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Rationale and Design for the Intramyocardial Injection of Autologous Bone Marrow Mononuclear Cells for Patients with Chronic Ischemic Heart Disease and Left Ventricular Dysfunction Trial (FOCUS)

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Abstract

Background—The increasing worldwide prevalence of coronary artery disease (CAD) continues to challenge the medical community. Management options include medical and revascularization therapy. Despite advances in these methods, CAD is a leading cause of recurrent ischemia and heart failure, posing significant morbidity and mortality risks along with increasing health costs in a large patient population worldwide.

Trial Design—The Cardiovascular Cell Therapy Research Network (CCTRN) was established by the National Institutes of Health to investigate the role of cell therapy in the treatment of chronic cardiovascular disease. FOCUS is a CCTRN-designed randomized Phase II, placebo-controlled clinical trial that will assess the effect of autologous bone marrow mononuclear cells delivered transendocardially to patients with left ventricular (LV) dysfunction and symptomatic heart failure or angina. All patients need to have limiting ischemia by reversible ischemia on SPECT assessment.

Results—After thoughtful consideration of both statistical and clinical principles, we will recruit 87 patients (58 cell treated and 29 placebo) to receive either bone marrow–derived stem cells or placebo. Myocardial perfusion, LV contractile performance, and maximal oxygen consumption are the primary outcome measures.

Conclusions—The designed clinical trial will provide a sound assessment of the effect of autologous bone marrow mononuclear cells in improving blood flow and contractile function of

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the heart. The target population is patients with CAD and LV dysfunction with limiting angina or symptomatic heat failure. Patient safety is a central concern of the CCTRN, and patients will be followed for at least 5 years.

The Rationale for FOCUS

Coronary artery disease (CAD) remains the single largest killer of Americans, producing myocardial infarctions and heart failure (HF).1 Recent research has delivered substantial improvements in medical therapy and coronary artery revascularization reducing coronary heart disease mortality.2 However, despite advances in medical and revascularization therapy, CAD is a leading cause of HF, as well as angina, bearing its own increased morbidity and mortality risks and health costs in an enlarging patient population. Seven million heart attack hospitalizations in the US have generated almost 5 million patients living with HF who face end-stage HF with its 5-year mortality of approximately 50%.3›4 Because of the burden faced by these patients with limited options, investigation of alternative treatments are needed, e.g., therapeutic angiogenisis, designed to improve myocardial perfusion and anginal symptoms, as well as left ventricular (LV) systolic function. One potential treatment strategy is the use of bone marrow–derived mononuclear cells (BMMNCs) in the treatment of patients with ischemic cardiomyopathy.

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) 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 Board (IRB) approvals and oversight. By recruiting from multiple centers, the Network accelerates the time for study completion, increases the generalizability of study findings, and improves dissemination of public health related findings.[5]

Background of Cardiovascular Cell Therapy and Myocyte Replacement

A type of “adult stem cells”, BMMNCs, have been intensively studied as potential therapy that could enhance perfusion in an injured area of the heart and help repair injured tissue in humans.[6] Early evidence revealed that bone marrow cells differentiate into endothelial cells associated with angiogenesis[7,8,9,10] and into cardiomyocytes.11›12›13 Others have shown the importance of cell isolation techniques.[14] In addition, isolation techniques optimizing functional viability (e.g., ability of cells to form colonies) critically affect the impact of the transplanted cells on LV ejection fraction (LVEF).13 Recently, Yeh, Willerson, et al. have demonstrated that fusion between myocytes and circulatory adult progenitor cells can produce a new generation of myocytes.15›16 Endothelial cell generation follows a separate, direct differentiation.17 In addition, other investigators have shown the benefit of using autologous human BMMNCs for treatment of patients with acute ST segment elevation

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myocardial infarction (STEMI) undergoing percutaneous coronary intervention.[18,19,20,21,22,23]

Preliminary clinical findings

The work of Assmus et al.[24] demonstrated the potential benefits of cell therapy in a population of HF patients (Table 1), and both Tse et al.25 and van Ramshorst et al.[26] demonstrated the feasibility of using a NOGA Myostar catheter (Cordis, Miami Lakes, Florida) for direct delivery of cells to the myocardium.

