PROTOCOL

TITLE: A randomized, Multi-center, Double-Blind, Placebo-Controlled Phase IIa Study of the Safety and Efficacy of Intravenous Delivery of Allogeneic Mesenchymal Stem Cells in Patients with a Cardiomyopathy and Severe Heart Failure who Required Implantation of a Left Ventricular Assist Device (LVAD)

IND NUMBER: **IND 18457**

PROTOCOL NUMBER: **STEMVAD-001**

INVESTIGATIONAL

PRODUCT: Allogeneic Mesenchymal Bone Marrow Cells, (MSCs) (adult human)

MEDICAL MONITOR: **William S. Weintraub, MD**

SPONSOR: MedStar Heart and Vascular Institute

CENTERS: Up to 2

CONTACT:

Samer Najjar, MD

MedStar Heart and Vascular Institute
110 Irving St NW, Suite 1F-19
Washington, DC 20010
Ph: 202-877-8085
Fax: 202-877-8032

PROTOCOL ACCEPTANCE FORM

TITLE:	A randomized, Multi-center, Double-Blind, Placebo-Controlled Phase IIa Study of the Safety and Efficacy of Intravenous Delivery of Allogeneic Mesenchymal Stem Cells in Patients with a Cardiomyopathy and Severe Heart Failure who Required Implantation of a Left Ventricular Assist Device (LVAD)
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I have read the protocol, including all appendices, and agree that it contains all necessary details for my staff and me to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I understand that I am not allowed to make changes to this protocol.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by CardioCell, Inc. I will discuss this material with them to ensure that they are fully informed about the investigational products and the study, and with Good Clinical Practice (GCP).

Principal Investigator's Name (print)
Samer Najjar, MD

_____ _____

Principal Investigator's Signature Date

PROTOCOL SYNOPSIS

TITLE: A randomized, Multi-center, Double-Blind, Placebo-Controlled Phase IIa Study of the Safety and Efficacy of Intravenous Delivery of Allogeneic Mesenchymal Stem Cells in Patients with a Cardiomyopathy and Severe Heart Failure who Required Implantation of a Left Ventricular Assist Device (LVAD)

PROTOCOL NUMBER: **STEMVAD-001**

INVESTIGATIONAL PRODUCTS: Allogeneic Mesenchymal Bone Marrow Cells, (MSCs) (adult human)

PHASE:	IIa
INDICATION:	heart failure with an implanted LVAD
IND/IDE NUMBER:	IND 18457
SPONSOR:	MedStar Heart and Vascular Institute

ABBREVIATIONS

Abbreviation	Definition
2 D	Two Dimensional
3 D	Three-Dimensional
ACE	Angiotensin-Converting Enzyme
AE	Adverse Event
aGVHD	Acute Graft-Vs-Host Disease
AHA	American Heart Association
ALT	Alanine Amino Transferase
AMI	Acute Myocardial Infarction Cardiomyopathy
AST	Aspartate Amino Transferase
Bi-VAD	Biventricular Assist Device
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CABG	Coronary Artery Bypass Graft Surgery
CAD	Coronary Artery Disease
CBC	Complete Blood Count
CD	Cluster Of Differentiation
CK	Creatine Kinase
COPD	Chronic Obstruction Pulmonary Disease
CRF	Case Report Form
CRP	C-Reactive Protein
CTA	Computed Tomography Angiography
CV	Curricula Vitae
DNA	Deoxyribonucleic Acid
DSMB	Data And Safety Monitoring Board
ECG	Electrocardiogram
Fas	First Apoptosis Signal
FDA	Food And Drug Administration
FEV1	Forced Expiratory Volume In One Second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GCSF	Granulocyte-Colony Stimulating Factor
HF	Heart Failure
HFrEF	HF With Reduced Left Ventricular Ejection Fraction
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HS-CRP	High Sensitivity CRP
ICAM	Intercellular Adhesion Molecule
ICD	Implantable Cardioverter-Defibrillator
ICF	Informed Consent Form
ICH	International Conference On Harmonization
ICM	Ischemic Cardiomyopathy
IFNγ	Interferon Gamma
IgA	Immunoglobulin A
IgE	Immunoglobulin E

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHF	Ischemic heart failure
IL	Interleukin
IND	Investigational New Drug
IP	Interferon Gamma-Induced Protein
IRB	Institutional Review Board
KCCQ	Kansas City Cardiomyopathy Questionnaire
LAD	Left Anterior Descending Coronary Artery
LFTs	Liver Function Tests
LRS	Lactated Ringer's Solution
LV	Left Ventricular
LVAD	Left Ventricular Assist Device
LVEF	Left Ventricular Ejection Fraction
MCP	Monocyte Chemoattractant Protein
MDRD	Modification of Diet In Renal Disease
MedDRA	Medical Directory For Regulatory Activities
MHC	Major Histocompatibility
MIF	Macrophage Migration Inhibitory Factor
MSCs	Mesenchymal Stem Cells
MUGA	Multiple Gated Cardiac Blood Pool Imaging
NICM	Non-Ischemic Cardiomyopathy
NK	Natural Killer
NOD/SCID	Severe Combined Immunodeficiency
NT-proBNP	N-Terminal Prohormone Of Brain Natriuretic Peptide
NYHA	New York Heart Association Classification
PAI	Plasminogen Activator Inhibitor
PCI	Percutaneous Coronary Intervention
QTc	Rate-Corrected QT Interval
RAGE	Receptor For Advanced Glycation End Products
RV	Right Ventricular
SAE	Serious Adverse Event
SDF	Stromal Derived Factor
SPECT	Single-Photon Emission Computerized Tomography
ST-2	Soluble Suppression Of Tumorigenicity 2
TNF alpha	Tumor Necrosis Factor Alpha
TREM	Triggering Receptor Expressed On Myeloid Cells
uPAR	Urokinase-Type Plasminogen Activator Receptor
VCAM	Vascular Cell Adhesion Molecule
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cells

BACKGROUND

1 Left ventricular assist device therapy for advanced ischemic heart failure

1.1 Heart failure

Ischemic heart failure

Coronary artery disease (CAD) is one of the two most common risk factors for heart failure (HF) and portends poor outcomes[1-5]. Ischemic etiology of HF is defined as 1) a prior history of myocardial infarction (MI), or 2) coronary revascularization (coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI)), or 3) obstructive CAD (≥75% stenosis of the left main or proximal left anterior descending coronary artery or ≥75% stenosis of two or more major epicardial coronary arteries).

Non-ischemic heart failure

Non-ischemic heart failure is defined as heart failure caused by mechanisms other than myocardial ischemia or infarction. Dilated cardiomyopathy is the most common cause of non-ischemic heart failure.

1.2 Left ventricular assist devices

Advanced HF is refractory HF in which the disease has progressed despite standard medications to a point where there is a need for cardiac replacement therapies. Approximately 10% of the 3 million patients with HF with reduced left ventricular ejection fraction (HFrEF) in the United States (US) have advanced (end stage or stage D) HF (AHF) and are in need for cardiac replacement therapy or end of life care[6-9]. Because of the limited supply of cardiac donors (approximately 3273 hearts transplanted in the US in the year 2017) and instability of some transplant candidates awaiting organs, durable left ventricular assist devices (LVADs) have emerged as an alternative or as a bridge to transplantation[6, 10]. Improvement of LVAD technology has revolutionized the field of AHF, with markedly improved survival and quality of life for thousands of individuals compared with medical therapy alone. Hence, the rate of LVAD utilization is steadily growing in the US and worldwide[10].

However, despite mechanical support of the left ventricle, approximately 50% of the patients die over the course of the 4 years following LVAD implantation[10]. Right ventricular dysfunction and failure frequently occurs in the LVAD population and contributes to the high incidence of re-hospitalization and mortality[10]. Adding to the complexity is the fact that progressive right HF augments susceptibility to developing one or more of the other

causes of death in these patients.

Multiple mechanisms are postulated to contribute to right HF after LVAD implantation. Inflammation contributes to progressive myocardial dysfunction in acute myocardial infarction and in chronic HF of ischemic or non-ischemic etiology[11-15] and inflammatory cytokines are higher in HF patients with LVAD than HF or healthy controls and remain persistently elevated during at least 9 months of follow up[16, 17]

1.3 Persistent inflammation as a cause of progressive cardiac dysfunction.

1.3.1 Overview. Considerable evidence has now emerged that is compatible with the concept that the progressive deterioration of cardiac function seen both in acute myocardial infarction (AMI) and ischemic cardiomyopathy (ICM)/non-ischemic cardiomyopathy (NICM) is in part caused by an excessive, persistent inflammatory response11, 13-15, 18 (Fig 1.1)

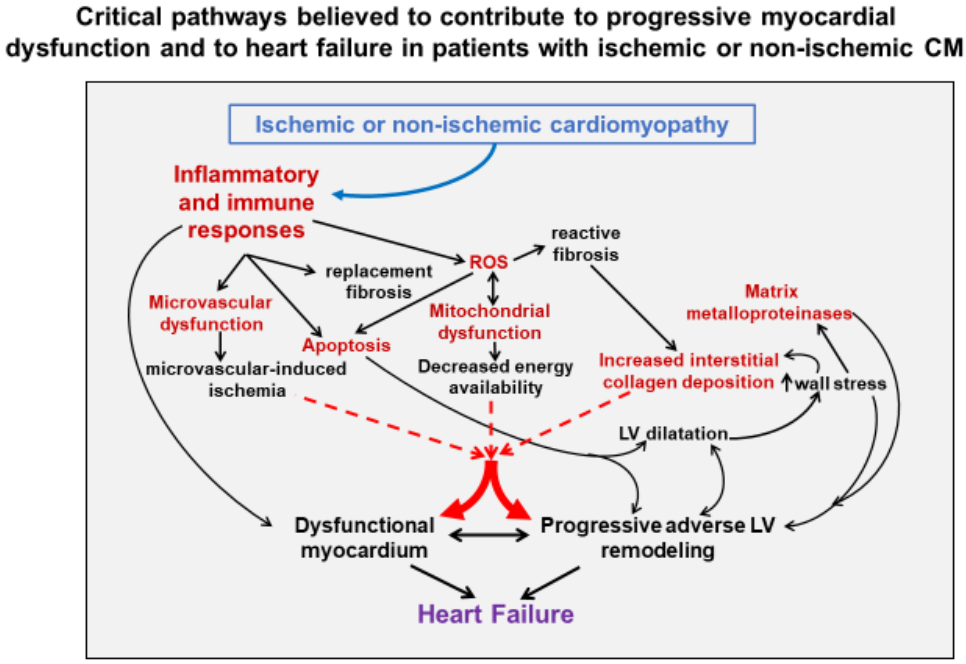

Figure 1.1. Inflammatory and immune responses, influencing a diverse array of pathways, importantly contribute to progressive myocardial dysfunction and adverse LV remodeling observed in patients post-AMI or with cardiomyopathy.

1.3. 2. Proof of the validity of this concept was recently provided by the results of investigations in mouse models of AMI and of ICM. We developed a monoclonal antibody approach to depleting natural killer (NK) cells, which are major orchestrators of the inflammatory response. Intravenous administration of the anti-NK antibody

depleted NK cells from the spleen and from the heart, and decreased myocardial neutrophils. Importantly, these anti-inflammatory effects led to marked improvements in LV remodeling and function (See figure 3.2, 3.3, and 3.5 and related text).

1.3.3 Specific data indicating the presence of a marked inflammatory response in patients with LVAD implantation.

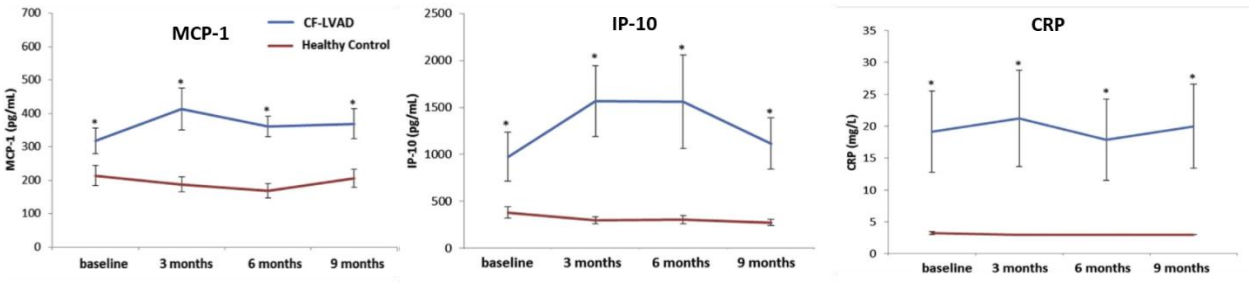

Figure 1.2. Evidence that a persistent inflammatory response exists in LVAD patients.

Figure 1.2 derives from the study by Grosman-Rimon and colleagues[17]. They investigated the levels of inflammatory markers in continuous flow-LVAD recipients before and after LVAD implantation. They found that, compared to normal, common inflammatory biomarkers (MCP-1, IP-10, and C-reactive protein (CRP)) are markedly elevated and that, despite improvements in LV dimensions and BNP levels after LVAD implantation, markers of inflammation remained markedly elevated in the LVAD recipients.

We therefore hypothesize that the same disease process that contributes importantly to progressive LV dysfunction in patients with AMI and with chronic HF--persistent inflammation--also contributes to progressive right ventricular (RV) dysfunction in LVAD patients.

2 MESENCHYMAL STEM CELLS

2.1 Modifying MSCs for enhanced production rate and enhanced functionality

Experimentally, several modifications in MSCs have resulted in their enhanced functionality[19-23]. The strategy we have adopted for optimization of MSC functionality *for preclinical studies and clinical translation is use of MSCs grown in hypoxic culture conditions*[24-26].

