CARDIOVASCULAR CELL THERAPY RESEARCH NETWORK

Protocol
Study Chair:

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

PRC/DSMB Approved Protocol 2: Late-TIME Protocol

A Phase II, Randomized, Controlled, Double-Blind Pilot Trial Evaluating the Safety and Effect of Administration of Bone Marrow Mononuclear Cells Two to Three Weeks Following Acute Myocardial Infarction

Supported by:

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

CCTRN Authorship Team

Jay H.Traverse, M.D.*	University of Minnesota
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.	University of Minnesota
Marc Penn, M.D.	Cleveland Clinic
Carl J. Pepine, M.D.	University of Florida
Emerson Perin, M.D., Ph.D.	Texas Heart Institute
Douglas Vaughan, M.D.	Vanderbilt University
James Willerson, M.D.	Texas Heart Institute
David Zhao, M.D.	Vanderbilt University
Robert Simari, M.D. (Study Chair)	Mayo Clinic College of Medicine
Dejian Lai, M.D.	Coordinating Center for Clinical Trials
Lemuel Moyé, M.D., Ph.D.	Coordinating Center for Clinical Trials

Supported by:

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

(Grant number U01HL087318-01)

*Principal author

Table of Contents

LIST OF ABBREVIATIONS

ACE – Angiotensin converting enzyme
AE – Adverse event
AEG – Ambulatory ECG
ALT – Alanine aminotransferase
AMI – Acute myocardial infarction
AST – Aspartate aminotransferase
Atm – Atmosphere
Bi-V – Bi-ventricular pacemaker
BM – Bone marrow
BMMNC – Bone Marrow Mononuclear Cell
BMS – Bare metal stent
BNP – B-type natriuretic peptide
BSA – Body surface area
CABG – Coronary artery bypass grafting
CBC – Complete blood count
cc – Cubic centimeter
CC – Clinical Center
CCTRN – Cardiovascular Cell Therapy Research Network
CD – Cluster differentiation
CFR – Code of Federal Regulations
CFU – Colony forming units
CK – Creatine kinase
CKMB – Creatine kinase-myocardial band
CPC – Circulating blood progenitor cells
CXCR – Chemokine receptor
CXR – Chest X-ray
cMRI – Cardiac magnetic resonance imaging
DCC – Data Coordinating Center
DES – Drug-eluting stent
DSMB – Data and Safety Monitoring Board
EC – Executive Committee
ECG – Electrocardiogram
EF – Ejection fraction
EPC – Endothelial progenitor cell
F – Female
F – Fahrenheit
FACS – Fluorescent activated cell sorting
FDA – Food and Drug Administration
FDG-PET – Fluoro-deoxyglucose Positron Emission Tomography
FFE – Fast field echo
FISP – Fast imaging with steady-state precession
Fr – French (size of catheter)
GHz – Gigahertz
GMP – Good manufacturing practice(s)
Hb - Hemaglobin

HBc – Hepatitis B core
HBs – Hepatitis B surface
HCV – Hepatitis C virus
HF – Heart failure
HGF – Hepatocyte Growth Factor
HIV – Human immunodeficiency virus
HSA – Human serum albumin
HTLV – Human T lymphotrophic virus
IABP – Intra-aortic balloon pump
ICD – Implantable cardiac defibrillator
ID – Identification
IGF – Insulin Growth Factor
IND – Investigational New Drug
INR—International Normalized Ratio
IRB – Institutional Review Board
IU – International unit
LAD – Left anterior descending coronary artery
LFT- Liver function test
LV – Left-Ventricle
LVEDV – Left-ventricular end-diastolic volume
LVESV – Left-ventricular end-systolic volume
LVEF – Left-ventricular ejection fraction
M – Male
MI – Myocardial infarction
ml – Milliliter
mm – Millimeter
MNC – Mononuclear cell fraction
MRI – Magnetic resonance imaging
ms – millisecond
MSC – Mesenchymal stem cell
MVO – Microvascular obstruction
MVO2 – Myocardial oxygen consumption
NC – Nucleated cells
NHLBI – National Heart, Lung and Blood Institute
NS – Normal saline
NYHA – New York Heart Association
NIDDM – Non-insulin dependent diabetes mellitus
OHRP – Office of Human Research Protection
PBS – Phospho buffered saline
PCI – Percutaneous coronary intervention
PI – Principal Investigator
PO – Project Office
PT—Prothrombin time
PTCA – Percutaneous transluminal coronary angioplasty
PTT—Partial thromboplastin time
RCT – Randomized clinical trial

SAE – Serious adverse event
SDF-1 – Stromal Derived Factor – 1
TB – Tuberculosis
TI – Time interval
TIA – Transient ischemic attack
TIMI – Thrombolysis in Myocardial Infarction
UP – Unexpected Problem
VEGF – Vascular Endothelial Growth Factor
VSD – Ventricular septal defect
VT – Ventricular tachycardia
WBC – White blood cell count

Executive Summary

Study Design: This is a Phase II, randomized, double-blind, placebo-controlled clinical trial that will assess the effect of delivery of bone marrow mononuclear cells (BMMNC) two to three (2-3) weeks post acute myocardial infarction (AMI) on global and regional left ventricular (LV) function determined by cardiac magnetic resonance imaging (cMRI).

Target population: 87 male and female subjects who have no contraindication to BMMNC delivery and who have: 1) moderate to large infarctions, 2) no prior history of coronary artery bypass graft (CABG) or myocardial infarction (MI) that resulted in left-ventricular (LV) dysfunction, and 3) initial ejection fraction (EF) following revascularization measured by echocardiography $\leq$45%.

Enrollment Period: All five centers of the Cardiovascular Cell Therapy Research Network (CCTRN) will enroll for two years.

Rationale: Following an AMI, a remodeling process is initiated that may ultimately lead to the development of congestive heart failure (HF), which is the leading admission diagnosis for hospitalization in the United States and carries a 50% five-year mortality rate. Experience with delayed cell delivery (two to three weeks post-AMI) is limited.

Therefore, Late-TIME should be considered a pilot study. The myocardial milieu several weeks post-AMI time may be quite different than in the acute setting and will provide an alternate time for cell delivery. This protocol will be the first study to deliver cells two to three weeks following AMI, a time frame bracketed by growing safety profiles of BMMNC delivery in both the acute and chronic phases following AMI. In addition, this delayed cell delivery time frame holds the potential for extending cell-based therapies to a broader population, specifically it permits patient transfer to tertiary sites for cell delivery and inclusion of a sicker cohort of patients with cardiogenic shock on presentation who would be too ill to enroll in the TIME trial (three days versus seven days post-MI).

Primary Response Variables: Two co-primary measures of interest

1. Global left venticular ejection fraction
2. Regional left ventricular ejection fraction

Secondary Response Variables

1. Combined outcome (first of) death, reinfarction, repeat revascularization, hospitalization for HF
2. LV mass
3. End diastolic volume
4. End systolic volume
5. Infarct size

Subgroup Analyses
Prespecified subgroup analyses in this pilot study will include an examination of the interaction between the effect of cell delivery and each of the following variables:

1. Age (<65 versus ≥65 years of age)
2. Gender
3. Race
4. Hypertension (history of hypertension)
5. Diabetes mellitus
6. Microvascular obstruction (MVO)
6. Statin use
7. Drug-eluting stent (DES) versus bare metal stent (BMS)
8. Stented vessel

Primary Hypothesis:
As compared with placebo, intracoronary administration of BMMNC two to three weeks following AMI will result in improved global and regional LV function.

Secondary Hypotheses:
As compared with placebo, the intracoronary administration of BMMNC will result in:

1. Smaller end-diastolic volume;
2. Smaller end-systolic volumes;
3. A lower incidence of death, reinfarction, repeat revascularization, or hospitalization for HF combined.

Relevance to the Goals of the CCTRN
This proposal is consistent with the rationale of the CCTRN, which is to investigate new cell delivery effects 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 reduce the time needed to complete the study. Use of Network core laboratories will standardize measures of interest. Finally, the regional distribution of the cell networks will broaden the dissemination of its results, thereby improving the general public health.

1.0 STUDY OBJECTIVES

1.1 Primary Objective

The primary objective of this Phase II, randomized, double-blinded, placebo-controlled pilot study is to determine if delayed (two to three (2-3) weeks) administration of intra-coronary autologous bone marrow mononuclear cells (BMMNC) versus placebo to patients following acute myocardial infarction (MI) can safely produce a measurable improvement in global and regional (border zone) left ventricular (LV) function as determined by cardiac magnetic resonance imaging (cMRI) at six (6) months compared with baseline.

Secondary responses of interest in both study groups will include change in each of LV mass, systolic and end-diastolic dimensions, and infarct size at six months. In addition, adverse clinical events including hospitalization for congestive heart failure (HF) will be assessed at two years.

1.2 Relevance to the CCTRN

This protocol is consistent with the scope of the 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 in BMMNC transplantation. Unlike the TIME study, the Late-TIME study reflects an earlier stage of clinical development since there are fewer studies which deliver cells in the sub-acute or chronic time frame following MI. In fact, it will be the first randomized trial to deliver cells two to three weeks following MI, a time frame bracketed by growing safety profiles of BMMNC delivery in both the acute and chronic phases following MI. Therefore, Late-TIME should be considered a pilot study. The myocardial milieu at this time will be quite different than in the acute setting and will provide an alternate time for cell delivery. An important reason for selecting this time frame is the potential to extend the study of cell-based therapies to a much larger population that allows for transfer to tertiary sites for cell delivery. Additionally, this later time frame for treatment may permit inclusion of sicker patients, such as those who arrive to hospital too late for optimal reperfusion and those with cardiogenic shock on presentation who might be too ill to treat earlier.

2.0 BACKGROUND

2.1 Rationale for the proposed trial

2.1.1 Unmet Clinical Needs

Following an acute MI (AMI), a remodeling process is initiated that results in replacement of myocytes by fibrotic tissue resulting in scar formation. Additionally, there may be significant and ongoing apoptotic loss of viable cardiac myocytes at the border zone of the infarct secondary to microvascular obstruction (MVO), recurrent ischemia or reperfusion injury. If the infarction is significant, then left-ventricular dysfunction may develop due to scar expansion and left-ventricular dilatation. This process may ultimately lead to the development of HF. Current-

ly, HF is the leading admission diagnosis for hospitalization in the United States and carries a 50%, 5-year mortality rate (1). Although medical therapy may improve symptoms and extend survival to a limited degree, cardiac transplantation remains the only curative procedure available. Unfortunately, its use is significantly limited due by the shortage of donor hearts. The development of new strategies to improve ventricular function following MI has been a prominent goal for cardiovascular investigation.

