For adverse events with a causal relationship to the investigational product, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and the DCC concurs with that assessment.

In the rare event that the investigator does not become aware of the occurrence of a serious adverse event immediately (i.e., if an outpatient study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the adverse event.

7.3.1 Severity Assessment

The investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the adverse event. For purposes of consistency, these intensity grades are defined as follows:

MILD	Does not interfere with subject's usual function.
MODERATE	Interferes to some extent with subject's usual function.
SEVERE	Interferes significantly with subject's usual function.

Note: A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with subject's usual function) but would not be classified as serious unless it met one of the criteria for serious adverse events, listed above.

7.3.2 Causality Assessment

If the investigator does not know whether or not investigational product caused the event, then the event will be handled as "possibly related to investigational product" for reporting purposes.

The investigator will use the adjectives below in the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an adverse event.	
PROBABLE	AEs that are considered, with a high degree of certainty, to be related to the study product.
POSSIBLE	AEs in which the connection with the study product administration appears unlikely but cannot be ruled out with certainty.
UNLIKELY	AEs that are likely produced by the patient's clinical state, environment, toxic factors or other modes of therapy administered to the patient.
UNRELATED	AEs that are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.)

7.3.3 Expectedness Assessment

EXPECTED	Any AE or SAE for which the nature or severity is consistent with information in the Investigator Brochure
UNEXPECTED	Any AE or SAE for which the nature or severity is not consistent with information in the Investigator Brochure

7.4 Reporting Responsibilities of the Sponsor (DCC)

7.4.1 Sponsor Reporting Requirements to the Executive Committee, NHLBI and DSMB

The DCC-PI will notify the Executive Committee, NHLBI and DSMB of the occurrence of any death or unexpected and associated SAE within 72 hours of the DCC receiving notification of the event. This will be followed by a written report no later than seven days after the DCC's initial notification of the event's occurrence. For all other SAEs, the DCC-PI will notify the Executive Committee, NHLBI, and DSMB no later than 15 days of the DCC receiving notification of the event. This will be followed by a written report no later than 30 days after the DCC's initial notification of the event's occurrence. The timing and contents of these reports are governed by the CCTRN *Guidelines for Reporting to Data Safety and Monitoring Board (DSMB)*.

7.4.2 Sponsor Reporting Requirements to FDA

Once the DCC has been notified of a SAE the following are the DCC's reporting requirements to the FDA:

- Fatal or life-threatening, unexpected SAE's and associated with the study drug must be reported to the FDA within 7 calendar days
- Other SAE's that are non-fatal or life-threatening, but are unexpected and associated with the study drug use must reported to the FDA with 15 calendar days

These 7-day and 15-day reports can be satisfied by completion of the FDA Form 3500A (MedWatch Form), as well as any source documents as they relate to the event.

7.5 Unanticipated Problems (UPs)

An UP is an incident, experience, or outcome that specifically causes increased risk to the study or to its participants which may be of medical or non-medical etiology, and meets the following criteria:

- Unexpected (in terms of nature, severity, or frequency), given (a) the research procedures that are described in the protocol-related docu-

ments, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- Definitely, probably or possibly related to participation in the research (i.e., there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures or materials involved in the research); and
- Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

All UP reporting will follow the same guidelines as noted above for SAE reporting, and must include a corrective action plan/measures to prevent recurrence.

7.6 Guidelines for Holding Product in the Event of a Catheterization Facility Event

The events listed below will follow the same reporting criteria for SAE's as it relates to the investigational sites as well as the DCC:

- 1) Hypotensive episode
- 2) Hemodynamically significant arrhythmia requiring antiarrhythmic therapy
- 3) Hemodynamically unstable
- 4) Fever (Temperature increase to $\geq 100.4^{\circ}\text{F}$)
- 5) Excessive bleeding from bone marrow harvest site
- 6) Cardiac perforation

7.7 Holding Rules

The expected number of SAEs in this small trial is expected to be too small to support formal statistical stopping rules. However, the following criteria are offered for guidelines under which this clinical trial may be put on hold pending a detailed investigation by the DSMB. The assessment of the AE shall include the relationship to the trial procedures as not related or possibly related. If possibly related, an indication of causality to a specific aspect of the study procedures will be determined: (e.g. bone marrow harvesting-related, vascular access related, cell delivery/catheter related, or other procedure related).

7.8 Holding Criteria

The study will be placed on hold if any of the following events occur during the course of the study.

1. Tumor growth in the heart at the site of injection in patients.

2. One (1) patient with arrhythmic sudden cardiac death within 30 days of treatment.
3. One (1) death with unexplained pathological evidence of severe inflammatory changes or infection at site of treatment
4. One (1) episode of clinically significant (i.e. requiring surgical intervention) myocardial perforation as a result of the injection procedure.
5. Two (2) patients with stroke within 24 hours after the injection procedure.

Once the findings have taken place, the centers will inform the DCC, and the DCC will inform the NIH and its DSMB as well as the FDA.

The above criteria are offered for consideration by the DSMB. Ultimately, it is this Board that will establish holding criteria. In addition, the DSMB will monitor the distribution of all SAE's, and the network will be responsive to all DSMB concerns concerning SAE's that are not part of 1-5 above.

In addition, the DSMB may also recommend stopping the trial for the performance-related issues that would prevent the study from meeting its scientific objectives, such as:

- Failure to recruit and enroll patients
- Inability to meet protocol requirements for delivery of cells at specified times
- Inability to prepare cells meeting study quality specifications

7.9 Guidance for NOGA Catheter Usage

If any of the following symptoms occur either during LV mapping with the NOGA XP System or during endocardial injections with the MyoStar Injection Catheter, they could indicate a serious clinical deterioration. If any of the following events / symptoms occur, the procedure should be temporarily halted and the patient should be reevaluated for suitability to continue with the treatment under investigation: product administration should be discontinued.

- persistent complaints of chest pain;
- complains of cardiac pain associated with injections;
- persistent hypotension;
- complaints of shortness of breath;
- ICD shocks to stop ventricular tachycardia (VT);
- DC cardioversion or defibrillation for VT;
- there is any question as to the location of the catheter tip in relation to vasculature or the LV; and
- any unanticipated change in level of consciousness or neurological status.

The procedure will be terminated in the event of:

- sustained hypotension not responsive to fluid administration;
- clinical signs and symptoms indicating acute coronary syndrome;
- clinical signs and symptoms indicating a cerebrovascular accident;
- cardiac tamponade is strongly suspected or confirmed;
- hemopericardium requiring pericardiocentesis;
- two episodes of sustained ventricular tachycardia requiring cardioversion or administration of an antiarrhythmic;
- the patient experiences one episode of ventricular fibrillation (VF);
- identification of thrombus in the LV or the aorta that was not previously present on echo or left ventriculogram;
- an aortic dissection is suspected or confirmed;
- cardiac perforation;
- excessive bleeding from the bone marrow harvest site;
- fever of 99.4 degrees or higher;
- hemodynamically unstable.

7.10 Monitoring of Liver Function Tests (AST/ALT):

Subjects with an AST and/or ALT elevation $> 2.0 \times$ ULN are permitted to continue in the study but are required to have a serum liver function test panel drawn at the earliest possible date to reconfirm the elevated value and to be monitored approximately every 2 weeks thereafter until elevated liver enzyme value(s) resolved or returned to Baseline values, whichever occurred sooner.

8.0 ENDPOINT EVALUATION AND CLASSIFICATION

See the MVO₂, SPECT Imaging, and MRI Core Lab Procedure Manuals.

9.0 STATISTICAL PROCEDURES

9.1 Randomization

Once informed consent has been obtained, eligible patients will be entered into the study randomly assigned to one of the selected treatment strategies in an interactive Web-based randomization session where exclusion and eligibility criteria will be assessed. Patients will be randomized to the therapy type (active or control therapy), using variable block sizes of six or nine, randomly selected. Pa-

tients will be stratified by center. When a patient is randomized, the clinic will be given an identification (ID) number and acrostic, specific information on the assigned treatment regimen, and a list of procedures to be completed at the baseline visit. A participant-specific schedule of visits and procedures will be displayed for printing locally. The DCC will monitor patient recruitment by providing reports to the Core Laboratories and Project Office (PO) as appropriate during the recruitment phase. Updated reports will be maintained on an Internet site accessible to all units of the study. The recruitment reports will provide data on recruitment of women and minorities (African-Americans, Hispanics, and Asians). Goals for recruitment will be set and will be reviewed by the DCC and PO.

