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CONFIDENTIAL AND PROPRIETARY

Organization: CARDIOVASCULAR CELL THERAPY RESEARCH NETWORK (CCTRN)

Supported by: The National Heart, Lung, and Blood Institute (NHLBI)

Network Chair: Robert Simari, M.D., Kansas University Medical Center

Sponsor: The University of Texas Health Science Center (CCTRN Data Coordinating Center)

Information Type: ☐ Initial Protocol ■ Protocol Amendment

Full Title: A Phase II, Randomized, Placebo-Controlled Study of the Safety, Feasibility, and Efficacy of Autologous Mesenchymal Stem Cells and c-kit$^+$ Cardiac Stem Cells, Alone or in Combination, Administered Transendocardially in Subjects with Ischemic Heart Failure

Short Title: ***C***ombination ***O***f mese***N***chymal and c-kit$^+$ ***C***ardiac st***E***m cells as ***R***egenerative ***T***herapy for ***H***eart ***F***ailure (CONCERT-HF)

IND Number: 16399

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CONCERT-HF Signature Page

I have read this protocol and any updates provided within and I 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.

If changes in personnel occur during completion of this protocol, I will be responsible for identifying appropriate, trained individuals to carry out the responsibilities of the protocol and will notify the Data Coordinating Center promptly of these changes.

_____

Investigator Name (print)

_____ _____

Investigator Name (signature) **Date**

Sign and return to the Data Coordinating Center (via fax at 713-486-0981)

Confidentiality and Non-Disclosure of Materials and Proceedings Agreement

I understand and agree that information disclosed orally or in written form or discussed in any meeting may include confidential information that is proprietary to agencies sponsoring the proposed research and/or involves the privacy rights of the individuals.

I agree that I will not disclose or divulge in any manner any confidential or private information provided in the protocol titled, "A Phase II, Randomized, Placebo-Controlled Study of the Safety, Feasibility, and Efficacy of Autologous Mesenchymal Stem Cells and c-kit$^+$ Cardiac Stem Cells, Alone or in Combination, Administered Transendocardially in Subjects with Ischemic Heart Failure." Additionally, any information revealed during meetings in any form or manner will not be provided by me to any third party for any purpose whatsoever. "Confidential or Private Information" as used in this Agreement shall not include:

1. Information or knowledge in my possession prior to disclosure at CCTRN meetings;
2. Information generally available to the public or thereafter becomes generally available to the public through a source other than the CCTRN;
3. Information that was rightfully obtained by me from a third party, who, I believe, is under no obligation of confidentiality to CCTRN with respect to such information.

Print Name:_____ Signature: _____

Date:_____

Certification of Conflicts of Interest

All Network members involved in the design, conduct, or analyses of this protocol must certify that they have read the "Cardiovascular Cell Therapy Research Network Conflict of Interest Disclosure Policy." (Located on the CCTRN website: www.cctrn.org)

(Check only one)

☐ I have read the above referenced policy and have reviewed the proposed research. I hereby certify that, based on the information provided to me, **I do not have a conflict of interest with the proposed work.**

☐ I have read the above referenced policy and have reviewed the proposed research. **I report that I have conflicts with the following companies/institutions/affiliations**:

_____ _____ _____

_____ _____ _____

My signature below indicates that, to the best of my knowledge, I have disclosed all conflicts of interest that I may have with this proposed research. Furthermore, I agree to promptly notify the CCTRN if my financial interests, or those of my spouse or dependent children, change during the course of the trial or within two years after trial completion.

Print Name:_____ Signature: _____

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List of Abbreviations and Definition of Terms

ACT	Activated Clotting Time
AE	Adverse Event
BM	Bone Marrow
BMA	Bone Marrow Aspiration
BMC	Bone Marrow Mononuclear Cell
cMRI	Cardiac MRI
CABG	Coronary Artery Bypass Graft
CAD	Coronary Artery Disease
CCMF	Central Cell Manufacturing Facility
CCS	Canadian Cardiovascular Society
CFR	Code of Federal Regulations
CFU-F	Colony Forming Units – Fibroblasts
COA	Certificate of Analysis
CPC	Cardiac Progenitor Cell
CPL	Cell Processing Lab
CPQAL	Cell Processing Quality Assurance Lab
DMSO	Dimethyl Sulfoxide
DEMRI	Delayed-Enhanced Magnetic Resonance Imaging
DSMB	Data and Safety Monitoring Board
EF	Ejection Fraction
ECG	Electrocardiogram
eGFR	Glomerular Filtration Rate
EPC	Endothelial Progenitor Cells
EMB	Right Ventricle Endomyocardial Biopsy
EMM	Left Ventricular Electromechanical Mapping
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GLP	Good Laboratory Practice
HARP	Harmonic Phase
HIPAA	Health Insurance Portability and Accountability Act
HSA	Human Serum Albumin
HTLV	Human T-cell Lymphotropic Virus
ICD	Implantable Cardioverter-Defibrillator

ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements of Pharmaceuticals for Human Use
ICM	Ischemic Cardiomyopathy
IDM	Infectious Disease Markers
IND	Investigational New Drug application
IRB	Institutional Review Board
IV	Intravenous
KDR	VEGF Receptor-2
LAD	Left Anterior Descending Artery
LAO	Left Anterior Oblique
LN_2	Liquid Nitrogen
LV	Left Ventricular
LVAD	Left Ventricular Assist Device
LVEDVI	Left Ventricular End Diastolic Volume Index
LVESVI	Left Ventricular End Systolic Volume Index
MACE	Major Adverse Cardiac Events
MDRD	Modification of Diet in Renal Disease formula
MEM	Minimum Essential Medium
MI	Myocardial Infarction
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MNC	Mononuclear Cell
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell (human)
NHLBI	National Heart, Lung, and Blood Institute
NOGA	NOGA® XP Mapping and Navigation System
NT-proBNP	N-Terminal pro-Brain Natriuretic Peptide
NYHA	New York Heart Association
PBS	Phosphate Buffered Saline
QA	Quality Assurance
QC	Quality Control
RAO	Right Anterior Oblique
RHC	Right Heart Catheterization
SAE	Serious Adverse Event
SDF-1	Stromal Cell Derived Factor 1
SOC	Standard of Care
SOP	Standard Operating Procedures

SPI	Study Product Injection
SW	Stroke Work
TTC	Triphenyltetrazolium Chloride
ULN	Upper Limit of Normal
UMMSM	University of Miami Miller School of Medicine
U.S.	United States
VEGF	Vascular Endothelial Growth Factor
VO_2 max	Maximal Oxygen Consumption
WBC	White Blood Count

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Executive Summary

Study Centers: Centers of the NHLBI CCTRN	**Phase of Development:** Phase II

Study Therapy: Autologous Mesenchymal Stem Cells (MSCs), c-kit$^+$ cells , or the combination of MSCs plus c-kit$^+$ cells produced from bone marrow aspirates and cardiac biopsies, respectively.

Study Title: A Phase II, Randomized, Placebo-Controlled Study of the Safety, Feasibility, and Efficacy of Autologous Mesenchymal Stem Cells and c-kit$^+$ Cardiac Stem Cells, Alone or in Combination, Administered Transendocardially in Subjects with Ischemic Heart Failure, a.k.a. ***C***ombination ***O***f mese***N***chymal and c-kit$^+$ ***C***ardiac st***E***m cells as ***R***egenerative ***T***herapy for ***H***eart ***F***ailure (CONCERT-HF)

Objectives: To assess feasibility, safety, and efficacy of autologous MSCs and c-kit$^+$ cells, alone or in combination, administered by transendocardial injection in subjects with heart failure (HF) of ischemic etiology.

Subject Population: One hundred sixty (160) subjects with HF of ischemic etiology.

Design and Investigational Plan: This investigation will be conducted in two stages.

Stage 1: An open label lead-in investigation will consist of sixteen (16) subjects randomized 1:1 to either a standard of care (SOC) control group (i.e. subjects do not undergo harvest, mapping, or injection procedures) or to the combination cell therapy group (as described below in Stage 2, Group A). All subjects will be followed for 3 months to complete safety and functional assessments. Resultant three month data from this phase will be reviewed by the Data and Safety Monitoring Board prior to initiation of Stage 2. Those subjects randomized to the combination cell therapy group will continue to be followed per protocol for 12 months. Those randomized to the SOC control group will have the option to be evaluated for enrollment in Stage 2.

Stage 2: A phase II, double-blind, placebo-controlled trial will consist of one hundred forty-four (144) subjects who meet all inclusion/exclusion criteria. All subjects will be randomized 1:1:1:1 to one of four treatment strategies:

1. Group A (36 subjects) – Combination of autologous MSCs and c-kit$^+$ cells ("Combo"): Target dose is a mixture of 150 x 10^6 (150 million) MSCs plus 5 x 10^6 (5 million) c-kit+ cells delivered in 15 injections each of 0.4 ml volume
2. Group B (36 subjects) – Autologous MSCs: Target dose is 150 x 10^6 (150 million) MSCs delivered in 15 injections each of 0.4 ml
3. Group C (36 subjects) – Autologous c-kit+ cells: Target dose is 5 x 10^6 (5 million) c-kit+ cells delivered in 15 injections each of 0.4 ml
4. Group D (36 subjects) – Placebo: 15 injections each of 0.4 ml cell-free PlasmaLyte-A medium

- Within 60 days of signing informed consent, all subjects will have iliac crest bone marrow aspiration and right heart catheterization, including transvenous right ventricle endomyocardial biopsy only for groups A and C.
- A central cell manufacturing facility will manufacture the target doses of MSCs and c-kit+ cells.
- After cell manufacturing, cells or placebo will be administered via the NOGA® XP Mapping and Navigation System (NOGA).
- Subjects will return to the cardiac catheterization laboratory to receive study product –approximately 14 weeks after harvest procedures.
- Injections will be administered transendocardially during left ventricular catheterization (NOGA) and will be targeted to the border zone and the adjacent scarred tissue.
- Following cell or placebo injections, subjects will be followed at day 1, week 1, and months 1, 3, 6, and 12 to complete safety and efficacy assessments.

Eligibility Criteria:

Inclusion Criteria

To participate, a subject MUST:

1. Be ≥ 21 and <80 years of age

2. Have documented coronary artery disease (CAD) with evidence of myocardial injury, LV dysfunction, and clinical evidence of HF
3. Have a "detectable" area of myocardial injury defined as ≥ 5% LV involvement (infarct volume) and any subendocardial involvement by cMRI
4. Have an EF ≤ 40% by cMRI
5. Be receiving guideline-driven medical therapy for heart failure at stable and tolerated doses for ≥ 1 month prior to consent. For beta-blockade "stable" is defined as no greater than a 50% reduction in dose or no more than a 100% increase in dose.
6. Be a candidate for cardiac catheterization
7. Have NYHA class I, II, or III HF symptoms
8. If a female of childbearing potential, be willing to use one form of birth control for the duration of the study, and undergo a pregnancy test at baseline and within 36 hours prior to injection

Exclusion Criteria

To participate, a subject MUST NOT HAVE:

1. Indication for standard-of-care surgery (including valve surgery, placement of left-ventricular assist device, or imminent heart transplantation), coronary artery bypass grafting (CABG), and/or percutaneous coronary intervention (PCI) for the treatment of ischemic and/or valvular heart disease. Subjects who require or undergo PCI should undergo these procedures a minimum of 3 months in advance of randomization. Subjects who require or undergo CABG should undergo these procedures a minimum of 4 months in advance of randomization. In addition, subjects who develop a need for revascularization following enrollment should undergo revascularization without delay. *Indication for imminent heart transplantation is defined as a high likelihood of transplant prior to collection of the 12 month study endpoint. Candidates cannot be UNOS status 1A or 1B, and they must have documented low probability of being transplanted.*
2. Valvular heart disease including 1) mechanical or bioprosthetic heart valve; or 2) severe valvular (any valve) insufficiency/regurgitation within 12 months of consent
3. Aortic stenosis with valve area ≤ 1.5 cm^2
4. History of ischemic or hemorrhagic stroke within 90 days of consent
5. History of a left ventricular remodeling surgical procedure utilizing prosthetic material
6. Presence of a pacemaker and/or ICD generator with any of the following limitations/conditions:
   - manufactured before the year 2000
   - leads implanted < 6 weeks prior to consent
   - non-transvenous epicardial or abandoned leads
   - subcutaneous ICDs
   - leadless pacemakers
   - any other condition that, in the judgment of device-trained staff, would deem an MRI contraindicated
7. Pacemaker-dependence with an ICD (*Note: pacemaker-dependent candidates without an ICD are not excluded)*
8. A cardiac resynchronization therapy (CRT) device implanted < 3 months prior to consent
9. Other MRI contraindications (e.g. patient body habitus incompatible with MRI)
10. An appropriate ICD firing or anti-tachycardia pacing (ATP) for ventricular fibrillation or ventricular tachycardia within 30 days of consent
11. Ventricular tachycardia ≥ 20 consecutive beats without an ICD within 3 months of consent, or symptomatic Mobitz II or higher degree atrioventricular block without a functioning pacemaker within 3 months of consent
12. Presence of LV thrombus (*See guidance in section 6.3.3*)
13. Evidence of active myocarditis
14. Baseline VO_2 max greater than 75% of age and gender based predictive values (see Section 6.3.7)
15. Baseline eGFR <35 ml/min/1.73m^2
16. Blood glucose levels (HbA1c) >10%

17. Hematologic abnormality evidenced by hematocrit < 25%, white blood cell < 2,500/ul or platelet count < 100,000/ul
18. Liver dysfunction evidenced by enzymes (AST and ALT) > 3 times the ULN
19. Coagulopathy (INR ≥ 1.3) not due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors) (see Sections 6.2.2 and 6.2.3 re: study procedures and anticoagulation therapy). Subjects who cannot be withdrawn from anticoagulation will be excluded.
20. HIV and/or active HBV or HCV
21. Allergy to radiographic contrast material that cannot adequately be managed by premedication
22. Known history of anaphylactic reaction to penicillin or streptomycin
23. Received gene or cell-based therapy from any source within the previous 12 months
24. History of malignancy within 5 years (i.e., subjects with prior malignancy must be disease free for 5 years), excluding basal cell carcinoma and cervical carcinoma in situ which have been definitively treated
25. Condition that limits lifespan to < 1 year
26. History of drug abuse (use of illegal "street" drugs except marijuana, or prescription medications not being used appropriately for a pre-existing medical condition) or alcohol abuse (≥ 5 drinks/day for > 3 months), or documented medical, occupational, or legal problems arising from the use of alcohol or drugs within the past 24 months
27. Participation in an investigational therapeutic or device trial within 30 days of consent
28. Cognitive or language barriers that prohibit obtaining informed consent or any study elements
29. Pregnancy or lactation or plans to become pregnant in the next 12 months
30. Any other condition that, in the judgment of the Investigator or Sponsor, would impair enrollment, study product administration, or follow-up

Hypotheses:

Feasibility: MSCs and c-kit+ cells, both alone and in combination, can be manufactured and delivered to subjects with ischemic cardiomyopathy

Safety: MSCs and c-kit+ cells, alone or in combination are well-tolerated by subjects with ischemic cardiomyopathy

Efficacy:

- Combo improves LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months
- MSCs alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months
- c-kit+ cells alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months
- Combo improves LV function and functional status when compared with MSCs from baseline to 6 months and baseline through 12 months
- Combo improves LV function and functional status when compared with c-kit+ cells from baseline to 6 months and baseline through 12 months
- MSCs or c-kit+ cells improve LV function and functional status when compared with each other from baseline to 6 months and baseline through 12 months

Feasibility Measures:
The number and percent of subjects who have:

- Events between randomization and study product injection (SPI) that precludes the subject from getting SPI
- Failed bone marrow aspiration procedure
- Failed endomyocardial biopsy procedure
- Failed release criteria (including minimum number of cells) for receiving the MSC product
- Failed release criteria (including minimum number of cells) for receiving the c-kit+ cell product
- Less than 15 injections during the SPI procedure
- At least one cardiac MRI endpoint measure that is uninterpretable due to issues related to the device, including, but not limited to, inability to undergo the procedure

Safety Measures:
The following safety data will be collected and analyzed by therapy group between baseline and a) 6 months and b) 12 months:
Major adverse cardiac events (MACE)[1] including: death, hospitalization for worsening HF, and/or exacerbation of HF (non-hospitalization)

- Other significant clinical events including: non-fatal stroke, non-fatal myocardial infarction, coronary artery revascularization, ventricular tachycardia/fibrillation, and/or pericardial tamponade
- All adverse events that are at least grade 2 in severity

Prospectively Declared Endpoint Measures:
Each of the following domains and measures has the same priority for the efficacy analyses to assess improvement in LV function and functional status:

- Myocardial evaluations by cMRI over time:
  - Function:
    - Change in LVEF
    - Change in global and regional strain (HARP MRI)
  - Structure:
    - Change in LVEDVI
    - Change in LVESVI
    - Change in LV Sphericity Index
  - Morphology:
    - Change in infarct/scar volume (DEMRI)
- Functional capacity over time:
  - Change in VO_2 max (treadmill)
  - Change in exercise tolerance (6MWT)
  - Change in MLHF Questionnaire (subject reported)
- Clinical outcomes over time:
  - MACE
  - Cumulative days alive and out of hospital for HF
- Biomarkers over time:
  - Change in NT-proBNP

Duration of Study Follow-Up: Following discharge from the hospital, subjects will be assessed at week 1, and months 1, 3, 6 and 12.

1.0 STUDY OBJECTIVES

1.1 Primary Objectives

The purpose of this study is to assess feasibility, safety, and efficacy of autologous bone marrow-derived mesenchymal stem cells (MSCs) and c-kit^{+} cells, alone or in combination, administered by transendocardial injection in subjects with heart failure (HF) of ischemic etiology.

1.1.1 Primary Feasibility Objective

To assess whether MSCs and c-kit+ cells, alone or in combination, can be manufactured and delivered to subjects with ischemic cardiomyopathy (ICM).

1.1.2 Primary Safety Objective

To assess the relative safety of c-kit+ cells and MSCs, delivered alone or in combination, when compared with placebo.

1.1.3 Primary Efficacy Objectives

- To assess whether the combination of autologous MSCs and c-kit+ cells (hereinafter referred to as “Combo”) improves left ventricular (LV) function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months.
- To assess whether MSCs alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months.
- To assess whether c-kit+ cells alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months.
- To assess whether Combo improves LV function and functional status when compared with MSCs from baseline to 6 months and baseline through 12 months.
- To assess whether Combo improves LV function and functional status when compared with c-kit+ cells from baseline to 6 months and baseline through 12 months.
- To assess whether MSCs or c-kit+ cells improve LV function and functional status when compared with each other from baseline to 6 months and baseline through 12 months.

1.2 Relevance to the Cardiovascular Cell Therapy Research Network (CCTRN)

This study is consistent with the overall mission of the CCTRN, which is to investigate the safety and effectiveness of stem cell therapy in subjects with cardiovascular disease. It is also consistent with the goal of accelerating research in the use of cell-based therapies for the management of cardiovascular disease. This study will collect important clinical and mechanistic information on the safety, feasibility and impact of administering adult stem cells, including a combined cell product, in subjects with HF of ischemic etiology, a population with limited treatment options.

2.0 BACKGROUND

2.1 Rationale

The field of stem cell-based cardiac repair has advanced rapidly since publication of the first adult human stem cell clinical trials in 2002-03[2,3]. At present, several categories of adult stem cells (possibly enhanced by concomitant strategies aimed at improved migration or survival) hold great promise to restore function in diseased hearts. CONCERT-HF will compare MSCs

alone, c-kit+ cells alone, and Combo as therapy for ischemic cardiomyopathy (ICM). MSCs have been chosen because of: 1) their efficacy in small and large animal models; 2) their safety and efficacy in clinical trials[4-8]; and 3) they offer the substantial advantage of already having approval from the FDA for investigational use in humans. c-kit+ cells have been shown to be safe and efficacious in preclinical models including large animal models[9-23]. In addition, recent data from animal models and mechanistic studies suggest that the combination of MSCs and c-kit+ cells has greater therapeutic efficacy than either cell type alone[17-19,23,24]. CONCERT-HF will assess the feasibility, safety, and clinical efficacy of combining c-kit+ cells with MSCs compared with each of placebo, MSCs alone, and c-kit+ cells alone in subjects with ICM.

2.2 Background Preclinical Studies

MSCs: A well-developed porcine model of anterior myocardial infarction (MI) has characterized the impact of cellular cardiomyoplasty on cardiac structure and function using hemodynamic, imaging, and histological analyses. Two distinct sets of studies have been conducted, representing the early treatment of acute MI, as well as the treatment of chronic ICM (see Table 1).

Published work demonstrates that autologous and allogeneic MSC transplantation in post-MI pigs improved cardiac function with histological evidence of robust engraftment at 8 weeks and differentiation to a myocyte-like phenotype[25,26]. Studies of MSC engraftment in rodent and swine models of MI have shown that the administration of MSCs produces: 1) functional benefit in post-MI recovery of ventricular function, 2) evidence of neoangiogenesis at the infarct, 3) decrease in collagen deposition in the region of scar, and 4) some evidence of cells expressing contractile and sarcomeric proteins but lacking true sarcomeric functional organization, although these cells are quantitatively insufficient to account for the functional improvement[25,27].

HF is characterized by mechanoenergetic uncoupling: decreased efficiency of work per unit oxygen consumption. In placebo-treated animals, stroke work (SW) decreased substantially during the 8 weeks following MI, with a paradoxical increase in myocardial oxygen consumption resulting in decreased ratio of SW/VO_2 max[28]. However, investigators observed that after a period of follow-up, MSC-injected animals demonstrated improved myocardial efficiency principally due to increasing SW (from 374.4±59.3 to 654.4±129.9 mmHg/mm at 8 weeks) and decreasing VO_2 max (from10.3±2 to 3.7±1.8 Joules/beat), both toward normal. Thus, MSC therapy exerts favorable effects on the damaged heart that extend to improvements in cellular energy metabolism.

In addition to the studies outlined above using models of acute MI in the pig, a model of chronic MI in the Gottingen mini-swine has also been developed [29-31]. Both autologous and allogeneic MSCs have been used, with surgical and catheter delivery strategies, and sufficient experience has been developed to translate the therapy from the laboratory bench to clinical trials[4-8,32]. Together the results indicate that bone marrow-derived MSCs stimulate cardiac recovery by engrafting, forming new blood vessels that increase tissue perfusion in hypoperfused areas, forming new cardiac myocytes, and importantly interacting with endogenous precursor cells (i.e., cardiac stem cells) to also contribute to new cardiac myocyte formation[6,24,29,33].

Table 1

Preclinical Studies: Autologous and Allogeneic Mesenchymal Stem Cells (MSCs) Administered Via Intramyocardial Injection

Study	Model	N	Cell Delivery	Cell Source & Type	Cell Doses (x 10^6)	Safety Results	Efficacy Results
Shake[25]	14-Day Post-MI Pig	14	Surgical (needle) IM Injection	Autologous, porcine MSCs	60.0	• No ectopic tissue formation • No MSC differentiation to non-cardiac tissue • No significant inflammatory infiltrates at site of MSC implantation	• MSC engraftment • ↑regional contractile function
Cattaneo[34]	1-Day Post-MI Pig	13	Surgical (needle) IM Injection	Allogeneic, porcine MSCs	200.0	• No ectopic tissue formation • No significant inflammatory response	• MSC engraftment • ↑EF and global wall motion score
Amado[28]	3-Day Post-MI Pig	14	PIM (catheter) Injection	Allogeneic, porcine MSCs	200.0	• No deaths; no malignant arrhythmias • No evidence of cardiac perforation during injection	• MSC engraftment • ↓infarct scar • Improved systolic and diastolic function
Amado[35]	3-Day Post-MI Pig	22	PIM (catheter) Injection	Allogeneic, porcine MSCs	200.0	• No difference in deaths between treated/placebo	• MSC engraftment • ↑Viable myocardium • ↓infarct scar
Schuleri[30]	90-Day Post-MI Pig	9	Surgical (needle) IM injection	Autologous, porcine MSCs	20.0 (Low) 200.0 (High)	• No difference in deaths between groups • No evidence of post-injection arrhythmias • No ectopic tissue formation	• ↓infarct scar at High Dose • ↑EF
Quevedo[29]	90-Day Post-MI Pig	6	PIM (catheter) Injection	Allogeneic, porcine MSCs	200.0		• MSC engraftment & differentiation • ↓infarct scar • ↑global LV function and EF
Hatzistergos[24]	3-Day Post-MI Pig	12	PIM (catheter injection)	Allogeneic porcine MSCs & concentrated condition medium	75 100 & 10 x concentrated condition medium		• MSC engraftment & differentiation • ↑EF

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

Finally, a review of the available preclinical work demonstrates no ectopic tissue formation, significant inflammatory responses, nor arrhythmias or deaths attributed to either allogeneic or autologous MSC therapy. Thus, preclinical work demonstrates that MSC injection can produce a wide range of benefits, including improved regional and global ventricular function, reduced myocyte apoptosis, and improved tissue perfusion[26].

c-kit+ cells: Cardiac stem cells have stimulated great interest as a potential therapy for patients with heart disease. In the past 15 years, the ability of rodent and porcine c-kit+ cells to alleviate LV dysfunction and remodeling has been repeatedly demonstrated by several independent laboratories in various preclinical animal models of acute MI[10]. A number of preclinical studies have also demonstrated their efficacy in subacute or chronic HF, and are summarized below (See Table 2).

Wysoczynski *et al*.[11] performed a study in a mouse model of left anterior descending coronary artery (LAD) ligation, in which echo-guided administration of 1 million c-kit^{pos} cardiac cells into the LV cavity at 2 days after MI significantly improved cardiac function and increased vascular density compared with vehicle treatment. The endothelial phenotype of the cells, coupled with the finding of increased vascular density, suggested a potential pro-vasculogenic action of c-kit+ cells.

In a study by Li *et al*.[2] mice underwent a 60-min LAD occlusion followed by reperfusion; at 2 days after reperfusion they received either 4 intramyocardial injections of 1 x 10^5 c-kit+ cells in the peri-infarct region or an intracoronary infusion (cells were infused into the aortic root during aortic clamping) of 4 x 10^5 c-kit+ cells. At 39 days after treatment, mice that received c-kit+ cells via either route exhibited significantly improved regional function in the infarcted region, improved global LV systolic and diastolic function, and decreased LV dilation. This study showed that intracoronary c-kit+ cell infusion is at least as effective as intramyocardial injection in limiting LV remodeling and improving both regional and global LV function.

Given the promising results of the mouse study by Li *et al.*[12], Hong *et al.*[13] developed a highly sensitive and accurate method to quantify the absolute number of male mouse c-kit+ cells in female recipients after transplantation. Using the same murine model of a 60-min LAD occlusion followed by reperfusion, 100,000 male c-kit+ cells were infused intracoronarily. Only 12.7% of the male c-kit+ cells present in the heart immediately (5 min) after infusion were still present at 24 h, and their number declined rapidly thereafter. By 35 days after infusion, only ~ 1,000 male c-kit+ cells were found in the heart. Despite the low retention and rapid disappearance of c-kit+ cells from the recipient heart, intracoronary delivery of c-kit+ cells significantly improved LV function at 35 days, as assessed by Millar catheter. These results suggest that direct differentiation of c-kit+ cells cannot account for the beneficial effects of c-kit+ cells on LV function, and that paracrine effects must be the major mechanism.