2.2 Development of cell-based therapies for cardiac repair

Preclinical Studies

Preclinical studies have demonstrated myocardial regeneration and improved myocardial function with delivery of BM-derived cells in animals following MI. In a study (47) 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.

In a study (48) 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*.

Drs. Geng and Willerson; UT99-117 study (49) 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 MSCs was not associated with cardiac tamponade or any clinical untoward effects at immediate and up to 15 days follow-up.

Clinical Studies

Although the data supporting significant myocardial regeneration in these preclinical studies have since been challenged (6, 7), a number of small clinical trials had already begun in Europe testing the strategy of delivering autologous bone marrow cells into the infarcted region following MI (8-10). Autologous BMMNC contain populations of endothelial, hematopoeitic and mesenchymal stem cells that are easily obtainable in patients and can be processed over several hours. The rationale for their intracoronary use in patients is based on the following: 1) The presence of a patent vasculature following reperfusion with percutaneous

transluminal coronary angioplasty (PTCA)/stenting of the infarct vessel to provide an avenue for cell delivery; 2) The up-regulation of certain chemokines such as stromal derived factor (SDF-1) which increases following an MI and may direct stem cell homing and differentiation (11); and 3) The enhancement of angiogenesis and cell survival by stem cell-secreted growth factors such as vascular endothelial growth factor (VEGF) and insulin growth factor (IGF)-1 may improve perfusion and reduce apoptotic cell death in the infarct border zone (12).

Following these small nonrandomized studies (8-10), several randomized controlled trials (RCTs) have been completed (13-17). These RCTs and others (18-20) have been recently analyzed by Abdel-Latif and colleagues (21) in a meta-analysis (Table 1) that included 18 trials of cell delivery of various bone marrow-derived cell types (BMMNC, mesenchymal stem cells (MSC), and endothelial progenitor cells (EPC)) following AMI and in chronic ischemia in 999 patients. They observed that on average, cell delivery significantly improved left-ventricular ejection fraction (LVEF) by 3.7%, reduced infarct size by 5.5% and decreased left-ventricular end-diastolic volume (LVEDV) by 4.8 ml. Their review supported the overall safety profile of cell delivery and suggested that the greatest improvement in LV function occurred when cells were administered 5-30 days following MI. They also observed that there was no significant difference in outcome in those patients that received less than the median number of cells compared to those who received greater than the median number of cells; however, dose effect has never been studied directly.

Table 1. Characteristics of Studies Included in the Meta-analysis

Source	Sample Size	Mean Follow-up Duration, mo	Study Design	Cell Type	No. of Cells Transplanted	Route of Injection	Clinical Scenario	Time From PCI and/or MI to Transplantation, d*
Bartunek et al., [18] 2005	35	4	Cohort	BMMNC (CD133*)	$12.6 \pm 2.2 * 10^6$	IC	AMI	11.6 ± 1.4
Ge et al., [19] 2006	20	6	RCT	BMMNC	$40 * 10^6$	IC	AMI	1
Janssens et al., [16] 2006	67	4	RCT	BMMNC	$172 \pm 72 * 10^6$	IC	AMI	1-2 (Range)
Lunde et al., [14] 2006	100	6	RCT	BMMNC	$87 \pm 47.7 * 10^6$	IC	AMI	6 ± 1.3
Meyer et al., [17] 2006	60	18	RCT	BMMNC	$24.6 \pm 9.4 * 10^8$	IC	AMI	4.8 ± 1.3
Ruan et al., [20] 2005	20	6	RCT	BMC	NR	IC	AMI	1
Schächinger et al., [15] 2006	204	4	RCT	BMMNC	$236 \pm 174 * 10^6$	IC	AMI	4.3 ± 1.3
Strauer et al., [8] 2002	20	3	Cohort	BMMNC	$28 \pm 22 * 10^6$	IC	AMI	8 ± 2

Abbreviations: AMI, acute myocardial infraction; BMC, bone marrow cell; BMMNC, bone marrow mononuclear cell; CPC, circulating progenitor cell; EPC, endothelial progenitor cells; IC, intracoronary injection; ICM, ischemic cardiomyopathy; IM, intramyocardial infection using electromechanical mapping system; MI, myocardial infarction; MSC, mesenchymal stem cell; NR, not reported; OMI, old myocardial infraction; PBSC, peripheral blood stem cells; PCI, percutaneous coronary intervention; RCT, randomized controlled trial.

*Values are given as mean ± SD unless otherwise specified.

2.3 Key questions generated by clinical studies to be addressed in this proposal

2.3.1 Mechanism of action

Although these cell delivery studies have generally confirmed the safety of this approach, the mechanism(s) responsible for the improvement in LV function in

humans has not been determined. Numerous basic and preclinical approaches are being used outside of this proposal to define the mechanism of benefit of BMMNC in this setting. As a network of clinical Investigators, the CCTRN is committed to provide as much mechanistic insight as possible through careful clinical investigation. In this Phase II, randomized, double-blind, controlled study, a well-defined and translatable cell product and dose will be utilized, a high risk population has been identified, careful storage and analysis of biospecimens is proposed and regional and global assessment of left ventricular function will be performed. The time of delivery will be addressed using time frames that are consistent with clinical applicability and an emerging safety profile. Additional human investigation in this and other clinical studies will provide a framework to complement ongoing basic science while further clarifying the therapeutic potential of cell delivery. It should be noted that this protocol is not designed to make head-to-head comparisons among cell types, but will instead generate a foundation for future studies to build upon within the CCTRN.

2.3.2 Effect of timing of cell administration

Although the timing of administration of cell delivery following MI may be a critical factor in dictating efficacy, this property of the intervention has never been directly addressed in a clinical study. The early inflammatory milieu and presence of MVO present in the first few days following an MI may create an adverse environment for delivery and survival of transplanted cells, which may have contributed to the negative findings of clinical studies that administered cells within one day following MI (16). Conversely, certain stem cell homing factors such as SDF-1 are elevated in the early post-infarction period (11, 22) that could benefit stem cell retention. In the mouse, myocardial expression of SDF-1, VEGF and hepatocyte growth factor (HGF) are maximally up-regulated at 48 hours post-MI and decline significantly by 96 hours following MI (23).

In a pre-clinical model, Ma et al.(22) administered 5 x 10^6 MSCs via the tail vein in rats at multiple time-points post-MI (12hrs, 1, 2, 4, 8, 16 days). They observed that the greatest number of labeled MSCs retained in the heart when measured three days following administration occurred in those animals that received cells one day post-MI. The number of retained cells declined significantly with each subsequent day of administration such that no cells were present when cell administration occurred at eight or sixteen days post-MI. Cell retention was highly correlated with improvement in fractional shortening and vessel density in the peri-infarct region. Unfortunately, no pre-clinical data have been published that has examined the effect of timing of stem cell administration post-MI in a larger animal model where the inflammatory and healing response may be significantly different (24).

In the previously published randomized clinical trials (13-17), BMMNC were administered between one and seven days but timing was never integrated into the randomization scheme (Table 2). Thus, the effect of timing of administration of BMMNC following AMI is not known. The potential benefit of cell administration several weeks following MI will be investigated in this study.

Table 2. Cell delivery and outcomes for major randomized stem cell clinical trials in acute MI

Study	Total Cells ($\times 10^6$)	$CD34^+$ ($\times 10^6$)	Outcome
REPAIR-AMI (15)	236 ± 174	6.1 ± 3.6	Positive
ASTAMI (14)	68 (54 to 130)*	0.7 (0.4 to 1.6)*	Negative
BOOST (13,17)	2,460 ± 940	9.5 ± 6.3	Pos(6mo)/Neg(18mo)
Janssens et al. (16)	172 ± 72	2.8 ± 1.7	Neg(LVEF) Pos(MRI)

interquartile range

Assmus et al. (26) compared administration of intracoronary BMMNC or cultured circulating blood progenitor cells (CPC) versus placebo in 75 patients at least three months following MI (mean=81 ± 72 months). They noted a small but statistically significant 2.9% improvement in LVEF by left-venticulography in the BMMNC group (n=35) but not in the CPC (-0.4%) or placebo group (-1.2%). In an expanded follow-up to that study (27), they administered 214 ± 98 x 10^6 BMMNC to 121 patients at a mean of seven years (range=4 months to 39 years) post-MI. They observed an LVEF increase from 39.9% to 41.7% (p <0.001) three months later. This was accompanied by significant reduction in N-terminal pro-ANP and -BNP levels and that those patients whose cells exhibited the highest levels of CFU and migratory capacity demonstrated a survival benefit at follow-up. These findings indicate that very late administration of BMMNC post-MI is safe and feasible and may result in clinical benefit in certain patients.

2.3.3 Effect of cell dose variability

In all of the randomized trials to date, there has been wide variation in the mean number of total BMMNC and $CD34^+$ cells administered between and within each study so that some patients in the treatment arm may have received up to three times as many cells as other patients (Table 2). This will be the first clinical trial to administer a single dose of cells to all the patients in the treatment group (150 x 10^6 cells). This dose was selected for the following reasons:

1. Achievable through a bone marrow aspiration with minimal risks and discomfort;
2. Excess cells will provide biospecimens for concurrent functional evaluation;
3. Within the range of prior studies.

2.3.4 Effect of cell preparation

Although BMMNC obtained from density centrifugation have been the principal cellular product delivered in the majority of the AMI clinical trials, there have been subtle variations in the cell preparation techniques that may have affected out-

come (27-29). Differences in density gradient centrifugation protocols and reagents as well as cell storage time and conditions exist among the studies. As such, correlations between procedures and clinical outcomes have been generated. Seeger and colleagues (28) suggest that isolation procedures used in the positive REPAIR-AMI study, including the use of X-VIVO 10 with 20% autologous serum resulted in a more potent cell population and phenotype compared to those used in the negative ASTAMI trial. Conversely, the ASTAMI Investigators in a recent editorial in *Lancet* (30) suggested that the cell medium used by the REPAIR-AMI Investigators may have harmed the placebo group and resulted in an increase in clinical events in that cohort which would explain their positive findings.

To address variation in cell preparation techniques, the CCTRN proposes to utilize a closed cell separation system (Sepax, *BioSafe*) that will provide for standardization among network sites and reproducible product generation. Data are provided, in a companion document, to compare Sepax product with that of traditional open systems as well as the effect of cell storage.