9.2 Statistical Analyses

Biostatisticians at the DCC, with the assistance of scientific programmers, have adapted or developed a number of statistical programs for analyzing study data. Data are analyzed for both data monitoring purposes, as described above, and for the purpose of detecting beneficial or adverse treatment effects. The DCC uses standard statistical packages such as SAS, S-PLUS, R and STATA to perform statistical analyses.

9.3 Baseline Analyses

Although the stratified random assignment of participants to the various treatments should ensure comparability with respect to known and unknown variables, imbalance may occur by chance. Descriptive statistics for baseline characteristics known or suspected to be associated with outcomes will be prepared for the various treatment groups. The variables considered in such a description can be categorized as follows: (1) demographic characteristics; (2) medical history; (3) physical examination; (4) laboratory data; and (5) quality of life and psychosocial data. Chi-square statistics and Student *t*-testing will be used to evaluate the differences between the treatment arms for both trials with reference to baseline characteristics between categorical and continuous variables, respectively. For categorical variables, exact tests will be performed when Chi-square approximation is in doubt.

9.4 Outcome Analyses

Despite the efforts of CCTRN Investigators to ensure that patients return to their center for follow-up evaluation, we anticipate that a small number of subjects will be unable to return for their follow-up endpoint assessment.

Anticipating this difficulty, the sample size for this study has been increased by a small percent, allowing the Investigators to capture complete data on a number

of patients as close to the pre-specified sample size as possible. However, for those patients who are missing the final six month endpoint data, we will carry out a Last Observation Carried Forward (LOCF) analysis. For a patient who is missing the follow up information, the value of the follow-up measure will be assumed to be equal to their baseline value. Thus the difference in the endpoint measure over the six month follow-up will be zero. While a large number of missing data points, corrected in this manner can produce a bias toward the null, this standard LOCF procedure will be adequate for the small number of patients with missing data.

Prospectively described details of the analyses of primary and secondary endpoints follow.

9.4.1 Primary endpoints

There are three proposed co-primary endpoints of LV function:

- a. Change in maximal oxygen consumption (MVO_2);
- b. Change in left ventricular end systolic volume (LVESV) as assessed by echocardiography with contrast;
- c. Change in reversible defect size as assessed by SPECT.

Each of these will be assessed individually using general linear model procedures. Unpaired t -tests will assess the effect of therapy on the change from baseline to six months on each of these three co-primary endpoints. General linear mixed modeling techniques will assess the effect of treatment on the continuous secondary outcomes of the study. A dichotomous variable will be created that assigns the value of zero to every patient in the control group and one to every patient in the cell delivery group. Both unadjusted and adjusted treatment effects will be computed; adjustments will be for baseline covariates whose association with the dependent variable is generally accepted. If we let y_{ijk} be the measurement or relative measurement of the outcome for treatment i of patient j at time k , then y_{ijk} can be represented as a general linear mixed model:

$$y_{ijk} = \mu + \alpha_i + \tau_k + (\alpha\tau)_{ik} + d_{ij} + e_{ijk}$$

where $\mu, \alpha_i, \tau_k, (\alpha\tau)_{ik}$ are fixed effects due to overall treatment, follow-up time, and interaction between treatment and follow-up time, respectively, and d_{ij} is the random effect due to patient j in treatment i group and e_{ijk} is the error term. Although we do not anticipate there will be interaction between treatment and follow-up time, we will perform the analysis. More generally, the above model can be written in the following matrix form

$$Y = X\beta + Zu + e$$

where X is the design matrix and $\mu, \alpha_i, \tau_k, (\alpha\tau)_{ik}$ are elements of β , Z is the design matrix for the random part, d_{ij} are elements of u . We can assume u to be a multivariate normally-distributed random variable with a mean vector of zero and variance-covariance matrix G , where e is the error vector with elements of e_{ijk} . Various forms of correlation structure for e will be investigated in statistical modeling.

Should it be necessary, transformations of the measurements will be considered (e.g., natural log, square root, and reciprocal transforms).

In this phase II study, there will be no corrections for multiplicity in these evaluations. P -values less than 0.05 will be reported as statistically significant.

9.4.2 Analyses of Secondary Endpoints

The secondary endpoints of the study will compare the changes in the following measures from baseline⁺ to six month follow-up.

1. Reduction in fixed perfusion defect(s) by SPECT.
 - a. Change in total defect size by SPECT
 - b. Change in fixed defect size by SPECT
 - c. Change in Sum Difference Score by SPECT
2. Progression to fixed defect by SPECT.
3. Regional wall motion by MRI (in eligible patients).
4. Regional blood flow improvement by MRI (in eligible patients).
5. Regional wall motion by echocardiography.
6. Clinical improvements at 6 months, including change in anginal score by the following measures:
 - a. Canadian Cardiovascular Society Functional Classification of Angina Pectoris (CCS) (Appendix 1).
 - b. NYHA class (Appendix 2).
 - c. Decrease in antianginal medication (nitrates needed weekly).
 - d. Exercise time and level.
 - e. Serum BNP levels in patients with CHF.
 - f. LV diastolic dimension by contrast ECHO.
 - g. Evaluation of the relationship between the degree of reversible ischemia at baseline and the effect of therapy on each of the primary endpoints and secondary endpoints 1-3.
7. MACE:
 - a. New MI.
 - b. Rehospitalization for PCI in coronary artery territories that were treated.
 - c. Death.
 - d. Rehospitalization for Acute Coronary Syndrome (ACS) and for CHF.

⁺ Baseline defined as time period from day consent signed to day of treatment (not to exceed 60 days).

Those secondary endpoints that are continuous will be analyzed first using an unpaired *t*-test to compare the change over time in the control group to the change over time in the active group. They will then be analyzed using a general linear model procedure as described above to determine whether (1) treatment affected the change in the variable over time, and (2) covariates such as age, gender, or diabetes mellitus influenced the relationship between treatment and the change in the variable over six months.

Dichotomous secondary endpoints (e.g., clinical improvement at six months, change in anginal score by CCS, NYHA Class, decrease in anti-anginal medication (nitrates needed weekly), exercise time and level), will be analyzed using a Chi-square test and Fisher's exact test.

The time-to-event endpoints (i.e., re-hospitalization secondary endpoints) will be evaluated using Kaplan-Meier survival curves. If the number of events permits, a Cox proportional hazard analysis will be carried out as well. Dichotomous endpoints will be assessed using logistic regression. Outcomes based on numbers of events such as numbers of re-hospitalizations will be analyzed using Poisson regression. *P*-values less than 0.05 will carry the label statistically significant.

9.4.3 Subgroup evaluations

Analyses will include an examination of the effects of cell administration on prespecified subgroups:

- 1) Age
- 2) Gender
- 3) Patients with diabetes
- 4) Race
- 5) Serum BNP levels in patients with CHF
- 6) Pre-existing comorbidity (e.g. MACE)
- 7) Baseline LVEF
- 8) Functional characteristics of the cells that are used, including colony forming capability and motility in individual patients compared to subsequent influence of the cells on myocardial perfusion and contractile performance in individual patients.

If a treatment effect is demonstrated, it is not likely to behave identically among all important subgroups. Therefore, the overall observed treatment effect would be the average of the effects in each of the component subgroups weighted by their distributions in the sample. We will use the general linear model to determine if the relationship between cell delivery and the endpoint is influenced by subgroup strata. For dichotomous endpoints, contingency table analysis and logistic regression analysis will be implemented to assess the influence of sub-

group strata on the relationship between cell delivery and the dependent variable. The general linear model will be used to assess if the effect of cell therapy on the pre-specified endpoints is modulated by the type of perfusion defect the patient had at baseline.

9.5 Multiple Comparisons

Multiple comparison procedures can be useful protective devices, guarding against type I error inflation. Such tools are a staple of Phase III confirmatory studies. The use of this tool in Phase II “proof-of-concept-studies” is problematic. At this level of investigation, tight control of the overall family wise type I error rate would increase the likelihood that the Investigators would attribute a potentially important treatment effect to the play of chance. Nevertheless, Investigators must be cognizant that chance effects occur commonly in Phase II trials. The Investigators have tried to strike a balance between the need to control the number of evaluations, on the one hand, and the need to identify new effects, on the other, by limiting the number of primary endpoints.

