Published in final edited form as:
Am Heart J. 2009 September ; 158(3): 356–363. doi:10.1016/j.ahj.2009.06.009.

Rationale and Design for TIME: A Phase-II, Randomized, Double-Blind, Placebo-Controlled Pilot Trial Evaluating the Safety and Effect of Timing of Administration of Bone Marrow Mononuclear Cells Following Acute Myocardial Infarction

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Abstract

Several previous studies have demonstrated that administration of autologous bone marrow-derived mononuclear cells (BMMNCs) improve cardiac function in patients following acute myocardial infarction (AMI). However, optimum timing of administration has not been investigated in a clinical trial. The Cardiovascular Cell Therapy Research Network (CCTRN) was developed and funded by the NHLBI to address important questions such as timing of cell delivery and to accelerate research in the use of cell-based therapies. The TIME trial is a randomized, Phase II, double-blind, placebo-controlled clinical trial. The five member clinical sites of the CCTRN will enroll a total of 120 eligible

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ClinicalTrials.gov#NCT00684021

patients with moderate-to-large anterior AMIs who have undergone successful PCI of the LAD coronary artery, and have an LVEF ≤45% by echocardiography. Participants will have bone marrow aspirations and intra-coronary infusions of 150×10^6 BMMNCs or placebo on day 3 or day 7 post-AMI. Objectives of this study are 1) To evaluate effects of BMMNCs on regional and global left-ventricular (LV) function compared to placebo therapy in patients with acute AMI as assessed by cardiac magnetic resonance imaging (cMRI) at 6 months, and 2) To assess whether effects of BMMNC infusion on global and regional LV function and safety are influenced by the time of administration. This study will provide further insight into the clinical feasibility and appropriate timing of autologous BMNNC therapy in high-risk patients following AMI and PCI.

Introduction

The development of new strategies to improve left-ventricular (LV) function following acute myocardial infarction (AMI) has been a prominent goal for cardiovascular investigation. Although endogenous repair mechanisms appear limited in humans, studies in animal models have demonstrated that myocardial function can be significantly improved with bone marrow-derived stem cells following experimental AMI (1–4). Although data supporting significant myocardial regeneration in these preclinical studies has not been uniform (5,6), it has led to a number of clinical trials testing the strategy that delivery of autologous bone marrow-derived mononuclear cells (BMMNCs) into the infarct region following AMI may improve LV function (7–10).

Meta-analyses of AMI stem cell trials (11,12) have confirmed that BMMNC administration appears safe over several years of follow-up, and results in a small, but statistically significant improvement in LV ejection fraction (LVEF). Additionally, it appears that cell therapy may attenuate LV remodeling to a limited degree providing hope that further improvements in this therapy could eventually reduce the incidence of heart failure. Despite this significant progress, answers to basic questions such as the effect of cell type and dose have not been addressed, and no trial to date has been sufficiently powered to determine the optimal time to administer cells in the post-AMI period.

Timing of cell administration may play a key role in determining the benefit of cell therapy given the temporal changes that occur in the myocardium in the days following AMI that may affect stem cell efficacy and survival. Increased expression of chemokines such as stromal derived factor one (SDF-1) immediately post-AMI may augment stem cell homing and differentiation (13). Conversely, development of a vigorous inflammatory response coupled with the release of reactive oxygen species and cytokines, such as TNF-alpha in the infarct region in the days following an AMI may adversely affect cell survival (14).

In light of the relative paucity of mechanistic studies into important questions, such as timing of cell delivery, the National Heart, Lung, and Blood Institute (NHLBI) established the Cardiovascular Cell Therapy Research Network (CCTRN) to accelerate research into the use of cell-based therapies for the management of cardiovascular diseases. The Transplantation in Myocardial Infarction Evaluation (TIME) study is a Phase II trial developed by the CCTRN to provide further research into the efficacy, safety, and most appropriate timing of autologous BMMNCs in high-risk, post-AMI patients.