To assess the utility of repeated treatments, Bolli and colleagues performed a study of multiple administrations of c-kit+ cells in a rat model of ICM[36]. Rats with a 30-day-old MI and LV dysfunction received one or three c-kit+ cell infusions (12 million cells each) into the LV cavity, 35 days apart. Compared with vehicle-treated rats, the single-dose group exhibited improved LV function after the 1st infusion (consisting of c-kit+ cells) but not after the 2nd and 3rd infusions (consisting of vehicle). In contrast, in the multiple-dose group regional and global LV function im-

proved by a similar degree after each c-kit+ cell infusion, resulting in cumulative effects. The single- and multiple-dose groups exhibited less scar and less collagen in the risk and noninfarcted regions. This study demonstrates that a single infusion of c-kit+ cells alleviates LV dysfunction in a model of chronic ICM, and that repeated c-kit+ cell infusions are even more effective than a single administration.

To address the question as to whether the beneficial effects of 3 repeated c-kit+ cell doses can be recapitulated by 1 large dose containing the same total number of cells, a study[37] was performed in the same model (rats with a 30-day-old MI). Rats received via infusion into the LV cavity either a single large dose of 36×10^6 c-kit+ cells followed by 2 doses of vehicle or 3 equal doses of 12×10^6 c-kit+ cells 35 days apart. Infusion of a single, large dose of c-kit+ cells improved LV function, but there was no further improvement after the 2 doses of vehicle. In contrast, the 3 doses of c-kit+ cells caused a progressive improvement in LV function, the cumulative magnitude of which was greater than achieved with a single dose. This study confirms that a single administration of c-kit+ cells improves LV function in a rat model of ICM and shows that dividing this single dose into 3 smaller doses is even more effective.

In a rat model of isoproterenol (ISO)-induced cardiomyopathy[14], intravenous administration of 5×10^5 clonogenic c-kit+ cells at 28 days after ISO injection significantly improved LV fractional shortening and dP/dt_{max} and reduced LV end-diastolic pressure and diameter at 28 days after treatment compared with vehicle-treated and cardiac fibroblast-treated rats. These beneficial effects of c-kit+ cells were maintained at 56 days after cell treatment.

To develop a safe and efficient delivery method for c-kit+ cell therapy, the efficacy of retrograde coronary vein (RCV) infusion of c-kit+ cells was studied[15]. Rats with ICM received an RCV infusion of c-kit+ cells at 21 days after MI. RCV-treated rats showed a significant improvement in cardiac function and exhibited an increase in capillary density, a decrease in total heart collagen, and a reduction in both infarct size and cardiomyocyte hypertrophy when compared with vehicle-treated rats.

To enhance the cardiogenic potential of c-kit+ cells, c-kit+ cells were treated with mocetinostat (MOCE), a specific class I HDAC inhibitor, and were retrogradely infused into the coronary vein of rats at 3 weeks after MI[16]. Transplantation of either control or MOCE/c-kit+ cells resulted in an improvement in cardiac function and retardation of LV remodeling, as evidenced by cardiomyocyte hypertrophy reduction. Compared with control cells, infusion of MOCE/c-kit+ cells resulted in a further reduction in LV end-diastolic pressure and myocardial collagen.

In a study by Bao *et al.*[17] in a rat model of ICM, 4 intramyocardial injections of 1×10^6 c-kit+ cells, 1×10^6 BM-MSCs, or 5×10^5 c-kit+ cells + 5×10^5 BM-MSCs into the infarct border zone were performed at 28 days after MI. c-kit+ cells and/or BM-MSCs improved cardiac function after MI and reduced infarct size despite the fact that *in vivo* cell tracking experiments showed minimal persistence of donor cells in the myocardium after transplantation. c-kit+ cells also enhanced the expression of pro-angiogenic factors and boosted post-MI angiogenesis in the myocardium in a paracrine manner. This study showed that transplantation of c-kit+ cells + BM-MSCs was superior to transplantation of either c-kit+ cells or BM-MSCs alone in promoting post-MI angiogenesis and improving cardiac function after MI.

Since these studies in rodents yielded promising results, the effects of c-kit+ cells were assessed in porcine models of ICM. In a study by Williams *et al*.[23] in an immunosuppressed swine model of ICM, human c-kit+ cells/MSCs (1 million cells/200 million cells), human c-kit+ cells alone (1 million cells), human MSCs alone (200 million cells), or placebo (phosphate-buffered saline) were injected into the infarct border zone at 14 days after MI. Swine in all 3 cell therapy groups showed significantly reduced infarct size and improved LV ejection fraction at 6 weeks after treatment compared with placebo. The infarct size reduction was 2-fold greater with combination therapy versus either cell therapy alone. A substantial improvement in left ventricular chamber compliance (end-diastolic pressure-volume relationship) and contractility (preload recruitable stroke work and dP/dt_{max}) were also observed in combination-treated swine. This study demonstrated that c-kit+ cells improve LV function in a porcine model of ICM, and that the combination of c-kit+ cells and MSCs is superior to either cell alone.

A subsequent study by Karantalis *et al*.[18] demonstrated that the combination of autologous MSCs and c-kit+ cells produces greater improvement in cardiac performance than MSCs alone in a nonimmunosuppressed swine model of ICM. In this study, Gottingen mini-pigs underwent myocardial ischemia/reperfusion to produce an MI, and cardiac tissue and bone marrow were obtained for isolation of autologous c-kit+ cells and BM-MSCs. Autologous MSCs alone (200 x 10^6 MSCs) or in combination with c-kit+ cells (1 x 10^6 c-kit+ cells) were injected transendocardially at 3 months after MI. At 3 months after treatment, both groups of cell-treated animals exhibited significantly reduced scar size, increased viable tissue, and improved wall motion relative to placebo. Significant improvement in ejection fraction, stroke volume, cardiac output, and diastolic strain was only seen in the combination-treated animals. This study is notable because it used autologous cells (thus mimicking CONCERT-HF); it demonstrates that the addition of c-kit+ cells to MSCs significantly improves the beneficial effects of MSCs.

In view of the finding that the combination of autologous MSCs and c-kit+ cells provides additive beneficial effects in ICM, Natsumeda *et al.*[19] further studied whether a combination of allogeneic stem cells promotes cardiac repair. Cardiac tissue and bone marrow were obtained from male Yorkshire swine for isolation of c-kit+ cells and MSCs. Nonimmunosuppressed female Göttingen pigs were subjected to MI and received transendocardial injections of either allo-MSCs + allo-c-kit+ cells (200 million MSCs/1 million c-kit+ cells), 200 million allo-MSCs, 1 million allo-c-kit+ cells, or placebo at 3 months post MI. All three treatments improved LV function but the combination exerted the greatest effect, improving the end-systolic pressure-volume relation. Only combination therapy, but not MSCs or c-kit+ cells, prevented ongoing negative LV remodeling by offsetting increases in chamber volumes. Both combination therapy and allo-MSCs reduced scar size and resulted in less immunotolerant regulatory T cells and low-grade inflammatory infiltrates in the myocardium. This study confirms that c-kit+ cells are effective in ICM but the combination of c-kit+ cells over MSCs is superior.

Kulandavelu *et al*.[21] studied whether overexpression of Pim1 in c-kit+ cells enhances their cardioreparative properties. Immunosuppressed Yorkshire pigs with ICM received intramyocardial injection of 1 x 10^6 human c-kit+ cells or human c-kit+ cells overexpressing Pim1 at 2 weeks after MI. At 8 weeks after treatment, both human c-kit+ cells reduced MI size compared with placebo, but $Pim1^+$ cells produced greater decrease in scar compared with human c-kit+ cells. $Pim1^+$ human c-kit+ cells also produced a greater increase in regional contractility in both infarct and border zones. Both c-kit+ cell types significantly increased LV ejection fraction at 4 weeks.

Kamata *et al.*[20] studied the beneficial effects of transplanting a sheet of c-kit+ cells in a porcine model of ICM. Immunosuppressed swine were subjected to MI induced by ameroid constrictor; a cell sheet with 1 x 10^8 human c-kit+ cells with or without 2.5 x 10^6 cells human EPCs was placed on the epicardium of the ischemic area at 4 weeks post ischemia. At 2 months after sheet transplantation, the epicardial radial strain (RS) in the ischemic area was similarly increased after treatment with c-kit+ cell-derived cell sheets alone or in combination with EPCs. The endocardial RS in the ischemic area was greatest after combined treatment compared with c-kit+ cells only. The authors concluded that transplantation of c-kit+ cell sheets induced significant functional recovery of the ischemic epicardium, and that concomitant EPC transplantation elicited transmural improvement in function in this porcine model of chronic ischemic injury.

In addition to the above-mentioned studies of ICM, Taghavi *et al.*[22] tested c-kit+ cell therapy in a cat model of ISO-induced cardiomyopathy. Approximately 1 × 10^6 autologous c-kit+ cells or MSCs were delivered by intracoronary injection at 10 days of ISO infusion. At 28 days after treatment, fractional shortening was found to improve with either c-kit+ cells and MSCs compared with vehicle. This study suggests that both c-kit+ cell and MSC therapy improves cardiac function and attenuates pathological remodeling.

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TABLE 2

Studies of c-kit+ cells in experimental settings of chronic ischemic cardiomyopathy

Authors	Model	Treatment	Delivery	Outcomes	Summary
Wysoczyn-ski[11]	Mice/Sub-acute MI	c-kit^{+} cells ●12 Slow adhering ●10 rapidly adher-ing ●15 vehicle	1 x 10^6 ●Intra LV admin ●2 days post-MI	35 days post treatment ●LVEF ●Infarcted wall thickness ● Histologic exam	SA group: significant ↑ LVEF ↑ infarcted wall thickness ↓ scar over vehicle
Li[12]	Mice/Sub-acute MI	c-kit+ vs. vehicle ●8 IC c-kit+ ●9 IC vehicle ●10 IM c-kit+ ●12 IM vehicle	4 x 10^5 for IC 1 x 10^5 for IM ●IC admin ●IM admin ●2 days post-MI	39 days post treatment ●LVEF ●LVFS ●LV volume ●LV diameter ●Infarcted wall thickness ●Infarcted wall thickening frac-tion ● Hemodynamics ● Histologic exam	Both c-kit+ groups: significant ↑ LVEF ↑ LVFS ↑ infarcted wall thickness ↑ infarcted wall thickening fraction ↓ LV volume ↓ LV diameter ↑dP/dt max&min ↑ elastance ↑ viable myocardium over vehicle
Hong[13]	Mice/Sub-acute MI	c-kit+ vs. vehicle ●7 c-kit+ ●9 vehicle	IM1 x 10^5 ●IC administration ●2 days post-MI	35 days post treatment ●Hemodynamics ●Histological cell characterization	c-kit+ group: significant ↑ LVEF ↑ Elastance ↓ LVEDP over vehicle
Tokita[36]	Rats/ICM (old MI)	c-kit+ vs. vehicle ●16 c-kit+ x 1 dose ●18 c-kit+15 x 3 doses ●16 vehicle	112 x 10^6 ●Intra LV admin ●30 days post-MI and 35 days post-1st and 35 days post 2nd dose	5 days post each treatment ●LVEF ●Infarcted LV wall thickening fraction ●Hemodynamics Histologic exam	1 and 3 doses c-kit+ groups: significant ↑ LVEF ↑ infarcted wall thickening fraction ↑ dP/dt max&min ↑ elastance ↓ LVEDP ↓ scar and collagen over vehicle
Tang[37]	Rats/ICM (old MI)	c-kit+ vs. vehicle ●16 c-kit+ x 1 dose ●18 c-kit+ x 3 doses ●16 vehicle	36 x 10^6 x 1 or 12 x 10^6 x 3 ●Intra LV admin ●30 days post-MI and 35 days post-1st and 35 days post 2nd dose	35 days post each treatment ●LVEF ●LV volume ●Infarcted LV wall thickening fraction ●Hemodynamics ●Histologic exam	I1 high and 3 repeated doses c-kit+ groups: significant ↑ LVEF ↓ LVESV ↑ Elastance ↓ scar over vehicle

Authors	Model	Treatment	Delivery	Outcomes	Summary
Ellison[14]	Rats/ ISO-induced CM	c-kit+ vs. cardiac fibroblasts and vehicle • 4 c-kit+ • 4 cFibro • 4 vehicle	15-245 x 10^5 c-kit+ • IV admin • 28 days post-ISO	628 days post treatment • GLVFS • Hemodynamics	c-kit+ group: significant ↑ LVFS ↑ dP/dt max ↓ LVEDP ↓ LVID over cFibro or vehicle
Zakharova[15]	Rats/ICM (old MI)	c-kit$^+$ cells vs. vehicle • 9 c-kit$^+$ cells • 9 vehicle	RCV infusion at 21 days post-MI	21 days post treatment • Hemodynamics • Histological exam	c-kit$^+$ cells group: significant ↑ LVEF ↑ dP/dt max&min ↑ SW ↑ CO ↓ LVEDP ↓ Tau ↓ infarct size ↓ fibrosis over vehicle
Zakharova[16]	Rats/ICM (old MI)	c-kit$^+$ cells, HDACI-c-kit$^+$ cells vs. vehicle • 8 c-kit$^+$ cells • 8 HDACI-c-kit$^+$ cells • 8 vehicle	RCV infusion at 21 days post-MI	21 days post treatment • Hemodynamics • Histological exam	c-kit$^+$ cells (and more so HDACI-c-kit$^+$ cells) significant: ↑ LVEF ↑ dP/dt max ↑ CO ↓ LVEDP ↓ infarct size ↓ fibrosis over vehicle
Bao[17]	Rats/ICM (old MI)	CPCs vs. vehicle • 6 c-kit+ • 6 vehicle	1 x 10^6 • IM admin • 4 weeks post-MI	28 days post treatment • LVEF • Fractional shortening • Histological exam	c-kit+ group: significant ↑ LVEF, ↑ fractional shortening, ↓ infarct size, ↓ fibrosis over vehicle
Williams[23]	Pigs/ICM (old MI)	hc-kit+ or hc-kit$^+$+hMSCs vs. placebo • 5 c-kit+ • 5 c-kit$^+$+MSCs • 5 vehicle	1 x 10^6 c-kit+ 1 x 10^6 c-kit+ + 2 x 10^8 MSCs • Intramyocardial • 14 days post-MI	4 weeks post treatment • LVEF • LV volume • PV loops • Histologic exam	c-kit+ and c-kit$^+$+MSC groups: significant ↑ LVEF, ↑ SV&CO, ↑ dP/dt min, ↓ tau, & ↓ scar over placebo
Karantalis[18]	Pigs/ICM (old MI)	c-kit$^+$+MSCs vs. placebo • 8 c-kit$^+$ +MSCs • 6 placebo	1 x 10^6 c-kit+ + 2 x 10^8 MSCs • Transendocardial • 3 months post-MI	3 months post treatment • LVEF • LV volume • Diastolic performance • Histologic exam	c-kit$^+$+MSC group: significant ↑ LVEF ↑ SV&CO ↑ endothelial function ↓ scar over placebo

Authors	Model	Treatment	Delivery	Outcomes	Summary
Kulandavelu[21]	Pigs/ICM (old MI)	hc-kit+, Pim$^+$ hc-kit+ vs. placebo • 10 c-kit+ • 9 Pim$^+$ c-kit+ • 10 placebo	1 x 10^6 c-kit+ or Pim$^+$ c-kit+ • Epicardium • 2 weeks post-MI	4 or 8 weeks post-treatment • LVEF • LV volume • PV loops • Histologic exam	c-kit+ and Pim$^+$ c-kit+ groups: significant ↑LVEF, ↑ SV&SW, ↓scar over placebo at 4 weeks post-treatment Pim$^+$ c-kit+ group extended significant ↑LVEF, ↑ SW, ↑ cardiac efficiency, ↓scar over placebo at 8 weeks post-treatment
Natsumeda[19]	Pigs/ICM (old MI)	Allo-c-kit+ +allo-MSCs vs. placebo • 7 c-kit+ +MSCs • 6 placebo	1 x 10^6 c-kit$^+$+2 x 10^8 MSCs Transendocardial injection • 3 months post-MI	3 months post treatment • Hemodynamics • LV volume • Morphometric exam	c-kit$^+$+MSC group: significant ↑ ESPVR ↓ LV EDV ↓ LV ESV ↓ scar over placebo
Kamata[20]	Pigs/ICM (old MI)	c-kit+ vs. sham • 6 c-kit+ • 6 sham	1 x 10^8 c-kit+ sheet • Epicardium • 21 days post ischemia	8 weeks post c-kit+ sheet • LVEF • LV volume • PV loops • dP/dt • LVEDP • LV strain analysis • Perfusion score • Histologic exam	c-kit+ group: significant ↑ LVEF ↓ EDV & ESV ↑ dP/dt max & dP/dt min ↑ Elastance ↑ dP/dt ↓ LVEDP ↑ LV wall motion ↑ Perfusion score ↓ fibrosis
Taghavi[22]	Cats/ISO-induced CM	c-kit+ vs. no treatment (sham) • 7 c-kit+ • 5 sham	1 x 10^6 • IC administration • 28 days post ISO	38 days post treatment • LVEF • Fractional shortening • LV volume • Transmitral E/A ratio • LV dP/dt • Contractility index • Relaxation index • Myocardial collagen content	c-kit+ group: significant ↑fractional shortening, ↑E/A ratio, ↑max LV dP/dt, ↑Contractility index; ↓Relaxation index (Tau), ↓collagen deposition when compared to sham group

In summary, the foregoing preclinical data demonstrate that delivery of c-kit+ cells is associated with improved LV function in various animal models (mice, rats, pigs, cats) of subacute MI, old MI, or ISO-induced cardiomyopathy. Among these, the studies in large (porcine) animal models of chronic ICM[18-21,23] have greater clinical relevance for the CONCERT-HF trial.

Combined MSCs and c-kit+ cells: Based on findings that MSCs induce proliferation of c-kit+ cells[24], an investigation was carried out to determine if the combination of both cell types produced a greater reduction in MI size and improvement in LV function than each cell type alone[23]. Yorkshire swine underwent balloon occlusion of the LAD coronary artery followed by reperfusion, and were immunosuppressed after MI with cyclosporine and methylprednisolone. Intramyocardial injection of either combination human c-kit+ cells/human MSCs (1M/200M, n=5), human c-kit+ cells alone (1M, n=5), human MSCs alone (200M, n=5), or placebo (PBS, n=5) was administered to the infarct border zones at 14 days post-MI. Each cell therapy group reduced MI size relative to placebo ($p<0.05$), and the MI size reduction was two-fold greater in combination vs. either cell therapy alone ($p<0.05$). Significant improvement in LV chamber compliance (end-diastolic pressure volume relationship, $p<0.01$) and contractility (preload recruitable SW and dP/dtmax, $p<0.05$) was also observed in combination treated swine. LV ejection fraction (LVEF) was restored to baseline in all the cell therapy groups, while pigs receiving placebo had persistently depressed LV function ($p<0.05$). The engraftment of stem cells was seven-fold greater in the combination group vs. either cell type alone ($p<0.001$). In summary, combining human MSCs and human c-kit+ cells as a cell therapeutic enhanced MI size reduction and restored diastolic and systolic function toward normal after MI.

The superiority of the c-kit+ cell + MSC combination over either cell alone was further demonstrated by two subsequent studies in porcine models of chronic ICM[18,21] and in a rat model of chronic ICM[17], as detailed above. Taken together, these studies[17-19,23] illustrate potentially important biological interactions between c-kit+ cells and MSCs that enhance cell therapeutic responses and support the conduct of human clinical trials.

Although the above study demonstrated the benefits of combining 1 million c-kit+ cells and 200 million MSCs, the dose of c-kit+ cells was relatively small and it is possible that greater efficacy could be achieved by increasing it. In this regard, evidence for the safety of intracoronary infusion of higher doses of c-kit+ cells has been provided by a recent study in pigs[38]. Fourteen normal pigs (i.e., pigs not subjected to myocardial infarction) received an intracoronary infusion (into the LAD, using a catheter without balloon inflation) of 20×10^6 human c-kit+ cells (n=9) or placebo (n=5, PlasmaLyte A solution). There was no significant difference between treated and control groups attributable to the cell product with respect to any of the tests of cardiac, renal, or liver injury. Furthermore, echocardiographic analyses did not reveal decline in myocardial function or change in cardiac dimensions.

2.3 Background Clinical Studies

Transplanting progenitor cells into a region of damaged myocardium, termed cellular cardiomyoplasty[39], is a potentially new therapeutic modality designed to repair necrotic, scarred, or dysfunctional myocardium[40-42]. Ideally, graft cells should be readily available, easy to culture to ensure adequate quantities for transplantation, and able to survive in host myocardium that can be a hostile environment of limited blood supply and hostile immune reaction. Although it was originally thought that effective cellular regenerative strategies require that the administered cells dif-

ferentiate into adult cardiomyocytes and couple electromechanically with the surrounding myocardium, recent evidence indicates that is not required for effective cardiac repair. More importantly, transplantation of graft cells should improve cardiac function and prevent adverse ventricular remodeling.

To date, a number of candidate cells have been transplanted in experimental models, including fetal and neonatal cardiomyocytes[43], pluripotent stem cell-derived cardiomyocytes[44,45], tissue engineered contractile grafts[46], skeletal myoblasts[47], several cell types derived from adult bone marrow[48-53], and cardiac progenitors residing within the heart itself. There has been substantial clinical development in use of whole bone marrow and skeletal myoblast preparations in studies enrolling both post-infarction patients, and patients with chronic ischemic left ventricular (LV) dysfunction and HF.

2.3.1 Bone marrow mononuclear cells

Evidence from trials investigating delivery of bone marrow or bone marrow-derived cells represents a highly promising modality for cardiac repair and suggests that cellular cardiomyoplasty is a safe and effective strategy to improve cardiac function in patients with acute MI or chronic ICM[54,55]. The clinical experience with the administration of cells using the IC route, peripheral IV injection, and transendocardial injection also provides substantial evidence of clinical safety at the cell doses administered. Although CONCERT-HF will not use these cells, a review of these studies (Table 3) is relevant since the mode of delivery for many of them (i.e., catheter-based IM injection) is the same as that proposed for CONCERT-HF.

For example, the FOCUS-CCTRN trial[56] (First Mononuclear Cells injected in the United States conducted by the CCTRN) employed transendocardial injections to assess the safety and effectiveness of bone marrow mononuclear cells in patients with chronic HF and ongoing myocardial ischemia. This phase II randomized double-blind, placebo-controlled trial recruited 92 patients who were symptomatic (New York Heart Association classification II-III or Canadian Cardiovascular Society classification II-IV) with a left ventricular ejection fraction of 45% or less, a perfusion defect by single-photon emission tomography (SPECT), and coronary artery disease not amenable to revascularization received maximal medical therapy. Cells were delivered by direct IM injection, and patients were followed for six months by clinic visit and for five years by telephone. No deaths, serious arrhythmias, or other serious adverse events were attributed to cell therapy, and the DSMB never placed the study on hold due to a safety signal.

This and other studies in Table 3 provide substantial evidence of: 1) clinical safety with the administration of cells by transendocardial injection; 2) clinical safety at the cell doses administered; and 3) preliminary support the potential for clinical efficacy.

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TABLE 3 CLINICAL STUDIES: AUTOLOGOUS BONE MARROW-DERIVED MONONUCLEAR CELLS (BMCs)						
Study	**N**	**Cell Delivery Method**	**Cell Source & Type**	**Cell Dose (x 10^6)**	**Safety Results**	**Efficacy Results**
Stamm[57]	6	Direct IM injection during CABG surgery	Autologous, AC133$^+$ BMCs	1.2 - 3.4	No arrhythmias; no neoplasia	↑ global contractility (EF)
Tse[58]	8	Catheter-based IM injection	Autologous BMCs	2.6 - 21.2	No arrhythmias	↑ wall motion & thickening
Fuchs[59]	10	Catheter-based IM injection	Autologous BMCs	32.6 ± 27.5	No arrhythmias or other SAEs	↓ angina score; ↓ ischemia
Perin[3]	14	Catheter-based IM injection	Autologous CD34$^+$ BMCs	25.5 ± 6.3	No arrhythmias at 6-mo. F/U	↑ global contractility (EF); ↓ ESV
Beeres[60]	25	Catheter-based IM injection	Autologous BMCs	84.1 ± 28.7	No arrhythmias or pericardial effusion	↑ global contractility (EF); ↓ ESV
Briguori[61]	10	Catheter-based IM injection	Autologous CD34$^+$ BMCs	4.6 ± 1.5	No arrhythmias or AMI	↑ quality of life; ↑ perfusion
de La Fuente[62]	10	Catheter-based IM injection	Autologous CD34$^+$ BMCs	86 ± 3	No arrhythmias at 12-mo. F/U	↑ global contractility (EF)
Mocini[63]	36	Direct IM injection during CABG surgery	Autologous CD34$^+$ BMCs	292 ± 232	No SAEs	↑ global contractility (EF)
Hendrikx[64]	20	Direct IM injection during CABG surgery	Autologous BMCs	60.1 ± 31.1	Possible inducible VT	↑ global contractility (EF)
Stamm[65]	55	Direct IM injection during CABG surgery	Autologous, CD133$^+$ BMCs	3.85 - 103.0	No arrhythmias	↑ global contractility (EF)
Li[66]	6	Direct IM injection during CABG surgery	Autologous BMCs	50 – 100	No arrhythmias; no neoplasia	Not assessed
Perin[56]	92	Catheter-based IM injection	Autologous BMCs	100	No arrhythmias; no neoplasia	small ↑ EF (exploratory)
Traverse[67]	87	IC infusion	Autologous BMCs	150	No arrhythmias; no neoplasia	No effect on LV function
Traverse[68]	120	IC Infusion	Autologous BMCs	150	No arrhythmias; no neoplasia	No effect on LV function
AMI: acute myocardial infarction; IM: intramyocardial; CABG: coronary artery bypass graft; BMC: bone marrow-derived mononuclear cells; EF: Ejection Fraction; ESV: end systolic volume; F/U: follow-up; SAE: serious adverse event; VT: ventricular tachycardia; IC: intracoronary						

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

MSCs are a particularly promising bone marrow-derived cell for cardiac regenerative therapy because of their availability, immunomodulatory properties, and track-record of safety and efficacy[5,8,32,42]. Although there is no agreed upon cell surface marker that characterizes MSCs, they appear related to c-kit+ cells as they pass through a stage of cardiac differentiation in

which they express this cell surface marker. C-kit is a tyrosine kinase receptor for stem cell factor[69].

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

Chen *et al.*[70] randomly assigned 69 patients to receive intracoronary (IC) infusions of autologous MSCs (average cell dose: 5.4×10^{10}) or placebo (saline) 18 days after the onset of acute MI symptoms. At the three-month follow-up visit, LVEF was significantly improved in the MSC-treated group (from 49% ± 9% at baseline to 67% ± 11%) compared to the placebo group (from 48% ± 10% at baseline to 53% ± 18%; $p<0.01$ for the between-group comparison). This improved EF was sustained at six months post-infusion. In addition, significant reductions in perfusion defect, LV End Diastolic Volume (LVEDV) and LV End Systolic Volume (LVESV) were reported in the MSC-treated group. No adverse events were reported in this study.