2.3.5 Importance of age and diabetes

Each of the BMMNC studies published to date contains a cohort of patients who received stem cell delivery, but failed to improve their LV function. It is crucial to determine the cellular or patient characteristics responsible for this in order to ensure proper patient selection. Admittedly, no study to date has been powered to comment on this group of patients. *In vitro* studies of BMMNC have documented an age-related decline in human bone marrow stem cell homing in response to SDF-1 that is associated with impaired neovascularization (31). Stem cells isolated from older patients demonstrate reduced secretion of cytokines such as VEGF that may impair angiogenesis (12) and EPCs isolated from diabetic subjects exhibit significantly reduced tubule formation in Matrigel (32).

2.3.6 Importance of microvascular obstruction (MVO)

Another critical factor is MVO as detected by cardiac magnetic resonance imaging (cMRI) (33, 34). MVO increases with ischemic duration (35), and frequently arises following PTCA revascularization during AMI as the result of embolization of a thrombus, deposition of platelet-fibrin clot, and endothelial cell sloughing within intramyocardial capillaries as a result of reperfusion injury (36). This is frequently manifested as reduced thrombolysis in myocardial infarction (TIMI) flow on the angiogram and portends a poor prognosis as it is associated with adverse left-ventricular remodeling and increased cardiovascular events (33).

The observation of TIMI 3 flow following revascularization for an AMI does not preclude the presence of significant MVO. In 110 patients with AMI and PTCA revacularization, MVO was observed in 46% of patients, yet 85% of patients had TIMI 3 flow (34). The presence of MVO in patients receiving intracoronary stem cells may impair BMMNC delivery to the areas of myocardium in greatest need of cell delivery. Because MVO resolves over several weeks, the administration of stem cells at a very early period post-MI when MVO is at its peak may impair mi-

crovascular delivery to the myocardium. This may have contributed to the negative findings of Janssens et al. (16), who delivered BMMNC one day following MI. Indeed, a subgroup analysis by them demonstrated that the presence of significant MVO precluded significant recovery with cell delivery. Those patients without significant MVO statistically improved their LVEF by 5.5%. In contrast, the REPAIR-AMI Investigators (15) noted in a subgroup analysis that those patients who received BMMNC five to seven days post-MI had a greater improvement in LVEF compared to those transplanted at an earlier time point when MVO may have been increased.

2.3.7 Effect of Drug Eluting Stents (DES) versus Bare Metal Stents (BMS)

The use of DES versus BMS for percutaneous revascularization of the infarct artery will be determined by the Institution's usual practice in which approximately 80% of patients receive DES during left anterior descending coronary artery (LAD) revascularization. However, it should be noted that >95% of stents placed to date in the European Trials were BMS. A recent study has demonstrated that patients who receive DES have impaired collateralization in the downstream myocardium six months following stent implantation (37). These findings raise the possibility that placement of a DES may impair EPC activity.

2.3.8 Effect of the Location of the Infarct Vessel

In the reperfusion era, measures of left ventricular function, heart failure, and age have been consistently related to 6-12 month mortality and development of heart failure. However, we now know that the location of the infarct vessel is an important determinant of morbidity and mortality rates. It is of interest to assess whether the location of the infarct vessel influences the effect of time on the role of cell therapy on the study endpoints.

2.4 Summary of rationale

The majority of the above studies demonstrate safety and feasibility of transplantation of BMMNC in patients following AMI. To date, there have been no significant findings of serious adverse events (SAEs) associated with this cell delivery and no evidence of increased arrhythmias or in-stent restenosis compared to placebo-assigned patients. There has been no reported increase in troponin levels following administration of BMMNC as previously suggested in the canine heart following intracoronary infusion of larger, cultured mesenchymal stem cells (38). Together with the safety issue, there is a suggestion of beneficial effects on LVEF. Thus, the field is well-situated to support additional clinical studies to test this strategy.

The established safety record, relative ease of cell acquisition and preparation of BMMNC have prompted many sites to develop cell delivery for patients with AMI. However, the CCTRN believes that widespread adoption of cell delivery is premature given the methodological limitations present in the previous trials (13-17). These include: 1) failure to randomize or inadequate method of randomization; 2) lack of blinding of patients and/or caregivers; 3) failure to include a true placebo group; 4) use of left-ventriculography for measurement of the primary response

variable (LVEF); 5) patient populations with ejection fractions frequently greater than 50% who are unlikely to develop significant LV dysfunction; 6) failure to give a uniform dose of BMMNC within each study; 7) failure to pre-specify the timing of administration of cells following MI; and 8) failure to ascertain the primary outcome blinded to treatment.

To address the above limitations we propose a randomized, blinded, placebo-controlled, pilot study of autologous BMMNC administration to 87 patients two to three weeks following AMI. The primary response variable will be change in regional and global LVEF at six months compared to baseline as measured by cMRI. Patients will be followed for two years to evaluate clinical outcomes such as death, repeat revascularization, MI and hospitalization for HF. Patients will be randomized in a 2:1 ratio of BMMNC treatment or placebo. The intention of this pilot is to provide objective data in a methodologically rigorous format on the effectiveness of BMMNC delivery two to three weeks post AMI, as a basis for further study using either BMMNC or more enriched cell types such as $CD34^+$.

2.5 Preliminary studies to support the protocol

2.5.1 Ongoing feasibility study

To support the rationale and safety of this trial a pilot study was initiated in December 2005 following Investigational New Drug (IND) approval by Food and Drug Administration (FDA) (BB-IND #12480) in September, 2005, at Minneapolis Heart Institute and Abbott Northwestern Hospital.

A total of 40 patients have been enrolled, an AMI and successful percutaneous revascularization of the LAD coronary artery as part of the *Level 1 AMI* program (39). Entry criteria included LVEF <45% measured by left-ventriculography or echocardiography. All patients underwent cMRI prior to receiving cells. Following informed consent, patients underwent bone-marrow aspiration (50-70 ml) under local anesthesia at the posterior iliac crest. Patients were randomized to receive intracoronary infusion of either BMMNC (100 x 10^6 cells) or placebo (5% albumin in normal saline (NS)).

The day of stem cell infusion was determined by the patient's clinical course, with administration of the cellular product occurring towards the end of expected hospitalization (3 to 11 days, mean=5.2 days). The aspirate was transported to the University of Minnesota Cell Therapy Lab, an FDA-approved GMP facility where the mononuclear cells were isolated by Ficoll density centrifugation. Cells were transported to the hospital in the afternoon following checks for sterility and viability measurements in sterile, labeled bags containing 100 million BMMNC in 5% human albumin solution.

Patients were transferred to the catheterization laboratory in the afternoon. Following placement of a 6 French (Fr) sheath in the right femoral artery, angiography was performed to document patency of the stented artery. The patient was given 3000U of heparin (iv) and a 3.5 Fr infusion catheter (*Tracker, Boston*

Scientific) was advanced over a guidewire and placed at the distal end of the stented segment. A total of 100 million BMMNC were infused over 20 minutes by hand injection at a rate of five million cells per minute. Following completion of the infusion, an angiogram was taken to document patency and TIMI 3 flow. Patients were discharged the following day.

2.5.1.1 Preliminary Results

Forty patients (31M, 9F) with moderate to large anterior infarctions have enrolled in the trial to date. Their average age was 54 years and seven had non-insulin dependent diabetes mellitus (NIDDM). Their average ischemic time (onset of pain to percutaneous coronary intervention (PCI)) was 7.2 hrs and seven required intra-aortic balloon pump (IABP) support. Two patients underwent hypothermia treatment following initial cardiac arrest. The average day of transplant was five ± two days following MI. The peak creatine kinase (CK) was 3074 IU and the CKMB was 282 IU. Average LVEF by echocardiography performed one day following MI was 37%. Their average LVEF by cMRI was 48% three to five days following MI. In the Minneapolis Heart Institute Foundation, the LVEF by cMRI is 10% higher than that measured by echocardiography. All patients had significant MVO on baseline MRI. Their average left-ventricular end-diastolic volume (LVEDV) was 189.5 ml and LVESV was 102.9 ml with an LV mass of 173 grams.

The average bone marrow aspirate collection was 65 ml and the mean BMMNC number was 170 million cells. The average percent $CD34^+$, $CD133^+$ and $CD34^+/CD133^+$ cell count in the delivered cell product were 2.01%, 0.22% and 1.09%, respectively. The viability of the isolated BMMNC was greater than 96% in all patients.

Four significant adverse events were reported during the trial. One patient in the placebo group received an ICD for palpitations and syncope. One patient in the BMC group underwent CABG 8 months following cell therapy for an anomalous right coronary artery that was found to course between the aorta and pulmonary artery. One patient in the placebo group underwent repeat stenting in the LAD for in-stent restenosis at 15 months followed by CABG one month later after admission for a NSTEMI due to stent thrombosis. One patient in the BMC group underwent stenting of the circumflex artery two months following cell therapy infusion due to a pre-existing stenosis. There were no serious, unexpected, events that were related or possibly related to the study product or procedure reported during the trial.

2.5.1.2 Complications and Adverse Events (AEs)

There have been no complications associated with the bone marrow aspiration or BMMNC infusion, and no quality issues associated with the cellular product. There have been three AEs, with none attributed to the intervention. One patient was readmitted overnight one month following cell infusion with chest pain. The cause of pain was determined to be gastrointestinal. One patient underwent repeat revascularization of the target vessel at four months due to stenosis proxim-

al to the LAD stent. This was successfully treated with a second drug eluting stent (DES) and he was discharged the following day. There have been no arrhythmias detected by serial ambulatory electrocardiogram (ECG/AEG) monitoring in any of the patients although one patient received an implantable cardiac defibrillator (ICD) three months following cell administration because of palpitations and light-headedness; however, no ventricular arrhythmias were ever documented in the patient.