10.0 TRIAL MANAGEMENT

10.1 Database

The DCC will maintain the CCTRN study database in a web-accessible electronic format. Detailed documentation of study variables will be prepared and available to study Investigators, and where necessary, to external scientists. Appropriate confidentiality and security of these files will be maintained at all times.

10.1.1 Framework

The DCC has developed a web-based online application for data entry using the state-of-the-art, Microsoft .NET framework. A secure environment, requiring user login and authentication, will be maintained for the entry of and/or access to patient data. The data collected from Clinical Centers will be stored on a secure database in the Coordinating Center computer facility. Training will be provided by the DCC and DCC staff will be available to answer questions and resolve issues. Extensive data verification and validation will be implemented on the web application to check for data accuracy, completeness, and consistency within patients.

10.2 Security

Several levels of security will be implemented to protect the confidentiality of the data. All authorized users will be provided a unique name/password and will be given access as identified by the Principal Investigator. Passwords will expire every ninety days and users will be required to change them. The server on which the data is stored will be behind a firewall and will be in the most secure zone (100) with no direct access to the internet. In addition, data will be protected through the use of Secure Socket Layers, (SSL), the current standard for encrypting data between a client and a server as it is passed across the Internet. In addition to these layers of security, every connection to a secured site will be recorded with data indicating which person connected, the time of the connection, and the area accessed. The user's password will be stored in binary, hashed format within the database for additional security. Access to secure areas of the website will be logged with the users ID and the date and time of access. This audit table will be maintained throughout the life of the studies. The servers that host the Network database are enrolled in the automated virus and operating system patch management system to protect against any virus attacks. The database will be backed up nightly, and rotational sets of these backup tapes will be stored at an off-site University archival storage facility that is secure and has restricted access.

10.3 Follow-up

The DCC will provide online web based forms for the follow-up data collection. All the standards and security guidelines that were set for baseline forms will be implemented for these forms as well. Data will be stored on a secure database and access will be limited and secure. Training and documentation will be provided by DCC staff to all the Clinical Centers (CCs) on the data entry process. DCC staff will also be available to answer questions and help resolve issues as necessary. Reports for follow-up data will also be made available.

10.4 Laboratory Data Processing Support

The DCC will develop and maintain online web forms for the laboratories for data collection, both for baseline and annual follow-up. The data will be validated with extensive edit rules and the CCs/Lab will be able to correct errors real time. Access will be limited and will require secure login authentication. The DCC will provide training and documentation to laboratory personnel on the data entry process and will be available to answer question and resolve issues as necessary. The data collected will be stored on a secure database in the DCC and will be backed up every night. The web application will be available 24/7. Reports will be generated as necessary with real time data.

10.4.1 File transfers

Provisions will be made for those core labs that prefer to transfer files in a batch mode. Files with data from the laboratory will be transferred to a secure server residing in the computer facility of the DCC. Users transferring this data will be provided with user identification numbers and passwords for restricted and secure access. Data transmitted will then be processed and checked for validity and completeness. Only data that passes these edits will be stored in the database. The rejected records will be sent back to the centers/lab for correction and re-transmittal.

10.5 Data Quality

The case report forms used for data entry are created by the DCC project and programming staff in conjunction with the research personnel at each clinical site. Once developed, individual forms are unit tested by the programming team and released to a test server. The forms are then tested by both DCC and clinical site personnel for accuracy and utility. Continuity and acceptance testing will be done by the clinical site research and laboratory personnel. An iterative process of suggestions/corrections/retesting will occur until the application is accepted. Personnel accessing the application for data submission will receive training on the web based system prior to the randomization of patients. There will be defined a minimum data set that constitutes completeness. All data will have to pass through range and logical checks in addition to intra- and inter-form checks for consistency. The sequence of events will be enforced by allowing subordinate forms to become accessible only after its primary form has been submitted. If a response to a question on a form requires ancillary forms to be completed, the user will receive reminder messages within the application to complete the proper form. Weekly reports on the CC's data entry and completeness will be generated. If a CC has problems, action will be taken from retraining through phone calls to a site visit if necessary.

10.6 Computing Infrastructure

The School of Public Health network consists of a fiber optic backbone using gigabit technology to provide the fastest and most state-of-the-art network communications possible. A backbone of Cisco switches provides for client access to backend resources and servers at 100 megabits per second. Aside from providing simple network access, Information Technology staff has real-time monitoring capabilities to diagnose and correct potential network problems. The campus has also implemented a four tier network firewall to protect all workstations and servers with varying degrees of security, based on the device's security level within the organization.

10.7 Backup Procedure

The study data will be backed up on a nightly basis and a set of these backup tapes will be stored offsite.

10.8 Site Visits

Each clinic will be site visited by members of the DCC, NHLBI, laboratory quality assurance personnel and member(s) of the Steering Committee. Routine monitoring visits occur one or more times during the period after the Initiation Visit but before the Study Closeout Visit. Guidelines for scheduling monitoring visits shall be determined according to the stage of development, complexity of the study, the rate of subject accrual and other factors.

These visits are conducted for routine monitoring only and are intended to ensure that the protocol and applicable regulatory requirements are being followed, that patients' rights and safety are protected, and to confirm data integrity and quality. The routine monitoring visits will occur in accordance with the following guidelines:

21 CFR 312.53	Selecting Investigators and Monitors
21 CFR 312.56	Review of Ongoing Investigations
ICH E6, 4.1	Investigator's Qualifications and Agreement
ICH E6, 5.5	Trial Management, Data Handling and Record Keeping
ICH E6, 5.18	Monitoring
FDA	Guidelines for the Monitoring of Clinical Investigations (January 1988)

11.0 HUMAN PATIENTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject and this fact will be documented in the subject's record.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All computer entry and networking

programs will be done using SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NHLBI, the OHRP, the sponsor, or the sponsor's designee. The confidentiality of the data will be maintained within legal limits, as required by law. This protocol conforms to the OSHA/HHS/HIPAA guidelines for HIV/HFV occupational safety.

11.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB, the NHLBI, the sponsor, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research patients are protected.

11.4 Informed Consent

11.4.1 Human Patients Involvement and Characteristics

Participants in this research trial will be recruited from the inpatient and outpatient cardiology services and their patient reference networks of the five clinical trial centers of the CCTR. The inclusion criteria have been described in the Research Design and Methods section. The age of the participants must be greater than 18 years of age. There is no upper age limit. The patients must be clinically stable, have CAD, and have LVEF \leq 45%. There is no exclusion of any subpopulation with regard to race or gender.

11.4.2 Sources of Material

See section 5.1.1.

11.4.3 Risks Associated with the Patient Population, Procurement, Processing, and Injection of the Study Product

Bone Marrow Aspiration

Possible risks of bone marrow aspiration include: bruising, bleeding, infection, hematoma (a swelling filled with blood) at site of aspiration, brief discomfort in the hip area and in rare instances death. There is also a possibility that the subject's heart failure may worsen for a short period of time.

Cell Processing

Processing the cells is done under strict sterile conditions; however, there is a rare chance that the cells could get contaminated while being harvested or processed. Testing will be done on the cells; however, it takes about 2 weeks to get the results. If the tests show the cells were contaminated, the subject will be

contacted and instructed about whether or not he/she should be treated with antibiotics. The subject will keep a daily temperature log to help determine the development of an infection before the test results are known. If the subject notes a fever, he/she will be requested to notify the investigator/study team.

NOGA Mapping

The possible risks of NOGA Mapping include, but are not limited to, damage to blood vessels, bleeding, infection, inflammation of the sac surrounding the heart, damage to kidneys, a small risk of heart attack, stroke, damage to the heart valves, perforation (a small hole) in the heart causing blood to accumulate around the heart, irregular heart rhythms (including ventricular tachycardia and ventricular fibrillation), dislodgement of material into other arteries leading to possible blockage, radiation exposure and a very small risk of death.