A multi-center, randomized, double-blinded, placebo-controlled study was performed in 53 patients who were treated 3-10 days post-MI[4]. Patients were administered with one of three cell-dose levels of allogeneic MSCs (0.5, 1.6 and 5.0 cells/kg; corresponding to 3.5×10^7, 1.1×10^8, and 3.5×10^8 cells per patient for a 70 kg body weight patient), or placebo administered via peripheral IV injection, and followed for six months. There were no deaths reported in the study; no toxicity was observed with the administration of the allogeneic MSCs (which were found to be well-tolerated at all dose levels administered, with 5.3 adverse events per patient in the MSC-treated group vs. 7.0 in the placebo group); and no serious adverse events were attributed to MSC administration.

A phase I/II randomized trial was conducted to assess the safety and efficacy of allogeneic and autologous MSCs delivered via transendocardial injection in patients with ICM[5]. In the PercutaneOus StEm Cell Injection Delivery Effects On Neomyogenesis (POSEIDON) trial (NCT01087996), 30 patients were divided into 6 subgroups based on type of MSC (allogeneic or autologous) and dose (20 million, 100 million, and 200 million). The primary outcome was 30-day post-catheterization incidence of predefined treatment-emergent serious adverse events (SAEs). Efficacy assessments included 6-minute walk test, exercise peak VO_2, Minnesota Living with Heart Failure Questionnaire (MLHFQ), New York Heart Association class, LV volumes, EF, early enhancement defect (EED), a measure of infarct size based on CT imaging techniques, and sphericity index. Within 30 days, the treatment-emergent SAE rate was 6.7% (one patient in each group), well below the pre-specified stopping event rate of 25%. The one-year incidence of SAEs was 33.3% (n=5) in the allogeneic group and 53.3% (n=8) in the autologous group ($p=0.46$). Relative to baseline, autologous but not allogeneic MSC therapy was associated with an improvement in the 6-minute walk test and the MLHFQ score, but neither improved exercise VO_2 max. Allogeneic and autologous MSCs reduced mean EED by −33.21% (95% CI, −43.61% to −22.81%; $p<0.001$) and sphericity index, but did not increase EF. Allogeneic MSCs reduced LVEDV. Low-dose concentration MSCs (20 million cells) produced greatest reductions in LV volumes and increased EF. Allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions. In summary, MSC injection favorably affected patient functional capacity, quality of life, and ventricular remodeling.

The Transendocardial Autologous Cells in ischemic Heart Failure Trial (TAC-HFT)[7] was conducted in order to evaluate the safety of transendocardial stem cell injection with autologous MSCs and bone marrow mononuclear cells (BMCs) in patients with ICM. This was a phase 1/2 randomized, blinded, placebo-controlled study involving 65 patients with ICM and left ventricular (LV) ejection fraction less than 50% The study compared injection of MSCs (n=19) with placebo (n = 11) and BMCs (n = 19) with placebo (n = 10) following patients for 1 year. No patient had a treatment-emergent serious adverse event at day 30. Over 1 year, the MLHFQ score improved with MSCs (-6.3; 95% CI, -15.0 to 2.4; *p*=0.02) and with BMCs (-8.2; 95% CI, -17.4 to 0.97; *p*=.005) but not with placebo (0.4; 95% CI, -9.45 to 10.25; p=0.38). The 6-minute walk distance increased with MSCs only (repeated measures model, $p = 0.03$). Infarct size as a percentage of LV mass was reduced by MSCs (-18.9%; 95% CI, -30.4 to -7.4; within-group, $p = 0.004$) Regional myocardial function as peak Eulerian circumferential strain at the site of injection improved with MSCs (-4.9; 95% CI, -13.3 to 3.5; $p = 0.03$) . The authors concluded that transendocardial stem cell injection with MSCs or BMCs appeared to be safe for patients with chronic ICM and LV dysfunction.

The Cardiopoietic stem Cell therapy in heart failURE (C-CURE) trial[71] assessed the feasibility and safety of autologous bone marrow-derived and cardiogenically oriented mesenchymal stem cell therapy to probe for signs of efficacy in patients with chronic HF. In the cell therapy arm, bone marrow was harvested and isolated mesenchymal stem cells were exposed to a cardiogenic cocktail. Derived cardiopoietic stem cells were delivered by map guided endomyocardial injections. The target dose was attained in 75% and delivery without complications in 100% of cases. There was no evidence of increased cardiac or systemic toxicity induced by cardiopoietic cell therapy. Left ventricular ejection fraction was improved by cell therapy (from 27.5 ± 1.0% to 34.5 ± 1.1%) versus standard of care alone (from 27.8 ± 2.0% to 28.0 ± 1.8%, $p < 0.001$) and was associated with a reduction in left ventricular end-systolic volume (-24.8 ± 3.0 ml vs. -8.8 ± 3.9 ml, $p < 0.001$). Cell therapy also improved the 6-min walk distance (+62 ± 18 m vs. -15 ± 20 m, $p < 0.01$) and provided a superior composite clinical score encompassing cardiac parameters in tandem with New York Heart Association functional class, quality of life, physical performance, hospitalization, and event-free survival.

A recent trial supported by Mesoblast Ltd. was performed at six centers in the US between 8/2008 and 5/2010 to assess the safety and efficacy of bone marrow-derived allogeneic mesenchymal precursor cells (MPCs) STRO-1bright cells on LV function (NCT00721045). This was a Phase II, single blind, randomized dose-escalation study of 60 subjects with fifteen subjects per group (10 treated and 5 controls in each group) exploring three doses of cells (i.e., 25 million, 75 million or 150 million). Subjects were injected via the transendocardial route utilizing the NOGA® XP Mapping and Navigation System (NOGA) into viable myocardium. Outcomes were a) immunological safety; b) safety of the injection procedure; c) 6-month surrogate efficacy findings of no change in LVEF and 6-minute walk test, significant decrease in both LVESV and LVEDV in the 150 million group; and d) MACE evaluations to 36 months in all groups. For ischemic MACE (i.e., death, MI and revascularization) pooled MPC had significantly lower events but no significant difference between any cell dose group and control patients. For HF MACE (i.e., cardiac death or HF re-hospitalization) there were no events in the 150 million cell group. HF-related MACE was seen in all other groups. These data were partially presented at AHA 2013 and published in Circulation Research in 2015[72].

2.3.3 Human experience with cardiac-derived cells

The first two trials employing cell products developed from human myocardium were SCIPIO[73] and CADUCEUS[74]; however, given concerns that have been raised regarding the work of Dr. Piero Anversa and colleagues, only the CADUCEUS study will be described.

CADUCEUS[74] *(CArdiosphere-Derived aUtologous stem CElls to reverse ventricular dysfunction)* was a phase I trial which utilized endomyocardial biopsy (EMB) specimens. The cardiosphere-derived cells were infused via intracoronary route at 1.5-3 months after myocardial infarction to assess safety and evaluate effects seen previously in pre-clinical models (i.e. reduced scarring, increased viable myocardium, improved cardiac function). No complications were associated with the infusion and, by six months, no deaths, cardiac tumors, or MACE were observed in either the active group (n=17) or standard care group (n=8). The active group demonstrated significant change over the standard care group in reduced scar mass (8.4g ± 5.1, p=0.001) and regional systolic wall thickening (7.7% vs -5.9%; p=0.045). Similarly, the active group showed increases in viable heart mass (13.0g ± 11.4 vs 0.9g ± 6.2; p=0.01) and improved regional contractility (-2.0% ± 6.3 vs 1.5% ±7.3; p=0.009) when compared to the standard care group. The trial demonstrates the safety of infusing cell products derived from human myocardium, while lending credence to pre-clinical findings of increased viable myocardium.

2.4 Advances in Cardiac Imaging

Members of the CCTRN clinical research team have extensive experience in the evaluation of myocardial scar and LV function. Using an IV injection of Gadolinium-DTPA, the study team is able to determine infarct size non-invasively with T1-weighted delayed-enhanced MRI (DEMRI). Specifically, the DEMRI data were analyzed to identify the area at risk in the peri-infarct region and evaluate the clinical impact of the heterogeneity of myocardial injury[75-77]. Several experiments have validated DEMRI as an accurate and viable method for quantification of myocardial fibrosis by comparing the measured scar area to those from thallium scintigraphy, electrocardiography, and histopathology in animals and humans[78-81]. The prognostic implications of DEMRI have been studied extensively in a variety of pathologies and have also been compared to and proven superior to other imaging modalities. DEMRI is the current gold standard to predict regional functional recovery. Another area of significant interest is the use of tagged MRI scanning to detect and quantify alterations in regional myocardial mechanics in animal models of ICM[82,83]. Non-surgical animal models have been developed for studying cardiac mechanics, perfusion, and interventional procedures[84-87]. New MR imaging and analysis methods that enable the rapid determination of myocardial function in infarction and ischemia have recently been developed[88,89]. This technique, Harmonic Phase (HARP) MRI[88,90,91] is based on tagged MRI techniques. Computationally, the analysis of HARP MRI can be performed much more rapidly than the traditional tag tracking MRI. HARP MRI, similar to tagged MRI, yields quantitative motion and strain parameters on a regional basis that can be used for comparison across patients or at serial time points after intervention. Thus, HARP MRI analysis represents a rapid and repeatable method to assess LV function serially in a quantitative manner. HARP provides fast, accurate assessment of myocardial strains in humans with and without coronary artery disease[92]. Similar techniques have been used to assess structural and functional changes after MI in rats[93,94].

Furthermore, T1 myocardial mapping techniques will be employed (at select qualified centers with such capabilities) as an essential adjunctive tool to garner a more complete picture of the

myocardial condition following stem cell therapy over DEMRI which will also be performed. Recently, T1 mapping has been shown to effectively demonstrate fibrotic changes in a myriad of pathologic conditions[95-97]. While the DEMRI is very sensitive to small areas of regional fibrosis, this method enables a comparison to the signal from the "normal" myocardial reference areas in relation to the fibrotic regions. In patients with more diffuse fibrosis, which include those with chronic ICM, T1 mapping will provide a more sensitive method by which both interstitial and replacement fibrosis can be quantified in the myocardium.

2.5 Rationale for Dual Therapy

This trial is based upon the hypothesis that the combination of two cell types will have a greater effect on LV function and functional status than either cell type alone or than placebo. The hypothesis arose from work showing that bone marrow-derived MSCs stimulate a number of endogenous cardiac c-kit cells in porcine models of infarction[24]. Additionally, ex vivo, MSCs enhance the survival and proliferation of c-kit+ cells. To test the therapeutic response of dual therapy, a prospective porcine study was designed and conducted to test whether dual therapy enhances the reduction in MI scar size[23]. As detailed above, this study, indeed, documented an enhanced reduction in the burden of MI scar and LV dysfunction with dual therapy vs. mono therapy, a concept that was confirmed by other studies in pigs and rats[17-19,23]. One of these studies[18] used a porcine model in which autologous c-kit+ cells and MSCs were delivered 3 months after MI using transendocardial injection, and thus is highly relevant to CONCERT-HF. Thus, the CONCERT-HF trial is designed to reproduce the effect seen in the porcine studies and to test the impact of cell therapy using dual therapy (MSCs plus c-kit+ cells) compared to placebo and to either cell type alone. The endpoints will allow us to determine if dual therapy has an enhanced effect relative to placebo, an enhanced effect relative to mono therapy, and if it is well-tolerated in humans with ICM.

2.6 Dose Rationale

Many considerations are involved in selecting optimal doses of cells. The idea that the combination of MSCs plus c-kit+ cells is effective is predicated on animal data[23]. The key principle for our selection of doses for the combined cell product is optimizing the dose of each individual cell rather than a ratio of the cells. We evaluated a significant amount of data from which we selected the individual cell doses.

MSCs: Clinical studies have utilized cell doses that were over 200 million MSCs[4,5] and doses of 1×10^{10} MSCs[70] – as much as sixty-five times greater than the MSC cell dose proposed herein (150×10^6). In two preclinical studies in a porcine model[61,98] and in the TAC-HFT[32] and POSEIDON trials[5] MSC therapy was safely administered via intramyocardial injection at doses of up to 200×10^6 cells. The MSC dose comes from data from the POSEIDON study[5] published two years ago by the University of Miami group (a CCTRN center) that compared autologous vs. allogeneic MSCs, as well as a trial supported by Mesoblat Ltd. which included subjects from two CCTRN centers and used multiple doses of Mesoblast's MPCs including 150×10^6 cells. In this dose escalation study[72] of 60 subjects, MSC therapy was safely administered via intramyocardial injection at doses of up to 150×10^6 cells. Specifically, increasing doses of MSCs were associated with decreasing LVEDV and decreasing LVESV. This benefit was maximized at the dose of 150 million MSCs. In addition, no serious adverse events were reported at the 150 million MSC dose. These studies support using the dose level of 150×10^6 for the MSC prepara-

tion in this clinical study. The target MSC dose was chosen based on data from previous studies, as well as practical considerations and the ability to grow this quantity of cells for most subjects within approximately 28 days.

c-kit+ cells: The entire body of pre-clinical work done on c-kit+ cells strongly supports the safety of a dose of cells greater than $1x10^6$ cells. Doses of c-kit+ cells used in animal studies,normalized to body weight, have ranged enormously, from 20 cells/gram body weight in pigs[21,23] to 40 cells/gram body weight in pigs[18,19] to as high as 2 x 10^5 cells/gram body weight in rats[37]; the corresponding total doses were 1 x 10^6 cells in pigs[21,23] , 1 x 10^6 cells in minipigs[18,19] and 36 x 10^6 in rats[37]. No adverse effects were noted in any study. The target dose used in CONCERT-HF (5 x 10^6 cells or 63 cells/gram body weight) is at the lower end of this range. It should be noted that in pigs, doses much higher than 1 x 10^6 have been found to be safe; Bolli et al. at the University of Louisville (a CCTRN center) performeda study in pigs in which they infused 20 x 10^6 human c-kit+ cells into the LAD, which is roughly equivalent to 40-60 x 10^6 cells in humans, and found no adverse safety signals[38]. No adverse effects of c-kit+ cells have been reported in published studies or seen in unpublished data, as assessed by cardiac enzymes, myocardial function or histology. In clinical trials, other similar sized cardiac-derived cells have been safely given at a dose of 25 x 10^6 [74]. For CONCERT-HF, the target dose of c-kit+ cells will be 5 x 10^6 c-kit+ cells. This dose was selected based on the safety data in pigs described above[38] and on our experience with successful isolation and expansion of c-kit+ cells from tissue samples (EMBs) acquired from a diverse population of patients with ICM. From a practical standpoint, our experience indicates that in many patients 5 x 10^6 c-kit+ cells is the highest number of cells that can be grown from the EMBs without resorting to more than six passages.

Combined MSCs and c-kit+ cells: Preclinical studies have demonstrated that the use of c-kit+ cells in combination with MSCs generates improved cardiac function in pigs[17-19,23] and support the hypothesis that dual therapy is well-tolerated and may signal improved heart function in subjects with ICM. In a pre-clinical swine study of intramyocardial injections at 14 days post-MI, the dose used was a combination of 1 x 10^6 human c-kit+ cells plus 200 x 10^6 human MSCs[23]. The same does have been used in two subsequent pig studies[18,19]. While these studies demonstrated improved cardiac function at these cell doses, the dose of c-kit+ cells was relatively small and it is possible that greater efficacy could be achieved by increasing it. The upper limit on the total number of all cells was carefully considered because: 1) data show efficacy can be reduced when doses are too high (possibly due to the viscosity or lack of oxygenation of the mixture); and 2) it is important to limit the concentration of cells that will be passed through the various catheter systems to avoid cell clumping.

In conclusion, the target cell doses for CONCERT-HF will be 150 x 10^6 MSCs and 5 x 10^6 c-kit+ cells, alone or in combination, striving to reach maximal cell numbers in each group. These doses have a strong safety profile and yield compelling evidence for the potential to improve LV function and functional status in subjects with HF of ischemic etiology.

3.0 INVESTIGATIONAL PLAN

3.1 Research Questions

- Can MSCs and c-kit+ cells, both alone and in combination, be manufactured and delivered to subjects with ischemic cardiomyopathy?

- Are MSCs and c-kit+ cells, alone or in combination, well-tolerated by subjects with ischemic cardiomyopathy?
- Do MSCs combined with c-kit+ cells, MSCs alone, or c-kit+ cells alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months post treatment with study product?
- Do MSCs combined with c-kit+ cells improve LV function and functional status when compared with either MSCs alone or c-kit+ cells alone from baseline to 6 months and baseline through 12 months post treatment with study product?
- When compared with each other, do either MSCs alone or c-kit+ cells alone offer a relative advantage in improving LV function and functional status from baseline to 6 months and baseline through 12 months post treatment with study product?

3.2 Study Design

This study will evaluate one-hundred sixty (160) subjects and will be undertaken in two stages.

Stage 1 (open label lead-in) consists of sixteen (16) subjects randomized 1:1 to either a standard of care (SOC) group (i.e., receive no study intervention procedures) or to Combo therapy. Data from this stage will be assessed for safety of the study procedures and bioactivity of the product. Following successful review of the lead-in data by the DSMB (see Section 7.7), this will be followed by the second stage consisting of a double-blind, placebo-controlled, clinical trial enrolling 144 subjects. Those subjects randomized to Combo therapy will continue to be followed per protocol for 12 months. Those randomized to the SOC control group will have the option to be evaluated for enrollment in the trial conducted in Stage 2.

Stage 2 is a phase II, randomized, placebo-controlled clinical trial designed to evaluate the feasibility, safety and effect of Combo, MSCs alone, and c-kit+ cells alone compared with placebo as well as each other in subjects with HF of ischemic etiology.

A total of one hundred forty-four (144) subjects will be randomized (1:1:1:1) to receive Combo, MSCs, c-kit+ cells, or placebo. Within 60 days of signing informed consent, all subjects will undergo bone marrow aspiration (BMA) and right heart catheterization (RHC). The RHC will include endomyocardial biopsy (EMB) for the 72 subjects randomized to the Combo and c-kit+ cell groups. All subjects will undergo study product injection using the NOGA® XP Mapping and Navigation System. After randomization, baseline imaging, harvest procedures, and study product injection, subjects will be followed up at 1 day, 1 week, 1 month, 3 months, 6 months and 12 months post study product injection (SPI). All subjects will have DEMRI scans to assess scar size and LV function and structure at baseline and at 6 and 12 months post study product administration. All endpoints will be assessed at the 6 and 12 month visits which will occur 180 ±30 days and 365 ±30 days respectively from the day of study product injection (Day 0). For the purpose of the endpoint analysis and safety evaluations, we will utilize an “intention-to-treat” study population. An as-treated analysis will also be conducted.

3.3 Study Treatment Assignments and Dosages

One hundred forty-four (144) subjects meeting all inclusion/exclusion criteria will be evaluated at baseline. Subjects will be randomized 1:1:1:1 to one of four treatment strategies:

1. Group A (36 subjects) – Combo: Target dose is a mixture of 150 x 10^6 (150 million) MSCs and 5 x 10^6 (5 million) c-kit+ cells delivered in 15 injections each of 0.4 ml volume
2. Group B (36 subjects) – Autologous MSCs: Target dose is 150 x 10^6 (150 million) MSCs delivered in 15 injections each of 0.4 ml volume

3. Group C (36 subjects) – Autologous c-kit+ cells: Target dose is 5 x 10^6 (5 million) c-kit+ cells delivered in 15 injections each of 0.4 ml volume
4. Group D (36 subjects) – Placebo: 15 injections each of 0.4 ml cell-free PlasmaLyte-A medium

Minimum doses are outlined in Section 5.8 of this protocol. In order to maintain study blinding, if a subject in group A does not meet the minimum dose of c-kit+ cells, the subject will receive MSCs and conversely, if they do not meet the minimum dose of MSCs, the subject will receive c-kit+ cells; should both products fail to meet release criteria, the subject would receive placebo. If a subject in group B or C does not meet the minimum dose, the subject will receive placebo. For statistical analysis purposes, all subjects will be analyzed in the group to which they were randomized in accordance to the "intention-to-treat" principle.

The study product for Group A will contain at most 155 million cells (150 million MSCs plus 5 million c-kit+ cells). The product delivery goal is to keep the number of injections, the volume per injection and the cell concentration per injection moderate. Administering 15 injections of 0.4 ml volume each provides 6.0 ml of total study product volume with a cell concentration of no more than 25.833 million total cells/ml (±10%), or a maximum of 10.333 million cells/0.4ml (±10%), per dose, per injection site. While the number of injections (n=15) and the volume per injection (0.4 ml) will remain unchanged, smaller numbers of cells will result in a lower concentration of cells per injection. See Section 5.8 for additional details.

3.4 Feasibility Assessment

To demonstrate that MSCs and c-kit+ cells, both alone and in combination, can be manufactured and delivered to subjects with ICM, the following measures will be reported.
The number and percent of subjects who have:

- Events between randomization and SPI that preclude the subject from getting SPI
- Failed bone marrow aspiration procedure
- Failed endomyocardial biopsy procedure
- Failed release criteria (including minimum number of cells) for receiving the MSC product
- Failed release criteria (including minimum number of cells) for receiving the c-kit+ cell product
- Less than 15 injections during the SPI procedure
- At least one cardiac MRI endpoint measure that is uninterpretable due to issues related to the device, including, but not limited to, inability to undergo the procedure

3.5 Safety Assessment

Adverse event monitoring is discussed in detail in Section 7. In order to assess the relative safety of MSCs and c-kit+ cells delivered alone or in combination when compared with placebo, the following safety data will be collected and analyzed by therapy group between baseline and a) 6 months and b) 12 months:

Major adverse cardiac events (MACE)[1] including: death, hospitalization for worsening HF, and/or exacerbation of HF (non-hospitalization)

- Other significant clinical events including: non-fatal stroke, non-fatal myocardial infarction, coronary artery revascularization, ventricular tachycardia/fibrillation, and/or pericardial tamponade
- All adverse events that are at least grade 2 in severity (see Section 7.3.1)

3.6 Study Endpoints

3.6.1 Efficacy Objective

To assess whether cell therapy (Combo, MSC, and c-kit+ cell) improves LV function and functional status when compared with placebo and whether the improvement in subjects receiving the combined cell product is greater than the improvement in those receiving either individual cell product alone. LV function and functional status are assessed at baseline, 6 months, and 12 months.

3.6.2 Prospectively Declared Efficacy Endpoint Measures

To assess the overall effect of the cell types used in CONCERT-HF, multiple endpoints have been selected from different categories of effects (domains)[99]. Each having the same priority for the efficacy analyses, the domains and endpoint measures for CONCERT-HF are:

- Myocardial evaluations by cardiac MRI (cMRI) over time:
  - Function:
    - Change in LVEF
    - Change in global and regional strain (HARP MRI)
  - Structure:
    - Change in LVEDVI
    - Change in LVESVI
    - Change in LV Sphericity Index
  - Morphology:
    - Change in infarct/scar volume (DEMRI)
- Functional capacity over time:
  - Change in VO_2 max (treadmill)
  - Change in exercise tolerance (6MWT)
  - Change in MLHF Questionnaire (subject reported)
- Clinical outcomes over time:
  - MACE
  - Cumulative days alive and out of hospital for HF
- Biomarkers over time:
  - Change in NT-proBNP

3.6.2.1 Comparisons

Each of the following comparisons will be made between:

1. Combo versus MSC
2. Combo versus c-kit+ cell
3. Combo versus placebo
4. MSC versus placebo
5. c-kit+ cell versus placebo
6. MSC versus c-kit+ cell

3.6.2.2 Analyses

The reported effect for each of the endpoints will be:

- Difference between baseline and 6 months when all subjects have completed their six month evaluations

And, when all subjects have completed their 12 month evaluation:

- Difference in the trajectories from baseline through 12 months
- Difference between baseline and 12 months
- Difference between 6 months and 12 months

3.6.2.3 Sub-study Analysis

Change in global diffuse fibrosis will be evaluated as a sub-study. This experimental endpoint will only be performed at clinical sites that have the necessary sequencing software and are qualified to participate in this sub-study by the MRI core lab.

3.6.2.4 c-kit+ cell Product Characterization Analysis

Characterization of c-kit+ cell products, i) hematopoietic and other stem/progenitor cell markers, ii) cardiogenic markers, and iii) senescence) will be reported. Means and medians will be used for central tendency and dispersion will be depicted with the standard deviation and interquartile range.

3.7 Sample Size Computations and Assumptions

The sample size computation is based on general linear model under the assumption of independence and normality of the observations. For the sample size computation, we assume that this four armed study is balanced.

We write y_k is the change between the baseline and follow-up measures in accordance with Section 3.6 above and let x_i =1 if the ith subject receives MSC alone, 0 otherwise, and w_i =1 if the ith subject receives c-kit+ cell alone, 0 otherwise and z_i =1 if the ith subject receives Combo, 0 otherwise. Then we construct the model

$$\mathbf{E}\left[y_i\right] = \beta_0 + \beta_1 x_i + \beta_2 w_i + \beta_3 z_i \,.$$

3.7.1 Efficacy Hypotheses and Testing

Using this model for a given endpoint in Section 3.6.2 and for a given analysis in Section 3.6.2.2, we can address each of the six comparisons in Section 3.6.2.1 as follows:

- **Hypothesis 1**: Combo improves LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months.
  Addressed by the hypothesis $\mathrm{H}_0 : \beta_3 = 0 \;\; vs. \; \mathrm{H}_a : \beta_3 \neq 0$
- **Hypothesis 2**: MSCs alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months.
  Addressed by $\mathrm{H}_0 : \beta_1 = 0 \;\; vs. \; \mathrm{H}_a : \beta_1 \neq 0$
- **Hypothesis 3**: c-kit+ cells alone improve LV function and functional status when compared with placebo from baseline to 6 months and baseline through 12 months.
  Addressed by $\mathrm{H}_0 : \beta_2 = 0 \;\; vs. \; \mathrm{H}_a : \beta_2 \neq 0$
- **Hypothesis 4**: Combo improves LV function and functional status when compared with MSCs from baseline to 6 months and baseline through 12 months.
  Addressed by $\mathrm{H}_0 : \beta_3 = \beta_1 \;\; vs. \; \mathrm{H}_a : \beta_3 \neq \beta_1$
- **Hypothesis 5**: Combo improves LV function and functional status when compared with c-kit+ cells from baseline to 6 months and baseline through 12 months.
  Addressed by $\mathrm{H}_0 : \beta_3 = \beta_2 \;\; vs. \; \mathrm{H}_a : \beta_3 \neq \beta_2$
- **Hypothesis 6**: MSCs or c-kit+ cells improve LV function and functional status when compared with each other from baseline to 6 months and baseline through 12 months.
  Addressed by $\mathrm{H}_0 : \beta_2 = \beta_1 \;\; vs. \; \mathrm{H}_a : \beta_2 \neq \beta_1$

Assume b_i is the least square estimator of β_i for each of the four parameters (i = 1,2,3,4) in the model above. Then, in general, we need to compute the variance of several linear combinations of the b_i's. We write the model above as $\mathbf{E}\left[\underline{\mathbf{y}}\right] = \mathbf{X}\underline{\mathbf{b}}$ where $\mathbf{y}$ is a 4n-tuple vector of the changes in the measurement over time, $\mathbf{X}$ is the design matrix for the model above, and $\underline{\mathbf{b}}$ is the 4 by 1 vector of parameter estimates. If we write this linear combination in general as $\underline{\mathbf{a}}'\underline{\mathbf{b}}$ where $\underline{\mathbf{a}}$ is a 4 by 1 vector of constants, then we may write the variance as $\mathrm{Var}\left(\underline{\mathbf{a}}'\underline{\mathbf{b}}\right) = \left[\underline{\mathbf{a}}'\left(\mathbf{X}'\mathbf{X}\right)^{-1}\underline{\mathbf{a}}\right]MSE$.