Organizational Structure and Oversight

CCTRN was established by the NHLBI to develop, coordinate, and conduct multiple collaborative protocols testing the effects of stem cell therapy on cardiovascular disease. The Network builds on contemporary findings of the cell therapy basic science community, translating newly acquired information to the cardiac clinical setting in the Phase I/II study

paradigm. The Network consists of five clinical research centers (Cleveland Clinic Foundation, University of Florida, Minneapolis Heart Institute Foundation / University of Minnesota, Texas Heart Institute and Vanderbilt University), a data coordinating center (DCC) (University of Texas School of Public Health) that provides trial management and data analysis, a cell processing quality control center and six core laboratories. Together, these Network components provide standardization of cell therapy preparation and endpoint measurements. All clinical centers participate in the selection and design of Network protocols that are also reviewed by an independent Protocol Review Committee (PRC) and a Gene and Cell Therapies Data Safety and Monitoring Board (DSMB) under the aegis of the NHLBI. Each clinical center and the DCC have independent Institutional Review Boards (IRB) approvals and oversight. By recruiting from multiple centers, the Network accelerates the speed with which its studies can be completed, increases the generalizability of study findings, and improves the dissemination of its findings to influence public health.

Objectives and Design

The objectives of this study are 1) to evaluate the effect of a single intracoronary infusion of autologous BMMNCs on regional and global LV function when compared to control therapy in patients with an acute anterior MI as assessed by cMRI, and, 2) to assess whether the effect of this BMMNC infusion on regional and global LV function is influenced by whether it is given at 3 versus 7 days post-AMI. The primary outcome will be change in regional function (i.e.segmental shortening, thickening and radial displacement) and global LV function (i.e., LVEF) at six months compared to baseline as measured by cMRI. Patients will be followed for two years to evaluate the effect of therapy on the clinical events of death, repeat revascularization, MI and hospitalization for CHF. A secondary objective will be to examine the effects of cell phenotype on therapeutic efficacy through ancillary studies at a Biorepository Core.

Hypotheses and Study Power

The primary hypotheses of the TIME study are that, as compared with placebo therapy, 1) administration of cell therapy will improve global and regional LV function, and 2) this improvement will depend on the timing of cell delivery. The secondary hypotheses are that, in comparison with control therapy, administration of cell therapy will result in smaller end-diastolic and end-systolic volumes, and a lower incidence of the composite adverse events of death, reinfarction, repeat revascularization, and hospitalization for CHF.

Enrollment and Study Population

This study is a randomized, double-blind, placebo-controlled clinical study of autologous BMMNC administration to 120 patients following AMI. Patients will be randomized to a 2:1 treatment versus placebo therapy ratio at each of the two time points. Patients enrolled in this study will be recruited from each of the five sites participating in the CCTRN. Enrollment will be limited to patients with moderate-to-large anterior infarctions with no prior history of coronary artery bypass grafting and whose LVEF following PCI is ≤45%. Because of the experimental nature of cell therapy and limited long-term safety data, only patients who are at increased risk of death or major adverse events, including recurrent MI or development of CHF, will be eligible for cell therapy administration. All prospective patients will be screened and enrolled in the trial after meeting inclusion / exclusion criteria (Table 1) and signing both the informed consent and HIPAA forms.

Randomization to the Timing of Administration

The clinical center will electronically transmit eligibility criteria to the DCC, at which time a computer-generated scheme will randomly allocate eligible patients (1:1) to an intervention time group (3 or 7 days post-PCI, with day zero defined as the day of incident PCI). This randomization will be not be blinded, and participants will be stratified by clinical center. Patients randomized to Day 3 therapy must receive cMRI, bone marrow aspirations, cell processing, and therapy infusions on the third day post PCI. Patients randomized to Day 7 therapy receive a Day 3 cMRI and have bone marrow aspirations, a repeat cMRI, cell processing, and cell therapy infusions on Day 7 post-PCI. An adjustment of one day is permitted should the assigned day of administration fall on a weekend or holiday.