2.5.2 Cell preparation

To address variation in cell preparation techniques, the CCTRN proposes to utilize a closed cell separation system (Sepax, *BioSafe*) that will provide for ease of utility among network sites and reproducible product generation. In order to demonstrate comparability of the Sepax density gradient cellular product with that of traditional open systems (Manual density gradient), a series of experiments were performed doing direct comparison, which are fully described in a separate document. Briefly, bone marrow aspirations (100ml) were obtained from normal donors and evenly split between the two systems. The final product from each method was compared with the starting population with regards to total nucleated cells (TNC), mononuclear cells (MNC), $CD34^+$ cells and Colony Forming Units (CFU) recovery. Results are summarized below:

Table 3. Sepax versus Manual Comparison Results

	Starting TNC ($x10^8$)	TNC Recovery (%±SD)	MNC Recovery (%±SD)*	$CD34^+$ Cell Recovery (%±SD)
n=12				
Manual	10.3±4.7	24.1±6.6	46.8±16.4	64.6±16.7
Sepax	13.9±3.7	19.5±4.4	46.5±16.2*	68.3±12.2

*n=6 only due to an attempt to reduce the wash volume from 50 ml to 30 ml and obtain the cells in the 30 ml final volume needed for the trial

In addition, flow cytometric analysis on the final cellular products demonstrated that there was not a preferentially enrichment of a particular subpopulation by one procedure compared with the other. Finally, cells were incubated overnight in X-VIVO 10 media as previously described by Seeger et al. (28). We did not see a significant difference in the cells obtained from either the manual or Sepax procedure after this culture period

2.5.3 Catheter compatibility

Three samples of bone marrow cells were passed through a PTCA catheter (Maverick, *Boston Scientific*). Fractions were collected and analyzed to determine if there are any adverse affects on the cells. The mononuclear cell (MNC) fraction was enriched from three bone marrow samples using the Sepax device and a Ficoll density gradient solution (*GE Healthcare*). The enriched cells were resuspended in a 30 ml sample (5% Albumin/PBS) and then passed through the catheter. Six fractions (each approximately 5 ml) were collected for analysis. Bone marrow was harvested from normal donors (*Cambrex*, Maryland) and shipped to MDACC overnight. The diameters of the three catheters were *2.5* mm, 3.0 mm,

and 3.5 mm. Samples were submitted from each fraction for total nucleated cell counts (TNC) and viability determination (7-AAD, Flow Cytometry) (Total nucleated cell counts = cell concentration x cell volume; Cell Recovery = Absolute cell number post-processing in each fraction/Absolute cell number pre-processing in each fraction; Total Cell Recovery = Total cells recovered after processing/Total cells pre-processing). The final fraction was submitted for 14-day sterility cultures, and a pooled sample from the fractions was submitted for CFU assay. Detailed numbers will be provided in a separate document, but the following conclusions were found:

- Individual TNC recovery was >60% for all fractions in each run
- Overall TNC recovery was 83% for each run
- Viability was >95% for all fractions in each run
- Sterility was negative for each run (based upon evaluation of the final fraction passed through the catheter)
- CFUs for each run demonstrated growth (based upon a pooled sample)
- Overall recovery of CFU was >96%

All expected outcomes were met for all three runs. Based upon these data, it has been concluded that there is no adverse effect on mononuclear cell-enriched bone marrow cells by passing them through the proposed delivery catheter.

3.0 STUDY DESIGN

3.1 Introduction

To answer the aforementioned questions of cell delivery two to three weeks post-MI we propose a Phase II, randomized, double-blinded, placebo-controlled, clinical trial of autologous BMMNC administration to patients following acute MI. This timing will allow for transfer of patients from distant sites for recruitment and thus markedly increase the number of patients that can have an opportunity for treatment.

Enrollment in each will be limited to patients with moderate to large infarctions and whose initial LVEF measured by echocardiography is less than 45%. Patients with a previous history of CABG or MI that resulted in LV dysfunction as defined as the presence of a regional wall motion abnormality are ineligible. Additionally, patients enrolled in Late-TIME will be required at the time of enrollment two to three weeks following their MI to have an LVEF $\leq$45% so that a group of patients who are at increased risk of LV-remodeling and the development of HF are studied.

3.2 Late-TIME Study

3.2.1 Specific objective

To evaluate the effect of a single dose of BMMNC cells on regional and global LV function when administered two to three weeks after MI onset compared with placebo following an MI.

This objective will be addressed by a Phase II, randomized, double-blinded, placebo-controlled pilot study.

3.2.2 Primary Endpoints

There are two co-primary endpoints: 1) change in global LV function from baseline to six months in the active cell delivery group as compared with the analogous change in the control group; and, 2) change in regional LV function from baseline to six months in the active cell delivery group when compared with the change in the control group. Each will be measured by cMRI, which is expected to be available on all patients.

3.2.3 Secondary Analyses

The interaction of the effect of cell delivery, and the influence of late timing on the effect of cell delivery will be evaluated on each of the following endpoints:

- Combined endpoint (first of) death, reinfarction, repeat revascularization, or hospitalization for HF
- All patients will be followed for overall survival
- LV mass
- LVEDV
- LVESV
- Infarct size

In addition, the influence of DES on the effect of timing will be evaluated. Because it is anticipated that a much greater proportion of patients in this trial will receive DES at the time of revascularization, a secondary evaluation of the trial will compare the response of cell delivery in the bare metal stent (BMS) versus DES patients. However, we recognize that the numbers with BMS will likely be small.

3.2.4 Intervention.

Active therapy consists of approximately 150 x 10^6 TNC (80% BMMNC). This dose was chosen based on our ability to consistently obtain at least 150 million cells with a 80-90 ml bone marrow aspirate using local anesthesia in a pilot study of 40 patients enrolled to-date.

Placebo patients will undergo bone marrow aspiration of the same volume, but receive only 5% human serum albumin/saline. Randomization will be a 2:1 (active:placebo) allocation. Patients will be stratified by Clinical Center.

3.2.5 Sample size

The Late-TIME study is a Phase II, randomized, double-blinded pilot clinical trial that assesses whether the delivery of BMMNC can ameliorate LV dysfunction when that delivery occurs two to three weeks (14-21 days) after the MI.

3.2.5.1 Assumptions

In the absence of efficacy monitoring by the DSMB, hypothesis testing for the primary endpoint(s) will be carried out at the 0.05 level. Assuming independence and normality of the observations, the sample size is calculated using a two-sample *t*-test statistic

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

where

- N = number of placebo patients + number of active group patients
- 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., difference between the change in the active group over time minus the change in the control group over time
- σ_{Δ}^{2} = the variance in the change over time (incorporates the correlation over time). Pooled between the active and placebo groups.
- k = ratio of number of active group to placebo group patients.
- f = expected proportion of patients anticipated to be lost to follow-up.

3.2.5.1.1 Global ejection fraction

The literature suggests (21) that the achievable absolute change in global ejection fraction is $\delta = 4$ and common group standard deviation of the difference of LVEF over time as $\sigma_{\Delta} = 6$. This produces a sample size of 86, administratively rounded up to 87:58 in the active group and 29 in the control group. The sensitivity of these sample sizes to the effect size and standard deviation of the difference are demonstrated (Table 4).

Table 4.Total sample size for the effect of dose in late time on global ejection fraction
Type I error = 0.05; power = 80%; followup losses = 5%

		Treatment Effect (δ)				
		4	5	6	7	8
Std Dev	5	60	39	28	21	17
of Diff	6	86	56	39	29	23
(σ)	7	116	75	53	39	30
	8	151	97	68	51	39

3.2.5.1.2. Regional ejection fraction assumptions

For regional LVEF we are assuming a $\delta = 6.7$ and a common group standard deviation, $\sigma_{\Delta} = 9.5$ from the 2004 Boost manuscript (13).This produces 77 patients: The sensitivity of these sample sizes to the effect size and standard deviation of the difference is demonstrated (Table 5).

Table 5. Total **s**ample size for effect of dose on regional ejection fraction in lateTIME
Type I error = 0.05; power = 80%; followup losses = 5%

		Treatment Effect (δ)				
		6	6.5	7	7.5	8
Std Dev	8	68	58	51	44	39
of Diff	9.5	95	81	70	62	54
(σ)	10	105	90	78	68	60
	10.5	116	99	86	75	66

3.2.5.2 Sample sizes for overall effect: regional and global function

A sample size of 87 patients is required for this study (Table 6).

Table 6. Sample Size for lateTIME Pilot
Based on Assumptions in Section 3.5

	Global LVEF Regional LVEF
Placebo	29
Active	58
Total Sample Size	87

3.2.5.3 The multiple testing issue

Type I error correction in the multiple testing environment 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 and the need to identify new effects, on the other, by limiting the number of primary endpoints.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

To carry out this study, 87 patients are required. Patients enrolled in this study will be recruited from all of the sites participating in the National Heart, Lung, and Blood Institute (NHLBI) CCTRN. Patients with an acute MI are admitted to the respective hospitals of the Network or transferred from local hospitals affiliated

with the Network hospitals. All patients will have undergone percutaneous revascularization of the infarct artery that has resulted in a moderate to large infarction with an LVEF ≤45% by echocardiography on presentation and demonstrate a persistently reduced LVEF (≤45%) two to three weeks later at enrollment by echocardiography. All prospective patients will be screened by the Investigators or study coordinators and will be enrolled in the trial after meeting inclusion/exclusion criteria and signing the informed consent and HIPAA forms.

4.1 Implantable Cardiac Defibrillator (ICD) Use

In 40 patients enrolled to date in our preliminary study, we have had no patients meet criteria for ICD /bi-ventricular pacemaker (Bi-V) therapy although one patient had an ICD placed for palpitations and light-headedness which was presumed to be ventricular in origin despite the absence of documentation. All patient's LVEF have been greater than 35% at three months and no patients have had ventricular arrhythmias (sustained or significant non-sustained) by Holter Monitoring or symptoms such as syncope. All patients with document ventricular tachycardia (VT) (sustained or non-sustained) on Holter monitoring or syncope will be referred to an electrophysiologist for evaluation of the need for an ICD. If a patient in the network requires ICD/Bi-V therapy before six-month cMRI than cMRI will be performed just prior to implantation and patient will be followed with serial echocardiograms in place of cMRI.

A total of 87 patients will be enrolled in this study; it is expected that they will be discharged and readmitted at a later date for the study.

4.2 Randomization

Randomization will occur at the Data Coordinating Center (DCC) (Details provided in section 9.1). The patients and research staff including the MRI physicians and interventional cardiologists will be blinded to the treatment group. All patients will be advised to take aspirin 325 mg and Plavix (clopidogrel) 75 mg for 24 months and will be advised to take statins, beta-blockers and angiotensin converting enzyme (ACE) inhibitors per guideline recommended care unless contraindicated. Patients with an LVEF <40% will be advised to take an aldosterone antagonist unless contraindicated by renal insufficiency or hyperkalemia. All Investigators are required to adhere to the standard medical management of acute myocardial infarction for all patients entering the trial.

Note to Investigator: The use of either DES or BMS for percutaneous revascularization of the infarct-artery is required. The revascularized vessel must be patent at the time cell administration is to be attempted.

