

Safety & Efficacy of Intramyocardial Injection of Mesenchymal Precursor Cells on Myocardial Function in LVAD Receptients (LVAD MPC II)

NCT02362646

Document Date: August 2018

Cardiothoracic Surgical Trials Network

SAFETY & EFFICACY OF INTRAMYOCARDIAL INJECTION OF MESENCHYMAL PRECURSOR CELLS ON MYOCARDIAL FUNCTION IN LVAD RECIPIENTS (LVAD MPC II)

Protocol



Sponsored By NHLBI, NINDS, and CIHR

CT Surgery Network Research Group

Data Coordinating Center
InCHOIR
Icahn School of Medicine at Mount Sinai
New York

October 2016
Revision 4.1

CONFIDENTIAL

TABLE OF CONTENTS

Abstract	11
Data Collection Schedule.....	13
Objectives	15
Background And Rationale.....	15
Study Design.....	23
Endpoints	23
Study Subjects.....	24
Eligibility Criteria	25
Treatment Assignments	26
Randomization	26
Treatment Intervention.....	26
LVAD Implant	27
Definition and Measurement of Endpoints.....	27
Adverse Events	33
Clinical Centers.....	44
Investigators.....	45
Site Initiation.....	46
Data Collection	46
Data Management	56
Statistical Analysis and Sample Size Justification.....	57
Organization of the Study	61
References.....	64

APPENDICES

Appendix I: Intramyocardial Injection Procedures

TABLE OF CHANGES

Version	Section	Change	Reason	Page
2.0	Title Page	Revised title page to indicate Rev 2.0 and date of August 2014.	Protocol update	1
2.0	Footer	Changed footer Rev 2.0 and date of August 2014.	Protocol update	All
2.0	Definitions, Acronyms & Abbreviations	Removed definitions, acronyms and abbreviations not found in the protocol and added Clinical Trial Application, Personal Information Protection and Electronic Documents Act and mechanical circulatory support to the list.	Internal consistency within protocol	6-7
2.0	Study Synopsis	Added Survival as a Secondary Endpoint	Protocol Update	8
2.0	Secondary Endpoints	Hospitalizations added as a secondary endpoint	Protocol update	9, 21, 29
2.0	Data Collection Schedule	Updated Data Collection Schedule	Internal consistency within protocol	10-11
2.0	Selected Inclusion Criteria	Updated Item #2 to indicate that FDA-approved LVADs will be utilized at US sites and Health Canada-approved LVADs will be utilized at Canadian sites.	Regulatory clarification	9
2.0	Exclusion Criteria	Concomitant arrhythmia ablation at the time of LVAD implantation added as an exclusion criterion.	Protocol Update	9, 22
2.0	Study Design	Updated to indicate that FDA-approved LVADs will be utilized at US sites and Health Canada-approved LVADs will be utilized at Canadian sites.	Regulatory clarification	19-20
2.0	Study Design	Clarified description of patient follow-up duration	For clarity	20
2.0	Physiological Assessments	Removed anti-HLA sensitization post-implant	Internal consistency within protocol	21
2.0	Study Subjects	Updated to indicate that FDA-approved LVADs will be utilized at US sites and Health Canada-approved LVADs will be utilized at Canadian sites.	Regulatory clarification	21
2.0	Inclusion Criteria	Updated Item #6 to indicate that FDA-approved LVADs will be utilized at US sites and Health Canada-approved LVADs will be utilized at Canadian sites.	Regulatory clarification	22
2.0	Exclusion Criteria	Anti-HLA antibody titer exclusion criteria footer revised to specify type of PRA documented by the clinical site laboratory and type documented by the core laboratory.	For clarity	22
2.0	Randomization	Revised Cohort 1 to MPCs in the first sentence of the section.	Internal consistency within protocol	23
2.0	LVAD Implant and Management	Updated to indicate that FDA-approved LVADs will be utilized at US sites and Health Canada-approved LVADs will be utilized at Canadian sites.	Regulatory clarification	24
2.0	Secondary Endpoints	Updated CTSN Neurocognition Committee to Neurocognitive Core lab.	Internal consistency within protocol	29
2.0	Secondary Endpoints	Added Spanish as a language validated for the neurocognitive batteries.	For consistency between protocol and core laboratory documents	29
2.0	Adverse Events	Revised second paragraph to include “in compliance with their institutional policies” at the end of the sentence.	Protocol update	30
2.0	Adverse Events	Added Research Ethics Boards	To reflect inclusion of Canadian sites in study	30, 31, 38-40, 55
2.0	Expedited Reporting	Added description of SAE reporting to Health Canada	To reflect inclusion of Canadian sites in study	31
2.0	Clinical Centers and Investigators	Added additional information on and documentation required of Canadian sites	To reflect inclusion of Canadian sites in study	38-39
2.0	Conflict of Interest	Added mention of Tri-Council Policy and removed “and no less than annually” from the last sentence.	To reflect inclusion of Canadian sites in study and for consistency with Network procedures	40

Version	Section	Change	Reason	Page
2.0	Patient Confidentiality/HIPAA Certification	Added Health Canada to list of possible reviewers of data and PIPEDA as governing regulation	For clarity	40
2.0	Site Initiation	Added "and other regulatory documents" to second sentence of section.	For clarity	40
2.0	Data Collection	Clarified which case report forms will capture each assessment.	For consistency between protocol and electronic data capture system	40-46
2.0	Pre-Screening Failure Form	Renamed section to Screening Failure	For consistency between protocol and electronic data capture system	40
2.0	Pre-Screening Failure Form	Clarified the HIPAA and PIPEDA compliant information collected.	For consistency between protocol and electronic data capture system	40
2.0	Informed Consent	Changed description of authorization for use and disclosure of private information	To reflect inclusion of Canadian sites in study	41
2.0	Demographics	Updated the demographic data collected.	For consistency between protocol and electronic data capture system	41
2.0	Physical Exam	Removed BSA as it will be automatically calculated from height and weight in the electronic data capture system.	For consistency between protocol and electronic data capture system	40
2.0	Medications	Clarified how medications will be collected and documented during the course of the trial.	For consistency between protocol and electronic data capture system	41 & 46
2.0	Laboratory Assessments	Removed HCO ₃ or CO ₂ (mM/L), total bilirubin (mg/dL), alkaline phosphate (IU/L), lactate dehydrogenase (LDH; U/L), coagulation profile, red blood cell (10 ³ /mL), neutrophils (%) and lymphocytes (%) laboratory assessments.	Protocol update	42 & 45
2.0	Immunologic Assessment	Removed baseline immunologic assessment sample obtained within 24 hours prior to randomization.	Protocol update	43
2.0	Echocardiography	Revised collection window to 14 days prior to randomization.	Protocol update	43
2.0	Echocardiography	Changed the method of submitting echocardiograms to the Core Lab from shipping to uploading.	For consistency between protocol and electronic data capture system	43
2.0	Modified Rankin Scale	Added Appendix VII as Modified Rankin Scale location.	Protocol update	43 & 48
2.0	Intervention Injection Verification	Revised method for verifying and submitting injection verification.	For consistency between protocol and electronic data capture system	44
2.0	Post-Intervention Data Collection	Removed hemodynamics data collection.	Internal consistency within protocol	46
2.0	Missed Visit	Revised how missed visits will be captured.	For consistency between protocol and electronic data capture system	49
2.0	Monitoring	Added age and removed initials and date of birth from the data points to be verified during a monitoring visit.	For consistency between protocol and electronic data capture system	51
2.0	Monitoring	Updated close-out visit description	For consistency between protocol and DCC procedures	51
2.0	Statistical Analysis and Sample Size Justification	Add analytical plan for hospitalization secondary endpoints.	Protocol update	54
2.0	Event Adjudication Committee	Updated description of EAC membership	For consistency between protocol and DCC procedures	55
2.0	DCC, Investigator and Mesoblast Sections	Updated to include Canadian documentation and requirements	For clarity	55-56

Version	Section	Change	Reason	Page
2.0	Appendix I	Revised to <i>Injection Verification Case Report Form</i> to <i>Treatment Intervention Case Report Form</i> and provided updated instructions for verifying injections.	Consistency between protocol and electronic data capture system	60-62
2.0	Appendix II	Early terminations guidelines for LVAD wean clarified.	Consistency within protocol	64
2.0	Appendix VII	Renamed Appendix from NIH Stroke Scale Administration Guidelines to Neurological Assessments.	Consistency within protocol	73
2.0	Appendix VII	Inserted NIH Stroke Scale Administration Guidelines header.	Internal consistency within protocol	73
2.0	Appendix VII	Added Modified Rankin Scale	Internal consistency within protocol	77
3.0	Title Page	Revised title page to indicate Rev 3.0 and date of March 2015	Protocol update	1
3.0	1° Endpoints and 2° Endpoints	Changed wean and functional status assessments to over 6 months (primary endpoint) and 12 months (secondary endpoint)	To align data collection schedule better with expected time on VAD for BTT patients	9, 21, 26-29
3.0	Weaning and Six Minute Walk Schedule	Added detail on timepoints for these assessments	Protocol clarification	9, 21, 26, 28
3.0	Exclusion Criteria	Added history of known or suspected hypercoagulable state as an exclusion criterion	Consensus of clinical investigators	10, 24
3.0	Duration, Study Design	Changed follow-up duration from 12 to 24 months	For collection of long term follow up data	9, 21
3.0	Data Collection Schedule	Changed 90 day wean and chemokine and cytokine assessment to 4 months ; added detail on LDH collection to Laboratory Assessments; added 24 month Vital Status Follow-up	For internal consistency within protocol	11-12
3.0	Rationale for Selection of the Efficacy Endpoint	Changed primary endpoint to weans conducted over 6 months	For internal consistency within protocol	18
3.0	Physiological Assessments	Updated WMSI assessment schedule	Protocol update	22, 29
3.0	Functional Status	Changed scheduled to 9 and 12 months	Protocol update	27
3.0	Cytokine Quantification	Changed assessment schedule to 120 days	Protocol update	31
3.0	Hemolysis	Added information on assessments to be conducted in event of hemolysis	Protocol update	36
3.0	Laboratory Assessment	Added 4 months to biospecimen collection, laboratory assessment, physical exam, medications and early stopping events schedule	Protocol update	46-48
3.0	Laboratory Assessments	Added LDH detail to laboratory assessment	Protocol update	46
3.0	LVAD Wean and Functional Assessment	Updated echo, 6MWT, wean and functional status data collection timepoints	To align data collection schedule better with expected time on VAD for BTT patients	46-48
3.0	Vital Status Follow-Up	Added 24 month follow-up for check on vital status	For collection of long term follow up data	49
3.0	Statistical Analysis and Sample Size Justification	Provided updated information on statistical analysis plan and sample size calculation	Protocol update	52-55
3.0	Peripheral Blood	Changed collection at 90 days to 120 days	Internal consistency within protocol	72
3.0	Tests to be Performed	Changed collection at 90 days to 120 days	Internal consistency within protocol	73
4.0	Title Page	Revised title page to indicate short name of the trial (i.e., LVAD MPC II), Rev 4.0 and date of January 2016	Protocol Update	1
4.0	Header & Footer	Updated header and footer to standard CTSN document control	Protocol Update	ALL

Version	Section	Change	Reason	Page
4.0	Table of Contents	Updated to reflect changes in protocol, including removal of Appendices II-IX	For consistency with all other CTSN protocols Appendices II-IX were moved to the appropriate Manual of Procedures/Manual	2
4.0	Abstract	Removed Anti-HLA sensitization post-transplant (for transplanted BTT patients)	Internal consistency within protocol as this activity is not part of the protocol	10
4.0	Abstract	Revised exclusion criteria	To clarify specific criteria for enrollment purposes and be consistent where possible with the industry partner's heart failure protocol	11
4.0	Data Collection Schedule	Removed Month 9 Immunologic Assessment and Biospecimen Assessment	Internal consistency within protocol	12
4.0	Data Collection Schedule	Added 4 Days Post RAND	Internal consistency within protocol	12-13
4.0	Data Collection Schedule, Cytokine Quantification, Laboratory Assessment, Biospecimen Analyses	Revised 7 Days Post RAND window to ± 2	To align data collection & avoid overlap with 4 Days post RAND assessment window	12-13, 31, 47-50,
4.0	Data Collection Schedule	Removed Post transplant Assessment row and corresponding footer for clinical biopsy and rejection monitoring results	Internal consistency within protocol as this activity is not part of the protocol	12-13
4.0	Objectives	Added short name of trial	For convenience and clarity, when referring to the trial or the protocol-specific Manual of Procedures (MOP)	14
4.0	Endpoints	Revised the reference as to where details concerning the LVAD wean are found, i.e., from Appendix II to relevant section of protocol	For consistency and clarity referred reader to the section within the protocol where this information is found.	22
4.0	Physiological Assessment	Removed 4 month WMSI assessment	This assessment was included in Version 3.0 in error. The data will not be used for either the primary or secondary endpoint analyses and is thus being removed to correct the error	23, 30
4.0	Physiological Assessments	Removed PTAV, TAPSE and RVIF Peak velocity and simplified section as Appendix V was moved to the LVAD MPC II MOP	Consensus of Echo Core Lab Director and clinical investigators after gaining experience under Version 3.0 and realizing that obtaining these values is burdensome for the site Echo labs; additionally, Appendix V was moved to the LVAD MPC II MOP for consistency with other CTSN protocols	23, 30
4.0	Study Subjects	Clarified monthly enrollment statement	For consistency with anticipated enrollment period	24

Version	Section	Change	Reason	Page
4.0	Eligibility Criteria	Modified Exclusion Criteria	To clarify specific criteria and be consistent where possible with the industry partner's heart failure protocol	24-25
4.0	Treatment Intervention	Added the relevant MOP title because there are multiple manuals for this protocol due to its complexity	For clarification & consistency across trial documents	26
4.0	Definition and Measurement of Endpoints	Clarified where wean guidelines are now found and deleted redundant references to Appendix II. Also clarified where information from Appendices III-IX are now found.	For consistency with other CTSN protocols, Appendices II-IX moved to LVAD MPC II MOP.	27-31
4.0	Definition and Measurement of Endpoints	Removed specific amount of time sustained signs and symptoms should be noted before early termination of wean	To clarify that the senior clinician overseeing the wean is to determine early termination of wean based on signs and symptoms; additionally, for consistency with LVAD Wean Procedure Guidelines	27, 28
4.0	Adverse Events	Modified Protocol-Defined Adverse Events	For consistency with current INTERMACS registry definitions to enable standardization by current recognized and accepted definitions	36-44
4.0	Adverse Events	Added Pleural Effusion as a Protocol-Defined Adverse Event	For consistency with other CTSN protocols	37
4.0	Clinical Centers	Increased the number of sites from 25 to 30 as a precautionary measure	To ensure that accrual milestones are met	44
4.0	Site Initiation	Updated this section to explain the site initiation process as well as the personnel required to attend the pre-enrollment conference call	For clarity and consistency with the LVAD MPC II MOP	46
4.0	Screening/Pre-Implant Data Collection	Revised section header to Pre-Implant (Screening and Baseline) Data Collection	For consistency across trial documents	46
4.0	Demographics	Removed handedness	For consistency between protocol and EDC	47
4.0	Medications	Clarified collection for Cardiovascular and Non-Cardiovascular Medications	For consistency between protocol and EDC	47
4.0	Laboratory Assessment	Revised collection windows based on eligibility and to align with standard of care	For consistency with clinical practice	47
4.0	Biospecimen Analyses	Revised collection window to within 72 hours prior to Treatment Intervention and simplified this section	For consistency with Laboratory Assessment Schedule and Biospecimen Core Laboratory Manual	47-48
4.0	Immunologic Assessment	Clarified collection window of anti-murine and anti-bovine samples and simplified this section	Clarification of protocol and for consistency with Biospecimen Core Laboratory Manual	48
4.0	Quality of Life	Removed reference to QoL Checklist	For consistency between protocol and EDC	49
4.0	Post Intervention Data Collection - Laboratory Assessment	Added LDH collection schedule	Consistency within protocol	51
4.0	Biospecimen Analyses	Simplified section	For consistency with Biospecimen Core Laboratory Manual	51
4.0	Immunologic Assessment	Simplified section	For consistency with Biospecimen Core Laboratory Manual and clarification of protocol	51