2.2 Potentiation of MSCs with Hypoxic Culture

Stem cells reside in defined microenvironmental niches and oxygen conditions critically regulate cellular responses. For example, oxygen tension in human bone marrow is ~3.0%. Hypoxic culture conditions can maintain MSCs in an undifferentiated state that favors stem cell self-renewal. It enhances expression of genes involved in developing mesodermal and non-mesodermal cell lines, increases multipotency and transdifferentiation, increases the half- life of vascular endothelial growth factor (VEGF) mRNA significantly (6–8h in low oxygen conditions compared to 30–40 minutes in normoxia), and decreases transversions from oxidative damage[25-27].

2.3 Potency of MSCs Cultured in a Hypoxic Environment

Stemedica has obtained a patent for culture of human donor MSCs in hypoxic conditions. The biological potency of these cells has been studied in *in vitro* experiments and compared to MSCs cultured in normoxic conditions. MSCs grown in hypoxic conditions formed more colonies in culture assays, had a greater proliferation rate, produced greater quantities of VEGF, Stromal derived factor 1 (SDF-1), HIF-1alpha, and angiopoeitin-1. These MSCs also demonstrated greater migratory capacity in response to chemokines and cytokines. In summary, MSCs grown under hypoxic conditions compared to those grown in normoxic conditions have 1) a higher proliferation rate, 2) higher clonogenicity as determined by colony forming unit assay, 3) increased production of cytokines participating in homing of host stem cells to the site of injury (SDF-1) and in neovascularization (VEGF), 4) higher resistance to factors adversely affecting performance of MSC at the site of injury such as tumor necrosis factor, and 5) increased migration towards the factors produced at the ischemic site such as Hepatocyte Growth Factor. *These properties of the MSCs we will be using in this trial—Stemedica's MSCs grown under hypoxic conditions from harvest until injection into the patient--will likely increase those activities involved in myocardial healing and thereby stand a greater chance of leading to beneficial effects on myocardial dysfunction. All of the following preclinical studies we report below that originated in our preclinical laboratory were performed using Stemedica's MSCs.*

3. MSCs improve LV function in experimental AMI and in ICM in large part through their paracrine-induced anti-inflammatory activities.

Until recently, the prevailing view of the mechanisms responsible for any potential benefit of stem cells in patients with acute myocardial infarction (AMI) or with HF was that benefit derived from local effects—once engrafted in damaged myocardium, the stem cells either transdifferentiate into functional myocardium, stimulate resident myocardial stem cells to expand and repopulate the heart with functioning myocytes, or secrete substances leading to myocardial healing. This mechanistic perspective implied that the greater the number of engrafted cells in the myocardium, the greater the cardiac benefit. Because few intravenously administered stem cells engraft in injured myocardium, invasive strategies providing direct delivery of stem cells to the heart were uniformly adopted. This necessarily involved either catheter-based delivery (intracoronary or transendocardial injection), or surgical delivery (direct intramyocardial injection).

A transformative concept relating to stem cell treatment of patients with AMI or with HF has recently evolved—i.e., that the *intravenous* administration of stem cells will improve left ventricular (LV) function in patients with AMI or with HF and that the major mechanism responsible for this resides in the *systemic anti-inflammatory effects of stem cells*[12].

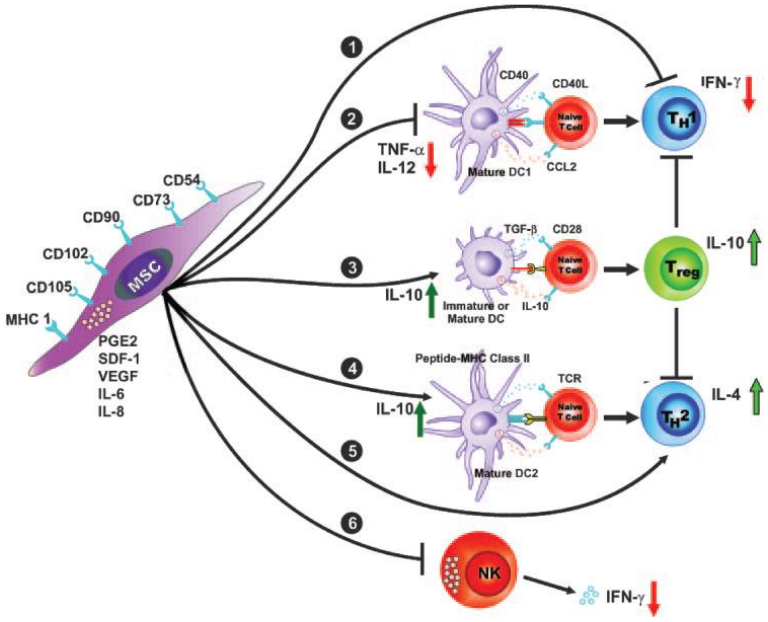

Figure 3.1. MSCs secrete multiple anti-inflammatory molecules (From Aggarwal et al[22]).

The concept's validity derives from compelling preclinical data and from two new mechanistic insights; first, that the progressive deterioration of cardiac function seen both in AMI and HF is, as noted above, in part caused by an excessive, persistent inflammatory response[7, 11, 13, 14, 28]; and second, that if stem cells improve cardiac function, improvement is not caused by repopulating the myocardium with new myocytes, but rather by stem cells secreting numerous molecules with a diverse array of activities[11, 13, 29-31]. These paracrine activities include those related to angiogenesis, tissue healing, apoptosis, mitochondrial dysfunction, microvascular dysfunction, collagen deposition[4] and, perhaps most importantly, through potent *systemic* anti-inflammatory actions[12, 11, 14, 32]

(Fig 3.1) These anti-inflammatory effects cause *in vivo* suppression of both innate and adaptive immune responses to AMI[11, 14, 15, 18, 32].

As examples of the anti-inflammatory effects of MSCs, *intramyocardial* injection of MSCs in a rat model of AMI reduces myocardial levels of TNF-α, IL-1β, IL-6, MMP-1, TIMP-1, and reduces collagen deposition. These changes are associated with reduced infarct size and improved LV function[33]. In addition, it was shown in mice with AMI that *intravenously* administered MSCs improve LV function and decrease inflammation through factors secreted by MSCs lodged in organs outside the heart[33]. Because immunocompromised (NOD/SCID) mice were used in this study, it was uncertain what the paracrine-mediated immunomodulatory and salutary cardiac effects of the MSCs would be in an immunocompetent animal.

Our group addressed this issue and demonstrated in immunocompetent mice that intravenously administered human MSCs 24h following AMI decreased splenic and myocardial NK cells, as well as myocardial neutrophils[14] (Fig 3.2).

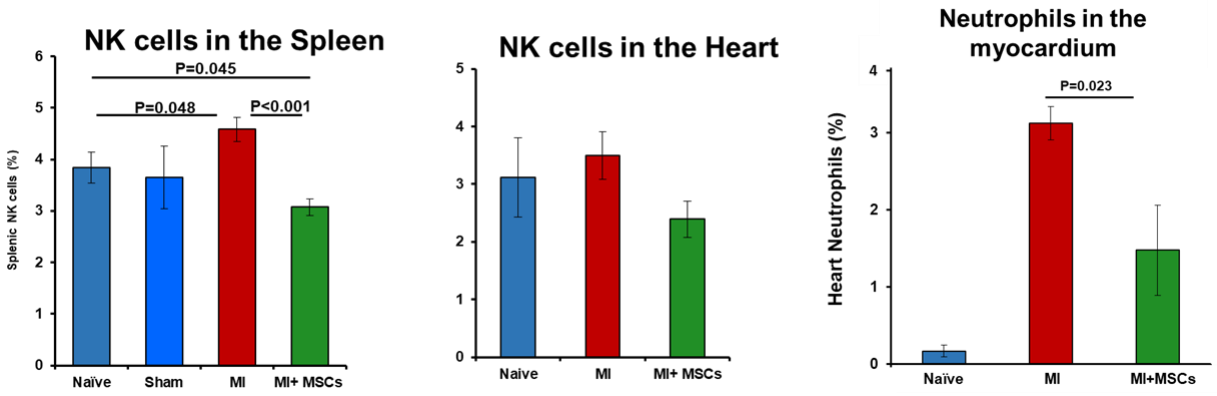

Figure 3.2. Anti-inflammatory effects of IV administration of MSCs. MSCs were injected IV 24h post AMI and hearts and spleens were harvested 7 days post AMI.

These anti-inflammatory effects were associated with significantly attenuated adverse LV remodeling in mice with large infarcts. We recently performed a similar experiment in mice with chronic ICM[14](Fig 3.3).

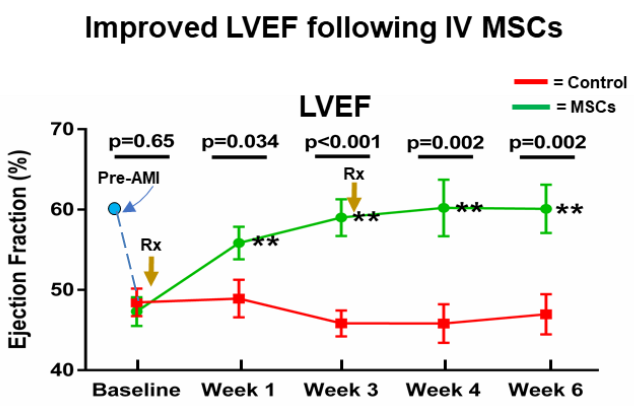

Figure 3.3. Effects of IV administration of MSCs in mice with ischemic cardiomyopathy.

Infarcts were produced by LAD ligation followed in 45 minutes by restoration of flow; by 4-weeks the mice developed LV dysfunction with decrease in left ventricular ejection fraction (LVEF) and adverse remodeling. IV injection of MSCs at 4 weeks after AMI (Time 0) and then repeated 3 weeks later led to marked improvements in ejection fraction and LV remodeling[14].

Thus, compelling preclinical evidence indicates that intravenously administered MSCs improve cardiac outcomes in AMI and in ICM, and appear to do so, at least in part, through anti-inflammatory activities. The inflammatory targets are in large part involved in systemic pathways originating in multiple tissues responsible for responding to tissue injury and generating inflammatory responses, such as the spleen.

In this regard, an important concept relating to the initiation and propagation of inflammatory responses resides in the concept of a cardiosplenic axis. Myocardial injury (indeed, injury involving any tissue) signals the spleen, either through neural or humoral pathways, causing increased splenic inflammatory cell populations, with subsequent release of these cells into the circulation where they then home to sites of injury[34]. A cardiosplenic axis may also participate in HF, as spleens of mice with ICM are enlarged, and splenectomy not only decreases cardiac macrophages and dendritic cells but also attenuates adverse LV remodeling[35].

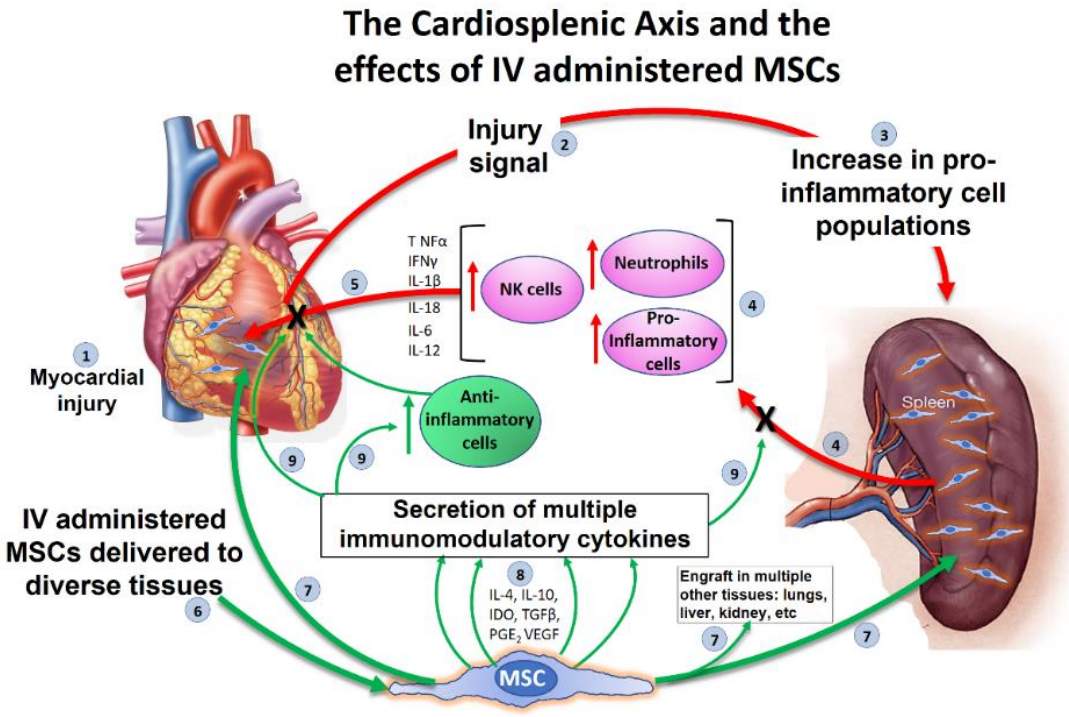

Figure 3.4. The cardiosplenic axis and the target of IV injected MSCs. (From Epstein et al[13]) Inflammatory and immune responses, influencing a diverse array of pathways, importantly contribute to progressive myocardial dysfunction and adverse LV remodeling. IV administered MSCs inhibit many of these proinflammatory pathways, an effect constituting one of the major mechanisms responsible for the beneficial myocardial effects of this intervention. [IL-1β indicates interleukin-1 beta; IL-6, interleukin-6; PGE2, prostaglandin E2; TNF-α, tumor necrosis factor alpha; and VEGF, vascular endothelial growth factor.]