4.3 Inclusion criteria

a) Patients at least 21 years of age.
b) Patients with first acute MI and subsequent successful primary percutaneous coronary intervention (PCI) in an artery at least 2.5 mm in diameter occurring two to three weeks before recruitment.
c) No contraindications to undergoing cell therapy procedure within two to three weeks following AMI and PCI.
d) Hemodynamic stability as defined as no requirement for IABP, inotropic or blood pressure supporting medications.
e) Ejection fraction following reperfusion with PCI $\leq$45% as assessed by echocardiography.
f) Consent to protocol and agree to comply with all follow-up visits and studies.
g) Women of child bearing potential willing to use an active form of birth control.

Note: The inclusion criteria require that only patients with a first q-wave infarction with resulting LVEF $\leq$ 45% will be enrolled. It is possible that a patient who meets this and all other entry criteria, but does not have ST segment elevation, may be enrolled.

4.4 Exclusion criteria

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

a) History of sustained ventricular arrhythmias not related to their AMI (evidenced by previous holter monitoring and/or medication history for sustained ventricular arrhythmias in patient's medical chart).
b) Require CABG or PCI due to the presence of residual coronary stenosis >70% luminal obstruction in the non-infarct related vessel (Additional PCI of non-culprit vessels may be performed prior to enrollment).
c) History of any malignancy within the past five years excluding non-melanoma skin cancer or cervical cancer in-situ.
d) History of chronic anemia (hemoglobin (Hb) <9.0 mg/dl).
e) History of thrombocytosis (platelets >500k).
f) History of thrombocytopenia in the absence of recent evidence that platelet counts are normal
g) Known history of elevated INR (PT) or PTT.
h) Life expectancy less than one year.
i) History of untreated alcohol or drug abuse.
j) Currently enrolled in another Investigational drug or device trial
k) Previous CABG.
l) Previous MI resulting in LV dysfunction (LVEF <55%)
m) History of stroke or transient ischemic attack (TIA) within the past six months.
n) History of severe valvular heart disease (aortic valve area <1.0 cm^2 or >3+ mitral regurgitation.

o) Pregnancy or breast feeding
p) Has a known history of HIV, or has active Hepatitis B, active Hepatitis C, or active TB
q) Patients with active inflammatory or autoimmune disease on chronic immunosuppressive therapy.
r) Contraindications to cMRI.
s) Previous radiation to the pelvis with white blood cell count (WBC) and platelet counts below hospital specific normal values.
t) Women child bearing potential not willing to practice an active form of birth control.
u) Chronic liver disease that might interfere with survival or treatment with cell therapy.
v) Chronic renal insufficiency as defined by a creatinine ≥2.0 mg/dL or requires chronic dialysis.

4.6 Anticoagulation management in the evaluation of patients

Anticoagulation therapy is a frequently present in the post MI environment. The decision to treat patients with anticoagulation therapy immediately post MI is made by the treating physician and not the CCTRN Investigator. The investigators will use the following guidelines for assessing the suitability of such patients for this protocol (14, 16, 51).

4.6.1. Atrial Fibrillation

Since the risk of an embolic event is very low on any given day for patients with chronic atrial fibrillation, these patients will be enrolled and their anticoagulation therapy held for the day of the procedure. Doing so would avoid the small but finite risks of bleeding during bone marrow aspiration or cell delivery while minimizing the very small risk of an embolic event.

4.6.2 LV Thrombus

If the patient has had an LV thrombus requiring anticoagulation therapy, we will proceed with recruiting the patient where the investigative team determines that proceeding on anticoagulation could be performed without undue risks (e.g., through a radial approach) which can be carried out without discontinuing anticoagulation therapy.

4.6.3 Other Indications for Anticoagulation

If the treating physician desires that the patient stay on continuous anticoagulation therapy, then the patient is removed from further consideration as a CCTRN subject. If the treating physician decides together with the patient that enrollment is in the patients best interest, this decision is noted and justified in the patient's hospital record by the physician. At the treating physician's discretion, and if the patient meets all other entry criteria for the study, the patient would have their anticoagulation therapy temporarily interrupted for bone marrow aspiration and cell infusion, and then reinitiated post procedure. This temporary interruption would be identical to the procedures employed daily at each of our center's cardiac ca-

theterization labs to perform cardiac catheterization or our bone marrow units to perform bone marrow aspirations on any anticoagulated patient (e.g. prosthetic mechanical heart valve, deep vein thrombosis, etc.).

5.0 INTERVENTION

The intervention is the intracoronary delivery of approximately 150 $x10^6$ TNCs.

5.1 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 2. 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.

5.2 BMMNC Characteristics

BMMNC 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; ≥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 infusion, 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 study identification number and acrostic. From our initial patient experience, 150-200 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 infused a within 12 hours of completing the bone marrow aspiration in each patient (total volume=30 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 an infusion of 5% HSA/Saline.

5.3 Infusion

Infusion of BMMNCs or placebo will be performed in the cardiac catheterization laboratory within 12 hours of completing the bone marrow aspiration and within the randomized time points following primary PCI of the infarct vessel. Investigators will administer the cellular product in syringes. A 6 Fr guiding catheter is ad-

vanced to the ostium of the appropriate coronary artery and the patients are administered heparin sufficient to achieve an ACT of at least 200 seconds. An angioplasty guide wire is advanced to the distal end of the infarct vessel beyond the stented site. An over-the-wire PTCA catheter (Maverick*, Boston Scientific*) equal to the stent diameter is advanced over the guidewire and the tip positioned in the stented region. Its length will be sized to the previously placed stent such that the inflated balloon length will not exceed the length of the stent. The wire is withdrawn and the catheter aspirated and then flushed with heparinized saline. The dead space of the catheter is 0.75 ml. The cells (approximate volume=30 ml) are sterilely withdrawn through a 6 or 12cc syringe. If a needle is required to withdraw the study product, a 20 gauge or larger gauge needle is to be used. The catheter is then primed with 0.75 ml of cells from the first infusate syringe. The cells will be infused in six aliquots (five ml) over two minutes each during balloon inflation at low pressure. Each of the first five infusions would contain 5ml each, with the remainder of the infusate (up to five ml) in the final infusion. Complete cessation of antegrade blood flow during balloon inflation will be confirmed with an initial contrast injection. Two minutes of reperfusion will occur following each cycle of cell infusion. It is expected that some patients may develop significant chest discomfort or significant ST-segment changes during balloon inflation as described in the European trials. The ischemic duration will be reduced as necessary to accommodate this, but the number of cycles will then be increased so that the total duration of ischemia will remain constant in each patient

5.4 Harvest, Isolation and Testing of BMMNC

5.4.1 General

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

5.4.2 Procurement

Approximately 80-90 ml (±10ml) of bone marrow will be collected from the posterior superior iliac spine of the patient using established, standard collection procedures by a trained physician. Only one bone marrow aspiration will be attempted. Sterile technique will be followed to prevent contamination of the marrow collection and infection at the site of collection. The details of the aspiration procedure are located in Appendix 2. 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. 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.

5.4.3 Infectious Disease Testing & Prevention of Cross-Contamination:
Although cells are autologous in this protocol, the standard tests for infectious diseases will be performed during the hematology baseline testing (as per the local site's standard operating procedure). 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 antibody (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.

5.4.4 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 MNC fraction using the Sepax instrument (*BioSafe*, 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 appropriate 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 MNCs 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 analyis will be performed (Cell Count (TNC), Viability, Flow Cytometry Analysis, Endotoxin testing, CFU Sterlity, 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 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.

5.4.5 Release Criteria

As noted the final product will be suspended in 5% HSA/saline. Analysis by Viability, Gram Stain, TNC and Entotoxin testing will be performed.

5.4.6 Post Release Analysis

Colony forming units (CFU), 14 day sterility, and analysis by flow cytometry (enumeration of CD34+ CD133+ and CD45+ cells) will serve as an *in vitro* surrogate potency assay, much like CD34+ cell enumeration for early (short-term) hematopoeitic engraftment in the setting of hematopoeitic 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.

5.4.7 Cell Dose

The maximum dose that will be administered to patients is approximately 150 x 10^6 total nucleated cells (TNC) in 30 ml of 5% HSA/saline solution. The total dose delivered during the infusion is recorded in the database. Any patient randomized to the cell therapy arm whose bone marrow aspiration produces less than the stipulated target dose will receive all the available cells as a second bone marrow aspiration will not be performed. Cell number (i.e., TNC count) will be determined using a hematology analyzer. 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.

All cells that exceed the administered dose of 150 million aliquots of BMMNC 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. This information will be used to examine the relationship between cell therapy outcomes and cell characteristics e.g., cell type consistency, cytokine and nitric oxide production, and genome-wide expression profile. As part of a nine marker stem/progenitor cell panel analyses, the following cell surface cluster of differentiation (CD) markers will be collected and reported (as percentages) to the DCC for each patient enrolled the protocol; AC133 antigen, CD34, VEGFR2(KDR), CD31, CD45 (from CD31/CD45 combination), CXCR4, CD14, CD11b, CD3. In addition, antibodies reflecting B-cell attributes, migration analyses and colony forming units-granulocyte/macrophage (CFU-GM) assays will be examined. The influence of these variables on the endpoints of this study will be examined using the general linear model for continuous endpoints and logistic regression for dichotomous clinical outcome measures.

5.4.8 Final Product Release Criteria Testing

Final product (lot) release criteria testing results (see table below) will be available prior to the BMMNC being transported to the hospital for administration.

Table 7. Product Release Specifications		
Assay	Test Method	Specification
Rapid Sterility	Gram Stain	No organisms
Viability	Trypan Blue	≥70%
Endotoxin	EndoSafe PTS	<5Eu/kg
TNC	Manual or Automated	$\leq 150 \times 10^6$

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 8).

Table 8. Post Production Monitoring		
Assay	Test Method	Specification
Immunophenotyping	Flow Cytometry	Report
CFU	Per Site SOP	Report
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. 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.

5.5 Randomization and Unblinding

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:

5.5.1 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. 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. The assignment of therapy (i.e., active or placebo) occurs after bone marrow aspiration and cell processing (discussed in section 5.5.2).

5.5.2 Unblinding of Randomization

Subsequently, the patient undergoes a bone marrow aspiration of approximately 80-90ml (±10ml), the aspirate is processed through the investigational Sepax system, and samples are drawn for rapid release and other testing. No patient will undergo more than one bone marrow aspiration. At this point, when the patient's processed cells have passed the bone marrow release criteria, the computer assigns the patient to active or placebo therapy, and the 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 infusion. 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 or the gram stain, then the patient cannot enter the study.

