Official Study Title: A Phase I, First-in-Human, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Study of the Safety and Efficacy of Allogeneic Mesenchymal Stem Cells in Cancer Survivors with Anthracycline-Induced Cardiomyopathy
Short Title: Stem Cell Injection in Cancer Survivors (SENECA)
NCT: NCT02509156
Date of Final Study Protocol: IRB approval date 10/19/2018

Organization: CARDIOVASCULAR CELL THERAPY RESEARCH NETWORK (CCTRN)

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

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

Information Type: ☐ Initial Protocol ■ Protocol Amendment

Full Title: A Phase I, First-in-Human, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Study of the Safety and Efficacy of Allogeneic Mesenchymal Stem Cells in Cancer Survivors with Anthracycline-Induced Cardiomyopathy

Short Title: ***S****t****E****m cell i****N****j****E****ction in* ***CA****ncer survivors (SENECA)*

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SENECA 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 I, First-in Human, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Study of the Safety and Efficacy of Allogeneic Mesenchymal Stem Cells in Cancer Survivors with Anthracycline-Induced Cardiomyopathy”. 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." (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 Definitions of Terms

6MWT	Six Minute Walk Test
ACE	Angiotensin-Converting Enzyme inhibitor
AE	Adverse Event
AIC	Anthracycline-Induced Cardiomyopathy
allo-MSC	allogeneic human Mesenchymal Stem Cells
ARB	Angiotensin Receptor Blocker
BMC	Bone Marrow Cell
CAD	Coronary Artery Disease
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CMP	Comprehensive Metabolic Panel
DCM	Dilated Cardiomyopathy
DSMB	Data and Safety Monitoring Board
EF	Ejection Fraction
EPC	Endothelial Progenitor Cells
ESR	Expedited Safety Report
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GVHD	Graft Versus Host Disease
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSC	Hematopoietic Stem Cells
HTLV	Human T-cell Lymphotropic Virus
ICAM	Intracellular Adhesion Molecule
ICD	Implantable Cardioverter Defibrillator
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements of Pharmaceuticals for Human Use
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
LAD	Left Anterior Descending
LFT	Liver Function Test
LV	Left Ventricle, Left Ventricular
LVEDV	Left Ventricular End Diastolic Volume

LVEF	Left Ventricular Ejection Fraction
LVESV	Left Ventricular End Systolic Volume
MACE	Major Adverse Cardiac Events
MHC	Major Histocompatibility Complex
MI	Myocardial Infarction
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
NAT	Nucleic-Acid-based Test
NMDP	National Marrow Donor Program
NT-proBNP	N-Terminal pro-Brain Natriuretic Peptide
NYHA	New York Heart Association
PCI	Percutaneous Coronary Intervention
PTT	Partial ThromboplastinTime
SAE	Serious Adverse Event
SCA-1	Stem Cell Factor Antigen
SCF	Stem Cell Factor
SDF-1	Stromal-Cell-Derived Factor 1
SENECA	StEm cell iNjEction in CAncer survivors
SPI	Study Product Injection
URL	Upper Reference Limit
VEGF	Vascular Endothelium Growth Factor
VEGFR2	Vascular Endothelial Growth Factor Receptor-2
WBC	White Blood Cell
WNV	West Nile Virus

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EXECUTIVE SUMMARY

Study Centers: Centers of the NHLBI CCTRN	**Phase of Development:** Phase I
Study Therapy: Transendocardial delivery of allogeneic human mesenchymal stem cells (allo-MSCs)	
Study Title: A Phase I, First-in-Human, Multicenter, Randomized, Double-Blinded, Placebo Controlled Study of the Safety and Efficacy of Allogeneic Human Mesenchymal Stem Cells in Cancer Survivors with Anthracycline-Induced Cardiomyopathy, a.k.a. ***S****t****E****m cell i****N****j****E****ction in ****CA****ncer survivors (SENECA)	
Objectives: To demonstrate safety and feasibility of allo-MSCs administered by transendocardial injection in subjects with anthracycline-induced cardiomyopathy (AIC).	
Subject Population: Subjects with AIC and who are eligible to undergo cardiac catheterization/NOGA.	

Design and Investigational Plan: Approximately thirty-six (36) subjects (including six actively treated lead-in participants and up to thirty-one randomized clinical trial participants) will be enrolled. The six subjects participating in the open-label lead-in will receive allo-MSC as described below in Group A. The remaining approximately subjects will be randomized 1:1 to one of two treatment strategies:

- Group A (15 subjects): 100 x 10^6 (100 million) allo-MSCs delivered in twenty 0.4 ml injections.
- Group B (15 subjects): 20 injections of 0.4 ml cell-free Buminate solution.

- For patients randomized to Group A, the allo-MSCs will be derived from allogeneic bone marrow isolated from normal, healthy donors by standard iliac crest aspiration.
- Cells or placebo will be administered via the NOGA Myostar injection system.
- The injections will be administered transendocardially during cardiac catheterization using the NOGA Myostar Injection System. Injections will be targeted throughout the left ventricle based on NOGA mapping and assessment of scar / fibrosis.
- All consented and eligible subjects will have study product injection within 45 days of signing informed consent.
- Following cardiac catheterization and allo-MSC or placebo injections, subjects will be followed at day 1, week 1, and months 1, 6, and 12 to complete all safety and efficacy assessments.

Eligibility Criteria:

Inclusion Criteria

To participate, a subject MUST:

1. Be ≥ 18 and < 80 years of age
2. Be a cancer survivor with diagnosis of AIC
3. Have an LVEF ≤ 45% by cMRI
4. Be in NYHA class II-III
5. Have received the initial diagnosis of AIC at least six months earlier and be on stable, optimally-tolerated therapy with beta-blockers, ACE inhibitors/ARBs, and/or aldosterone antagonists for 3 months, unless contraindicated
6. Have a period of at least two years of clinical cancer-free state* and low likelihood of recurrence (a five-year risk of recurrence estimated at 30% or less), as determined by an oncologist, based on tumor type, response to therapy, and negative metastatic work-up at the time of diagnosis
   exceptions to this are carcinoma in situ or fully resected basal and squamous cell cancer of the skin.
7. Be a candidate for cardiac catheterization

Exclusion Criteria

To participate, a subject MUST NOT HAVE:

1. A life expectancy <12 months
2. A CT scan or baseline cardiac MRI showing new tumor or suspicious lymphadenopathy raising concern of malignancy
3. Presence of obstructive CAD as determined via imaging within 5 years prior to study enrollment provided there have been no symptoms or evidence of CAD since the test *(see section 4.1 for*

imaging guidance))

4. Had a previous myocardial infarction
5. A history of radiation therapy AND evidence of constrictive physiology and/or evidence of other patterns of non-ischemic cardiomyopathy on cardiac MRI (e.g., amyloidosis, sarcoidosis, hemochromatosis, pure radiation-induced cardiomyopathy, etc.) not consistent with AIC being the dominant etiology of heart failure
6. Valvular heart disease including 1) mechanical or bioprosthetic heart valve; or 2) severe valvular (any valve) insufficiency/regurgitation within 12 months of consent
7. Aortic stenosis with valve area ≤ 1.5cm^2
8. A history of LV reduction surgery or cardiomyoplasty
9. Evidence of cardiogenic shock
10. A history of ischemic or hemorrhagic stroke within 90 days of baseline testing
11. Liver dysfunction during baseline testing, as evidenced by enzymes (e.g., AST, ALT, alkaline phosphatase) greater than 3 times upper limit of normal
12. Diabetes with poorly controlled blood glucose levels (HbA1c > 8.5%)
13. An underlying autoimmune disorder or current immunosuppressive therapy (e.g., chronic corticosteroid, rheumatologic or immune modulating therapy) or likelihood of use of immunosuppressive therapy during participation in the trial *(medications will be considered on a case by case basis)*
14. A baseline eGFR <35 ml/min/1.73m^2
15. A contrast allergy that cannot adequately be managed by premedication
16. Received gene or cell-based therapy from any source within the previous 12 months
17. A hematologic abnormality during baseline testing as evidenced by hemoglobin < 9 g/dl; hematocrit < 30%; absolute neutrophil count < 2,000 or total WBC count more than 2 times upper limit of normal; or platelet values < 100,000/ul
18. Evidence of active systemic infection at time of study product delivery
19. HIV and/or active HBV or HCV
20. Coagulopathy (INR > 1.5) not due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors) (see Section 6.4 re: injection procedure and anticoagulation therapy) *Note: Subjects who cannot be withdrawn from anticoagulation will be excluded.*
21. Presence of LV thrombus *(see guidance in Section 6.6.3)*
22. 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
23. Pacemaker-dependence with an ICD (*Note: pacemaker-dependent candidates without an ICD are not excluded)*
24. A cardiac resynchronization therapy (CRT) device implanted < 3 months prior to consent
25. Other MRI contraindications (e.g. patient body habitus incompatible with MRI)
26. An appropriate ICD firing or anti-tachycardia pacing (ATP) for ventricular fibrillation or ventricular tachycardia within 30 days of consent
27. 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
28. A 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
29. Cognitive or language barriers that prohibit obtaining informed consent or any study elements (interpreter permitted)
30. Participation (currently or within the previous 30 days) in a cardiac related investigational therapeutic (including stem cell based therapies) or device trial
31. Pregnancy, lactation, plans to become pregnant in the next 12 months, or is unwilling to use acceptable forms of birth control during study participation
32. Any other condition that, in the judgment of the Investigator or Sponsor, would be a contraindication to enrollment, study product administration, or follow-up

Primary Endpoints

Safety: To demonstrate the relative safety of allo-MSCs 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:

1. Major adverse cardiac events (MACE) including: death, hospitalization for worsening heart failure (HF), and/or other exacerbation of HF (non-hospitalization)
2. Other significant clinical events including: non-fatal stroke, non-fatal myocardial infarction, coronary artery revascularization, ventricular tachycardia/fibrillation, pericardial tamponade, infectious myocarditis, hypersensitivity reaction, neoplasm, and/or any other potential deleterious late effects detected and corroborated by clinical presentation, laboratory investigations, image analysis, and when necessary with biopsy from suspected target sites in the body
3. All adverse events (AEs) at least grade 2 in severity

Feasibility: To assess the practicality of study procedures, the following measures will be reported. The number and percent of subjects who:

1. Have events between randomization and SPI that preclude the subjects from getting SPI
2. Receive less than 20 injections during the SPI procedure
3. Do not receive the intended dose of cells (100 million), reason, and actual dose delivered
4. Have 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
5. Fail to complete follow-up

Secondary Endpoints

Prospectively Declared Efficacy Endpoint Measures:

To explore whether allo-MSCs produce a trend toward improved LV function and functional status when compared with placebo, the following domains and measures will be evaluated:

- 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 area of injury (e.g., inflammation, edema, fibrosis)
- Functional capacity over time:
  - Change in exercise tolerance (6MWT)
  - Change in MLHF Questionnaire (subject reported)
- Clinical outcomes over time:
  - MACE
  - Cumulative days alive and out of hospital
- 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, 6, and 12 (including assessment for cancer recurrence).

1.0 STUDY OBJECTIVES

1.1 Primary Objective

The primary purpose of this study is to examine the safety and feasibility of delivering allogeneic human mesenchymal stem cells (allo-MSCs) by transendocardial injection to cancer survivors with left ventricular (LV) dysfunction secondary to anthracycline-induced cardiomyopathy (AIC).

1.2 Secondary Objective

The secondary purpose of this study is to obtain preliminary evidence for therapeutic efficacy of allo-MSCs delivered by transendocardial injection to cancer survivors with LV dysfunction secondary to AIC.

1.3 Hypotheses

Ho 1: Allo-MSCs delivered transendocardially in subjects with LV dysfunction, secondary to AIC, are well-tolerated.