The Perin, Willerson, et al. evaluation was the first study in patients with chronic and multivessel CAD and severe HF in which patients were treated with autologous BMMNCs administered by direct transendocardial injections into reversibly injured myocardium using a NOGA catheter.[27] The NOGA system allows electromechanical maps of the LV to be obtained, thereby permitting online diagnosis of myocardial viability by measuring endocardial electrical activity. This study demonstrated that the delivery of 30 million BMMNCs was associated with improvements in myocardial perfusion, maximal oxygen consumption [VO_2(max)], and end systolic volume (ESV) in treated patients.[27] These same authors have also completed a randomized trial in patients with severe HF and multivessel CAD providing safe transendocardial injections of cells with a NOGA catheter,28 representing the first FDA approved trial in patients with severe HF in the U.S. These preliminary studies demonstrated the safety of NOGA catheter use in this population. The results of cell delivery in the early clinical experience and previous preclinical studies suggest a benefit on ventricular function and myocardial perfusion that may result from some combination of direct and paracrine effects. The current protocol represents an extension of the former studies using higher doses of cells and refined endpoints in a multicenter environment.

Design of FOCUS

Objectives and hypotheses

The objective of **F**irst M**o**nonulcear **C**ells injected in the **US** (FOCUS) is to evaluate the safety and efficacy of NOGA guided autologous BMMNC injections in adult patients with CAD and ischemic LV dysfunction and angina, HF, or both, who are not candidates for other revascularization procedures. The hypothesis is that BMMNCs injected directly into reversibly injured areas of the heart in patients with ischemic LV dysfunction (LVEF ≤45%), and either limiting angina (Class II to IV) or symptomatic HF (NYHA Class II to III), will improve myocardial perfusion (using VO_2[max]), regional ventricular function (using echocardiography and single-photon emission computed tomography [SPECT]), and symptoms (Table 2). This clinical trial is registered at http//:www.clinicaltrials.gov (NCT00824005).

Study population

FOCUS is a randomized, Phase II, double-blind clinical trial. Hemodynamically stable patients age ≥18 years with significant CAD, reduced LV systolic function (LVEF ≤ 45%) and either limiting angina (Canadian Class II to IV) and/or symptomatic CHF (NYHA class II-III) will be eligible for enrollment (Tables 3a, 3b). Participants must be symptomatic despite maximal medical therapy. Participants must also have documented evidence of reversible myocardial ischemia by SPECT. Finally, participants must be determined not to be suited to any other type of coronary revascularization therapy (percutaneous or surgical) by a cardiovascular surgeon and interventional cardiologist who are not trial investigators.

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Baseline testing, randomization and blinding

The baseline assessment for all patients will include a complete history and physical examination; laboratory examinations including complete blood count, measures of hepatic function, infectious disease serologies, and cardiac biomarkers; adenosine myocardial perfusion SPECT; exercise treadmill test with measurement of VO_2(max) and six minute walk test; 2-dimensional echocardiography (including myocardial contrast and strain and strain rate measurements); and cardiac magnetic resonance imaging (MRI). In addition, the 36-item Short form Health Survey Questionnaire, Minnesota Living with Heart Failure Questionnaire, and ICD interrogation will be performed.

After informed consent, patients will be randomly assigned (2 active:1 control), center-stratified to one of the selected treatment strategies in a web-based session. All patients will be blinded. However, as the study product cannot effectively be masked to the interventionalists, the process of blinding resides with endpoint and adverse event determination. Specifically, each of the three primary endpoints of the study will be determined by a core laboratory whose personnel are blinded to therapy assignment. In addition, each of the clinical centers will take steps to ensure that adverse event assessments are carried out by investigators who are blinded to therapy.