Version	Section	Change	Reason	Page
4.0	Medications	Clarified collection for Cardiovascular and Non-Cardiovascular Medications	For consistency between protocol and EDC	51
4.0	Wean Assessment	Revised sentence on hypoperfusion assessment	For consistency between protocol and EDC	53
4.0	Quality of Life	Removed reference to QoL Checklist	For consistency between protocol and EDC	53
4.0	Appendices II - IX	Appendices II through IX were moved to the MOP and any references to these Appendices were updated throughout protocol.	For consistency with all other CTSN protocols	68-106
4.1	Title Page, Header & Footer	Revised title page and header and footer on all pages to indicate Rev 4.1 and date of October 2016	Protocol Update	1
4.1	Abstract	Changed number of subjects from 120 to 159 (Groups 1 and 2) and treatment arms from 80 to 106 (Group 1) and from 40 to 53 (Group 2)	Sample size correction	11
4.1	Study Subjects	Changed number of subjects from 120 to 159	Sample size correction	24
4.1	Treatment Assignments	Changed treatment arm sample sizes from 80 to 106 (Group 1) and from 40 to 53 (Group 2)	Sample size correction	26
4.1	Laboratory Assessment	Added Day 30 collection of hematology and chemistry samples	To correct internal inconsistency in protocol	51
4.1	Statistical Analysis And Sample Size Justification	Revised Sample Size Justification and moved figure to the paragraph to which it is referenced.	Modification corrects original sample calculations and page formatting	58

DEFINITIONS, ACRONYMS & ABBREVIATIONS

6MWT	Six Minute Walk Test
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BTT	Bridge to transplant
C	centigrade
CABG	Coronary Artery Bypass Grafting
CAD	coronary artery disease
CFR	Code of Federal Regulations
CHF	congestive heart failure
CI	cardiac index
CI	confidence interval
CO	cardiac output
CTA	Clinical Trial Application
CTSN	Cardiothoracic Surgical Trials Network
DCC	Data Coordinating Center
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
DT	Destination therapy
eCRF	Electronic case report form
EAC	Event Adjudication Committee
EDC	Electronic Data Capture System
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Medical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HLA	Human leukocyte antigen
ICH	International Conference on Harmonization
IDC	Idiopathic dilated cardiomyopathy
IgG	Immunoglobulin G
InCHOIR	<u>I</u> nternational <u>C</u> enter for <u>H</u> ealth <u>O</u> utcomes and <u>I</u> nnovation <u>R</u> esearch
IND	Investigational New Drug
IRB	Institutional Review Board
LDH	lactate dehydrogenase
LV	left ventricle
LVAD	left ventricular assist device
LVEF	left ventricular ejection fraction
MCSD	Mechanical circulatory support device
MOP	Manual of Procedures
mRNA	messenger ribonucleic acid
MPC	mesenchymal precursor cells
NHLBI	National Heart, Lung and Blood Institute
OHT	orthotopic heart transplantation

PIPEDA	Personal Information Protection and Electronic Documents Act
PB	Peripheral blood
PRBC	packed red blood cells
PTAV	Peak tricuspid annular velocity
REB	Research Ethics Board
REMATCH	<u>R</u> andomized <u>E</u> valuation of <u>M</u> echanical <u>A</u> ssistance for the <u>T</u> reatment of <u>C</u> ongestive <u>H</u> eart Failure
RNA	ribonucleic acid
rpm	revolutions per minute
RV	right ventricle
RVIF	Right ventricular inflow
RVSP	RV systolic pressure
SOP	Standard Operating Procedures
TAPSE	Tricuspid annular plane systolic excursion
TR	Tricuspid regurgitation
TTE	Transthoracic echocardiogram
UNOS	United Network for Organ Sharing
VAD	ventricular assist device
VEGF	vascular endothelial cell growth factor
WMSI	Wall motion score index

ABSTRACT

Objectives	<p>The <i>primary objectives</i> of this trial are to evaluate the safety and efficacy of injecting MPCs (150 million dose) into the native myocardium of LVAD recipients.</p> <p>The <i>secondary objectives</i> are to explore the functional and physiologic effects of injecting MPCs (150 million dose) into the native myocardium of LVAD recipients.</p>
Study Design	Prospective, multi-center, double-blind, randomized, single dose cohort, sham procedure controlled trial
Target Population	Patients with end-stage heart failure, either ischemic or non-ischemic etiology, who are being evaluated for LVAD implantation as a bridge-to-transplant (BTT) or destination therapy (DT)
Rx arms	<p>Patients will be enrolled in a single dose cohort randomized in a 2:1 allocation to intramyocardial injection of study product or control (cryoprotective media alone) at the time of LVAD implantation:</p> <ul style="list-style-type: none"> ○ Group 1 (n=106): 150 million allogeneic MPCs (Mesoblast, Inc) ○ Group 2 (n=53): 50% Alpha-MEM/42.5% ProFreeze NAO Freezing Medium/7.5% DMSO (control)
Sample Size	159 patients
Duration	<p>All patients will be followed until cardiac transplantation (for bridge to transplant patients) or until 24 months post randomization, whichever comes first.</p> <ul style="list-style-type: none"> ○ Myocardial sample donation (at OHT or post mortem as relevant)
1° Endpoints	<p>The <i>primary safety endpoint</i> is the incidence of study intervention-related adverse events (i.e., infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization).</p> <p>The <i>primary efficacy endpoint</i> is the number of temporary weans from LVAD support tolerated over 6 months following randomization.</p>
2° Endpoints	<p><i>Secondary Endpoints</i> include:</p> <p><i>Safety</i></p> <ul style="list-style-type: none"> ○ Incidence of all serious adverse events ○ Anti-HLA antibody sensitization while on LVAD support <p><i>Survival</i></p> <ul style="list-style-type: none"> ○ Survival ○ Survival to cardiac transplantation <p><i>Functional Status (over 6 months)</i></p> <ul style="list-style-type: none"> ○ Duration of ability to tolerate wean from LVAD support- ○ 6 Minute walk test (6MWT) as tolerated at 20 (± 10) minutes following initiation of wean <p><i>Functional Status (over 12 months)</i></p> <ul style="list-style-type: none"> ○ Ability to tolerate each wean from LVAD support for 30 minutes ○ Duration of ability to tolerate wean from LVAD support ○ 6MWT as tolerated at 20 (± 10) minutes following initiation of wean <p><i>Physiologic Assessments</i></p> <ul style="list-style-type: none"> ○ Echocardiographic assessments of the myocardial size and function by transthoracic echocardiography with LVAD at full support, and as tolerated following 6MWT while weaned from LVAD support ○ Myocardial neovascularization at time of explant ○ Cardiomyocyte regeneration at explant ○ Cell Engraftment and fate at explant

	<p><i>Quality of Life & Neurocognition</i></p> <ul style="list-style-type: none"> ○ Quality of Life (QoL) as assessed by SF12 and Kansas City Cardiomyopathy Questionnaire (KCCQ) at 6 & 12 months ○ Neurocognition at 90 days and 12 months post randomization <p><i>Hospitalizations</i></p> <ul style="list-style-type: none"> ○ LOS of index hospitalization ○ Frequency (<30 days and ≥30 days following index hospital discharge) and cause of readmissions ○ Hospital resource use (or costs)
Weaning and 6-minute Walk Schedule	60 days, 4 mos, 6 mos, 9 mos, and 12 mos (or until transplant, whichever comes first)
Selected Inclusion Criteria	<ol style="list-style-type: none"> 1. Admitted to the clinical center at the time of randomization 2. Clinical indication and accepted candidate for implantation of an FDA-approved (US sites only) or Health Canada-approved (Canadian sites only) implantable, non-pulsatile LVAD as a bridge-to-transplant or for destination therapy
Selected Exclusion Criteria	<ol style="list-style-type: none"> 1. Planned percutaneous LVAD implantation; 2. Anticipated requirement for biventricular mechanical support; 3. Arrhythmia ablation at the time of LVAD implantation; 4. Planned aortic valve intervention for aortic insufficiency at the time of LVAD implantation; 5. Cardiothoracic surgery within 30 days prior to randomization; 6. Spontaneous myocardial infarction related to ischemia due to a primary coronary event such as unstable plaque rupture, erosion or dissection within 30 days prior to randomization; 7. Prior cardiac transplantation, LV reduction surgery, or cardiomyoplasty 8. Acute reversible cause of heart failure (e.g. myocarditis, profound hypothyroidism); 9. Stroke within 30 days prior to randomization; 10. Platelet count < 100,000/mcL within 24 hours prior to randomization; 11. Acute infectious process: acute bacterial, fungal or viral disease OR acute exacerbation of chronic infectious disease such as hepatitis ; 12. Presence of > 10% anti-HLA antibody titers with known specificity to MPC donor HLA antigens; 13. A known hypersensitivity to dimethyl sulfoxide (DMSO), murine, and/or bovine products; 14. History of a known active malignancy within the past 3 years except for localized prostate cancer, cervical carcinoma in situ, breast cancer in situ, or nonmelanoma skin cancer that has definitively been treated; 15. Presence of human immunodeficiency virus (HIV); 16. Received investigational intervention within 30 days of randomization; 17. Treatment and/or an incomplete follow-up treatment of any investigational cell based therapy within 6 months prior to randomization; 18. Active participation in other research therapy for cardiovascular repair/regeneration; 19. Prior recipient of stem precursor cell therapy for cardiac repair; 20. Pregnant or breastfeeding at time of randomization; 21. History of known or suspected hypercoagulable state in the opinion of the investigator

DATA COLLECTION SCHEDULE

Assessment	Pre-Implant	Randomization	LVAD Implant/ Intervention (INT)	12 hours post INT	1 Day post INT	4 Days post RAND	7 Days post RAND	30 Days post RAND	60 Days post RAND	90 Days post RAND	Month 4 post RAND	Month 6 post RAND	Month 9 post RAND	12 Months post RAND [‡]	24 Months post RAND	Event Driven
Window				± 4 hours	+1		± 2	± 3	± 7	± 14	± 14	± 14	± 14	± 14	± 30	
General																
Informed Consent	x															
Pre-Screening Failure	x															
Demographics	x															
Medical History	x															
Physical Examination	x				x		x		x	x	x	x	x	x		
Medications	x				x		x		x	x	x	x	x	x		x
Immunotherapy Medication	x															x
Laboratory Assessment ⁺	x			x		x ⁺	x	x	x	x	x	x	x	x		x
Immunologic Assessment	x							x		x		x		x		
Biospecimen: Chemo and Cytokine Analyses	x				x		x	x	x		x	x		x		
Eligibility Evaluation	x															
Randomization & Treatment Assignment		x														
Hospitalization			x													x
Surgical Procedure			x													x
QoL (SF12 & KCCQ)	x											x		x		
Vital Status Follow-Up															x	
Cardiac																
Hemodynamics	x															
Echocardiography	x								x		x	x	x	x		
Cardiac Histology [#]			x													x [#]
Six Minute Walk Test (6MWT)									x		x	x	x	x		
Intervention Injection Verification			x													
Wean Assessment									x		x	x	x	x		
Neurological																
NIH Stroke Scale ^{**}	x															x
Modified Rankin Scale ^{**}	x															x
Neurocognitive Testing	x									x				x		

DATA COLLECTION SCHEDULE (continued)

Assessment	Pre-Implant	Randomization	VAD Implant/ Intervention (INT)	12 hours post INT	1 Day post INT	4 Days post RAND	7 Days post RAND	30 Days post RAND	60 Days post RAND	90 Days post RAND	Month 4 post RAND	Month 6 post RAND	Month 9 post RAND	12 Months post RAND [‡]	24 Months post RAND	Event Driven
Window				± 4 hours	+1		± 2	± 3	± 7	± 14	± 14	± 14	± 14	± 14	± 30	
Event Driven Data																
Adverse Events																x
Early Stopping Events			x	x	x		x	x	x	x	x	x	x	x		x
Mortality																x
Pump Retrieval and Explant (&/or Postmortem) Examination																x
Missed Visit							x	x	x	x	x	x	x	x		x
Study Completion/Early Termination																
End of Study/Investigator Statement																

[‡] Final data collection
^{**} Event driven within 72 hours of a neurological event, and at 30 (±10) and 60 (±10) days post neurological event
[#] At native heart explantation for cardiac transplantation or at autopsy (if applicable)
⁺ LDH to be collected at Days 4, 7, 30, 60 and 90; at 4, 6, 9 and 12 months; and as an event-driven data point.

OBJECTIVES

The *primary objectives* of this trial are to evaluate the safety and efficacy of injecting higher dose allogeneic mesenchymal precursor cells (MPCs) into the native myocardium of LVAD recipients.

The *secondary objectives* of the LVAD MPC II trial are to explore the functional and physiologic effects of intramyocardial injection of the higher dose MPCs in LVAD recipients.

BACKGROUND AND RATIONALE

LVADs and Stem Cells: a Novel Strategy for Myocardial Recovery

Left ventricular assist devices (LVADs) have well-documented survival and quality of life benefits in patients with advanced heart failure both as a bridge to cardiac transplantation (BTT) and as a long-term therapy, so-called Destination Therapy (DT), in patients who are not transplant candidates.¹⁻⁴ Reports of improved myocardial function have motivated investigation of the use of LVADs as a bridge to recovery, but while most LVAD recipients do show some indications of reverse remodeling of the left ventricle (LV) as evidenced by salutary changes in ventricular structure, myocyte contractile strength⁵, normalization of extracellular matrix and tissue and circulating neurohormones⁶, and programs of gene expression,⁷⁻¹⁰ these improvements are rarely sufficient to allow removal of the device.¹¹ The disconnect between reverse remodeling and recovery of cardiac function, or “passive recovery”, have prompted our efforts to investigate “active recovery” to augment ventricular recovery with adjunctive therapies to LVAD support, such as the administration of stem cells at the time of LVAD implantation.

Recent pre-clinical and clinical evidence suggests that myocardial transplantation of allogeneic mesenchymal stem cells, in particular, can enhance cardiac performance in settings of acute and chronic functional impairment.¹²⁻¹⁴ Unlike whole organ transplantation or many other allogeneic cell transplants, mesenchymal stem cell transplants do not appear to cause rejection and instead may be associated with evidence of induced tolerance to the donor.^{15,16} While our ultimate goal is the achievement of bridging to myocardial recovery and successful LVAD explantation, allosensitization could adversely impact donor suitability in LVAD recipients who are transplant candidates. To this end, the Cardiothoracic Surgical Trials Network (CTSN) recently conducted, in collaboration with the Cardiovascular Cell Therapy Research Network (CCTRN), an exploratory trial of a single low dose (25 million [M]) allogeneic MPCs (Mesoblast, Inc.) injected into the native myocardium at the time of LVAD implantation. The objectives of this trial were to provide evidence of safety as well as explore the efficacy of the intervention. If safety were established, the pre-specified plan was to conduct a follow-up trial with higher dose MPCs.

Study Product: Mesenchymal Precursor Cells

Mesoblast’s allogeneic, immunoselected, *ex vivo* expanded MPCs are derived from bone marrow mononuclear cells, which are obtained from the posterior iliac crest of healthy human donors. The allogeneic MPCs are produced in a Good Manufacturing Practice (GMP) facility and cryopreserved until use. The allogeneic MPCs are a STRO-3 immuno-selected, culture-expanded, immature sub-fraction of adult bone marrow-derived mononuclear cells.^{14,17} They are formulated and cryopreserved in 7.5% DMSO/50% Alpha Modified Eagle’s Medium (MEM) and 42.5% ProFreeze® and stored in the vapor phase of liquid nitrogen until use. Cell procurement, processing, cryopreservation, and storage procedures are performed by a contract manufacturing

facility under GMP conditions. Donor and process testing are conducted for transmissible infectious diseases, karyotype, tumorigenicity, sterility, endotoxins, and mycoplasma. The product is characterized by cell count, viability, surface antigen expression of STRO-1, CC-9, and HLA class I and II. Cryopreserved products are shipped to clinical sites for local storage, and cells are thawed and injected according to study procedures. Mesoblast, Inc. contributed its MPCs for the initial trial conducted and will provide them for this current trial (see section on Study Administration). Further information on manufacturing and product characterization is presented in the Investigator's Brochure.