Ho 2: Allo-MSCs can be delivered to subjects with LV dysfunction, secondary to AIC, and the outcome measures can be collected.

Ho 3: Compared with placebo, allo-MSCs promote myocardial repair, thereby producing a trend toward reduced scar burden and improved cardiac performance, functional capacity, and quality of life from baseline to six months and/or from baseline to 12 months post treatment with study product.

1.4 Relevance to the CCTRN

This study is consistent with the goal of the Cardiovascular Cell Therapy Research Network (CCTRN), which is to investigate the safety and effectiveness of new cell therapies in cardiovascular disease. It is also consistent with the scope of the CCTRN, which is to accelerate research in 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 stem cells in cancer survivors with AIC. AIC is a growing problem that is associated with a grim prognosis and disproportionately affects women and young adults. Currently, no effective treatment exists for this lethal syndrome.

2.0 BACKGROUND

2.1 Population

Anthracycline-based chemotherapies remain common and effective treatments for breast cancer, lymphomas, leukemias, and sarcomas. Unfortunately, the use of anthracyclines is limited by their cardiotoxic effects, which may manifest as late as 20 years from initial exposure[1]. Studies in cells and animals suggest that the mechanism of anthracycline-induced cardiomyopathy (AIC) is multifactorial[2]. Anthracyclines induce multiple forms of cellular injury by free radical production[2]. In addition, anthracyclines alter nucleic acid biology by intercalation into DNA and modulate intracellular signaling, leading to cell death and the disruption of homeostatic

processes such as sarcomere maintenance[2]. In an effort to decrease AIC, many strategies have been tested, but no specific therapies are universally acknowledged to prevent or treat anthracycline-induced cardiac dysfunction. Moreover, cardioprotective agents available in the market such as dexrazoxone and liposomal delivery systems of doxorubicin have shown disappointing results, diminishing tumor response or causing debilitating hand-foot-and-mouth disease[3]. Current therapies are palliative, and AIC remains an incurable and often fatal disease for which no effective treatment is available.

The broad use of anthracyclines for over 50 years has dramatically improved cancer survival statistics. For example, prior to the 1960s, children with leukemia had an overall 5-year survival of 30%. With the increased use of anthracycline-based chemotherapy and early detection, 5-year survival in children is now 80%[4]. Unfortunately, though mortality rates decrease with anthracyclines, life-altering cardiac sequelae from anthracyclines remain a problem, with a range of 5–23% of patients developing late-onset heart failure secondary to anthracycline-induced cardiotoxicity[5,6].

The risk of AIC is primarily related to the lifetime cumulative dose of anthracyclines. At a cumulative dose of 400 mg/m^2, there is a 5% risk of developing heart failure, which increases to 25% at 700 mg/m^2 [7]. An empiric cutoff of 500 mg/m^2 has been suggested to minimize the cardiotoxic effects of doxorubicin[7]. If patients have other predisposing risk factors for AIC such as age at two extremes (older age >65 years, and young age < 18 years), prior cardiac pathology (hypertension, LV hypertrophy, coronary artery disease), diabetes, or prior radiation exposure, the recommended total cumulative exposure is decreased to 450 mg/m^2. Even at low cumulative doses, however, such as standard-dose therapy for breast cancer, 5% of patients will have evidence of AIC[8]. **Thus, every exposure to an anthracycline carries some risk of inducing cardiac dysfunction.**

While ongoing trials suggest that the addition of Angiotensin-Converting Enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB), beta-blockers, and statins may help treat AIC, there continues to be a subset of patients that will develop progressive symptoms and end-stage heart failure despite optimal medical therapy[6,9-11]. Despite the use of optimal medical therapy for heart failure, AIC is often irreversible and carries a grim prognosis, to the point that an increasing number of AIC patients have required heart transplantation over the last 13 years (Figure 1, see below)[12]. **The prognosis for AIC remains worse than that of ischemic or other forms of idiopathic cardiomyopathy, with a 3.5 higher relative risk of dying within five years**[13]. Many patients with AIC are relative young, and approximately 70% of them are women[14].

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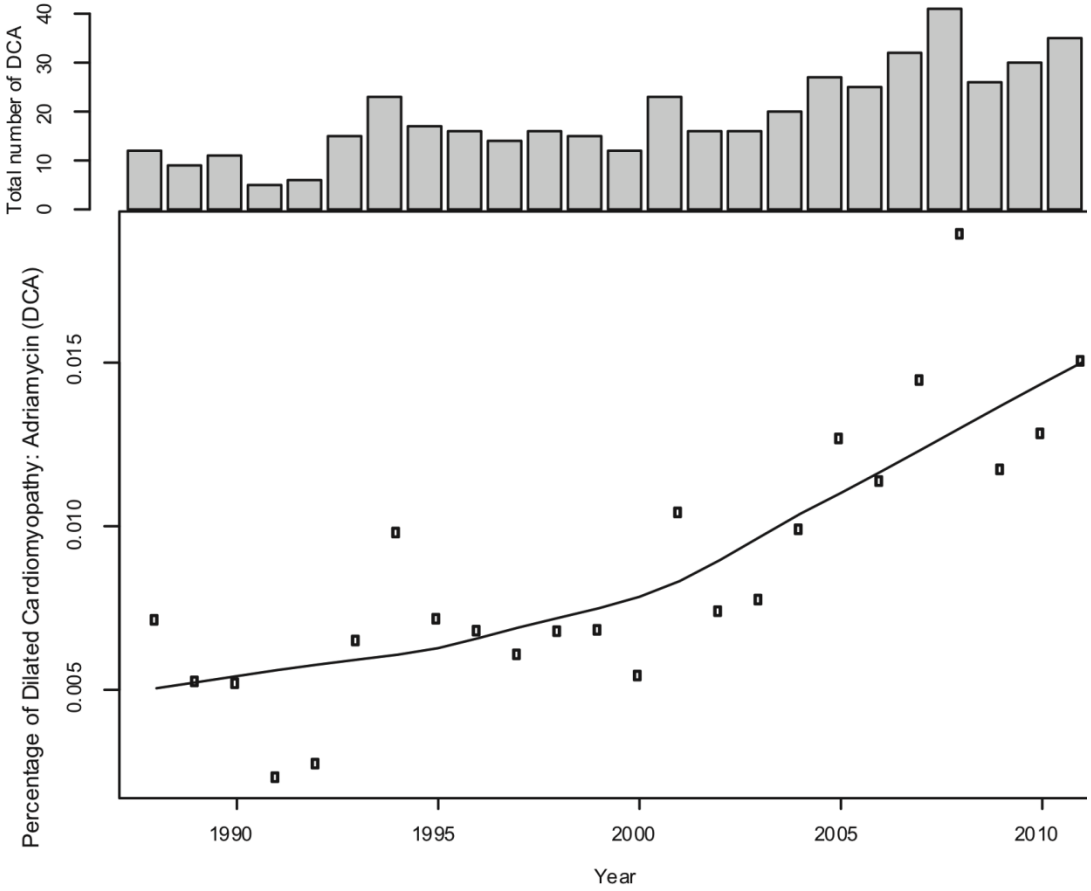

Figure 1. Statistically significant increase in percentage of orthotopic heart transplantations annually from 1987 to 2011 for the diagnosis of DCA ($p < 0.001$)[12]

2.2 Study Rationale

The technique of transplanting progenitor cells into a region of damaged myocardium, termed cellular cardiomyoplasty[15], is a potential, new therapeutic modality designed to replace or repair necrotic, scarred, or dysfunctional myocardium. Ideally, graft cells should be readily available, easy to culture to ensure adequate quantities for transplantation, and able to survive in host myocardium[16-18], often a hostile environment of limited blood supply and immune rejection. Whether effective cellular regenerative strategies require that administered cells differentiate into adult cardiomyocytes and couple electromechanically with the surrounding myocardium is increasingly controversial and recent evidence suggests that this may not be required for effective cardiac repair. Most 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[19], embryonic stem cell-derived myocytes[20,21], tissue-engineered contractile grafts[22], skeletal myoblasts[23], several cell types derived from adult bone marrow[24-29], and cardiac precursors residing within the heart itself[30]. There has been substantial clinical development in the use of whole bone marrow, select bone marrow cells, skeletal myoblasts, adipose derived cells, and cardiac stem cells in studies enrolling both post-infarction patients and patients with chronic

ischemic left ventricular dysfunction and heart failure. The effects of allogeneic bone marrow derived mesenchymal stem cells (MSCs) have also been studied clinically.

Currently, bone marrow or bone marrow-derived cells represent a promising modality for cardiac repair. The totality of evidence from trials investigating autologous whole bone marrow infusions into patients following myocardial infarction supports the safety of this approach[31]. In terms of efficacy, increases in LVEF are reported in meta-analyses of the randomized controlled trials[31]. Chronic ischemic left ventricular dysfunction resulting from heart disease is a common and problematic condition; definitive therapy in the form of heart transplantation is available to only a tiny minority of eligible patients. Cellular cardiomyoplasty for chronic heart failure has been studied less than for acute myocardial infarction (MI), but represents a potentially important alternative for this disease.

Therapies for AIC remain limited; other than heart transplant, therapy is largely palliative. Given the limited availability of donor hearts and the bleak prognosis of this disease, **new strategies for the treatment of AIC refractory to optimal heart failure therapy are sorely needed**. This is indeed an important unmet need in contemporary cardiovascular medicine. Accordingly, this trial will test the safety and feasibility of administering allogeneic MSCs in subjects with AIC and focus on the impact of the cells for treating heart failure in this population.

2.3 Background of Regenerative Therapy in Cancer

A major concern in the application of cell-based regenerative therapies after cancer is whether these new treatments will increase the risk of tumor recurrence. Below is a review of what is known concerning interactions between stem cells / regenerative tissue and the potential risks of regenerative therapy after cancer treatment.

The interaction of proliferating multipotent mesenchymal stem cells MSCs with tumor cells have been addressed in co-culture demonstrating that the chemokine CCL5 by bone marrow-derived MSC (BM-MSCs) increase the motility of breast cancer cell lines in *in vitro* models of tumor invasion[32-34]. Because these studies utilized immortalized cancer cell lines that grow rapidly in culture, they fail to model the crucial aspects of tumor heterogeneity, tumor dormancy, and reactivation of occult tumor cells.

While research shows that MSC's promote tumor growth in immortalized cell lines there are other contradictory studies, which demonstrate that MSCs inhibit tumor growth. For example MSCs reportedly inhibit tumorgenesis from Kaposi's sarcoma cells[35] and hepatoma cells[36], bone metastasis of prostate cancer[37], and in vitro growth of hepatoma, lymphoma, and insulinoma cell lines[38]. Multiple mechanisms have been invoked to relate these findings to the cytokines, chemokines, and prostaglandins secreted by MSCs[39]. Since the data with MSCs with various tumor cell lines is conflicting, more data is needed on MSCs and phenotypically similar cells lines in other experimental systems and clinical studies to determine the tumorigenic potential of MSCs.
There is significant similarity between MSCs and adipose-derived stem / stromal cells (ASCs) [40]. ASCs phenotypically resemble bone marrow derived MSCs (BM-MSCs) and share their multi-potentiality and many surface markers (predominantly CD105+ / CD73+ / CD90+ / CD44+) [41,42]. Like BM-MSCs, ASCs are able to give rise to differentiated progeny with characteristics of bone, cartilage, fat, and blood vessels. ASCs and BM-MSCs are both promising candidates for cellular therapy, but the potential risk of promoting tumor reactivation remains controversial. The utility

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of ASCs, as cellular therapy after breast tumor resection, is germane since autologous fat transfers with ASCs have been used for breast reconstruction post mastectomy[43,44]. Despite the potential concern that self-renewing adipose tissue may promote tumor recurrence by reactivation of occult breast cancer cells, studies have not shown that stem cell-augmented adipose transplantation for breast reconstruction increases cancer reoccurrence[45,46].