Bone Marrow Aspiration and Cell Processing

On the morning of the study product administration, patients will undergo bone marrow aspiration in accordance with standard operating procedures developed by each CCTRN site. Approximately 80–90 mls of bone marrow are aspirated under appropriate anesthesia from the iliac crest using standard techniques and transported to the institution's cell processing lab. Each site will utilize the Sepax System (Biosafe, Eysins, Switzerland) for BMMNC isolation. This approved closed system device for cord blood processing produces a faster isolation and more uniform cellular product (15). This will be the first cardiovascular stem cell trial to utilize this method of cell isolation.

The cells will be harvested and washed three times in heparinized phosphate buffered saline (PBS) before resuspension in PBS supplemented with 5% human albumin. The composition of $CD34^+$, $CD45^+$ and $CD133^+$ cells will be determined by fluorescent activated cell sorting (FACS) analysis. Viability of the cells will be determined by Trypan Blue exclusion; and $\geq$70% viability will be required before transplantation. A 14-day sterility culture, CFU Assay and endotoxin analysis will be performed on the final product. Because 14-day sterility testing will not be available prior to the product's infusion, a negative Gram stain will be required before the product is released (Table 3). Products are labeled and tracked with adhesive labels containing the patient's name and hospital identification number.

Approximately 150–200 million nucleated cells can be routinely harvested with this volume of bone marrow. Target dose will be determined using a hematology analyzer. Because the specific cell type(s) responsible for the previously observed biologic effect in the infarct zone has not been identified, unfractionated BMMNC will be used. Characteristics of the specific population of cells administered in this study will be investigated by the CCTRN Biorepository and correlated with major outcomes to help address this question. Although autologous cells will be used in this protocol, standard tests for infectious diseases, including 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) will be performed. Cells testing positive for infectious disease markers will be labeled as infectious and quarantined while in the Clinical Cell Therapy Laboratory facilities. If any test is positive, the patient will be notified of the result via the Medical Director, Principal Investigator (PI), and patient physician within 48 hours for appropriate clinical action.

After the product has passed the prospectively described release criteria, an unblinded cell processor will enter the data into a web-based computer application that transmits the data to the DCC. The patient will then be assigned to cell therapy or placebo intervention. Patients randomized to cell therapy will receive 150 million cells. Any patient randomized to the cell therapy arm whose BM aspiration produces less than the target dose will receive all the available cells harvested. Cells in excess of 150 million will be sent to the CCTRN Biorepository core labs at the University of Florida and the University of Minnesota for cell

characterization. Patients who are randomized to receive placebo therapy will receive 5% Human Serum Albumin in an identical volume of saline with a 100 μl of blood matching the appearance of an active cell preparation and thereby blinding the identity of the infusate being delivered. Their cells will be sent to the Biorepository core lab.

Infusion of Cellular Product

The final cellular product or placebo will be infused within six to twelve hours after bone marrow aspiration (total volume=30 ml). The patients are heparinized to an ACT > 200 seconds, and the infusate is delivered via an over-the-wire PTCA catheter in six aliquots (five ml), each delivered over two minutes of balloon inflation within the previously placed stent at low pressure (3–4 atm). Two minutes of reperfusion will occur following each cycle of cell infusion. Patients are routinely discharged the following day and they are advised to take aspirin and 75 mg of clopidogrel for 24 months, as well as the usual post-AMI care medications. Patients with LVEFs <40% will be advised to take an aldosterone antagonist unless contraindicated by creatinine ≥ 2.5 or potassium ≥ 5.0. Follow-up schedules will follow a pre-specified plan (Table 2).

Safety Monitoring

All CCTRN participants will be closely monitored for adverse events and this information will be transmitted to Institutional Review Boards (IRBs) of each center by the DCC; to the FDA, through the University of Texas Health Science Center (UTHSC)-held IND, and by the DSMB. The DSMB will meet at least twice yearly to review performance of the participating sites, to assess accruing safety data, and ascertain feasibility of continuation of the study. Monthly safety and performance reports will be provided to the DSMB chair, the NHLBI Program Office, and the CCTRN Steering Committee Chair.