Pre-Clinical Studies

An extensive nonclinical program has been conducted to explore and confirm the safety and efficacy of administration of allogeneic MPCs for the treatment of patients with ischemic or nonischemic heart disease and heart failure. Mesenchymal precursor cells have been evaluated in vitro as well as in vivo in nonclinical studies with rodents and sheep (acute and chronic myocardial ischemia and doxorubicin-induced nonischemic cardiomyopathy).

Results from several in vitro studies, which included mixed lymphocyte cultures and T-cell proliferation assays, demonstrated that immunoselected and culture-expanded MPCs are nonimmunogenic in allogeneic settings and demonstrate immunosuppressive activity.

In nude rat models of myocardial ischemia, implantation of allogeneic MPCs into the acutely ischemic myocardium was associated with arteriogenesis at 2 weeks following administration of MPCs and improvement in global parameters of systolic and diastolic function at both 2 and 6 weeks after implantation.

The ovine models of chronic ischemic and nonischemic cardiomyopathy (CM) demonstrated a dose-related improvement in left ventricular end-diastolic pressure (LVEDP) and left ventricular ejection fraction (LVEF) (ischemic model) and prevention of deterioration of LVEF and stabilization of ventricular remodeling (nonischemic model) with transendomyocardial delivery of allogeneic ovine MPCs. In contrast, the control groups for the chronic ischemic and nonischemic ovine models of CM showed time-related worsening of critical cardiac parameters.

Cell-tracking studies have shown that less than 1% of injected human MPCs persist at 24 hours after being injected into ischemic rat heart tissue, yet functional cardiac recovery is observed at 6 weeks after implantation. Arteriole proliferation in infarcted areas of the myocardium in the ovine model is documented at 7 days following ovine MPC implantation and is evident at the 2-month endpoint. Improvement in vascular perfusion is seen at 4 weeks, whereas cardiac functional recovery is seen at 8 weeks. These observations indicate that the effects of MPCs on functional cardiac recovery occur shortly following administration and may result from beneficial and sustained effects on endogenous cells and tissues. The neovascularization observed with MPCs reflect protective mechanisms. These observations indicate that the protective and reparative mechanisms of MPCs do not require long-term MPC survival or engraftment.

A total of 7 studies conducted in sheep included histopathologic evaluations for organ toxicity. Collectively, the results from these studies indicate that an allogeneic MPC product can be delivered safely by surgical epicardial injection, transendocardial injection by catheter, and

intracoronary infusion. The findings from these studies also indicate that a one-time dose of MPCs ranging from 25 to 225 M MPCs is well tolerated.

A summary of completed nonclinical studies of mesenchymal precursor cells (MPCs) in cardiovascular disease models is below. Further information on preclinical findings is presented in the Investigator’s Brochure.

Summary of Completed Nonclinical Studies of Mesenchymal Precursor Cells in Cardiovascular Disease Models

Study Number	Indication/Purpose	Product/Dose/Carrier	Time Of Cell Injection Following Myocardial Damage	Control	Route	Method	Species # of animals	Duration of Follow-Up (Post-treatment)
AB001	Acute myocardial infarction	Allogeneic ovine MPCs 25, 75, 225 or 450 million	Delivered to non-ischemic border zone immediately after MI	Pro-freeze	Epicardial injection	Ligation of LAD and its 2 nd diagonal branch	Sheep (46)	8 weeks
AB003	Acute myocardial infarction	Xenogeneic human MPCs 0.2 or 1 million	Delivered to non-ischemic border zone immediately after MI	Saline	Epicardial injection	Ligation of LAD	Nude rat	2-6 weeks
AB004	Acute myocardial infarction	Allogeneic ovine MPCs 225 million	Treatment 5 days post MI	Profreeze	Trans-endocardial injection via NOGA	90-min balloon occlusion at distal LAD (after D2)	Sheep (9)	55±4 days
AB005	Acute myocardial infarction	Allogeneic ovine MPCs 25 or 225 million	Treatment 10 days post MI	None	Trans-endocardial injection via NOGA	90-min balloon occlusion at distal LAD (after D2)	Sheep (4)	7 days
AB006	Acute myocardial infarction	Allogeneic ovine MPCs 225 million	Delivered to non-ischemic border zone immediately after MI	Profreeze	Epicardial injection	60-min occlusion at D1 branch of LAD	Sheep (8)	4 weeks
AB010	Acute myocardial infarction	Allogeneic ovine MPCs 225 million	Treatment 10 days post MI	None	Trans-endocardial injection via	90-min balloon occlusion at distal LAD (after	Sheep (10)	50±4 days

Study Number	Indication/ Purpose	Product/ Dose/ Carrier	Time Of Cell Injection Following Myocardi al Damage	Control	Route	Method	Species # of animals	Duration of Follow-Up (Post- treatment)
AB021	Chronic myocardial infarction, ischemic-reperfusion model	Allogeneic ovine MPCs 225 million	4 weeks after infarction	Profreeze	NOGA	Epicardial Injection D2)	Sheep (10)	8 weeks
AB100	Safety of intracoronary infusion of MPCs in healthy sheep	Allogeneic ovine MPCs 25, 37.5 or 50 million	n/a	None	Intracoronary infusion	Proximal LAD infusion (Twin Pass Catheter)	Sheep (12)	2 days
AB101	Chronic myocardial infarction, ischemic-reperfusion model	Allogeneic ovine MPCs 25, 75 or 225 million	4 weeks after MI	Profreeze	Endocardial injection	90-minute balloon occlusion of LAD	Sheep (40)	8 weeks
AB102	Acute myocardial infarction	Allogeneic ovine MPCs 12.5, 25 or 37.5 million Ringer's lactate	Immediately after MI	Vehicle (Ringer's lactate)	Intra-coronary infusion	90-min balloon occlusion directly behind D1 or septal branch of LAD	Sheep (30)	2 months

D2= second diagonal branch; MPCs=mesenchymal precursor cells; MI=myocardial infarction; LAD=left anterior descending artery.

Clinical Studies of Cell-based Myocardial Repair

Mesoblast is conducting several clinical trials involving the administration of allogeneic MPCs by a number of routes and in various cardiovascular and non-cardiovascular indications.

As of 19 March 2013, 291 patients have been enrolled in Mesoblast sponsored clinical trials with allogeneic MPCs administered to 183 subjects and a further 40 subjects enrolled in investigator-sponsored cardiovascular clinical studies. As of August 26, 2013, MPCs have been well tolerated in dose ranges of up to 246 Million (M) allogeneic cells.

The adverse events reported in these studies have been generally mild to moderate in severity. Across all indications evaluated, the most frequently reported adverse events that occurred at a rate of $\geq 5\%$ in the combined MPC treatment group and higher than controls, were chest pain (7.1% vs. 5.5%), fatigue (6.0% vs. 5.5%), upper respiratory tract infection (6.6% vs. 2.2%), neck pain (5.5% vs. 4.4%), dizziness (7.1% vs. 2.2%) and hypotension (7.7% vs. 2.2%). There has been no dose-limiting toxicity observed to date. In all studies to date, antibody responses to MPC

donor-specific HLA have been observed in approximately 10-15% of treated patients. No clinical events or adverse sequelae have been attributed to this sensitization to date.

Based on review of cumulative and aggregate safety data to date the systemic safety and local tolerability of MPCs has been acceptable.

The allogeneic MPCs used in each of the ongoing studies are identical with respect to manufacturing, characterization and lot release testing.

Immediately below is a table listing Mesoblast's sponsored clinical trials with allogeneic MPCs.

Mesoblast, Inc. Sponsored Clinical Trials with Allogeneic MPCs

Protocol Number	Location	Indication	Route of Administration	MPC Treated	Control Treated	Total Enrolled
EMT-AB001	USA	Acute MI	Transendocardial injection	1	1	2
HF-AB002	USA	Heart failure	Transendocardial injection	45	15	60
MSF-0106	USA	Lumbar spinal fusion	Surgical implantation	4	2	6
MSB-SF003	USA	Lumbar interbody fusion	Surgical implantation	16	8	24
MSB-CAR001*	Australia	Prevention of osteoarthritis	Intra-articular injection	*	*	17
MSB-CF001	Australia	Cervical fusion	Surgical implantation	8	4	12
MSB-CF002	USA	Cervical fusion	Surgical implantation	16	8	24
MSB-DR001	USA/ Australia	Disc repair	Intra-disc injection	60	40	100
MSB-DM003	USA	Type 2 Diabetes	IV Infusion	30	11	41
ANG-AMD002	Singapore /Australia	Wet AMD	Intravitreal Injection	3	2	5
MSB-DN001*	Australia	Diabetic nephropathy and Type 2 diabetes	IV Infusion	-	-	-
ANG.AMI-IC001*	Australia/ Europe	Acute MI	Intracoronary Infusion	-	-	-
MSB-RA-001*	USA/ Australia	Rheumatoid Arthritis	IV Infusion	-	-	-
MSB-RA-002**	Europe	Rheumatoid Arthritis	IV Infusion	-	-	-
CB-AB006	USA	Hematopoietic reconstitution of hematological malignancies	Infusion [^]	-	-	-
Total				>183	>91	291
Enrollment as of March 19, 2013 * Blinded study, enrollment on-going Note: For blinded studies with ongoing enrollment allocation of subjects to active or Placebo arms is unknown ** Planned blinded study, enrollment not yet started						

^Umbilical cord blood expanded with allogeneic MPCs co-culture

The clinical trials and a detailed summary of the clinical safety outcomes to date are provided in more details in the Investigator's Brochure.

Safety Considerations

In pre-clinical animal studies, it has been found that less than 5-10% of stem cells applied directly to the myocardium engraft and remain long-term at this site. The other cells either die through apoptosis or disseminate systemically through the circulation, and can be found in the spleen, liver, bone marrow and other organs. Consequently, any stem cell treatment that is delivered to the native heart of an LVAD recipient will be expected to enter the systemic circulation, potentially resulting in direct exposure of the injected stem cells to biomaterials on the LVAD surface.

No patients in the initial CTSN LVAD/MPC trial, evaluating 25 million MPCs in LVAD recipients, experienced any of the key safety endpoints, defined as infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization syndrome. While there were three deaths (30%) in the control group and none in the MPC group at 90 days, 6 MPC patients (30%) died over the 12 months, such that the 12 month mortality rates were identical between groups. The serious adverse event rates seen in the initial trial were similar between the MPC and control groups at both 90 days and 12 months, and the AE profiles were consistent with those seen in the LVAD population.¹⁸

Importantly, the initial CTSN trial (n=30) demonstrated safety with regard to allosensitization. Of patients randomized to the MPC group, 10% developed donor specific antibodies (DSA) compared to 30% of the control group, and DSA resolved by 12 months in all who survived to the end of the trial. As of August 26, 2013, up to 246 M cell dose of Mesoblast's allogeneic MPCs manufactured under identical conditions as contemplated in the present study (150 million) have been safely administered although not in this particular patient population.

Rationale for Selection of the Efficacy Endpoint

The field of mechanical circulatory support, particularly in the context of evaluating myocardial function and recovery in the setting of adjunctive interventions, remains nascent. Standard heart failure efficacy endpoints such as 6-Minute Walk Test (6MWT), NYHA class or HF hospitalizations, are of limited use in this population because of the presence of the LVAD itself and the physiologic state of off-loading the left ventricle. It is becoming increasingly accepted clinical practice, however, to periodically evaluate the ability of LVAD recipients to tolerate a temporary wean from LVAD support and to assess their functional capacity off support for evidence of myocardial recovery. LVAD explantation, however, regardless of the results of the wean assessments, remains rare. Whereas the initial CTSN LVAD/MPC trial was designed primarily to assess the safety of intramyocardial injection of MPCs in LVAD recipients, the secondary objectives were selected to explore a clinically meaningful effect of the intervention within the constraints imposed by the implanted LVAD in this unique population. The current assumption remains that the most meaningful cardiac assessments in LVAD patients are those performed on little or no LVAD support. Accordingly, the key efficacy outcome for initial CTSN

LVAD/MPC trial was defined as the ability to tolerate a wean from LVAD support for 30 minutes. The utility of the ability to tolerate a wean as a predictor of long-term recovery has yet to be established, however, it is generally accepted that those unable to tolerate a wean (in the absence of non-cardiac causes) have persistent and profound cardiac debilitation. The preliminary trial experience, however, demonstrated that non-cardiac factors, such as intermittent bleeding which may prompt clinical decisions to avoid adjusting anticoagulation necessary for the safety of a wean, impact the ability to wean from non-cardiac causes at a particular time point. In order to mitigate the impact of the inability to tolerate the wean at a single time point for non-cardiac causes, the primary efficacy endpoint selected for this next trial is the *number of weans tolerated over 6 months following randomization*. This efficacy endpoint will also reflect disease burden over time.

Additional assessments of LV performance during the temporary intervals of reduced LVAD support will also be performed over the one year period of observation post-implantation, to further assess any impact on reverse remodeling, and explore alternative predictors of myocardial recovery.

The clinical management of the patients enrolled in the trial will be based on the judgment of the multidisciplinary heart failure and transplant teams, and not on the results of the study-related evaluations. It is increasingly accepted, however, that the ability to tolerate a wean is influenced by optimal medical therapy while on LVAD support, including aggressive off-loading of the LV. As such, guidance for optimal medical therapy on LVAD support is included in this protocol.

Rationale for Dose Selection

Given that the initial LVAD/MPC trial did not demonstrate any safety concerns, including allosensitization, the Network investigators decided to proceed, as originally planned, with the follow-up trial in a larger cohort of patients. Despite the low dose of cells deployed in the initial trial, a potential efficacy signal was observed in that a greater proportion of MPC patients experienced successful temporary weans at 90 days. Moreover, the total number of temporary weans tolerated by MPC patients was double that of the control group. Similarly, the significantly lower early mortality rate and fewer hospitalizations in MPC patients compared to control were promising. However, the treatment effect was not seen at one-year. This argues for evaluating higher doses in this population, especially since sensitization concerns were addressed at the lower dose.

Preclinical studies conducted by Mesoblast found that a dose of 150 M cells was well tolerated and effective. The first of the pre-clinical studies was a dose escalation study in a sheep model of ischemic cardiomyopathy (ICM) with progressive HF. The results indicated that transendocardial delivery of allogeneic MPCs was safe. Progressive deterioration of cardiac function and LV remodeling were prevented in the high-dose MPC (225 M) treatment group, but not in the lower-dose MPC (75 M and 25 M) groups and the control group. The second pre-clinical evaluation of transendocardial delivery of MPCs (mean \pm SD, $109 \times 10^6 \pm 5 \times 10^6$ cells per animal) in a sheep HF model demonstrated improvement in LV systolic function, stabilization of left ventricular remodeling, and histologic improvements in the non-ischemic cardiomyopathic left ventricle. The burden of fibrosis was lower after transendocardial delivery of MPCs compared to the placebo arm, although it was still greater than that found in normal healthy sheep hearts.

In addition to the results from the animal studies, the dose of 150 M MPCs to be injected into the endomyocardium was selected for this study because it was shown to be safe and effective in the Mesoblast Phase II trial HF-AB002, and was not associated with a clinically significant immune response. The Mesoblast trial enrolled 60 patients with chronic systolic heart failure and randomized them to 25 M, 75 M, or 150 M MPCs dose cohorts (patients were randomized 3:1, MPC to control within each cohort). A blinded post-hoc analysis of the data was performed to evaluate the HF-major adverse cardiac event (HF-MACE) rate, defined as cardiac death, hospitalization for decompensated HF, or successful resuscitation of documented ventricular fibrillation. The analysis demonstrated that the 150 M dose group was statistically significantly superior to all other treatment groups after 36 months of follow-up.