Both autologous fat transfers and autologous tissue flaps pose little risk of reoccurrence in breast cancer patients who are adequately treated with surgery, chemotherapy, and radiation due to the notion that the cancer cells / tumor beds are dormant and thus low potential for transformation. The concept of dormancy, although ill-defined in oncology literature, refers to cells that are out of cell cycle (i.e., G0), or persisting in a dynamic state of balanced proliferation and death. The same can be said for normal tissue stem cells[47]. Dormancy is an intrinsic characteristic of the resting tumor cell, perhaps imposed by epigenetic programming[48]. As such, transition between dormant and active states requires genetic reprogramming and not merely the presence of signals from hormones or angiogenic factors. If increased local vascularization with adipose tissue flaps at the site of a tumor bed was an independent risk factor for recurrence, this fact would have become clear after observing former breast cancer patients with reconstructed tissue flaps. Immediate autologous tissue flap reconstruction has not been correlated with increased recurrence rates[49]. It is believed that patients who are appropriately treated for breast cancer and selected for adipose tissue flap are in a state of remission (tumor dormancy) and thus at a low risk for reoccurrence. Current data on adipose tissue flap reconstruction supports this notion.

During the 1990s, autologous hematopoietic transplantation was widely used to rescue the bone marrow of breast cancer patients undergoing dose-intensive chemotherapy. This practice was discontinued when randomized trials failed to show superiority over conventional therapy[50]. However, the massive damage to all proliferative tissues by high-dose therapy and the ensuing tissue regeneration did not promote breast cancer relapse. Therefore, when a solid tumor malignancy such as breast cancer was treated with regenerative cell-based therapy the data demonstrated safety without increasing the risk of local tumor reoccurrence.

A similar parallel can be drawn from the allogeneic and autologous hematopoietic stem cell transplantation for hematologic malignancies, where there are decades of experience[51,52]. When patients are given a bone marrow transplant during first remission (tumor dormancy) patients often have extended periods of disease free survival. In contrast, when hematologic malignancy is active (i.e., the patient has had multiple remissions or has active disease at the time of myeloablative therapy), relapse often occurs early in the peri-transplant period when bone marrow regeneration is at its peak[53]. A similar argument can be made for cytokine mobilization during autologous hematopoietic stem cell transplantation, where patients in remission are treated with consolidation chemotherapy and the hematopoietic growth factor granulocyte colony stimulating factor (G-CSF)[54]. The patients experience bone marrow suppression followed by explosive regeneration without triggering early relapse. In all these examples of breast cancer tissue flap reconstruction, bone marrow transplantation, and cytokine-mediated marrow stimulation, appropriate patient selection with good tumor response (remission / tumor dormancy) is essential to minimize the risk of cancer reoccurrence.

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Available evidence from cell lines, case reports, and clinical studies favors the interpretation that regenerating cell / tissue based therapy stimulates the growth of active, high-grade tumors. In contrast, dormant cancer / cancer in remission does not appear to be activated by cell / tissue regeneration. However, the data does support that regenerative-based therapies should be deferred until cancer remission has been firmly established and the patients are felt to have low risk of reoccurrence.

2.4 Therapy Rationale

Since therapy with stem cells promises to be a safe and effective treatment for regenerating myocardium and improving cardiac function in ischemic cardiomyopathy[55,56], we propose that stem cells may also be beneficial in AIC. In both cases, the underlying cause of HF is loss of viable myocytes with replacement fibrosis. Cancer survivors, however, differ importantly from patients with ischemic heart failure with respect to the potential source of stem cells. **In patients with AIC, the use of allogeneic cells is necessitated by the concern that autologous cells may lead to dissemination of the original malignancy for which patients were treated with anthracyclines; causing recurrence or dissemination of the original malignancy would be so catastrophic that this risk prohibits the use of autologous cell therapy.** Therefore, in this trial, we will use allo-MSCs rather than autologous MSCs.

The bone marrow harbors a variety of cells that may contribute to vasculogenesis or cardiomyogenesis, either directly, or by facilitating endogenous repair mechanisms. Bone marrow cells have been prepared on the basis of being: 1) endothelial precursor cells that are $CD34^+$; 2) MSCs purified without an antigen panning technique on the basis of their fibroblast morphology, ability to divide in culture, and to differentiate into mesodermal lineages[57]; and 3) cells that express the stem cell factor receptor, c-Kit[58]. Endothelial progenitor cells (EPCs) express the surface markers CD34, CD133, c-kit, and the vascular endothelial growth factor receptor-2 (VEGFR2; KDR; Flk-1)[59-64]. Hematopoietic stem cells (HSCs) exhibit self-renewal and differentiation. Their cell-surface phenotype is $CD34^+$, stem cell factor antigen $(SCA\text{-}1)^+$, c-kit^+, and Lin^- (review[65]). While there has been controversy regarding the ability of bone marrow-derived cells to trans-differentiate into cardiomyocytes[66], clinical trials of whole bone marrow therapies continue to suggest potential benefit in terms of improving cardiac function or reducing the burden of scarred myocardium[31].

2.4.1 Mesenchymal Stem Cells (MSCs)

MSCs are a particularly promising bone marrow-derived cell for cardiac regenerative therapy because of their availability, immunologic properties, and track-record of safety and efficacy[18]. Studies of MSC engraftment in rodent and swine models of myocardial infarction have shown that the administration of MSCs produces: 1) functional benefit in post- MI recovery of ventricular function; 2) evidence of neo-angiogenesis at the site of the infarct; 3) decrease in collagen deposition in the region of the scar; and 4) some evidence of cells expressing contractile and sarcomeric proteins but lacking true sarcomeric functional organization[67,68]. Moreover, MSCs are thought to be ideal candidate cells for allogeneic transplantation because they show minimal major histocompatibility complex (MHC) class II and intercellular adhesion molecule (ICAM) expression and lack B-7 co-stimulatory molecules necessary to cause a T-cell mediated immune response[18,69].

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[70]. Some, but not all, groups have purified MSCs expressing c-kit directly from bone marrow that have the capacity to form cardiac myocytes[71]. This is of functional significance given the demonstration that stem cell factor stimulates cardiac repair post-MI[72].

2.4.2 Immunological Properties of MSC and Human Leukocyte Antigen (HLA)-Matching

The use of allogeneic cellular products typically requires matching of the graft HLA to the donor. Mismatched grafts can result in graft rejection and can induce graft versus host disease (GVHD). However, MSCs represent a unique cell population for allogeneic cellular therapy. MSCs have been shown to exert anti-proliferative, immune-modulatory, and anti-inflammatory effects. Human MSCs fail to induce proliferation of allogeneic lymphocytes in vitro[73]. Also, MSCs suppress proliferation of T cells activated by allogeneic cells or mitogens[74]. The suppression appears to be mediated, at least in part, by soluble factors and affects several types of immune cells.

Several hundred patients have received MSCs in clinical trials that used an allogeneic source for the cell product. Infusion of MSCs was well tolerated and no side effects were noted[75]. Several patients have been treated with MSCs to treat severe graft versus host disease (GVHD). In a 9-year old boy who received a matched, unrelated donor hematopoietic stem cell transplant for leukemia, severe acute steroid-resistant GVHD of the gut and liver was reversed by infusion of haplo-identical MSCs derived from the patient's mother[76].

These data suggest that human MSCs may be a unique cell population for regenerative medicine with minimal immune reactivity, which decreases the potential of graft rejection and/or GVHD.

2.4.3 Potential Mechanisms for MSC Mediated Improvements in Cardiac Function

As noted above, prior studies have shown that a variety of cellular sources are capable of differentiating into phenotypes that strongly resemble the three principal cell types in the myocardium: cardiomyocytes, smooth muscle, and vascular endothelium. Preliminary data from Hare's group, and reports from other labs cited above, suggest that MSCs have the potential to form all three cell types within infarcted myocardium *in vivo*. Nevertheless, it is important to consider that MSCs may exert other favorable effects on cardiac repair above and beyond differentiation[18]. For example, these cells may also participate in the recruitment and/or stimulation of other cells to differentiate into a cardiac phenotype.

There is a wealth of evidence suggesting that stem cell homing to damaged myocardium is directed by injury signal(s) emanating from the area within or surrounding the infarct. For example, stromal-cell-derived factor 1 (SDF-1), a chemokine that is a natural ligand for the CXCR4 receptor, is crucial for bone marrow retention of hematopoietic stem cells[77,78], cardiogenesis[79], recruitment of endothelial progenitor cells to sites of ischemic tissue[80] and, potentially, migration of tissue-committed stem/progenitor cells[81]. Interestingly, it was recently shown that the CXCR4 receptor is strongly expressed by a subset of MSCs and plays an important role in MSC mobilization[82]. Expression of SDF-1 dramatically increases over the first week following infarction, and exogenous expression of SDF-1 increases the number of mobilized bone marrow cells (BMCs) homing to the heart at time periods remote from infarction[83]. These findings suggest that MSCs participate in the complex autocatalytic cascade of cytokines and growth factors that is activated following MI. Indeed, human MSCs are capable

of secreting several cytokines, including stem cell factor (SCF) and G-CSF[80], and intramyocardial administration of MSCs is associated with increases in vascular endothelium growth factor (VEGF) levels[29]. Furthermore, it has been shown that MSCs participate in angiogenesis and arteriogenesis, differentiating into endothelium and vascular smooth muscle in a VEGF-dependent manner[84].

Once cells successfully home and engraft in the heart, they must survive in a hostile environment if they are to effect successful cardiac repair. It is thought that apoptosis within the infarct region is responsible for the fact that only a fraction of cells injected directly into the heart will engraft and survive, and that such cell death reduces the efficacy of cellular cardiomyoplasty. In a dramatic proof of principle study, Mangi et al. genetically engineered rat MSCs using *ex vivo* retroviral transduction to overexpress the anti-apoptotic protein Akt1, a serine-threonine kinase[67]. Transplantation of $5x10^6$ cells overexpressing Akt into the ischemic rat myocardium led to dramatic improvements in structure and function that far exceeded those seen with injection of control MSCs transduced with Lac-Z. MSCs reduced inflammation, collagen deposition and cardiomyocyte hypertrophy, regenerated 80-90% of lost myocardium, and completely normalized systolic and diastolic cardiac function in a dose-dependent fashion.

2.5 Background Preclinical

De Angelis et al. induced AIC in rats with 3 weeks of doxorubicin treatment; these investigators showed that subsequently treatment of these rats with syngeneic c-kit+ cardiac stem cells resulted in a 66% decrease in mortality at 6 weeks and a significant improvement in multiple parameters of LV function compared with control rats treated with vehicle[85].