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. Goals for recruitment will be set and will be reviewed by the investigators, DCC, PO and DSMB.

Intervention

FOCUS will have a control group and a single, active intervention group. Each patient will receive 15 intramyocardial electromechanical-guided needle insertions and injections. The intervention group will receive 100×10^6 BMMNCs as the target dose; placebo patients will receive 5% human albumin.

Bone marrow aspiration and cell processing

On the morning of the study product administration, approximately 80-90 ml of bone marrow will be 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, Switzerland) for BMMNC isolation, generating a uniform cellular product[29]. The final product will be resuspended in normal saline containing 5% human serum albumin and adjusted to a concentration of 1.0×10^8 cells in 3 ml in each of three 1 ml syringes. Criteria for release consist of a negative Gram stain, endotoxin limit of ≤5.0EU/kg recipient weight, total cells dose of up to 1.1×10^8 and viability ≤70% as assessed by Trypan Blue exclusion. Supplementary testing includes 14-day aerobic and anaerobic sterility testing (BACTEC or BacT/Alert), CFU-GM colony growth and flow cytometric analysis for CD34+ and CD45+ cells (Table 4). These assessments will be performed at the cell processing centers, in addition to a more extensive antibody panel used by the Biorepository. The cell and /control products are labeled identically and must be delivered for administration within 12 hours of the marrow harvest.

Upon completion of cell processing, the patient will be admitted to the cardiac catheterization lab for the injection procedure.

Study product delivery

Cell insertion will follow with the NOGA injection catheter. Following insertion through a femoral artery sheath, the injection catheter will be placed into the LV in a retrograde

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fashion through the aortic valve. Coronary and LV angiography and LV electromechanical mapping (EMM) will be performed to locate the target area to be injected, and all injections will occur in areas of a SPECT defect associated with viability. The target myocardial area must display points that show electrical viability, defined as a unipolar voltage of >6.9 mV. After proper placement of the catheter tip is confirmed, participants randomized to the active intervention will have needle injection of 0.2 ml of cells (5.3 to 7.3 million cells per injection site). Those randomized to the control product will receive a cell-free solution of 5% albumin. Both treatment groups will receive injections of the product at each of 15 different sites. Among those receiving the active treatment the total dose delivered will be 100 million autologous BMMNCs. Each injection site will be evaluated prior to cell injection, enhancing safety and ensuring intramyocardial delivery of the cellular product using these criteria: (1) perpendicular position of the catheter to the LV wall; (2) excellent loop stability (4 mm); (3) underlying voltage >6.9 mV; and (4) presence of a premature ventricular contraction on extension of the needle into the myocardium.

Regardless of group assignment, participants will be monitored overnight in the cardiac telemetry unit (simple telemetry) after the injection procedure. A transthoracic echocardiogram will be performed immediately after the procedure to detect possible pericardial effusion, and serial biomarker measurements of myocardial injury (CK, CK-MB, and troponin) will be obtained after the procedure.

Biorepository core laboratory

In addition to core laboratories for each of the primary outcomes, a central CCTRN Biorepository will be maintained. Its mission will be to provide storage of critical biomaterial derived from patients enrolled in clinical protocols within the CCTRN, and to maintain the long-term integrity of these specimens, progenitor cell profiles, and cytokine analyses obtained during the clinical protocol. Cell number (i.e., total nucleated cell count) will be determined using a hematology analyzer.

The dose of 100 million cells is based upon previously reported clinical trials of the safe intracoronary delivery of BMMNC in AMI patients. All cells that exceed the target dose of 100 million BMMNCs for active group patients and all cells for those patients assigned to the placebo group will be provided to the CCTRN biorepository core. With appropriate patient consent, these samples will be used to analyze the phenotypic and functional characteristics of bone marrow and peripheral blood vascular progenitor cells. The data will be correlated with clinical results to investigate possible correlations between cell therapy outcomes and cell characteristics.