If the cell product passes the release criteria, then the technician who is to be unblinded enters the information that the patient has passed their release criteria into the computer. The computer then makes the therapy assignment and reveals that therapy assignment to the unblinded technician. The infusate should be indistinguishable as to active or placebo when it is delivered to the Investigator who will be providing the infusate to the patient. Thus the person bringing the infusate to the cath lab and the Investigator who injects the infusate in the patient will remain blinded. Therefore, to ensure compliance with cGCPs, the Network proposes the addition of 100 microliters of autologous blood to placebo for blinding purposes. The product that the patient will receive is a placebo consisting of HSA/Saline containing 100 microliters of whole blood collected from the marrow donor (i.e. the patient him/herself). The placebo material will not require release testing provided that it is the same lot of HSA/Saline that was used to prepare the cells and that the cells passed endotoxin and Gram stain testing.

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
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 Schedule and Timing of Follow-up Visits and Testing

All patients enrolled in this trial will undergo serial follow-up examination and testing to determine the long-term safety of this cell delivery as mandated by the FDA. Patients will take twice-daily measurements of temperature for one month following infusion of product. The patients will be required to see their primary physician or one of the Investigators within 48 hours if the patient develops a persistent fever greater than 100.0° F.

6.2 Consent Visit (Two to Three Weeks Post-MI)

- Consent signed
- Inclusion/Exclusion Review (LVEF ≤45% by echo)
- Complete Medical History and Medication Review
- Assessment of New York Heart Association (NYHA) Class (see Appendix 1)
- Physical Exam, including vital signs, height and weight
- Laboratory Tests (include complete blood count (CBC/diff, lipid panel, renal panel, hepatic panel, troponin I or T, CK, CK-MB, hsCRP, BNP level, and pregnancy test (women of childbearing age))
- Assays for the detection of HIV and HCV (by nucleic acid testing), anti-HIV I/II, anti-HTLV I/II, anti-HBc antibody (Ab), HBsAg, anti-HCV, and Treponema pallidum (by serology) are collected per local site's standard operating procedure[¥]
- Echocardiogram (***send to echo core lab following study product randomization***)*
- 12-lead ECG

[¥] Infectious disease testing can be done on the day of infusion.

NOTE: The treatment checklist must be completed and submitted prior to the bone marrow aspiration. Subjects who fail the checklist will be excluded from the study.

6.3 Day 0 (Study Product Infusion)

- Incremental medical history
- Physical Exam, including vital signs, height and weight

- Assessment of NYHA class
- Bone marrow aspiration for stem cell harvest
- Study product infusion in catheterization laboratory
- Vital signs pre- and post- bone marrow harvest and cellular product infusion
- Troponin I or T, CK, CK-MB collected one time on the morning following infusion
- Telemetry after procedure (18-24hrs)
- Cardiac MRI (baseline) (***send to MRI core lab following study product randomization***)
- Five 10 ml venous blood (purple top tubes) for biorepository FACS and migration analysis
- 10 ml venous blood (green top heparin tubes) for biorepository plasma cryostorage
- Review of medications for changes
- Assess for AEs/SAEs

6.4 Day 1

- Incremental medical history
- Physical Exam, including vital signs, height and weight
- Assessment of NYHA class
- 12-lead ECG
- Review of past 18-24 hours of Telemetry
- Laboratory Testing (CBC/diff, renal and hepatic panel)
- Two 10 ml venous blood (purple top tubes) for biorepository FACS analysis
- 10 ml venous blood (green top heparin tubes) for biorepository plasma cryostorage
- Review of medication for changes
- Assess for AEs/SAEs

6.5 Month 1

- Incremental medical history
- Physical Exam, including vital signs, height and weight
- Assessment of NYHA class
- 12-lead ECG
- 24-hour Holter
- Laboratory Testing (CBC/diff and hepatic panel)
- Two 10 ml venous blood (purple top tubes) for biorepository FACS analysis
- 10 ml venous blood (green top heparin tubes) for biorepository plasma cryostorage
- Review of medication for changes
- Assess for AEs/SAEs

6.6 Month 3

- Incremental medical history

- Physical Exam, including vital signs, height and weight
- Assessment of NYHA class
- Laboratory Testing (CBC/diff and hepatic panel)
- Two 10 ml venous blood (purple top tubes) for biorepository FACS analysis
- 10 ml venous blood (green top heparin tubes) for biorepository plasma cryostorage
- Review of medication for changes
- Assess for AEs/SAEs

6.7 Month 6

- Incremental medical history
- Physical Exam, including vital signs, height and weight
- Assessment of NYHA Class
- Laboratory Testing (CBC/diff and hepatic panel)
- 12-lead ECG
- Cardiac MRI ***(send to MRI core lab)***
- Echocardiogram (Limited)* ***(send to echo core lab)***
- Two 10 ml venous blood (purple top tubes) for biorepository FACS analysis
- 10 ml venous blood (green top heparin tubes) for biorepository plasma cryostorage
- BNP level
- Review of medication for changes
- Assess for AEs/SAEs

6.8 Month 12

- Incremental medical history
- Physical Exam, including vital signs, height and weight
- Assessment of NYHA Class
- Laboratory Testing (CBC/diff and hepatic panel)
- Cardiac MRI
- BNP level
- Review of medication for changes
- Assess for AEs/SAEs

6.9 Month 24

- Incremental medical history
- Physical Exam, including vital signs, height and weight.
- Assessment of NYHA Class
- Laboratory Testing (CBC/diff and hepatic panel)
- Cardiac MRI
- BNP level
- Review Medication for changes
- Assess for AEs/SAEs

* **Echo Contrast Information for Baseline and Month 6**
The site will use its clinical judgment to determine if echo contrast (as an aid in visualization of the ventricular endocardial border definition) will be obtained, following these guidelines

A - All echos must include a non contrast component, including collection of data measures before the addition of contrast.
B - If not contraindicated, a contrast component will be obtained
C - If contrast is included in the baseline echo then the 6 month echo visit must also add echo contrast.

6.10 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 Section 5.4.7.

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.

Table 9 provides a summary of the schedule.

Table 9. Schedule of Procedures in Late TIME (Day 0 is the day of study product infusion)

	Consent	Day 0 (SPI)	Day 1	Mo 1	Mo 3	Mo 6	Mo 12	Mo 24
Complete Medical History	X							
Incremental Medical History		X	X	X	X	X	X	X
Informed Consent	X							
Physical Exam	X	X	X	X	X	X	X	X
Laboratory Tests	X	X	X	X	X	X	X	X
Pregnancy Test*	X							
Echo	X					X		
ECG	X		X	X		X		
Bone Marrow Aspiration		X						
Biorepository Blood Draws		X	X	X	X	X		
Cardiac MRI		X				X	X	X
Study Product Infusion (SPI)		X						
Medication Review	X	X	X	X	X	X	X	X
AE/SAE Evals		X	X	X	X	X	X	X
Telemetry (18-24 hrs post SPI)		X						
Holter				X				

* In women of childbearing age

ECHO will be performed at 12, and 24 months if MRI becomes contraindicated
The cardiac MRI's obtained at 12 and 24 months are collected to identify safety findings such as changes in myocardial perfusion, wall motion abnormalities, and the presence of left ventricular thrombus

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 find-

ings, 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 transfer 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 (i.e. associated with the study product or study procedures) 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 From 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 documents, 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 ≥100.4^{o}F)
5) Excessive bleeding from bone marrow harvest site
6) Cardiac perforation

7.7 Monitoring of Liver Function Tests (AST/ALT)

Subjects with an AST and/or ALT elevation >1.5 x 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

A cardiac MR 1.5 T scanning unit (Avanto, Cardiac MR Scanner, Siemens Medical Systems or equivalent) will be used for cardiac MR images. The exact cMRI Scanner will vary at each institution; however, the exact imaging protocols will be established by the MRI Core Lab. Cardiac MRI was chosen by the Network to evaluate the primary and secondary endpoints because it is independent of the geometric assumptions required for calculations of ejection fraction compared with echocardiography or left-ventriculography. Furthermore, the interstudy reproducibility for cMRI is significantly better than echocardiography for measurements such as LVEF, LVEDV, LVESV and cardiac mass (40, 41). However, the patient's measurements of global LVEF from entry into the study until completion will be assessed by echocardiography in place of cMRI when MRI is contraindicated.

A series of scout images will be required with a pulse sequence that collects an image in a fraction of the RR interval. Scout imaging will start with a transverse view at the mid-ventricular level. Using the first scout as a localizer, the technologist will acquire the next scout image for the image plane that intersects with the long axis of the LV. A horizontal long axis cine series (four-chamber view) will be acquired first followed by the long axis cine (two-chamber view). The image planes for the short axis studies will start at the base of the heart, at least 2 cm above the mitral-tricuspid valve plane to achieve maximal LV coverage. Using Tru-FISP (balanced- FFE) cineangiography, the slices will be consecutively positioned every 7 mm from the base to apex with a 3 mm interslice gap. The acquisition window for retrospectively-triggered cine sequence will be obtained. End-expiratory breath-hold with sampling throughout the entire cardiac cycle will be employed because of better reproducibility of each slice position for more accurate left ventricular volume analysis. A minimum of 20 cardiac phases will be used to cover the RR interval.

8.1 Functional Data Analysis

Commercial Siemens *Argus* analysis software will be used for measurement of global left myocardial mass, volumes, and ejection fraction. Short axis cine images will be placed in the 17 segment model (42) and a five-point scale will be applied for a qualitative assessment of regional function. Segmental functional recovery will be measured in the defined infarct zone and each contiguous segment (border zone) and will be defined as an increase from akinetic to hypokinetic or normal; hypokinetic to normal; or dyskinetic to akinetic, hypokinetic or normal (13). Endocardial and epicardial borders will traced in the short-axis slices in end-diastolic and end-systolic views for determination of LVEDV, LVESV and global LV mass. Global LVEF is calculated as (LVEDV-LVESV) / LVEDV x 100%. These measurements will also be reported on a normalized scale for body surface area (m^2 BSA). Regional systolic wall motion in the infarct and border zones will be expressed in mm of radial displacement of the endocardial con-

tour. Regional wall thickening is defined as the percent increase of LV wall thickness during systole compared with diastole (40). The infarct zone is defined as all myocardial segments that contain late enhancements with gadolinium, and the border zone is defined as the first adjacent normal segment.