Cardiac Catheterization/Coronary Angiography (CAG):

Potential risks associated with this procedure are oozing of blood around where the catheter (small hollow tube) goes into the skin, collection of blood (hematoma) under the skin, allergic reaction to the dye that is injected when the doctor looks at the heart vessels (angiography) either during or following the study product injection procedure, or formation of a blood clot (a blockage) at the catheter insertion site. A blood clot could stop the flow of blood or hurt the blood vessel. If blood flow is stopped or slowed a lot, the body parts that rely on that blood could also be damaged which could lead to loss of function or surgical removal of the body part, or could worsen the subject's heart condition and symptoms. Other problems that could happen because of this test include local nerve damage (loss of feeling), infection, changes in how the heart beats, stroke, and heart attack. Some temporary problems that might happen are temporary movements (spasm) of a muscle, vein, or artery; pulling apart of blood vessel walls (separation of the layers of the walls of a blood vessel); or sudden blockage (closure) of a blood vessel; a very rare complication could result in death or a need for an urgent coronary artery bypass graft (open heart surgery). Serious complications including death happen in less than 1 in every 1,000 tests that are performed.

The risks of the use of the iodine that is in the contrast media for the heart angiography procedure are rare. Some problems that might occur are hypersensitivity or even severe allergic reactions, or decreased kidney function, particularly if the patient has underlying kidney problems.

Study Product Injection

The catheter used to inject the study product is investigational. Some problems that might happen include (but others could occur) are: irregular heartbeats, damage to the heart muscle, perforation of the heart causing blood to accumulate around the heart, bleeding, heart attack, stroke, dislodgement of material into

other arteries (possibly causing blockage), need for emergency surgery and death. It is possible that a small amount of cells will enter the bloodstream of the heart rather than the heart muscle. If the injection catheter penetrates through the heart (from inside to outside) and cells appear in the fluid filled area surrounding the heart which cushions the heart as it moves (pericardial space) there is a possibility of potentially harmful effects which could cause an inflammatory response. Injection directly into the heart muscle also may cause inflammation or irritability.

There may be some circumstances where the research team is unable to give the subject the study product (cells or placebo); such as change in the coronary anatomy, equipment failure, or poor quality of the stem cells. If any such event occurs *before* the subject has been randomized into the study, he/she will not be able to continue in the study. The option of cell donation will be discussed with the subject by the research team. If events such as this occur and the subject has *already* been randomized into the study, the research team will request the subject continue with follow up in the study and the harvested stem cells would be stored in the biorepository (with appropriate consent form on file).

In the rare event that 100 million cells cannot be obtained, we will give the cells available (≥ 30 million cells) to patients randomized to the active group. This is based on the study carried out by Drs. Willerson and Perin in Brazil (1) in which 30 million cells were given to people from the same population as FOCUS. Many of these patients experienced improved blood flow and heart function after this 30 million cell infusion. We do not want to deny these patients the potential benefit of this therapy, nor would we want them to undergo a bone marrow procedure for no useful purpose. To the extent possible, the FOCUS Investigators will carry out a dose-response analysis, in which we will evaluate the relationship between cell dose delivered and effect size on each of our three primary endpoints.

Radiation Risks

This research study involves exposure to radiation from nuclear medicine, chest x-ray, and cardiac catheterization laboratory x-ray procedures. The expected total amount of radiation exposure to the subject in this study is approximately 5 rem.

Risks in those with Coronary Artery Disease

Coronary artery disease is a progressive disease; therefore whether or not a subject participates in this study, he/she may have worsening of their condition. This may include continued or worsening chest pain, development of new blockages, worsening heart failure and/or the possible need for further treatment, heart at-

tack, stroke and death. These treatments may include medication, additional procedures to place stents or pacing devices, or bypass surgery.

11.4.4 Adequacy of Protection Against Risks

The following steps have been taken to minimize the risk to patients in this study.

11.4.4.1 Recruitment and Informed Consent

All patients will be informed of the risks of therapy in the informed consent form.

11.4.4.2 Protection Against Risk

The potential risks of this study and the subsequent interventions by the patient's health care professionals are described in detail in the Research Design and Methods section of this application. Risks of breach of confidentiality will be reduced by keeping all records of the patient in a secured location in the hospital or research offices and access will be limited to their direct health care providers or research staff. All personnel involved in this study have undergone appropriate training in the protection of human participants regarding security measures and confidentiality in research trials.

11.4.5 Potential Benefits of the Proposed Research to the Patients and Others

The administration of autologous bone marrow-derived stem cells offers a new therapeutic option to patients with left ventricular dysfunction whose goal is to not just reduce the rate of LV deterioration but to actually ameliorate congestive heart failure.

11.4.6 Importance of the Knowledge to be Gained

The knowledge to be gained from this clinical trial is significant in that this will demonstrate whether there significant response to 100 million bone marrow-derived mononuclear cells and intramyocardial stem cell delivery and measures of myocardial function. The trial has been designed to address critical limitations in the previous published trials by including patients with moderate to severe left-ventricular dysfunction, a group of patients who are most likely to benefit from this form of therapy. The risks to the patients are reasonable in relation to the knowledge gained from this study since this therapy may potentially reduce the incidence of congestive heart failure which is a leading cause of morbidity and mortality throughout the world.

11.4.7 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan has been outlined in Section 7 above. This research protocol will be approved by the IRB's of all participating centers. This IND builds on a previous FDA approved IND (BB #11044) held by Dr. Emerson Perin at the Texas Heart Institute for treating heart failure patients with bone marrow derived adult stem cells. Monitoring of the trial will be performed by the DCC per ICH, FDA regulations and 21 CFR 312.

11.4.8 Risk-Benefit Analysis

Patients with profound left ventricular dysfunction and heart failure are at risk for significant morbidity and mortality. This study has the potential to improve cardiac function by preserving or recovering functional myocardial tissue. Having highly trained experts deliver and oversee the therapy, in conjunction with close study monitoring substantially reduces the likelihood of adverse events. In addition, demonstrating that the regional delivery of cells is associated with global improvement in function has the potential to change the current practice of delivering therapy, producing improved cell viability and engraftment. The risk/benefit ratio is considered acceptable by the Investigators, with a history of over 500 EMM procedures done with few complications.

The potential risks to the patients remain reasonably low in relation to the anticipated benefit of improving cardiac function in patients with heart failure whose present state with maximal medical therapy places them at very considerable risk for near-term future adverse events.

11.5 Recruitment Principals and Strategies

Each of the five CCTRN centers is committed to recruiting patients for this protocol, accessing a large number of patients from a variety of community resources.

The Greater Cleveland Area has four proposed network sites and will recruit from a population of 5,047 patients with heart failure. The Minnesota center will recruit from a population of 6,601 patients with CHF, 2,240 with a hospital admission for CHF. Vanderbilt can recruit from a population of 7876 outpatients with CHF including 4,590 patients with a known discharge diagnosis of CHF. The Texas Heart Institute will enroll from the entire Texas Medical Center Hospitals (annual patient visits of 5.5 million) through referrals from physicians to Drs. Willerson and Perin. In addition, THI has a track record of recruiting patients from across the United States. The University of Florida will draw from 5,565 patients with CHF.

The study will be open to men and women of all race/ethnicities. At THI, the expected population of patients will be approximately 12% Hispanic, 10% African-American, 72% Non-Hispanic White, 1% Asian, and 5% of other ethnic backgrounds, reflecting the ethnic diversity of the patient population seen in the THI Heart Failure Clinic. Every effort will be made to recruit males and females of all races and background with appropriate inclusion criteria. Cleveland Clinic will recruit approximately 62% male, 75% Caucasian, 20% African-American. Vanderbilt will recruit approximately 50% female, 15% Hispanic or Latino; Approximately 15% will be African-American. The DSMB will monitor recruitment of minorities and females at each of the study centers, and if this falls below the expected levels at any center, will interact with the CCTRN executive leadership committee and with that center's leaders to exert every effort to further enhance recruitment of women and minorities at that center.