The capacity of MSCs to decrease NK cell activity and other components of the innate immune system in the setting of AMI is well known. We were particularly interested in the question of whether MSC-induced reduction in NK cells played a *causal* role in improving myocardial function following AMI, as NK cells play a major role in coordinating the innate immune response. We proved the validity of this hypothesis by demonstrating that when NK cells were depleted not by MSCs but by an anti-NK cell antibody administered 24h prior to AMI, infarct size significantly decreased and LV function significantly improved[14] (Fig 3.5).

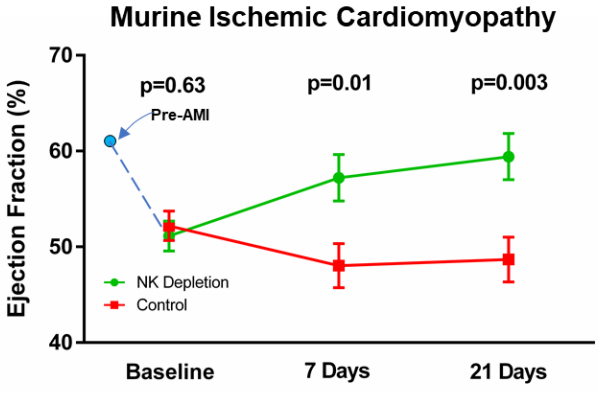

Figure 3.5 Proof that the anti-inflammatory effects of MSCs are critical to their ability to improve LV function in ischemic cardiomyopathy: effects of primary antibody-induced NK cell depletion. (From Luger et al[14].) Injection of an antibody 4 weeks post AMI that induces NK cell depletion improves LV function and adverse remodeling.

Of note, improved LVEF occurs within 7 days of administration, suggesting that one mechanism contributing substantively to the impaired LV function is caused by the presence of dysfunctional, but still viable, myocardium. We hypothesize that the dysfunctional but viable myocardium responds to the anti-inflammatory activities of the MSCs (and perhaps to other MSC-based effects) such that contractile activity is restored.

4. ***Anti-inflammatory effects of IV administration of MSCs in patients with NICM and correlation with the magnitude of MSC-induced decrease in NK cells with improved left ventricular ejection fraction.***

One of the investigators of the present trial was co-PI of a trial testing the effects of IV administration of MSCs in patients with NICM[36]. The MSCs used in the present trial will derive from the same donor and will have undergone the same growth and expansion methods (undertaken by Stemedica) as were used in the prior trial. The study was a Phase 2a randomized, single blind, placebo controlled, crossover, multicenter trial consisting of 23 patients with NICM. Crossover occurred at 90 days after study initiation and study termination occurred at 180 days.

Compared to placebo, IV MSCs:

- Significantly increased 6-minute walk distance.
- Significantly improved Kansas City Cardiomyopathy scores.
- However, there were no significant changes from baseline in LV function between the two groups.

Of great interest, IV administration of MSCs decreased circulating NK cells, and *the magnitude of MSC-induced decrease in NK cells correlated with the magnitude of improvement in LV function*[36] (Fig 4.1).

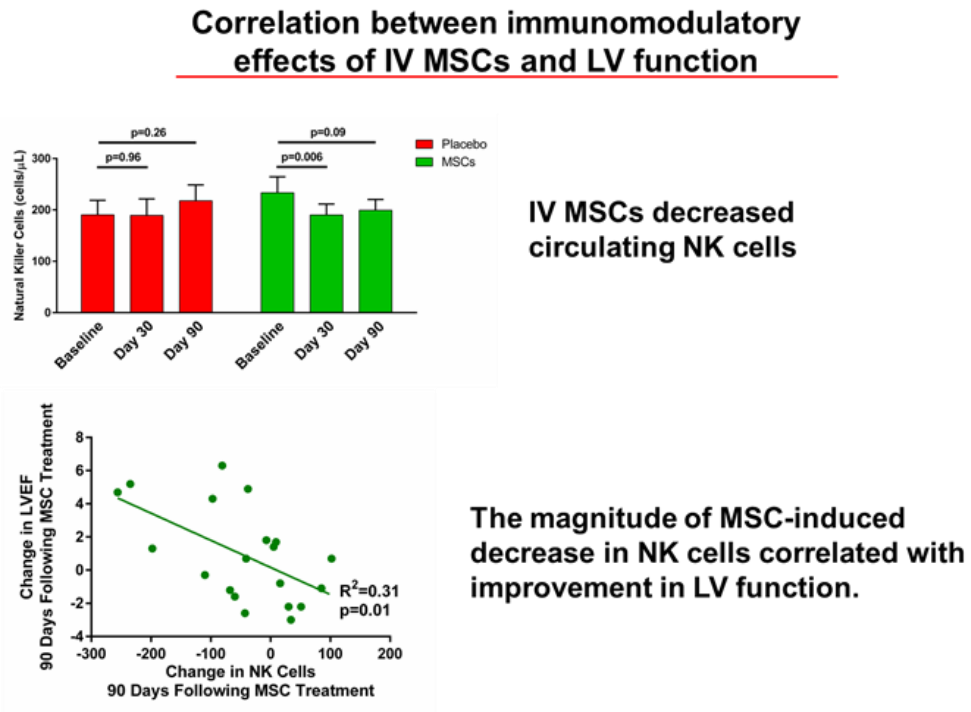

Figure 4.1. Effects of IV administration of patients with non-ischemic cardiomyopathy[36].

These results provide evidence compatible with the concept that in patients with HF intravenous administration of MSCs decreases circulating NK cells, and further suggest that the magnitude of the anti-inflammatory effect produced in the individual patient relates to the magnitude of the beneficial effect exerted on myocardial function.

5. *Suggestion that MSCs residing in inflamed tissue exert less efficacious paracrine activity than MSCs derived from non-inflamed tissue; evidence suggesting greater efficacy derived from IV injected MSCs (residing mainly in lungs, liver, and spleen) vs. MSCs injected directly into ischemic myocardium.*

5.1. An important study relating to the immunomodulatory effects of MSCs was published by Naftali-Shani et al[37]. These investigators demonstrated that MSCs derived from the hearts of mice with persistent LV dysfunction, when tested in vitro, changed to an inflammatory phenotype, and when administered into the periinfarction region of mice 28 days following AMI did not improve LV function--they actually worsened anterior wall thinning. Importantly, MSCs derived from a non-inflamed tissue--subcutaneous fat--did not switch to an inflammatory phenotype. Of relevance to this is the fact that the vast majority of MSCs administered IV to mice with AMI

embed in tissues other than the heart[14]. Although speculative at this point, these observations suggest that intravenously administered MSCs actually may lead to greater myocardial benefit than would direct delivery of the cells to the myocardium.

5.2. Moreover, evidence suggests that MSCs residing in inflamed tissue will be detected more easily by the immune surveillance system, a change that would lead to shorter half-lives. Thus, Le Blanc et al[38] demonstrated that undifferentiated MSCs express HLA class I but not class II molecules, leading to the conclusion these cells are less likely to be eliminated by the immune surveillance system. However, addition of IFNγ for 48 hours induced greater than 90% of cells to express HLA class II molecules. This finding suggests that MSCs administered directly into an inflamed area, such as the myocardium in patients with AMI or HF, will begin expressing HLA class II molecules and will be more effectively eliminated by the immune surveillance system. In contrast, it appears that when MSCs are administered IV, where most of the cells reside in non-cardiac, non-inflamed tissue, they will be more immune evasive and persist longer, thereby having a greater opportunity to exert their beneficial effects on inflammation and myocardial function. In this regard, it is possible that prolonged persistence of MSCs, once injected, is not necessary to result in beneficial effects, but that efficacy results from a "hit-and-run" mechanism mediated by the secretion, through paracrine activities, of immunomodulatory factors during the initial days following MSC injection[39-41].

6*. Need for repeated administration of stem cells*

While chronic inflammation existing in many patients with HF might be transiently suppressed by a single injection of a therapeutic agent, it will almost certainly not be curative. These patients probably would experience recrudescence of the inflammation, with continuing long-term myocardial damage.

The concept of the need for repeated administration of stem cells was recently demonstrated in preclinical models of ICM[41-44]. These investigators hypothesized that in the setting of ICM, greater stem cell efficacy would be achieved by injecting the cells multiple times, thereby providing more prolonged exposure of diseased myocardium to the paracrine actions of the cells than could be achieved by a single injection. They demonstrated that cardiac progenitor cells administered 3 times, 35 days apart, into the LV cavity of rats with 30-

day old myocardial infarctions improved myocardial function more than did a single injection. Similar results were observed in mice in which cardiac mesenchymal cells were injected.

This concept is illustrated by the following figure:

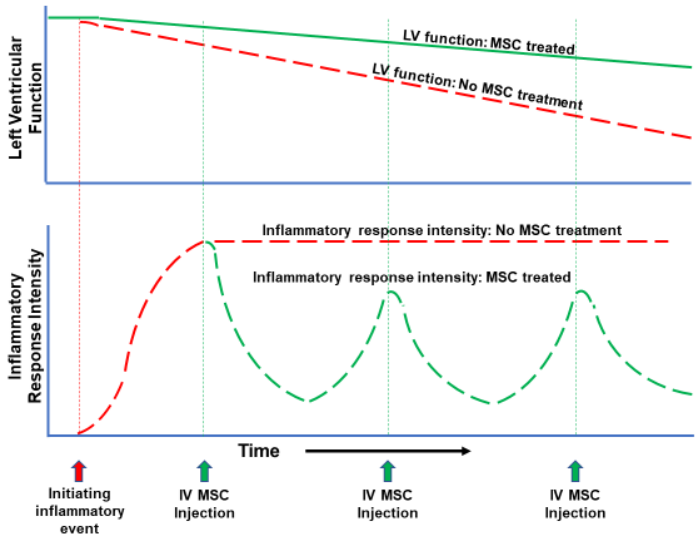

Figure 6.1. Proposed concept of the need for repeated intravenous MSC injections to maintain anti-inflammatory effects and thereby to modulate, over time, the progressive deterioration of myocardial function that otherwise occurs in patients with heart failure. (From Epstein et al[45]) The upper graph represents ventricular function plotted against time. The lower graph represents the intensity of the inflammatory response plotted against time. The graphs depict the hypothetical response to a single injection of MSCs (in red) vs. the hypothetical response to repeated MSC injections (in green).

If the concept is valid that treatment of HF patients will require repeated injections of MSCs, the strategy of intravenous delivery of MSCs provides a safe and efficient means for repeated administration of the MSCs, a fact that we believe will be transformative to the field of cardiovascular stem cell therapeutics.

7. *Safety of MSC administration*

In a 2016 publication Ankrum et al[39] noted that nearly 350 clinical trials had been, or were being, conducted with MSC preparations; 190 of these used allogeneic cells. These authors concluded that "Importantly, allo-MSC therapy has consistently been shown to be safe…" In 2012 Lalu and associates published a meta-analysis of clinical trials examining the safety of systemic MSC administration[46]. These authors concluded that: "Our analysis was unable to detect associations between MSC treatment and the development of acute infusional toxicity, organ system complications, infection, death, or malignancy. There was, however, a significant association between MSC administration and transient fever….aside from fever, the published current clinical trials suggest that the administration of MSCs is safe". Importantly, hyperacute rejection-like symptoms have not

been reported in human patients who receive allogeneic MSC therapy[47]

Of relevance, the MSCs being used in the present study are the same (CardioCell/Stemedica) as used in a study of patients with NICM[36]. This was a Phase 2a study in which MSCs were administered intravenously to 23 patients, the results of which are outlined in the Background, Section 2. There were no clinically significant adverse effects observed.

8. *Safety issues surrounding development of immune responses to allogeneic MSCs with repeated injections.*

The safety of a single dose of MSCs has been compellingly demonstrated. Although the safety of multiple injections of MSCs has as yet to be *definitively* determined, multiple intravenous injections have frequently been given to patients with no, or minimal serious sequela. This experience is summarized in the following paragraphs.

Patients with graft vs. host disease (an immunocompromised cohort of patients).

Chen et al reported a systematic review and meta-analysis of patients with steroid-refractory acute graft-vs-host disease (aGVHD) given multiple intravenous allogeneic MSC injections[48]. A total of 301 patients from thirteen studies were included. Of these, 9 patients (3%) developed responses that were deemed either not likely, or possibly, related to the MSC infusion. Of particular note is the study by Kurtzberg et al[49]. Seventy-five pediatric patients were studied. The mean total number of infusions received was 9.7 ± 3.97, with a median of 10.0 (range, 1 to 20), with 40 patients (53.3%) receiving >8 infusions. Only 1 patient (1.3%) experienced an infusion-related reaction (ie, rise in body temperature, increased breathing, and decreased oxygen saturation) after the third and fourth infusions, which resolved without sequela. The authors concluded that MSC infusion "…appears to have a benign safety profile". Infusions were well tolerated, with only 2 reported reactions occurring in the same patient out of more than 500 infusions administered during the course of the study. There were no cases of ectopic tissue formation. The number and type of events reported are consistent with a severely immunocompromised aGVHD patient population. At study entry, these patients had a complicated history and suffered from a variety of severe medical conditions. Treatment…did not lead to apparent additional toxicities and was well tolerated in this population. Furthermore, this therapy was not associated with hematologic or renal

toxicity."