Psaltis et al. examined the safety and utility of allogeneic MSCs delivered transendocardially in a sheep model of AIC[86]. In this study, MSCs were prepared from ovine bone marrow by immunoselection using the tissue nonspecific alkaline phosphatase, or STRO-3, monoclonal antibody. Fifteen sheep with AIC were assigned to catheter-based, transendocardial injections of ovine MSCs (n = 7) or placebo (n = 8), under electromechanical mapping guidance. Each animal received 20 injections of 0.2ml/injection of placebo or cell product distributed throughout the left ventricle 10 weeks after first exposure to doxorubicin. Follow-up was for 8 weeks, with end points assessed by cardiac MRI, echocardiography, and histology. Intramyocardial injections of MSCs with the NOGA MyoStar injection system were distributed similarly throughout the left ventricle in both groups. Cell transplantation was associated with 1 death late in follow-up, compared with 3 early deaths among control animals. At 8 weeks after treatment, LV end-diastolic volume increased in both cohorts; however, MSC therapy attenuated the increase in end-systolic volume, which was not statistically significant, whereas in the control group there was a statistically significant increase in end-systolic volume ($P< 0.01$). Importantly, LV ejection fraction increased from 37.3 ± 2.8% before cell therapy to 39.2 ± 1.4% at 8 weeks after cell therapy (P=NS) in treated sheep, whereas in control animals it decreased from 38.8 ± 4.4% to 32.5 ± 4.9% ($P<0.05$) at corresponding time points. Histological analysis showed that MSC therapy was associated with less fibrosis burden than in the control group and an increased density of karyokinetic cardiomyocytes and arterioles ($P< 0.05$ for each). These changes occurred in the presence of modest cellular engraftment after transplantation. **This study, conducted in a large preclinical animal model of AIC, provides robust evidence for the therapeutic utility of allogeneic MSCs in the treatment of AIC, and thus supports the rationale for the present study.**

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2.6 Background Clinical

2.6.1 Previous Clinical Trials of Allogeneic Mesenchymal Stem Cells (allo-MSCs)

Allo-MSCs have been used in several recent FDA-approved trials. **The cumulative experience in these studies demonstrates an outstanding safety margin and a significant potential for cardiac regeneration.**

In a multi-center, randomized, double-blinded, placebo-controlled clinical study by Hare et al. 53 patients with recent acute MI (3 to 10 days) were treated with allo-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 intravenous injection, and followed for six months[87]. There were no deaths reported in the study; no toxicity was observed with the administration of the allo-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. The group of patients treated with MSCs demonstrated reduced ventricular tachycardia episodes (P=0.025), and pulmonary function testing demonstrated improved forced expiratory volume in 1 s (P=0.003) in the MSC-treated patients. In addition, left ventricular ejection fraction (LVEF) in the important subset of anterior MI patients was significantly better in MSCs versus control subjects, with a 7.3% absolute improvement in EF at 6 months over baseline. In the cardiac MRI substudy, MSC treatment, but not placebo, increased LVEF and led to reverse remodeling.

The POSEIDON trial was a phase 1/2 randomized controlled study in which 30 patients with chronic ischemic cardiomyopathy were randomized to 20 million, 100 million, or 200 million autologous MSCs or allo-MSCs (5 patients were given each cell type for each dose of cells)[56]. The cells were delivered by transendocardial injection into 10 LV sites in a volume of 0.5 mL for each injection. The primary outcome was incidence of severe adverse events at 30 days. At 1 year, there was no difference between allogeneic and autologous MSCs with regards to serious adverse events. In addition, at 1 year, there were no ventricular arrhythmia serious adverse events (SAEs) observed among allo-MSC recipients compared with 4 patients (26.7%) in the autologous MSC group (P = .10). Although there was no significant improvement in LVEF in any group, there was a sizeable reduction in scar size in both allogeneic and autologous groups. Importantly, allo-MSCs did not stimulate significant donor-specific allo-immune reactions.

The CTSN LVAD study[88] was an NHLBI-sponsored multi-center, double-blind, sham-procedure controlled trial to assess the safety and feasibility of MSC implantation into the LVAD-assisted heart. Thirty patients were randomized (2:1) to intramyocardial injection of 25 million mesenchymal precursor cells (MPCs) (Mesoblast, Inc.) or medium during LVAD implantation and followed until transplant or 12 months whichever came first. No safety events were observed. At 90 days, successful temporary LVAD weaning was achieved in 50% of MPC and 20% of control patients (P=0.24). Three deaths (30%) occurred in control patients and none occurred in MPC patients. Mean left ventricular ejection fractions after successful wean were 24.0% (MPC=10) and 22.5% (control=2; P=0.56). At 12 months, 30% of MPC patients and 40% of control patients were successfully temporarily weaned from LVAD support (P=0.69), and 6 deaths occurred in MPC patients. Donor-specific HLA sensitization developed in 2 MPC and 3 control patients and resolved by 12 months.

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In addition, Perin et al. (NCT00721045) investigated the safety of allo-MSCs in the setting of acute MI. This study was supported by Mesoblast and performed at six centers in the US between 8/2008 and 5/2010 to assess the safety and effect of bone marrow derived allogeneic MPC's STRO-1bright cells on LV function. This was a Phase II, single-blind, randomized, dose-escalation study of 60 subjects with fifteen subjects per 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 system 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 (control corrected 34 meters' improvement but not significant in the 150 million cell group), significant decrease in both left ventricular end systolic volume (LVESV) and end diastolic volume (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 heart failure (HF) MACE (i.e., cardiac death or HF re-hospitalization) there were no events in the 150 million cell group, and there were no significant differences between any cell dose group and control patients. For all events, there were 12 events in total combined three cell groups (n=45) compared with 11 events in the control group (n=15). These are unpublished data, partially presented at AHA 2013 and currently submitted and under review for publication.

Taken together, these trials of allo-MSCs demonstrate not only an excellent safety profile of this therapy but also a considerable potential for allo-MSCs to exert beneficial effects in patients with heart failure[56,87].

2.6.2 Ongoing/Future Clinical Trials of Allogeneic Mesenchymal Stem Cells

There are several ongoing studies of allo-MSCs in cardiac diseases. The safety study of *Allogeneic Mesenchymal Precursor Cell Infusion in MyoCardial Infarction (AMICI)* (NCT01781390) is a multi-center randomized clinical trial in which 225 patients with new acute MI in the left anterior descending (LAD) artery territory are randomized to intracoronary infusion of 12.5 or 25 million allo-MSCs or placebo (cells are injected at the time of primary coronary intervention).

Allogeneic stem cells are also under investigation in patients with dilated cardiomyopathy (DCM). *PercutaneOus StEm Cell Injection Delivery Effects On Neomyogenesis in Dilated CardioMyopathy (the POSEIDON-DCM Study)* (NCT01392625) is an ongoing study that is actively recruiting 36 patients with DCM and randomizing to receive autologous MSCs vs. allo-hMSCs via intramyocardial injection; patients will receive 100 million MSCs (autologous or allogeneic) delivered in 10 injections via the transendocardial route utilizing the NOGA system.

2.6.3 Previous Clinical Experience with Intramyocardial Delivery of Stem Cells

There is substantial clinical experience with the transendocardial delivery of autologous bone marrow-derived mononuclear cells (BMCs) as well as autologous MSCs and allo-MSCs in the setting of chronic LV dysfunction. The aforementioned POSEIDON trial[56], the TAC-HFT trial[89], the FOCUS-CCTRN trial[90] and the RENEW trial (NCT01508910), are only a few examples of the application of this delivery method for cell therapy in patients with cardiovascular disease. In addition, multiple ongoing trials are using intramyocardial injection as the mode of delivery for stem cells. Table 1 (see below) lists the 11 studies in which intramyocardial delivery of BMCs was performed[91-101]. Cell delivery has been performed via either i) direct intramyocardial (IM) injection during coronary artery bypass graft (CABG) surgery, or ii) catheter-based

intramyocardial injection (transendocardial delivery). Taken together, these studies clearly support the clinical safety of the intramyocardial injection delivery method.

Table 1. Studies utilizing Intramyocardial Delivery of BMCs

Study	N	Cell Delivery Method	Cell Source & Type	Cell Doses (x 10^6)	Safety	Efficacy Results
Stamm[99]	6	Direct IM injection during CABG surgery	Autologous, AC133* BMCs	1.2 - 3.4	No arrhythmias; no neoplasia	↑ global contractility (EF)
Tse[101]	8	Catheter-based IM injection	Autologous BMCs	2.6 – 21.2	No arrhythmias	↑ wall motion & thickening
Fuchs[100]	10	Catheter-based IM injection	Autologous BMCs	32.6 ± 27.5	No arrhythmias or other SAEs	↓ angina score; ↓ ischemia
Perin[97]	14	Catheter-based IM injection	Autologous CD34* BMCs	25.5 ± 6.3	No arrhythmias at 6-mo. F/U	↑ global contractility (EF); ↓ ESV
Beeres[91]	25	Catheter-based IM injection	Autologous BMCs	84.1 + 28.7	No arrhythmias or pericardial effusion	↑ global contractility (EF); ↓ ESV
Briguori[92]	10	Catheter-based IM injection	Autologous CD34* BMCs	4.6 ± 1.5	No arrhythmias or AMI	↑ quality of life; ↑ perfusion
de La Fuente[93]	10	Catheter-based IM injection	Autologous CD34* BMCs	86 ± 3	No arrhythmias at 12-mo. F/U	↑ global contractility (EF)
Mocini[96]	36	Direct IM injection during CABG surgery	Autologous CD34* BMCs	292 + 232	No SAEs	↑ global contractility (EF)
Hendrikx[94]	20	Direct IM injection during CABG surgery	Autologous BMCs	60.1 ± 31.1	Possible inducible VT	↑ global contractility (EF)
Stamm[98]	55	Direct IM injection during CABG surgery	Autologous, CD133* BMCs	3.85 – 103.0	No arrhythmias	↑ global contractility (EF)
Li[95]	6	Direct IM injection during CABG surgery	Autologous BMCs	50 - 100	No arrhythmias; no neoplasia	Not Assessed

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

The proposed transendocardial injection system, the NOGA MyoStar injection system, has been utilized in multiple clinical trials of transendocardial injection including, but not limited to, the ACT34-CMI[102], FOCUS-HF[103], FOCUS-CCTRN[90], RENEW (NCT01508910), and NOGA-DCM[104] studies. Again, this vast experience has shown this system to be safe, with minimal SAEs. In a retrospective safety study of the NOGA MyoStar injection system, data from 71 patients who had undergone intramyocardial mapping and/or injection in the context of clinical trials were pooled and analyzed[105]. Although there were no me[illegible]omplications, NOGA

mapping followed by direct intramyocardial injections led to CK-MB rises exceeding three times the upper limit of normal in 8 patients (17%) who received a volume of 0.3 mL per injection, compared with none in NOGA mapping alone[105].

2.7 Dose and Delivery Rationale

2.7.1 Dose Rationale

The clinical experience with the administration of MSCs in the trials performed to date provides substantial evidence of safety at cell doses up to 200 million MSCs as well as preliminary evidence of efficacy. In SENECA, we propose to administer a total of 100 million allo-MSCs, divided into 20 injections of 5 million cells each. A dose of 100 million MSCs delivered transendocardially has already been used in clinical trials, namely, the POSEIDON[56] and TAC-HFT trials[89], and found to be safe. This dose is within the range of doses (20 to 200 million MSCs) used in previous human studies of MSCs[56,89]. In the ongoing POSEIDON-DCM trial (NCT01392625), a dose of 100 million allo-MSCs is being used. The rationale for selecting 100 million cells is that in the POSEIDON trial[56], there was an inverse dose response, leading the authors to speculate that higher doses may be less efficacious due to deleterious effects on cell retention, survival, or performance.

Preclinical investigations are also consistent with an inverse dose response. Hamamoto et al. demonstrated in an ovine model of acute MI treated with transendocardial injection of 25, 75, 225, and 450 million STRO-3 MSCs that, compared with control animals, low doses (25 and 75 × 10^6 cells) of MSCs significantly attenuated the increase in LV end diastolic (LVEDV) and end systolic (LVESV) volumes; the 225 million dose improved only the LVESV over control, whereas the 450 million dose did not produce any salutary remodeling effect relative to controls[106]. In addition, infarct expansion was attenuated only in the 25, 75, and to some extent the 225 million groups although EF improved at all cell doses. CD31 and smooth muscle actin (SMA) immunohistochemical staining demonstrated increased vascular density in the border zone only at the two lower cell doses (more so in the 75 million group albeit, statistically insignificant between 25 and 75 million cell groups)[106]. Another ovine study used a protocol and allo-MSC doses similar to the above study and found that the LVEDV increase was significantly attenuated only in the 25 and 75 million groups compared with controls whereas the drop in EF was uniform across all doses of allo-MSCs in comparison to the control animals ($P<0.05$)[107]; both findings in Dixon et al.[107] were analogous to the findings by Hamamoto et al[106].