Outcomes

To evaluate the efficacy of autologous bone marrow mononuclear cell injections in patients with ischemic LV dysfunction and symptomatic angina, FOCUS will assess three co-primary endpoints (Table 5):

(1) Change in VO_2(max) from baseline to 6-month follow-up;

(2) Change in LVESV as assessed by echocardiography with contrast from baseline to 6-month follow-up;

(3) Change in reversible defect size as assessed by SPECT from baseline to 6-month follow-up.

All primary endpoint measurements will be performed in a core laboratory by investigators who remain blinded to treatment assignment. VO_2(max) will be assessed using the Naughton treadmill protocol. Echocardiographic measurements will be performed by an

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echocardiographic core lab according to published guidelines[30] and LV end systolic volumes calculated by the Modified Biplane Simpson's method, using myocardial contrast to enhance endocardial definition. Adenosine myocardial perfusion (SPECT) tests will be performed at baseline and 6 months to identity change in ischemic (reversible) defects. Baseline SPECT imaging will be performed at rest and following adenosine infusion over 4 minutes (or if contraindicated, regadenoson administered as a bolus) using standardized protocols. To enhance the detection of viability on resting images, sublingual nitroglycerin will be administered 15 minutes prior to injecting 99mTc-Sestamibi for the resting image. The same procedure will be repeated at 6 months. Additional secondary endpoints are described in Table 5. These assessments include regional wall motion and regional blood flow improvement by MRI, regional wall motion by echocardiography, clinical improvement, major adverse cardiovascular events, and reduction in fixed perfusion defect(s) by SPECT.

Statistical Analysis

The evaluation for each of the three primary endpoints—VO_2(max), LVESV, and reversible defect size—will compare the change (6-month follow-up minus baseline) in the measure of the control group to change in the measure of the BMMNC group.

For each of the three co-primary endpoints, a sample size was computed based on estimates of the effect size and standard deviation of the difference. Type I error is apportioned at the 0.05 level to be carried out at 80% power. All testing is two-sided. Due to the interest in this protocol by patients with heart failure unamenable to currently accepted treatment modalities, there will be twice as many patients in the active group as in the control group. We anticipate 10% follow-up losses. To ensure adequate power for each of the three endpoints, the sample size was computed for each of them, and the maximum sample size was selected (Table 6). This produced a sample size of 86 patients, administratively increased to 87 patients (29 in the control group, 58 in the active group). Thus, FOCUS provides adequate power to examine the effect of cell therapy for VO_2(max), LVESV, and reversible defect size. In our computation, no type I error adjustment for multiple comparisons was incorporated since this is a phase 2 study.

Statistical Analyses of the Primary and Secondary Endpoints

Descriptive statistics for baseline characteristics known or suspected to be associated with outcomes will be prepared for the various treatment groups, including, but not limited to: (1) demographic characteristics; (2) medical history; (3) physical examination; (4) laboratory data; and (5) quality of life and psychosocial data. Chi-square statistics and Student *t*-testing will be used to evaluate the differences between the treatment arms. General linear mixed modeling techniques will assess the effect of treatment on the continuous primary and secondary outcomes of the study. Both unadjusted and baseline covariate-adjusted treatment effects will be computed. Dichotomous secondary endpoints (e.g., clinical improvement at 6 months, change in anginal score by CCS, NYHA Class, decrease in anti-anginal medication [nitrates] needed weekly, exercise time and level), will be analyzed using a Chi-square test and Fisher's exact test. The time-to-event endpoints (i.e., re-hospitalization secondary endpoints) will be evaluated using Kaplan-Meier survival curves. If the number of events permits, a Cox proportional hazard analysis will be carried out as well. Dichotomous endpoints will be assessed using logistic regression. Outcomes based on numbers of events, such as numbers of re-hospitalizations, will be analyzed using Poisson regression. *P*-values less than 0.05 will carry the label statistically significant.