Of note, the post-hoc HF-MACE outcomes were consistent with the outcomes observed with the pre-specified endpoint assessments, including 6MWT, left ventricular end systolic volume (LVESV), and left ventricular end diastolic volumes (LVEDV). These surrogate endpoints have previously been associated with clinical outcomes and have been demonstrated to correlate with morbidity and/or mortality in patients with chronic HF due to LV systolic dysfunction. Kramer et al., for example, demonstrated a significant correlation between long-term clinical outcomes and the shorter term changes in LVESV, LVEDV and LV ejection fraction (LVEF).¹⁹

In the Phase II HF-AB002 trial, the 150 M MPC dose group demonstrated a placebo corrected improvement in LVESV and LVEDV of -11.5 ml and -17.7 ml, respectively. Based on a recent meta-analysis by Kramer and colleagues, the magnitude of effect in LVESV and LVEDV reported in the 150 M MPC group would be associated with an approximate 20-30% relative risk reduction in HF-MACE. Changes from baseline LVESV and LVEDV observed were consistent with clinically meaningful reverse remodeling (improvement) in the 150M dose cohort. No significant changes in LVEF were observed in any of the dose treatment groups over time.

Compared with control patients, the 150 M group showed a trend toward improvement in exercise capacity as assessed by the 6MWT at 6 and 12 months. The control-corrected change from baseline at 12 months (52.5 meters; P=0.062) exceeded the 30-meter value considered to be clinically important. While Opasich and colleagues concluded that “when the 6-MWT is used as an end-point in intervention studies, the clinical relevance of the results must be considered cautiously if the variation in distance is less than 10% in individuals even though the results are statistically significant”²⁰, the treatment effect of 14.5% (52.5 m) observed in the Mesoblast HF-AB002 trial surpasses the 10% threshold considered clinically relevant.

In aggregate, the concordance of results observed for these three important efficacy outcomes (i.e., HF-MACE, LV remodelling, and exercise capacity) indicate that the 150 M MPC dose is likely to be an effective dose. Consequently, the FDA recently approved a Mesoblast-sponsored Phase III trial evaluating a dose of 150 M MPCs in medically managed HF patients.

Based on Mesoblast’s experience, the current protocol will evaluate a single dose of 150 million MPCs in LVAD recipients.

STUDY DESIGN

This is a prospective, multi-center, double-blind, randomized (2:1), single dose cohort, sham procedure controlled trial to evaluate the safety and efficacy of injecting a dose of 150 M allogeneic MPCs into the native myocardium of LVAD recipients. Patients with advanced heart failure, implanted with an LVAD as either BTT or DT may be eligible to participate in the trial. FDA-approved LVADs will be utilized at US sites, and Health Canada-approved LVADs will be utilized at Canadian sites. All patients will be followed until cardiac transplantation or 24 months post randomization, whichever comes first.

Cardiac transplantation must not be delayed for any study related reasons when a donor organ becomes available for a patient participating in this trial.

ENDPOINTS

Primary Endpoints

The primary *safety* endpoint of this study is the incidence of potential study intervention-related adverse events (infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization syndrome).

The primary *efficacy* endpoint of this study is functional status, while temporarily weaned from LVAD support, over the 6 months post randomization. Functional status is defined by the ability to tolerate wean from LVAD support to low flow for 30 minutes. See the Definition and Measurement of Endpoints section below for details.

Secondary Endpoints

Safety

- Incidence of all serious adverse events
- Anti-HLA antibody sensitization while on LVAD support

Survival

- Survival
- Survival to cardiac transplantation

Functional Status (over 6 months)

- Duration of tolerated wean from LVAD support-
- **6MWT** as tolerated at 20 (\pm 10) minutes following initiation of wean

Functional Status (over 12 months)

- Ability to tolerate each wean from LVAD support for 30 minutes
- Duration of ability to tolerate wean from LVAD support
- **6MWT as tolerated at 20 (\pm 10) minutes following initiation of wean¹**

¹ Weaning and 6-minute walk schedule: 60 days, 4 mos, 6 mos, 9 mos, and 12 mos (or until transplant, whichever comes first)

Physiologic Assessments

For patients who tolerate wean from LVAD support for 30 minutes

- Echocardiographic assessments of the myocardial size and function by transthoracic echocardiography with LVAD at full support, and as tolerated following 6MWT while weaned from LVAD support, including:
 - Left ventricular ejection fraction;
 - Left ventricular end-diastolic and end-systolic dimensions;
 - Left ventricular fractional shortening;
 - Regional wall motion score index (WMSI) (*Comprehensive post 6MWT echo at 6 and 12 months*)
 - RV function (normal, mild, moderate, severe) by Integrative Assessment;
 - RVSP from tricuspid regurgitation (TR) jet;

For all patients (including those unable to tolerate LVAD wean)

- Echocardiographic assessments of the myocardial size and function by transthoracic echocardiography with LVAD at full support,
 - Left ventricular ejection fraction;
 - Left ventricular end-diastolic and end-systolic dimensions;
 - Left ventricular fractional shortening;
 - RV function (normal, mild, moderate, severe) by Integrative Assessment;
- Myocardial neovascularization at time of explant
- Cardiomyocyte regeneration at explant
- Cell Engraftment and fate at explant

Quality of Life & Neurocognition

- Quality of Life (QoL) at 6 & 12 months post randomization
- Neurocognition at 90 days & 12 months post randomization

Hospitalizations

- LOS of index hospitalization
- Frequency (<30 days and ≥30 days following index hospital discharge) and cause of readmissions
- Hospital resource use (or costs)

Tertiary Endpoints

The following mechanistic endpoints will be assessed:

- Chemo/cytokine quantification of plasma derived from peripheral blood samples will be performed. Assessments include TNF- α , IL-6, IL-10, IL-17, TGF- β , RANTES, and SDF-1.

STUDY SUBJECTS

A total of 159 patients with advanced heart failure who are scheduled to be implanted with an LVAD as a BTT or DT will be enrolled in this trial. FDA-approved LVADs will be utilized at US sites, and Health Canada-approved LVADs will be utilized at Canadian sites. We anticipate

recruiting approximately 6 patients/month and that enrollment will be completed within 20 months.

ELIGIBILITY CRITERIA

Patients with end-stage heart failure, of either ischemic or non-ischemic etiology, who are being evaluated for LVAD implantation as a BTT or DT, are candidates for this study. Candidates who meet all inclusion criteria and no exclusion criteria will be eligible for the trial regardless of gender, race, or ethnicity.

Inclusion Criteria

1. Signed informed consent, inclusive of release of medical information, and Health Insurance Portability and Accountability Act (HIPAA) documentation;
2. Age 18 years or older;
3. If the subject or partner is of childbearing potential, he or she must be willing to use adequate contraception (hormonal or barrier method or abstinence) from the time of screening and for a period of at least 16 weeks after procedure;
4. Female subjects of childbearing potential must have a negative serum pregnancy test at screening;
5. Admitted to the clinical center at the time of randomization;
6. Clinical indication and accepted candidate for implantation of an FDA-approved (US sites only) or Health Canada-approved (Canadian sites only) implantable, non-pulsatile LVAD as a bridge to transplantation or for destination therapy.

Exclusion Criteria

1. Planned percutaneous LVAD implantation;
2. Anticipated requirement for biventricular mechanical support;
3. Concomitant arrhythmia ablation at time of LVAD implantation;
4. Planned aortic valve intervention for aortic insufficiency at the time of LVAD implantation;
5. Cardiothoracic surgery within 30 days prior to randomization;
6. Spontaneous myocardial infarction related to ischemia due to a primary coronary event such as unstable plaque rupture, erosion or dissection within 30 days prior to randomization;
7. Prior cardiac transplantation, LV reduction surgery, or cardiomyoplasty;
8. Acute reversible cause of heart failure (e.g. myocarditis, profound hypothyroidism);
9. Stroke within 30 days prior to randomization;
10. Platelet count < 100,000/mcL within 24 hours prior to randomization;
11. Acute infectious process: acute bacterial, fungal, or viral disease OR acute exacerbation of chronic infectious disease such as hepatitis;
12. Presence of >10% anti-HLA antibody titers² with known specificity to MPC donor HLA antigens³;

² Documented by clinical site laboratory for sites that measure PRA. Core Lab will assess screening PRA for all sites that use calculated PRA values.

³ Documented by Core Lab

13. A known hypersensitivity to dimethyl sulfoxide (DMSO), murine, and/or bovine products;
14. History of a known active malignancy within the past 3 years except for localized prostate cancer, cervical carcinoma in situ, breast cancer in situ, or nonmelanoma skin cancer that has been definitively treated;
15. Presence of human immunodeficiency virus (HIV);
16. Received investigational intervention within 30 days prior to randomization;
17. Treatment and/or an incomplete follow-up treatment of any investigational cell based therapy within 6 months prior to randomization;
18. Active participation in other research therapy for cardiovascular repair/regeneration;
19. Prior recipient of stem precursor cell therapy for cardiac repair;
20. Pregnant or breastfeeding at time of randomization;
21. History of known or suspected hypercoagulable state in the opinion of the investigator.

TREATMENT ASSIGNMENTS

Patients will be enrolled in a single dose cohort and randomized in a 2:1 allocation to intramyocardial injection of study product or control (cryoprotective media alone [sham procedure]) at the time of LVAD implantation:

- Group 1 (n= 106): 150 million allogeneic MPCs (Mesoblast, Inc)
- Group 2 (n= 53): 50% Alpha-MEM/42.5% ProFreeze NAO Freezing Medium/7.5% DMSO (Control)

RANDOMIZATION

Patients will be enrolled in a single dose cohort and randomized in a 2:1 allocation to receive either intramyocardial injection of study product (MPC) or cryoprotective media alone (Control), during LVAD implantation. Randomization will take place only after it has been determined that all study eligibility criteria have been met. **LVAD implantation and study intervention must be administered within 24 hours following randomization.**

MASKING

This is a double-blind, sham procedure controlled trial. In order to maintain blinding of investigators, the study intervention solutions will be thawed and prepared for intramyocardial injection by designated personnel at the site who must have no interaction with the patients, and limited interaction with the study investigators and coordinators, as necessary to conduct the trial. The syringes, once prepared will appear identical for both groups. Site personnel and patients, as well all core lab study personnel will be blinded to treatment assignment throughout the trial.

TREATMENT INTERVENTION

Treatment intervention (and LVAD implantation) must occur within 24 hours following randomization. The treatment intervention will be performed in the following manner:

Four 1 cc syringes will be prepared and maintained in the sterile field as detailed in Appendix I. Study product (MPC or Control) will be thawed and prepared as detailed in the Study Product Technician Manual of Procedures (MOP). The injection process should take place within 15 minutes, from time of first injection to last injection, and must take place no longer than 90 minutes following thawing of the study product. A total of 16-20 epicardial injections of 0.2 mL

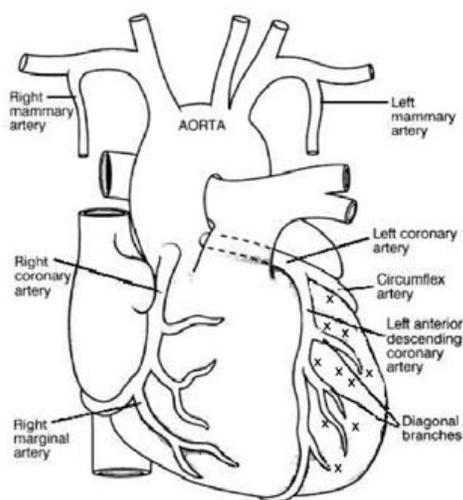
each (not to exceed a total of 4.0 mL) will be performed as detailed in Appendix I. Injections should target as much left ventricular myocardium as possible, as suggested in the diagram below (Figure 1). The location of each injection delivered will be documented on the Intervention Injection Verification Case Report Form (CRF) by the surgical team during the operation. See the Study Product Technician MOP for detailed study product (MPC or Control) handling and preparation. See Appendix I (Intramyocardial Injection Procedures) for the injection procedures.

Of note, study product thawed longer than 90 minutes must be discarded.

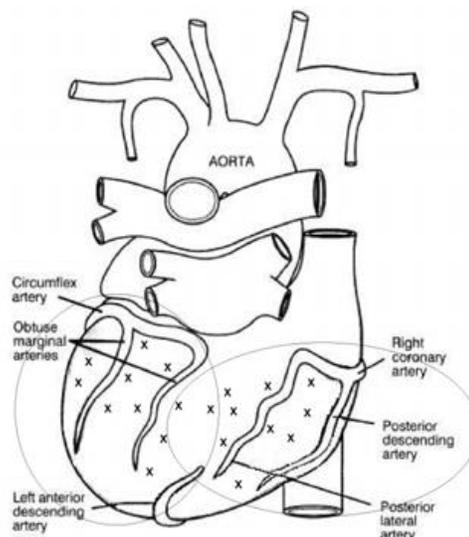
LVAD IMPLANT AND MANAGEMENT

Any permanent surgically implanted non-pulsatile **LVAD approved by the FDA or Health Canada for BTT or DT** for end-stage heart failure may be implanted at the discretion of the surgeon for patients enrolled in this trial. NOTE: Only FDA-approved LVADs will be utilized at US sites, and only Health Canada-approved LVADs will be utilized at Canadian sites. Implantation and management will be performed according to the **Directions for Use for the specific LVAD.** Long term LVAD management should include optimization of hemodynamic off-loading of the left ventricle to target: (1) reduction of LVEDd to 5.5-6 cm; (2) reduction of mitral regurgitation to, at most, mild regurgitation; and (3) reduction in mean blood pressure to < 100 mmHg.

Figure 1.
Anterior View



Posterior View



DEFINITION AND MEASUREMENT OF ENDPOINTS

Primary Endpoints

The Primary Safety Endpoint

The primary *safety* endpoint of this study is the incidence of potential study intervention-related adverse events, defined as (a) infectious myocarditis, (b) myocardial rupture, (c) neoplasm, (d) hypersensitivity reaction, and (e) immune sensitization syndrome, will be determined.

The Primary Efficacy Endpoint

The primary *efficacy* endpoint of this study is functional status, defined by the number of temporary weans from LVAD support tolerated over 6 months post randomization. A successful wean is the ability to tolerate temporary weaning from LVAD support for 30 minutes without sustained symptoms of worsening heart failure. Wean failures are defined as follows:

- Inability to tolerate the temporary wean for 30 minutes;
- Death;
- Patient too unstable, in the judgment of the primary heart failure cardiologist, to tolerate the wean attempt (in ICU, on inotropic support or having persistent heart failure symptoms despite LVAD support).

LVAD weans will be performed at 60 days and at 4 and 6 months (or until transplant, whichever comes first).

LVAD Wean

The continuous flow LVAD wean guidance was adopted from the Harefield Hospital Protocol with permission of Dr. Emma Birks.

Non-pulsatile LVAD (e.g., HM II, HVADTM) Weaning Guidelines: Patients with an INR < 2.0 at the time of wean should receive a 5,000 to 10,000 unit dose of intravenous heparin at least 5 minutes prior to reducing the pump speed. The LVAD flow will then be reduced as detailed in the CTSN LVAD MPC II Trial LVAD Wean Procedure Guidelines, which are located in the LVAD MPC II MOP. Blood pressure measurements will be taken prior to wean and monitored throughout the wean.

Early termination of LVAD Wean

The guidelines for early termination of device turn down during echocardiography and the 6MWT dictate that the LVAD will be returned to full LVAD flow if the patient develops persistent signs or symptoms of low output or vascular congestion as determined by the senior clinician overseeing the wean. Signs and symptoms may include: light headedness, dyspnea, fatigue, chest pain, or pulmonary edema.

Based on the collective experience of its multi-center international collaborators and experience for the first CTSN LVAD MPC Trial, an absolute change in blood pressure alone, in the absence of the clinical signs and symptoms listed above, will not necessitate terminating the LVAD wean.