In addition, the DCC, under the direction of its Medical Director, will oversee and coordinate collection, standardization, integration, and analysis of study data from the various study components (enrolling sites and core facilities) and the preparation and distribution of the required reports to each of the safety oversight entities. The DCC will facilitate and monitor regulatory and safety compliance at each site and core laboratory and will conduct site visits to each site and core laboratory to assure protocol adherence and regulatory compliance, both on a regular basis and for cause.

Determination of Outcomes

A 1.5T cMRI scanner will be used, with precise magnetic resonance imaging protocols developed by the MRI core laboratory. Since resolution of myocardial stunning and improvement in global and regional LV function often continues to occur between day 3 and day 7, all patients will undergo baseline cMRI measurements at day 3. Commercial Siemens *Argus* analysis software will be used for measurement of global left ventricular myocardial mass, volumes, and LVEF. Regional systolic wall motion, thickening and radial displacement in the infarct and border zones will be determined. Areas of microvascular obstruction (MVO), infarct size and degree of transmurality will be quantified by delayed, contrast (gadolinium) enhanced MR imaging.

Wall Motion Imaging

Both global and segmental left ventricular function will be obtained using a steady-state free-precession (SSFP) or fast gradient echo technique. Long axis cine images in the 2-chamber and 4-chamber projections will be acquired. In addition, a set of contiguous short axis slices (8–10mm thick) will be obtained from the mitral valve annulus through the apex of the left ventricle thoughout the cardiac cycle. Data will be analyzed using the Cardiovascular

Angiography Analysis System/Magnetic Resonance Ventricular analysis (CAAS/MRV) software (PIE Medical Imaging BV, Maastricht, The Netherlands). Global parameters assessed will include: end-diastolic volume, end-systolic volume, stroke volume, ejection fraction, and left ventricular mass. Volumetric measurements will be performed by direct planimetry on the contiguous short axis images at both end-systole and end-diastole. Regional measurements will include wall thickening and wall motion, and will be calculated using 100 chords spaced every 3.6° originating from the centroid of the left ventricle. Regional data will be reported using the AHA 17-segment model. The minimum spatial and temporal resolution requirements of the SSFP sequence are 2.5 × 2.5 millimeter voxels and 40 milliseconds, respectively.

Baseline Perfusion Imaging

A 2-chamber long-axis cine image will be obtained based on axial scout images with the imaging plane spanning the center of the mitral valve coaptation point and through the apex of the left ventricle. Based on this, a 4-chamber long-axis cine image will be obtained. Subsequently a T1-weighted gradient-echo baseline perfusion sequence will be performed using intravenous Gadolinium (e.g., Gadolinium-DTPA). Three short-axis slices will be obtained (positioned from the 2-chamber and 4-chamber cine images) to encompass the basal, middle, and apical thirds of the left-ventricle during a bolus administration of Gadolinium (0.15 – 0.2 mmol / kg). Imaging will be acquired for a total of 60 dynamics per slice ensuring that the passage of contrast material through the myocardium is captured for semi-quantitative analysis.

Viability Imaging

Fifteen to twenty minutes following administration of gadolinium contrast agent, delayed-enhancement imaging will be performed with a T1-weighted inversion-recovery prepared gradient-echo sequence (DE-MRI). The inversion delay time will be iteratively adjusted for optimal nulling of normal myocardium. Contrast-enhanced viability imaging will be performed with two techniques: the standard 2D technique, which acquires a single slice each breath hold, will be performed in the short-axis projections using the same plane prescription as the functional short axis cine series; and a high-resolution 3D technique will be used to acquire 10 short-axis slices during a single breath hold. Regions of irreversible myocardial damage are manifested by “hyperenhancement” (bright white areas) on the images, while normal and/or viable tissue is “nulled” (black) on the acquired images. The presence, location, and extent of irreversibly damaged tissue will be qualitatively and quantitatively assessed on a segmental basis. Pre- and post-therapy imaging, both cine wall motion and DE-MRI, will be carefully matched for consistency and accuracy using internal landmarks including the insertion sites of the right ventricular freewall and the papillary muscle insertions.