Patients with Chronic Obstruction Pulmonary Disease (an immunocompetent cohort of patients).

Sixty-two patients with chronic obstruction pulmonary disease (COPD) were randomized to double-blinded IV infusions of either allogeneic MSCs or vehicle control. Patients received four monthly infusions (100 x 10^6 cells/infusion) and were subsequently followed for 2 years. The authors report that "There were no infusional toxicities and no deaths or serious adverse events (SAEs) deemed related to MSC administration. There were no significant differences in the overall number of adverse events, frequency of COPD exacerbations, or worsening of disease in patients treated with MSCs."

In our study, during MSC infusion, we will carefully observe our patients to determine whether clinically important safety-related immune response issues arise acutely.

We will also test for long-term development of immune cell reactivity, development of anti-HLA antibodies, and whether any positive test responses are associated with adverse clinical outcomes.

9. *Issue of development of immune responses to allogeneic MSCs interfering with potential beneficial effects of MSCs.*

Because it had been demonstrated that MSCs do not constitutively express Major Histocompatibility Class (MHC) II antigens or the B7 and CD40 ligand costimulatory molecules, and because MSCs have immunosuppressive and immunomodulatory properties, it became commonly accepted that MSCs are immunoprivileged and not eliminated by the immune surveillance system when administered to HLA mismatched recipients[39]. However, studies also demonstrated that while MSCs do not constitutively express HLA class II molecules, the addition of IFNγ for 48 hours induced greater than 90% of cells to express HLA class II[29,40]. This finding constitutes one of a large number of studies demonstrating that MSCs are not immunoprivileged—rather, they do generate immune responses when administered to HLA mismatched recipients and are cleared more rapidly than syngeneic MSCs[39, 47, 50].

Despite this lack of persistence, it appears MSCs are relatively protected from immune detection. Thus, Ankrum and colleagues presented the results of a study in which the relative persistence of allo-fibroblasts vs. allo-MSCs

was compared[39]. The study showed that fibroblasts died by day 10 and MSCs by day 20—in other words, although the MSCs were not immune privileged, they had some protection against immune rejection (the authors refer to this as *immune evasive*) resulting in longer persistence than the non-MSCs. This concept was also documented by Galipeau et al[51] who used murine MSCs transfected with the gene expressing erythropoietin as a reporter for persistent MSC functionality. MSCs were injected subcutaneously in either MHC-mismatched allogeneic or matched syngeneic mice. Although expression of erythropoietin lasted for a longer time in the syngeneic mice, the mismatched MSCs still produced erythropoietin for over 30 days

It therefore has been unequivocally demonstrated that MSCs are not immune-privileged, as originally proposed. They eventually are eliminated from mismatched hosts through immune responses. However, MSCs do persist for a limited time (longer than other types of mismatched cells) and are, thus, immune evasive. The issue relating to potential efficacy therefore becomes whether MSCs persist long enough to exert a therapeutic effect. The literature supports a positive response to this question, as the concept has been advanced that the relevant activities of the MSCs need only a limited time to produce their effects. Such a mechanism has been referred to as a “hit-and-vanish” or “hit and run” mechanism[39, 40].

The results of our own studies in mice with either AMI or ICM[14] (reviewed in part; see Background, Section 3, and Figure 3.2 and 3.3) relate to this issue. AMI or ICM was created and the mice were treated by intravenous administration of *human* MSCs. This therefore was a *xenograft* model, susceptible to the immune surveillance issues discussed above. We found:

- Human MSCs, injected IV into mice with AMI, persist in a viable state in the heart for at least 7 days, and when injected into mice with ICM, persist in a viable state in the heart for at least 20 days.
- The number and duration of the persisting MSCs are sufficient to exert biologically important effects—IV administered MSCs led to a) significant immune modulatory effects (among these, and perhaps most important, a decrease in spleen and heart NK cells) and b) marked improvement in LV function in both AMI and in ICM models.

- Importantly, in the ICM model MSCs were injected at time 0 and a repeat dose was administered 3 weeks later. No acute adverse effects occurred following the second injection. Additionally, the double injection led to a marked beneficial effect in myocardial function as measured by echocardiography at weekly intervals, with a final measurement at 7 weeks (see Background, Section 2).

It therefore appears that a beneficial myocardial functional effect is not dependent on MSCs persisting indefinitely in the host—benefit appears to accrue from MSCs secreting anti-inflammatory and other factors early after their injection. Perhaps intravenous administration is a *desirable* strategy, as most of the MSCs injected intravenously embed in non-cardiac tissue (lungs, spleen, kidneys)—i.e., in tissues that are not the site of inflammation, a factor that avoids exposing the MSCs to activation factors, such as IFNγ, that are present at high levels in inflamed tissues and that lead to the expression of HLA class II molecules, which would lead to more rapid immune-mediated removal of the cells.

This intravenous strategy, along with the potential efficacy of a "hit and vanish" mechanism, could effectively reduce the deleterious effects of persistent inflammation (as we postulate exists in LVAD patients) even without long-term persistence of a single MSC injection. Our trial is also important from the perspective of testing the efficacy and safety of multiple IV injections of MSCs, as it will be the first study undertaken in cardiac patients to test a multi-injection strategy.

And while safety of MSC administration has been compellingly demonstrated (at least over the short term), the long-term effects have not been extensively documented. It is perhaps encouraging to know that because MSCs do elicit immune responses that eliminate the cells over weeks or months, long term problems would not be anticipated.

10*. Central hypotheses for carrying out the proposed study:*

Compelling preclinical data indicate that chronic inflammation contributes to the progressive myocardial dysfunction occurring post-AMI and in HF. It has now also been definitively demonstrated in preclinical models that intravenously delivered MSCs to mice with either AMI or ICM improves myocardial function, an effect

caused at least in part by systemic MSC-induced anti-inflammatory effects. And a recently published phase 2a clinical study provides evidence suggesting that intravenous administration of MSCs to patients with NICM decreases circulating NK cells; importantly, the magnitude of the MSC-induced decrease in NK cells was significantly associated with the magnitude of improvement in LV function. Experimental data are also compatible with the conclusion that the beneficial anti-inflammatory effects are transient, and that multiple injections would likely be necessary to achieve persistent beneficial effects on myocardial function. Finally, LVAD patients have persistent, and strikingly, elevated markers of inflammation.

We hypothesize that the same disease processes—in particular, persistent inflammation—that contribute to progressive LV dysfunction in mice with ICM and in patients with chronic HF, whether of ischemic or non-ischemic etiology, also contribute to progressive RV dysfunction found in many LVAD patients.

Therefore…

- **We further hypothesize that *intravenous delivery of MSCs to patients with LVADs and RV failure will improve both LV and RV function, and therefore patient outcomes.***
- ***In addition, by reducing the excessive systemic inflammatory responses that exist in HF, the MSC-induced decrease in inflammation may improve multiple non-cardiac mechanisms that contribute to poor outcomes.***

3 STUDY OBJECTIVES

3.1 Primary

To assess the safety of human allogeneic MSCs derived from bone marrow and administered intravenously—3 times at monthly intervals—to subjects with advanced HF of ischemic etiology (ischemic heart failure; IHF) or non-ischemic etiology (NICM) who underwent implantation of LVAD.

3.2 Secondary

To assess the efficacy of human allogeneic MSCs administered intravenously—3 times at monthly

intervals—to subjects with advanced HF who underwent implantation of LVAD : Specifically, effect on 1) right (RV) and left ventricular (LV) structure and function, 2) inflammatory and cardiac biomarkers, and 3) clinical status.

4 STUDY DESIGN

4.1 Description of the Study

This is a Phase IIa, double blind, placebo-controlled, multi-center, randomized study in subjects with advanced HF who are supported by LVADs.

The study will enroll 30 subjects and will consist of 2 cohorts. Enrolled subjects will be stratified by LVAD device type (HeartWare HVAD, HeartMate II or HeartMate III) and randomized at 1:1 into an experimental group (n=15) or a placebo group (n=15), respectively. Subjects in the experimental group will receive 1.5 million cells per kg, and subjects in the placebo group will receive 1.5 mL/kg Lactated Ringer's Solution at baseline, 1 month and 2 months. All study participants will receive the MSCs or placebo within ±7 days of the specified timing.

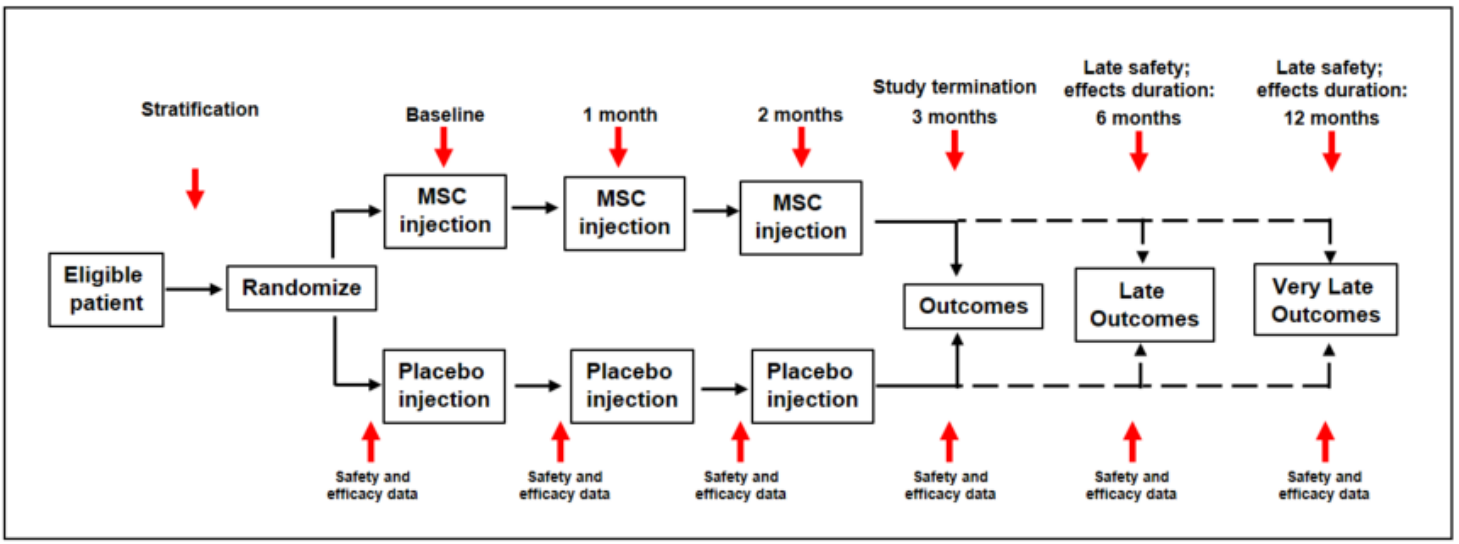

Figure 4.1 Study Design

Safety of study participants will be evaluated after each visit, as detailed in Appendix A. Physical examination and concomitant medications will be recorded at each infusion visit as well 90, 180, and 360 days. Complete examinations will be used to assess disability and functional status at baseline (prior to treatment) and at the visits scheduled at 1, 2, and 3 months after the initial administration. A baseline 2D and 3D and Doppler

transthoracic echocardiography with speckle-tracking and gated blood pool SPECT ventriculography for assessment of right and left ventricular systolic function and volumes will be performed at baseline and at specific time points during follow-up. All echocardiograms will be assessed in a blinded core lab. All SPECT ventriculograms will be assessed by a blinded observer.

Subjects will continue all of their regular medications unless contraindicated. Subjects may be administered beta-blockers, ACE inhibitors, angiotensin receptor blockers or angiotensin receptor neprilysin inhibitor, mineralocorticoid receptor antagonists, isosorbide, hydralazine, mineralocorticoid receptor antagonists, statins, or other medications, as tolerated. Goal mean arterial blood pressure will be <85 mmHg. LVAD pump speed will not be adjusted unless the rationale for speed change is discussed with and approved by the principal investigator with the exception of an emergency situation.

SUBJECT PARTICIPATION

Eligible participants must have advanced HF of ischemic etiology, defined as 1) a prior history of myocardial infarction and/or 2) angiographically (invasive or cardiac CT angiography) documented significant CAD (defined as a prior history of coronary revascularization (CABG or PCI) and/or obstructive CAD (≥75% stenosis of the left main or proximal LAD or ≥75% stenosis of two or more major epicardial coronary arteries)) or non-ischemic cardiomyopathy etiology.

Enrollment window will open after discharge from index implant hospitalization and once the patient has been deemed stable with their LVAD implantation.

The screening evaluation will include obtaining written informed consent, collection of a complete medical history, including current and past medications, physical examination and laboratory tests. LVAD implantation is required for inclusion in the study.

A comprehensive 2D and 3D and 3D and Doppler transthoracic echocardiography with speckle tracking and gated blood pool SPECT ventriculography for assessment of right and left ventricular systolic function and volumes will be performed at baseline and at the specific time points during follow-up.

Eligible subjects will receive repeated infusions of either MSCs or placebo based on randomization at time 0 and at day 30 (±7 days) and day 60 (±7 days).

Follow-up visits will occur at days 7 (±2 days), 30 (±7 days), 37 (±2 days), 60 (±7 days), 67 (±2 days), 90 (±7 days), 180 (±7 days), and 360 (±7 days). Information on AEs, concomitant medications, vital signs, clinical laboratory tests and physical examination will be collected according to the schedule of assessments (Appendix A). All guideline-based medical care will continue as prescribed by the subject's personal physician(s). Use of other investigational agents or treatments is not allowed during this study.