Note that

$$\mathbf{X}'\mathbf{X} = \begin{bmatrix} 4n & n & n & n \\ n & n & 0 & 0 \\ n & 0 & n & 0 \\ n & 0 & 0 & n \end{bmatrix}$$ is a function of n, the number of subjects in each treatment group.

The power of the hypothesis test

$$\mathrm{H}_0 : \underline{\mathbf{a}}'\underline{\boldsymbol{\beta}} = 0 \quad \text{vs} \quad \mathrm{H}_a : \underline{\mathbf{a}}'\underline{\boldsymbol{\beta}} \neq 0$$

is then computed as

$$1-\beta = 1 - F_Z\left[Z_{1-\alpha/2} - \frac{\underline{\mathbf{a}}'\underline{\mathbf{b}}}{\sqrt{\left[\underline{\mathbf{a}}'\left(\mathbf{X}'\mathbf{X}\right)^{-1}\underline{\mathbf{a}}\right]\frac{MSE}{1-f}}}\right]$$

where

α = Type I error
β = Type II error
Z_c = the cth percentile from the standard normal probability distribution
MSE = the variance in the change over time (incorporates the correlation over time) pooled between the active and placebo groups.
F_Z = Cumulative distribution function of the standard normal distribution
$\mathbf{X}$ = the 4n by 4 design matrix for this model
$\underline{\mathbf{b}}$ = the 4 x 1 vector of parameter estimates.
$\underline{\mathbf{a}}$ = 4 x 1 vector of constants in which terms the statistical hypothesis $\underline{\mathbf{a}}'\underline{\boldsymbol{\beta}} = 0$ is expressed.
f = percentage of subjects lost to follow-up.

In this model assuming a balanced design,

$$\mathbf{X}'\mathbf{X} = \begin{bmatrix} 4n & n & n & n \\ n & n & 0 & 0 \\ n & 0 & n & 0 \\ n & 0 & 0 & n \end{bmatrix} = \begin{bmatrix} 4 & 1 & 1 & 1 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \end{bmatrix} n: \quad (\mathbf{X}'\mathbf{X})^{-1} = \begin{bmatrix} 1 & -1 & -1 & -1 \\ -1 & 2 & 1 & 1 \\ -1 & 1 & 2 & 1 \\ -1 & 1 & 1 & 2 \end{bmatrix} \frac{1}{n}.$$

To compute the variance of, for example, the hypothesis that $\mathrm{H}_0 : \beta_3 = 0 \ \ vs. \ \mathrm{H}_a : \beta_3 \neq 0$, we write that if $\underline{\mathbf{a}}' = \begin{bmatrix} 0 & 0 & 0 & 1 \end{bmatrix}$ so $\underline{\mathbf{a}}'\underline{\boldsymbol{\beta}} = \begin{bmatrix} 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} = \beta_3$. Thus,

$$\left[\underline{\mathbf{a}}'(\mathbf{X}'\mathbf{X})^{-1}\underline{\mathbf{a}}\right] = \begin{bmatrix} 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & -1 & -1 & -1 \\ -1 & 2 & 1 & 1 \\ -1 & 1 & 2 & 1 \\ -1 & 1 & 1 & 2 \end{bmatrix} \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \end{bmatrix} \frac{1}{n} = \frac{2}{n}$$

Where *n* is the number per therapy group and the trial size $N = 4n$

All models will be run adjusting for baseline values allowing for a smaller mean square error and slightly greater power. In the following tables, the standard error $\sigma_\Delta = \sqrt{MSE}$.

Evaluations of efficacy are literature based. Measures of ejection fraction variability are from LateTIME[67]. Effect size measures of LVESVI, LVEDVI, VO_2 max, and 6MWT are from FOCUS[56]. Infarct size estimates are from personal communication with the lab of Dr. Roberto Bolli. Sphericity Index estimates come from TAC-HFT[100]. The following computations demonstrate the power available from thirty-six subjects in each group.

3.7.2 Left Ventricular Ejection Fraction (LVEF)

We estimate the standard deviation of the difference (σ_Δ) as 8. Based on Williams et al.[23], the expected change in the LVEF for the Combo group over time compared with the change over time in the placebo group is 13.4, providing power > 99%. We conservatively estimate the effect size of the Combo vs. MSC comparison as 7 generating 91% power assuming the standard deviation of the difference over time is 8 (Table 4). We do not expect that the Combo vs. c-kit+ cell comparison will produce this level of effect, instead anticipating that this comparison will produce an effect size of 5.5 which can be detected with 74% power.

3.7.3 Infarct Size

We expect a change in infarct size (Dr. Bolli, personal communication), of 8 grams in the Combo group with a standard deviation of 7.0 when compared with the MSC group. This produces a power of greater than 99% (Table 5). The expected change in infarct size of Combo vs. the change in c-kit+ cell over time is anticipated to be 6 detectable at a power of 97%. The comparisons of cell types with placebo will produce greater power since the effect of Combo vs. placebo is greater than the effect seen from cell to cell comparisons.

3.7.4 Left Ventricular End Systolic Volume Index (LVESVI)

The standard deviation of the change in LVESVI is estimated to be 20 ml. Based on personal communication from Dr. Bolli and an article by Williams et al.[23], the expected change in Combo when compared with MSC is estimated to be 24 ml producing greater than 99% power (Table 6). The expected change in Combo compared with c-kit+ cell is 18 ml which is detectable with 93% power. The comparisons of cell types with placebo will produce greater power since the effect of Combo vs. placebo is greater than the effect seen from cell to cell comparisons.

3.7.5 Left Ventricular End Diastolic Volume Index (LVEDVI)

The standard deviation of the change in LVEDVI is estimated to be 40 ml. The expected change in LVEDVI of Combo is 45 ml (Dr. Bolli, personal communication) when compared with MSCs alone, producing 99% power (Table 7). The expected change in Combo vs. the c-kit+ cell alone group is 30 ml generating 81% power. The comparisons of cell types with placebo will produce greater power since the effect of Combo vs. placebo is greater than the effect seen from cell to cell comparisons.

3.7.6 VO_2 max

FOCUS[56] produced a small change 1 ml/kg/min increase in VO_2 max when compared with placebo and a standard deviation of 2.9. This small difference is not likely to be detectable and would be of less interest to the clinical community. However, given that this estimate of effect size could be wrong there is value in stating our prospective interest in analyzing this variable. Based on a standard deviation of 2.9, the smallest difference that we could detect for any treatment group comparisons would be a 2.5 ml/kg/min increase with 91% power (Table 8).

3.7.7 Six Minute Walk Test (6MWT)

The effect size will be based on the mean of two measures at each time point per subject rather than on a single measurement. The standard deviation for the change in 6MWT is anticipated to be approximately 121.92 meters based on the FOCUS[56] data with its intrasubject correction of 0.44 and incorporation of the intraclass correlation structure. We anticipate that cell therapy will produce an increase of 28.96 meters in the Combo vs. MSC comparison (Table 9). The comparisons of cell types with placebo will produce greater power since the effect of Combo vs. placebo is greater than the effect seen from cell to cell comparisons. Power will be low for each of the evaluations, but we nevertheless have a prospective interest in assessing the effect of cell therapy on the 6MWT.

3.7.8 Minnesota Living with Heart Failure Questionnaire (MLHFQ)

We anticipate that the standard deviation of the change in the mean MLHFQ is 22. We expect that the change in Combo compared with MSC will produce a 20 point change, generating a power of 93% (Table 10). We believe that Combo compared with c-kit+ cell will produce a 16 unit difference (79% power). The comparisons of cell types with placebo will produce greater power since the effect of Combo vs. placebo is greater than the effect seen from cell to cell comparisons.

3.7.9 Sphericity Index

Based on TAC-HFT[100] and assuming a standard error 0.10, we anticipate a 0.14 increase in sphericity index in Combo when compared with MSC (greater than 99% power) and 0.10 when Combo is compared with c-kit+ cells alone (97% power) (Table 11). The comparisons of cell

types with placebo will produce greater power since the effect of Combo vs. placebo is greater than the effect seen from cell to cell comparisons.

3.8 Interim Analysis

A formal interim efficacy analyses will be conducted after the first 50% (72/144) of subjects have had, or should have had, 6 months of follow-up. Details are provided in section 8.

Table 4. Power for LVEF Change Over Time
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect										
		4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9
Std Dev	6.0	**0.72**	**0.81**	**0.89**	**0.94**	**0.97**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
of Diff	7.0	**0.58**	**0.68**	**0.77**	**0.85**	**0.90**	**0.94**	**0.97**	**0.98**	**0.99**	**1.00**	**1.00**
(σ_Δ)	8.0	**0.48**	**0.57**	**0.66**	**0.74**	**0.81**	**0.87**	**0.91**	**0.94**	**0.97**	**0.98**	**0.99**
	9.0	**0.39**	**0.48**	**0.56**	**0.64**	**0.72**	**0.78**	**0.84**	**0.89**	**0.92**	**0.95**	**0.97**

Table 5. Power for Infarct Size Change Over Time
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect									
		4	4.5	5	5.5	6	6.5	7	7.5	8	8.5
Std Dev	5.0	**0.86**	**0.93**	**0.97**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
of Diff	6.0	**0.72**	**0.81**	**0.89**	**0.94**	**0.97**	**0.98**	**0.99**	**1.00**	**1.00**	**1.00**
(σ_Δ)	7.0	**0.58**	**0.68**	**0.77**	**0.85**	**0.90**	**0.94**	**0.97**	**0.98**	**0.99**	**1.00**
	8.0	**0.48**	**0.57**	**0.66**	**0.74**	**0.81**	**0.87**	**0.91**	**0.94**	**0.97**	**0.98**

Table 6. Power for LV End Systolic Volume Change
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect										
		6	8	10	12	14	16	18	20	22	24	26
Std Dev	15.0	**0.33**	**0.53**	**0.72**	**0.86**	**0.94**	**0.98**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
of Diff	20.0	**0.21**	**0.33**	**0.48**	**0.62**	**0.76**	**0.86**	**0.93**	**0.97**	**0.99**	**1.00**	**1.00**
(σ_Δ)	25.0	**0.15**	**0.23**	**0.33**	**0.44**	**0.57**	**0.68**	**0.78**	**0.86**	**0.92**	**0.95**	**0.98**
	30.0	**0.11**	**0.17**	**0.24**	**0.33**	**0.43**	**0.53**	**0.62**	**0.72**	**0.79**	**0.86**	**0.91**

Table 7. Power for LV End Diastolic Volume Change
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect									
		10	15	20	25	30	35	40	45	50	55
Std Dev	31.3	**0.23**	**0.44**	**0.68**	**0.86**	**0.95**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
of Diff	35.8	**0.18**	**0.36**	**0.56**	**0.76**	**0.89**	**0.96**	**0.99**	**1.00**	**1.00**	**1.00**
(σ_Δ)	40.2	**0.15**	**0.29**	**0.47**	**0.65**	**0.81**	**0.91**	**0.96**	**0.99**	**1.00**	**1.00**
	44.7	**0.13**	**0.25**	**0.40**	**0.56**	**0.72**	**0.84**	**0.92**	**0.97**	**0.99**	**1.00**

Table 8. Power for VO2max Change
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect										
		0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5
Std Dev	2.7	**0.10**	**0.29**	**0.56**	**0.80**	**0.94**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
of Diff	2.8	**0.10**	**0.27**	**0.53**	**0.77**	**0.92**	**0.98**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
(σ_Δ)	2.9	**0.10**	**0.26**	**0.50**	**0.74**	**0.91**	**0.98**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	3	**0.09**	**0.24**	**0.48**	**0.72**	**0.89**	**0.97**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**

Table 9. Power for Six Minute Walk Change
Type I error = 0.05; N = 144; 20% followup loss; 2 meas per time pt.

		Treatment Effect									
		70	75	80	85	90	95	100	105	110	115
Std Dev	120	**0.60**	**0.66**	**0.72**	**0.77**	**0.81**	**0.85**	**0.89**	**0.91**	**0.94**	**0.95**
of Diff	125	**0.57**	**0.62**	**0.68**	**0.73**	**0.78**	**0.82**	**0.86**	**0.89**	**0.92**	**0.94**
(σ_Δ)	150	**0.43**	**0.48**	**0.53**	**0.58**	**0.62**	**0.67**	**0.72**	**0.76**	**0.79**	**0.83**
	175	**0.33**	**0.37**	**0.41**	**0.45**	**0.50**	**0.54**	**0.58**	**0.62**	**0.66**	**0.70**

Table 10. Power for MLHF Change
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect										
		8	10	12	14	16	18	20	22	24	26	28
Std Dev	21	**0.30**	**0.44**	**0.58**	**0.72**	**0.82**	**0.90**	**0.95**	**0.98**	**0.99**	**1.00**	**1.00**
of Diff	22	**0.28**	**0.41**	**0.54**	**0.68**	**0.79**	**0.87**	**0.93**	**0.97**	**0.99**	**0.99**	**1.00**
(σ_Δ)	23	**0.26**	**0.38**	**0.51**	**0.64**	**0.75**	**0.84**	**0.91**	**0.95**	**0.98**	**0.99**	**1.00**
	24	**0.24**	**0.35**	**0.48**	**0.60**	**0.72**	**0.81**	**0.89**	**0.94**	**0.97**	**0.98**	**0.99**

Table 11. Power for Sphericity Index Change
Type I error = 0.05; N = 144; 20% followup loss

		Treatment Effect									
		0.04	0.06	0.08	0.1	0.12	0.14	0.2	0.18	0.2	0.22
Std Dev	0.08	**0.48**	**0.81**	**0.97**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
of Diff	0.10	**0.33**	**0.62**	**0.86**	**0.97**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
(σ_Δ)	0.12	**0.24**	**0.48**	**0.72**	**0.89**	**0.97**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**
	0.14	**0.19**	**0.37**	**0.58**	**0.77**	**0.90**	**0.97**	**0.99**	**1.00**	**1.00**	**1.00**

4.0 IDENTIFICATION AND ENROLLMENT OF SUBJECTS

Details of Stage 1: Instructions for enrollment of Stage 1 (open label lead-in) subjects are included in Appendix E. Instructions include all activities and the visit schedule from consent through follow-up.

4.1 Recruitment and Screening (prior to consent)

The study Sponsor (Data Coordinating Center) will provide participating clinical centers with a variety of materials to aide in recruitment. This may include, but is not limited to, informational DVDs and brochures which provide education about heart failure and include information about the study; physician referral letter templates which can be used to promote awareness of the

study in the cardiovascular community; templates for flyers which can be utilized at approved clinic locations and as part of health fair materials; templates for print advertisements which can be utilized in newsprint and media campaigns; Research Match, the non-profit free, secure registry, may also be utilized to identify potential candidates for trial participation. Not all materials have been developed prior to trial initiation; however each of these methods (the templates, final products, and services) will be reviewed and approved by both the Sponsor IRB and the clinical center IRB prior to use.

Screening of subjects includes reviewing medical records and imaging studies for inclusion/exclusions prior to consent. From the review of subjects' medical records and imaging studies on file, subjects who are determined to have a diagnosis of chronic ischemic LV dysfunction secondary to MI, have EF ≤ 40%, are candidates for cardiac catheterization, and have NYHA class of II or III, as stated in Section 4.3, and also do not have evidence in their medical record of study exclusions stated in Section 4.4, are eligible to be consented to the study.

At the time of screening, subjects must have 1) documented CAD with evidence of myocardial injury, LV dysfunction, and clinical evidence of HF and 2) have a "detectable" area of myocardial injury defined as ≥ 5% LV involvement (infarct volume) and any subendocardial involvement by cMRI. In addition, EF ≤ 40% can be defined by gated blood pool scan (MUGA), cMRI, left ventriculogram, or EF ≤ 35% by two-dimensional echocardiogram.

Note: Imaging studies (cMRI, ECG, SPECT, MUGA, echo, and/or left ventriculogram) are acceptable within 12 months prior to consent, and the PI will determine the need to repeat any standard of care imaging modalities to use for screening.

4.2 Consent

Before being enrolled, all subjects must consent in writing to participate. An informed consent form (ICF) will be given to each subject. The ICF will contain all United States federally required elements, all International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)-required elements, and Health Insurance Portability and Accountability Act Authorization (HIPAA) information in language that is understandable to the subject. The informed consent includes descriptions of all study related procedures, all possible risks to participant, and the time commitment involved with participating. All consent forms will have IRB approval. The ICF and review must be in a form understandable to the subject. Translation of ICFs will be done in accordance with local IRB procedures.

Potential participants will be approached by one of the study investigators or research coordinators. Information regarding study participation will be provided to the potential participant prior to consent. Subjects will be given ample time to review the ICF and ask questions before signing. The Investigator or designee and the subject must both sign and date the ICF after review, and before the subject can participate in the study. The subject will receive a copy of the signed and dated form, and the original will be retained in the site study files. The research staff member obtaining consent will document the informed process in the subject's chart for monitoring purposes. The Investigator or his/her designee must emphasize to the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

4.3 Inclusion Criteria

To participate, a subject MUST:

1. Be ≥ 21 and <80 years of age
2. Have documented CAD with evidence of myocardial injury, LV dysfunction, and clinical evidence of HF
3. Have a "detectable" area of myocardial injury defined as ≥ 5% LV involvement (infarct volume) and any subendocardial involvement by cMRI
4. Have an EF ≤ 40% by cMRI
5. Be receiving guideline-driven medical therapy for heart failure at stable and tolerated doses for ≥ 1 month prior to consent. For beta-blockade "stable" is defined as no greater than a 50% reduction in dose or no more than a 100% increase in dose.
6. Be a candidate for cardiac catheterization
7. Have NYHA class I, II, or III heart failure symptoms (see Appendix C)
8. If a female of childbearing potential, be willing to use one form of birth control for the duration of the study, and undergo a pregnancy test at baseline and within 36 hours prior to injection

4.4 Exclusion Criteria

To participate, a subject MUST NOT HAVE:

1. Indication for standard-of-care surgery (including valve surgery, placement of left-ventricular assist device, or imminent heart transplantation), coronary artery bypass grafting (CABG) procedure, and/or percutaneous coronary intervention (PCI) for the treatment of ischemic and/or valvular heart disease. Subjects who require or undergo PCI should undergo these procedures a minimum of 3 months in advance of randomization. Subjects who require or undergo CABG should undergo these procedures a minimum of 4 months in advance of randomization. In addition, subjects who develop a need for revascularization following enrollment should undergo revascularization without delay. *Indication for imminent heart transplantation is defined as a high likelihood of transplant prior to collection of the 12 month study endpoint. Candidates cannot be UNOS 1A or 1B, and they must have documented low probability of being transplanted.*
2. Valvular heart disease including: 1) mechanical or bioprosthetic heart valve; or 2) severe valvular (any valve) insufficiency/regurgitation within 12 months of consent
3. Aortic stenosis with valve area ≤ 1.5 cm^2
4. History of ischemic or hemorrhagic stroke within 90 days of consent
5. History of a left ventricular remodeling surgical procedure utilizing prosthetic material
6. Presence of a pacemaker and/or ICD generator with any of the following limitations/conditions:
   - manufactured before the year 2000
   - leads implanted < 6 weeks prior to consent
   - non-transvenous epicardial or abandoned leads
   - subcutaneous ICDs
   - leadless pacemakers

- any other condition that, in the judgment of device-trained staff, would deem an MRI contraindicated

7. Pacemaker-dependence with an ICD (*Note: pacemaker-dependent candidates without an ICD are not excluded)*
8. A cardiac resynchronization therapy (CRT) device implanted less than 3 months prior to consent
9. Other MRI contraindications (e.g. patient body habitus incompatible with MRI)
10. An appropriate ICD firing or anti-tachycardia pacing (ATP) for ventricular fibrillation or ventricular tachycardia within 30 days of consent
11. Ventricular tachycardia ≥ 20 consecutive beats without an ICD within 3 months of consent, or symptomatic Mobitz II or higher degree atrioventricular block without a functioning pacemaker within 3 months of consent
12. Presence of LV thrombus *(See guidance in section 6.3.3)*
13. Evidence of active myocarditis
14. Baseline VO_2 max greater than 75% of age and gender based predictive values (see Section 6.3.7)
15. Baseline eGFR < 35 ml/min/1.73m^2
16. Blood glucose levels (HbA1c) >10%
17. Hematologic abnormality evidenced by hematocrit < 25%, white blood cell < 2,500/ul or platelet count < 100,000/ul
18. Liver dysfunction evidenced by enzymes (AST and ALT) > 3 times the ULN
19. Coagulopathy (INR ≥ 1.3) not due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors) (see Sections 6.2.2 and 6.2.3 re: study procedures and anticoagulation therapy). Subjects who cannot be withdrawn from anticoagulation will be excluded.
20. HIV and/or active HBV or HCV
21. Allergy to radiographic contrast material that cannot adequately be managed by premedication
22. Known history of anaphylactic reaction to penicillin or streptomycin
23. Received gene or cell-based therapy from any source within the previous 12 months
24. History of malignancy within 5 years (i.e., subjects with prior malignancy must be disease free for 5 years), excluding basal cell carcinoma and cervical carcinoma in situ which have been definitively treated
25. Condition that limits lifespan to < 1 year
26. History of drug abuse (illegal “street” drugs except marijuana, or prescription medications not being used appropriately for a pre-existing medical condition) or alcohol abuse (≥ 5 drinks/day for > 3 months), or documented medical, occupational, or legal problems arising from the use of alcohol or drugs within the past 24 months
27. Participation in an investigational therapeutic or device trial within 30 days of consent

28. Cognitive or language barriers that prohibit obtaining informed consent or any study elements
29. Pregnancy or lactation or plans to become pregnant in the next 12 months
30. Any other condition that, in the judgment of the Investigator or Sponsor, would impair enrollment, study product administration, or follow-up

4.5 Baseline Testing

The baseline testing period extends from the date the ICF is signed until completion of both of the BMA and RHC with and without EMB (RHC/EMB) procedures (see Appendices A & B). This period will not exceed 60 days.

The following evaluations will be carried out at baseline:

- Comprehensive medical and surgical history, vital signs and physical examination (Section 6.3.1)
- Current use of prescription and OTC medications (Section 6.3.1)
- Baseline blood tests (Section 6.3.2)
- Infectious disease panel (Section 6.3.2)
- Pregnancy test (for women of childbearing potential) (Section 6.3.2)
- cMRI imaging (Section 6.3.3, MRI Core Lab Manual of Operations) and ICD interrogation (Section 6.3.8) (if applicable)
- Six-minute walk test (Section 6.3.4, Protocol Manual of Operations)
- Questionnaires including MLHFQ and sexual function (Section 6.3.5)
- 12 lead ECG (Section 6.3.6)
- Treadmill-based VO_2 max (Section 6.3.7)

4.6 Post-Consent Review and Randomization

Prior to randomization, baseline evaluations and eligibility criteria will be reviewed by Network investigators. The purpose of the post-consent case review is to determine if a combination of factors suggests the subject may be a poor research risk beyond whether or not they meet individual eligibility criteria. In addition, if a change in the subject's status has occurred such that the subject no longer meets all of the eligibility criteria, randomization will be postponed, or if the condition is not resolvable, the individual will be excluded from participation. Following successful completion of baseline testing and favorable case review, subjects will be randomized either to A) Combo (MSCs plus c-kit+ cells), B) MSCs alone, C) c-kit+ cells alone, or D) placebo (see Section 8.1).

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5.0 CELL PRODUCTION AND DELIVERY

5.1 Procurement and Shipment of Bone Marrow for MSC Production

The Central Cell Manufacturing Facility (CCMF) will receive email notifications from the DCC when a subject has been enrolled (consented), when a subject has been determined eligible to participate in the study (upon completion of baseline testing), and when the bone marrow aspiration (BMA) procedure has been scheduled.

Approximately 90ml (±10ml) of bone marrow (BM) will be harvested from the posterior iliac crest of all subjects by a trained physician as described in Appendix A. The marrow will be aspirated into pre-heparinized syringes containing preservative-free heparin for a final effective dose of 100 units of heparin / ml of BM. Specific guidelines are provided in Appendix A. Subjects on aspirin and Plavix (Clopidogrel) at the time of consent should remain on these medications for the harvest procedure.

The harvested material will be transported to each local cell processing laboratory (CPL) facility according to its standard operating procedure (SOP) for transfer of fresh BM.

Upon arrival at the local CPL, samples will be taken for the following QC tests (see Table 12): Sterility using aerobic, anaerobic and fungal cultures, nucleated cell count, and viability.

Table 12. QC Tests on BM at Local CPL

Assay	Test Method	Specification
Viability	Trypan Blue	Report Results
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth

BM Release test results prior to shipping to CCMF:

- Viability of the cells will be measured and documented.
- In the event that sterility testing (culture) becomes positive, the CPL staff will immediately inform the CCMF, the CPQAL, and the Biorepository of the positive result.

The marrow will be split and samples shipped to both the CCMF for production of MSCs and to the CCTRN biorepository with appropriate consent (see Section 6.4.1). Sixty-five mL (+/- 5 mL) of bone marrow will be sent to the CCMF per manufacturer's SOP, and the remainder of the bone marrow suspension (~25 mL), will be shipped to the CCTRN biorepository with appropriate consent (see Section 6.4.2). Personnel from the local CPL will communicate with the CCMF regarding subject information, exact time of harvest, volume of BM suspension shipped, QC test results, time of shipment, carrier, estimated delivery, tracking information, etc.

5.2 Procurement and Shipment of Endomyocardial Biopsy (EMB) for c-kit+ cell Production

Individuals meeting enrollment criteria will be scheduled to undergo right heart catheterization (RHC) with or without right ventricle endomyocardial biopsy (EMB), depending on randomization assignment. Subjects on aspirin and Plavix (Clopidogrel) at the time of consent should remain on these medications for the procedure. Up to six EMB samples will be harvested as described

in Appendix B. Site harvesters will attend a best practices session prior to study initiation to review EMB procedures to optimize safety and assure that they are standardized across sites.

The biopsied material will be transported to the local CPL on wet ice, or equivalent, to be prepared for overnight shipment to the CCMF per manufacturer's SOP. Personnel from the CPL will communicate with the CCMF for subject information, exact time of harvest, number of cryovials shipped, time of shipment, carrier, estimated delivery, tracking information, etc.