Secondary Endpoints

Safety

The following additional secondary endpoints will be assessed for all patients (including those who do not tolerate LVAD wean):

Serious & Study-Intervention Related Adverse Events

The incidence of potential study intervention-related adverse events, defined as (a) infectious myocarditis, (b) myocardial rupture, (c) neoplasm, (d) hypersensitivity reaction, and (e) immune sensitization syndrome, will be determined. In addition, the incidence and frequency

of all anticipated and unanticipated serious adverse events will be determined. Moreover, the incidence of all adverse events classified by the clinical site investigator or independent Event Adjudication Committee as “possibly” or “probably” study-intervention related (to either the study product or to the intramyocardial injection procedure itself) will be determined. See Causality and Expected Serious Adverse Event sections that follow. The study intervention-related events above, or mortality in excess of rates expected for the BTT and DT LVAD populations will trigger cessation of enrollment (see Statistical Analysis Early Stopping Rules section).

Anti-HLA Antibody Sensitization

The incidence of anti-HLA antibody sensitization will be measured by complement dependent cytotoxic assay at regular intervals post-study intervention, and will be compared among groups. Immune reactivity results for % IgG Class I and IgG Class II, will be assessed. All measurements will be performed by the Immunologic Core Lab (See the LVAD MPC II MOP for details).

Overall Survival & Survival to Cardiac Transplantation

Overall survival and survival to cardiac transplant (in the BTT patients enrolled) will be assessed and compared between groups.

The following secondary endpoints will be assessed during the temporary LVAD wean from full support for patients able to tolerate the wean:

Functional Status (over 6 months and over 12 months)

Ability to Tolerate Wean from LVAD Support for 30 Minutes

The ability to tolerate the temporary wean from full LVAD support to target low flow for 30 minutes will be assessed at 60 days and at 4, 6, 9 & 12 months, and will be compared between groups. See LVAD MPC II MOP for LVAD Wean Procedure Guidelines.

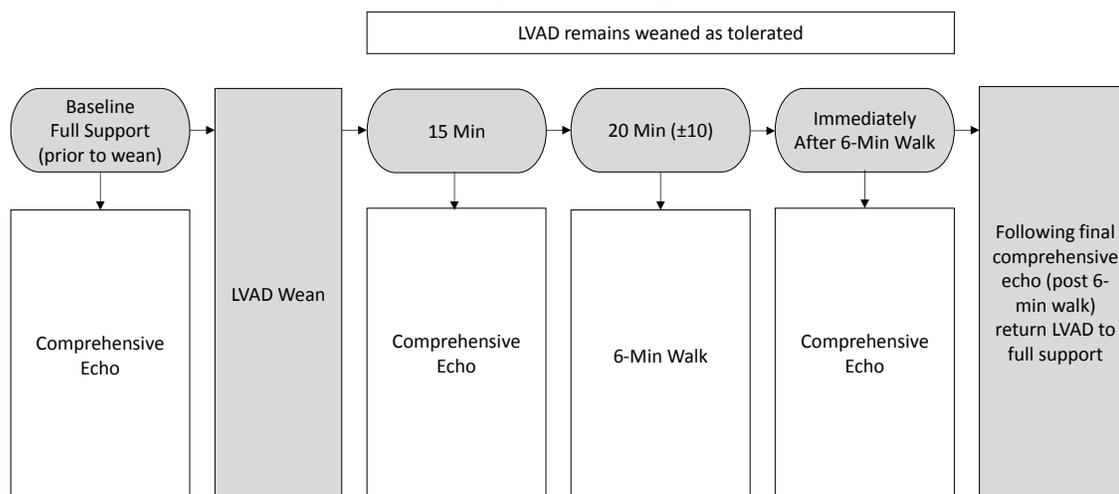
Duration of Ability to Tolerate Wean

The duration of ability to tolerate wean from LVAD support will be measured until the patient:

- (a) Reaches 30 minutes at target low speed LVAD support, **and**
- (b) Completes the echo and 6MWT (if this takes longer than 30 minutes and the patient remains stable), **or**
- (c) Requires early termination of device turn down (e.g., during echocardiography and/or the 6MWT) and resumption of LVAD at full support, for persistent signs or symptoms of low output or vascular congestion as determined by the senior clinician overseeing the wean. Signs and symptoms may include: light headedness, dyspnea, fatigue, chest pain, or pulmonary edema.

These signs and symptoms will be assessed at each time point post intervention.

Timeline of Functional Assessments Completed During LVAD Wean:



The LVAD wean will continue until the patient (a) reaches 30 minutes at low target speed of LVAD support and completes the functional assessments, or (b) fails the wean. The LVAD will then be increased to the pre-wean (baseline) full support.

6 Minute Walk Test

The total distance walked in six minutes will be assessed. For those who tolerate the LVAD wean, the 6MWT will be performed immediately following the 15 minute full echo assessment (at 20 (\pm 10) minutes following initiation of wean). The distance will be measured (in feet), regardless of the number of times stopped or the rate of ambulation. The 6MWT instructions are detailed in the LVAD MPC II MOP.

Weaning And 6-Minute Walk Schedule: 60 days, 4 mos, 6 mos, 9 mos, and 12 mos (or until transplant, whichever comes first).

Physiologic Assessments

Echocardiographic Assessments of Myocardial Function

Echocardiographic parameters (refer to the LVAD MPC II MOP) will be assessed at each wean assessment time point at (a) baseline with the LVAD on *full support* prior to wean, (b) 15 minutes following initiation of wean from LVAD support (while LVAD flow remains weaned), and (c) immediately following the 6MWT, as tolerated by the patient.

Echocardiograms will be read by the Echocardiography Core Lab. The following values will be calculated from the measurements: left ventricular fractional shortening, left ventricular mass by the formula of Devereux (only full support, comprehensive echo study). Regional wall motion assessment will be performed using the American Society of Echocardiography scoring system, modified to exclude the 4 apical segments because of the presence of the LVAD cannula in the LV apex. Therefore, 12 segments (basal and mid-ventricular segments) will be utilized instead of 16.

The following echo assessments will be recorded at each time point before and after the LVAD wean as per protocol:

- Left ventricular (LV) end-diastolic and end-systolic dimensions
- LV fractional shortening
- LVEF by Simpson's Rule (when possible)
- LVEF by visual assessment
- Regional WMSI (*Comprehensive post 6MWT echo at 6 and 12 months*). WMSI assessment will be performed using the American Society of Echocardiography scoring system, modified to exclude the 4 apical segments because of the presence of the LVAD cannula in the LV apex. Therefore, 12 segments (basal and mid-ventricular segments) will be utilized instead of 16.
- RV function (normal, mild, moderate, severe) by Integrative Assessment
- RV systolic pressure (RVSP) from tricuspid regurgitation jet

The following secondary endpoints will be assessed in ALL patients, unrelated to the ability to tolerate the temporary wean from LVAD support:

Neovascularization and Cardiomyocyte Proliferation

Myocardium from the apical core removed at LVAD implant and cardiac samples taken at the time of explant for cardiac transplantation or any other indication, if applicable, will be categorized with regard to region, including anterior, anterolateral, lateral, inferior, midventricular and basilar specimens. All samples will be evaluated for neovascularization, quantification of cardiomyocyte proliferation by immunohistochemistry or molecular analyses, general histology, and DNA analysis (Detailed in the LVAD MPC II MOP).

Cell Engraftment and Fate

Myocardium from multiple samples taken at the time of explant for cardiac transplantation or any other indication, if applicable, will be evaluated for cell engraftment. Relative number of donor cells in the recipient heart will be estimated by real time PCR method measuring copy numbers of donor HLA-DRB alleles different from the recipient in the myocardial samples. (Detailed in LVAD MPC II MOP).

QoL and Neurocognition

Quality of life data will be collected and evaluated as a secondary endpoint. Quality of life will be assessed with the Kansas City Cardiomyopathy Questionnaire (KCCQ), a widely used tool in heart failure populations, and the Short Form 12 (SF12), a widely used overall health status measure (refer to the LVAD MPC II MOP for questionnaires). QoL will be compared between treatment groups at 6 months (± 14) and 12 months (± 14 days) post randomization.

Neurocognition will be compared between treatment groups at 90 (± 14) days and 12 months (± 14 days) post randomization, but not during LVAD wean. Cognitive performance will be assessed using the following battery of tests: Hopkins Verbal Learning Test; Trailmaking Tests A and B; MCG Complex Figures; Digit Span; Digit Symbol Substitution Test; and Controlled Oral Word Association (refer to the LVAD MPC II MOP). Neurocognitive testing will be administered by clinical site personnel who have been trained and certified for test administration by the

Neurocognitive Core lab personnel. All neurocognitive test scoring will be performed centrally by the Neurocognitive Core lab.

The neurocognitive battery used in this trial has been validated in English, Spanish and French. For patients who do not speak English, Spanish or French as a first language and therefore cannot perform the battery, this will not preclude them from participating in the trial and completion of the battery for these patients will not be required.

Hospitalizations

Length of Index Hospitalization

Overall length of stay for the index hospitalization will be measured (and broken down by days spent in the ICU versus days spent on telemetry/step down unit and regular floors). In addition, discharge location will be captured.

Hospital Readmissions

Readmission rates will be calculated for hospitalizations within the first 30 days following index hospitalization discharge, and for the duration of follow-up thereafter. All readmissions will be classified by the investigator and adjudicated by the Event Adjudication Committee (EAC).

Hospital Resource Use

Inpatient resource utilization and costs will be measured through UB-92 forms and hospital billing forms for clinical sites located in the United States. Transfusion requirements will be calculated from the medical record. In addition to index hospitalization costs, costs associated with subsequent readmissions will also be included in the study. Patients will also be asked at each follow-up visit if they have been hospitalized at another hospital and if yes for how long. Costs related to hospitalization at non-Network institutions will be imputed based on the average per day cost of hospitalization of study patients at Network hospitals. Outpatient costs incurred at non-Network and non-US hospitals will not be captured.

Tertiary Endpoints

The following *tertiary endpoints* will be assessed for all patients (including those who do not tolerate LVAD wean):

Cytokine Quantification

Plasma samples will be processed at the site at baseline (prior to study intervention), at day 1 (+1) post intervention, and at days 7 (± 2), 30 (± 3), 60 (± 7), and 120 (± 14) days post randomization, and at 6 and 12 months (± 14 days) post randomization. Chemo/cytokine quantification of plasma derived from peripheral blood samples will be performed by Luminex multiplex assay and include TNF- α , IL-6, IL-10, IL-17, TGF- β , RANTES, SCF and SDF-1.

ADVERSE EVENTS

An adverse event is any unfavorable, harmful or pathological change in the health of a research subject. It may be indicated by physical signs, symptoms, clinically significant laboratory abnormalities, and/or disease temporally associated with the use of a medical (investigational) treatment, procedure, or product, whether or not related to the medical (investigational) treatment, procedure, or product. This definition includes inter-current illnesses, injuries, exacerbations of pre-existing conditions and events occurring as a result of product abuse or overdose. Stable pre-existing conditions and elective procedures to address such conditions are not adverse events. A change in a laboratory variable is considered an adverse event if it leads to a change in the patient's functional status, or is considered by the attending physician to be clinically significant or if it caused (or may have caused) the investigator to reduce or discontinue the use of the product or to institute therapy.

All investigators conducting clinical studies supported by the NHLBI must report both expected and unexpected serious adverse events to the Data Coordinating Center (DCC) and their individual Institutional Review Boards (IRBs)/Research Ethics Boards (REBs) in compliance with their institutional policies.

All protocol-defined and serious adverse events will be collected from the time of randomization into this trial until induction of anesthesia for cardiac transplant or 12 months, whichever occurs first. Any condition that was pre-existing is *not* an adverse event unless there is a change in the nature, severity or degree of the condition. In addition, information regarding the occurrence of the events that trigger the Early Stopping Rules will also be collected as they occur, and their absence will be confirmed at each study visit.

Early Stopping Rules

An early stopping event is an event that results in a halt to enrollment. *These events include: infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization syndrome.* (See Statistical Analysis Section, Early Stopping Rules).

Copies of source documentation related to reported adverse events will be collected. All patient identifiers will be removed from the source documents, and will be replaced with the patient's study identification number.

Serious Adverse Events

Serious adverse events are defined as any experience that results in a fatality or is life threatening; results in significant or persistent disability; requires or prolongs a hospitalization; results in a congenital anomaly/birth defect; or represents other significant hazards or potentially serious harm to research subjects or others, in the opinion of the investigators. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.

Unexpected serious adverse events are defined as any experience that has not been described in the package insert for a given product/device or investigator's brochure, protocol, or the informed consent document. Expedited reporting is required for serious adverse events that are unexpected.

Expedited Reporting (Procedure for Reporting Serious Adverse Events)

Protocol defined (expected) and unexpected serious adverse events, as well as all events that trigger the Early Stopping Rules, must be reported to the DCC and entered into the electronic data capture (EDC) system within 24 hours of discovery of the event. All serious adverse events must be reported to the respective IRB/REB in accordance with the clinical center's policies. The DCC will notify the NHLBI program officials of any unexpected serious adverse events (including death), and events that trigger the Early Stopping Rules, via e-mail within 24 hours of receipt of the event. The DCC will report all expected deaths (regardless of relationship to intervention), as well as all adverse events that are unexpected, serious and intervention-related to the NHLBI program officials within 72 hours of notification. The NHLBI program officials, in turn, will notify the DSMB chair of these events. All serious adverse events will be reported to the DSMB at least semi-annually at the discretion of the DCC medical monitor. The IND Sponsor will promptly upon discovery, report serious and unexpected adverse events for which there is a reasonable possibility that the cell therapy (i.e., study product and/or injection procedure) caused the events, to the FDA in accordance with 21 CFR 312.32 regulations and ICH E2A guidelines. The Clinical Trial Application (CTA) Sponsor will also inform Health Canada, in an expedited manner, of any serious unexpected adverse drug reaction that has occurred inside or outside Canada in accordance with Canadian Food and Drug Regulations Division 5, Section C.05.014.

Causality

The investigator will assess the relationship of an adverse event to the treatment intervention. If possible, the investigator should distinguish the relationship between the event and (a) the LVAD implant and (b) the investigational intervention. Causality will be defined as follows:

Probable

Adverse events that, after careful medical evaluation, are considered with a high degree of certainty to be related to the LVAD implant or investigational intervention. The following characteristics will apply:

- A reasonable temporal relationship exists between the event and the LVAD implant or investigational intervention, and
- The event is a known reaction to the LVAD implant or investigational intervention, which cannot be explained by an alternative etiology commonly occurring in the population/individual.

Possible

Adverse events that, after careful medical evaluation, do not meet the criteria for a probable relationship to the LVAD implant or investigational intervention, but for which a connection has reasonable certainty. The following characteristics will apply:

- The event occurs after exposure to the LVAD implant or investigational intervention, and
- The event is not a known reaction to the LVAD implant or investigational intervention but cannot be explained by a commonly occurring alternative etiology, or

- In the absence of a temporal relationship, the event cannot reasonably be explained by an alternative etiology.

Unlikely

Adverse events that, after careful medical evaluation, do not meet the criteria for a possible or probable relationship to the LVAD implant or investigational intervention and for which a connection is unlikely but cannot be ruled out. The following characteristics will apply:

- The event does not follow a reasonable temporal sequence from administration of the LVAD implant or investigational intervention, or
- May be explained by a commonly occurring alternative etiology in the population/individual, or
- May have been produced by environmental factors, and there is no apparent pattern of response to the LVAD implant or investigational intervention.

Expected Serious Adverse Events

There are certain known and expected risks associated with products that are used in the production of MPCs. These risks include:

Reaction to Fetal Calf Serum or Murine Mouse Antibody

For immunoselection of the allogeneic MPCs, the technology incorporates an antibody based sorting process using murine derived antihuman antibody. In the cell expansion process, fetal calf serum is used. The risk of sensitization from this formulation is unknown, but expected to be extremely rare.

Reaction to Dimethyl Sulfoxide

Dimethyl sulfoxide 7.5% is used as part of the MPCs cryopreservation process. The therapeutic and toxic effects of DMSO include its own rapid penetration and enhanced penetration of other substances across biologic membranes, free radical scavenging, and effects on coagulation, anticholinesterase activity, and DMSO-induced histamine release by mast cells. The systemic toxicity of DMSO is considered to be low. The DMSO exposure in this therapy is minimal and is locally applied.