Statistical Methods

The TIME trial is a two-factor experiment. The two factors are therapy (active versus placebo therapy) and timing (3 days versus 7 days). The principal interest is whether the effect of cell administration timing influences the relationship between BMMNC infusion and cardiac function. Hypothesis testing for each of the primary endpoints will be carried out at the 0.05 level in this Phase II study. A total of 120 patients provides satisfactory power for the assessment of the overall effect of BMMNC administration compared to control for each of the two components of the primary endpoint (global and regional function) and permit an adequately powered inquiry into the influence of timing for each of the two co-primary endpoints. Assuming independence and normality of the observations, the sample size is calculated using the normal approximation to the two sample *t*-test statistic. Final sample sizes were increased by 5% to compensate for patients who are lost to follow-up. The sample size of 120 patients is required to detect an absolute change of 5 absolute units in global ejection

fraction, and 7 absolute units in regional ejection fraction with 80% power at the 0.05 level. No correction for multiple comparisons will be made in this Phase II study.

Analyses

The compatibility of baseline characteristics between the two treatment groups will be ascertained using standard normal tests (including t-tests) for continuous variables and exact testing for categorical variables. The analysis variable will be the change in LVEF (one primary endpoint is global, the second is regional) from the immediate pre-infusion level (Day 3 MRI in patients randomized to Day 3 therapy, and Day 7 in patients randomized to Day 7 therapy). Since each primary endpoint, global LVEF(%) and regional LV function is a continuous variable, general linear mixed modeling will assess the effect of treatment on the primary endpoint of the study. In keeping with standard methodology for clinical trials, the primary analysis will compare the randomized study groups. 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. Last observation carried forward (LOCF) procedures will be followed for patients who do not have a six month evaluation.

Both the effect of cell administration and the effect of the timing of cell administration will be evaluated for each of the secondary endpoints using procedures carried out for the primary evaluation. Logistic regression will be used to assess the effect of cell administration on the combined endpoint of death, reinfarction, repeat revascularization, and hospitalization for CHF.

The effect of subgroup stratum on the relationship between timing and timing's influence on the cell delivery-endpoint (both primary and secondary endpoint) relationship 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, hypertension, diabetes mellitus, MVO, statins, stent type (drug eluting stent versus bare metal stent) and LVEF (Table 4).

Discussion

Recent findings in animals that stem cell therapy significantly reduces the development of LV dysfunction following MI (1–4) have been rapidly translated into clinical trials using a variety of cell types including intra-coronary BMMNC administration (7–10). Importantly, these trials have demonstrated that cell delivery following AMI is safe over several years of follow-up and meta-analyses review of these trials have found a small, but significant improvement in LV function (11,12). However, despite these encouraging findings, many fundamental questions in cell therapy have not been addressed, including the important questions of optimal cell type, dose, and the timing of cell delivery post-AMI (16). The CCTRN was formed to address many of these unresolved issues, and this is the first clinical cell therapy trial sufficiently powered to focus on the important question of timing of cell delivery post-AMI.

The timing of cell delivery following AMI is likely to be a critical factor in determining the efficacy of cell therapy due to the temporal changes that occur in the myocardium in the early days following AMI. These include the expression of growth factors and cytokines that may both promote cell survival and angiogenesis, or conversely, encourage myocyte apoptosis and adverse LV remodeling. Expression of chemokines, such as SDF-1 (13) that may aid in stem cell homing, are up-regulated in the infarct zone in the first few days following AMI. This is consistent with recent findings in patients demonstrating the homing of radiolabelled progenitor cells to the infarct zone being greatest in the first few days following AMI (17) where their secretion of vascular endothelial growth factor (VEGF) and insulin growth factor (IGF)-1 may improve perfusion and reduce apoptotic cell death in the infarct border zone

(18) Conversely, a strong inflammatory reaction and release of reactive oxygen species in the infarct zone may adversely affect survival of the injected cells. Additionally, there may be changes in the quality and quantity of harvested stem cells between the Day 3 and 7 time-point given the egress of cells from the bone marrow that occurs in this time window following AMI (19). These factors among many, suggest that timing of stem cell administration post-AMI may be a critical component in dictating efficacy.