8.2 Myocardial Infarction (MI) Data

Infarct size will be quantified by delayed, contrast-enhanced MR imaging, which currently represents the most accurate method of assessment. Following left ventricular function assessment, gadolinium will be given using a 0.2 mmol/kg dose. Two minutes after gadolinium administration, single shot Tru-FISP short axis images will cover the left ventricle in two to three breath-holds for evaluation of MVO. The TI of this sequence will be set to the lengthy value of 450 ms (in order to make normally perfused myocardium gray and areas of MVO black). After 20 minutes, the presence of hyperenhancement will be evaluated with diastolic 2D flash imaging. The TI will be adjusted to "null" normal myocardium. The entire LV will be covered following multiple breath holds using a slice thickness of 5 mm (to minimize partial volume averaging) and no interslice gap. The transmural extent of late hyperenhancement (infarct) will be defined as: 0-25%, 26-50%, 51-75% and >75%.

8.3 Myocardial Mass and Microvascular Obstruction (MVO) Data Analysis

The flash protocol for assessment of hyperenhancement will be used and myocardial mass will be planimetered using the Siemens *Argus* analysis software. A second analysis will planimeter only the areas of hyperenhancement on each 2D slice. The total mass of hyperenhanced tissue will then be reported as a percentage of the entire myocardial mass or the percentage of myocardium infarcted. MVO is manually calculated as the hypoenhanced region within the delayed hyperenhanced infarct region.

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 active or control group, using variable block sizes of six or nine, randomly selected. Patients 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. 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 Analysis

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 (by clinical center) 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: 1) demographic characteristics; 2) medical history; 3) physical examination; and 4) laboratory data. Exact testing for categorical variables and Student *t* testing for continuous variables will be used to evaluate the differences in baseline variables between treatment groups.

9.4 Analyses of Primary Outcome

9.4.1 Baseline evaluations

The compatibility of baseline characteristics between the two treatment groups will be ascertained using standard normal tests for continuous variables and Fisher's exact tests for categorical variables. All hypotheses testing, and all effect sizes and their 95% confidence intervals will be evaluated using the general linear mixed model. Nonparametric techniques will also be used in the statistical analysis.

9.4.2 Co-primary endpoint evaluations

The primary endpoint, global LVEF (%) and regional LV function, is a continuous variable. General linear mixed modeling techniques will be utilized to assess the effect of treatment on the primary endpoint of the study. Both unadjusted and adjusted treatment effects will be computed; adjustments will be for clinical site as well as for baseline covariates whose association with the dependent variable is generally accepted. In keeping with standard methodology for clinical trials, the primary analysis will compare the randomized study groups.

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. Last observation carried forward analyses will be carried out as supportive evaluations.

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 matter can produce a bias toward the null, this standard LOCF procedure will be adequate for the small number of patients with missing data.

9.4.3 Secondary analyses

The effect of timing of cell adminsration will be evaluated for each of the secondary endpoints. Using general linear model procedure, the effect of cell adminstration on LV mass, end diastolic volume, end systolic volume, and infarct size will be assessed. The analysis variable will be the change in LVEF (either global or regional) from the immediate pre-infusion level to six months. Both unadjusted and adjusted treatment effects will be computed; adjustments will be for baseline covariates whose association with the dependent variable is generally accepted. Logistic regression will be used to assess the effect of cell administration on the combined endpoint of death, reinfarction, repeat revascularization, and hospitalization for HF.

9.4.4 Subgroup evaluations

The effect of subgroup stratum on the relationship between cell delivery and the endpoints (both primary and secondary) will be assessed. If a treatment effect is demonstrated, it is not likely to behave identically among all important subgroups. The subgroups of interest are age, gender, race, LVEF, hypertension, statins, stented vessel, and antiplatelet agents. If we have sufficient patients, an evaluation of the effect of cell delivery within each of the two strata: 1) DES and 2) BMS, will be carried out. These additional analyses can sometimes be helpful in identifying extreme differences in the effects of treatment among subgroups, although the literature wisely warrants that caution be used in interpreting subgroup analyses.

9.5 Additional analyses and new endpoints

The development of cardiovascular cell delivery protocols in CCTRN requires an intelligent choice of an endpoint. The endpoint selections involve choosing from among dichotomous endpoints (e.g., total mortality, nonfatal MI, recurrent cardiovascular hospitalization) and a continuous endpoint that provide a direct clinical assessment (e.g., ejection fraction).

However, the small sizes of these studies, combined with this early, mechanistic examination of the effects of stem cell effects on heart function requires that the Investigators also focus on variables that measure mechanisms of action, e.g., left ventricular ejection fraction (LVEF), end systolic volume, end diastolic volume

and infarct size. While each of these measures meets the pathophysiologic rationale for the selection as an endpoint, and each of them will be measured in their own right, the selection from among them is complicated, as each has advantages and disadvantages.

The DCC is developing new statistical methodologies that each construct a single omnibus statistic measuring the combined effect of cell delivery on both the dichotomous clinical variables and the continuous ones. Each of these procedures: 1) adaptation of multivariate analysis, and 2) modified score statistic following the initial development work (43-45) is being pursued in a separate sub-study.

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 will develop and maintain 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 DCC computer facility. Training will be provided 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.

10.1.2 Access

The DCC will recommend a desktop or a laptop, such as a DELL Inspiron 710M with an Intel Pentium M processor 2.1 GHz, I GB DDR SDRAM memory, 80 GB hard drive, with a Combo DVD + RW and wireless networking and make available, upon approval, software and hardware that will be necessary for the Clinical Center (CC) staff to access and to enter data into the web-based application as well as to generate necessary reports. The system will be available at all times except for occasional systems maintenance.

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 pro-

tected 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 back-up 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 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 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. Reports will be generated as necessary with real-time data.

10.4.1 File transfers

Provisions will be made for those sites 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 Clinical Center's data entry and completeness will be generated. If a Clinical Center has problems, action will be taken from retraining through phone calls to a site visit, if necessary.

10.6 Computing Infrastructure

The University of Texas 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 SC annually.

11.0 HUMAN SUBJECTS

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 study identifiers to maintain subject confidentiality. All computer entry and networking programs will be done using study identifiers 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 subjects are protected. The DSMB reviews recruitment and safety events on a semi-annual basis and provides recommendations to the NHLBI regarding modification or discontinuation of the protocol.

11.4 Informed Consent

11.4.1 Human Subjects Involvement and Characteristics

Participants in this research trial will be recruited from the inpatient cardiology services of the five clinical trial centers of the CCTRN. All patients enrolled in this clinical trial will have presented with an AMI and will have undergone successful percutaneous revascularization of the infarct-related artery. The inclusion/exclusion criteria for the 87 patients to be enrolled in this trial have been described in the Research Design and Methods section. The age of the participants must be greater than 21 years of age. There is no upper age limit. The patients must be clinically stable following their infarction and have developed at least moderate left-ventricular dysfunction. There is no exclusion of any subpopulation with regard to race or gender.

11.4.2 Sources of Material

Eighty to ninety ml (±10 ml) of bone marrow with be harvested from each patient and transported to the Cellular Therapeutic Facility of each center for isolation of the BMMNC (stem cells) and returned to the hospital for administration to the patient on the same day. Data to be recorded 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.

11.4.3 Potential Risks

Alternative forms of stem cell administration to patients following AMI include intravenous administration or direct intramyocardial injections. The intravenous administration of stems cells results in suboptimal retention of stem cell in the

myocardial region (46). The direct myocardial intramyocardial injection of stem cells in humans following AMI has not been performed, in part, due to the potential increased risk of myocardial rupture or pericardial effusion. In the event that a bone marrow aspiration procedure does not yield the target number of mononuclear cells, the cells that are obtained will be injected according to protocol. The same viability and sterility testing will be done on these cells and they will be injected into the patient. Subsequently, the same endpoint analyses will also be done in the patients with less than the target number of cells injected as they will be included in the total cell treated group, and if the numbers are adequate, they will also be evaluated separately.

11.4.3.1 Risks Associated with the Patient Population, Procurement, Processing, and Infusion of the Study Product

Bone Marrow Aspiration

Possible risks of bone marrow aspiration include: bruising, bleeding, infection, hematoma at site of biopsy, brief discomfort in the hip area, and faintness from the procedure.

Patients taking anticoagulation medications at the time of the bone marrow aspiration may experience a temporary interruption in their administration during which time the patient may be at an increased risk of a clinical event (e.g., stroke). The patient should be advised to inform the research team immediately of any symptoms of dizziness, light-headedness, blurred vision, slurred speech, facial drooping, decrease sensations anywhere on his/her body, or weakness or a decrease in strength of the extremities. The patient should be closely monitored during any interruption in anticoagulation therapy, such as the bone marrow aspiration, angiogram, and delivery of study product for the events described below.

Cell Processing Procedure

Processing the cells is done under strict sterile conditions; however, there is a rare chance that the cells could become contaminated while being processed. Testing will be done on the cells, and if the tests reveal contamination, the patient will be notified and instructed on 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 patient notes a fever, he/she will be requested to notify the investigator/study team.

Coronary Angiography Procedure (Cardiac Catheterization)

This procedure includes both risks associated with the use of contrast (dye), the use of radiation, and the insertion of the catheter (tube).

> ***Risks associated with the dye*** include allergic reaction to the chemical dye (although rare, this can include rash or sudden dangerous drop in blood

pressure), kidney failure related to the chemical dye, or emboli from the aorta.

Risks associated with radiation
The amount of research related radiation exposure received from taking part in this study is about the same as that normally received by patients having such cardiac procedures for non-research purposes (approximately 33% of the radiation dose allowed to radiation workers in one year).

Risks associated with the insertion of the catheter include bruising, bleeding, and pseudoaneursm formation. Treatment of a pseudoaneurysm may include blood transfusion, ultrasound guided compression with medication to aid in resolution or surgery. The patient may experience a brief sensation of discomfort or numbness at the catheter insertion site with the insertion of an arterial sheath or catheter. Similar discomforts may be experienced with the removal of the sheath or catheter. In rare cases, injury to the blood vessel where the catheter is placed resulting in infection at the site or a possible loss of function can occur. In addition, arterial dissection, hemorrhage, or thrombosis (requiring repeat angioplasty or stenting) may occur as a result of inserting or removing the catheter.

Study Product Infusion
Risks associated with the study product and its infusion include ECG changes (, potentially requiring medications or electrical shock to the heart to correct), electrical abnormalities (that could require placement of a temporary or permanent pacemaker), significant chest discomfort, pain, or significant ST-segment changes during balloon inflation as described in the European trials. The ischemic duration will be reduced as necessary to accommodate this, but the number of cycles will then be increased so that the total duration of ischemia will remain constant in each patient. There may be decreased blood flow in the small vessels of the heart. More serious risks include myocardial infarction, cerebrovascular accident, emergency open-heart surgery, and death.