11.6 Monitoring

11.6.1 Pre-Investigation Visits

The monitor assures the Investigator clearly understands and accepts the obligations incurred in undertaking a clinical investigation:

Prior to the initiation of a clinical investigation, the monitor should visit the site of the clinical investigation to assure that the Investigator:

1. Understands the investigational status of the test article and the requirements for this accountability.
2. Understands the nature of the protocol or investigational plan.
3. Understands the requirements for an adequate and well-controlled study.
4. Understands and accepts his or her obligations to obtain informed consent in accordance with 21 CFR Part 50. The monitor should review a specimen of each consent document to be used by the Investigator to assure that reasonably foreseeable risks are adequately explained.
5. Understands and accepts his or her obligation to obtain IRB review and approval of a clinical investigation before the investigation may be initiated and to ensure continuing review of the study by the IRB in accordance with 21 CFR Part 56, and to keep the sponsor informed of such IRB approval and subsequent IRB actions concerning the study.
6. Has access to an adequate number of suitable patients to conduct the investigation.
7. Has adequate facilities for cell preparation and conducting the clinical investigation.
8. Has sufficient time from other obligations to carry out the responsibilities to which the Investigator is committed by applicable regulations.
9. Understands periodic monitoring visits will occur.

11.6.2 Scheduled monitor visits

The monitor should visit the Investigator at the site of the investigation frequently enough to assure that:

1. The facilities used by the Investigator continue to be acceptable for purposes of the study.
2. The study protocol or investigational plan is being followed.
3. Changes to the protocol have been approved by the IRB and/or reported to the sponsor and the IRB.
4. Accurate, complete, and current records are being maintained.
5. Accurate, complete, and timely reports are being made to the sponsor and IRB.
6. The Investigator is carrying out the agreed-upon activities and has not delegated them to other previously unspecified staff.
7. Review of Subject Records will take place.

11.6.3 Monitor Role Continued

The monitor should compare a representative number of subject records and other supporting documents with the Investigator's reports to determine that:

1. The information recorded in the Investigator's report is complete, accurate, and legible.
2. There are no omissions in the reports of specific data elements such as the administration to any subject of concomitant test articles or the development of an intercurrent illness.
3. Missing visits or examinations are noted in the reports.
4. Patients failing to complete the study and the reason for each failure are noted in the reports.
5. Informed consent has been documented in accordance with 21 CFR Parts 50 and 56.

11.6.4 Monitor Recording

The monitor should maintain a record of the findings, conclusions, and action taken to correct deficiencies for each on-site visit to an Investigator. Such a record may enable the FDA to determine that a sponsor's obligations in monitoring the progress of a clinical investigation are being fulfilled. The record may include such elements as:

1. The date of the visit;
2. The name of the individual who conducted the visit;
3. The name and address of the Investigator visited;
4. A statement of the findings, conclusions and any actions taken to correct any deficiencies noted during the visit.

11.7 Investigator responsibilities

11.7.1 Investigator Performance

Prior to enrolling the first subject, each Investigator must read and understand the protocol and sign the Investigator Protocol Signature Page. Additional requirement that must be met are:

1. Signed Confidentiality Agreement
2. Signed Protocol Signature Page
3. Current medical license
4. Financial disclosure
5. CV, signed and dated, for all primary Investigators and sub-Investigators
6. Completed NOGA training
7. Local stem cell processing lab certified
8. Completed site training
9. Follow all Good Clinical Practice requirements for clinical research

11.7.2 Site Requirements:

Prior to enrollment of the first patients, the Investigator and institution will be asked to provide the following documents:

1. Executed clinical study agreement
2. IRB approved informed consent form
3. IRB approved final protocol
4. Current laboratory certification
5. Current laboratory normals

11.7.3 Institutional Review Board Approval

Prior to enrolling the first subject, the Investigator must obtain written approval from the IRB. The approval must contain the date the study was approved, the version of the informed consent that was approved and the signature of the IRB chairperson. The primary investigator will follow all Good Clinical Practice requirements.

11.7.4 Informed Consent

The DCC must review and approve all informed consent forms prior to submitting to the IRB. All study patients must provide written informed consent using an IRB-approved informed consent document.

11.7.5 Reporting Requirement of the Sites

See section 7.2.2.

11.8 Sponsor Responsibilities

11.8.1 Introduction

The DCC will act as the study Sponsor, and thus have overall responsibility for the conduct of the study, including assurance that the study follows all standards and regulatory requirement of the U.S. Food and Drug Administration. The DCC will adhere to Sponsor general duties as outlined by 21 CFR Subpart D; Part 312.50-312.70.

11.8.2 Routine Duties

The DCC is responsible for obtaining and reviewing copies of IRB approvals. They are responsible for setting up all training for each site for NOGA training and checking off the certification of their local laboratories for processing of stem cells. The DCC will ensure that the study is conducted according to Good Clinical Practice (GCP), the Declaration of Helsinki, the Study Protocol, and any other applicable regulatory agency requirement. The DCC will also ensure proper clinical site monitoring.

11.8.3 Site Training

The DCC will be responsible for the setting up all training required in the protocol and will establish a schedule for site initiation visits.

Training for the Sepax system will be twofold. Equipment training will be provided by the manufacturer (Biosafe) both centrally for the installation qualification, as well as the operational qualification and upon delivery of equipment to the cell processing labs the performance qualification at the five sites. The UT MD Anderson Stem Cell Laboratory (Blood & Marrow Transplantation Department, Cord Blood Bank) will provide central procedural training using the Sepax system. Baylor College of Medicine Center for Cell and Gene Therapy will provide quality control oversight (including site visits), technical expertise, and consultation.

Training to use the NOGA XP® System for Investigators at centers where there is no current experience will travel to the Texas Heart Institute in Houston, Texas for hands on training with the NOGA System. When their proficiency has been shown and certified by Dr. Emerson Perin at the Texas Heart Institute, they will be allowed to proceed to use the investigational NOGA injection catheter in this study protocol at their own institution.

The Texas Heart Institute® has been appointed as the National training site for certification of physicians performing NOGA® mapping in the United States. A training manual and video will be supplied to all trainees. The training will consist of up to four phases which include: a) initial didactic and practical animal lab experience; b) advanced mapping techniques; and c) a follow-up program for continued assessment of mapping quality by Investigators.

11.8.4 Site Monitoring

The DCC will be responsible for monitoring each site throughout the course of the study by following the FDA Guidelines for monitoring of a clinical trial (revised 1998). Source document review will be performed against entries on the CRF and a quality assurance check will be performed to ensure that the Investigator is complying with the protocol and regulations. At the time of the completions of the study, a close out monitoring visit will take place to ensure all trial materials and subject data are properly documented.

11.8.5 Reporting to the FDA

The DCC will hold the IND for this study and submit proper filings to obtain and maintain the IND. The DCC will submit all appropriate reports and fillings to the FDA as required by regulations. This includes unanticipated adverse events, any item listed on the “Stopping Rules”, withdrawal of IRB approval, withdrawal of FDA approval, annual progress reports to the FDA and all final reports. The DCC will maintain all records according to requirements set form by current Good Clinical Practice Guidelines (cGCP).

All Core Laboratories and clinical sites will maintain study records for at least 3 years after the study is terminated.

12.0 FUTURE STUDIES

Texas Heart Institute, a Clinical Center of CCTRN, has two ongoing pilot stem cell clinical trials presently; one study using Aldehyde Dehydrogenase-Bright Stem Cells (ALDH^{br}), and the other study using autologous mesenchymal cells obtained from adipose tissue. It is our intent to identify the best of these two cell types in humans, a cell that promotes angiogenesis and improves cardiac regional ventricular function.

We anticipate that near the end of this protocol, and working with the CCTRN and NIH, we will add another arm to this study utilizing different cell types. We will use intramyocardial injection with the NOGA MyoStar catheter for comparison in exactly the same protocol we are using with the BMMNC studies. We plan

to compare at least one novel cell type with the optimal numbers of the BMMNC for treatment of patients with ischemic cardiomyopathy later in these studies. We recognize this will require additional protocol review and consent by the appropriate advisory committees.

13.0 DISSEMINATION

The overall usefulness of scientific research depends not only on the importance of the findings, but also on its eventual reach and effect on population health. Therefore, research projects must integrate ways to promote the eventual diffusion of the results into their research plans. We will work with professional associations to access health care providers like the NHLBI has done for a number of initiatives including asthma and hypertension. We will use three general dissemination methods that will be tailored for the target audiences.

13.1 Web Site

The web site will be created from the beginning of the project with objectives targeted to the three audiences. The CCTRN website will serve as one method of distribution of information about stem cell research in cardiovascular disease in general and about the specific study protocols. For the general lay public, the goal is to promote a hospitable context for the research by informing the public about the kinds of research being done, including the source of the stem cells; what this research is and what it is not; plans for studies; study findings; and the potential for new treatments. Physicians need information about the research that is closely tied to clinical trial opportunities and potential treatments for patients. This information should be tied to the normal places practitioners seek such resources. For the researcher audience, the web site will provide more in-depth technical information and published works.