In the majority of published randomized clinical trials (7–10), BMMNCs were administered between one and seven days post-AMI, but timing was not integrated into the randomization scheme. As a result, the designs of these trials were not sufficient to define the optimum timing of cell delivery following AMI. In a subgroup analysis, the REPAIR-AMI trial (7) suggested that cell administration between 5 and 7 days was optimal in regards to recovery of LVEF. However, the ASTAMI trial (10) administered BMMNCs in a similar time window and found no improvement in left-ventricular ejection fraction. However, this difference in outcomes may have also occurred due the different isolation procedure utilized in the ASTAMI trial (Lymphoprep) that may have resulted in reduced stem cell efficacy (20).

The number of infused cells administered to patients post-AMI has varied significantly between the randomized trials, with differences up to several orders of magnitude (7–10). Furthermore, the number of delivered cells within each trial has rarely been uniform, with some patients receiving up to three times the number of cells compared to other patients within the same trial. The failure to deliver a consistent cell dose remains a limitation of these trials. Only one published trial has attempted to address the issue of dose on its effects on LVEF (21). In that trial, 44 patients were randomized to 10 versus 100 million BMMNCs administered 5 to 9 days post-AMI where a slightly greater improvement in LVEF was observed in the high-dose cohort (5% vs 3%). A recent meta-analysis found no effect between the number of cells infused and recovery of LVEF (11). However, a second meta-analysis (12) suggested that improvement in LVEF with BMMNC administration was dependent on the infusion of at least 100 million cells. Although the TIME trial is not a dose-ranging study, it will be the first major trial to administer the same number of cells to all of its patients (150 million), thus eliminating a potential variable that has not been controlled for in earlier trials.

The near exclusive use of autologous cells in cardiovascular cell therapy trials offers many important advantages from an immunologic and safety standpoint. However, recent research also suggests that the intrinsic efficacy of the cellular product may decline with age and important co-morbidities such as diabetes, etc. (22,23) that are common to the population being studied. The CCTRN is committed to the systematic exploration of cell phenotypes by the establishment of a Biorepository that will perform migration assays, measure nitric oxide and cytokine production and characterize important receptor subtypes. In this randomized, Phase II, double-blind, controlled study, a well-defined and translatable cell product and dose will be utilized in a relatively high risk population. The timing of cell 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. Although this study is not designed to make head-to-head comparisons among cell types, it will generate a foundation for future studies to build upon within the CCTRN.

Acknowledgments

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents. This study is supported by the National Heart, Lung, and Blood Institute (NHLBI), U01 HL087318-01, and by the Production Assistance for Cellular Therapies (PACT), N01-HB-37164. PTCA and guide catheters and wires were generously supplied by the Boston Scientific Corporation (Natick, MA). Technical support for the Sepax System was provided by Biosafe (Eysins, Switzerland).

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Table 1

Inclusion and Exclusion Criteria for TIME

Inclusion Criteria

1) Patients at least 21 years of age
2) Patients with first acute anterior MI with successful primary percutaneous coronary intervention (PCI) in the LAD artery at least 2.5 mm in diameter within 24 hours of onset of symptoms.
3) Hemodynamic stability as defined as no requirement for IABP, inotropic or blood pressure supporting medications.
4) Ejection fraction following reperfusion with PCI ≤45% as assessed by echocardiography.
5) Consent to protocol and agree to comply with all follow-up visits and studies.
6) Women of child bearing potential willing to use an active form of birth control.