2.7.2 Delivery Rationale

The choice of the transendocardial delivery route is based on both preclinical and clinical data. In a preclinical study, Psaltis et al. (2010) demonstrated in an ovine model that cell therapy delivered by the NOGA device is safe and effective in the treatment of doxorubicin-induced cardiomyopathy[86]. Moreover, there is substantial clinical experience in humans with the transendocardial delivery of autologous bone marrow-derived mononuclear cells (BMCs) as well as autologous MSCs and allo-MSCs in the setting of chronic LV dysfunction. POSEIDON[56], TAC-HFT[89], FOCUS-HF[103], FOCUS-CCTRN[90], RENEW (NCT01508910), NOGA-DCM[104], and Perin et al.'s study (NCT00721045) clearly demonstrate that NOGA can be carried out safely in humans with cardiomyopathy. Table 1's tabulation of the numerous studies utilizing direct intramyocardial injection adds to the weight of favorable clinical experience with this technique**.** Intracoronary delivery of allo-MSCs is not recommended in our protocol because of the likelihood, or at least the possibility, of producing microvascular obstruction with resultant dose dependent myocardial damage[108,109]. In addition, no published clinical trial supports the efficacy

of intracoronary MSC delivery, in contrast to the copious literature supporting the efficacy of transendocardial MSC delivery as discussed previously.

Studies by Tham[110] and Steinherz[5] demonstrate that fibrosis in AIC patients is diffuse with a unique cardiac MRI profile. Our injections will target areas of diffuse fibrosis. The use of NOGA technology in preclinical models of anthracycline-induced cardiomyopathy, as well as idiopathic dilated cardiomyopathy, justifies its use in this protocol. Taken together, the available literature and ongoing experience support the clinical safety of the intramyocardial injection delivery method and suggest that this route of cell administration is efficacious.

3.0 STUDY DESIGN

3.1 Research Questions

- **Question 1**: Are allo-MSCs well-tolerated by subjects with LV dysfunction secondary to AIC?
- **Question 2**: Can allo-MSCs be delivered to subjects with LV dysfunction secondary to AIC, and can the outcome measures be collected?
- **Question 3**: Do allo-MSCs produce a trend toward improved LV function and functional status when compared with placebo from baseline to six months and/or from baseline to 12 months post treatment with study product?

3.2 Study Design

This study will evaluate approximately thirty-six subjects. The first six subjects will receive allo-MSC therapy (open label) and will be assessed for safety and feasibility of the study procedures. Following 1 month data review of each of the six subjects by the NHLBI Gene and Cell Therapy Data and Safety Monitoring Board; this will be followed by a randomized, double-blind clinical trial enrolling approximately thirty subjects (see Section 7.7). These subjects will be randomized 1:1 to receive allo-MSCs or placebo. All subjects will undergo cardiac catheterization and study product administration using the NOGA Myostar catheter injection system. Subjects will be followed at 1 day, 1 week, 1 month, 6 months, and 12 months post study product injection. All endpoints will be assessed at the 6 and 12 month visits which will occur 180 ±30 days and 365 ±30 days, respectively, after the day of study product injection (Day 0).

For the purpose of the safety evaluations and endpoint analysis, we will utilize an “intention-to-treat” study population. In addition, because this phase I study is the first cell therapy study in this population, at 12 months all available standard-of-care medical records for cancer surveillance will be reviewed for cancer recurrence. Approximately thirty-six subjects will be enrolled over a 12-18 month accrual period.

3.3 Study Treatment Assignments and Dosages

Approximately thirty-six (36) subjects (including six actively treated lead-in participants and up to thirty-one randomized clinical trial participants) will be enrolled. The six subjects participating in the open-label lead-in will receive allo-MSC as described below in Group A. The remaining subjects will be randomized 1:1 to one of two treatment strategies:

- Group A – Receive allo-MSCs: 20 endomyocardial injections of 5 million cells each (for a total of 100 million allo-MSCs); with each injection administered in a volume of 0.4ml

- Group B – Receive placebo: 20 endomyocardial injections of cell-free Buminate solution; with each injection administered in a volume of 0.4 ml.

The study product for Group A will be supplied from an allogeneic human MSC source (Appendix A); cells will be isolated from bone marrow of normal human donors and manufactured at a CCTRN cell manufacturing facility (CMF). Administering 20 injections of 0.4 ml each provides 8.0 ml of total study product volume with a cell concentration of 12.5 million total cells per ml, or 5 million cells per injection site. Twenty injections will be performed to treat the entire left ventricle (see section 5.4).

3.4 Study Endpoints

3.4.1 Safety Assessment

To demonstrate the relative safety of allo-MSCs 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:

1. Major adverse cardiac events (MACE) including death, hospitalization for worsening heart failure (HF), and/or exacerbation of HF (non-hospitalization)
2. Other significant clinical events including non-fatal stroke, non-fatal myocardial infarction, coronary artery revascularization, ventricular tachycardia/fibrillation, pericardial tamponade, infectious myocarditis, hypersensitivity reaction, neoplasm, or any other potential deleterious late effects detected and corroborated by clinical presentation, laboratory investigations, image analysis, and when necessary with biopsy from suspected target sites in the body
3. All adverse events (AEs) at least grade 2 in severity (see section 7.3.1)

3.4.2 Feasibility Measures

To assess the practicality of study procedures, the following measures will be reported.
The number and percent of subjects who:

1. Have events between randomization and SPI that preclude the subjects from getting SPI
2. Receive less than 20 injections during the SPI procedure
3. Do not receive the intended dose of cells (100 million), reason, and actual dose delivered
4. Have 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
5. Fail to complete follow-up

3.4.3 Prospectively Declared Efficacy Endpoint Measures:

To explore whether allo-MSCs produce a trend toward improved LV function and functional status when compared with placebo, the following domains[111] and measures will be evaluated:

- 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 area of injury (e.g., inflammation, edema, fibrosis)
- Functional capacity over time:

  - Change in exercise tolerance (6MWT)
  - Change in MLHF Questionnaire (subject reported)
- Clinical outcomes over time:
  - MACE
  - Cumulative days alive and out of hospital
- Biomarkers over time:
  - Change in NT-proBNP

The comparisons above will be made between the allo-MSC and placebo groups.

3.4.4 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.

3.5 Sample Size

This phase I study will recruit approximately 36 subjects who will have baseline and follow-up assessments to assess the changes over time in LV function and functional status. It is hoped that we will establish the precision of these estimates [111]. These measures will then be used to adequately power subsequent studies that will be able to determine the effect of therapy with statistical regularity and precision. The following measures will be assessed:

1. LV End Diastolic Volume (LVEDV) Index
2. LV End Systolic Volume (LVESV) Index
3. LV Ejection Fraction (LVEF)
4. Area of Injury (e.g., inflammation, edema, fibrosis)
5. Sphericity Index criteria
6. MLHF Questionnaire
7. Exercise tolerance (6MWT)
8. Global and regional strain

This study will recruit 31 patients with the addition of 6 patients to account for loss-to-follow-up. Subjects will be followed for one year; this is six months more than our FOCUS study which experienced a 10% follow-up loss rate. Increasing the estimation for loss-to-follow-up in SENECA to 15% over the 12 month course of follow-up would produce 4.5 patients potentially lost, administratively rounded up to 6 patients.

4.0 IDENTIFICATION AND ENROLLMENT OF SUBJECTS

Potential subjects will be identified by the study team and medical records will be reviewed for possible consent to the study.

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 subjects includes reviewing medical records for inclusion/exclusions prior to consent, as well as subjects' imaging studies. Subjects who are determined to have a diagnosis of AIC, have NYHA class of II or higher, have LVEF ≤ 45% by cMRI, and are a candidate for cardiac catheterization, and do not have evidence in their medical record of study exclusions, should be approached about consenting to the study. EF ≤ 45% can be defined by gated blood pool scan (MUGA), cMRI, left ventriculogram, or EF ≤ 40% by two-dimensional echocardiogram.

In order to exclude those with ischemic heart disease, available imaging in the patient's chart will be reviewed. Acceptable imaging for the detection of obstructive CAD includes coronary arteriogram or any non-invasive test within five years prior to enrollment in the study provided that there have been no symptoms or evidence of CAD since the test. If the subject has not had one of these tests done in the corresponding timeframe, then the individual should have one of these tests as standard of care (institutional choice) to be reviewed for entry into the study. If a test is not otherwise authorized under a patient's insurance under standard of care, a stress echo will be included as part of the baseline testing and paid for by the study, to be reviewed for eligibility.

Prior to study entry an oncological assessment form will be completed by the subject's oncologist to assess the risk of cancer recurrence. Current medical records will be reviewed to verify the absence of any new metastatic disease, stage 4, or recurrence.

4.2 Consent

Before being admitted to the clinical study, all subjects must consent in writing to participate. An informed consent form (ICF) will be given to each subject which 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.

A separate consent form will be utilized for six subjects participating in the open-label lead-in. These subjects will undergo the same screening and baseline procedures as those who will participate in the randomized phase of the study (see Section 6.0 below); however, all lead-in subjects will receive the allo-MSC product.

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. The ICF and review must be in a form understandable to the subject. 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 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.
All consent forms will have IRB approval. Individuals who agree to participate will receive a copy of the signed informed consent. The research staff memb ng 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.

4.3 Inclusion Criteria

To participate, a subject MUST:

1. Be ≥ 18 and < 80 years of age
2. Be a cancer survivor with diagnosis of AIC
3. Have an LVEF ≤ 45% by cMRI
4. Be in NYHA class II-III
5. Have received the initial diagnosis of AIC at least six months earlier and be on stable, optimally-tolerated therapy with beta-blockers, ACE inhibitors/ARBs, and/or aldosterone antagonists for 3 months, unless contraindicated
6. Have a period of at least two years of clinical cancer-free state* and low likelihood of recurrence (a five-year risk of recurrence estimated at 30% or less), as determined by an oncologist, based on tumor type, response to therapy, and negative metastatic work-up at the time of diagnosis
   exceptions to this are carcinoma in situ or fully resected basal and squamous cell cancer of the skin.
7. Be a candidate for cardiac catheterization

4.4 Exclusion Criteria

To participate, a subject MUST NOT HAVE:

1. A life expectancy <12 months
2. A CT scan or baseline cardiac MRI showing new tumor or suspicious lymphadenopathy raising concern of malignancy
3. Presence of obstructive CAD as determined via imaging within 5 years prior to study enrollment provided there have been no symptoms or evidence of CAD since the test (see section 4.1 for imaging guidance)
4. Had a previous myocardial infarction
5. A history of radiation therapy AND evidence of constrictive physiology and/or evidence of other patterns of non-ischemic cardiomyopathy on cardiac MRI (e.g., amyloidosis, sarcoidosis, hemochromatosis, pure radiation-induced cardiomyopathy, etc.) not consistent with AIC being the dominant etiology of heart failure
6. Valvular heart disease including 1) mechanical or bioprosthetic heart valve; or 2) severe valvular (any valve) insufficiency/regurgitation within 12 months of consent
7. Aortic stenosis with valve area ≤ 1.5cm^2
8. A history of LV reduction surgery or cardiomyoplasty
9. Evidence of cardiogenic shock
10. A history of ischemic or hemorrhagic stroke within 90 days of baseline testing
11. Liver dysfunction during baseline testing, as evidenced by enzymes (e.g., AST, ALT, alkaline phosphatase) greater than 3 times upper limit of normal
12. Diabetes with poorly controlled blood glucose levels (HbA1c > 8.5%)
13. An underlying autoimmune disorder or current immunosuppressive therapy (e.g., chronic corticosteroid, rheumatologic or immune modulating therapy) or likelihood of use of immunosuppressive therapy during participation in the trial *(medications will be considered on a case by case basis)*
14. A baseline eGFR <35 ml/min/1.73m^2
15. A contrast allergy that cannot adequately be managed by premedication
16. Received gene or cell-based therapy from any source within the previous 12 months