13.2 E-network

To develop a dissemination network or linkage system for the beginning of the research, the CCCT will recruit participation in two networks. These interactive networks will build support for distribution of information as it becomes available. The first is the public-serving network. These participants would be liaisons from voluntary health associations such as the American Heart Association. This type of organization has a mission of public information and can serve as an effective link to public media sources. The second network will comprise liaisons from professional health care provider associations. These organizations will be identified by the NHLBI and project committees based on the model of successful programs at the NHLBI such as Asthma Education and Prevention Program. The organizations will recruit liaisons who will receive periodic updates about ongoing

studies and results and who will be available to provide feedback about the implications of study findings for practitioners and the barriers to patient participation in protocols. As studies are initiated and as results become available, the CCTRN will work with the clinical sites and the NHLBI press office to coordinate the release of this information.

13.3 Manuscripts and Presentations

A primary task of the DCC will be to provide data analyses for all manuscript proposals and presentations approved by the SC. The CCTRN Investigators will take the lead in presenting study data at major scientific meetings and in the writing, preparation, and submission of manuscripts to appropriate peer-reviewed journals. In addition, the Network Investigators will actively enlist the participation of junior Investigators in manuscript writing and presentations at scientific meetings. The DCC will also make data sets available to the CCs, Cell Processing and other Cores, will provide consultation and assistance to the CCs regarding the appropriate data analysis methods, and will perform independent data analysis in order to verify the Investigators' findings.

The DCC will play an active role in preparing study publications in collaboration with other study Investigators and the NHLBI Project Office. The DCC will prepare all manuscripts for submission to the journals and will serve as the liaison between the lead author, and the journal. A Publications and Ancillary Studies Committee will organize and monitor writing committees and provide oversight on what presentations and publication have priority within the study. The DCC will maintain and distribute a progress report on the status of all active papers, as well as a study bibliography including abstracts, presentations, letters, editorials, etc.

13.4 Methodologic Developments

In addition to providing statistical support to PIs at CCs and NHLBI, Investigators at DCC will take leading role in developing possible new statistical methods that may have the potential to improve statistical analysis for projects in CCTRN and beyond. These new discoveries will be presented to scientific meetings and in statistical journals as peer-reviewed articles.

14.0 REFERENCES

1. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, Rossi MI, Carvalho AC, Dutra HS, Dohmann HJ, and others. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;107(18):2294-302.
2. Hughes S. Cardiac stem cells. *J Pathol* 2002;197(4):468-78.
3. Krause DS. Plasticity of marrow-derived stem cells. *Gene Ther* 2002;9(11):754-8.
4. Choi D, Oh HJ, Chang UJ, Koo SK, Jiang JX, Hwang SY, Lee JD, Yeoh GC, Shin HS, Lee JS, and others. In vivo differentiation of mouse embryonic stem cells into hepatocytes. *Cell Transplant* 2002;11(4):359-68.
5. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275(5302):964-7.
6. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001;107(11):1395-402.
7. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7(4):430-6.
8. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, and others. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410(6829):701-5.
9. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;279(5356):1528-30.
10. Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, Jia ZQ. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999;100(19 Suppl):II247-56.

11. Tomita S, Mickle DA, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y, Liu P, Li RK. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J Thorac Cardiovasc Surg* 2002;123(6):1132-40.
12. Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, Weissman NJ, Leon MB, Epstein SE, Kornowski R. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol* 2001;37(6):1726-32.
13. Hamano K, Li TS, Kobayashi T, Hirata K, Yano M, Kohno M, Matsuzaki M. Therapeutic angiogenesis induced by local autologous bone marrow cell implantation. *Ann Thorac Surg* 2002;73(4):1210-5.
14. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, and others. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001;104(9):1046-52.
15. Gunsilius E, Duba HC, Petzer AL, Kahler CM, Grunewald K, Stockhammer G, Gabl C, Dirnhofer S, Clausen J, Gastl G. Evidence from a leukaemia model for maintenance of vascular endothelium by bone-marrow-derived endothelial cells. *Lancet* 2000;355(9216):1688-91.
16. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;109(3):337-46.
17. Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34⁺-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 2003;108(17):2070-3.
18. Zhang S, Shpall E, Willerson JT, Yeh ET. Fusion of human hematopoietic progenitor cells and murine cardiomyocytes is mediated by alpha 4 beta 1 integrin/vascular cell adhesion molecule-1 interaction. *Circ Res* 2007;100(5):693-702.
19. Zhang S, Wang D, Estrov Z, Raj S, Willerson JT, Yeh ET. Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes in vivo. *Circulation* 2004;110(25):3803-7.

20. Losordo DW, Schatz RA, White CJ, Udelson JE, Veereshwarayya V, Durgin M, Poh KK, Weinstein R, Kearney M, Chaudhry M, and others. Intramyocardial transplantation of autologous CD34⁺ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation* 2007;115(25):3165-72.
21. Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S. Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. *Eur Heart J* 2007;28(6):766-72.
22. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106(15):1913-8.
23. Assmus B, Fischer-Rasokat U, Honold J, Seeger FH, Fichtlscherer S, Tonn T, Seifried E, Schachinger V, Dimmeler S, Zeiher AM. Transcatheter transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure: results of the TOPCARE-CHD Registry. *Circ Res* 2007;100(8):1234-41.
24. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, and others. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 2002;106(24):3009-17.
25. Erbs S, Linke A, Schachinger V, Assmus B, Thiele H, Diederich KW, Hoffmann C, Dimmeler S, Tonn T, Hambrecht R and others. Restoration of Microvascular Function in the Infarct-Related Artery by Intracoronary Transplantation of Bone Marrow Progenitor Cells in Patients With Acute Myocardial Infarction. The Doppler Substudy of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) Trial. *Circulation* 2007.
26. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, and others. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006;355(12):1210-21.
27. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D and others. Intracoro-

- nary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004;364(9429):141-8.
28. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, and others. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. *Circulation* 2006;113(10):1287-94.
 29. Vale PR, Lorsodo DW, Tkebuchava T, Chen D, Milliken CE, Isner JM. Catheter-based myocardial gene transfer utilizing nonflourosopic electromechanical left ventricular mapping. *J Am Coll Cardiol* 1999;34 (1):246-54.
 30. Perin EC, Willerson JT, and others. Randomized trial of bone marrow-derived mononuclear cells in the treatment of patients with chronic CAD, LV dysfunction, and HF. Manuscript in preparation 2007.
 31. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, and others. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002;360(9331):427-35.
 32. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber Cam Freund M, Steinhoff G. Autologous bone marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003;362(9351):45-6.
 33. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003;361(9351):47-9.
 34. Perin EC, Silva GV, Sarmiento-Leite R, Sousa AL, Howell M, Muthupillai R, Lambert B, Vaughn WK, Flamm SD. Assessing myocardial viability and infarct transmuralty with left ventricular electromechanical mapping in patients with stable coronary artery disease: validation by delayed-enhancement magnetic resonance imaging. *Circulation* 2002;106(8):957-61.
 35. Vale PR, Losordo DW, Milliken CE, McDonald MC, Gravelin LM, Curry CM, Esakof DD, Maysky M, Symes JF, Isner JM. Randomized, single-