Table 13. Sterility Testing of Ham's F-12 Medium & Complete Growth Medium for EMB Tissue Processing and c-kit+ cell procurement		
Assay	**Test Method**	**Specification**
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth

5.3 Receipt of harvest samples at the CCMF

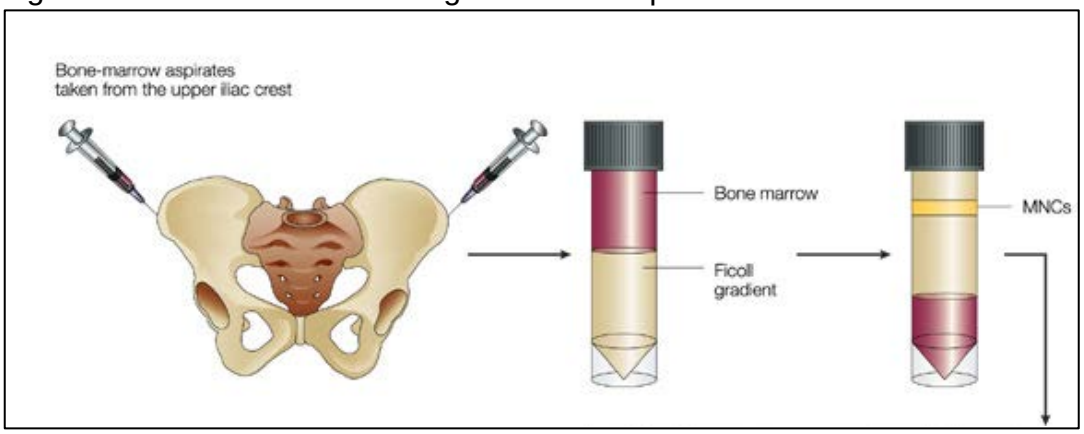
Upon arrival at the CCMF, the products will be inspected for quality and maintenance of temperature during shipping. A temperature deviation will be reported and investigated. After receipt and inspection, the products will be taken to the clean room laboratory for further processing per manufacturer's SOP. Samples will be taken for the following QC tests (see Table 14): Sterility using aerobic, anaerobic, and fungal cultures and cell viability (BM only).

Table 14. QC Tests on BM at the CCMF		
Assay	**Test Method**	**Specification**
Viability	Trypan Blue/Crystal Violet	Report Results
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT or equivalent or conventional culture assay	No Growth

5.4 Production and Cryopreservation of MSC Product

BM products meeting specifications will be processed to obtain a mononuclear cell (MNC) enriched fraction as illustrated in Figure 1.

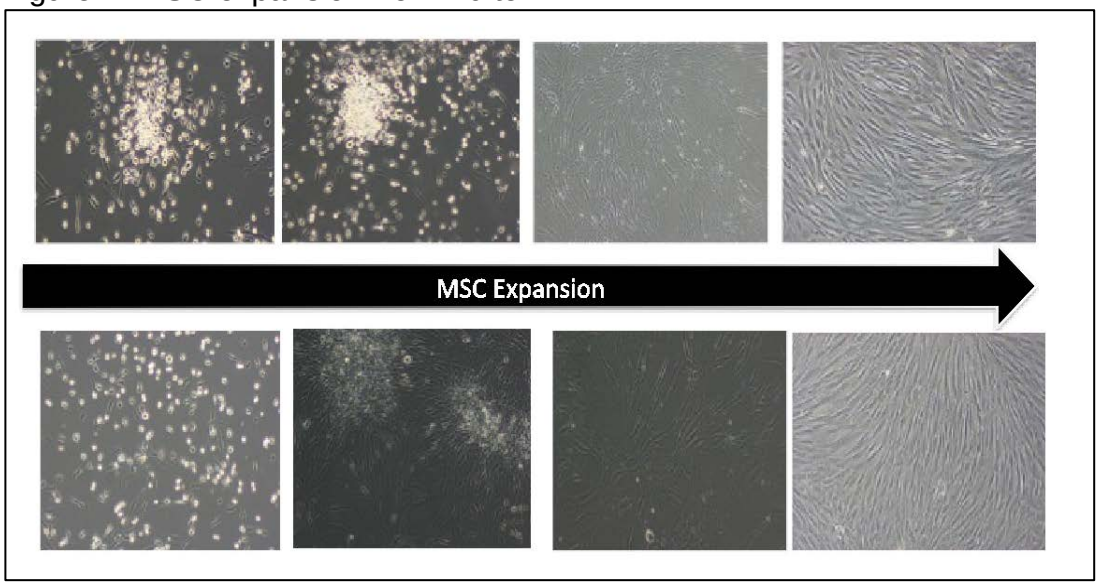
Figure 1. MNC enrichment using Ficoll technique



After Ficoll separation, the cells will be washed twice and cultured in heparin-free media. MNCs of subjects randomized to either Combo or MSC alone treatment group will be cultured to generate the designated target dose of 150 million MSCs.

After 14 days of culture, the cells are harvested. Figure 2 below shows how the cells look as they expand from Passage 0 (P0) to Passage 1 (P1).

Figure 2. MSC expansion from P0 to P1



Samples of MSC products will be characterized by the CCMF to assure that they meet predetermined specifications. Cell count, phenotype, potency, mycoplasma testing, and other QC tests, as indicated in Table 15, are performed on a sample of cell suspension taken from the last passage which represents the harvest of the final product prior to cryopreservation.

Table 15. QC Testing of MSC Cell Culture Prior to Cryopreservation

Assay	Test Method	Specification
Mycoplasma PCR*	VenorGeM® (Cells in Conditioned Medium prior to cryopreservation)	Negative
Viability*	Trypan Blue	≥70%
CFU-F	Colony Formation, 14 Days	Growth
Phenotype/Cell Characterization CD 105^+ $CD45^+$	Flow Cytometry	$CD105^+$ >80% $CD45^+$ <2%
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth
Cell Count – MSCs	Hemocytometer	$>75x10^6$

* Tests done prior to cryopreservation

The MSC products are frozen and stored in liquid nitrogen (LN_2) vapor phase at the CCMF until ready to be shipped. Sterility testing for aerobic, anaerobic, and fungal cultures is performed on a sample of product after adding cryopreservation medium immediately before freezing, as indicated below in Table 16.

Table 16. Sterility Testing of MSC Final Product Post Adding Cryopreservation Medium

Assay	Test Method	Specification
Endotoxin	EndoSafe PTS	≤ 5EU/ml
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth

Bacteriostasis and Fungistasis (B&F) testing will also be performed on the first 3 MSC products and/or when any significant changes are made in the manufacturing process or materials of an existing product. B&F testing is designed to validate the procedure used to test a given product for sterility by demonstrating that microorganisms present on the product can be detected in the course of the sterility test.

MSC products will be held frozen in LN_2 vapor phase and will not be released for injection until final results of endotoxin and sterility testing are obtained by the CCMF. The release will require negative sterility test results before and after adding cryopreservation medium, as well as negative mycoplasma by PCR before adding cryopreservation medium, as indicated above in Tables 14 and 15. Products with positive sterility results will be discarded and a complete investigation report will be generated by CCMF according to the facility SOP. A copy of the report will be sent to DCC and CPQAL, as well as to local CPL.

5.5 MNCs for non-MSC Treatment Groups

MNCs of the subjects randomized to c-kit+ cells alone or to placebo will be cryopreserved by the CCMF. With appropriate subject consent, cryopreserved MNC products from subjects randomized to c-kit+ cells alone or to placebo will be shipped in a LN_2 dry shipper to the CCTRN biorepository (see Section 6.4).

5.6 Production and Cryopreservation of c-kit+ cells

EMB samples will be received at the CCMF. For subjects randomized to Combo or c-kit+ cell alone treatment groups, culture and subsequent production of c-kit+ cells will follow a modified version of established protocols for cell expansion on file with the FDA (IND 14647) to generate the designated target dose of 5 million c-kit+ cells.

Cells are expanded in culture for approximately 3 to 4 weeks and then enriched for c-kit$^+$ cells using immune-magnetic beads[73] (CD117 MicroBead Kit, Miltenyi Biotec). The c-kit$^+$ CPCs are further expanded to produce the final target dose range of 0.8×10^6 to 5×10^6 c-kit+ cells.

Samples of the c-kit+ cells will be characterized by the CCMF with respect to 7AAD, CD45, CD31, CD34, CD133, CD14, CD16, CD11b, CD19, CXCR4, CD90 (or 105), NKx2.5, GATA-4, MEF2c, a-sarcomeric actin, alpha myosin heavy chain for myocyte lineage, smooth muscle actin for VSMC lineage, vWF for endothelial lineage, p16INK4a expression, doubling time, telomere length, and telomerase activity.

To assure they meet all predetermined specifications, cell count, phenotype (CD117 positivity), mycoplasma testing, and other QC tests as indicated in Table 17 are performed on a sample of the cell suspension taken from the last passage which represents the harvest of the final product prior to cryopreservation.

Table 17. QC Testing of c-kit+ cell Cell Culture Prior to Cryopreservation		
Assay	**Test Method**	**Specification**
Mycoplasma PCR*	VenorGeM® (Cells in Conditioned Medium prior to cryopreservation)	Negative
Viability*	Trypan Blue	≥70%
Phenotype/Cell Characterization CD 117^{+} CD45^{+}	Flow Cytometry	CD117^{+} ≥70% CD45^{+}<2%
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent (supernatant medium)	No Growth
Cell Count – c-kit+ cells	Manual	>0.8 x10^{6}

* Tests done prior to cryopreservation

The c-kit+ cell products are frozen and stored in LN_2 vapor phase at the CCMF until ready to be shipped. Sterility testing for aerobic, anaerobic, and fungal cultures is performed on a sample of product after adding cryopreservation medium immediately before freezing as indicated below in Table 18.

Table 18. Sterility Testing of c-kit+ cell Final Product Post Adding Cryopreservation Medium		
Assay	**Test Method**	**Specification**
Endotoxin	EndoSafe PTS	≤ 5EU/ml
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth

B&F testing will also be performed on the first 3 c-kit+ cell products and/or when any significant changes are made in the manufacturing process or materials of an existing product.

c-kit+ cell products will be frozen in LN_2 vapor phase and will not be released for injection until final results of endotoxin and sterility testing are obtained by the CCMF. The release will require negative sterility test results on c-kit+ cell products before and after adding cryopreservation medium, as well as negative mycoplasma by PCR before adding cryopreservation medium, as indicated above in Tables 16 and 17. Products with positive sterility results will be discarded and a complete investigation report will be generated by CCMF according to the facility SOP. A copy of the report will be sent to DCC and CPQAL, as well as to the local CPL.

5.7 Shipment of Cryopreserved MSC and c-kit+ cell Products

The CCMF will use validated liquid nitrogen dry shippers (a.k.a. cryoshipper) with built-in data loggers to monitor temperatures. Cryopreserved MSC and/or c-kit+ cell products will be shipped to the local CPLs within one week before the scheduled injection procedure. The CCMF will ensure that the cryoshipper is charged for at least 24 hours prior to each shipment. Labeling requirements mandated by regulatory agencies will be fulfilled. All required documents as described in shipping SOPs of the CCMF will accompany the cryoshipper.

The CCMF will also provide all relevant SOPs, worksheets, forms, labels, etc. for product preparation for administration. The local CPL, DCC and CCMF will work with the clinical research team to coordinate the injection date and product shipment according to the randomization schedule outlined in Section 8.1.

Since MSCs and c-kit+ cell cultures require different times for manufacturing, all subjects will receive study product approximately 14 weeks from the date of the harvest procedures.

5.8 Preparation for Administration of MSC and c-kit+ cell Products

The products will undergo cell counts and QC testing before injection as indicated in Table 19.

Table 19. QC Tests on Thawed Washed MSC and c-kit+ cell products at CPL

Assay	Test Method	Specification
Rapid Sterility*	Gram Stain	No organisms seen (negative)
Viability*	Trypan Blue	≥70%
Endotoxin*	EndoSafe PTS	≤ 5EU/kg **
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth
Cell Count – c-kit+ cells*	Manual	Minimum 0.8×10^6 final dose Maximum 5×10^6 final dose
Cell Count – MSCs*	Manual	Minimum 75×10^6 final dose Maximum 150×10^6 final dose

* Tests done for release criteria specification prior to injection; for cell counts, the product must meet the minimum count for release

** Based on recipient weight and product volume

5.8.1 Final Release Criteria Specifications for Autologous MSCs and c-kit+ cells

As stated above in Table 19, all MSC and c-kit+ cell thawed washed products must meet Gram stain, viability, endotoxin, and cell count release criteria specifications prior to injection. In addition, all MSC and c-kit+ cell cell cultures prior to cryopreservation (see Tables 14 and 16) must meet Mycoplasma PCR specifications prior to injection. Finally, all MSC and c-kit+ cell final products after adding cryopreservation medium (see Tables 15 and 17) must meet aerobic, anaerobic and fungal sterility testing specifications prior to injection.

5.8.2 Cell Suspensions

5.8.2.1 MSCs alone

MSCs (150 x 10^6) are suspended in a final volume of 6.1 ml (±0.5 ml) of thaw medium (Plasma-Lyte-A 1%HSA) to suspend the MSC pellets in 0.4 ml injection volume, times 15 injections for a total of 150 million MSCs. The physician will administer 15 individual doses of 10 x 10^6 cells/0.4ml (±10%), per dose, per injection site. The 15th injection will be followed by 0.1ml of saline to flush any residual cells from the line into the 15th injection site. Any cell suspension remaining after delivery of the 15 injections must not be injected and shall be discarded according to institutional guidelines.

5.8.2.2 c-kit+ cells alone

c-kit+ cells (5.0 x 10^6) are suspended in a final volume of 6.1 ml (±0.5 ml) of thaw medium (PlasmaLyte-A 1%HSA) to suspend the c-kit+ cell pellets in 0.4 ml injection volume, times 15 injections for a total of 5 million c-kit+ cells. The physician will administer 15 individual doses of approximately 0.333X10^6 cells/0.4ml (±10%), per dose, per injection site. Any cell suspension remaining after delivery of the 15 injections must not be injected and shall be discarded according to institutional guidelines.

5.8.2.3 Combo

The mixture of MSCs (150 x 10^6) and c-kit+ cells (5 x 10^6) is suspended in a final volume of 6.1 ml (±0.5 ml) of thaw medium (PlasmaLyte-A 1%HSA) to suspend the combined cell pellets in 0.4 ml injection volume, times 15 injections for a total of 150 million MSCs and 5 million c-kit+ cells. The physician will administer 15 individual doses of a maximum of 10.333 x 10^6 cells/0.4ml (±10%), per dose, per injection site. Any cell suspension remaining after delivery of the 15 injections shall not be injected and shall be discarded according to institutional guidelines.

5.9 Placebo Group

Subjects randomized to the placebo group will undergo baseline testing, BMA and RHC procedures. Approximately 14 weeks after the BMA and RHC procedures, the placebo group will receive transendocardial injections of 0.4ml PlasmaLyte-A supplemented with 1% HSA in 15 injection sites. The placebo product will undergo QC testing before injection as indicated in Table 20.

Table 20. Sterility Testing of Placebo for Injection

Assay	Test Method	Specification
Rapid Sterility*	Gram Stain	No organisms seen (negative)
Endotoxin*	EndoSafe PTS	≤ 5EU/kg**
Aerobic, Anaerobic, and Fungal	14 day Bactec/BacT/ALERT assay or equivalent	No Growth

* Tests done for release criteria specification prior to injection
** Based on recipient weight and product volume

5.10 Positive 14-day Sterility After Injection

Should the final 14-day sterility culture produce a positive result after the study product has been administered to the subject, then the following steps will take place:

a) The local CPL staff will immediately contact CPQAL and the local research team. The local CPL will remain in contact with the CPQAL and local research team regarding the identity of the organism and its antibiotic sensitivities as soon as this information is available for the local team to consider antibiotic prophylaxis.
b) CPQAL will contact the DCC immediately to notify of a positive result on a sterility test sample of an administered product. CPQAL will also inform the CCMF.
c) The subject will remain in the study and be monitored for clinical signs of infection. The local research team will report to the DCC any resultant adverse events per protocol (see Section 7.3).
d) The CCMF will conduct a complete laboratory investigation according to the facility SOP and generate a report that will be sent to the DCC, CPQAL, and local CPL.
e) The DCC will report the sterility failure, results of the investigation of the cause, and a corrective action plan to the FDA within 30 days after the initial receipt of the positive culture test result.

Every effort will be made to protect the blinding of those involved in the study endpoint and safety event collection.

5.11 Blinding and Study Teams

The study will remain double-blind by having both blinded and unblinded study teams at each center. All subjects, cell and cell-free groups, will undergo BMA, RHC with or without EMB, as well as injections by NOGA® XP Mapping and Navigation System (NOGA). The only unblinded research team members are those performing activities directly related the RHC/EMB procedure and possibly the interventionalist delivering injections if they also peform the RHC/EMB procedure, or if they are able to discern the study product. In the interest of safety, the interventionalist must be able to visualize the study product to inspect that it looks safe (e.g., free of air bubbles, clumping, etc.). Cardiac MRI and VO_2 max endpoints will be determined by core laboratories. Core laboratory evaluation of endpoint measures will be conducted by personnel who are blinded to therapy assignment. Coordinators and other personnel participating in the collection of cMRI, 6MWT, VO_2 max, and the MLHFQ endpoint data also will be blinded to the therapy assignment. In addition, each of the clinical centers will take steps to ensure that adverse event assessments are carried out in a blinded fashion.

Local CPL and CCMF staff will be unblinded in order to assist with sample and product preparation (see Section 5.8). With the exception of the unblinded research team members described above, the majority of the research team (investigators, coordinators and other staff involved with subject recruitment, baseline testing, endpoint data collection and event data) will be blinded to the treatment assignment.

If for important medical reasons unblinding of additional team members is thought to be necessary, the Investigator may identify the treatment assignment by contacting the DCC who is responsible for maintaining randomization records for all subjects.

5.12 NOGA Catheterization Procedure

Intramyocardial cell delivery by NOGA® XP Mapping and Navigation System (NOGA) will be used in this trial. All investigators performing the catheter-based study procedures will receive appropriate training in the use of the catheters by the catheter manufacturer (Biologic Delivery Systems). All interventionalists will be certified under this training program. Site interventional cardiologists will meet routinely by teleconference to review injection procedures to assure that they are standardized across sites.

LV cine angiocardiography will be performed in orthogonal planes (typically right anterior oblique (RAO) 30 and left anterior oblique (LAO) 60 degree projections). End-diastolic endocardial contours will be saved as angiographic image recordings in both projections, to provide guidance regarding location of LV borders during fluoroscopic manipulation of the injection catheter.

LV electromechanical mapping (EMM) will be performed using NOGASTAR® Mapping Catheter(s), sized appropriately for the LV dimension. An 8 French femoral artery sheath of sufficient length will be chosen to aid operators in negotiating pelvic vasculature with the NOGA catheter. Unfractionated Heparin will be used to maintain an activated clotting time (ACT) between 200 and 250 seconds during NOGA mapping and injection. EMM will be performed according to standard clinical practice, with attention to achieving a smooth endocardial contour and accurate representations of the long and short axes of the LV chamber.

For the cell or cell-free transendocardial study product injection (SPI) procedure, the MYOSTAR™ Injection Catheter (MyoStar) will be prepared by adjusting the needle extension at 0° and 90° flex and by placing 0.1 cc of study product in the needle dead space (e.g. priming). All study product will be retained to complete the 15 required injections. To ensure safety and limit potential for extracardiac administration of the injectate, the needle extension/wall thickness will be set at a ratio of ≤0.5. The needle length should not exceed 50% of target tissue thickness when assessed at both 0° and 90°. The injection catheter will be advanced to the aortic valve, and retrograde into the LV. The catheter tip will then be positioned against the endocardium at the target area.

Priority for injection site selection will be based upon the objective of encircling an area of myocardium in one (or more) infarct territory, selected by the investigator to be a territory which is: a) safely treatable with low risk of perforation or other complications – avoiding the true LV apex and other areas known to be <6mm in myocardial thickness, and avoiding the left bundle branch site of earliest activation; b) clinically important based on viability assessments provided below; and, c) accessible with the tip of the selected MyoStar catheter.

Correlation with other imaging modalities (including, when clinically available, electrocardiography, echocardiography, coronary angiography, MRI, scintigraphy, PET, and LV angiography) is encouraged in selecting the target territory. Boundaries of myocardial scar will be demonstrated in the NOGA map using a color coding scheme to easily identify areas of unipolar local voltage of greater or less than 7 mV. Transendocardial injections will be placed so as to encircle the scar, with injection sites in both a) the viable border zone encircling the area of scar (characterized by the presence of unipolar voltage ≥ 7mV), and b) scar adjacent to the viable border zone.

Sites for injection should satisfy the following criteria: 1) perpendicular position of the catheter to the LV wall, and 2) loop stability <4mm. Ideally the majority of injections would be placed in viable zone with a voltage threshold of ≥7mV with the demonstration of a premature ventricular contraction on extension of the needle. Some injections will be placed in scar border zone with a voltage threshold of ≥ 4mV without the requirement of a premature ventricular contraction. *When selecting sites for injection in the border zone, those with unipolar voltage in the upper range of 4-7 mV would generally be preferred to those in the lower range, and those with a voltage <4 mV would generally be avoided.*

Each of the 15 injections will contain 0.4 ml of cell suspension with up to 10 million MSCs and/or up to 0.333 million c-kit+ cells for the MSC alone, c-kit+ cell alone, or Combo cell treatment arms. For the final injection, 0.1 cc of saline should be placed in the catheter and injected. This will allow the 0.1 cc already in the catheter (from priming) to be administered into the myocardium, constituting the 15th injection. Each injection should be infused over 60 seconds.

Sites of injection will be marked and recorded by a solid circular tag on the NOGA map accompanied by a reference to injection number and presence or absence of needle-induced extrasystole. At the investigator's discretion, sites of injection may also be recorded on cine fluoroscopy, and demarcated as a dot with the corresponding injection series number on the overlay tracings in both RAO and LAO projections.

After the injection procedure, subjects will be monitored overnight. A 2-D echocardiogram will be performed post SPI procedure (within 6 hours). Additional echocardiographic assessments for pericardial effusion will be done only as clinically indicated. Myocardial necrosis markers (Troponin I or T) will be collected 8 (+/- 2) hours post-injection catheter procedure and again prior to discharge.

Following the cardiac catheterization procedure and the cell or cell-free injections, all subjects will be followed at Day 1, Week 1, and months 1, 3, 6 and 12 to complete all safety and efficacy assessments.

5.13 Circumstances that may affect study product delivery

If any of the following symptoms occur before or during SPI, they could indicate a serious clinical deterioration. If any of the following events/symptoms occurs, the procedure should be temporarily halted and the patient should be reevaluated for suitability to continue with the treatment under investigation:

1. Hypotensive episode defined as a sustained drop in blood pressure exceeding 20mm/Hg not responsive to fluid administration
2. Hemodynamically significant arrhythmia requiring anti-arrhythmic therapy
3. Two episodes of sustained ventricular tachycardia/ventricular fibrillation requiring cardioversion
4. Hemodynamic instability
5. Fever (Temperature increase to ≥100.4^{o}F)
6. Cardiac perforation
7. Clinical signs and symptoms indicating a cerebrovascular accident

6.0 CLINICAL AND LABORATORY EVALUATIONS

Instructions for enrollment of Stage 1 (open label lead-in) subjects are included in Appendix E. Instructions include all activities and the visit schedule from consent through follow-up

6.1 Schedule of Procedures Stage 2 (Table 21b)

CONCERT-HF Study Procedures	Base-line Testing	Harvest	MRI Visit[5]	D0 (SPI)	D1	Wk1 (+/-3 days)	M1 (+/-7 days)	M3 (+/-14 days)	M6 (+/-30 days)	M12 (+/-30 days)	M24 (+/-30 days) Call[8]
Informed Consent	X										
Complete Medical History	X										
Physical Exam	X	X		X	X	X	X	X	X	X	
Vital Signs	X	X		X[1]	X	X	X	X	X	X	
Adverse Events	X	X		X	X	X	X	X	X	X	
Con Medications	X	X		X	X	X	X	X	X	X	
NYHA & CCS	X	X		X			X	X	X	X	
MLHFQ	X							X	X	X	
Sexual Function Surveys	X								X	X	
12-lead ECG	X			X[2]	X[2]	X			X	X	
2D Echoes		X[8]		X[2]							
Telemetry				X[3]							
Laboratory Testing[4]	X	X	X	X	X	X	X	X	X	X	
Cardiac MRI	X		X						X	X	
ICD Interrogation[6]	X		X	X					X	X	
Treadmill (VO_2 max)	X								X	X	
6 Minute Walk	X								X	X	
Randomization	X										
Bone Marrow Aspiration		X									
Sham/Heart Biopsy (RHC/EMB)		X									
Catheterization (NOGA)				X							
Temp. Log					X[7]						

Baseline testing and harvest procedures will take place within 60 days of ICF, and SPI will occur approximately 14 weeks after harvest procedures.

1. Subjects will have assessments of vitals (BP, temperature, pulse rate) pre- and post-procedure.
2. ECGs will be performed within 6 hours following the SPI catheterization procedure and again before discharge; a 2-D echocardiogram will be performed post-SPI (within 6 hours).
3. Subjects will be monitored on simple telemetry up to 24 hours post-procedure or until discharge, whichever is sooner.
4. See Section 6.3.2 for specific tests done at each time point.
5. A cardiac MRI will be performed within 30 days prior to SPI (baseline measure; see Section 6.2.3).
6. ICD interrogation: (if applicable) done before and after every MRI as part of MRI protocol, as well as before the SPI procedure.
7. Temperature log 2x/day x 7 days.

8. Two 2-D echocardiograms will be performed on all subjects on the day of harvest: 1) a pre-RHC procedure echo to assess for pre-existing pericardial effusion, and 2) a post-RHC (with or without EMB) procedure echo within 6 hours to ensure no post-procedure effusion (even if subject is stable).

6.2 Study Phases and Visits

6.2.1 Baseline Phase

After the subject has consented to the study, the subject will have a series of assessments to establish eligibility to receive treatment.

All baseline tests and procedures, and harvest procedures, will occur within 60 days of signing informed consent form (ICF), with the exception of the cMRI to be used for the baseline measurement (see below). No baseline exams will take place until the subject is fully informed of the research and signs the ICF. BMA (see Appendix A) and RHC/EMB procedures (see Appendix B) will take place after the subject has been determined eligible and has been randomized (see Section 4.6).

6.2.2 BMA and RHC Procedures

After randomization, all subjects will undergo BMA and RHC. For subjects randomized to the MSCs alone and placebo groups, only a RHC will be performed (a.k.a. “sham biopsy”). A RHC will include EMB for subjects randomized to the Combo and c-kit+ cells alone groups.

Subjects determined to be eligible during baseline testing will be scheduled for their BMA (see Section 5.1 and Appendix A) and RHC with or without EMB (RHC/EMB) (see Section 5.2 and Appendix B), depending on randomization assignment. The RHC will include EMB for the 72 subjects randomized to the Combo and c-kit+ cell only groups. A script for the sham biopsy can be found in the Manual of Operating Procedures. BMA and RHC/EMB procedures will be performed on the same day. Before the procedures, all subjects will have a physical exam including vital signs, weight, assessment of NYHA class (Appendix C), CCS class (Appendix D), review of AEs and concomitant medications and laboratory assessments (see Section 6.3.2). Two 2-D echocardiograms will be performed on all subjects on the day of harvest: 1) a pre-RHC/EMB procedure echo to assess for pre-existing pericardial effusion, and 2) a post-RHC/EMB procedure echo within 6 hours to ensure no post-procedure effusion (even if subject is stable). For subjects receiving systemic anticoagulation therapy, an INR measurement will be performed on the morning of the planned procedures. Aspirin therapy or dual antiplatelet therapy will not be interrupted for BMA and RHC/EMB procedures. Pressures collected during the procedure should be measured at end-expiration at end diastole.