17. A hematologic abnormality during baseline testing as evidenced by hemoglobin < 9 g/dl; hematocrit < 30%; absolute neutrophil count < 2,000 or total WBC count more than 2 times upper limit of normal; or platelet values < 100,000/ul
18. Evidence of active systemic infection at time of study product delivery
19. HIV, and/or active HBV or HCV
20. Coagulopathy (INR > 1.5) not due to a reversible cause (e.g., warfarin and/or Factor Xa inhibitors) (see Section 6.4 re: injection procedure and anticoagulation therapy) *Note: Subjects who cannot be withdrawn from anticoagulation will be excluded.*
21. Presence of LV thrombus *(See guidance in Section 6.6.3)*
22. 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
23. Pacemaker-dependence with an ICD (*Note: pacemaker-dependent candidates without an ICD are not excluded)*
24. A cardiac resynchronization therapy (CRT) device implanted less than 3 months prior to consent
25. Other MRI contraindications (e.g. patient body habitus incompatible with MRI)
26. An appropriate ICD firing or anti-tachycardia pacing (ATP) for ventricular fibrillation or ventricular tachycardia within 30 days of consent
27. 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
28. A 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
29. Cognitive or language barriers that prohibit obtaining informed consent or any study elements (interpreter permitted)
30. Participation (currently or within the previous 30 days) in a cardiac related investigational therapeutic (including stem cell based therapies) or device trial
31. Pregnancy, lactation, plans to become pregnant in the next 12 months, or is unwilling to use acceptable forms of birth control during study participation
32. Any other condition that, in the judgment of the Investigator or Sponsor, would be a contraindication to enrollment, study product administration, or follow-up

4.5 Baseline Testing Window

The baseline testing period extends from the date the ICF is signed until the study product injection (SPI) procedure. This period will not exceed 45 days. Should subjects fail baseline testing for a reason that is likely to change over time, they may be considered for re-testing. See section 6.2 for procedures and assessments to be performed during the baseline testing period.

Individuals meeting enrollment criteria will be scheduled to undergo the SPI procedure (see section 5.4) and randomized to cells or placebo (see section 4.6). The SPI procedure will occur no less than three days after randomization and no more than 45 days from signing the ICF.

4.6 Randomization Procedures and Timing

Randomization to treatment assignment (cells or placebo) will be conducted using a web access database created and maintained by the Data Coordinating Center (DCC). The research coordinator (RC) will randomize the subject via the CCTRN web-based database which will perform the computer-generated randomization assignment and send automatic emails notifying the study team that the treatment assignment can be accessed by unblinded study team members with appropriate security credentials. The RC will not randomize the subject until the following criteria are met: i) the subject has provided written informed consent, ii) baseline evaluations are complete, iii) the inclusion and exclusion criteria for the study have been satisfied, and iv) MSC product is available at the cell manufacturing facility (CMF) should the subject be randomized to the cell group. Randomization should occur no less than 3 days prior to the scheduled SPI procedure (to allow time for cell shipping and local product preparation) and no more than 7 days prior to the scheduled SPI procedure (to minimize the risk of randomizing subjects who do not receive treatment).

5.0 STUDY PRODUCTS AND DELIVERY

The study products (allo-MSCs and placebo) are each individually described but shall be referred to throughout the protocol as “study product”.

5.1 Allogeneic Mesenchymal Stem Cells (allo-MSCs)

The MSC cell bank will be prepared at the Center for Cell and Gene Therapy (CAGT) GMP Facility at Baylor College of Medicine which will be referred to as the Central Cell Manufacturing Facility (CCMF). The CCMF has a Facility Masterfile at the FDA describing its infrastructure and operations. In brief, the facility consists of 22 ISO 7 clean rooms in which manufacturing is performed. The MSC will be manufactured using the Terumo Quantum Cell Expansion system. This is a functionally-closed computer-controlled bioreactor. The cells adhere to hollow fibers that are pre-coated with recombinant Fibronectin. The cells are fed by continuous flow of medium through the bioreactor. After 48 hours the non-adherent cells are flushed from the bioreactor and feeding continues. Cell growth is assessed by measurement of glucose consumption and lactate production. When lactate levels reach 4mM/liter the flow of medium is doubled to 0.2 ml/min. This process is repeated until the flow rate has reached 0.4ml/min and the lactate levels are above 4mM. At this point the bioreactor is flushed with 2.5 times the reactor volume of phosphate- buffered saline. 180ml of recombinant trypsin (TrypLE Select) is added to the reactor for 15min to detach the cells. These are then automatically harvested into a bag containing growth medium.

For passage two the cells are seeded into the Fibronectin coated bioreactor cartridge at ~$1x10^4$ cells/ cm^2 . Growth kinetics are monitored as described above. Harvesting is performed when the flow rate reaches 1.6ml/min and the lactate concentration reaches 4mM/liter.

All operations are programmed into the bioreactor and reagent transfers are performed using sterile connect devices or aseptic connectors. Samples are taken for testing aseptically within an ISO 5 biological safety cabinet. This procedure has been reviewed by the Food and Drug Administration and approved for use in a separate IND submission.

For subjects randomized to receive allo-MSCs, allogeneic bone marrow donor-derived MSCs will be provided (Appendix A), according to established protocols, by the CCMF. The availability of allo-MSCs offers the potential for an "off the shelf" product for patients. Significant data have been generated (see section 2.4) to demonstrate that allo-MSCs are immunosuppressive[73-75]. In addition, allo-MSCs are immune-privileged and can be infused without immune rejection despite disparate HLA phenotypes[56].

5.1.1 Shipment of Cryopreserved allo-MSC Allografts

The cells will be cryopreserved at the CCMF according to their standard operating procedures (SOPs) using a controlled-rate freezer and stored in the vapor-phase of liquid nitrogen in continuously monitored cell storage banks. Twenty-four hours prior to shipment, a validated liquid nitrogen dry shipper will be charged with liquid nitrogen. The following morning any remaining non-absorbed liquid nitrogen will be decanted from the dry shipper and the vials containing the frozen MSC will be transferred to the shipper. The Data Logger (temperature monitor) located in the lid will be activated and the shipper sent via Federal Express to the local cell processing laboratories (CPLs) following CCMF SOPs within one week prior to the scheduled injection procedure. The CCMF will also provide all relevant SOPs for product preparation for administration. Certificates of Analysis (COA) will be provided by the CCMF with each product shipped to the local CPL.

5.1.2 Preparation for Administration of-MSC Allografts

Relevant SOPs and worksheets used for thawing, washing, and testing of allo-MSC product will be provided by the CCMF. The allo-MSC product will undergo QC testing before injection as indicated in Table 2a.

$1X10^8$ allo-MSCs will be provided in a final volume of 8.0 ml of Buminate solution 5% HSA, at a cell concentration of approximately $12.5X10^6$ total cells / ml. The physician will administer 20 individual doses of $5X10^6$ cells/0.4ml, per dose, per injection site. The 20^{th} injection will be followed by 0.1 ml of saline to flush any residual cells from the line into the 20^{th} injection site. It is permissible to prepare more than 8.0 ml of cell suspension, at a maximum concentration of $12.5X10^6$ cells / ml. However, any excess volume remaining after delivery of the 20 injections shall not be injected and shall be discarded according to institutional guidelines.

Table 2a. Testing of allo-MSCs for Injection

Assay	Test Method	Specification
Viability*	Trypan blue	≥70
Cell Count*	Manual	$1x10^8$
Rapid Sterility*	Gram Stain	No organisms
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

5.2 Placebo Group

Subjects randomized to the placebo group will undergo the same baseline testing and imaging as those in the allo-MSC group. The placebo will be kept on site at the local CPLs. The fifteen

patients in the placebo group will receive transendocardial injections of cell-free Buminate solution in 20 injection sites. The placebo product will undergo QC testing before injection as indicated in Table 2b.

Table 2b. Testing of Placebo for Injection		
Assay	**Test Method**	**Specification**
Rapid Sterility*	Gram Stain	No organisms
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

5.3 Blinding of the Interventionalist and Study Team

With regard to 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.). As the study product cannot be effectively masked, the process of blinding resides with endpoint and adverse event determination. The cMRI endpoint 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, and the MLHFQ endpoint data will also 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 (see section 4.6) in order to assist with product preparation; however, the research teams (investigators, coordinators and other staff involved with endpoint data collection and event data) will be blinded to the treatment assignment. If for important medical reasons unblinding 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.

5.4 NOGA Catheterization Procedure

Intramyocardial cell delivery by 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.

Left ventricular (LV) cine angiocardiography will be performed in orthogonal planes (typically RAO 30 and 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 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. The mapping catheter is retrogradely

advanced across the aortic valve into the LV cavity. 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 NOGA injection catheter will be prepared by adjusting the needle extension at 0° and 90° flex and priming the catheter by injecting the study product until a drop is observed at the extended needle tip (approximately 0.1 cc). All study product will be retained to complete the 20 required injections. To ensure safety and limit potential for extracardiac administration of the injectate, the needle extension will be adjusted to ensure that the needle length is ≤ 0.5 mm and will 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 placed into the LV in retrograde fashion.

Priority for selecting the 20 injection sites will be based upon the objective of delivering the study product in a minimum of four myocardial segments according to the AHA 17 segment polar map) selected by the investigator to be a territory which is: a) safely treatable with low risk of perforation or other complications; b) avoiding the true LV apex and other areas known to be < 6 mm in myocardial thickness, and avoiding the left bundle branch site of earliest activation; c) clinically important based on viability assessments provided below; and, d) accessible with the tip of the selected MyoStar catheter.

The NOGA map obtained will be unique in that it will display heterogeneous distribution of unipolar voltage in the LV, typical of patients with dilated cardiomyopathy. This is thought to represent areas of relative fibrosis and will serve as the basis for selection of the target injection area. The operator will proceed to delineate an injection area using the criteria mentioned above and ideally select injection sites that demonstrate intermediate level unipolar voltage (4 to 8mV). This range can be expanded to include areas with unipolar voltage of up to 12mV if necessary.

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 will be placed at a site with a voltage threshold of ≥ 4mV and ≤ 8mV. *When selecting sites for injection generally those with a voltage < 4mV will be avoided unless there are no higher unipolar voltage values present on the unipolar voltage map.*

Each of the 20 injections will contain either 0.4 ml of cell suspension with 5 million allo-MSCs per injection site for the active cell treatment arm or 0.4 ml of cell-free Buminate solution for the placebo group. 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 20th 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. Sites of injection may also be recorded on cine fluoroscopy, and demarcated as a dot with the corresponding injection series number on 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 (within 6 hours). Additional echocardiographic assessments for pericardial effusion will be done only as clinically indicated. Troponin I or T will be collected 8 (+/- 2) hours post-injection catheter procedure and again prior to discharge. An analysis of the impact of biomarker elevations on measures of LV function and MACE will be carried out. We believe this will demonstrate that biomarker elevations are not related to study outcomes.