Administration of Human Allogeneic MSCs

The study will enroll a minimum of 30 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=15) or a placebo group (n=15). Subjects in the experimental group will receive 1.5 million cells per kg, and subjects in the placebo group will receive 1 mL/kg LRS at days 0, 30 and 60.

After the subject is randomized into one of the two cohorts, the final formulation of MSC or placebo will be prepared. Within 8 hours of infusion, the appropriate number of cells will be thawed at the study site pharmacy and re-suspended in LRS at a concentration of $1x10^6$ cells/mL Subjects in placebo group will receive LRS at a volume of 1.5 mL/kg. The study solution (MSCs or placebo) will be labeled to ensure investigator blinding. The solution will be labeled to indicate that it is an investigational agent and will contain subject’s identifying information, date, and time of formulation.

An intravenous line will be placed into a peripheral vein, and 0.9% sodium chloride solution will be run to keep the vein open. The study product will be drawn using an 18-gauge needle into a 60 mL syringe with an eccentric tip. The needle will be removed, and infusion tubing will be attached to the syringe. The syringe with infusion tubing will be placed in a metered dose syringe pump positioned horizontally with the syringe rotated such that the syringe tip is at the lowest position. The product will be infused intravenously into the subject’s arm at a constant rate of approximately 2 mL/min. The applicable volume for the target dose will be delivered within ±2 mL. Depending on subject’s weight, up to three 60 ml syringes of product may be infused. After the entire product volume is delivered, 25 mL of LRS (without cells) will be infused at 2 ml/min to deliver cells from the infusion line for more accurate dosing. Subjects in the placebo group will receive intravenous LRS. Their syringes will be labeled as above to preserve blinding and followed by 25 ml of LRS infusion as above.

If the subject has an adverse reaction during the infusion, the infusion may be interrupted or slowed according to the severity of the reaction.

The following symptoms, if noted, during infusion may lead to slowing or cessation of the infusion session:

- Temperature ≥39°C.
- Unexplainable shaking chills.
- Angioedema.
- Respiratory distress.
- Wheezing/bronchospasms.
- Hypotension (Drop in MAP by ≥20 mmHg to an absolute MAP<60 mmHg).
- Hypoxemia (persistently reduced SpO2 of <88%).
- Any symptoms of signs, which in the investigator's opinion indicate clinical instability or a significant infusion reaction.

In the event the infusion session is prematurely stopped the investigator may opt to resume if the symptoms subside, however this will be at the discretion of the investigator. Adverse reaction duration the infusion, slowing or cessation of the infusion and any resuming of the infusion will be recorded on the case report form. If a session is prematurely stopped for adverse reaction, the patient will continue with the next interval follow up per the schedule of events.

All appropriate treatment will be given to reduce any discomfort and ensure the subject's safety. The infusion may be restarted, if interrupted, if the subject is not considered to be at risk by the Investigator and the subject consents.

All subjects will receive the follow-up tests and evaluations as detailed in Appendix A.

CONCOMITANT THERAPY AND CLINICAL PRACTICE

Subjects will continue all of their regular medications unless contraindicated. Subjects may be administered beta-blockers, ACE inhibitors, angiotensin receptor blockers or angiotensin receptor neprilysin inhibitor, mineralocorticoid receptor antagonists, isosorbide, hydralazine, statins, or other medications, as tolerated. Goal mean arterial BP will be <85 mmHg. LVAD speed will be maintained, unless there is a compelling clinical indication to change it. Changes in pump speed should be approved by the PI, unless emergency.

4.2 Endpoints

PRIMARY ENDPOINT

Safety of MSCs assessed at the following time-intervals:

Baseline and days 30, 60, 90, 180, and 360 post-initial infusion

1. Procedural complications
2. Vital signs
    a. Temperature
    b. Mean arterial blood pressure
    c. Heart rate
    d. Respiratory rate
    e. Weight
    f. LVAD parameters: Pump speed, flow, pulsatility and power
3. Uncontrolled systemic infection.
4. Hemodynamically unstable clinical arrhythmias that require appropriate defibrillator shock, anti-tachycardia pacing or initiation or escalation of antiarrhythmic medications.
5. Laboratory tests
    a. Complete blood count (CBC)/ differential.
    b. Comprehensive Metabolic Panel: Sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, glucose, calcium, magnesium and liver function tests (ALT, AST, alkaline phosphatase, bilirubin and albumin)
    c. Troponin-I.
    d. Allosensitization (change in antibodies against HLA (panel reactive antibody test) from baseline for strong HLA antibody specificities (MFI>2000))
6. Electrocardiogram.
7. All-cause mortality. This will be reviewed by the DSMB. The LVAD population is a high risk group with very

high mortality, which will be taken into account when reviewing safety. Survival rate among HF patients with LVAD is 95% at 1 month, 81% at 1 year, 70% at 2 years, 59% at 3 years and 49% at 4 years. For patients enrolled within our study window, we would expect a 20% mortality rate in our patient population.

SECONDARY ENDPOINT

All secondary end points will be assessed pre-MSC infusion at days 0, 30, and 60, and also at days 90, 180, and 360. In addition, blood will be drawn 1 week after each infusion for potential biomarker analysis, depending on analysis of pre-infusion data. These 1-week post infusion blood samples will be frozen and stored.

Efficacy

1. Change in the following inflammatory cytokines at 90 days:

   CD14, CD163, CD40, CRP, E-Selectin, Fas, (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1 alpha (IL-1 F1), IL-1 beta, IL-1 R4, IL-10, IL-12 p70, IL-13, IL-18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8, Lipocalin-2, MCP-1, MCP-2, MIF, MIP-1 alpha, MIP-1 beta, Osteopontin, PAI-1, Platelet Factor 4, Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1, VEGF-A using a biomarker array kit.
2. NK cell depletion, defined as % reduction in NK cells from baseline to day 90.

EXPLORATORY ENDPOINTS

1. Change in the following cardiac biomarkers: N-Terminal Prohormone of Brain Natriuretic Peptide (NT-proBNP) and soluble suppression of tumorigenicity 2 (sST2) between baseline and day 90 post initial infusion.

2. Change in RV systolic function and volumes assessed by 2D and 3D echocardiography and gated blood pool SPECT ventriculography scan between baseline and day 90 post initial infusion.

3. Hospitalizations due to right HF (as defined by INTERMACs) at day 90.

4. 6 minute walk distance changes between baseline and day 90 post initial infusion.

5. Change in Quality of life (Kansas City Cardiomyopathy Questionnaire; KCCQ) between baseline and day 90 post initial infusion.

6. Frequency and severity of gout flares at day 90.

4.3 Safety Plan

The safety of subjects will be carefully monitored by the investigators and reviewed by an independent Data and Safety Monitoring Board (DSMB). Details of AE description and procedures for reporting are detailed in section 8.

4.4 Compliance with Laws and Regulations

This study will be conducted in accordance with the International Conference on Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP), the principles of the Declaration of Helsinki (October 1996) and applicable local, state, and federal laws, as well as other applicable country laws.

5 STUDY ENROLLMENT

Subjects will participate in this study for about 360 days as follows: Patients will be deemed enrolled once the patient is randomized.

5.1 SUBJECT SELECTION

The study will enroll at least 30 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=15) or a placebo group (n=15), stratified by LVAD type. Subjects in the experimental group will receive 1.5 million cells per kg, and subjects in the placebo group will receive 1.5 mL/kg Lactated Ringer's Solution at day 0 (±7 days), day 30 (±7 days) and day 60 (±7 days).

Subjects will be selected using the following inclusion and exclusion criteria:

INCLUSION CRITERIA

1. Age ≥18 years.
2. One of the two following HF etiologies:

a. Ischemic HF etiology, defined as 1) a prior history of myocardial infarction and/or 2) angiographically or cardiac CTA documented significant CAD (defined as a prior history of coronary revascularization (CABG or PCI) and/or obstructive CAD (≥75% stenosis of the left main or proximal LAD or ≥75% stenosis of two or

more major epicardial coronary arteries)) prior to LVAD implantation (any coronary angiogram ever prior to LVAD implantation can be used, but an angiogram is not required to confirm non-ischemic etiology, imaging is acceptable).

b. Non-ischemic cardiomyopathy

3. Advanced HF defined as HF requiring LVAD implantation and deemed stable on his/her LVAD.
4. On stable medical therapy (per the discretion of the treating physician) including beta-blockers, ACE-inhibitors, angiotensin receptors blockers, angiotensin receptor neprilysin inhibitor, mineralocorticoid receptor antagonists, isosorbide, hydralazine, and mineralocorticoid receptor antagonists) and optimized pump speed for at least a month prior to randomization.
5. HS-CRP level≥2 mg/l.
6. NYHA class II-III symptoms.
7. Ability to understand and provide signed informed consent.
8. Reasonable expectation that patient will receive standard post-treatment care and attend all scheduled safety follow-up visits.

EXCLUSION CRITERIA

1. Women of childbearing potential. Postmenopausal women or women with permanent contraception method (defined as total hysterectomy) will not be excluded.
2. History of debilitating stroke (modified Rankin Score > 3) within 3 months.
3. The likelihood of requirement of cardiac surgery during the study period.
4. Presence of clinically significant, uncorrected left sided valvular heart disease, active acute myocarditis, or uncontrolled hypertension defined as Persistently elevated mean arterial blood pressure (>100 mmHg). Echocardiography within 12 months of screening. Patients can be re-evaluated, at the discretion of the investigator.
5. QTc >550 ms (in the absence of bundle branch block, interventricular conduction delay or ventricular pacing). Electrocardiogram (ECG) within 60 days.

6. History of cardiac arrest within 3 months.
7. Hypertrophic or infiltrative cardiomyopathy.
8. Considered or listed for organ transplantation or history of organ transplantation
9. Illness other than HF with life expectancy less than 12 months.
10. Enrolled in an interventional trial or received an experimental drug or device within 30 days of randomization.
11. Biventricular assist device (Bi-VAD) support.
12. Severe COPD defined by FEV1<1L, and FEV1/FVC<70% within 12 month if known history of COPD, otherwise FEV1<1L, and FEV1/FVC<70% within 24 months
13. Uncontrolled seizure disorder.
14. Clinically significant hematologic, hepatic, or renal impairment as determined by screening clinical laboratory tests within the last 30 days:
    - Liver disease = ALT or AST > 3x normal, alkaline phosphatase or bilirubin >2x normal
    - Renal disease = End stage renal disease on long term dialysis Hematologic = Unexplained persistent leukocytosis (WBC >11 K/UL) or hemoglobin < 8.5 gm/dl
15. Presence of any other clinically-significant medical condition, psychiatric condition, or laboratory abnormality, that in the judgment of the investigator or sponsor may affect compliance with the study protocol or pose a safety risk to the subject.
16. Inability to comply with the conditions of the protocol.
17. Acute coronary syndrome within 4 weeks (clinical diagnosis, confirmed by electrocardiographic abnormalities and elevation of troponin-I).
18. Malignancy within the previous five years, except adequately treated basal cell carcinoma, provided that it is neither infiltrating nor sclerosing, and carcinoma in situ of the cervix
19. Active uncontrolled systemic infection. Positive blood or deep tissue cultures or clinical or imaging evidence of systemic infection despite complete course of effective antimicrobial therapy as determined by infectious diseases. Localized (non-systemic) infection is not an exclusion criterion.

Patients can be re-evaluated, at the discretion of the investigator.

20. Early postpartum cardiomyopathy (within six months of diagnosis).
21. Presence of inherited or acquired immune deficiency or human immunodeficiency virus infection (HIV). Negative HIV test within the preceding 12 months is required.
22. Systemic corticosteroids, immunosuppressive drug therapy (cyclophosphamide, methotrexate, cyclosporine, tacrolimus, azathioprine, mycophenolate, sirolimus, etc.), and DNA depleting or cytotoxic drugs taken within four weeks prior to study treatment.
23. Known Porphyria.
24. Allergy to sodium citrate or any “caine” type of local anesthetic.
25. Patient enrolled in hospice care.

5.2 Method of Treatment Assignment and Blinding

This study is a Phase IIa, double-blind, placebo-controlled, multi-site (up to 2), randomized trial comprised of 30 subjects with an experimental group (stem cell infusion; n=15) and a placebo group (n=15; LRS at a volume of 1.5 mL/kg). Each group will receive 3 repeated doses of the assigned treatment (no cross-over) at times 0, 30 and 60 days. The infusion bags of stem cells or placebo will be identical.

In the event that a patient is unable to receive an infusion on or within the window of 30 or 60 days, the earliest infusion will be scheduled once it has been deemed safe for infusion. The primary endpoint measurement at day 90 (or within window) will remain irrespective to the number of infusions completed. More specifically, if infusion #2 (day 60) is missed then it will be schedule as soon as the patient is deemed stable. The infusion #3 will occur 30 days from infusion # 2 but before day 90 (plus window) primary endpoint imaging evaluation. In the event that infusion # 3 would fall after the upper bound of the 90 day primary endpoint window then this infusion will be skipped.

5.3 Subject Discontinuation and Withdrawal

Subjects have the right to withdraw from the study at any time. Subjects will be given the option to provide a reason for withdrawal. In case of early withdrawal, the reason for withdrawal will be recorded on the appropriate Case report form (CRF). Attempts to complete early termination exam as defined in 7.4 will be made. The Investigator has the right to discontinue a subject from the study for any medical reason that the investigator determines may jeopardize the subject's safety if he or she continues in the study, or for reasons of noncompliance (e.g., missed visits, use of other investigational drugs, etc.).

5.4 Study Termination

The Sponsor has the right to terminate the study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to subjects.
- Unsatisfactory subject enrollment.
- Rate of enrollment of < one subject per 3 months.
- An excess drop out of subjects (a total of ≥5 subjects per each arm, but not due to VAD –related mortality).
- Inaccurate or incomplete data recording.
    - ≥20% missing data for the primary safety endpoints.
    - ≥30% missing data for the efficacy endpoints.