Risks in Those with Coronary Artery Disease

Coronary artery disease is a progressive disease. Subjects in these trials may experience worsening of their condition and the possible need for additional medical or surgical intervention. This may include continued or worsening angina, development of new stenosis, congestive heart failure, myocardial infarction, cerebrovascular accident, and death.

11.4.4 Adequacy of Protection Against Risks

11.4.4.1 Recruitment and Informed Consent
Participants in this research trial will be recruited from the inpatient cardiology services of the five clinical trial centers participating in the CCTRN and from affi-

liated sites associated with the Network hospitals. All patients enrolled in this clinical trial will have presented with an AMI and will have undergone successful percutaneous revascularization of the infarct-related artery. Potential subjects will be approached by one of the Investigators or research nurses after discussion with the patient's primary physician. The information provided to the patient is included in the informed consent form. The informed consent will include all of the above mentioned potential risks to participants.

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 Subjects and Others

The administration of autologous BMMNC offers a new therapeutic option to patients following an AMI, the goal of which is to improve LV function and reduce the incidence of developing HF. This proposal offers several significant improvements over the previously published clinical trials in Europe describing this treatment in approximately 900 patients. Importantly, no significant safety issues have been raised with this cell delivery, and thus we believe that the potential risks to the patients remains reasonable in relation to the anticipated benefit of improving cardiac function above which can be obtained with maximal medical therapy.

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 be the first randomized, placebo-controlled trial of cellular delivery following moderate to large acute MI in the United States to assess the role of dosing and late-timing of administration. 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 cell delivery. The risks to the subjects are reasonable in relation to the knowledge gained from this study since this cell delivery may potentially reduce the incidence of HF, 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.

11.4.8 Risk-Benefit Analysis

The administration of autologous BMMNC offers a new therapeutic option to patients following AMI. The goal of this cell delivery is to improve LV function and

reduce the incidence of new HF, a leading cause of morbidity and mortality throughout the world. Having highly trained experts deliver and oversee the cell delivery with close study monitoring substantially reduces the likelihood of AEs. The potential risks to the patients remain reasonably low in relation to the anticipated benefit of improving cardiac function above which can be obtained with maximal medical therapy.

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.

Specifically, Cleveland Clinic has access to 1,479 patients with MI from four proposed network sites. The Minnesota center will recruit from a population of 1,812 patients with AMI. Vanderbilt can recruit from 1,007 patients with AMI. Besides recruiting from itself, the Texas Heart Institute (THI) can recruit from Ben Taub Hospital, DeBakey VA Hospital, Texas Children's Hospital, Herman Memorial Hospital, MD Anderson Hospital, Methodist Hospital, Kelsey-Seybold Hospital, and Baylor Clinic. In addition, THI has a track record of recruiting patients from across the United States.

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. Half the patients will be female. Cleveland Clinic will recruit approximately 62% male, 75% Caucasian, 20% African-American. Vanderbilt will recruit approximately 50% female, 15% Hispanic or Latino, and 15% 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.

12.0 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. CCTRN will work with professional associations to access health care providers like the NHLBI has done for a number of initiatives including asthma and hypertension. CCTRN will use three general dissemination methods that will be tailored for the target audiences.

12.1 Web Site

The web site will be created from the beginning of the project with objectives targeted to the three audiences. The CCTRN web site 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 isn't; 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.

12.2 E-network

To develop a dissemination network or linkage system for the beginning of the research, the Coordinating Center for Clinical Trials (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-service 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.

12.3 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 Clinical Centers (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 pre-

pare 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.

12.4 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.

13.0 REFERENCES

1.) Braunwald E. Congestive heart failure: a half century perspective. Eur Heart J 2001;22:825-836.
2.) Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-705.
3.) Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nature Med 2001;7:430-436.
4.) Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107:1395-1402.
5.) Yoon YS, Wecker A, Heyd L, et al. Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. JCI 2005;115:326-338.
6.) Murry CE, Soopa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428:664-668.
7.) Balsam LB, Wagers AJ, Christensen JL, et al. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004;428:668-673.
8.) Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913-1918.
9.) Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106:3009-3017.
10.) Fernández-Avilés F, San Roman JA, Garcia-Frade J, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circ Res 2004;95:742-748.
11.) Askari AT, Unzek S, Popovic ZB, et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697-703.
12.) Kinnaird T, Stabile E, Burnett MS, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promotes *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. Circ Res 2004;94:678-685.
13.) Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004;364:141-148.
14.) Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute wall infarction. N Engl J Med 2006;355:1199-1209.

15.) Schächinger V, Erbs S, Elsässer A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 2006;355:1210-1221.
16.) Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 2006;367:113-121.
17.) Meyer GP, Wollert KC, Lotz J, et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST Trial. Circulation 2006;113:1287-1294.
18.) Bartunek J, Vanderheyden M, Vandekerckhove B, et al. Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. Circulation 2005;112(Suppl I):I178-I183.
19.) Ge J, Li Y, Qian J, et al. Efficacy of emergent transcatheter transplantation of stem cells for treatment of acute myocardial infarction (TCT-STAMI). Heart 2006;92:1764-1767.
20.) Ruan W, Pan C, Huang G, et al. Assessment of left ventricular segmental function after autologous bone marrow stem cells transplantation in patients with acute myocardial infarction by tissue tracking and strain imaging. Chin Med J. 2005;118:1175-1181.
21.) Abdel-Latif A, Bolli R, Tleyjeh IM, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 2007;167:989-997.
22.) Ma J, Ge J, Zhang S, et al. The time course of myocardial stromal cell-derived factor 1 expression and beneficial effects of intravenously administered bone marrow stem cells in rats with experimental myocardial infarction. Basic Res Cardiol 2005;100:217-223.
23.) Kucia M, Dawn B, Hunt G, et al. Cells expressing early cardiac markers reside in the bone marrow and are mobilized into the peripheral blood after myocardial infarction. Circ Res 2004;95:1191-1199.
24.) Dewald O, Ren G, Duerr GD, et al. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am J Pathol 2004;164:665-677.
25) Assmus B, Honold J, Schächinger V, et al. Transcoronary Transplantation of Progenitor Cells after Myocardial Infarction. N Eng J Med 2006;355:1222-1232.
26.) Assmus B, Fischer-Rasokat U, Honold J, et al. Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic post-infarction heart failure:results of TOPCARE-CHD registry. Circ Res 2007;100:1234-1241.
27.)Schächinger V, Erbs S, Elässer A, et al. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J. 2006;27:2775-2783.

28.) 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;28:766-772.
29.) Dawn B, Bolli R. Bone marrow for cardiac repair: the importance of characterizing the phenotype and function of injected cells. Eur Heart J 2007;28:651-652.
30.) Arnesen H, Lunde K, Aakhus S, Forfang K. Cell therapy in myocardial infarction. Lancet 2007;369:2142-2143.
31.) Heeschen C, Lehmann R, Honold J, et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation 2004;109:1615-1622.
32.) Tepper OM, Galiano RD, Capla JM et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002;106:2781-2786.
33.) Wu KC, Zerhouni EA, Judd RM, et al. Prognostic significance of microvascular obstruction by magnetic resonance imaging in patients with acute myocardial infarction. Circulation 1998;97:765-772.
34.) Homboch V, Grebe O, Merkle N, et al. Sequelae of acute myocardial infarction regarding cardiac structure and function and their prognostic significance as assessed by magnetic resonance imaging. Eur Heart J. 2005;26:549-557.
35.) Tarantini G, Cacciavillani L, Corbetti F, et al. Duration of ischemia is a major determinant of transmurality and severe microvascular obstruction after primary angioplasty: a study performed with contrast-enhanced magnetic resonance. JACC 2005;46:1229-1235.
36.) Schwartz RS. Microvascular obstruction in acute coronary syndromes: onward to a new therapeutic target. Catheter Cardiovasc Interv 2005;66:170-172.
37.) Meier P, Zbinden R, Togni M, et al. Coronary collateral function long after drug-eluting stent implantation. JACC 2007;49:15-20.
38.) Vulliet PR, Greely M, Halloran SM, et al. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. Lancet 2004;363:783-784.
39.) Henry TD, Sharkey SW, Burke N, et al. A regional system to provide timely access to percutaneous coronary intervention for ST-elevation myocardial infarction. Circulation 2007;116:721-728.
40.) Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol 2002;90:29-34.
41.) Natori S, Lai S, Finn JP, et al. Cardiovascular function in Multi-Ethnic Study of Atherosclerosis: normal values by age, sex, and ethnicity. Am J Roentgenol 2006;186:S357-S365.
42.) Cerquiera MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: a statement for healthcare professionals from the cardiac imaging committee

of the Council on Clinical Cardiology of the American Heart Association. Circulation 2002;105:539-542.

43.) Moyé LA for the SAVE Cooperative Group. Rationale and design of a trial to assess patient Survival and Ventricular Enlargement after myocardial infarction. Am J Cardiol 1991;68:70D-79D.

44.) Moyé LA, Davis BR, Hawkins CM. Analysis of a Clinical Trial Involving a Combined Mortality and Adherence Dependent Interval Censored Endpoint. Stat Med 1992;11:1705-1717.

45.) Pfeffer MA, Braunwald, E, Moyé LA, et al. on behalf of the SAVE Investigators (1992). Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction - results of the Survival and Ventricular Enlargement Trial. N Eng J Med 1992;327:669-677.

46.) Hofmann M, Wollert KC, Meyer GP, et al. Monitoring of bone marrow cell homing into infarcted human myocardium. Circulation 2005;111:2198-2202.

47.) Center for Cardiovascular Biology and Atherosclerosis, University of Texas Health Science Center at Houston; Heart Failure Research Lab, Texas Heart Institute.

48.) 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.

49.) Drs. Geng and Willerson; UT99-117 study.

50.) Thygesen et al. Universal definition of myocardial infarction. J Am Coll Cardiol 2007;50:2173-2195.

51.) Huikuri H, Kervinen K,Niemelä M,Ylitalo K,Säily M, Koistinen P, Savolainen E-R, Ukkonen H, Mikko Pietilä, Airaksinen JKE, Knuuti J, and Mäkikallio TH for the FINCELL Investigators (2008). Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction.29: 2723-2732

APPENDIX 1

New York Heart Association (NYHA) Classification

Class	Patient Symptoms
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 2 -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, Betadine 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 (±10ml) 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 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 takes 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 infusion site.

Late TIME 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 **Date**
CCTRN Data Coordinating Center