Research teams should follow institutional and interventionalist standard of practice for managing anticoagulation before the BMA and RHC/EMB procedures. The following guideline could also be used. *Anticoagulation management guideline:* Stop warfarin 4 days prior to the date of planned BMA and RHC/EMB. Careful consideration for bridging anticoagulation should be given to assess risk of occurrence of thrombotic events for subjects off anticoagulation.[101] Perform INR measurement on morning of planned procedures. Require an INR of < 1.6 for these subjects to proceed with BMA and RHC/EMB. For subjects on Factor Xa inhibitors, stop 2 days prior to planned BMA and RHC/EMB.

Following successful procedures, the bone marrow and EMB tissue (if procured) will be prepared and sent to the CCMF for processing. BMA and RHC/EMB procedures will occur within 60 days from subject signing informed consent and approximately 14 weeks prior to SPI.

6.2.2.1 Circumstances that would halt or terminate the RHC/EMB procedure

If any of the following occur before or during RHC/EMB, they could indicate a serious clinical deterioration.

- If any of these conditions/events occur, the procedure should be temporarily halted and the subject should be reevaluated for suitability to continue with the procedure:
  - the systolic blood pressure (SBP) taken the day of harvest is <80 mmHg and constitutes a significant change from baseline, e.g., SBP change from ≥ 100 mmHg (at baseline) to <80 mmHg (at harvest);
  - the heart rate (HR) taken the day of harvest is >100 and constitutes a significant change from baseline, e.g., HR change from ≤ 80 (at baseline) to >100 (at harvest).
  - the baseline pulmonary artery (PA) systolic pressure taken during the RHC is 50-59 mmHg;
  - the baseline right ventricle (RV) systolic pressure taken during the RHC is 50-59 mmHg;
  - the baseline wedge pressure taken during the RHC is 30-34 mmHg; (note: if wedge pressure is >30 mmHg, collect an O2 sat measure);

- If any of these conditions/events occur, the procedure should be terminated (subject can be evaluated for rescheduling if condition/event resolves):
  - change in NYHA Class to Class IV;
  - the baseline PA systolic pressure is ≥ 60 mmHg;
  - the baseline RV systolic pressure is ≥ 60 mmHg;
  - the baseline wedge pressure is ≥ 35 mmHg.

6.2.3 MRI Evaluation and Day 0 Study Product Injection (SPI)

MRI Evaluation Visit

Within the 30 days prior to the scheduled NOGA procedure (Day 0), a cMRI will be done and compared with the initial cMRI done during baseline testing to demonstrate any change in EF between baseline testing and the intervention. The initial cMRI will be used to determine eligibility. The cMRI just prior to treatment will be used for the baseline measurement. A local read of this cMRI is required as part of a pre-SPI safety review. If MACE occurs (see Section 3.5) between baseline testing and Day 0, a cMRI will be performed and can be used for the baseline measurement.

Day 0 (SPI)

Before the SPI, all subjects will have a physical exam including vital signs, weight, assessment of NYHA class (Appendix C), CCS class (Appendix D), review of AEs and concomitant medications and laboratory assessments (see Section 6.3.2) including a pregnancy test (females of childbearing potential) within 36 hours prior to SPI. For subjects receiving systemic anticoagulation therapy, an INR measurement will be performed on the morning of the planned procedure.

Research teams should follow institutional and interventionalist standard of practice for managing anticoagulation before the SPI procedure. The following guideline could also be used. *Anticoagulation management guideline:* Stop warfarin 4 days prior to the date of planned SPI. Careful consideration for bridging anticoagulation should be given to assess risk of occurrence of thrombotic events for subjects off anticoagulation.[101] Perform INR measurement on morning of planned procedure. Require an INR of < 1.6 for these subjects to proceed with SPI. For subjects on Factor Xa inhibitors, stop 2 days prior to planned SPI procedure.

With appropriate biorepository consent, peripheral blood will be drawn before sedation for the SPI procedure. Subjects will have assessments of vitals (BP, temperature, pulse rate) immediately pre- and post-procedure. After the injection procedure, subjects will be monitored overnight. Subjects will be monitored on simple telemetry up to 24 hours post-procedure or until discharge, whichever is sooner. A 2-D echocardiogram will be performed post SPI (within 6 hours). Additional echocardiographic assessments for pericardial effusion will be done as clinically indicated.

6.2.4 Day 1 Post Catheterization

All subjects will have laboratory assessments (see Section 6.3.2), with Troponin I or T performed 8 (+/- 2) hours post cardiac catheterization/NOGA and again prior to discharge. Subjects will also have ECGs performed within 6 hours following the catheterization procedure and again before discharge. The subject will keep a daily temperature log for seven days following the catheterization procedure to help assess the early development of an infection.

6.2.5 Week 1 Evaluations

After one week, subjects will return to the clinic for a physical exam and laboratory assessments including Troponin I or T (see Section 6.3.2), as well as collection of the temperature log.

6.2.6 Month 1 – Month 12 Visits

Outpatient visits should be completed as close to the scheduled visit dates as possible adhering to the visit schedule and follow-up windows stated in Section 6.2.8. If required, outpatient visit procedures may take place over more than one day. If procedures are performed on more than one day, the date of the physical exam will be considered the visit day.

6.2.6.1 Transplants, CRT, or LVADs before Months 6 or 12

If subjects are transplanted, receive CRT, or receive an LVAD prior to the month 6 or 12 visits, every attempt should be made to collect the endpoint measures (see Section 3.6.2) before transplant, CRT, or LVAD procedure. Labs and pathology (explanted heart) will be collected from those providing appropriate consent (see Section 6.4). Participants will continue to be followed for safety (provided they are not withdrawn from the study) and seen for study visits, but no further endpoint collection (MRI, 6MWT, MVO2, MLHFQ, NT-proBNP) will be completed after the transplant, CRT, or LVAD procedure. Adverse event reporting will continue as per section 7.3 of the protocol.

6.2.7 Follow-up windows

The timeline for follow up will initiate with the day of SPI (Day 0). The time windows for each of the subsequent follow up visits will be as follows:

1. The 1-week visit will be 7 ±3 days (from day of SPI).
2. The 1-month visit will be at 30 ±7 days.
3. The 3-month visit will be at 90 ±14 days.

4. The 6-month visit will be at 180 ±30 days.
5. The 12-month visit will be at 365 ±30 days.

6.2.8 Lost to Follow-up

Randomized subjects will be followed for up to two years. Subjects will be considered lost to follow-up after 3 consecutive failed telephone contacts AND one certified letter returned to the site. Contact attempts will be documented in the subject's study chart.

6.3 Procedure Details

The timing of procedures is based on Day 0 being the day of SPI. Follow-up procedures take place at Day 1, Week 1, and at 1, 3, 6, and 12 months following the NOGA catheterization procedure to deliver the study product.

6.3.1 Medical History and Physical Exam

A complete medical history will be conducted during baseline testing including: vital signs, height and weight; assessment of NYHA classification (Appendix C) and CCS classification (Appendix D); medical, surgical, and smoking history; and review of current use of prescription and OTC medications. Similar physical exams will be conducted at each additional clinic visit during the study including vital signs, weight, assessment of NYHA and CCS classifications, review of AEs, and concomitant medications.

NOTE: Laboratory Testing for enrollment of Stage 1 subjects is included in Appendix E.

6.3.2 Schedule of Laboratory Testing Stage 2 (Table 22b)

CONCERT-HF Laboratory Testing	BSL	Harvest	MRI visit	Day 0 (SPI)	Day 1[11]	Wk 1	M 1	M 3	M 6	M 12
Chemistry Tests[1]	X			X	X	X	X	X	X	X
CBC with Differential[2]	X		X[13]				X	X	X	X
Liver Function Tests[3]	X							X	X	X
Pregnancy (childbearing women)	X			X[4]			X	X	X	X
NT-proBNP[5]	X							X	X	X
Troponin I or T	X			X[6]	X	X				
HbA1c	X							X	X	X
PT, INR, PTT[7]	X	X[12]		X[12]						
Infectious Disease Tests[8]	X									
Biomarkers (PB) (Biorepository)[9]				X[10]	X	X	X		X	

1. Chemistry Tests - sodium, potassium, chloride, bicarbonate (CO2), glucose, blood urea nitrogen (BUN), creatinine, and eGFR.
2. Complete Blood Count with Differential - CBC: WBC, RBC, hemoglobin, hematocrit, MCV, and platelets; Diff: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.
3. Liver Function Tests - albumin, alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, direct bilirubin, and total protein.
4. Will be completed within 36 hours prior to injection.
5. NT-proBNP required; send to outside lab if applicable.
6. Will be performed once in the morning and once 8 (+/- 2) hours post cardiac catheterization/NOGA.
7. Later time points if indicated.

8. Infectious disease tests should be the bone marrow donor panel used per local institutional guidelines, including HIV, Hep B (HBsAG, Anti-HBs, Anti-HBc), and Hep C (Anti-HCV), and results must be known prior to harvest. Donor tests are conducted within 60 days of harvest procedures. If for some reason these tests expire prior to either harvest, they will be performed again.
9. With appropriate consent, twenty (20) mL of peripheral blood (PB) will also be collected and transported to the CCTRN biorepository for scientific study (See Section 6.4 Collection of Biospecimens). Stage 1 participants will not be offered biorepository participation.
10. Collected prior to sedation for SPI procedure.
11. Day 1 labs will be collected 24 hours post-procedure or immediately prior to discharge, whichever is sooner.
12. For subjects receiving systemic anticoagulation therapy, an INR measurement will be performed on the morning of the planned procedure; must be <1.6 to proceed with procedure.
13. CBC drawn on day of MRI with hematocrit recorded on the MRI worksheet.

6.3.3 cMRI Procedure

All subjects will undergo cMRI during baseline testing, within 30 days prior to the NOGA procedure, and at 6 and 12 months (+/- 30 days) post study product injection. The cMRI within 30 days prior to SPI will be the baseline measure.

Cardiac MRI will be performed using 1.5 T scanners with multi-channel (8, 16, or 32 channels) cardiac coil, simultaneous ECG recording, and gadolinium. Prior to the cMRI examination, subjects will be screened for contraindication and coached on performing adequate breath-holds. Electrocardiographic leads and a blood pressure cuff will be positioned. The cardiac phased-array coil will be wrapped around the subject's chest and correctly positioned over the precordium. Participants will lie supine on the magnet table and enter feet-first into the center of the scanner. All cardiac images will be obtained during approximately 12-15 heartbeat breath- hold at end-expiration, averaging 10-15 seconds with adequate rest periods between the breath-holds (about 10-15 seconds). The imaging protocol will first include sagittal, axial and oblique scout images to localize the heart. It is anticipated that each cMRI session will last 60 minutes in duration. See MRI Core Lab Manual of Operations for acquisition details.

Guidance to the Principal Investigator: MRI was selected as the imaging modality for endpoint collection due to its excellent capability to identify scar and changes in LV function. The resolution of the imaging is such that it can also detect LV thrombi that may not otherwise be found using the standard echocardiogram. This includes many old, small, organized, endothelialized, mural thrombi that may or may not rise to the level of clinical significance.

Identification and clinical evaluation of LV thrombus: The MRI core lab will provide their interpretation about the presence of a thrombus to the local center. The MRI core lab will differentiate between the LV thrombi that meet the definition of an exclusion on the one hand from the smaller LV thrombi that would likely not appear on echo. The MRI core lab will describe the latter by stating, "*cannot rule out small LV thrombus*". For these, it will then be up to the site study PI and the harvester and/or NOGA operator to follow up in making the determination of clinical significance of any finding and whether (and when) they would proceed with study procedures. This leaves the decision to proceed in the presence of the smaller LV thrombi identified by MRI in the hands of the site study PI based on their assessment of clinical significance. If they proceed, documentation of that determination must be included in the participant's chart.

The MRI core lab provides the statement of any findings and also provides to the clinical team a weblink to the actual MRI images with instruction on the location of the finding(s) (i.e., which series/views will highlight their review). This allows the PI/interventionalist at the center to readily identify the location, view the images, and request additional info if needed. All studies are reviewed by the local radiologist for clinical findings for the PI's consideration before enrollment. The site is responsible for having a local read (safety or professional) of baseline MRIs prior to SPI and to ensure there is local documentation on file regarding presence of thrombus.

6.3.3.1 Performance of cMRI in subjects with implantable cardiac devices (ICD/pacemakers)

The presence of a pacemaker or implanted defibrillator device is not a contraindication to MRI scanning[102,103].

The procedures use are based upon the safety recommendations as listed in "Safety of Magnetic Resonance Imaging in Subjects With Cardiovascular Devices: An American Heart Association Scientific Statement"[102]. In particular, the recommendations include:

1. Written and verbal informed consent is obtained. Specific risks are documented, including
   a. pacemaker/ICD dysfunction and/or damage
   b. arrhythmia
   c. device dislodgement
   d. thermal injury
   e. death
2. There is direct involvement of a cardiologist or specifically trained Registered Nurse with pacemaker/ICD expertise, to oversee pre-scan device measurements, device changes including therapy (ICD) disabling for the duration of the scan, and post-scan measurements and re-enabling of therapy and other device parameters.
3. Advanced Cardiovascular Life Support (ACLS) trained personnel and a "crash cart," including defibrillator, are available throughout the procedure to address an adverse event.
4. Maintenance of visual and voice contact with the subject throughout the procedure.
5. At all times during which the device is disabled, continuous ECG telemetry and pulse oximetry, blood pressure measurements every 5 minutes, and symptoms are monitored including throughout the scan.

Regarding specific implanted device parameter measurements and programming changes, protocols of Johns Hopkins University[104] is used as follows:

1. Exclusion of subjects whose devices were manufactured before 2000
2. Exclusion of subjects with nontransvenous epicardial, or abandoned (capped) leads
3. Exclusion of pacemaker-dependent subjects with ICDs
4. ICD therapies are disabled during the study to avoid unwarranted anti-tachycardia pacing or shocks
5. Limitation of the estimated whole-body averaged SAR to <2.0 W/kg for MR scan acquisition
6. Exclusion of subjects with leads implanted less than six weeks prior to study enrollment

As a part of the training process, personnel who are highly experienced with MR imaging on patients with pacemakers and ICDs will oversee the initial cases at each site until adequate proficiency is reached by each site's imaging staff members.

6.3.4 Six-minute Walk Test (6MWT)

6MWTs will be performed to assess functional capacity. Subjects will perform a 6MWT at baseline and at 6 and 12 months. See Protocol Manual of Operations for acquisition details.

To minimize external sources of test-retest variability, 6MWTs will be administered by testers blinded to treatment arm.

Two tests will be done at each of the 3 visits (baseline, 6 and 12 months) separated by at least 30 minutes. If the variance between test 1 and test 2 for total distance walked is >10%, a third test will be done. If 6MWTs and VO_2 max test are done on the same day, they will be done in consistent order at each visit and at consistent intervals, with 6MWTs completed either at least 30 minutes before or starting at least 1 hour after the VO_2 max test.

6.3.5 Questionnaires

The subjects' quality of life will be assessed with the MLHFQ at baseline and at 3, 6, and 12 months. The subjects' sexual function will be assessed for males with the International Index of Erectile Function (IIEF) and for females with the Female Sexual Function Index (FSFI) at baseline and at 6 and 12 months.

6.3.6 Electrocardiograms (ECGs)

A 12-lead ECG will be performed at baseline, on Day 0 (4-6 hours post procedure), on Day 1 before discharge, at 1 week, and at 6 and 12 months. If there is a sustained run of ventricular tachycardia (≥20 beats) on the 12-lead ECG obtained during baseline testing, the subject will be removed from the study.

6.3.7 Treadmill Testing (VO_2 max)

Treadmill testing with VO_2 max assessment will be performed at baseline and at 6 and 12 months to assess whether cell therapy improves aerobic capacity.
During baseline testing, the subject must not have a VO_2 max greater than 75% of age and gender based predicted values in order to be eligible for the trial. The values for VO_2 max in normal persons[105] and the values reflecting 75% of normal (exclusion criteria) are as follows:

Age	Males	Females
20-29 years old	Normal 43±7.2 ml/kg/min; Exclusion criteria ≥ 32.3. ml/kg/min	Normal 36±6.9 ml/kg/min; Exclusion criteria ≥ 27.0 ml/kg/min
30-39 years old	Normal 42±7.0 ml/kg/min; Exclusion criteria ≥ 31.5 ml/kg/min	Normal 34±6.2 ml/kg/min; Exclusion criteria ≥ 25.5 ml/kg/min
40-49 years old	Normal 40±7.2 ml/kg/min; Exclusion criteria ≥ 30.0 ml/kg/min	Normal 32±6.2 ml/kg/min; Exclusion criteria ≥ 24.0 ml/kg/min
50-59 years old	Normal 36±7.1 ml/kg/min; Exclusion criteria ≥ 27.0 ml/kg/min	Normal 29±5.4 ml/kg/min; Exclusion criteria ≥ 21.8 ml/kg/min
60-69 years old	Normal 33±7.3 ml/kg/min; Exclusion criteria ≥ 24.8 ml/kg/min	Normal 27±4.7 ml/kg/min; Exclusion criteria ≥ 20.3 ml/kg/min
70-79 years old	Normal 29±7.3 ml/kg/min; Exclusion criteria ≥ 21.8 ml/kg/min	Normal 27±5.8 ml/kg/min; Exclusion criteria ≥ 20.3 ml/kg/min

6.3.8 Implantable Cardioverter Defibrillator (ICD) Interrogation

An ICD interrogation is a standard non-invasive assessment of the function of the ICD. This assessment (interrogation) identifies the occurrence of any significant ventricular arrhythmias over a certain time period and identifies any potential therapeutic interventions (such as shocks or antitachycardia pacing) that were used by the ICD to treat any ventricular arrhythmia. ICD interrogation will occur before and after every cMRI, as well as before the SPI procedure. Reports will be generated for the interrogations conducted before the MRIs at baseline (image collected

within 30 days of SPI) and at the 6 month and 12 month visits. Local electrophysiology personnel will review the device report for the presence of reportable clinical events. Copies of the reports should remain on site as source documentation (de-identified copies may be requested by the Sponsor for endpoint adjudication).

6.3.9 Echocardiograms

Two 2-D echocardiograms will be performed on all subjects the day of harvest: 1) a pre-RHC procedure echo to assess for pre-existing pericardial effusion, and 2) a post-RHC (with or without EMB) procedure echo within 6 hours to ensure no post-procedure effusion (even if subject is stable). A 2-D echocardiogram will also be performed post-SPI procedure (within 6 hours) to assess for adverse events potentially related to study product delivery (e.g. pericardial effusion, wall motion abnormalities, etc.). Additional echocardiographic assessments for pericardial effusion will be done as clinically indicated.

6.4 Collection of Biospecimens

A central CCTRN biorepository will be utilized in this study. Discussion of biorepository sampling is included in the consent form and subjects will have the option of participating in sample donation. Participation in the study does not equate with participation in donating to the biorepository; subjects can decline the biorepository donation and still participate in the trial.

The goals of this biorepository are: 1) to provide storage of critical biomaterials derived from subjects enrolled in clinical protocols within the CCTRN 2) to provide long-term integrity (up to 10 years) of these biospecimens and samples, and 3) to provide management of samples for ancillary studies of immunologic, immunohistochemical, cellular, and molecular analyses of collected samples; as well as phenotypic and functional analyses of cells and plasma samples with an aim toward gaining insight into diagnostics of disease progression and prognostics of successful intervention.

These biospecimens 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 and/or of cardiovascular disease. All such biospecimens will be destroyed after 10 years.

6.4.1 Bone Marrow

During the bone marrow aspiration procedure, approximately 90 mL (± 10 ml) of bone marrow will be harvested from active and placebo subjects. The bone marrow will be then transported to the local cell processing lab for preparation, packaging, and shipping to the CCMF or when appropriate to the CCTRN biorepository. Sixty-five mL (+/- 5 mL) of bone marrow will be sent to the CCMF. The remainder of the bone marrow suspension (~25 mL) will be shipped to the CCTRN biorepository with appropriate consent.

In addition, cryopreserved MNC products of subjects randomized to both the placebo and to the c-kit+ cell only groups will be shipped using a LN_2 dry shipper from the CCMF to the CCTRN biorepository with appropriate consent.

6.4.2 Peripheral Blood (PB)
Twenty mL of PB will be collected on Day 0 (day of injection, prior to sedation), on Day 1, Week 1, and at the 1, and 6 month visits. This blood will be sent to the CCTRN biorepository for additional characterization studies.

6.4.3 MSC and/or c-kit+ cell Autografts
Remaining MSC and/or c-kit+ cell product in excess of the target doses for the subject will be provided to the CCTRN biorepository (with appropriate consent).

6.4.4 Explanted hearts
Included in the informed consent is a request for the explanted heart in cases of transplant or in the event of the participant's death. These donated hearts will be studied to assess the result of study product delivery (e.g. cell proliferation, increased capillary density). With appropriate consent, the explanted heart will be sent to the CCTRN biorepository for further study.

7.0 EVENT MONITORING AND REPORTING
The safety monitoring program is a comprehensive, data driven program that provides ongoing capture and analyses of safety data and issues timely notifications, event specific reports, and scheduled cumulative trial reports of safety issues to appropriate study personnel, the NHLBI Program Director, the Data and Safety Monitoring Board (DSMB), and the Food and Drug Administration (FDA). The program complies with applicable U.S. law, regulations, and guidance.

7.1 Definitions Related to Adverse Events
The following definitions arise from recently modified FDA reporting regulations and International Conference on Harmonization (ICH) guidelines for use in this study:

7.1.1 Adverse Events (AEs)
An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study. The event does not need to have a causal relationship with treatment.

7.1.2 Suspected Adverse Reaction (SARs)
A suspected adverse reaction (SAR) is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the study product/procedures and the adverse event.

7.1.3 Serious Adverse Events (SAEs) or Serious Suspected Adverse Reaction (SSAR)
A serious adverse event (SAE) or serious suspected adverse reaction (SSAR) is defined as an AE/SAR which, in the view of the Investigator or Sponsor, results in: 1) Death; 2) a life-threatening event (i.e. an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe); 3) inpatient hospitalization of > 24 hours or prolongation of existing hospitalization; 4) a significant disability/incapacity; or 5) a congenital anomaly/birth defect. Other important medical events may be considered SAEs/SSARs if, in the opinion of the Investigator or DCC, they jeopardize the subject or require intervention to prevent one of the other outcomes listed above.

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 AE/SAR by the Investigator or Sponsor 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 AE/SAR. Any abnormal test result that is determined to be an error does not require reporting as an AE/SAR.

7.2.2 Hospitalizations

AE/SARs associated with hospitalization, or prolongation of hospitalization, are classified as 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, or same day surgeries (as outpatient/same day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical AE/SAR is not in itself an SAE/SSAR.

7.3 Reporting Responsibilities of the Investigator

For all events (AE/SAR and SAE/SSAR), 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 12 month clinic visit. Events should be recorded on the Adverse Event eCRF. Do not delay the initial reporting of an event in order to obtain resolution or follow up information.

For all events, the Investigator must pursue and obtain adequate information both to determine the severity and causality of the event. For 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 event that the Investigator does not become aware of the occurrence of a SAE/SSAR 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 event.

7.3.1 Severity Assessment

The DCC uses the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, for detailed descriptions of Severity Grades. The CTCAE schema is classified by body system and event using the MedDRA hierarchy and provides descriptions of events that qualify under each severity rating. The following table contains general descriptions of Adverse Event Severity Grades.

Please note: Grade 1 (Mild) AE/SARs are not entered in the electronic CRF in the CCTRN database.

Severity Grade	Description
1	Mild. Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
2	Moderate. Minimal, local, or non-invasive intervention indicated or limiting activities of daily living (i.e. preparing meals, shopping for groceries/clothes, managing money, using telephone, etc.)
3	Severe or medically significant but not immediately life-threatening. Hospitalization or prolongation of hospitalization indicated OR disabling OR limiting self-care (e.g. bathing, dressing, feeding self, using toilet, taking medications, etc.)
4	Life-threatening consequences; urgent intervention indicated.
5	Death. Death related to adverse event.

Notice that severity and seriousness are different concepts. 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 SAE/SSARs (see Section 7.1.3 above).

7.3.2 Causality Assessment

The DCC nomenclature for assessing the causal relationship between the study product/procedure and an event is listed in the following table.

Adverse Event/Suspected Adverse Reaction Relationship to Study Product/Procedure

Unrelated	No temporal association to study product/procedure. An alternate etiology has been established.
Unlikely	Clinical events which are likely to be caused by subject's clinical state, environment or administration of other therapies or exposure to toxins.
Possibly related	Reasonable temporal relationship to study product/procedure. Connection to study product/procedure cannot be ruled out.
Probably related	There is a reasonable temporal association with the study product/procedure. There is a high degree of certainty that the event is related to the study product/procedure.
Definitely related	There is a direct temporal relationship to the study product/procedure. The event follows a known pattern of response to the study product/procedure.

The Investigator chooses the category that overall best describes the relationship between the event and the study product/procedure and records the evaluation on the Adverse Event eCRF. Note: If the Investigator does not know whether or not the study product/procedure caused the event, then the event will be handled as "possibly related to investigational product" for reporting purposes.

7.3.3 Expectedness Assessment

The DCC nomenclature for assessing whether an event is expected or unexpected with regard to the study product/procedure is listed in the following table.

Expected	Any event for which the nature or severity is consistent with information in study Investigator Brochure
Unexpected	Any event for which the nature or severity is not consistent with information in study Investigator Brochure

7.4 Reporting Responsibilities of the Sponsor (DCC)

7.4.1 Safety Monitoring Program and Reporting

The Safety Monitoring Program uses a combination of, email notifications, event specific reports, and scheduled cumulative trial reports to keep the NHLBI Program Director and NHLBI DSMB informed about real and potential safety issues.

Email Notifications are comprised of an email to the NHLBI Program Director and NHLBI DSMB Executive Secretary with available information on the date and nature of the event, the site Investigator's evaluation of the severity, expectedness, and relatedness to study product/procedure; and a Sponsor assessment of the event given the information known at the time of the initial reporting.

Event specific reports are formal written reports providing the details of the event (including circumstances surrounding the event, laboratory testing, concomitant medications, and any formal diagnoses made via medical intervention). These reports include a full sponsor assessment of the severity, expectedness, and relatedness to study product/procedure as well as any available status update on the subject.

Scheduled cumulative trial reports are prepared semi-annually by the DCC. These are used by the NHLBI DSMB to assess recruitment, subject safety, and continued trial feasibility. These reports include total numbers of AE/SARs and SAE/SSARs experienced in the overall trial. The information provided includes both new events reported since the last DSMB meeting and cumulative events reported during the life of the trial.

7.4.2 Sponsor Reporting Requirements to the EC, NHLBI and DSMB

Once the event has been reported to the DCC by the Investigator, the DCC uses the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Classification (SOC) to classify all AEs/SARs (including SAEs/SSARs assessed by Investigator or DCC). Additional supporting documentation may be requested from the site Investigator and his/her team to enable the DCC Safety Officer to accurately assess the event for reporting.