We will use the general linear model to determine if the relationship between cell delivery and the endpoint is influenced by subgroup strata. Specific subgroup analyses will include

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an examination of the effects of cell administration stratified by the following variables: age, gender, race, diabetes, serum B-type natriuretic peptide (BNP) levels in patients with HF, pre-existing comorbidity, baseline LVEF, and functional characteristics of the cells that are used, including colony forming capability and motility.

Follow-up visits and surveillance

The timeline for follow up will initiate with the day of injection (day 1). Subsequent follow up visits will include 1-week, 4-week, 3-month, 6-month, and 12-month visits. At each visit (through the 6-month visit), the participant will undergo a history for ascertainment of adverse events, a physical exam, electrocardiogram, and laboratory evaluations (including cardiac biomarkers). Limited 2-dimensional echocardiograms will be performed at week 1 and week 4 visits for safety reasons. 24-hour Holter monitoring will be performed at week 1, week 4, month 3, and month 6 visits to assess for possible arrhythmias. Similarly, ICD interrogation (if applicable) will occur at 3-month and 6-month visits. Quality of life questionnaires will be assessed at 3-month and 6-month visits.

As described above, the 6month visit will include measurements of the three primary outcomes including adenosine SPECT imaging, treadmill exercise test with VO_2(max), and complete echocardiogram (with myocardial contrast and strain analyses). In addition to these studies, cardiac MRI will be performed in participants without contraindications. A 6-minute walk test will also be performed. The 12-month visit will include a medical history, physical exam, and administration of a quality of life questionnaire. Thereafter, participant will receive yearly phone calls for up to 5 years.

Participant safety of cell therapy is a prime concern and will be assessed. Adverse cardiac events will be ascertained. Potential arrhythmias will be assessed with Holter monitoring and ICD interrogation.

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 and of the DCC; to the FDA, through the University of Texas Health Science Center (UTHSC)-held Investigator New Device (IND) application; and to 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 study continuation. Monthly safety and performance reports will be provided to the DSMB chair, NHLBI Program Office, and CCTRN Steering Committee Chair. A set of clinically based stopping rules have been developed in consultation with the FDA (Table 7). 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.

Discussion

FOCUS will measure the response to the intramyocardial cell delivery of 100 million BMMNCs on measures of myocardial function and perfusion. The trial has been designed to address critical limitations in the previous published trials by including patients with moderate to severe LV dysfunction, a group of patients who are most likely to benefit from this form of therapy. Risks to patients are reasonably low in relation to knowledge to be gained from this study since this therapy may potentially reduce the severity of HF in patients whose present state with maximal medical therapy places them at very considerable risk for near-term and future adverse events.

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Heart failure affects 4.7 million Americans, with 550,000 newly diagnosed cases per year, resulting in annual costs of 10 to 40 billion dollars for treatment.[31] Approximately 50% of hospital admissions have HF have normal ejection fractions.[32,33] These patients are more likely to be elderly and women.[34] Patients with end-stage HF have a 5-year mortality of approximately 50%. With the advances of acute care of heart attack patients, more patients survive but have the threat of future long-term development of HF. Despite many successful technological breakthroughs, the limitations of these strategies have become clear, as the presence of myocardial scar tissue and/or an unsuitable coronary anatomy may preclude the opportunity to perform subsequent revascularization procedures.

A unique aspect of our study is the broad inclusion criteria, which permit enrollment of patients with LV systolic dysfunction secondary to CAD who manifest with symptoms of ischemia (e.g., angina) as well as those with HF symptoms. This broad population eases generalizability to a larger population. The reversible ischemia and VO_2(max) measures are sensitive to changes in these two populations. This study has the potential to improve cardiac function by preserving or recovering functional myocardial tissue, and/or by improving myocardial blood flow. In addition, demonstrating that the controlled delivery of cells to specifically targeted LV regions can safely improve global function establishes the feasibility, safety, and effectiveness of this procedure. The risk/benefit ratio is considered acceptable by the investigators with a history of over 500 EMM procedures done with few complications.