6. INVESTIGATIONAL PRODUCT

6.1 Formulation, Packaging, Preparation, Randomization of MSCs or Placebo

The final product will be stored and handled as described in Stemedica's Pharmacy Manual (referenced in their Investigator's Brochure).

The product being administered to the patient is the exact product being referenced in IND 14328 and will not be altered prior to use.

The following tests--gram stain, endotoxin, cell count, viability, and appearance--will be conducted with final results available prior to administration of the drug per Stemedica's formulation protocol. Sterility testing is

conducted per USP requirements by an approved testing lab for sterility testing after thawing the product, with results available post administration to the patient.

Endotoxin, appearance, and gram testing are done by our collaborators at the Cell Processing Lab at Children's Hospital (Cellular Therapy Lab) prior to administration. This Laboratory is located across the street from the MedStar Washington Hospital Center, where patient processing is performed and the cells will be infused.

The only test that will not be available prior to patient administration is the sterility test.

The following steps will be followed:

1. The Cellular Therapy Lab will send samples to an approved testing lab for sterility testing by BacT/Alert (culture for 14 days bacterial, 21 day fungal) immediately after thawing the product.
2. Retention samples will be sent from the Cellular Therapy Lab to Medstar when the product is transported. The Medstar clinical trial team will keep the retention samples (in case sterility results come back positive).
3. If sterility test is negative the retained sterility sample will be destroyed.
4. If sterility test is positive (i.e. the sample is not sterile) the following action plan will be followed.

Action plan for a positive sterility testing:

1. Microbiology testing lab will notify the PI, the clinical trial manager or designee, and the Cellular Therapy Lab of any positive cultures immediately.
2. Further testing for organism speciation and antibiotic sensitivity will be conducted by the microbiology lab and reported to the PI in a timely fashion to guide patient management.
3. The PI is responsible for ensuring patient safety and will ensure that the patient is notified of the test results, assessed for adverse events and treated in a timely fashion as medically appropriate.
4. The PI/designee will notify Stemedica point person within 24 hours of receipt of report from lab.
5. The PI will determine whether the FDA and IRB need to be notified immediately (e.g. if there is a serious adverse event) or if this will be included in the progress report. Standard reporting of adverse events will be followed as specified in the protocol.
6. The notification and workup for positive culture form will be completed (form attached).
7. All products with positive cultures will be marked with a biohazard label.

8. The retention sample will be sent to the microbiology lab for testing.

9. All the materials will be maintained until further instruction is received from Stemedica.

10. Stemedica staff to investigate failure and determine if further formulation activities or testing is required to support investigation of the issue.

The study will enroll a minimum of 30 subjects and will consist of 2 cohorts. Enrolled subjects will be randomized at 1:1 into an experimental group (n=15) or a placebo group (n=15). Subjects in the experimental group will receive 1.5 million cells per kg and subjects in the placebo group will receive 1.5 mL/kg Lactated Ringer's Solution.

Once the subject is randomized into one of the two cohorts, the cells or placebo will be prepared. Within 8 hours of infusion, the appropriate number of cells will be thawed at the study site pharmacy and re-suspended in Lactated Ringer's Solution (LRS) at a concentration of $1x10^6$ cells/mL Subjects in placebo group will receive LRS at a volume of 1.5 mL/kg. The study solution (cells or placebo) will be labeled to indicate that it is an investigational agent and will contain a subject's information, date, and time of formulation.

6.2 IV Administration of MSCs or Placebo

An intravenous line will be placed into an appropriate peripheral vein with 0.9% sodium chloride solution running to keep the vein open. The study solution described above will be taken up in an 18 gauge needle into a 60 mL syringe with an eccentric (offset) tip. The needle will be removed and infusion tubing will be attached to the syringe. The syringe with infusion tubing will be placed in a metered dose syringe pump positioned horizontally with the syringe rotated such that the syringe tip is at the lowest position. The product will be infused intravenously into the subject's arm at a constant rate of approximately 2 mL/min. The applicable volume for the target dose will be delivered within 2 mL. Depending on subject's weight, up to three 60 mL syringes of product may be infused. After the entire product volume is delivered, 25 mL of LRS (without cells) will be infused at 2 mL/min to deliver cells from the infusion line for more accurate dosing. Subjects in the placebo group will receive intravenous LRS. Their syringes will be labeled as above to preserve blinding and followed by 25 mL of LRS infusion as above.

If the subject has an adverse reaction during the infusion, the infusion may be interrupted or slowed according to the severity of the reaction. All appropriate treatment will be given to reduce any discomfort and ensure the subject's safety. The infusion may be restarted, if interrupted, if the subject is not considered to be at risk by the Investigator and the subject consents.

6.3 Product Storage and Stability

Once the cell suspension is prepared for administration to a subject as described above, the dose of allogeneic mesenchymal bone marrow cells will be stored at +4ºC until the time of administration. All cells will be administered to the subject within 8 hours of preparation. Cells that are not used within this time period will be discarded.

6.4 Concomitant Medications and Rehabilitation

Concomitant medications (any prescription and/or over-the-counter preparations) and therapies (nondrug or procedures) used by a subject while participating in this clinical trial will be optimized per the AHA Guidelines and recorded at screening and until the end of the study or the end of a subject's participation in the study. Subjects may be administered beta-blockers, ACE inhibitors, angiotensin receptor blockers or angiotensin receptor neprilysin inhibitor, mineralocorticoid receptor antagonists, isosorbide, hydralazine, statins, or other medications, as tolerated. All other prescription and over-the-counter preparations approved by the patients' physician will be permitted.

7. STUDY ASSESSMENTS AND SCHEDULE

7.1 General Assessments

Clinical Assessments

- Complete medical history and concomitant medications.
- Physical and cardiac examination, including vital signs (temperature, mean arterial blood pressure, weight, heart rate, respiratory rate, oxygen saturation, LVAD parameters: pump speed, power, flow,

pulsatility).

- All-cause mortality, admission for worsening HF and all-cause admissions.
- Exercise capacity: 6 minute walk distance.
- Kansas City Cardiomyopathy Questionnaire (KCCQ) (Appendix B).
- New York Heart Association (NYHA) Classification (Appendix B).

Clinical Laboratory Assessments

- Hematology (CBC): hemoglobin, hematocrit, platelet count, red blood cell count, white blood cell count, automated differential and platelet count.
- Serum Chemistry: includes sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, phosphorous, total bilirubin, albumin and total protein, and the following liver function tests (LFTs): alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)
- Immunoglobulin A (IgA), immunoglobulin E (IgE), immunoglobulin G (IgG), immunoglobulin M (IgM),
- Troponin I, NT-pro Brain Natriuretic Peptide (NT-proBNP) and ST 2
- CK and CK-MB
- Uric Acid
- HLA
- hsCRP
- Inflammatory/immune biomarkers:

  In-vivo immune responses.

1.1 Quantitative Immune Response - biomarkers Array.

At all time points blood samples from all patients will be analyzed for the following 40 immune response biomarkers. Samples from normal donors will serve as reference.

Quantibody® Human Immune Response Biomarker Array 1 Kit (RayBiotech).

CD14	CD163	CD40	CRP	E-Selectin

Fas (TNFRSF6/Apo-1)	**Fas Ligand (TNFSF6)**	**GCSF**	**ICAM-1 (CD54)**	**IL-1 alpha (IL-1 F1)**
IL-1 beta	**IL-1 R4**	**IL-10**	**IL-12 p70**	**IL-13**
IL-18	**IL-2**	**IL-2 R alpha**	**IL-4**	**IL-6**
IL-8	**Lipocalin-2**	**MCP-1**	**MCP-2**	**MIF**
MIP-1 alpha	**MIP-1 beta**	**Osteopontin**	**PAI-1**	**Platelet Factor 4**
Procalcitonin	**RAGE**	**Resistin**	**Thrombomodulin**	**TNF alpha**
TREM-1	**Troponin I**	**uPAR**	**VCAM-1**	**VEGF-A**

1.2. Antibody Isotype assay – Analyzing the effect of treatment with MSCs on the humoral response; levels of antibodies and isotype switch.

2. Cellular response

At all-time points leukocytes will be isolated from blood samples and will be frozen for further analysis. Leukocytes will be analyzed for:

Immune phenotyping by flow cytometry, to determine the effect of treatment with MSCs on the cellular immune response. (Expected effect - changing the balance from pro to anti-inflammatory phenotype).

Functional assays.

Proliferation assay – evaluating the suppressive effect of MSCs on T cells.

In-vitro cytokine secretion – testing the effect of MSCs treatment on leukocytes potential to secret cytokines following general stimulation.

Cytotoxicity assay – co-culture of leukocytes with MSCs will test the cytotoxic effect of leukocytes toward MSCs on the axis of time and repeated treatments. This assay will help evaluating the effectiveness of repeated treatments with MSCs.

B. Future assays (Biobanking)

It is to be expected that from the blood collected (listed above in 1.1-2) for all of the analyses detailed above, approximately 20ml of serum will remain unused. We plan to bank this serum for future use. Future analyses of the serum will derive from questions that are unanticipated at the initiation of the study but that usually arise once the analyses as per protocol are performed. The availability of serum to perform these

new analyses provide the potential to importantly supplement insights we can derive from this study.

ECG

A 12-lead ECG will be performed. Standard interval assessments, including QT and QTc, will be calculated from a single lead (typically lead II). QTc will be calculated on the screening ECG in order to determine if the subject meets enrollment criteria. QTc must be <550 ms in order for the subject to be randomized.

Echocardiography

- A full 2D, 3D and Doppler echocardiogram will be performed at baseline and day 30, 60, 90, 180, and 360. Images will be acquired with the patient positioned in the recumbent lateral position. Left ventricular volumes will be determined at end-diastole and end-systole by quantitative biplane assessment. Endocardial borders will be manually traced from apical four-chamber and two-chamber views. Left ventricular volumes will be used to calculate LVEF using the biplane modified Simpson's summation-of-disks method recommended by the American Society of Echocardiography.
- High frame-rate images of the LV in the three apical views (4-chamber, 2-chamber, and apical long axis) and the RV in the RV focused apical long axis will be acquired for speckle tracking echocardiography. Images will be analyzed offline on a dedicated workstation using TOMTEC software (TOMTEC Corporation) for calculation of segmental and global longitudinal strain and strain rate. Global longitudinal strain and strain rate will be calculated from all 18 segments of the LV and the 6 segments of the RV. Segmental strain and strain rate will be calculated for each segment separately.

Gated blood pool SPECT ventriculography

Gated blood pool SPECT ventriculography will be performed at baseline and days 90 and 180. Efficacy endpoints evaluated by quantitative 3D gated blood pool SPECT ventriculography will include: LV and RV end diastolic volumes, LV and RV end systolic volumes and global LV and RV ejection fractions.

Zio patch monitoring

Patients without an ICD will undergo periods of 7 day monitoring for arrhythmias using a Zio patch (iRhythm

Technologies, Inc). For the purposes of this study, life-threatening arrhythmias are defined as hemodynamically unstable sustained ventricular tachycardia or new- onset complete heart block.

7.2 Schedule of Assessments

7.2.1 Screening, Baseline and Randomization (Day -12- -11)

- Study participants must meet eligibility criteria (inclusion/exclusion criteria review).
- Sign the written informed consent.
- Complete medical history, including medications history.
- Complete physical and cardiac examination, including vital signs (temperature, mean arterial blood pressure, weight, heart rate, respiratory rate, peripheral arterial oxygen saturation, LVAD parameters: pump speed, power, flow, pulsatility), weight, and height.
- 12-lead ECG.
- Kansas City Cardiomyopathy Questionnaire (KCCQ).
- New York Heart Association (NYHA) Classification.
- 6 minute walk test
- Clinical labs: serum chemistry including BUN & creatinine, CBC with differential, LFTs, total protein, and albumin and LDH.
- Troponin, NT-pro Brain Natriuretic Peptide (NT-proBNP)
- Uric acid
- Biomarkers of inflammatory/immune response
- HIV test
- HS-CRP
- Zio patch recording or ICD interrogation
- Gated blood pool SPECT ventriculography
- Full 2D and 3D and Doppler transthoracic echocardiography with speckle-tracking.
- Randomization into experimental or control group

7.2.2 Assessments and treatment

Day 0 ± 7 Days, day 30 ± 7 Days and Day 60 ± 7 Days (day of first, second and third infusion, respectively)

Pre-infusion:

- Review and record any changes in medical history, including concomitant medications from before visit(s).
- Measure and record weight and vital signs (temperature, mean arterial blood pressure, weight, heart rate, respiratory rate, oxygen saturation, LVAD parameters: speed, power, flow, pulsatility) after subjects rest quietly in a supine or semi-recumbent position.
- Physical and cardiac examination.
- Draw and process blood samples for IgA, IgE, IgG, IgM
- Troponin, NT-pro Brain Natriuretic Peptide (NT-proBNP) and SST2.
- CK and CK-MB
- Zio patch or ICD assessment if not still on or done from baseline visit for the Zio patch patients
- Kansas City Cardiomyopathy Questionnaire (KCCQ). (30 and 60 day only)
- New York Heart Association (NYHA) Classification. (30 and 60 day only)
- 6 minute walk test (30 and 60 day only)
- Full 2D and 3D and Doppler transthoracic echocardiography with speckle-tracking (30 and 60 day only)
- Place an IV line in the upper extremity or hand with 0.9% sodium chloride running to keep the vein open
- CBC with differential
- CMP
- Uric Acid
- HLA and HS-CRP
- Biomarkers of inflammatory/immune response: A panel of inflammatory biomarkers: CD14, CD163, CD40, CRP, E-Selectin, Fas, (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1 alpha (IL-1 F1), IL-1 beta, IL-1 R4, IL-10, IL-12 p70, IL-13, IL-18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8,

Lipocalin-2, MCP-1, MCP-2, MIF, MIP-1 alpha, MIP-1 beta, Osteopontin, PAI-1, Platelet Factor 4, Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1, VEGF-A using a biomarker array kit and other markers as previously detailed using a biomarker array kit.