Potential Cell Contamination

MPCs are an allogeneic, immunoselected, ex vivo expanded cell product, which has the potential to become contaminated and subsequently cause infection in the study patient at the time of surgical implantation. This risk is greatly minimized by the use of a Good Manufacturing Practice (GMP)-compliant production facility. As with any blood or marrow-derived biological agent, infectious risks from unknown pathogens are possible.

Potential Inflammatory Responses

The addition of allogeneic MPCs may exacerbate local inflammatory responses resulting from the allogeneic exposure to the MPC donor. The risks of exposure are unknown but will be monitored throughout the study duration. If sensitivity occurs, persistent expression of anti-HLA antibodies could limit subsequent allogeneic transplant donor selection. Subjects will be monitored for these responses by performing inflammatory marker tests at follow-up visits.

Possible Effects of Cells on Fetus

Because of potential or unknown side effects of the study on the fetus, if the patient is a female of childbearing potential, the patient must have a negative serum pregnancy test prior to study entry. In the event that the study patient is confirmed to be pregnant during the study, the Principal Investigator must immediately notify the DCC and the DCC's Medical Monitor about the pregnancy and record it on the Adverse Event CRF. In addition, the Principal Investigator must report to the DCC follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be observed and monitored through the first year of life.

LVAD-related adverse events in this study will collect data using updated definitions consistent with the INTERMACS registry definitions to enable standardization by currently recognized and accepted definitions.

Protocol-Defined Adverse Events

Neoplasm

Any new uncontrolled growth of aberrant cells.

Major Bleeding (Non-Intra-operative)

An episode of suspected internal or external bleeding that results in one or more of the following: (a) death, (b) re-operation, (c) hospitalization; or (d) transfusion of red blood cells as follows:

- ≥ 50 kg: ≥ 4U packed red blood cells (PRBC) within any 24 hour period *during the first 7 days post-implant* (once the patient has left the operating room)
- ≤ 50 kg: ≥ 20 cc/kg packed red blood cells (PRBC) within any 24 hour period *during the first 7 days post-implant* (once the patient has left the operating room)
- Any transfusion of PRBC *after 7 days following implant*. (The number of units received/24 hour period will be recorded).

Note: Hemorrhagic stroke is considered a neurological event and not a separate bleeding event.

Intra-operative Bleeding

Bleeding that results in death, re-operation, or transfusion of more than 4 units of packed red blood cells during an operative event (while the patient remains in the operating room).

Cardiac Arrhythmias

Any documented arrhythmia that results in clinical compromise (e.g., abnormal VAD function [e.g., diminished LVAD flow or suction events], oliguria, pre-syncope or syncope, angina, dyspnea), or requires hospitalization or treatment (drug therapy, defibrillation, cardioversion, ICD therapy (e.g., shock or anti-tachycardia pacing) or arrhythmia ablation procedure).

Cardiac arrhythmias are classified as follows:

- Sustained ventricular arrhythmia resulting in clinical compromise, or requiring hospitalization or drug treatment, defibrillation or cardioversion, ICD therapy, or arrhythmia ablation procedure.
- Sustained supraventricular arrhythmia resulting in clinical compromise, or requiring hospitalization or drug treatment, cardioversion, ICD therapy or arrhythmia ablation procedure.

Pericardial Fluid Collection

Accumulation of fluid or clot in the pericardial space that requires surgical intervention or percutaneous catheter drainage. This event will be subdivided into those with clinical signs of tamponade (e.g. increased central venous pressure and decreased cardiac/LVAD output) and those without signs of tamponade.

Pleural Effusion

Accumulation of fluid or clot in the pleural space documented by chest radiogram or chest CT that requires evacuation with surgical intervention or chest tube placement.

Vasodilatory State

Episode of vasodilation documented by hemodynamic evidence, without obvious underlying cause such as, but not limited to, sepsis or pharmacotherapy, lasting ≥ 24 hours.

Inflammatory Reaction

Two types of inflammatory reactions will be identified as follows:

Hypersensitivity Reaction

Clinical syndrome including but not limited to fever, leukocytosis, or rash with onset ≤ 2 hours post treatment intervention and lasting < 24 hours, in the absence of clinical signs of concomitant infection.

Immune Sensitization Syndrome

Clinical syndrome including but not limited to fever, leukocytosis, rash or arthralgias with onset ≥ 7 days post treatment intervention *and* subsequent detection of anti-HLA antibodies against the donor cells detected ≤ 30 days following onset of syndrome, in the absence of clinical signs of concomitant infection.

Device Malfunction

Device malfunction occurs when any component of the mechanical circulatory support device (MCSD) system ceases to operate to its designed performance specifications or otherwise fails to perform as intended. Performance specifications include all claims made in the Instructions for Use for the device.

Device malfunctions are defined as **major** or **minor**:

1. **Major device malfunction**, otherwise known as failure, occurs when one or more of the components of the MCSD system either directly causes or could potentially induce a state of inadequate circulatory support (low cardiac output state) or death. A failure that was iatrogenic or recipient-induced will be classified as an Iatrogenic/Recipient-Inducted

Failure. A device malfunction or failure is considered major when one of the following conditions occurs:

- a. *Suspected or Confirmed Pump Thrombus* (see below)
- b. *Urgent Transplantation* (immediate 1A listing for transplant)
- c. *Pump Replacement*
- d. *Pump Explant*
- e. *Breach of Integrity of Drive Line Requiring Repair*
- f. *Death*

2. **Minor device malfunction** includes inadequately functioning external components which require repair or replacement but do not result in 1a-f under major device malfunction. It does not apply to “routine” maintenance which includes repair/replacement of external controller, pneumatic drive unit, electric power supply unit, batteries, interconnect cable.

Pump Thrombus represents a special case of major device malfunction and will be classified as “Suspected” based upon clinical, biochemical, or hemodynamic findings or “Confirmed” based upon device inspection or incontrovertible radiologic studies or absence of appropriate Doppler flow signals that confirms thrombus within the device or its conduits that results in or could potentially induce circulatory failure.

- **Suspected pump thrombus** is a pump-related malfunction in which clinical MCS D parameters suggest thrombus on the blood contacting components of the pump, cannulae, or grafts. Signs and symptoms should include at least 2 of the 3 following criteria:
 - **Presence of hemolysis**
 - **Presence of heart failure not explained by structural heart disease**
 - **Abnormal pump parameters**

Suspected pump thrombus should be accompanied by 1 or more of the following events or interventions:

- Treatment with intravenous anticoagulation (e.g., heparin), intravenous thrombolytics (e.g., tPA) or intravenous antiplatelet therapy (e.g., eptifibatid, tirofiban)
 - Pump replacement
 - Pump explantation
 - Urgent transplantation (UNOS 1A)
 - Stroke
 - Arterial non-CNS thromboembolism
 - Death
- **Confirmed pump thrombus** is a major pump-related malfunction in which thrombus is confirmed within blood contacting surfaces of the device inflow cannula or outflow conduit or grafts. This can be reported via direct visual inspection or by incontrovertible radiologic studies or absence of appropriate Doppler flow signals that results in or could potentially induce circulatory failure or result in thromboembolism.

Hemolysis

Minor Hemolysis: A plasma-free hemoglobin value greater than 20 mg/dL or serum lactate dehydrogenase (LDH) level greater than two and one-half times (2.5x) the upper limits of normal range at the implanting center occurring after the first 72 hours post-transplant **in the absence** of clinical symptoms or findings of hemolysis or abnormal pump function.

Major Hemolysis:

A plasma-free hemoglobin value that is greater than 20 mg/dL or serum lactate dehydrogenase (LDH) level greater than two and one-half times (2.5x) the upper limits of the normal range at the implanting center occurring after the first 72 hours post-implant **and associated with** clinical symptoms or findings of hemolysis or abnormal pump function. Major Hemolysis requires the presence of one or more of the following conditions:

- Hemoglobinuria (“tea-colored urine”)
- Anemia (decrease in hematocrit or hemoglobin level that is out of proportion to levels explainable by chronic illness or usual post-VAD state)
- Hyperbilirubinemia (total bilirubin above 2 mg/dL, with predominately indirect component)
- Pump malfunction and/or abnormal pump parameters

Subjects will undergo routine surveillance for hemolysis using serum lactate dehydrogenase (LDH). LDH to be collected at Days 4, 7, 30, 60 and 90; at 4, 6, 9 and 12 months; and as an event-driven data point.

Hepatic Dysfunction

An increase in any two of the following hepatic laboratory values (total bilirubin, aspartate aminotransferase/AST and alanine aminotransferase/ALT) to a level greater than three times the upper limit of normal for the hospital, for 14 consecutive days post-implant (or if hepatic dysfunction is the primary cause of death).

Hypertension

New onset BP elevation greater than or equal to 140 mmHg systolic or 90 mmHg diastolic (pulsatile pump) or 110 mmHg mean pressure (rotary pump).

Major Infection

A clinical infection accompanied by pain, fever, drainage and/or leukocytosis that is treated by anti-microbial agents (non-prophylactic). A positive culture from the infected site or organ should be present unless strong clinical evidence indicates the need for treatment despite negative cultures.

The general categories of infection are listed below:

Localized Non-Device Infection

Infection localized to any organ system or region (e.g. mediastinitis) without evidence of systemic involvement (see sepsis definition below), ascertained by standard clinical methods and either associated with evidence of bacterial, viral, fungal or protozoal infection, and/or requiring empirical treatment.

Percutaneous Site Infection

A positive culture from the skin and/or tissue surrounding the drive line or from the tissue surrounding the external housing of a pump implanted within the body, coupled with the need to treat with antimicrobial therapy when there is clinical evidence of infection such as pain, fever, drainage, and/or leukocytosis.

Internal Pump Component, Inflow or Outflow Tract Infection

Infection of blood-contacting surfaces of the LVAD documented by positive site culture, including valve endocarditis and pump housing infection.

Sepsis

Evidence of systemic involvement by infection, manifested by positive blood cultures and/or hypotension.

Myocardial Infarction (MI)

Two categories of myocardial infarction will be identified:

Peri-operative Myocardial Infarction

The clinical suspicion of myocardial infarction, together with CK-MB or Troponin >10 times the local hospital's upper limits of normal, found within 7 days following LVAD implant, together with ECG findings consistent with acute myocardial infarction. (This definition uses the higher suggested limit for serum markers due to apical coring at the time of LVAD placement and does not use wall motion changes because the apical sewing ring inherently creates new wall motion abnormalities).

Non-Perioperative Myocardial Infarction

The presence >7 days post-implant, of 2 of the following 3 criteria:

- Chest pain which is characteristic of myocardial ischemia;
- ECG with a pattern or changes consistent with a myocardial infarction;
- Troponin or CK (measured by standard clinical pathology/laboratory medicine methods) greater than the normal range for the local hospital with positive MB fraction ($\geq 3\%$ total CK). This should be accompanied by a new regional LV or RV wall motion abnormality on a myocardial imaging study.

Myocardial Rupture

The presence of myocardial rupture as evidenced by direct visualization (intra-operative or at autopsy) or by standard diagnostic modalities including, but not limited to echocardiography or ventriculography, with or without hemodynamic instability.

Neurological Dysfunction

Any new, temporary or permanent, focal or global neurological deficit ascertained by a standard neurological examination (administered by a neurologist or other qualified physician and documented with appropriate diagnostic tests and consultation note); or an abnormality identified by surveillance neuroimaging. The examining physician will classify the event as a cerebrovascular event as defined below or as a non-vascular acute neurologic event. A neurologic event may be recognized by a clinically evident sign or symptom, or by clinically-silent

electrographic seizure activity, or as a clinically silent lesion detected by surveillance neuroimaging. Each neurologic event should be classified by the clinical provider following complete neurologic assessment as one of the following event types:

- *Transient Ischemic Attack*: Acute transient neurologic deficit conforming anatomically to arterial distribution cerebral ischemia that resolves completely within 24 hours with no evidence of infarction on brain imaging.
- *Ischemic Stroke*: Acute neurologic deficit (or acute encephalopathy) of any duration associated with acute infarction on imaging corresponding anatomically to the clinical deficit. Classified as due to arterial-distribution ischemia or due to venous thrombosis.
- *Acute Symptomatic Intracranial Hemorrhage*: New acute neurologic deficit (or acute encephalopathy) attributable to Intracranial hemorrhage (ICH). ICH subtype are specified as one or a combination of the following types: subarachnoid, intraventricular, parenchymal, subdural.
- *Clinically Covert Ischemic Stroke or ICH*: infarction or ICH seen by surveillance imaging, without clinical findings or stroke or ICH at the time of event recognition.
- *Hypoxic-Ischemic Encephalopathy*: Acute new encephalopathy due to hypoxic-ischemic injury (HIE), manifest as clinically-evident signs and symptoms, or subclinical electrographic seizures found by complete neurological diagnostic evaluation to be attributable to acute global or focal hypoxic or ischemic brain injury not meeting one of the ischemic stroke or ICH events as defined above.
- *Other*: Acute new encephalopathy due to other causes, manifested as clinically-evident signs and symptoms or subclinical electrographic seizures found by complete neurological diagnostic evaluation to be attributable causes other than stroke, ICH or HIE as defined above.

The anticoagulation and antiplatelet medication regimen at the time of the neurological event will be recorded. In addition, the NIH Stroke Scale and the Modified Rankin Scale must be administered at the time of event (within 72 hours following the event), and at 30 days (± 10 days) and 60 days (± 10 days) post neurological event to document the presence and severity of neurological deficits. Each neurological event must be subcategorized as:

Psychiatric Episode

Disturbance in thinking, emotion or behavior that causes substantial impairment in functioning or marked subjective distress requiring intervention. Intervention is the addition of new psychiatric medication, hospitalization, or referral to a mental health professional for treatment. Suicide is included in this definition.

Renal Dysfunction

Two categories of renal dysfunction will be identified:

Acute Renal Dysfunction

Abnormal kidney function requiring dialysis (including hemofiltration) in patients who did not require this procedure prior to implant, or a rise in serum creatinine of greater than 3 times baseline or greater than 5mg/dL sustained for over 48 hours.

Chronic Renal Dysfunction

An increase in serum creatinine of 2 mg/dL or greater above baseline or requirement for hemodialysis, either of which is sustained for at least 90 days.

Respiratory Failure

Impairment of respiratory function requiring intubation or tracheostomy, or the inability to discontinue ventilatory support within 6 days (144 hours) post-LVAD implant. This excludes intubation for re-operation or temporary intubation for diagnostic or therapeutic procedures.

Right Heart Failure

Symptoms and signs of persistent right ventricular failure characterized by both of the following:

- Documentation of elevated central venous pressure (CVP) by:
 - Direct measurement (e.g., right heart catheterization) with evidence of a CVP or right atrial pressure > 16 mmHg.
 - or**
 - Findings of significantly dilated inferior cava with absence of inspiratory variation by echocardiography.
 - or**
 - Clinical findings of elevated jugular distension at least half way up the neck in an upright position.
- Manifestation of elevated CVP characterized by:
 - Clinical findings of peripheral edema (≥ 2+ either new or unresolved),
 - or**
 - Presence of ascites or palpable hepatomegaly on physical examination (unmistaken abdominal contour) or by diagnostic imaging,
 - or**
 - Laboratory evidence of worsening hepatic (total bilirubin > 2.0 mg/dL) or renal dysfunction (creatinine > 2.0 mg/dL)

The severity of right heart failure will be graded according to the below scale. **Direct measurement of CVP or right arterial pressure must be one of the criteria to grade the severity as severe or severe acute.**

Right Heart Failure Severity Grade		
Severity	Definitions	
	VAD Implant Admission	Surveillance Period*
Moderate	Post-implant inotropes, inhaled nitric oxide or intravenous vasodilators continued beyond post-op day 7 and up to post-op day 14 following VAD implant	Limited to 1 readmission for intravenous diuretics/vasodilators to treat RHF since last surveillance visit AND

Right Heart Failure Severity Grade		
Severity	Definitions	
	VAD Implant Admission	Surveillance Period*
		No inotropes since last surveillance visit
Severe	CVP and right arterial pressure greater than 16mmHg AND Prolonged post-implant inotropes, inhaled nitric oxide or intravenous vasodilators continued beyond post-op day 14 following VAD implant	Need for inotropes at any time since last surveillance period OR 2 or more readmissions for intravenous diuretics/vasodilators to treat RHF since last surveillance period OR Requiring RVAD support at any time after hospital discharge OR Death any time following discharge from the LVAD implant hospitalization with RHF as the primary cause
Severe-Acute	CVP or right arterial pressure greater than 16 mmHg AND Need for RVAD at any time following LVAD implant OR Death during LVAD implant hospitalization with RHF as primary cause	Not applicable

Arterial Non-CNS Thromboembolism

An acute systemic arterial perfusion deficit in any non-cerebrovascular organ system due to thromboembolism confirmed by one or more of the following:

- Standard clinical and laboratory testing
- Operative findings
- Autopsy findings

This definition excludes neurological events.