5.5 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 20 mmHg not responsive to fluid administration
2. Hemodynamically significant arrhythmia requiring antiarrhythmic therapy
3. Two episodes of sustained ventricular tachycardia/ventricular fibrillation requiring cardioversion
4. Hemodynamically unstable
5. Fever (temperature increase to ≥100.4°F)
6. Cardiac perforation
7. Clinical signs and symptoms indicating a cerebrovascular accident

6.0 CLINICAL AND LABORATORY PROCEDURES

6.1 Schedule of Procedures (Table 3)

SENECA Study Procedure	BSL	D0 (SPI)	D1	Wk1 (+/-3 days)	M1 (+/-7 days)	M6 (+/-30 days)	M12 (+/-30 days)	M24 (+/-30 days) Call$_7$
Informed Consent	X							
Complete Medical History	X							
Physical Exam	X	X	X	X	X	X	X	
Vital Signs	X	X$_1$	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	
Con Medications	X	X	X	X	X	X	X	
NYHA	X				X	X	X	
MLHFQ	X					X	X	
12-lead ECG	X	X$_2$	X$_2$	X		X	X	
Stress Echo	O							
2D Echoes		X$_2$						
Telemetry		X$_3$						
Labs and Biomarkers (PB)$_4$	X	X	X	X	X	X	X	
Cardiac MRI	X					X	X	
ICD Interrogation$_5$	X	X				X	X	
6 Minute Walk	X					X	X	
Randomization	X							
Catheterization / NOGA		X						
Temp. Log			X$_6$					

Telephone Interview								X

O= indicates optional test; dependent upon available imaging in the patient chart to assess CAD.
Baseline testing, randomization, and SPI will take place within 45 days of ICF.

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 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 for specific tests done at each time point.
5. ICD interrogation (if applicable) done before and after every MRI as part of MRI protocol as well as before the SPI procedure.
6. Temperature log 2x/day x 7 days.
7. 24-Month contact is a telephone visit only.

6.2 Baseline Testing

See Table 3 above for the procedures and assessments to be performed during this phase of the study. All baseline tests, procedures, and randomization, will occur within 45 days of signing informed consent form (ICF). No baseline testing will take place until the subject is fully informed of the research and signs the ICF unless the evaluations were being performed as part of routine medical care. After the subject has consented to the study, the subject will have a series of assessments to establish eligibility to receive study product. Randomization will take place after subject eligibility has been determined.

Subjects will undergo the following tests and procedures during the baseline testing period:

1. Comprehensive medical and surgical history, including smoking history and notation of the cumulative dose of anthracycline, time from anthracycline exposure to development of HF, and the time from diagnosis of HF to study enrollment
2. Concomitant medication and adverse event assessment
3. Physical examination including systems review
4. Vital signs (blood pressure [BP], temperature, heart rate, respiratory rate)
5. Height and weight
6. NYHA class assessment (Appendix B)
7. Questionnaire: Minnesota Living with Heart Failure Questionnaire (MLHFQ) (Section 6.6.2)
8. cMRI (Section 6.6.3, MRI Core Lab Manual of Operations)
9. ICD interrogation (if applicable). Reports will be generated for the interrogations conducted before the MRIs at baseline 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).
10. A 6-minute walk test (6MWT) (Section 6.6.1, Protocol Manual of Operations)
11. 12-lead ECG
12. Laboratory evaluations (Section 6.3) including pregnancy test on women of childbearing potential only
13. For participants with no recent (within past 5 years) available imaging to assess CAD, and for which an updated test is not otherwise authorized under a patient's insurance under standard of care, a stress echo will be obtained as part of the baseline testing and paid for by the study, to be reviewed for eligibility. This test should only be done, if recent imaging is not otherwise available or obtainable.

6.3 Schedule of Laboratory Examinations (Table 4)

Table 4. Laboratory Testing	BSL	Day 0 (SPI)	Day 1[11]	Wk 1	M 1	M 6	M 12
CMP/Chemistry Tests[1]	X	X	X	X	X	X	X
CBC with Differential[2]	X	X[6]	X	X	X	X	X
Panel of reactive antibodies (PRA)	X				X	X	X
Liver Function Tests[3]	X					X	X
Pregnancy (childbearing women)	X	X[4]				X	X
NT-proBNP[5]	X					X	X
Troponin I or T	X	X[6]	X	X			
HbA1c	X					X	X
PT, INR, PTT[7]	X	X[12]					
Infectious Disease Tests[8]	X						
Peripheral Blood Collection (Biorepository)[9]		X[10]	X	X	X	X	

1. Complete Metabolic Panel/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 – HBc, HBsAg, HBV, HCV, HIV-1 groups M&O RNA, and Anti-HIV
9. With appropriate consent, twenty (20) mL of peripheral blood will also be collected and transported to the CCTRN biorepository for scientific study (See Section 6.7 Collection of Biospecimens)
10. Collected prior to sedation for the 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 SPI

6.4 Day of Study Production Injection (SPI) – Day 0

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.[112] 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.

1. Incremental medical history
2. Physical exam including vitals (BP, temperature, pulse rate) and weight
3. Laboratory examinations (see section 6.3)
4. ICD interrogation prior to SPI
5. Cardiac catheterization and injection of study product (see section 5.4) including vitals (BP, temperature, pulse rate) immediately pre- and post-

6. 12-lead ECG will be performed within 6 hours following the catheterization procedure and again before discharge
7. A 2-D echocardiogram will be performed post SPI (within 6 hours). Additional echocardiographic assessments for pericardial effusion will be done only as clinically indicated.

6.5 Follow-up Tests Performed After SPI

Randomized subjects will be followed up at 1 week and 1, 6, and 12 months after study product injection for regular history and clinical examination. The MLHFQ will be repeated at 6 and 12 months after SPI. To further evaluate their functional capacity, subjects will also undergo a 6MWT at 6 and 12 months after SPI. Subjects will be assigned a functional class during the month 1, 6, and 12-month follow-up visits based on the standard NYHA classification. Patients treated with allo-MSCs or placebo will undergo the following tests at various time points after the procedure (Table 3):

6.5.1 Day 1 (within the first 24 hours after SPI)

1. Subjects will be monitored on simple telemetry up to 24 hours post-procedure or until discharge, whichever is sooner.
2. Incremental medical history
3. Physical exam including vitals (BP, temperature, pulse rate)
4. Laboratory examinations (Section 6.3)
5. 12-lead ECG will be performed within 6 hours following the catheterization procedure and again before discharge
6. The subject will keep a daily temperature log for seven days following the catheterization procedure to help assess the early development of an infection.

6.5.2 Week 1 (± 3 days after SPI)

1. Incremental medical history
2. Physical exam including vitals (BP, temperature, pulse rate) and weight
3. Laboratory examinations (Section 6.3)
4. 12-lead ECG
5. Collect temperature log from subject

6.5.3 Month 1 (± 7 days after SPI)

1. Incremental medical history
2. Physical exam including vitals (BP, temperature, pulse rate) and weight
3. Laboratory examinations (section 6.3)
4. NYHA classification (Appendix B)

6.5.4 Month 6 (± 30 days after SPI)

1. Incremental medical history
2. Physical exam including vitals (BP, temperature, pulse rate) and weight
3. Laboratory examinations (see section 6.3)
4. NYHA classification (Appendix B)
5. cMRI (Section 6.6.3, MRI Core Lab Manual of Operations)
6. Questionnaire: MLHFQ (Section 6.6.2)
7. 12-lead ECG
8. 6MWT (Section 6.6.1, Protocol Manual of Operations)

6.5.5 Month 12 (± 30 days after SPI)

1. Incremental medical history

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2. Physical exam including vitals (BP, temperature, pulse rate) and weight
3. Laboratory examinations (Section 6.3)
4. NYHA classification (Appendix B)
5. cMRI (Section 6.6.3, MRI Core Lab Manual of Operations)
6. Questionnaire: MLHFQ (Section 6.6.2)
7. 12-lead ECG
8. 6MWT (Section 6.6.1, Protocol Manual of Operations)
9. A standard of care visit with subject oncologist (or post-cancer follow-up care provider) within 3 months after study completion (to assess cancer recurrence during the study period).

6.5.5.1 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 6.7.3).

6.5.6 Month 24 Telephone Contact

A Month 24 telephone contact will be made to assess current medications, as well as morbidity and mortality in study subjects.

6.5.7 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 6-month visit will be at 180 ±30 days.
4. The 12-month visit will be at 365 ±30 days.
5. The 24-month phone call will be at 730 ±30 days.

6.5.8 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.

6.6 Collection of Endpoints

6.6.1 Six-minute Walk Test (6MWT)

6MWTs will be performed to assess functional capacity. Subjects will perform a 6MWT during 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. The tests will be done at consistent intervals.

6.6.2 Minnesota Living with Heart Failure Questionnaire (MLHFQ)

The subjects' quality of life will be assessed with the MLHFQ during baseline and at 6, and 12 months. The questionnaire includes details of subjects' symptoms specifically related to functional status and development of HF.

6.6.3 Cardiac Magnetic Resonance Imaging (cMRI) Evaluation

Cardiac MRI is a new diagnostic modality that provides superior assessment of the cardiac function, myocardial scar size measurement, and myocardial perfusion evaluation. Steady state free precession (SSFP) cardiac MR imaging will provide valuable information regarding cardiac anatomy and will assess the myocardium, including myocardial mass and zones of acute infarction. Cine MRI will also be performed on study patients and it is used to evaluate left and right ventricular function and size, and valvular and shunt lesions. Contrast-enhanced MRI will also be used to assess myocardial viability and scar quantification.

In addition to these more traditional cardiac MR techniques, we will be using T1 myocardial mapping techniques (at centers which have this capability and are qualified by the MRI Core Lab) to help garner a more complete picture of the myocardial condition. Recently, T1 mapping has been shown to effectively demonstrate fibrotic changes in myriad pathologic conditions[114-116]. While the delayed gadolinium enhancement is very sensitive to small areas of regional fibrosis, the method is dependent on the comparison to the signal from “normal myocardial” reference areas. In patients with more diffuse fibrosis (such as those with AIC), T1 mapping will be an essential adjunctive tool. Preliminary studies involving AIC and T1 mapping are promising[117].

In addition to the evaluation of myocardial fibrosis, the utility of cardiac MR studies for gauging functional indices is also quite robust. However, while LVEF has been used as a “gold standard” of therapeutic response, it may lack the sensitivity required to measure subtler, yet clinically relevant changes. Conceptually, this correlation between decreased strain and clinical events has been well described. One seminal study included 147 patients with heart failure with LVEF ≤ 45% (mean age, 64 ± 14 years; 74% men; mean LVEF, 29.9 ± 8.9%)[119]. Clinical events were observed in 20% of patients during the 12-month follow-up period and global longitudinal strain was shown to have the highest prognostic value over conventional EF measurements with an area under the curve of 0.83 using a cutoff of -7%[119]. Specifically, in chemotherapy-induced cardiomyopathies, strain has been shown to have greater sensitivity and have similar predictive value[120]. Hence, using cardiac MR studies to measure these more sensitive indices of function may allow for a more informative and conclusive study of cell therapy mechanisms and benefits in this setting. Tagged MRI will be performed, and global and regional myocardial strains and strain rates will be computed using the harmonic phase method.
This information, obtained before and after study product injection, will be crucial in estimating the benefit gained by injecting stem cells as well as evaluating the potential side effects.

Cardiac MRI will be performed in all patients at baseline and at 6 and 12 months after SPI. 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.

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 it for 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.

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 Principal Investigator and the interventionalist 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.

6.6.3.1 Performance of MR examinations in subjects with implantable cardiac devices (ICD/pacemakers)

The presence of a pacemaker or implanted defibrillator device is not a contraindication to MRI scanning[121,122].

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"[121]. 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 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[123] 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, experienced personnel with large volume experience in MR imaging 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.

6.7 Collection of Biospecimens

A central CCTRN biorepository will be utilized in this study. The biorepository will be included in the consent form and subjects will have the option of participating in the 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 overall trial.

The goal of this biorepository is three-fold: 1) to provide storage of critical biomaterials (i.e., subject peripheral blood and donor allo-MSC product) 2) to provide long-term integrity (up to 10 years) of these biospecimens and products, and 3) to provide management of samples for ancillary studies of immunologic, immunohistochemical, cellular, and molecular analyses of donor allo-MSC product and subject serum 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 biomaterials 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. Biomaterials will be destroyed after 10 years.

6.7.1 Peripheral Blood

Twenty ml of peripheral blood will be collected on Day 0 (day of injection, before sedation for SPI procedure), Day 1 (within 24 hours after SPI), Week 1, 1 month, and 6 months. This blood will be sent to the CCTRN biorepository per core lab SOP.