- blind, placebo-controlled pilot study of catheter-based myocardial gene transfer for therapeutic angiogenesis using left ventricular electromechanical mapping in patients with chronic myocardial ischemia. *Circulation* 2001;103(17):2138-43.
36. Otterstad JE, Froeland G, St.John Sutton M, Holme I. Accuracy and reproducibility of biplane two-dimensional echocardiographic measurements of left ventricular dimensions and function. *Eur Soc Cardiology* 1997; 18:507-513.
 37. Alazraki NP, Krawczynska EG, Ziffer JA, Vansant JP, Rettigre RI, Taylor A, King III SB, Garcia EV. Reproducibility of Thallium-201 Exercise SPECT Studies. *J Nucl Med* 1994; 35 (8):1237-1244.
 38. Mahmarian JJ, Shaw LJ, Filipchuk NG, Dakik HA, Iskander SS, Ruddy TD, Henzlova MJ, Keng F, Allam A, Moyé LA, Pratt CM. An initial strategy of intensive medical therapy is comparable to that of coronary revascularization for suppression of scintigraphic ischemia in high-risk but stable survivors of acute myocardial infarction. *J Amer Cardiol Soc*;48:2458-67.
 39. Rosner B., *Fundamentals of Biostatistics*, 6th Edition, Boston, Brookes Cole 2006, p 416.
 40. Casagrande JT, Pike MC, Smith PG. *Applied Statistics*; 27(2):176-180.
 41. Center for Cardiovascular Biology and Atherosclerosis, University of Texas Health Science Center at Houston; Heart Failure Research Lab, Texas Heart Institute
 42. Departments, of Cardiology, Blood and Marrow Transplantation, and Bioimmunotherapy, The University of Texas-M.D. Andersen Cancer Center, Houston, The University of Texas Houston Health Science Center, and the Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas
 43. Drs. Geng and Willerson; UT99-117 study
 44. Perin and Willerson et al

Appendix 1

Canadian Cardiovascular Society (CCS) Functional Classification of Angina Pectoris

<u>Class</u>	<u>Definition</u>	<u>Specific Activity Scale</u>
I	Ordinary physical activity, (e.g., walking and climbing stairs) does not cause angina; angina occurs with strenuous, rapid, or prolonged exertion at work or recreation. Ability to ski, play basketball, light jog (5 mph), or shovel snow without angina	
II	Slight limitation of ordinary activity; angina occurs on walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold, in wind, or under emotional stress; or only during the few hours after awakening; when walking > 2 blocks on level ground; or when climbing more than 1 flight of stairs at a normal pace and in normal conditions. Ability to garden, rake, roller skate, walk at 4 mph on level ground, and have sexual intercourse without stopping	
III	Marked limitation of ordinary physical activity; angina occurs on walking 1 to 2 blocks on level ground or climbing 1 flight of stairs at a normal pace in normal conditions. Ability to shower or dress without stopping, walk 2.5 mph, bowl, make a bed, and play golf	
IV	Inability to perform any physical activity without discomfort; anginal symptoms may be present at rest. Inability to perform activities requiring 2 or fewer metabolic equivalents (METs) without discomfort	

Adapted from Goldman L, Hashimoto B, Cook EF, Loscalzo A. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: advantages of a new specific activity scale. *Circulation*. 1981;64:1227-1234.

Appendix 2

New York Heart Association (NYHA) Classification

<u>Class</u>	<u>Patient Symptoms</u>
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

Appendix 3 – Cell Processing

Sepax isolation of BM-MNCs

INTERVENTION

The intervention is the intracoronary delivery of BMMNCs.

Administration

On the morning of the study product administration, patients will undergo bone marrow aspiration by a trained physician with substantial experience in carrying out bone marrow harvesting procedures. The details of the aspiration procedure are located in Appendix 4. Once harvested, the cells will be transported to the institution's cell therapy lab. Each site will utilize the investigational Sepax System for BMMNC isolation. This closed system allows for faster isolation and potentially increased patient safety. Furthermore, the use of this system will allow standardization across the Network to ensure a more uniform cellular product.

BMMNC Characteristics

BMMNCs containing a subpopulation of stem cells are isolated by the Sepax System. The cells are harvested and washed three times in Human Serum Albumin (HSA)/saline buffer before re-suspension in 5% HSA/saline. The composition of CD34⁺ and CD133⁺ cells is determined by fluorescent activated cell sorting (FACS) analysis. Viability of the cells will be determined by Trypan Blue exclusion; $\geq 70\%$ viability will be required before transplantation. A 14-day sterility culture, CFU Assay and Endotoxin analysis will be performed on the final product. Because 14-day sterility testing and CFU assay will not be available prior to the product's injection, a negative Gram stain will be required before the product is released. Product will be labeled and tracked with adhesive labels containing the patient's name and hospital identification number.

From our initial patient experience, 100 million TNC can be routinely harvested with this volume of bone marrow aspirate that contains a small fraction ($< 4\%$) of CD34⁺, CD45⁺ and CD133⁺ cells. The cellular product or placebo will be injected a minimum of four hours after bone marrow aspiration in each patient (total volume = 3 ml). We have chosen to use unfractionated BMMNC since the specific cell type(s) responsible for the previous observed biologic effect in the infarct zone has not been identified. The specific population of cells administered in this study will be monitored as a research tool to help address this question. Those patients randomized to placebo will receive injections of 5% HSA/Saline at each of the 15 injection sites in volumes identical to those used for the cell injections.

Injection Procedure

All cell and placebo injections will be done with the NOGA catheter. The details of its use are described in Section 5.1.1 of this protocol.

Harvest, Isolation and Testing of BMMNCs

General

Autologous BMMNC's will be manufactured at the individual CCTRN sites using the *Sepax System (Biosafe Geneva, Switzerland)*.

Procurement

Details of the aspiration procedure are located in Appendix 4. Upon completion of the bone marrow aspiration, the marrow will be transported to the Clinical Cell Therapy Laboratory. Marrow will be transported in a validated shipping container (room temperature) by a designated medical courier immediately to the Clinical Cell Therapy Laboratory at each CCTRN facility.

Infectious Disease Testing & Prevention of Cross-Contamination

Although cells are autologous in this protocol, the standard tests for infectious diseases will be performed. Testing will include assays for the detection of HIV and HCV (by nucleic acid testing), anti-HIV I/II, anti-HTLV I/II, anti-HBc Ab, HBsAg, anti-HCV, and Treponema pallidum (by serology). Additional testing deemed necessary by regulations and/or institutional policy will be performed. If a test is positive, the patient will be notified of the result, and the need for further testing will be determined through consultation with the patient's physician. Cells that test positive for infectious disease markers will be labeled appropriately as infectious and quarantined while in the Clinical Cell Therapy Laboratory Facilities. Standard (universal) precautions are practiced, and cells are maintained in closed-systems throughout processing. Standard operating procedures for the prevention of cross-contamination are established.

Cell Processing

Each Network laboratory will use their Standard Operating Procedures for accession, processing, transportation and issuing. Briefly, when the bone marrow arrives in the laboratory, samples will be removed for Quality Control (cell counts and viability at a minimum).

The laboratory will then perform a density gradient enrichment of the mononuclear cell fraction (MNC) using the Sepax instrument (BioSafe, SA, Geneva Switzerland). The Ficoll based separation protocol for the Sepax is an automated MNC isolation from blood products in a closed system using a density gradient technique followed by washing to remove Ficoll and concentrate the cells. The BioSafe instrument has FDA 510(k) clearance for Cord Blood Processing. Briefly, the single use disposable set is placed under the Biological Safety Cabinet and 100ml of cGMP grade Ficoll (GE Healthcare, New York) is added to the ap-

propriate bag. The bone marrow cells are attached to the input line and the disposable is loaded onto the Biosafe instrument per Manufacturer's recommendations. The instrument will then automatically load first the Ficoll and then the bone marrow cells into the chamber. After a set time, the MNC enriched cells are automatically collected into a temporary storage bag and the red cells/granulocytes and Ficoll are directed to the waste container.

The MNC's are then added back to the chamber and the cells are washed in Human Serum Albumin (HSA)/Saline buffer. After washing of the cells, the instrument signals to the operator that the procedure is complete. Quality control analysis will be performed (Cell Count, Viability, Flow Cytometry Analysis, Endotoxin testing, CFU Sterility, and gram stain at a minimum). Once the laboratory has determined that the cells have met the release criteria, they will be issued to the physician per standard procedures. For patients that have been randomized to the placebo arm, the MNCs will be frozen according to the Clinical Laboratory Standard Operating Procedures.

Immediately after processing, the BMMNC's will be transported to the cardiac catheterization laboratory at room temperature where they will be administered to the patient. It is estimated that the total out-of-body time will be no more than 12 hours.

Release Criteria

As noted the final product will be suspended in 5% HSA/saline. Analysis of Viability, Gram Stain, Endotoxin testing, and verification of TNC Dose will be performed. See Table A 3.1 for Final Product Release Criteria.