Venous Thromboembolism

Evidence of venous thromboembolic event (e.g. deep vein thrombosis, pulmonary embolism) by standard clinical and laboratory testing.

Wound Dehiscence

Disruption of the apposed surfaces of surgical incision, excluding infectious etiology, and requiring surgical repair.

Unexpected (Other) Serious Adverse Events

Other Serious Adverse Event

An event that causes clinically relevant changes in the patient's health or any event that is life-threatening, results in a fatality, results in permanent disability, requires hospitalization, or prolongs an existing hospital stay.

CLINICAL CENTERS

The trial will be conducted in up to 30 clinical centers that have been selected in conjunction with the NHLBI. The selected centers are all highly experienced LVAD and heart failure centers with a proven track record of conducting clinical trials in this area.

Each clinical center will be required to obtain IRB/REB approval for the protocol and consent revisions in a timely fashion, to recruit patients, to collect data and enter it accurately into the electronic data capture (EDC) system, to faithfully follow the protocol and adhere to the standards of Good Clinical Practice (GCP) and HIPAA regulations (US sites) or the Personal Information Protection and Electronic Documents Act (PIPEDA) regulations (Canadian sites). In addition, centers will be required to provide the DCC the information necessary for interim, annual, and final reports, to provide source documents, data and regulatory documents to study monitors, to respond promptly to DCC inquiries, and, to participate in analyses and reporting of study results.

Site Approval

The following documents are required for all sites approved to participate in the trial:

- Clinical Study Agreement with the CTSN DCC: InCHOIR, Department of Health Policy, Icahn School of Medicine at Mount Sinai
- Signed Conflict of Interest Statements
- Completed Form FDA 1572
- Signed and dated CVs for all staff on Delegation of Authority Log
- Completed Delegation of Authority Log
- Privacy training (HIPAA or PIPEDA) and Human Subjects training documentation (as required by local institutional guidelines) for all staff on Delegation of Authority Log
- Current licenses for all staff on Delegation of Authority Log
- IRB/REB roster
- IRB/REB approval for protocol, informed consent document, HIPAA authorization
- Clinical Center Laboratory Certification
- Dangerous Goods Certification Training for appropriate staff
- NIH Stroke Scale and Modified Rankin Scale Training Certification for appropriate staff
- Laboratory Normal Ranges
- Certification forms for Surgeons, Investigators, Unblinded Staff members
- Signed Document Approval Form for protocol

- Study-specific training documents

Canadian sites must provide:

- Health Canada Clinical Trial Site Information Form
- Qualified Investigator Undertaking Form
- Research Ethics Board Attestation Form

INVESTIGATORS

All surgeons, cardiologists, coordinators and other investigators involved in the trial must complete the Investigator Contact Form with their hospital affiliation, address, contact numbers (phone, fax, cell, pager), and email address. All investigators must send their CV, Clinical Study Agreement/Conflict of Interest Statement, completed Form FDA 1572, Qualified Investigator Undertaking Form (Canadian sites only), Good Clinical Practice Certificates, and HIPAA or other Privacy Protection certification as required by the local institution to the DCC.

Qualifications and Training

Clinical investigators will be cardiothoracic surgeons with expertise in cardiac transplant surgery and heart failure/transplant cardiologists. To qualify as a participating surgeon, the surgical investigators must have performed at least 10 LVAD implantation procedures annually (averaged over a 2 year period) and a minimum of 25 LVAD implants to date. The clinical center must perform a minimum of 10 LVAD implants annually. The heart failure/transplant cardiology investigator must have a minimum of 5 years of experience as an attending physician caring for critically ill cardiac patients and patients on LVAD support. Surgical and cardiology qualifications for all participating investigators will be collected on the Certification Form and faxed to the DCC prior to accreditation. The clinical site Principal Investigator will be responsible for overseeing the ongoing performance of the other participating surgical and cardiology investigators at that site over the course of the study. In addition, each investigator will participate in at least one of the bi-annual meetings of the Clinical Management Committee.

Each clinical site echocardiography lab involved in image acquisition for this trial will be certified by the Echocardiography Core Lab.

All clinical site investigators and coordinators will be trained by the DCC in the specifics of the protocol and the intramyocardial injection procedure at a site initiation visit in advance of patient enrollment. The study coordinators will be trained by the Neurocognition Core lab to administer the neurocognitive testing. In addition, the investigators and coordinators will undergo a separate training session to gain familiarity with the electronic data capture system.

Good Clinical Practices (GCP) Certificate

All investigators and coordinators who are involved in care of study patients, and/or research data collection must provide certification that they have successfully completed their institutionally required GCP or other Human Subjects Protection courses.

Conflict of Interest

A conflict of interest statement will be collected from all study investigators to ensure that no investigator may exert undue influence that may bias the trial. Any conflict of interest identified

will be reviewed by the NIH and managed in compliance with 21 CFR 54 and 42 CFR 50(f) and the Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans. Conflict of interest statements will be updated as changes occur.

Patient Confidentiality

Confidentiality of all patient records will be maintained according to HIPAA and PIPEDA guidelines. Study Investigators, site IRBs, REBs, the DCC (InCHOIR), the Event Adjudication Committee (EAC), the NHLBI, Mesoblast, Inc., Health Canada and the FDA may review source documentation for enrolled patients as necessary, but all unique patient and hospital identifiers will be removed prior to review. If the results of this study are published, the data will be presented in aggregate, with all patient identifiers removed.

HIPAA or other Privacy Training Certification

All investigators and coordinators must provide documentation that they have successfully completed the institutional requirements to ensure patient rights, privacy and security under HIPAA or PIPEDA.

SITE INITIATION

Site initiation involves multiple trainings, which will occur in-person, via teleconference, webinar, video and/or through on-line training courses, depending on one's role and responsibilities in the study, and culminates with a pre-enrollment teleconference for key study personnel. IRB/REB approval and the clinical study agreement between the clinical site and the DCC must be signed and executed prior to the completion of the site initiation process. Additionally, the completed Form FDA 1572, applicable CVs and other regulatory documents must be on file with the DCC prior to completing site initiation. Representatives from the DCC will conduct a pre-enrollment teleconference prior to site activation and enrollment of the first patient. The PI, lead heart failure cardiologist, study coordinator, LVAD coordinator, and study product technicians will be required to attend the pre-enrollment teleconference. All other health care professionals who may be involved in the trial (e.g. echocardiographers, neuropsychologists, engineers, social workers, in-patient staff) will be encouraged to attend.

DATA COLLECTION

Pre-Implant (Screening & Baseline) Data Collection

Screening Failure

Prior to informed consent

Prior to approaching a patient to begin the informed consent process, the study personnel will review data on prospective patients to determine eligibility for inclusion in the trial. All pre-screened patients (patients who are not consented) who are not enrolled are recorded as pre-screening failures on the Screening Registration form in the EDC. The data collected is HIPAA and PIPEDA compliant and does not include patient identifiers but does include screening quarter, screening year, age, sex, racial category, and reason not eligible or not enrolled.

Informed Consent

Prior to screening data collection and all protocol defined procedures

The investigator is responsible for ensuring that the informed consent process is conducted and documented appropriately by trained study staff. A signed informed consent form, which has been approved by the DCC and the individual IRBs/REBs, is required. The consent form must incorporate a clinical research authorization for use and disclosure of private health information and Release of Medical Information that authorizes release of medical records to the trial investigators, monitors, sponsors (NIH, NINDS, CIHR), Mesoblast, Inc. (the MPC manufacturer) and the DCC. The investigators or a designated and qualified individual, will provide a thorough explanation of the objectives, patient responsibilities, risks and benefits of the study, and will fully address all concerns raised by the patient and/or family. After all issues have been adequately resolved, and the investigator has confirmed that the patient has been fully consented, the patient will be asked to sign the informed consent. The consent process must be documented in the medical chart, and a signed copy of the consent must be given to the patient.

For the purpose of primary analysis, patients meeting the eligibility criteria are considered enrolled in the study at time of randomization.

Demographics

During screening, following informed consent

For all patients screened, age, racial category, sex, and education will be captured on the Screening Registration form.

Medical History

Within 7 days prior to randomization

This form captures information pertaining to the etiology of heart failure, the history of cardiovascular disease and other co-morbidities.

Physical Examination

Within 7 days prior to randomization

This form documents a physical examination including anthropometrics (height and weight), vital signs including temperature, and a cardiovascular, pulmonary, abdominal, and extremity exam.

Medications

Within 7 days prior to randomization

All cardiovascular medications that the patient receives during the pre-implant period (within 7 days prior to randomization) through study completion will be captured on the Medication Log in the electronic data capture (EDC) system. All non-cardiovascular medications will be captured on the Concomitant Medication CRF in the EDC.

Immunotherapy Medication

Within 30 days prior to randomization

This form captures all immunosuppressive medications/procedures that the patient received within 30 days prior to randomization.

Laboratory Assessment

Within 24 hours prior to randomization

- Urine or serum beta HCG (IU/L) for women who have the potential to become pregnant

- Platelet count ($10^3/\text{mL}$)

Within 72 hours prior to treatment intervention

- Blood Type and Cross
- Chemistry Panel to include:
Sodium (mM/L), Potassium (mM/L), Blood Urea Nitrogen (mg/dL), Creatinine (mg/dL), Alanine Aminotransferase (ALT; U/L), Aspartate Aminotransferase (AST; U/L)
- Hematology:
White blood cell count ($10^3/\text{mL}$), Hemoglobin (g/dL), Hematocrit (%)

Biospecimen Analyses

Within 72 hours prior to treatment intervention

A baseline peripheral blood sample will be obtained for chemo- and cytokine quantification (detailed in the Biospecimen Core Laboratory Manual). Isolated plasma will be stored frozen at the clinical site and shipped to the Biospecimen Core Lab as directed in the Manual.

Immunologic Assessment

Screening PRA will be obtained within 6 months prior to randomization (must be at least 2 weeks following receipt of PRBC or platelet administration, if applicable).

A screening anti-HLA antibody serum sample will be obtained and analyzed as described in the Biospecimen Core Laboratory Manual. Immune reactivity results for % IgG Class I and IgG Class II will be recorded. Presence of > 10% anti-HLA antibody titers triggers a comparison of recipient antibodies to the MPC donor HLA antigen profiles performed by the Immunologic Core Laboratory. ***Known specificity to the MPC donor HLA antigens will exclude participation in this trial*** (See Eligibility Criteria section).

Within 72 hours prior to treatment intervention

A baseline peripheral blood sample will be obtained to measure anti-murine and anti-bovine antibodies (detailed in the Biospecimen Core Laboratory Manual). The sample will be processed, frozen and stored at the clinical site and shipped to the Biospecimen Core Lab as directed in the Manual.

Hemodynamics

Within 7 days prior to randomization

Right heart catheterization should be performed within 7 days prior to randomization, if clinically indicated. In the absence of clinical indication, the hemodynamics must be obtained in the operating room *prior to treatment intervention* and LVAD implantation. Hemodynamics including central venous pressure (CVP), pulmonary artery pressures including systolic, diastolic and mean pulmonary artery pressures (PA_S , PA_D , PA_M), pulmonary capillary wedge pressure (PCWP), transpulmonary gradient, cardiac output (CO), cardiac index (CI), pulmonary artery oxygen saturation (PAO_2 sat), and pulmonary vascular resistance (PVR measured in Wood Units) will be assessed by right heart catheterization.

Echocardiography

Within 14 days prior to randomization

A complete echocardiogram will be performed prior to LVAD implantation to assess ventricular size, function and regional wall motion (See the LVAD MPC II MOP for details). The echo will be analyzed by the Echocardiography Core Lab. The form also captures information pertaining to echocardiography image acquisition including the date and time of the study, and date uploaded to the Echocardiography Core Lab. If an image was not performed, a protocol deviation will be documented.

NIH Stroke Scale

Within 7 days prior to randomization

The NIH Stroke Scale will be performed prior to LVAD implant for baseline neurological assessment () by a certified staff member (See the LVAD MPC II MOP).

Modified Rankin Scale

Within 7 days prior to randomization

The Modified Rankin Scale will be performed prior to LVAD implant for baseline neurological assessment () by a certified staff member (See the LVAD MPC II MOP).

Neurocognitive Testing

Within 7 days prior to randomization

Cognitive performance prior to LVAD implant will be assessed using the following battery of tests: Hopkins Verbal Learning Test; Trailmaking Form A and B; Digit Span; Digit Symbol; MCG Complex Figures; Controlled Oral Word Association. The testing will take a total of 45 minutes, will involve a trained technician but will not require a neurologist or neuro-psychologist and can be performed with a minimal amount of special equipment. Results from these tests will be independently scored by investigators from the CTSN Neurocognitive Core Lab. All neurocognitive batteries will be tape recorded and the de-identified recordings sent to the Duke University neurocognitive core lab for quality assurance evaluation. (See the LVAD MPC II MOP)

Quality of Life

Within 7 days prior to randomization

QoL will be assessed by the KCCQ and SF12, which will be completed by the patient. (See the LVAD MPC II MOP for questionnaires)

Eligibility Evaluation

Prior to randomization

This checklist of inclusion and exclusion criteria will be completed and signed by the investigator to verify that the patient meets all eligibility requirements for this trial.

Randomization Procedure

A DCC representative will be available to discuss any questions regarding patient eligibility. Once the site investigator has confirmed that the patient meets all eligibility criteria for participation in the trial, and has completed the eligibility forms in the EDC, randomization will be performed electronically. Only a research staff member trained on the protocol and designated by the PI to do so may perform the randomization.

Randomization

Must be completed within 24 hours prior to planned LVAD implantation and treatment intervention

The randomization procedure will be performed within 24 hours prior to the LVAD implantation and treatment intervention. Randomization to the study assignment will be generated by the Electronic Data Capture (EDC) system once the checklist of inclusion and exclusion criteria has been completed and verified. For the purpose of the primary analysis, patients are considered enrolled in the study once they are randomized and an identification code is generated.

Treatment Intervention

LVAD implantation with the ***assigned treatment intervention must be administered within 24 hours following randomization*** (See Randomization section).

Post Intervention Data Collection

In the following section of post intervention data collection, “post intervention” is defined as the time of LVAD implantation and intramyocardial study product injection.

Intervention Injection Verification

Must be completed within 24 hours following myocardial injection procedure

Immediately following the treatment intervention, the operating surgeon will verify the number and location of injection sites as well as the total volume implanted. The diagram will be signed by the surgeon and uploaded into the EDC. This form also captures the date and time of intramyocardial injections.

Hospitalization

At index hospitalization for LVAD implant

Information pertaining to the baseline hospital admission will be collected including date of hospitalization, date of hospital discharge, number of cumulative days in an intensive care unit setting following LVAD implant (i.e., OHRR, CCU, MICU, SICU) throughout the hospitalization, and disposition at time of discharge (home, skilled nursing facility, rehabilitation facility, death).

Surgical Procedure

Must be completed within 24 hours of initial LVAD implant procedure

Data associated with the initial LVAD surgery and type of LVAD implanted will be captured on this form.