Type of SAE/SSAR		Type of Report	Reporting Timeframe
Event is **NEITHER** grade 3 or higher, **NOR** unexpected, **NOR** related	➡	Cumulative DSMB report	Every six months
Event is **ONE OF:** grade 3 or higher **OR** unexpected **OR** related	➡	Email notification to DSMB	Within 15 days
Event is grade 3 **AND EITHER** unexpected **OR** related	➡	Email notification to DSMB	Within 15 calendar days
		Event specific report to DSMB	Within 30 calendar days
Event is unexpected **AND** related **AND** (grade 2-grade 5)	➡	Email notification to DSMB	Within 72 hrs
		Event specific report to DSMB	Within 7 calendar days

7.4.3 Sponsor Reporting Requirements to FDA

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

Type of SAE/SSAR	Report to	Timeframe
Fatal or life-threatening, unexpected, and associated with study product/procedure	FDA	MedWatch submitted within 7 calendar days of learning of event
Other SAE/SSARs that are non-fatal or life-threatening but are unexpected and associated with study product/procedure	FDA	MedWatch submitted within 15 calendar days of learning of 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 subjects 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/SSAR reporting, and must include a corrective action plan/measures to prevent recurrence.

7.6 NHLBI DSMB Criteria

All SAEs and SSARs will be evaluated by the NHLBI DSMB in accordance with its charter and review procedures. This includes an assessment of expectedness, relationship to the study product or procedures, and severity. The CCTRN Study Sponsor will rely on the NHLBI DSMB to identify conditions or events that would trigger further action, including a temporary halt, modification, or termination of the study for safety reasons.

7.7 Review of Open Label Lead-in (16 subjects)

The following outlines the process for the collection and review of data for the 16 subjects participating in the open label lead-in portion of the study:

1. Sixteen subjects who consent and meet all inclusion and exclusion criteria will be randomized 1:1 to SOC vs. Combo cell therapy.
2. The eight subjects in the Combo group will undergo a bone marrow aspiration and endomyocardial biopsy, and after cell manufacturing, receive the study product via transendocardial injections using NOGA (per protocol).

3. All subjects will be observed for complications or adverse effects of baseline testing and non-intervention study procedures. Subjects in the Combo group will also be observed for any complications or adverse effects of the bone marrow or endomyocardial harvests, electromechanical mapping, or delivery of cell therapy.
4. Events will be reported to regulatory oversight groups as outlined above in section 7.4.
5. The DSMB will review safety and bioactivity data (LV function and functional status) at three months for the sixteen subjects. This data will also be forwarded to the FDA in a subsequent amendment.
6. Upon successful DSMB review of the lead-in data, the study will be permitted to recruit subjects into both the active and placebo groups to the specified protocol sample size of 144.

8.0 STATISTICAL PROCEDURES

8.1 Randomization Strategy

8.1.1 Randomization in Stage 1 (open label lead-in)

Randomization to treatment assignment will be conducted using a web access database created and maintained by the Data Coordinating Center (DCC). Following successful completion of baseline testing, clinical sites will enter results of the testing in the web-based program. Subjects will be randomized either to 1) SOC (no study procedures) or 2) Combo (MSCs plus c-kit+ cells). We will use a block size of 2.

8.1.2 Randomization in Stage 2 (double-blind, placebo-controlled phase)

The size of this study requires explicit consideration of the losses to follow-up after the subject has been randomized. In CONCERT-HF, two periods of time are vulnerable to subject attrition: 1) during the approximately 14 week period after subjects are randomized but before they are treated; and 2) the period that begins when the subject receives study treatment and is subsequently followed for one year.

Despite investigators' best efforts, some subjects who are randomized will not continue in the study (e.g., clinical events between randomization and end of follow-up, inability to complete follow-up measures, etc.). Based on the ACCLAIM[106] and FOCUS[56] experiences, we anticipate 20% follow-up loss between randomization and the end of the one year follow-up, and we have therefore set our sample size at 144 subjects. The ACCLAIM[106] investigators evaluated the effect of a non-specific immunomodulation therapy in subjects with NYHA functional class II-IV chronic heart failure, LV systolic dysfunction, and hospitalization for heart failure or intravenous drug therapy in an outpatient setting within the past 12 months. During a mean follow-up of 10.2 months, out of 2226 patients a total of 341 patients (15.3%) were unable to complete the study. In FOCUS[56], out of 92 randomized patients, 13 were excluded post treatment (14.1% at the six month follow-up). The attrition rate for these two trials that enrolled a patient population comparable to the CONCERT-HF population was approximately 15%. Since we are following subjects for 12 months, we increased the CONCERT-HF attrition rate to 20%, and we increased the sample size and power.

Randomization, or the random allocation of therapy, is a well-accepted mechanism for reducing potential bias in evaluating treatment effects. Randomization to treatment assignment will be conducted using a web access database created and maintained by the Data Coordinating Center (DCC). Subjects will be randomized either to 1) Combo (MSCs plus c-kit+ cells), 2) MSCs

alone, 3) c-kit+ cells alone, or 4) placebo. Subjects will be stratified by center, and randomly selected variable block sizes of 4 or 8 will be implemented.

When a subject is pre-screened and entered in the web-based program, an identification (ID) number will be assigned. When the subject is consented and demographic information entered to the web-based program, an acrostic will be generated. The DCC will monitor recruitment by providing reports to the NHLBI Project Office as appropriate during the recruitment phase. Updated reports will be maintained on an Internet site accessible to Network members. Goals for recruitment will be set and will be reviewed by the DCC and the NHLBI Project Office.

8.2 Guiding Analysis Plan

The primary analyses in CONCERT-HF will be intention-to-treat for all endpoints.

8.3 Delays

Circumstances that may result in delaying SPI could be related to an issue with cell processing or to a change in the clinical condition of the subject.

- If a fatal cell processing related issue or contamination occurs during the window between randomization and the scheduled SPI procedure, the subject may undergo a second bone marrow aspiration and/or endomyocardial biopsy at a time to be determined by the Medical Monitor and CONCERT-HF Clinical Center PI upon review of the case details.
- If a cell processing related issue arises on the day of the SPI procedure and additional product can be thawed and delivered at a later time, the subject will have their SPI postponed.

A randomized subject who has a resolvable change in their clinical condition and their cells not yet prepared for injection will be allowed to have their SPI postponed to a time to be determined by the Medical Monitor and CONCERT-HF Clinical Center PI upon review of the case details. The subject must remain eligible for the study in order to proceed with SPI, which may include repeat baseline testing. A subject who cannot be treated will no longer qualify for inclusion in the study, though will be included in all analyses for which they have data in accordance with the "intention-to-treat" principle.

8.4 Statistical Analyses

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

8.4.1 Baseline Analyses

Although the stratified random assignment of participants to the various treatments should ensure comparability with respect to known and unknown variables, imbalance may occur by chance. Descriptive statistics for baseline characteristics known or suspected to be associated with outcomes will be prepared for the treatment groups. The variables considered in such a description can be categorized as: 1) demographic characteristics; 2) medical history; 3) physical examination; 4) laboratory data; and (5) quality of life / psychosocial data. Exact testing for

categorical variables and Student *t* testing (or Wilcoxon rank sum test for non-normally distributed variables) for continuous variables will be used to evaluate the differences in baseline variables between treatment groups.

8.4.2 Outcome Analyses

Safety data from Stage 1 will consist of clinical events that occur between randomization and the three month follow-up period and will be included in the formal analysis of the study. Feasibility data (as referenced in section 3.4) will be collected on all treated patients in Stage 1 and will be included in the formal analysis of the study. Outcome data from Stage 1 will consist of LV function and functional status measures collected at baseline and the three month follow-up time point on all patients; however these subjects will not be included in the formal analysis for efficacy as described below for Stage 2.

8.4.2.1 Feasibility Evaluations

To assess the practicality of study procedures, the feasibility assessments outlined in Section 3.4 will be reported by the distribution of occurrences across subjects. The number of uninterpretable cMRI endpoint measures will be assessed at baseline, and at 6 and 12 months.

8.4.2.2 Safety Evaluations

The safety assessments outlined in Section 3.5 will be carried out. For MACE and other significant clinical events defined in Section 3.5, we will carry out a time-to-first-event analysis using standard life table techniques. For all categories of events, the total numbers of each event will be tabulated and evaluated using Fisher's Exact Test.

8.4.2.3 Prospectively Declared Endpoints

To meet the efficacy objective stated in Section 3.6.1 and assess the prospectively declared efficacy endpoint measures outlined in Section 3.6.2, the analyses will be carried out as follows.

All endpoints listed in Section 3.6.2 except MACE are continuous and will be assessed individually using a general linear mixed model in an intention-to-treat analysis. The within subject component will reflect the measures obtained at baseline, six months, and twelve months. The between subject component will reflect the effects of the cell types or placebo. The model to be assessed will be an effect modification or interaction model

$$E[y_i] = \beta_0 + \sum_{j=1}^{k} \alpha_j z_{ij} + \sum_{j=1}^{m} \gamma_j u_{ij} + \beta_1 w_i + \beta_2 x_i + \beta_3 z_i$$

where the dependent variable, y_i is the dependent variable, The predictor variables are a battery of covariate variables obtained at baseline variables ($z_{j\,j}$=1 to k), the effect of MSCs w_i (dichotomous) the effect of c-kit+ cells x_i (dichotomous) and the effect of Combo z_i (dichotomous). The random effects are contained in u_{ij} j= 1 to m. This permits a test of the effect of each of these study products both against placebo, and against each other.

We will also carry out an analysis that tests whether there is true synergy produced by Combo (that is, whether the effect of the combined cell product improves LV function and functional status above and beyond that expected by the sum of the c-kit+ cells and MSCs alone) even though this assessment is underpowered. Both unadjusted and adjusted treatment effects will

be computed; adjustments will be for baseline covariates whose association with the dependent variable is generally accepted. Adjustments will also be made for clinical center.

The MACE endpoint is dichotomous and these data will be analyzed and reported by both the time to first occurrence of event and the total number of each event that contributes to the MACE endpoint. These data will be analyzed by therapy group between baseline and a) 6 months and b) 12 months.

8.4.2.4 Variability Management

The power of CONCERT-HF is sensitive to the variability of its endpoint measure. For example, from Table 4 a standard deviation of 8 provides 81% power in order to detect a 6 absolute unit increase in LVEF (36 subjects per treatment arm). An increase in the standard deviation from 8 to 9 decreases the power to 72%. It is important for CONCERT-HF to maintain control of the standard deviation.

For cMRI based endpoints, the DCC will work with the MR core lab to monitor the standard deviations of randomizing centers using only placebo data. The DCC will issue reports to the SC to review the standard deviations of endpoints by center. Center outliers will be identified and an investigation will ensue to determine factors that are likely contributing to the variability. The DCC and MR core lab will work with the center(s) to employ strategies to reduce variability (e.g., assess technicians' abilities to obtain imaging measures and provide additional training when required, evaluate changes in equipment at centers and/or need for software/hardware updates, monitor rate of staff turnover and impact, etc.).

8.4.2.5 Adaptive Design and Sample Size Adjustments

The assumptions used to compute the sample size may result in an overestimation or an underestimation (e.g., if there were more deaths). In order to maintain a sample size for CONCERT-HF that achieves our statistical goals, CCTRN proposes an adaptive design for finalizing the sample size. While we will manage variability in CONCERT-HF through quality control as outlined above, we also propose to monitor and possibly alter the sample size based on observed standard deviation (s_d) of change in LVEF and change in infarct size for the placebo group.

See Table 23 (LVEF) and Table 24 (infarct size) below for information to guide the recommendations and decisions to adapt the CONCERT-HF sample size. Evaluations will take place when 34% and 66% of the placebo cohort has completed Month 6 data collection, labeled information time (IT) in the tables below. It is anticipated that one change might be recommended at the first time point, i.e., 34% IT, and be confirmed at the second time point, i.e., 66% IT. The interim analyses will be submitted to the NHLBI DSMB for review and recommendation for actions to be taken if warranted.

The decision to decrease the sample size is based on the upper bound of the confidence interval (CI), while the decision to increase the sample size is based on the lower bound of the CI. The table for LVEF follows:

Table 23 Draft Proposal to Monitor and Adjust Sample Size LVEF

Findings to Decrease Sample Size

% IT	N per arm	CI	s_d	Confidence Interval LB	UB
34	12	0.99	3.5	2.3	7.3
66	24	0.99	4.5	3.3	7.3

Findings to Increase Sample Size

% IT	N per arm	CI	s_d	LB	UB
34	12	0.99	13.0	8.4	27.0
66	24	0.99	12.1	8.8	19.6

The table for infarct size follows:

Table 24 Draft Proposal to Monitor and Adjust Sample Size Infarct Size

Findings to Decrease Sample Size

% IT	N per arm	CI	s_d	Confidence Interval LB	UB
34	12	0.99	3.0	1.9	6.2
66	24	0.99	4.0	2.9	6.5

Findings to Increase Sample Size

% IT	N per arm	CI	s_d	LB	UB
34	12	0.99	11.5	7.5	23.9
66	24	0.99	10.0	7.3	16.2

The CI upper and lower bounds are computed from the approximate 99% CI for σ based on s_d as follows:

$$\sqrt{\frac{(n-1)s_d^2}{\chi^2_{0.995}(n-1)}} \le \sigma \le \sqrt{\frac{(n-1)s_d^2}{\chi^2_{0.005}(n-1)}}$$

These tables provide thresholds that when crossed suggest an action might be warranted. The simple observation of an s_d of the difference between baseline and Month 6 measures that appears “too large” or “too small” would not result in an automatic change in CONCERT-HF’s sample size. The following should also be considered upon NHLBI DSMB review of the data for recommendation and action to be taken:

- Was training in obtaining MR images sufficiently in place at each of the recruiting centers?
- Has there been recent staff turnover at least one center?
- Has there been a change in equipment for at least one center?
- Is there new literature on the topic of variability of MR measured LVEF or infarct size variability?
- Have other clinical trials reported relevant values of s_d for MR measured LVEF or infarct size?
- Are the recommendations for adapting the sample size based on change in LVEF and change in infarct size coherent?

These sources of information can provide a broader perspective on the interim findings of CONCERT-HF and will be considered before the sample size is changed. In addition, a decision at 34% IT may be preliminary to a confirmatory final decision at 66% IT.

8.4.2.5.1 c-kit+ cell growth failure

The CCTRN will be informed by the CCMF about c-kit+ cell manufacturing performance using aggregate data that do not unblind the Steering Committee to any subject's treatment assignment. If the number of subjects with final c-kit+ cell counts less than 800,000 c-kit+ cells is prohibitively large, CCTRN will consult with NHLBI on the feasibility of adding additional subjects to the study (i.e., randomized to either the Combo arm or the c-kit+ cells only arm) to ensure minimum power (80%) in assessing the prospectively specified hypotheses for the effects of cell therapy on study endpoints.

8.4.2.6 Subgroup Evaluations

The effect of subgroup stratum on the relationship between cell delivery and the endpoints 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 the following: age; gender; race; diabetes; hypertension; extent of left ventricular disease (single vessel vs diffuse chronic disease); presence of a cardiac device; and characteristics of the bone marrow and c-kit+ cell and MSC products, including function of progenitor cells (hematopoietic and mesenchymal), expression of surface markers, RNA and protein expression, growth kinetics and metabolic patterns, and the number of cells delivered. 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.

8.4.2.7 Sub-study Evaluation

Centers qualified to assess change in global diffuse fibrosis (via T1 mapping) through the MRI Core Lab will collect the requisite sequences for this sub-study evaluation. The analysis plan is as stated in 8.4.2.3 above.

8.4.2.8 As-Treated Evaluation

In addition to the intention-to-treat analysis, an as-treated analysis will be conducted. In this evaluation, subjects will be placed in treatment groups based on what they received, not in the group to which they were randomized. The primary endpoint analysis will then be repeated with these reassignments.

8.4.2.9 Dose-Response Evaluation

Anticipating that there will be variability in c-kit+ cell dose, an analysis of the relationship between the effect of therapy and c-kit+ cell dose received will be conducted for all efficacy endpoints.

8.4.2.10 Multiplicity Issues

In this phase II study, no adjustments are made for multiple comparisons. The reported measures of effect will be effect size, the standard error of the effect size, the 95% confidence interval for the effect size, and the p-value. *P*-values will be interpreted at nominal 0.05 levels in accordance with Hare et al.[99].

8.4.3 Interim Analysis

Interim analyses are a well accepted procedure required by many DSMB's[107]. One formal interim efficacy analyses will be conducted after the first 50% (72/144) of subjects have reached or exceed their six month evalution. Consideration of the current drop out rate suggests that this will produce 58 evaluable subjects (i.e., individuals with six month MR results). The interim analyses will give the DSMB an opportunity to conduct unblinded reviews of interim results and to use the information as the basis for recommendations to NHLBI regarding the study.

For efficacy/harm significance tests, Lan-DeMets alpha-spending function based on O'Brien-Fleming[108] boundaries will be used to control overall Type I error rate at $\alpha = 0.05$[109]. The advantage of the Lan-DeMets approach is that it can be used even when the looks are not equally spaced. In this case the boundaries would change somewhat. For looks at exactly 50% and 100% of the completed intention-to-treat evaluable subjects, the cumulative α will equal 0.003 and 0.05, respectively.

Another extremely useful monitoring tool is conditional power[110,111]. The conditional probability of obtaining a statistically significant result at the end of the trial is computed under different hypothesized treatment effects. Unlike the O'Brien-Fleming or Lan-DeMets boundaries, conditional power is usually used to justify terminating a trial which has no realistic chance of producing a statistically significant result. If one uses such a rule, the chance of a type 2 error (accepting the null hypothesis when it is false) is slightly greater than it would be without stochastic curtailment. This is because one could accept the null hypothesis at the end of the study or at an interim point.

8.4.3.1 Plan for CONCERT Interim Monitoring

In Section 3.7 of the CONCERT-HF protocol, there are 6 proposed hypotheses involving 8 different outcomes reflecting the change in (LVEF, infarct size, LVESV, LVEDV, VO2max, six minute walk distance, MLHFQ score, sphericity index) . For a given hypothesized change, with assumed standard deviation of change, the study has 80-90% power, for the evaluable subject sample size of 116, for any given hypothesis. There were no adjustments of multiple testing.

Guidelines proposed to the DSMB:

- *Futility:* It would seem reasonable to examine futility for the 3 major comparisons of Combo vs. placebo, MSCs vs. placebo, and c-kit+ cells vs. placebo for each of the 8 outcomes. If all are futile, i.e., for each comparison using the hypothesized change for 90% power for each outcome yields a probability of < 0.05 that the test statistic would fall into the critical region, then the trial would seem futile.

- *Efficacy-based termination:* To declare efficacy, it would seem that the Combo vs. placebo would have to yield a significant result (p<0.003) for at least the first 4 declared outcomes (placebo-adjusted changes in LVEF, LVESV, LVEDV, and infarct size).
- *Harm-based termination:* Similar to efficacy-based termination. It would seem that the Combo vs. placebo would have to yield a significant result (p<0.003) for harm for at least the first 4 declared outcomes (placebo-adjusted changes in LVEF, LVESV, LVEDV, and infarct size).

If the criteria for each of 1) futility-termination, 2) efficacy-termination, and 3) harm-termination are not met, then the trial would be permitted to continue.

The proposed stopping guidelines are:

1) Use Lan-DeMets version of O'Brien-Fleming for harm/benefit.
2) The boundaries will be symmetric.
3) Information time will be calculated as proportion of expected evaluable subjects.
4) Examine the data at 50% information time.
5) Use conditional power under the protocol-specified alternative hypotheses for futility.

9.0 TRIAL MANAGEMENT

9.1 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.

9.1.1 Informed Consent Process

Potential participants will be approached by one of the study investigators or research coordinators. Information regarding study participation will be provided to the potential participant. The informed consent includes descriptions of all study related procedures, all possible risks to participant, and the time commitment involved with participating. All consent forms will have IRB approval. Individuals who agree to participate will receive a copy of the signed informed consent. The research staff member obtaining consent will document the informed process in the subject's chart for monitoring purposes. Translation of ICFs will be done in accordance with local IRB procedures.

9.1.2 Risks Associated with the Procurement, Processing, Injection, and Assessment of the Study Product

9.1.2.1 Harvest Procedures

Anticoagulation Medications

For subjects taking anticoagulation medications (i.e. blood thinners) at the time of the harvest procedures (bone marrow aspiration and right heart catheterization), some of these medications

(i.e., warfarin and/or Factor Xa inhibitors) may be stopped for a short period before the procedures; during which time the subject may be at an increased risk of a stroke. It is very important that subjects be instructed to inform the research team immediately of any symptoms indicative of this (e.g. headache, dizziness, light-headedness, blurred vision, slurred speech, facial drooping, decrease sensations anywhere on the body, or weakness or a decrease in strength of the arms or legs). Subjects will be closely monitored during any interruption in anticoagulation therapy for the events listed above.

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. There is a possibility the subject could experience worsening of heart failure symptoms. There is a possibility of a fat embolism leading to shortness of breath, confusion, drowsiness, rash, fever, or seizure.

Right Heart Catheterization with/without Endomyocardial Biopsy
During the right heart catheterization the subject will receive some radiation (see the Radiation Risks Section below). Possible discomforts include stinging from the numbing medicine (topical cream), bruising, and discomfort from lying flat on the exam table for 20-30 minutes. Possible risks include decreases in blood pressure and ICD firing due to abnormal heart rhythms that last only a few seconds and go away. Less likely risks include bleeding, infection, serious and long-lasting heart rhythm problems, injury to the pulmonary artery, blood clots in the lungs, damage to the walls of the heart, and puncture of the heart wall with a risk of death. One patient in this study has died from a puncture of the heart wall following biopsy.

9.1.2.2 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 subject 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 subject notes a fever, he/she will be requested to notify the investigator/study team.

9.1.2.3 Cardiac Catheterization and NOGA mapping
Potential risks of this procedure include bleeding, hematoma at catheter insertion site, allergic reaction to the angiography dye, abdominal pain, formation of a blood clot which could lead to loss of function or surgical intervention. It is possible the subject may experience worsening heart failure symptoms. Other problems that could happen are: local nerve damage, infection, arrhythmias, stroke, and heart attack. Some temporary problems that might happen are: temporary movements (spasm) of a muscle, vein, or artery; separation of the layers of the walls of a blood vessel; or sudden blockage of a blood vessel. A very rare complication could result in death or a need for cardiac procedures such as percutaneous coronary intervention (with or without stent placement) or an urgent coronary artery bypass graft (open heart surgery). Serious complications, including death, happen in less than 1 in every 1,000 tests that are performed.

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

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

9.1.2.4 Study Product Injection

Some problems that might happen include (but others could occur): decreased blood pressure, irregular heartbeats, chest pain or discomfort, possible firing of ICD, damage to the heart muscle, perforation of the heart causing blood to accumulate around the heart, bleeding, heart attack, stroke, dislodgement of material into other arteries (possibly causing blockage), need for emergency surgery, and death. It is possible that a small amount of cells will enter the bloodstream of the heart rather than the heart muscle. If the injection catheter penetrates through the heart (from inside to outside) and cells appear in the fluid filled area surrounding the heart which cushions the heart as it moves (pericardial space) there is a possibility of potentially harmful effects which could cause an inflammatory response. Injection directly into the heart muscle also may cause inflammation or irritability.

There may be some circumstances where the research team is unable to give the subject the study product (cells or placebo); such as change in the coronary anatomy, equipment failure, or poor quality of the stem cells. The option of cell donation will be discussed with the subject by the research team.

9.1.2.5 cMRI Procedure

Risks associated with administration of contrast dye are nausea, vomiting, and headache. Allergic reactions to contrast dye are rare, but there are extremely rare instances of reactions causing death. The contrast dye used in the cMRI procedure is referred to as a gadolinium based contrast agent (GBCAs); after administration, GBCAs leave the body mostly through the kidneys. Recent publications report some deposits from GBCAs remain in the brains of some patients who undergo four or more contrast MRI scans, long after the last dose is received. Recent studies conducted in humans and animals have confirmed that these deposits can remain in the brain, even in people with normal kidney function. It is unknown whether these deposits are harmful or can lead to adverse health effects. Available information does not identify any adverse health effects. However, this issue continues to be studied by the FDA and subjects will be informed should any new specific adverse health concerns emerge.

Less common risks of contrast dye are kidney damage or nephrogenic systemic fibrosis (NSF), allergic reactions (rare), and death (extremely rare). The risk of kidney damage or NSF is increased with patients who already had some evidence of kidney disease or diabetes, or are dehydrated.

There is no radiation exposure from cMRIs. There is a risk of heat injury from radiofrequency coils and the cables to the coil and monitoring equipment. There may be some discomfort with placement of an IV line, administration of medications or blood draw, and lying down in the MRI machine.

MRIs on subjects with pacemakers and ICDs
The powerful magnetic fields and radio waves that are part of MRI scans could cause the ICD wires to overheat, potentially damaging heart tissue. MRIs can induce unwanted currents that could cause arrhythmias, or in the case of ICDs, cause an unnecessary shock. Pacemakers and ICDs can be temporarily reprogrammed so they do not react to an MRI's magnetic field and will be monitored during the MRI scan. During the scan there is an increased risk of experiencing an arrhythmia, which could be life threatening or fatal. A cardiologist or specifically trained Registered Nurse with pacemaker/ICD expertise will check the device before, during, and after the scan, and trained life support staff and a defibrillator will be present and available during the procedure.

9.1.2.6 Radiation Risks
This research study involves exposure to radiation from cardiac catheterization laboratory x-ray procedures. The expected total amount of radiation exposure to the subjects in this study is approximately 2.4 rem.

9.1.3 Adequacy of Protection Against Risks
The precautionary measures mentioned in the above sections will minimize the risk associated with bone marrow aspiration, right heart catheterization with/without endomyocardial biopsy, study product injection, and cMRI procedures for subjects. The procedures for bone marrow aspiration, right heart catheterization, endomyocardial biopsy, and study product delivery using NOGA mapping and injection are well known, and in the hands of skilled interventionalists with proper training, events remain low. A favorable risk profile for the use of autologous cell therapy continues to emerge as new clinical trials demonstrate safety of these procedures. The use of established protocols in the MR imaging of subjects with devices, along with standardized training of technicians by experienced core lab personnel, will minimize the risk of the cMRI procedure in those with devices. Subjects will be monitored throughout imaging and safety precautions are included as described previously.

9.1.4 Potential Benefits of the Proposed Research to the Subjects and Others
Subjects with ICM are at risk for significant morbidity and mortality. This study has the potential to improve cardiac function by preserving or recovering functional myocardial tissue. This project will also provide mechanistic insight into cell therapy which will be useful for finding new treatments for other diseases.

9.1.5 Risk Benefit Analysis
The administration of MSCs and c-kit+ cells, alone or in combination, offers an additional therapeutic option to subjects with ICM whose goal is to not just reduce the rate of LV deterioration but to actually stabilize and ameliorate HF. Having highly trained experts deliver and oversee the therapy, in conjunction with close study monitoring substantially reduces the likelihood of adverse events. The potential risks to the subjects remain reasonably low in relation to the possible benefit of improving their heart function above which can be obtained with standard of care treatment regimens.