6.7.2 Allo-MSC Allografts

A sample of the allo-MSC cell bank will be provided to the CCTRN biorepository for additional characterization studies.

6.7.3 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. With appropriate consent, the explanted heart will be sent to the CCTRN biorepository for further study.

All surplus biospecimens (bone marrow and blood) will be cryopreserved up to 10 years.

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 and 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 electronic case report form (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 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 (six subjects)

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

1. Six subjects who consent and meet all inclusion and exclusion criteria will receive allo-MSC cell therapy per protocol.
2. All subjects will be observed for any complications or adverse effects of electromechanical mapping or delivery of cell therapy.
3. Events will be reported to regulatory oversight groups as outlined above in section 7.4.
4. The DSMB will review 1 month safety and feasibility data from each of the six subjects. These data will also be forwarded to the FDA in a subsequent amendment.
5. Upon successful DSMB review of the lead-in data, the study will be permitted to recruit the remaining approximately thirty subjects into both the active and placebo groups to the specified protocol sample size.

8.0 STATISTICAL PROCEDURES

8.1 Randomization Strategy and Monitoring

Randomization, or the random allocation of therapy, is a well-accepted mechanism for reducing potential bias in evaluating treatment effects. Randomization process, timing, and notification details are provided in section 4.6 above. Subjects will be randomized either to allo-MSCs or to placebo stratifying by center and using block sizes of 2.

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 Delays and Early Withdrawal from the Study

There may be circumstances in which randomized subjects experience a delay in receipt of study product. There may also be circumstances that do not allow for randomized subjects to receive study product. These circumstances are discussed further below. All randomized subjects who do not receive SPI in the expected timeframe will have their case reviewed by the Medical Monitor and SENECA Center PI to assess whether the subject should be withdrawn immediately or delay study procedures for another attempt.

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

1. A randomized subject who has a resolvable change in their clinical condition, and for whom the product has not yet been prepared for injection, will be allowed to have their SPI postponed.

2. If a cell processing related issue arises on the day of the SPI procedure and additional product can be delivered at a later time, the subject will have their SPI postponed.

Length of postponement will be determined by the Medical Monitor and SENECA 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.
In this first-in-human, phase I trial, SENECA's principal analysis will be "intention-to-treat". The anticipated small number of patients who are randomized but do not receive treatment will have the reason for non-treatment tabulated.

8.3 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. The specific analysis plans follow:

8.3.1 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 and psychosocial data. Exact testing for categorical variables and Wilcoxon two sample rank sum test for continuous variables will be used to evaluate the differences in baseline variables between treatment groups.

8.3.2 Outcome Analyses

All analyses will be performed on two cohorts; (1) the randomized cohort, and (2) the total cohort (randomized plus the six patients who received open label therapy in a nonrandomized fashion).

8.3.2.1 Safety Evaluations

To demonstrate the relative safety of allo-MSCs when compared with placebo, the following analyses will be carried out, and the data tabulated and analyzed by therapy group (Fisher's Exact Test) between baseline and a) 6 months and b) 12 months:

1. Major adverse cardiac events (MACE) including death, hospitalization for worsening heart failure (HF), and/or exacerbation of HF (non-hospitalization)
2. Other significant clinical events including non-fatal stroke, non-fatal myocardial infarction, coronary artery revascularization, ventricular tachycardia/fibrillation, pericardial tamponade, infectious myocarditis, hypersensitivity reaction, neoplasm, or any other potential deleterious late effects detected and corroborated by clinical presentation, laboratory investigations, image analysis, and when necessary with biopsy from suspected target sites in the body
3. All adverse events (AEs) at least grade 2 in severity (see section 7.3.1)

8.3.2.2 Feasibility Evaluations

To assess the practicality of study procedures, the following measures will be reported.

The number and percent of subjects who:

1. Have events between randomization and SPI that preclude the subjects from getting SPI
2. Receive less than 20 injections during the SPI procedure
3. Do not receive the intended dose of cells (100 million), reason, and actual dose delivered
4. Have 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
5. Fail to complete follow-up

8.3.2.3 Prospectively Declared Efficacy Endpoints

To explore whether allo-MSCs produce a trend toward improved LV function and functional status when compared with placebo, the following domains[111] and measures will be evaluated:

- 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 area of injury (e.g., inflammation, edema, fibrosis)
- Functional capacity over time:
  - Change in exercise tolerance (6MWT)
  - Change in MLHF Questionnaire (subject reported)
- Clinical outcomes over time:
  - MACE
  - Change in days alive and out of hospital
- Biomarkers over time:
  - Change in NT-proBNP

All endpoints listed above are continuous and will be assessed with the following approaches.

1. Examination of departures from normality will be assessed. A general linear mixed model approach: The within subject component of the twelve-month evaluation will reflect the measures obtained at baseline, six months, and twelve months. The between subject component will reflect the effects of the cell types (allo-MSCs versus placebo). We are interested in testing whether the trajectory over time of the change in each of the endpoints above is modified by treatment (allogeneic versus placebo.)
2. The Wilcoxon two sample rank sum test, comparing the changes in the endpoint variables from a) baseline six months and b) baseline to twelve months.

8.4 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 age, gender, race, diabetes, hypertension, presence of a cardiac device, type of malignancy, baseline LVEF≤40% vs >40%, and number of cells delivered. For each endpoint, the effect of therapy will be determined within each subgroup stratum using the mixed general linear model with the subgroup strata as an explainer variable collection (i.e., a subgroup with *p* stratum will have *p*-1 indicator variables in the model

models are likely to be underpowered in this relatively small study. 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.

8.5 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 in 8.3 above.

8.6 Multiplicity Issues

This is the first study evaluating the effect of mesenchymal cells in patients with anthracycline-induced cardiomyopathy. No adjustments will be 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. Because of the high false discovery rate in our study, the findings from our study cannot be accepted by the research community as confirmatory, but must be treated as preliminary, requiring an additional study to confirm the SENECA results.

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 Study Product Injection

9.1.2.1 Cardiac Catheterization and NOGA Mapping

Potential risks of this procedure include bleeding, hematoma at catheter insertion site, allergic reaction to the angiography dye, formation of a blood clot which could lead to loss of function or surgical intervention. It is possible the patient 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 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 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. Some radiation is involved as part of the NOGA study.

9.1.2.2 Cell Processing

For those patients receiving the allo-MSC cell product, it is possible that the body might react and reject the allo-MSC cells. Human clinical research studies with allo-MSCs in heart patients have provided evidence against the occurrence of rejection of the transplanted cells and also against the occurrence of GVHD, where healthy infused cells recognize the tissues of the person receiving the cells as "foreign" and mount an immunologic attack.
Processing the cells is done under strict sterile conditions; however, there is a rare chance that the cells could get contaminated while being processed. Testing will be done on the cells; however, it takes about 2 weeks to get the final results. If the tests show cells were contaminated, the treatment team will be notified so that the patient may be treated with antibiotics. Subjects will be taking their temperature twice a day for one week, which may help determine infection before the test results are known.

There may be some circumstances where the patient is unable to receive the processed cells; such as a processing failure or poor quality of the cells. If events such as these occur, the patient will not receive the allo-MSC cells but will be asked to continue with follow up in the study. Processes in place to prevent these failures include: the use of standard operating procedures for preparing the allo-MSC cell product, which will identify any problems with the study product before it is released, and continuous temperature monitoring of the product during shipment to the treatment facility.

9.1.2.3 Study Product Injection

Possible problems that might happen include (but others could occur): decreased blood pressure, irregular heartbeats, possible firing of ICD, chest pain or discomfort, 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 quantity 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.

9.1.2.4 MRI Procedure

Risks associated with administration of contrast dye are nausea, vomiting, and headache. 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 MRIs. 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 don't react to an MRI's magnetic field and will be monitored during the MRI scan. There is now enough experience with this strategy, which has proven to be safe. 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.

9.1.2.5 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 1.2 rem.

9.1.3 Adequacy of Protection Against Risks

Overall the study procedures are low risk and in our previous similar trials there were no complications related to study product delivery.

9.1.4 Potential Benefits of the Proposed Research to the Subjects and Others

Subjects with AIC 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 allo-MSCs offers a new therapeutic option to subjects with LV dysfunction secondary to AIC; the goal of this new treatment is not just to reduce the rate of LV deterioration but to actually ameliorate heart failure. Having highly trained ex[illegible]ver 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 that 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 whether a promising cell type that heretofore has demonstrated the likelihood of few risks to the subject is well-tolerated by subjects with LV dysfunction secondary to AIC; and 2) determine if a dose of 100 million allo-MSCs delivered intramyocardially produces improvement in measures of myocardial function. The trial has been designed to address a novel area of study for a no-option group with a grim prognosis. The risks to the subjects are reasonable in relation to the knowledge gained from this study since this therapy may potentially reverse AIC, which is a leading cause of morbidity and mortality throughout the world.

9.1.6.1 Data and Safety Monitoring Board (DSMB)

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

9.2 Clinical Monitoring

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 follow

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 requirements 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 laborato

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 is 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 IND for this study and submit proper filings to obtain and maintain the IND. The DCC will submit all appropriate reports and fillings to the FDA as required by regulations. This includes unanticipated adverse events, 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 secure login authentication. The DCC will provide training and documentation to laboratory personnel on the data entry process and will be available to answer question and resolve issues as necessary. The data collected will be stored on a secure database in the DCC and will be backed up every night. Reports will be generated as necessary with real-time data.

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

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

9.6.1 Web Site

The web site has been created with objectives targeted to the study audience. The CCTRN web site will serve as one method of distribution of information about stem cell research in cardiovascular disease in general and about the specific study protocols. For the general lay public, the goal is to promote a hospitable context for the research by informing the public about the kinds of research being done, including the source of the stem cells; what this research is and what it isn't; plans for studies; study findings; and the potential for new treatments. Physicians need information about the research that is closely t ical trial opportunities

and potential 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.

9.6.2 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.

9.6.3 Methodologic Developments

In addition to providing statistical support to PIs at CCs and NHLBI, Investigators at 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: Normal Donors for Generation of Allo-MSCs

Procurement of Bone Marrow for allo-MSC Production

Bone marrow (BM) will be obtained from a normal volunteer, aged between 18 and 45, undergoing bone marrow harvest for transplantation to an allogeneic stem cell transplant recipient. 25-50 ml (+/-10 ml) additional marrow will be aspirated from the posterior iliac crest. The BM will be aspirated into pre-heparinized syringes containing preservative-free heparin for a final effective dose of ~100 units of heparin / ml of BM. The MSCs will be isolated and expanded to a final dose of $1x10^8$ allo-MSCs according to standard operating procedures (SOPs) of the CCMF.

Eligibility Criteria

1. The donor must meet the eligibility criteria for normal allogeneic bone marrow donors established by the FACT accredited Stem Cell Transplant Program at Baylor College of Medicine, Houston, Texas.
2. In addition, the donor must meet the eligibility requirements established under the Code of Federal regulations Title 21 CFR Part 1271 Subpart C – Donor Eligibility, which require official documentation of donor eligibility for donors of HCT/P based upon infectious disease testing, risk behaviors and clinical examination.

Informed Consent

Informed consent to donate additional bone marrow shall be obtained and documented. The procedure shall be explained in terms the donor can understand, and shall include information about the significant risks of the procedure. The donor shall have the right to review the results of tests. The donor shall have the opportunity to ask questions and the right to refuse to donate.

Follow-up Schedule

After discharge from the hospital, the BM donor will be followed up according to standard operating procedures in place at the Stem Cell Transplant Program at Baylor College of Medicine, Houston, Texas and described in the informed consent documentation. The donor will be provided with contact telephone numbers in the consent form for any questions or comments.

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Appendix B: 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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