There are several limitations of our study. The small (87) sample size defines it as a feasibility study; nevertheless, appropriate power is preserved for three prospectively declared endpoints. For a combination of logistical and financial reasons, we were unable to assess the baseline SPECT stress test for reversibility at the core lab as an entry criterion. Instead we rely on the local interpretation of this test. Due to the clinical requirement of an implantable cardiac defibrillator in a subset of our patients, we expect that 40% (35 of 87 patients) will have a contraindication for MRI. However, none of the primary outcome measures require MRI. Finally, due to the nature of the study product, we were unable to blind the interventionalist to the BMMNC product's identity. However, we have ensured that no one who has been unblinded by the procedure can assess adverse events or be involved in endpoint measurement. Having highly trained experts deliver and oversee the therapy, in conjunction with close study monitoring, substantially reduces the likelihood of adverse events.

Each of the five CCTRN centers is committed to recruiting patients for this protocol by accessing a large number of patients from a variety of community resources. All five clinical centers are now actively recruiting patients, and with 29 patients randomized as of January 14, 2010, the Network will complete recruitment to FOCUS by early 2011.

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.

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Table 1

Summary of relevant clinical trial experience

Principal Authors	Year	Design	N	Dose	Follow up	Findings
Tse, Kwong, et al. [26]	2003	Open	8		3 months	imp symptoms imp myocardial perfusion imp myocardial function
Willerson, Perin et al. [23]	2003	Randomized	21	30 million	2 months	Imp in ESV, LVEF, Rev Def
Assmus et. al.[25]	2007	Randomized	121	170 million	1.6 years	Reduced proBNP and proANP
Ramshorst, Bax et. al. [27]	2009	Randomized	50	100 million	6 month	Imp SSS and LVEF

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Table 2

Hypotheses to be tested in FOCUS

Primary Hypothesis:	
	As compared to placebo, the administration of bone marrow mononuclear cells to patients with CAD, LV dysfunction and limiting HF and/or angina will enhance myocardial perfusion, reduce LV end systolic volume, or enhance myocardial oxygen consumption.
Secondary Hypotheses:	
1	As compared to placebo, administration of bone marrow mononuclear cells will enhance regional myocardial function.
2	As compared to placebo, the administration of bone marrow mononuclear cells will diminish future MACE (new myocardial infarcts and rehospitalization for CHF).
3	As compared to placebo, administration of bone marrow mononuclear cells will enhance exercise ability.

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Table 3a

Inclusion Criteria for FOCUS

1	Patients >18 yo with significant (≥70 % luminal diameter narrowing of at least one major coronary artery) coronary artery disease.
2	LVEF ≤45% (by echocardiogram) and limiting angina (Class II to IV) and /or HF (NYHA class II - III).
3	Patients should be on maximal medical therapy.
4	Presence of reversibility by SPECT (adenosine stress) and/or viability as identified by NOGA.
5	Coronary artery disease not well suited to any other type of revascularization procedure (percutaneous or surgical) in the target region of the ventricle.
6	Hemodynamic stability as defined by systolic BP ≥80 mmHg without IV pressors or support devices.
7	Females of childbearing potential must be willing to use two forms of birth control for the duration of the study.
8	A signed consent form approved by the institutional review board.