During infusion:

- IV infusion of the study product or placebo
- Monitoring for any signs of adverse reaction, record AE/s and concomitant medications
- Record vital signs and LVAD parameters every 2 hours, and as clinically indicated
- Continuous ECG monitoring during infusion of study product
- Pulse oximetry continuously during and for 2 hours post infusion

Post- infusion:

- 12-lead ECG
- Monitoring for any signs of adverse reaction, record AE/s and concomitant medications
- Record vital signs and LVAD parameters every 2 hours till discharge
- Continuous ECG monitoring for 2 hours
- Pulse oximetry continuously for 2 hours
- Subjects will be discharged from the hospital when he/she is stable in the opinion of the investigator
- Physical exam, prior to discharge if clinically stable
- Provide subject or responsible individual accompanying the subject with the phone number for contacting the study nurse and/or investigator for any questions, concerns or changes in health status.

7.3 Follow-up Assessments

One week post infusion (7 days ± 2 days)

- Biomarkers of inflammatory/immune response: A panel of inflammatory biomarkers: CD14, CD163, CD40, CRP, E-Selectin, Fas, (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1

alpha (IL-1 F1), IL-1 beta, IL-1 R4, IL-10, IL-12 p70, IL-13, IL-18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8, Lipocalin-2, MCP-1, MCP-2, MIF, MIP-1 alpha, MIP-1 beta, Osteopontin, PAI-1, Platelet Factor 4, Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1, VEGF-A using a biomarker array kit and other markers as previously detailed using a biomarker array kit.

Day 90, 180, and 360 (±7) days post initial infusion

- Measure and record vital signs
- 12-lead ECG
- Pulse Oximetry
- Zio patch or ICD assessment
- Record all-cause mortality, admission for worsening HF and all-cause admissions, AEs and concomitant medication use
- Physical and cardiac examination
- Changes in medical history
- Clinical labs: serum chemistry including BUN & creatinine, CBC with differential, LFTs, total protein, albumin and LDH.
- HS-CRP
- Cardiac Biomarkers:NT-pro Brain Natriuretic Peptide (NT-proBNP) and ST 2
- Biomarkers of immune response: A panel of inflammatory biomarkers: CD14, CD163, CD40, CRP, E-Selectin, Fas, (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1 alpha (IL-1 F1), IL-1 beta, IL-1 R4, IL-10, IL-12 p70, IL-13, IL-18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8, Lipocalin-2, MCP-1, MCP-2, MIF, MIP-1 alpha, MIP-1 beta, Osteopontin, PAI-1, Platelet Factor 4, Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1, VEGF-A using a biomarker array kit using a biomarker array kit. Antibody Isotype assays, leukocyte cytokine secretory assays, and leukocyte cytotoxic effects toward MSCs. Blood sample for IgA, IgE, IgG, IgM,

- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- 6 minute walk test
- Full 2D and 3 D and Doppler transthoracic echocardiography with speckle-tracking.
- Gated blood pool SPECT ventriculography (only 90 and 180 days)
- Uric Acid
- HLA

7.4 Early Termination Visit

- Measure and record weight and vital signs
- LVAD parameters
- 12-lead ECG
- Pulse Oximetry
- Zio patch or ICD assessment
- Record the all-cause admission for worsening HF and all-cause admissions, AEs and concomitant medication use
- Physical and cardiac examination
- Changes in medical history
- Clinical labs: serum chemistry including BUN & creatinine, CBC with differential, LFTs, total protein, albumin, LDH, and uric acidBiomarkers of immune response: A panel of inflammatory biomarkers: CD14, CD163, CD40, CRP, E-Selectin, Fas, (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1 alpha (IL-1 F1), IL-1 beta, IL-1 R4, IL-10, IL-12 p70, IL-13, IL-18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8, Lipocalin-2, MCP-1, MCP-2, MIF, MIP-1 alpha, MIP-1 beta, Osteopontin, PAI-1, Platelet Factor 4, Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1, VEGF-A using a biomarker array kit. Antibody Isotype assays, leukocyte proliferation and cytokine secretory assays, and leukocyte cytotoxic effects toward MSCs. Hs-CRPBlood sample for IgA, IgE, IgG,

IgM 6 minute walk test

- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- New York Heart Association (NYHA) Classification
- Full 2D and 3D and Doppler transthoracic echocardiography with speckle-tracking
- Cardiac Biomarkers:NT-pro Brain Natriuretic Peptide (NT-proBNP) and ST 2
- Gated blood pool SPECT ventriculography

8 ASSESSMENT OF SAFETY

8.1 Safety Parameters

Safety assessments will consist of monitoring and recording adverse treatment events. Changes in clinical laboratory, clinical status and physical examination values will be used for evaluation.

To allow for the assessment of potential infusion toxicity at least 1 day will pass between the infusions of the Investigational Product/Placebo for each of the first five (5) subjects.

8.1.1 Adverse Event (AE)

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study stem cell administration. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis) which would include MD, DO, PA or NP and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

Severity of Event: All AEs will be assessed by the clinician using the following grading system of AE intensity.

- Mild: events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life threatening: any adverse drug experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician's assessment of an AE's relationship to investigational product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to investigational product assessed. In a clinical trial, the investigational product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a plausible temporal relationship between the onset of the AE and administration of the investigational product, and the AE cannot be readily explained by the subject's pre-existing clinical state,

intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the investigational product; and/or the AE abates or resolves upon discontinuation of the investigational product and, if applicable, reappears upon re-challenge.

- Not Related – There is good evidence that the AE has an etiology other than the investigational product (e.g., pre-existing medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to administration of the investigational product (e.g., cancer diagnosed 2 days after treatment).

8.1.2 Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes:

- Death (i.e., the AE actually causes or leads to death)
- Life threatening event (i.e., the AE, in the view of the Investigator, places the subject at immediate risk of death)
- Requires or prolongs inpatient hospitalization
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- A congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product(s)

Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of relatively minor medical significance (such as severe

headache). "Serious" is a regulatory definition and is based on subject or event outcome or action criteria usually associated with events that pose a threat to a subject's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness will be independently assessed when recording AEs and SAEs on the CRF.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The Investigator is responsible for ensuring that all study-emergent AEs and SAEs are recorded on the CRF and reported to the sponsor in accordance with protocol instructions. For each SAE observed, the investigator will obtain all of the information available about the event, including (but not limited to): hospital discharge diagnoses, hospital discharge note, death certificate, appropriate laboratory findings (including autopsies and biopsy results), and clinical examinations (including radiological examinations and clinical consultations).

8.2.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after initiation of study treatment and ends at study completion or study discontinuation/termination, whichever is earlier. Any condition present between consenting and time of treatment will be considered as baseline and not recorded as an AE. After this period, investigators should report only SAEs that are attributed to study treatment.

All AEs that are observed or reported prior to initiation of study treatment will be recorded as unrelated to treatment and those occurring after initiation of treatment will be recorded as treatment-emergent.

8.2.2 Assessment of Adverse Events

Investigators will seek information on AEs and SAEs at each subject contact by specific questioning and, as appropriate, by examination. All AEs and SAEs, whether spontaneously reported by the subject or noted by authorized study personnel, will be recorded in the subject's medical record and on the appropriate AE or SAE

CRF page.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, regulatory seriousness criteria if applicable, suspected relationship to the investigational product, and actions taken.

Note: The investigator's assessment of causality for individual AE reports is part of the study documentation process. Regardless of the "Yes" or "No" causality assessment for individual AE reports, the sponsor will promptly evaluate all reported SAEs against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators and applicable regulatory authorities. Attribution of SAEs will be reviewed on an ongoing basis, and may be changed as additional clinical data emerge (e.g., reversibility of AE, new clinical findings in subject with AE, effects of retreatment, AEs in other subjects).

8.2.3 Recording Adverse Events on the CRF

Investigators will use correct medical terminology/concepts when recording AEs or SAEs on the CRF. Avoid colloquialisms and abbreviations.

AEs will be recorded either on an AE CRF page (if no serious criteria are met) or SAE CRF page, but not both.

For each SAE observed, the investigator must report the triggering event for that particular episode of illness as the primary event.

Only one medical concept will be recorded in the event field on the AE or SAE CRF page.

8.3 Expedited Reporting Requirements for Serious Adverse Events

Any life-threatening (i.e., imminent risk of death) or fatal AE that is attributed by the investigator to the investigational product will be **telephoned to the Medical Monitor (or alternate) immediately**.

Medical Monitor: TBD

For initial SAE reports, investigators will record all case details that can be gathered within 24 hours on an SAE CRF page. The completed SAE CRF page will be faxed immediately upon completion to the attention of:

Dr. Nikolai I. Tankovich, M.D., Ph.D.,

President and Chief Medical Office, Stemedica Cell Technologies, Inc

Office Telephone No.:

Mobile Phone: 858 610-2588

Email: ntankovich@stemedica.com

Relevant follow-up information will be submitted as soon as it becomes available and/or upon request.

8.4 Type and Duration of Follow-Up of Subjects After Adverse Events

The Investigator will follow all unresolved study-emergent AEs and SAEs until the events are resolved, stabilized, the subject is lost to follow-up, or until it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AEs and SAEs (with dates) will be documented on the appropriate AE or SAE CRF page and in the subject's medical record to facilitate source data verification. For some SAEs, the sponsor or its designee may follow-up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

8.5 Data Safety and Monitoring Board (DSMB)

An independent DSMB appointed by the Sponsor will review the protocol and will thereafter provide medical and ethical guidance related to the conduct of this trial. During the study, the board will conduct ongoing review of the nature, frequency and severity of safety data. The DSMB Chairperson has the option to recommend suspension of enrollment in the study if, upon initial deliberation by the DSMB, urgent safety issues or alarming trends in summary safety data are identified that require further investigation.

The study sponsor will report all SAEs to the study DSMB. The DSMB will determine whether enrollment will be

continued, suspended, or terminated. In the event that the DSMB determines that the study will be suspended or terminated, the study sponsor will notify the Institutional Review Boards and the appropriate regulatory agencies.

9 STATISTICAL CONSIDERATIONS

Statistical analyses will be descriptive. Summary statistics will consist of sample size (N), means, standard deviations, medians, and minimum and maximum values for continuous variables, and counts and percentages for categorical variables. Tables will summarize all safety and efficacy outcome measures by actual treatment received. Summary tables will indicate the number of subjects with complete data for each measurement, event or outcome. No substitutions will be made for missing data. All analyses will be based on available data.

A detailed Statistical Analysis Plan will be written prior to data analysis. Significant deviations from the original statistical analysis plan will be detailed in the final study report.

This study intentionally is not powered and will enrolled 30 patients with 15 in each of the two randomization arms.

9.1 Randomization

Once eligibility and consent are confirmed, participants will be randomly assigned to the experimental or placebo group in a 1:1 allocation ratio, stratified by LVAD type.

9.2 Primary Safety Analysis

All safety outcomes will be summarized in tables and listings presented by experimental group. Continuous measures will be summarized with descriptive statistics (N, mean, SD, median, min, max). Categorical measures will be summarized using counts and percentages. The incidence of AEs will be presented by

toxicity grade, MedDRA system organ class and preferred term, and by relationship of the AEs to the study treatment and compared using a Fisher's Exact Test. Laboratory and vital signs data will be presented using shift tables and summaries of change from baseline to next infusion or follow up visit.

9.3 Determination of Sample Size

This Study is exploratory and its sample size is not determined by statistical power considerations, but is considered appropriate for an early phase safety trial.

9.4 Interim Analysis

No formal interim analysis for efficacy is planned, as this is a study with careful monitoring of subject safety in an ongoing manner by the principal investigator, Sponsor and independent Data Safety Monitoring Board.

9.5 Data Quality Assurance

The following steps will be taken to ensure the accuracy, consistency, completeness, and reliability of the data:

- Routine study site monitoring
- CRF review against source documents
- Data management quality control checks

10 INVESTIGATOR REQUIREMENTS

10.1 Study Initiation

Before the release of investigational products to the study site, the following documents must be on file with the sponsor:

- U.S. Food and Drug Administration (FDA) Form 1572 signed by the Principal Investigator or equivalent for non-U.S. sites if applicable.
    - The names of any sub-investigators must appear on this form. Investigators must also complete all regulatory documentation as required by local and national regulations.