9.1.6 Importance of the Knowledge to be Gained
The knowledge to be gained from this clinical trial is significant in that this will 1) demonstrate if promising cell types can be manufactured and delivered alone or in combination to subjects with ICM; 2) demonstrate whether promising cell types that heretofore have demonstrated the likeli-

hood of few risks to the subject are well-tolerated, both individually and in combination, by subjects with ICM; and 3) determine if the target doses of 150 million MSCs, 5 million c-kit+ cells, or the combination of these delivered transendocardially produces improvement in measures of myocardial function and/or quality of life. The trial has been designed to address critical limitations in the previous published trials by including subjects with moderate to severe LV dysfunction, a group of subjects who are most likely to benefit from this form of therapy. The risks to the subjects are reasonable in relation to the knowledge gained from this study since this therapy may potentially reduce the incidence of ICM which is a leading cause of morbidity and mortality throughout the world.

9.1.7 Data and Safety Monitoring Board (DSMB)

The Data and Safety Monitoring Plan has been outlined in Section 7 above.

9.2 Clinical Monitoring

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

9.2.1 Pre-Investigation Visits

The DCC team assures that the Investigator clearly understands and accepts the obligations incurred in undertaking a clinical investigation.

Prior to the initiation of a clinical investigation, the DCC team will train the site of the clinical investigation to assure that the Investigator:

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

9.2.2 Interim site visits

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

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

9.2.3 Monitor Role

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

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

9.2.4 Monitor Recording

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

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

9.3 Investigator Responsibilities

9.3.1 Investigator Performance

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

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

9.3.2 Site Requirements:
Prior to enrollment of the first subject, the Investigator and institution will be asked to provide the following documents:

1. Executed study contract between NHLBI and the clinical center
2. IRB approved informed consent form
3. IRB approved final protocol
4. Current laboratory certification for all associated laboratories
5. Current laboratory normal ranges

9.3.3 Institutional Review Board Approval
Prior to enrolling the first subject, the Investigator must obtain written approval from the IRB. The approval must contain the date the study was approved, the version of the informed consent that was approved and the signature of the IRB chairperson. The primary investigator and their staff will follow all Good Clinical Practice (GCP) requirements.

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

9.3.5 Reporting Requirement of the Sites
See Investigator reporting responsibilities in Section 7 above.

9.4 Sponsor Responsibilities

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

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

9.4.3 Site Training
The DCC will be responsible for the setting up all training required in the protocol.

9.4.4 Reporting to the FDA
The DCC will hold the study IND and submit proper filings to obtain and maintain the IND. The DCC will submit all appropriate reports and fillings to the FDA as required by regulations. This includes unanticipated adverse events, withdrawal of IRB approval, and withdrawal of FDA approval, annual progress reports to the FDA and all final reports. The DCC will maintain all records according to Good Clinical Practice Guidelines (GCP).

All Clinical Centers (CCs) and Core Laboratories (Cores) will comply with 21 CFR, part 312.62 with regard to record retention.

9.5 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.

9.5.1 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 subject data. The data collected from CCs and Cores 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 subjects.

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

9.5.3 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.

9.5.4 Laboratory Data Processing Support

The DCC will develop and maintain online web forms for the CCs and Cores for data collection, both for baseline and annual follow-up. The data will be validated with extensive edit rules and the CCs/Cores will be able to correct errors real time. Access will be limited and will require se-

cure 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.

9.5.4.1 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.

9.5.5 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 subjects. 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 and automatic email notifications on the CC's data entry and completeness will be generated. If a CC has problems, action will be taken ranging from retraining through phone calls to a site visit, if necessary.

9.5.6 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.

9.5.7 Backup Procedure

The study data will be backed up on a nightly basis and the backup will be stored offsite at a University on-line storage facility that is secure and has restricted access.

9.6 Dissemination

Dissemination is the process by which the results of research efforts and their implications are promulgated to the target communities. The dissemination process begins early in the study.

Study Investigators will present regularly the design, progress, and results of the investigations at annual meetings at symposia and annual meetings such as the American Heart Association and the American College of Cardiology.

9.6.1 www.ClinicalTrials.gov

The DCC has a standard operating procedure (SOP) for the registration, posting, and uploading of trial information and results to www.ClinicalTrials.gov. Per 42 CFR part 11, the DCC serves as the responsible party for these responsibilities on behalf of the CONCERT-HF study. The trial is currently registered (NCT02501811). The informed consent document includes corresponding language with respect to inclusion of trial data in the registry.

9.6.2 NHLBI's Data Repository (BIOLINCC)

The DCC will also be the responsible party for preparing the CONCERT-HF dataset in accordance with NHLBI data repository requirements for uploading to BIOLINCC. This will include a limited data set to be used for other research purposes. Following submission of trial results to www.ClinicalTrials.gov, the DCC generates a "package" of trial data (per BIOLINCC protocol) and supporting materials for upload to the Data Repository.

9.6.3 Website

A website has been created with objectives targeted to the study audience. The CCTRN website serves 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 treatments for subjects. This information should be tied to the normal places practitioners seek such resources. For the researcher, the website provides more in-depth technical information and published works. For the CCTRN investigators, the website provides a central location for meeting information, clinical trial information, and other resources.

9.6.4 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 plays an active role in preparing study publications in collaboration with other study Investigators and the NHLBI Project Office. The DCC collaborates with CCTRN investigators to prepare all manuscripts for submission to the journals and will serve as the liaison between the lead author, and the journal. A Publications and Ancillary Studies Committee organizes and monitors writing committees and provides oversight on what presentations and publication have priority within the study. The DCC maintains and distributes progress reports on the status of all

active papers, as well as a study bibliography including abstracts, presentations, letters, editorials, etc.

9.6.5 Methodologic Developments

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

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Appendix A: Bone Marrow Aspiration Standard Operating Procedure

The following Standard Operating Procedure (SOP) is for carrying out bone marrow aspirations for subjects recruited in the Cardiovascular Cell Therapy Research Network (CCTRN) protocols.

CCTRN subjects will undergo bone marrow aspiration to harvest cells for the 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. physician assistants, 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 cell therapy for cardiovascular conditions.

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

1. Texas Heart Institute Stem Cell Center
2. Minneapolis Heart Institute Foundation
3. University of Florida Department of Medicine
4. Stanford University School of Medicine
5. University of Miami Miller School of Medicine
6. Indiana University School of Medicine
7. University of Louisville School of Medicine

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. The CCMF will be notified at the following time points: 1) when a subject is enrolled and the bone marrow aspiration/heart biopsy has been scheduled, 2) when the bone marrow aspiration/heart biopsy procedure has begun, 3) and when the bone marrow aspiration/heart biopsy samples have been shipped.
4. Bone marrow aspiration and heart biopsy specimen transportation to the cell processing laboratory will be treated as a STAT procedure.

Subject preparation and specimen collection performed by Physician:

1. Refer to Section 6.2.2 regarding anticoagulation management prior to the procedure.
2. Verify subject identification with the subject.
3. Explain the risks and benefits of bone marrow aspiration and anesthesia. Give subjects an opportunity to ask questions and verbalize understanding. Document the informed consent process by having the subject sign informed consent forms for bone marrow aspiration and

anesthesia.

4. Sedative and analgesic medication for the bone marrow aspiration procedure, including conscious sedation will be left to the discretion of the performing or supervising physician per institutional guidelines for procedures of this volume with the exception of general anesthesia which will not be paid for by the study.
5. Subjects on aspirin and/or Plavix (clopidogrel) at the time of consent should remain on aspirin and/or Plavix (clopidogrel) for the bone marrow aspiration procedure.
6. All collection procedures must be performed with universal precautions and sterile aseptic technique.

Bone marrow aspiration procedures:

1. The media containers and/or heparin vials must be opened with sterile technique and medium prepared with the appropriate amount of anticoagulant. The final concentration of heparin will be 100 units of heparin/mL of bone marrow.
2. Position the subject in a prone or partial prone position. Evaluate pressure points with special attention to avoid pressure on arms, brachial plexus, breasts, genitalia, knees, vascular structures or other body parts.
3. Verify location of posterior iliac crest.
4. Prep and drape the location in sterile manner using institutionally approved preoperative skin antiseptic (e.g., chlorhexidine gluconate (ChloraPrep ®), betadine, isopropyl alcohol, alcohol 60) and sterile draping. Allow for the antiseptic to dry before applying local anesthesia.
5. Begin induction sedation and analgesic medications (e.g. Propofol, Versed, Ativan, or morphine).
6. After evidence of induction effect, apply local anesthesia (e.g., lidocaine 1% or bupivacaine) to the skin above the posterior iliac crest.
7. With a longer needle (e.g., spinal needle) apply local anesthesia (e.g., lidocaine 1% or bupivacaine) to the periosteum of the posterior iliac crest region
8. Holding the bone marrow aspiration needle and stylet in place, puncture skin and advance through subcutaneous tissue, periosteum and into the marrow cavity using a steady, controlled pressure with a twisting motion. When the needle is firmly in the bone and slight give in pressure is felt, the cavity has been entered.
9. Remove the stylet and quickly attach the prepared syringe to the needle hub.
10. Apply a strong, quick suction and obtain 5-10 mL of bone marrow.
11. Rotate the aspiration needle by 60 to 90 degrees, and aspirate 5-10 mL of bone marrow**.**
12. Repeat the rotation a total of two to six times per puncture, totaling 20 – 30 mL of bone marrow aspirate per puncture.
13. Re-insert the stylet and remove the needle from the bone with a light twisting motion.
14. While keeping the aspiration needle in the subcutaneous tissue, reposition the aspiration needle in an adjacent site of the posterior iliac crest.
15. The target aspiration volume is approximately 90 mL (± 10 mL). Therefore a total of 10-20 aspirations (5-10 mL) will be made. Since 2-6 aspirations are made in each puncture, then a total of 2-5 punctures will be required. Typically this is performed with only 1 to 2 skin punctures.
16. Physicians will perform the aspiration on one side. The only time aspiration will take place in the contralateral site is if the initial site produces a dry tap.
17. In the event that no marrow is aspirable, then pressure will be applied to the injection site until hemostasis is achieved.

18. A sterile dressing will be applied to all puncture sites. A sterile pressure dressing (e.g., Elastoplast) will be applied if persistent venous oozing is present.
19. Rotate the subject into a supine position and maintain that position for a minimum of 30 minutes.
20. The dressing over the puncture site should be checked after the 30 minutes of supine positioning to ensure no hemorrhage. The dressing may be removed 24 hours after the procedure and the subject should observe for signs of infection, bleeding or any other drainage. The subject should notify the study coordinator if evidence for these signs. It is usual for the subject to observe bruising and feel aching for several days after the procedure. This may be relieved with a warm pack. The subject should notify the study coordinator if the pain persists beyond several days or worsening pain.
21. Documentation of the procedure should be made by the Physician.
22. 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 Injection form and all specimens with the subject 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 local CPL to the CCMF and back.

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Appendix B: Standard Operating Procedure for Right Heart Catheterization with and without Endomyocardial Biopsy (RHC/EMB)

The following Standard Operating Procedure (SOP) is for carrying out right heart catheterization (RHC) with and without endomyocardial biopsies (EMB) for subjects recruited in CONCERT-HF.

Purpose:
RHC with right ventricle EMB (for subjects randomized to Combo or c-kit+ cell only arms) or RHC without EMB (for subjects randomized to placebo or MSC only arms) are scheduled procedures performed by a trained Physician (e.g. cardiologist, heart failure specialist, or interventionalist). Only physicians with specific training and substantial experience in carrying out RHCs with right ventricular EMBs (more than 100 successful procedures) will perform the procedure. Other medical personnel trained in EMB procedures (e.g. cath lab technologists, registered nurses, and medical technologists) will assist in the collection to ensure proper sample collection, preparation and processing of the specimen. The RHC with right ventricle EMB is indicated for research regarding cell therapy for cardiovascular conditions. RHC with EMB is also an indicated medical procedure for patients with heart failure and heart transplants. The RHC without EMB is a sham procedure (see Manual of Operations for details of sham procedure including example of sham EMB script) for subjects randomized to placebo or MSC only arms.

Scope:
This SOP refers to RHCs with and without right ventricle EMB collections at the seven CCTRN cell therapy centers and their associated satellite facilities. The seven centers are as follows:

1. Texas Heart Institute Stem Cell Center
2. Minneapolis Heart Institute Foundation
3. University of Florida Department of Medicine
4. Stanford University School of Medicine
5. University of Miami Miller School of Medicine
6. Indiana University School of Medicine
7. University of Louisville School of Medicine

PROCEDURE

Preparation of all subjects prior to RHC/EMB procedure:

1. Refer to Section 6.2.2 regarding anticoagulation management prior to the procedure. **Subjects' INR must be <1.6 on the day of procedure to proceed with the procedure.**
2. **All subjects will have two 2-D echocardiograms**: 1) a pre-RHC/EMB procedure echo to assess for pre-existing pericardial effusion, and 2) a post-RHC/EMB procedure echo within 6 hours to ensure no post-procedure effusion (even if subject is stable) (see section 6.3.9).
3. Verify subject identification with the subject.
4. Explain the risks and benefits of RHC and right ventricle EMB. Give subjects an opportunity to ask questions and verbalize understanding. Document the informed consent process by having the subject sign informed consent forms for RHC and right ventricle EMB.
5. Subjects on aspirin and/or Plavix (clopidogrel) at the time of consent should remain on aspirin and/or Plavix (clopidogrel) for the RHC and right ventricle EMB procedure.

6. **Pressures collected during the procedure should be measured at end-expiration at end diastole**.
7. The CCMF will be notified when 1) a subject is randomized and 2) the RHC/EMB has been scheduled (automated emails sent from database upon form entry).

For subjects having RHC with EMB (randomized to Combo or c-kit+ cells only arms):

Additional guidances for physician consideration:

1. Review of previous available imaging (e.g. screening MRI) pre-procedure to assess the cardiac anatomy prior to initiating the EMB procedure.
2. Use of biplane imaging or echocardiography to assist with identifying location of bioptome on the septum.
3. Have additional operators present for the procedure to provide ongoing independent assessment.
4. Patients with left bundle branch block and neither a pacer nor an ICD may have a temporary pacer at the discretion of the attending physician.

Supplies and transportation for EMB tissue:

1. Right ventricle EMB supplies will comply with the site-specific institutional procedures and practices, but will adhere to certain common principles across the sites.
2. All equipment, supplies, and reagents used in the process of EMB must be sterile with a lot number and date of expiration noted and able to be recorded on site-specific institutional data forms.

3. The CCMF will be notified by the local team when 1) the heart biopsy procedure has begun and 2) when the heart biopsy samples have been shipped.
4. Heart biopsy specimen transportation to the cell processing laboratory will be treated as a STAT procedure.

Specimen collection:

1. All collection procedures must be performed with universal precautions and sterile aseptic technique.
2. Prep and drape the sample collection table.
3. Place the EMB media container on the sample collection table.
4. Prep and drape right neck of patient for jugular access or right upper thigh for femoral access.
5. Position monitors either front facing at foot of table or side facing depending on access route.
6. Single-use bioptomes are to be used; reusable bioptomes are not permitted.

   Supplies:
   - Biopsy Forceps (Argon Jawz™, Cordis, or St. Judes)-*physician's discretion*.

- Size: 5-7F Maxi-Curve (*dependent upon access route utilized-see below*)
- Swan Ganz thermodilution catheter-*physician's discretion per institutional guidelines*
- Micropuncture kit

For subjects having RHC without EMB (randomized to placebo or MSCs only arms):
Script:

1. Cath lab personnel should follow a script (see Manual of Operations for example) that mimics what a subject would hear and feel if they were experiencing an EMB procedure.

ACCESS ROUTES:

Jugular:
The 50 cm disposable Radiopaque Endomyocardial Biopsy Forceps are designed for Right ventricular biopsies using the Jugular Approach.

Femoral:
The 105 cm disposable radiopaque endomyocardial biopsy forceps are designed for Right or Left Ventricular biopsies using the femoral approach.

CIRCUMSTANCES THAT WOULD HALT OR TERMINATE THE RHC/EMB PROCEDURE:

If any of the following occur before or during RHC/EMB, they could indicate a serious clinical deterioration.

- If any of these conditions/events occur, the procedure should be temporarily halted and the subject should be reevaluated for suitability to continue with the procedure:
  - the baseline pulmonary artery (PA) systolic pressure taken immediately prior to biopsy procedure is 50-59 mmHg;
  - the baseline right ventricle (RV) systolic pressure taken immediately prior to biopsy procedure is 50-59 mmHg;
  - the baseline wedge pressure taken immediately prior to biopsy procedure is 30-34 mmHg; (note: if wedge pressure is >30 mmHg, collect an O2 sat measure);
  - the systolic blood pressure (SBP) taken the day of harvest is <80 mmHg and constitutes a significant change from baseline, e.g., SBP change from ≥ 100 mmHg (at baseline) to <80 mmHg (at harvest);
  - the heart rate (HR) taken the day of harvest is >100 and constitutes a significant change from baseline, e.g., HR change from ≤ 80 (at baseline) to >100 (at harvest).

- If any of these conditions/events occur, the procedure should be terminated (subject can be evaluated for rescheduling if condition/event resolves)*:
  - change in NYHA Class to Class IV;
  - the baseline PA systolic pressure is ≥ 60 mmHg;
  - the baseline RV systolic pressure is ≥ 60 mmHg;
  - the baseline wedge pressure is ≥ 35 mmHg.

*If a second procedure is undertaken and the conditions again meet the termination threshold, provided the subject is stable, a sham procedure will be performed to maintain subject and staff blinding.

METHOD:

RHC: Upon arrival at the catheterization laboratory, the subject will be placed on the catheterization table in supine orientation and draped in the usual fashion. Percutaneous intravenous access, utilizing the internal jugular or femoral vein, will then be established using sterile technique. After insertion of an appropriately sized intravenous sheath, baseline intracardiac hemodynamic measures, including: right atrial, right ventricular, pulmonary artery, and pulmonary capillary wedge pressures will be recorded. Both phasic and mean pressures will be obtained. The type of catheter used to monitor heart function and blood flow (e.g., Swan-Ganz catheter) will be left to the discretion of the performing physician per institutional guidelines. The RHC without EMB is a sham procedure (see Manual of Operations for details of sham procedure including example of sham EMB script) for subjects randomized to placebo or MSC only arms.
EMB: Following removal of the pressure transducer, a bioptome will be inserted and positioned adjacent to the distal interventricular septum. The catheter will be positioned with the jaws of the bioptome closed. Positioning of the bioptome will be confirmed visually by fluoroscopy (this can be supplemented with ultrasound 2-D echo), along with evidence of ventricular ectopy. After confirmation of correct positioning of bioptome, up to 6 EMB samples (a.k.a. “bites”) of heart tissue will be harvested. **Confirm the position of the long sheath introducer or guiding catheter in the ventricle prior to each and after each specimen collection.**

The jaws of the Biopsy Forcep should be opened only after the forcep has emerged from the distal end of the long sheath introducer or guiding catheter, and correct placement against the interventricular septum has been determined. Following correct positioning the bioptome is withdrawn approximately 1 cm away from the septum, the jaw opened under fluoroscopic visualization and gently advanced against the septum.

The open jaws of the biopsy forcep should be positioned at the heart wall, closed firmly and sufficient pressure should be maintained on the handle to assure retention of the specimen during withdrawal through the sheath introducer or guiding catheter.

This procedure is repeated up to 6 times until adequate tissue is obtained for the cell expansion procedure. The bioptome is rinsed in heparinized saline prior to each advancement into the sheath for a repeat biopsy.

Following tissue acquisition, the aforementioned intracardiac hemodynamic measures will be repeated to ensure the absence of immediate complications related to the EMB procedure. If no complications occur as a result of the procedure, the sheath will be removed and the percutaneous access site will be bandaged using standard of care measures.

At the conclusion of the procedure the sheath is withdrawn, gentle pressure is applied to achieve hemostasis.

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Appendix C: NYHA Classification

New York Heart Association (NYHA) Classification

Class	Participant Symptoms
Class I (None)	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.

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Appendix D: CCS Classification

Canadian Cardiovascular Society (CCS) Functional Classification of Angina Pectoris

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

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Appendix E: Stage 1- (Open label lead-in) Enrollment and Activities

I. Recruitment and Screening

Recruitment and screening of subjects in Stage1 will follow the same chart review and imaging evaluation as outlined in Section 4.1.

II. Consenting

A separate consent form will be utilized for Stage 1. Please note there is no optional biorepository for the the Stage 1 subjects. The Stage 1 consent form will describe the timeline and activities for the SOC control group versus the Combo therapy group.

III. Activities and Follow Up

a. Baseline Testing

The baseline testing period will not exceed 60 days (from the date the ICF is signed until randomization). See below for activities during baseline.

b. Randomization and Treatment

Prior to randomization, eligibility criteria will be reviewed. If a change in the subject's status has occurred such that the subject no longer meets all of the eligibility criteria, randomization will be postponed, or if the condition is not resolvable, the individual will be excluded from participation. Following successful completion of baseline testing, clinical sites will enter results of the testing in the web-based program (Section 8.1.1). Subjects will be randomized either to 1) SOC (no study procedures) or 2) Combo (MSCs plus c-kit+ cells). Combo subjects will undergo harvest procedures per Sections 5.1 and 5.2 (*Note: As there is no biorepository for stage 1 subjects, all 90ml of bone marrow will be forwarded to CCMF for cell manufacturing*). Cell manufacturing will follow and is outlined in relevant subsections of 5.8. Study product injection in subjects receiving Combo product is outlined in Sections 5.13 and 5.14.

c. Visits Schedule and Evaluations

Subjects in the SOC group will attend 5-7 total visits (Baseline, Day 0, Week 1, Month 1, and Month 3. Subjects in the Combo group will attend 10-11 total visits (Baseline, harvest, MRI visit, Day 0, Day 1, Week 1, Month 1, Month 3, Month 6, and Month 12). For both groups, baseline testing (and 3 month visit) can be split over multiple days as needed.

Please refer to the corresponding protocol sections for details related to the evaluations described in Table 21a below:

- Comprehensive medical and surgical history, vital signs and physical examinations (Section 6.3.1)
- Current use of prescription and OTC medications (Section 6.3.1)
- Blood tests (Table 22a below)
- Infectious disease panel (Table 22a below)
- Pregnancy test (for women of childbearing potential) (Table 22a below)
- cMRI imaging (Section 6.3.3, MRI Core Lab Manual of Operations) and ICD interrogation (Section 6.3.8) (if applicable)
- Six-minute walk test (Section 6.3.4, Protocol Manual of Operations)
- Minnesota Living with Heart Failure Questionnaire
- 12 lead ECG (Section 6.3.6)
- Treadmill-based VO_2 max (Section 6.3.7, CPET Core Manual of Operations)

Stage 1: Open label lead-in (Table 21a)

Stage 1 (open label)	SOC Group					Combo Group									
Procedure	Baseline	D0*	W1	M1	M3	Baseline	Harvest	MRI Visit[5]	D0 (SPI)*	D1	W1	M1	M3	M6	M12
Informed Consent	X					X									
Complete Medical History	X					X									
Physical Exam	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X		X_1	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Con Medications	X	X	X	X	X	X	X		X	X	X	X	X	X	X
NYHA and CCS	X	X		X	X	X			X			X	X	X	X
MLHFQ	X				X	X							X		
12-lead ECG	X	X	X		X	X			X_2	X_2	X		X	X	X
2D Echoes									X_2						
Telemetry									X_3						
Laboratory Testing[4]	X	X	X	X	X	X	X	X	X		X	X	X	X	X
Cardiac MRI	X	X			X	X		X					X		
ICD Interrogation[6]	X	X			X	X		X	X				X		
Treadmill (VO2 max)	X				X	X							X		
6 Minute Walk	X				X	X							X		
Randomization	X					X									
Bone Marrow Aspiraton							X								
Heart Biopsy							X								
Catheterization/NOGA									X						
Temp Log										X_7					

* Visit is scheduled approximately 10 weeks after completion of baseline testing, randomization, (and completion of harvest procedures in the Combo group).

1. Subjects will have assessments of vitals (BP, temperature, pulse rate) immediately pre- and post-procedure.
2. For the Combo group-ECGs will be performed within 6 hours following the SPI catheterization procedure and again before discharge; a 2-D echocardiogram will be performed post SPI procedure (within 6 hours).
3. Subjects will be monitored on simple telemetry up to 24 hours post-procedure or until discharge, whichever is sooner.
4. See Table 22a for specific tests done at each time point.
5. A cardiac MRI will be performed within 30 days prior to SPI (baseline measure).
6. ICD interrogation: (if applicable) done before and after every MRI as part of MRI protocol, as well as before the SPI procedure (in combo group). Reports will be generated for the interrogations conducted before the MRIs at baseline (image collected within 30 days of SPI) and at the 3 month visit. Local electrophysiology personnel will review the device report for the presence of reportable clinical events. Copies of the reports should remain on site as source documentation (de-identified copies may be requested by the Sponsor for endpoint adjudication).
7. Temperature log 2x/day x 7 days.

Stage 1 Laboratory Testing (Table 22a)

CONCERT-HF Laboratory Testing	BSL	Harvest	MRI visit	Day 0 (SPI)	Day 1_9	Wk 1	M 1	M 3	M 6_{10}	M 12_{10}
Chemistry $Tests_1$	X			X	X	X	X	X	X	X
CBC with $Differential_2$	X		X_{11}				X	X	X	X
Liver Function $Tests_3$	X							X	X	X
Pregnancy (childbearing women)	X			X_4			X	X	X	X
NT-$proBNP_5$	X							X	X	X
Troponin I or T	X			X_6	X	X				
HbA1c	X							X	X	X
PT, INR, PTT_7	X	X_{12}		X_{12}						
Infectious Disease $Tests_8$	X									

1. Chemistry Tests - sodium, potassium, chloride, bicarbonate (CO2), glucose, blood urea nitrogen (BUN), creatinine, and eGFR.
2. Complete Blood Count with Differential - CBC: WBC, RBC, hemoglobin, hematocrit, MCV, and platelets; Diff: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.
3. Liver Function Tests - albumin, alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, direct bilirubin, and total protein.
4. Will be completed within 36 hours prior to injection.
5. NT-proBNP required; send to outside lab if applicable.
6. Will be performed once in the morning (both groups) and once 8 (+/- 2) hours post cardiac catheterization/NOGA (Combo only).
7. Later time points if indicated.
8. Infectious disease tests should be the bone marrow donor panel used per local institutional guidelines, including HIV, Hep B (HBsAG, Anti-HBs, Anti-HBc), and Hep C (Anti-HCV), and results must be known prior to harvest. Donor tests are conducted within 30 days of harvest procedures. If for some reason these tests expire prior to either harvest, they will be performed again.
9. Day 1 labs will be collected 24 hours post-procedure or immediately prior to discharge, whichever is sooner. These labs are only collected on the Combo group.
10. Month 6 and 12 labs are only collected on the Combo group.
11. For either group (Combo or SOC), CBC drawn on day of MRI with hematocrit recorded on the MRI worksheet.
12. For subjects receiving systemic anticoagulation therapy, an INR measurement will be performed on the morning of the planned procedure; must be <1.6 to proceed with procedure.

IV. Event Monitoring and Reporting

Sites will use the same process for subjects in Stage 1 as outlined in Section 7.

V. DSMB Data Review

Following successful three month data evaluation by the DSMB, those subjects randomized to Combo therapy will continue to be followed per protocol for 12 months. Those randomized to the SOC control group will have the option to be evaluated for enrollment in the trial conducted in Stage 2. Interested subjects will be consented under the trial consent form outlining all study related procedures, all possible risks, the time commitment, and the potential for randomization to a placebo group.