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Table 3b

Exclusion Criteria:

1	Atrial fibrillation, atrial flutter and/or significant uncontrolled arrhythmias.
2	Unstable angina
3	LV thrombus, as documented by echocardiography or LV angiography.
4	Vascular anatomy that precludes cardiac catheterization.
5	Severe valvular disease or mechanical aortic valve that would preclude safe entry of the catheter into the left ventricle.
6	Pregnant or lactating status. Pregnancy as determined by a positive pregnancy test at baseline.
7	Platelet count <100 K/mm3. †
8	WBC <2 K/mm3. †
9	Revascularization within 30 days.
10	TIA or stroke within 60 days of study enrollment.
11	ICD shock within 30 days of baseline screening.
12	Presence of sustained ventricular tachycardia .
13	Bleeding diathesis defined as an INR ≥ 2.0 in the absence of warfarin therapy.
14	History of non-basal cell carcinoma malignancy in the last 5 years
15	Infectious-disease test result positive for human immunodeficiency virus (HIV), hepatitis B, or hepatitis C.
16	Any previous transplant requiring immunosuppressive medication.
17	High-risk acute coronary syndrome (ACS) or a myocardial infarction in the month prior to evaluation.
18	Left ventricular wall thickness of <8 mm (by echocardiogram) at the target site for cell injection.
19	Inability to walk on a treadmill, except for class IV angina patients who will be evaluated separately.
20	Enrollment in an investigational device or drug study within previous 30 days.
21	Hepatic dysfunction as defined by: AST and ALT levels.
22	Chronic renal insufficiency
23	Any other contraindication to enrollment or follow-up.

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Table 4

Final BMMNC Product Release Criteria Testing

1. Product Release Criteria
Negative Gram stain
Viability: >70% by Trypan Blue exclusion
Target cell dose: 0.8 −1.1 ×10e8
Endotoxin: <5.0EU/kg recipient weight
2. Supplementary Testing by Cell Processing Centers
Aerobic and Anaerobic sterility by blood culture method
CFU-GM colony growth
Immunophenotyping CD34+/CD45+ cells

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Table 5

Outomes of the FOCUS trial

Primary Endpoints:

1. Change in maximum oxygen consumption (VO2(max))*
2. Change in left ventricular end-systolic volume as assessed by echocardiography
3. Change in ischemic (reversible) defect size as assessed by SPECT

Secondary Endpoints

The secondary endpoints of the study will compare the changes in the following measures from baseline+ to 6 month follow-up.

1. Regional wall motion by MRI (in eligible patients).
2. Regional blood flow improvement by MRI (in eligible patients).
3. Regional wall motion by echocardiography.
4. Clinical improvements at 6 months, including change in anginal score by the following measures:
   - **a.** Canadian Cardiovascular Society Functional Classification of Angina Pectoris (CCS) (Appendix 1).
   - **b.** NYHA class (Appendix 2)
   - **c.** Decrease in antianginal medication (nitrates needed weekly).
   - **d.** Exercise time and level.
   - **e.** Serum BNP levels in patients with CHF.
   - **f.** LV diastolic dimension by contrast ECHO.
5. MACE:
   - **a.** New MI
   - **b.** Rehospitalization for PCI in coronary artery territories that were treated.
   - **c.** Death.
   - **d.** Rehospitalization for Acute Coronary Syndrome (ACS) and for CHF.
6. Reduction in fixed perfusion defect(s) by SPECT.

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Table 6

Sample Size Parameters for Primary Endpoints. Power = 0.8; 10% follow-up loss is assumed.

Measure	Mean Difference between Groups	SD of the Change	Sample Size
VO2(max)	5	7	77
LVESV	22	32.5	86
Reversible Ischemia	10	11	48

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Table 7

Holding Criteria

The study will be placed on hold if any of the following events occur during the course of the study.	
1	Tumor growth in the heart at the site of injection in patients.
2	One (1) patient with arrhythmic sudden cardiac death within 30 days of treatment.
3	One (1) death with unexplained pathological evidence of severe inflammatory changes or infection at site of treatment
4	One (1) episode of clinically significant (i.e. requiring surgical intervention) myocardial perforation as a result of the injection procedure.
5	Two (2) patients with stroke within 24 hours after the injection procedure.