- Current curricula vitae (CV) of the Principal Investigator and all subinvestigators
- Complete financial disclosure forms for the Principal Investigator and all subinvestigators
- Written documentation of Institutional Review Board (IRB)/Ethics Committee (EC) approval of the protocol (identified by protocol number or title and date of approval) and Informed Consent Form (ICF; identified by protocol number or title and date of approval)
- A copy of the IRB-approved ICF
- IRB composition or assurance letter as applicable
- Current laboratory certification of the clinical laboratory performing sample analysis, as well as current references ranges for all laboratory tests (*if applicable)*
- A Clinical Trial Agreement signed and dated by the study site
- Investigator Brochure receipt signed and dated by the Principal Investigator *(if applicable)*
- A Protocol Acceptance Form signed and dated by the Principal Investigator

10.2 Study Completion

The following data and materials are required before a study can be considered complete or terminated:

- All essential documents (e.g., curriculum vitae for the Principal Investigator and subinvestigator, U.S. FDA Form 1572 or equivalent (non-U.S.)
- Copies of protocol amendments, IRB/EC approval/notification, and signed and dated Protocol Amendment Acceptance Form(s) *(if applicable)*
- All data collection and query resolution complete Original, final SAE reports and all supporting documentation (i.e., discharge summaries, laboratory results)
- Original, final Pregnancy Information forms and all supporting documentation *(if applicable)*
- Complete and accurate investigational product accountability records and inventory log
- Laboratory results, clinical data, and all special test results from screening through the end of the study
- A summary of the study prepared by the principal investigator (IRB/EC summary close out letter is acceptable)

10.3 Informed Consent

A sample ICF is provided as an attachment. In conformance with Federal Regulations governing informed consent, the STEMVAD study informed consent form that is given to the subject or authorized representative must be in a language understood by the patient or his/ her legal representative.

Prior to participation in the study, each patient must give written informed consent after having the study fully explained to him/her in the language that is most easily understood by the patient. All patients must be given the opportunity to ask questions and to have those questions answered to his/her satisfaction. All informed consent forms must be approved by the IRB before use and no patient may be consented unless such approval has been granted. All ICFs must be written in accordance with the current guidelines as outlined by GCP, and ICH.
All informed consent forms must be signed and dated by the person providing explanation of the study and signed and dated by the patient or his/her legal representative prior to participation in this research study. A signed and dated copy of the informed consent form will be given to each patient.

10.4 Institutional Review Board

A copy of the protocol, proposed ICFs, all written patient information, and any proposed advertising material must be submitted to the institution's IRB for written approval. No site may initiate study procedures/activities until such IRB approval had been granted in writing. A copy of the written IRB approval of the protocol and ICF must be received by the Sponsor of the trial before study supplies are shipped to the investigative site. The Investigator must submit and, where necessary, obtain IRB approval for all protocol amendments and changes to the ICF. If any investigator, co-investigator or sub-investigator participating in this study is a member of the IRB approving the study for the site, documentation must be provided by the IRB showing that the investigator did not vote on the approval of the study.

10.5 Study Monitoring Requirements

The study site will be monitored by the Sponsor's designee. The Principal Investigator will permit said designee, the U.S. FDA or other regulatory agencies, Ethics Committees and the respective national or local

health authorities to inspect facilities and records relevant to this study, in accordance with GCP and the ICH. If the investigator is notified of an audit pertaining to this study by the U.S. FDA or other applicable regulatory authorities, the Investigator must notify the Sponsor immediately.

The Investigator must provide sufficient space and allocate sufficient time for the monitor to inspect subject source records, CRFs, investigational product accountability records, and regulatory documents.

10.6 Case Report Forms

All CRFs will be filled out completely by designated personnel.

10.7 Source Data Documentation

Study monitors will perform ongoing source data verification to confirm that critical protocol data (i.e., source data) transcribed on the CRFs by authorized site personnel are accurate, complete, and verifiable from source documents. Source documents are where subject data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, laboratories, and medico-technical departments involved in a clinical trial. Source documents that are required to verify the validity and completeness of data transcribed on the CRFs must never be obliterated or destroyed. To facilitate source data verification, the investigator(s) and institution(s) must provide the sponsor direct access to applicable source documents and reports for trial-related monitoring, sponsor audits, and IRB review. The investigational site must also allow inspection by applicable regulatory authorities.

10.8 Investigational Product Accountability

All investigational products required for completion of this study will be provided by CardioCell, LLC. The recipient will acknowledge receipt of the drug by returning the appropriate documentation form indicating

shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study drug received at, dispensed from, returned to and disposed of by the study site will be recorded.

Investigational product will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to CardioCell, LLC with the appropriate documentation, as determined by the study site. If the study site chooses to destroy investigational product, the method of destruction must be documented.

CardioCell, LLC must evaluate and approve the study site's drug destruction standard operating procedure prior to the initiation of drug destruction by the study site.

10.9 Retention of Records (for approval enabling studies only)

United States FDA regulations (21 CFR §312.62[c]) and the ICH Guideline for GCP require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after the last marketing application approval in an ICH region or after at least 2 years have elapsed since formal discontinuation of clinical development of the investigational product. All state and local laws for retention of records also apply.

No records will be disposed of without written approval from the Sponsor. Written notification will be provided to Sponsor for transfer of any records to another party or moving them to another location.

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APPENDIX A: SCHEDULE OF ASSESSMENTS

Visit	Screening	Baseline	Randomization	Infusion 1	Post Infusion 1	Infusion 2	Post Infusion 2	Infusion 3	Post infusion 3	3 months	6 months	12 months	Early Term
Day	**Days -12 to -11**	**Day -5 to -1**		**Day 0**	**1 Week**	**Day 30±7**	**1 Week**	**Day 60±7**	**1 Week**	**Day 90±7**	**Day 180±7**	**Day 360±7**	
Informed Consent Form	X												
IV Infusion				**X**		**X**		**X**					
HIV Test	X												
Randomization			X										
Vital Signs and LVAD parameters**	X			X		X		X		X	X	X	X
Pulse Oximetry				X		X		X		X	X	X	X
Medical History	X			X		X		X		X	X	X	X
Concomitant medications	X			X		X		X		X	X	X	X
Physical Examination	X			X		X		X		X	X	X	X
6 minute walk test		X				X		X		X	X	X	X
Health Assessment, KCCQ, NYHA		X		X		X		X		X	X	X	X
12-lead ECG	X	X		X		X		X		X	X	X	X
Full 2D and 3 D Doppler transthoracic echocardiography with speckle-tracking		X				X		X		X	X	X	X
Zio patch (for patients who do not have an ICD)*		X		X		X		X		X	X	X	X
Gated blood pool SPECT ventriculography		X								X	X		X
ICD device check		X		X	X	X	X	X	X	X	X	X	X
CBC with differential				X		X		X		X	X	X	X
Serum Chemistry with BUN/Creatinine, LFT, total protein, albumin				X		X		X		X	X	X	X
LDH				X		X		X		X	X	X	X
HLA				X		X		X		X	X	X	
NT-proBNP				X		X		X		X	X	X	X
SST2				X		X		X		X	X	X	X
Troponin I				X		X		X					
IgA, IgE, IgG, and IgM				X		X		X		X	X	X	X

Biomarkers of immune response: CD14 CD163 CD40 CRP E-Selectin, Fas, (TNFRSF6/Apo-1) Fas Ligand, (TNFSF6) GCSF ICAM-1, (CD54) IL-1 alpha, (IL-1 F1), IL-1 beta IL-1 R4 IL-10 IL-12 p70 IL-13, IL-18 IL-2 IL-2 R alpha IL-4 IL-6, IL-8 Lipocalin-2 MCP-1 MCP-2 MIF, MIP-1 alpha MIP-1 beta Osteopontin PAI-1 Platelet Factor 4, Procalcitonin RAGE Resistin Thrombomodulin TNF alpha, TREM-1 Troponin I uPAR VCAM-1 VEGF-A, Antibody Isotype, Immune phenotyping, Proliferation Assay, In-vitor cytokine secretion, Cytokine assay (to be collected in 20 mL of blood—4 lavender tubes)				X	X	X	X	X	X	X	X	X	X
Total CK				X		X		X					
CK-MB				X		X		X					
Uric Acid				X		X		X		X	X	X	
HS-CRP				X		X		X		X	X	X	
Adverse Events				X		X		X		X	X	X	X

‡ A coronary angiogram performed prior to LVAD implantation may be used to determine eligibility.
*Zio patch recording or ICD device check must be completed prior to IV infusion.
**Vital signs and LVAD parameters taken every 2 hours after infusion and pulse oximetry taken continuously for 2 hours after IV infusion. Vital signs include temperature, blood pressure, heart rate respiratory rate and weight, LVAD parameters include pump speed, power, flow and pulsatilityNeed continuous ECG monitoring during infusion

APPENDIX B: QUESTIONNAIRES

THE KANSAS CITY CARDIOMYOPATHY QUESTIONNAIRE (KCCQ)

The following questions refer to your **heart failure** and how it may affect your life. Please read and complete the following questions. There are no right or wrong answers. Please mark the answer that best applies to you.

1. **Heart Failure** affects different people in different ways. Some feel shortness of breath while others feel fatigue. Please indicate how much you are limited by **heart failure** (shortness of breath or fatigue) in your ability to do the following activities over the past 2 weeks.

Place an X in one box on each line

Activity	**Extremely limited**	**Quite a bit limited**	**Moderately limited**	**Slightly limited**	**Not at all limited**	**Limited for other reasons or did not do the activity**
Dressing yourself	†	†	†	†	†	†
Showering/Bathing	†	†	†	†	†	†
Walking 1 block on level ground	†	†	†	†	†	†
Doing yardwork, housework or carrying groceries	†	†	†	†	†	†
Climbing a flight of stairs without stopping	†	†	†	†	†	
Hurrying or jogging (as if to catch a bus)	†	†	†	†	†	†

2. Compared with 2 weeks ago, have your symptoms of **heart failure** (shortness of breath, fatigue or ankle swelling) changed? My symptoms of **heart failure** have become…

Much worse	Slightly worse	Not changed	Slightly better	Much better	I've had no symptoms over the last 2 weeks
†	†	†	†	†	†

3. Over the past 2 weeks, how many times did you have **swelling** in your feet, ankles or legs when you woke up in in the morning?

Every morning	3 or more times a week, but not every day	1-2 times a week	Less than once a week	Never over the past 2 weeks
†	†	†	†	†

4. Over the past 2 weeks, how much has **swelling** in your feet, ankles or legs bothered you? In has been…

Extremely bothersome | Quite a bit bothersome | Moderately bothersome | Slightly bothersome | Not at all bothersome | I've had no swelling

Protocol:

† † † † † †

5. Over the <u>past 2 weeks</u>, on average, how many times has **fatigue** limited your ability to do what you want?

All of the time | Several times per day | At least once a day | 3 or more times per week but not every day | 1-2 times per week | Less than once a week | Never over the past 2 weeks

6. Over the <u>past 2 weeks</u>, how much has your **fatigue** bothered you? It has been…

Extremely bothersome | **Quite a bit bothersome** | **Moderately bothersome** | **Slightly bothersome** | **Not at all bothersome** | I've had **no fatigue**

† † † † † †

7. Over the <u>past 2 weeks</u>, on average, how many times has **shortness of breath** limited your ability to do what you wanted?

All of the time | Several times per day | At least once a day | 3 or more times per week but not every day | 1-2 times per week | Less than once a week | Never over the past 2 weeks

† † † † † † †

8. Over the past 2 weeks, how much has your shortness of breath bothered you?

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no shortness of breath**
†	†	†	†	†	†

9. Over the past 2 weeks, on average, how many times have you been forced to sleep sitting up in a chair or with at least 3 pillows to prop you up because of **shortness of breath**?

Every night	3 or more times a week, but not every day	1-2 times a week	Less than once a week	Never over the past 2 weeks
†	†	†		

10. **Heart Failure** symptoms can worsen for a number of reasons. How sure are you that you know what to do, or whom to call, if your **heart failure** gets worse?

Not at all sure	**Not very** sure	**Somewhat** sure	**Mostly** sure	**Completely** sure
†	†	†	†	†

11. How well do you understand what things you are able to do to keep your **heart failure** symptoms from getting worse? (for example, weighing yourself, eating a low salt diet, etc.)

Do not understand at all	Do not understand well	Somewhat understand	Mostly understand	Completely understand very
†	†	†	†	†

12. Over the past 2 weeks, how much has your **heart failure** limited your enjoyment of life?

It has **extremely** limited my enjoyment of life	It has limited my enjoyment of life **quite a bit**	It has **moderately** limited my enjoyment of life	It has **slightly** limited my enjoyment of life	It has **not limited** my enjoyment of life at all

13. If you had to spend the rest of your life with your **heart failure** the way it is right now, how would you feel about this?

Not at all satisfied	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
†	†	†	†	†

14. Over the past 2 weeks, how often have you felt discouraged or down in the dumps because of your

heart failure?

I felt that way **all of the** time	I felt that way **most of the** time	I **occasionally** felt that way	I **rarely** felt that way	I **never** felt that way
†	†	†	†	†

15. How much does your **heart failure** affect your lifestyle? Please indicate how your **heart failure** may have limited your participation in the following activities <u>over the past 2 weeks</u>?
Please place an X in one box on each line

Activity	**Severely** limited	Limited **quite a bit**	**Moderately** limited	**Slightly** limited	**Did not** limit at all	Does not apply or did not do for <u>other reasons</u>
Hobbies, recreational activities	†	†	†	†	†	†
Working or doing household chores	†	†	†	†	†	†
Visiting family or friends out of your home	†	†	†	†	†	†
Intimate relationships with loved ones	†	†	†	†	†	†

Developed by John Spertus et al., Mid America Heart Institute, Saint Luke's Hospital, Kansas City, MO.

NEW YORK HEART ASSOCIATION (NYHA)

Protocol Number	Site Number	Subject Number	Subject Initials	Visit Date (dd-MMM-yyyy)
	☐☐☐	☐☐☐☐	☐☐☐	_ _-_ _ _-_ _ _ _

Examination	Not Done	Result		If abnormal findings, describe
		Yes	No	
Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities	☐	☐	☐	
Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion	☐	☐	☐	
Class III: patients marked with limitation of activity; they are comfortable only at rest	☐	☐	☐	
Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest	☐	☐	☐	