Exclusion Criteria

1) History of sustained ventricular arrythmias not related to their AMI (evidenced by previous Holter monitoring and/or medication history for sustained ventricular arrhythmias in patient's medication chart).
2) Requires CABG or PCI due to the presence of residual coronary stenosis >70% luminal obstruction in the non-infarct related vessel (Additionally PCI of non-culprit vessels may be performed prior to enrollment).
3) History of any malignancy within the past five years excluding non-melanoma skin cancer or cervical cancer in-situ.
4) History of chronic anemia (hemoglobin (Hb) <9.0 mg/dl).
5) History of thrombocytosis (platelets >500k).
6) Baseline platelet count (prior to revascularization) < 120,000 or known history of thrombocytopenia.
7) Known history of elevated INR (PT) or PTT.
8) Life expectancy less than one year.
8) History of untreated alcohol or drug abuse.
9) Currently enrolled in another investigational drug or device trial
10) Previous CABG.
11) Previous MI or history of non-ischemic cardiomyopathy resulting in LV dysfunction (LVEF <55%)
12) History of stroke or transient ischemic attack (TIA) within the past six months.
13) History of severe valvular heart disease (aortic valve area < 1.0 cm^2 or > 3+ mitral regurgitation).
14) Pregnancy or breast feeding
15) Subjects with a known history of HIV, hepatitis B or C infection, or TB positive therapy.
16) Patients with active inflammatory or autoimmune disease on chronic immunosuppressive therapy.
18) Contraindications to cMRI.
19) Previous radiation to the pelvis with white blood cell count (WBC) and platelet counts below hospital specific normal values.
20) Women of child-bearing potential not willing to practice an active form of birth control.
21) Hepatic dysfunction as defined by: Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥3 times the upper limit of normal (ULN); or, Total bilirubin ≥2 times ULN with AST or ALT ≥2 times ULN.
22) Chronic renal insufficiency as defined by a creatinine ≥ 2.0 mg/dl or requires chronic dialysis.
23) The revascularized vessel is not patent at the time cell administration is to be attempted.
24) Patient with two or more of the following criteria will be excluded from the trial, unless the LVEF is less than 30%:
1. Onset of symptoms to treatment PCI< 2 hours
2. Peak CK < 1500 IU/ml
3. Absence of q-wave on Day 1 EKG (post stenting).
4. Age < 45 years

Table 2

Schedule of Procedures in TIME (Day 0 is the day of revascularization)

	Day 1 or 2 (Consent)	Day 3/7 (SPI)	Day 4/8	Month 1	Month 3	Month 6	Month 12	Month 24
Complete medical hx	X							
Incremental medical hx		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			X	X	X	X	X
ECHO	X					X		
EKG	X		X	X		X		
Bone marrow aspiration		X						
Biorepository blood draws		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 evaluations		X	X	X	X	X	X	X
Telemetry (18–24 hrs post SPI)		X						
Holter				X				

* In women of childbearing age

Table 3

Final Product Release Criteria Testing

Assay	Test Method	Specification
1. Product Release Specifications		
Rapid Sterility	Gram Stain Endotoxin	No organisms
Viability	Trypan Blue	≥70%
Endotoxin	EndoSafe PTS	< 5 EU/kg
TNC	Manual or Automated	Not more than 150 × 10^6
2. Post-Production Monitoring		
Immunophenotyping	Flow Cytometry	Report
CFU	Per Site SOP	Report
Sterility	14 day culture	No Growth

Table 4

Outcomes in TIME; active versus placebo comparisons

			Measurement	Method of Measurement
I.	**CO-PRIMARY**			
			Δ Global LV Function	cMRI
			Δ Regional LV Function	cMRI
II.	**SECONDARY**			
		A.	Composite of :	
			Death	Clinical outcome
			Reinfarction	Clinical outcome
			Repeat revascularization	Clinical outcome
			Hospitalization for HF	Clinical outcome
		B.	LV Mass*	cMRI
		C.	LVEDV*	cMRI
		D.	LVESV*	cMRI
		E.	Infarct size	cMRI
III.	**Additional/ Subgroups**	Age, gender, race, hypertension history, diabetes mellitus, statin use, DES/BMS use		Baseline characteristic
		MVO		cMRI