Post Release Analysis

Colony forming units (CFU), 14 day sterility, and analysis by flow cytometry (enumeration of CD34⁺ cells and CD133⁺ cells) will serve as an in vitro surrogate potency assay, much like CD34⁺ cell enumeration for early (short-term) hematopoietic engraftment in the setting of hematopoietic stem cell transplant. Neither in vitro assay will serve as lot release. In vivo assessment of cardiac function (e.g., measurement of ejection fraction) also provides an evaluation of potency and is described in the clinical study protocol.

Cell Dose

Target dose will be approximately 100×10^6 total nucleated cells (TNCs) in 3 mL of 5% HSA/saline solution. Cell number (i.e., TNC count) will be determined using a hematology analyzer. Should there be less than 30 million cells, the subject will not be randomized to therapy condition and no cells will be provided. This dose is based upon previously reported clinical trials of the safe intracoronary delivery of BMMNC in patients with an AMI and our animal studies.

Biorepository core

All cells that exceed the administered dose of 100 million aliquots of BMMNC's will be provided to the CCTRN biorepository core. With appropriate patient consent, these samples will be used to analyze the phenotypic characteristics of therapeutic BMMNC's. The data will be correlated with clinical results to investigate possible correlations between cell therapy outcomes and cell characteristics.

Final Product Release Criteria Testing

Final product (lot) release criteria testing results (Table A 3.1) will be available prior to the BMMNCs being transported to the hospital for administration.

Table A4.1 Product Release Specifications		
Assay	Test Method	Specification
Rapid Sterility	Gram Stain	No organisms
Viability	Trypan Blue	≥70%
Endotoxin	EndoSafe PTS	< 5Eu/kg
Cell Dose	Hematology counter	≥30 x 10 ⁶ ≤ 100 x 10 ⁶ cells

Additional, final product testing that will not be completed prior to release includes Immunophenotyping by flow cytometry testing, CFU, and sterility testing, as outlined in the following table (Table A 3.2).

Table A 3.2 Post Production Monitoring		
Assay	Test Method	Specification
Immunophenotyping	Flow Cytometry	Report
CFU	Per Site SOP	Growth
Sterility	14 day culture	No Growth

In the event that sterility testing becomes positive, the Clinical Microbiology Laboratory will immediately report the result to the Clinical Cell Therapy Laboratory staff who will immediately notify the Medical Director and Facility Quality Assurance person. The Medical Director will contact the Principal Investigator (PI) and patient physician within 48 hours, for appropriate clinical action. Sterility tests will be done on both the cells and the placebo and in reporting to the Medical Director and Facility Quality Assurance person, every effort will be made to protect the blinding of those involved in the study and the patient.

Appendix 4- CCTRN Bone Marrow Aspiration Standard Operating Procedure

The following standard operating procedure (SOP) is for carrying out bone marrow aspirations for patients recruited in the Cardiovascular Cell Therapy Research Network (CCTRN) protocols.

CCTRN patients will undergo one and only one bone marrow aspiration to harvest cells for a protocol.

Purpose:

Bone marrow aspiration is a scheduled procedure performed by a trained Physician (e.g., hematologist, pathologist, or hematopathologist). Only physicians with substantial experience in carrying out bone marrow harvesting procedures (more than forty previous successful procedures) will perform the procedure. Other medical personnel trained in bone marrow aspiration procedures (e.g. registered nurses, nurse practitioners, and medical technologists) will assist in the collection to ensure proper sample collection, preparation and processing of the specimen. The bone marrow aspiration is indicated for research regarding stem cell therapy for cardiovascular conditions.

Scope:

This SOP refers to bone marrow collections at the five stem cell therapy centers and their associated satellite facilities involved in the CCTRN. The five centers are as follows:

1. Texas Heart Institute Stem Cell Center
2. Minneapolis Heart Institute Foundation
3. University of Florida Department of Medicine
4. Cleveland Clinic Lerner College of Medicine
5. Vanderbilt University Medical Center

PROCEDURE

Supplies and transportation:

1. Bone marrow aspiration supplies will comply with the site-specific institutional procedures and practices.
2. All equipment, supplies, and reagents used in the process of bone marrow collection must be sterile with a lot number and date of expiration noted and able to be recorded on site-specific institutional data forms.
3. Study personnel will notify the site-specific cell processing lab at the following time points: 1) when a patient is enrolled and randomized, 2) when a patient's bone marrow aspiration has been scheduled, 3) when the bone marrow aspiration has begun.
4. Bone marrow aspiration specimen transportation to the cell processing laboratory will be treated as a STAT procedure.

Patient preparation and specimen collection performed by Physician:

1. Verify patient identification with the patient.
2. Explain the risks and benefits of bone marrow aspiration. Give patients an opportunity to ask questions and be able to verbalize understanding.

3. A separate consent form specific for the bone marrow aspiration procedure is signed by patients to document the informed consent process and to permit the physician to perform the aspiration.
4. Medication of patients for the bone marrow aspiration will be left to the discretion of the performing or overseeing Physicians with the exception of general anesthesia which will not be covered by the study.
5. Patients on aspirin and Plavix (clopidogrel) at the time of consent should remain on aspirin and Plavix (clopidogrel) for the bone marrow aspiration procedure. Continuance or discontinuance of other medications at the time of bone marrow aspiration, (e.g. Coumadin) are left to the discretion of the Study Physician.
6. All collection procedures must be performed with universal precautions and sterile aseptic technique.

Bone marrow aspiration procedures:

1. The media container and/or heparin vials must be opened with sterile technique and media prepared with the appropriate amount of anticoagulant. The final concentration of heparin will be 10-25 units of heparin/ml of bone marrow.
2. After the administration of medication (sedatives and/or analgesics) and prior to collection, the donor will be evaluated while in the prone position to be safely positioned without pressure compromise on arms, brachial plexus, breasts, genitalia, knees, vascular structures or other body parts.
3. The donor shall be prepped and draped in the usual manner using alcohol, Beta-dine and sterile draping.
4. Prior to insertion of collecting needles, the landmarks and sites of aspiration shall be reviewed and confirmed by both the Physician and Assistant.
5. A total of 80-90 mls (± 10 ml) of bone marrow product will be obtained. So that the samples are comparable across the five centers, physicians will aspirate no more than 5 ml of product per needle puncture into the marrow space. Approximately 5 mls of marrow is aspirated with each aspirate. Although there are multiple needle punctures in the bone marrow spaces, there are generally 1-2 skin punctures on the iliac crest.
6. An incision is made in the iliac crest and a needle is advanced through the periosteum and into the marrow space. A minimum of one skin puncture and 16 needle punctures into the marrow space are required to aspirate 80-90 ml (± 10 ml) of bone marrow. The number of skin punctures or needle punctures must not be so frequent as to require general anesthesia.
7. Physicians will perform the aspiration on one side. The only time aspiration will take place in the contralateral site is if the initial site produces a dry tap.
8. In the event that no marrow is aspirable, then pressure will be applied to the injection site until hemostasis is achieved. A dressing will then be applied.
9. Patients will be on anticoagulant medications, thus pressure will be applied to the injection site until hemostasis is achieved. A sterile dressing will be applied. A pressure dressing will be applied if persistent venous oozing is present.
10. All bone marrow collections will be sent to the site's cell processing laboratory using site-specific institutional transportation procedures. Bone marrow aspiration transportation to the cell processing laboratory will be treated as a STAT procedure and arrive at the cell processing lab as soon as possible following the bone marrow aspiration procedure.

Reporting requirements:

1. Label the CCTRN Study Product Infusion form and all specimens with the patient acrostic, study ID, date and time of collection, and label the form with the amount aspirated.
2. Site-specific chain of custody forms must be used to document the chain of custody of the bone marrow aspirate from the site of the procedure to the cell processing laboratory to the study product injection site.

FOCUS Protocol Signature Page:

I have read this protocol and agree to conduct the study as described and in accordance with other material supplied to me. In addition, I agree to conduct the study in compliance with all applicable regulations and guidelines.

Investigator Name (print)

Investigator Name (signature)

Date

On behalf of the Data Coordinating Center (DCC) of the Cardiovascular Cell Therapy Research Network, I confirm that the DCC will comply with all obligations detailed in all applicable regulations and guidelines. In addition, I will ensure that the Investigator is informed of all relevant information that becomes available during the conduct of the study.

Safety Officer's Signature
CCTRN Data Coordinating Center

Date