Cardiac Histology

At LVAD implantation, at native heart explant for cardiac transplantation, and at autopsy (if applicable)

Histologic samples will be forwarded to the Immunohistochemistry Core Laboratory for analyses, according to the specifications outlined in the LVAD MPC II MOP and Biospecimen Core Laboratory Manual. The form also records whether specimens for cardiac histology analyses were obtained and shipped to the Core Laboratory. If specimens were not collected or able to be analyzed, reasons for failure will be documented.

Laboratory Assessment

12 (± 4) hours post intervention; days 7 (± 2), 30 (± 3), 60 (± 7), and 90 (± 14) post randomization, and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

- Hematology:
White blood cell count ($10^3/\text{mL}$), Hemoglobin (g/dL), Hematocrit (%), Platelet count ($10^3/\text{mL}$)
- Chemistry Panel to include:
Sodium (mM/L), Potassium (mM/L), Blood Urea Nitrogen (mg/dL), Creatinine (mg/dL), Alanine Aminotransferase (ALT; U/L), Aspartate Aminotransferase (AST; U/L)

Days 4, 7 (± 2), 30 (± 3), 60 (± 7), and 120 (± 14) days, and 6, 9 and 12 months (± 14 days) post randomization, or until cardiac transplantation, whichever comes first

- Lactate dehydrogenase (LDH; U/L)⁴

Biospecimen Analyses

Day 1 (+1) post intervention, days 7 (± 2), 30 (± 3), 60 (± 7), and 120 (± 14), and 6 and 12 months (± 14 days) post randomization

A peripheral blood sample will be obtained for chemo-and cytokine quantification (detailed in the Biospecimen Core Laboratory Manual). Isolated plasma will be stored frozen at the clinical site and shipped to the Biospecimen Core Lab as directed in the Manual.

If the specimen was not collected, prepared, shipped, or able to be analyzed the reason for the failure will be specified (e.g. reason specimen not obtained, issue with shipping, etc).

Immunologic Assessment

At days 30 (± 3) and 90 (± 14), and at months 6 (± 14 days) and 12 (± 14 days) months post randomization, or until cardiac transplantation, whichever comes first

- Anti-HLA antibody serum sample
- Anti-murine and anti-bovine antibodies sample

Serum sample will be collected, processed and shipped as outlined in the Biospecimen Core Laboratory Manual. These samples will be stored frozen at the clinical site and shipped to the Biospecimen Core Laboratory as directed in the Manual.

If the specimen was not obtained or is unable to be analyzed, the reason for the failure will be specified (e.g. reason specimen not obtained, issue with shipping, etc) on the Specimen Checklist CRF in the EDC.

Physical Examination

At day 1(+1) post intervention; days 7 (± 2), 60 (± 7), and 90 (± 14) post randomization, and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

⁴ LDH to be collected also as an event-driven data point.

This form documents a physical examination including anthropometrics (height, weight and BSA), vital signs including temperature, and a cardiovascular, pulmonary, abdominal, extremity exam. A research clinician trained on the protocol and designated by the PI to do so will conduct all follow-up physical examinations.

Medications

At day 1(+1) post intervention; days 7(± 2), 60 (± 7), and 90 (± 14) post randomization, and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

All cardiovascular medications the patient receives from the pre-implant period (within 7 days prior to randomization) through study completion will be captured on the Medication Log in the EDC. Modifications to the cardiovascular medication regimen will be confirmed at a minimum for each time point defined above.

All non-cardiovascular medications will be captured on the Concomitant Medication CRF in the EDC at each of the listed study visits.

Early Stopping Events

At LVAD implantation, 12 (± 4) hours and days 1(+1) post intervention; days 7 (± 2), 30 (± 3), 60 (± 7), and 90 (± 14), and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

Information regarding the occurrence of any event that triggers the early stopping rule, regardless of the seriousness of the event, will be collected as they occur (event driven), and will be confirmed at each study visit. These events include: infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization syndrome. Only research physicians listed on the delegation of duties log may evaluate early stopping events.

LVAD Wean and Functional Assessment

Echocardiography

At 60 days (± 7) and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

An echocardiogram to assess myocardial function will be performed at each time point with LVAD at full support and again following LVAD wean as tolerated (See the LVAD MPC II MOP). The tests will be forwarded to the Echocardiography Core Lab for analysis as outlined in the Echo Core Lab Operation Manual for the LVAD MPC II Trial. Only the baseline (pre-wean) echocardiogram will be performed for patients who do not tolerate LVAD wean.

The Echocardiography & Collection form also captures information pertaining to echocardiography image acquisition including the date and time of the study, and date shipped to the CTSN Echocardiography Core Lab. If an image was not performed, a protocol deviation will be documented.

Six Minute Walk Test

At 60 days (± 7) and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

The 6MWT will be performed at 20 (± 10) minutes following initiation of wean (immediately following the 15 minute echocardiogram), if the patient remains clinically stable (See the LVAD Wean Procedure Guidelines in the LVAD MPC II MOP). This form captures the distance in feet walked on a level hallway in six minutes (See the LVAD MPC II MOP for further details). A research clinician trained on the protocol and designated by the PI will oversee the 6MWT.

Wean Assessment

At 60 days (± 7) and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first

The duration of ability to tolerate wean from LVAD support will be assessed. Signs or symptoms of hypoperfusion including, but not limited to, symptoms of low output (e.g., light headedness, dyspnea, fatigue) or signs of vascular congestion (e.g. pulmonary edema) will be assessed during the wean (See the LVAD MPC II MOP for LVAD Wean Procedure Guidelines). A research physician trained on the protocol and delegated by the PI will oversee the wean assessment.

The LVAD wean will continue until the patient:

- (a) Reaches 30 minutes off LVAD support, *and*
- (b) Completes the echo and 6MWT (if this takes longer than 30 minutes and the patient remains stable), *or*
- (c) Fails the wean

Following the wean, the LVAD will be restarted at the previous baseline mode and rate.

The functional assessments (echocardiography, 6MWT, and ability to tolerate wean) will be performed at 60 days (± 7) and at 4, 6, 9 and 12 months (± 14) post randomization, or until cardiac transplantation, whichever comes first.

Neurocognitive Testing

At 90 days (± 14) and 12 months (± 14 days) post randomization

Cognitive performance prior to LVAD implant will be assessed using the following battery of tests: Hopkins Verbal Learning Test; Trailmaking Form A and B; Digit Span; Digit Symbol; MCG Complex Figures; Controlled Oral Word Association. Study personnel, trained in accordance with the respective neurocognitive tool, must conduct these tests and document the results on the appropriate forms. The testing will take a total of 45 minutes, will involve a trained technician but will not require a neurologist or neuro-psychologist and can be performed with a minimal amount of special equipment. Results from these tests will be independently scored by investigators from the CTSN Neurocognitive Core Lab. The neurocognitive battery will be tape recorded and the de-identified recordings sent to the Duke University neurocognitive core lab for quality assurance evaluation. (See the LVAD MPC II MOP)

Quality of Life

At 6 months (± 14 days) and 12 months (± 14 days) post randomization

QoL will be assessed by the KCCQ and SF12, which will be completed by the patient. Data regarding completeness of QOL data collection and reasons for missing responses to questionnaires will be collected on the the KCCQ and SF12 CRFs. (See the LVAD MPC II MOP)

Vital Status Follow-Up

24 months (± 30 days) post randomization

A vital status follow-up assessment will be conducted via telephone (or via medical record review if necessary) by investigative site personnel to document that the patient is alive and to determine if the patient has undergone transplant or explant.

Event Driven Data Collection

Adverse Events

Event Driven within 24 hours of knowledge of event

The event classification, as well as detailed information regarding the event will be recorded. Information regarding all adverse events, including study-intervention specific adverse events will be collected on this form. Research staff trained on the protocol and delegated by the PI may identify and collect information on adverse events. The PI will evaluate all adverse events for expectedness, seriousness and relatedness.

Early Stopping Events

At every study visit and event driven

Information regarding the occurrence of any event that triggers the early stopping rule, regardless of the seriousness of the event, will be collected as they occur (event driven), and will be confirmed at each study visit. These events include: infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization syndrome. Only research physicians listed on the delegation of duties log may evaluate early stopping events.

NIH Stroke Scale

Event driven within 72 hours of a neurological event, and at 30 (± 10) days and 60 (± 10) days post neurological event

The NIH Stroke Scale will be performed by a certified staff member in the case of a neurological adverse event (See the LVAD MPC II MOP).

Modified Rankin Scale

Event driven within 72 hours of a neurological event, and at 30 (± 10) days and 60 (± 10) days post neurological event

The Modified Rankin Scale will be performed by a certified staff member in the case of a neurological adverse event. (See LVAD MPC II MOP)

Hospitalization

Event driven for any hospitalization following the initial LVAD implant

Information pertaining to any hospital admission following the initial LVAD implant hospitalization will be collected including date of hospitalization, date of hospital discharge, number of cumulative days in an intensive care unit setting (i.e., OHRR, CCU, MICU, SICU)

throughout the hospitalization, and disposition at time of discharge (home, skilled nursing facility, rehabilitation facility, death).

Surgical Procedure

Event driven within 24 hours of procedure

Data associated with an operation for any reason, including the initial LVAD implantation and treatment intervention surgery, all re-implants, cardiac transplantation, and re-operations, will be captured on this form.

Medication

Event driven with the report of one of the following adverse events: Right heart failure, Bleeding, Pump Thrombus, Stroke, Arterial Non-CNS Thromboembolism, and Venous Thromboembolism Event

All prescribed anticoagulation, antiplatelet and other medications as indicated that the patient is receiving at the time of the adverse event will be captured.

Immunotherapy Medication

Event driven

All prescribed immunosuppressive medications that the patient receives following LVAD implantation must be documented on this form.

Mortality

Event Driven within 24 hours of knowledge of event

The investigator will record the date of death, immediate cause of death, primary underlying cause of death, notation of autopsy being performed, and clinical narrative of the event.

Pump Retrieval and Explant (and/or Postmortem) Examination

Event Driven

All attempts to obtain permission for a full body autopsy should be made. At a minimum, autopsies will be requested of patients who participated in the study in order to evaluate the device, heart and other major organs. Photographs will be taken of the pump and all related components in situ before removal, and of all areas of evident pathology suspected to be device related. The LVAD should be opened upon removal and inspected. Histologic samples of the myocardium will also be obtained and forwarded to the Biospecimen Core Laboratory for analyses, according to the specifications outlined in the Biospecimen Core Lab Manual.

Missed Visit

Event Driven

If a patient is unable to return for follow-up before closure of a study visit window, a missed visit will be documented and reason why the visit was not conducted will be collected.

End of Study

Study Completion/Early Termination

Event Driven

This form records the date and reason for study completion or early termination.

Investigator Statement

At the end of study after eCRF data completion and review

After a complete review of the eCRFs and patient summaries, the investigator will sign this form to attest to the accuracy and completeness of the data collected.

DATA MANAGEMENT

In order to capture the highest quality data, we will use a web-based system with electronic validation. In addition, we will cross-validate the data for complex errors. Ongoing review of data collection by the DCC will ensure that the quality and completeness of the data will be reflective of the state of the art in clinical trials.

Electronic Data Capture

All study data will be entered in the electronic data capture (EDC) system. Study personnel requiring access will have their own Login/Password. Access to clinical study information will be based on individuals' roles and responsibilities. The application employs fine-grained role-based access control for data entry, viewing, and reporting options. All study data will be transmitted over an encrypted SSL (Secure Sockets Layer) connection that requires user authentication. This application is designed to be in full compliance with the International Conference on Harmonization and Good Clinical Practices (ICH-GCP), the FDA's Code of Federal Regulations (CFR) 21 Part 11 Electronic Record and Electronic Signatures, the FDA's "Guidance: Computerized Systems Used in Clinical Trials," and the Privacy Rule of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

EDC supports efficient data collection and management and facilitates rapid data closure. A strong advantage of web-based design is that the DCC has immediate and ongoing access to the data from all clinical centers so that queries can be generated and distributed to the sites in real-time, the frequency of missing data can be reduced by two mechanisms, the coordinators will receive a list of queries generated by the study monitors upon logging into the system, and any data required during a visit is immediately evident through the system and can be collected before closure of the visit window. The EDC will be a vital part of the centralized monitoring planned for this study.

Monitoring

The DCC will employ a risk-based approach to monitoring for this study. This will be accomplished via centralized or remote monitoring of data via the EDC with a focus on safety, study endpoints, data completion and data outliers. Clinical centers will provide source documentation (with patient-identifying information redacted) to the DCC for remote monitoring via upload to the EDC or remote access to electronic medical records. The DCC will also centrally monitor study logs including the Informed Consent Log, the Protocol Violation/Deviation Log and the Serious Adverse Event/IND Safety Report Log periodically to ensure that the sites are adhering to the study protocol and procedures. The DCC will generate performance metrics to analyze site characteristics such as recruitment rates and timeliness of data entry. This will allow the DCC to identify trends across sites and to address low-performing sites appropriately.

The DCC will also perform on-site monitoring visits at each study site at least once a year. The monitoring visits will be an opportunity to verify a minimum of the following data points for all patients: age, sex, signed informed consent, eligibility criteria, medical history, date of enrollment, protocol-defined serious adverse events (SAEs) and mortalities. The monitors will also conduct a review of the regulatory documents for the study.

The frequency of site visits will be based on the following considerations:

- The first monitoring visit will be scheduled soon after the first patient has been enrolled.
- Routine visits will be scheduled based upon enrollment rate, site experience, previous performance and information collected via centralized monitoring.
- Additional visits are scheduled as needed to resolve study-related problems including query resolution.
- The DCC will conduct close-out activities with each site as an on-site visit or via teleconference.

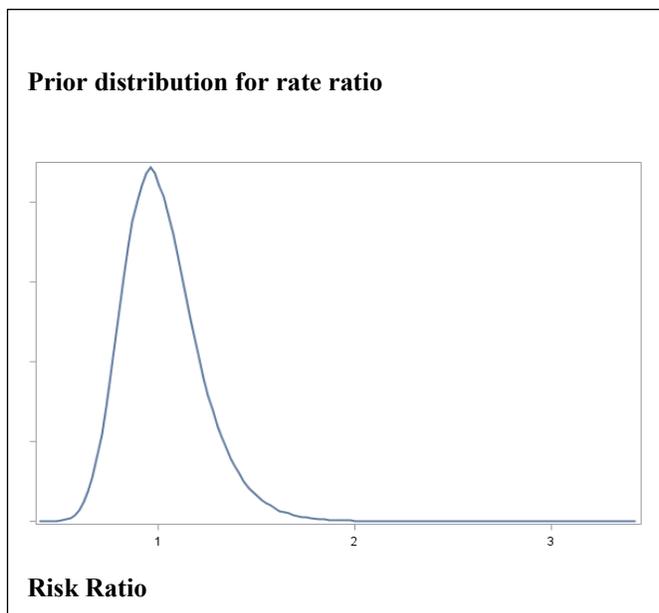
The primary objectives of the DCC are to educate, support, identify and resolve issues related to the clinical trial. The monitors will discuss the protocol in detail, and clarify any areas of uncertainty. At initiation of the study, the monitors will conduct a tutorial on the EDC system. The coordinators will practice entering data so that the monitors can confirm that the coordinators are proficient in all aspects of data entry, query response, and communication with the data management team.

Through the combination of centralized and on-site monitoring, the EDC system, instantaneous electronic validation, and visual cross-validation by the DCC to detect complex errors, it is anticipated that the best possible quality and most complete data will be collected.

STATISTICAL ANALYSIS AND SAMPLE SIZE JUSTIFICATION

The primary aims of this trial are determining safety and potential efficacy of MPC therapy at the studied dose. Safety will be based on the absence of all of the following: infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization; and on mortality. Efficacy will be based on measuring functional status; the number of successful temporary weans achieved out of three planned over the first six months of follow-up. Patients who expire prior to being assessed, or whose morbidity precludes assessment, will be considered weaning failures. Few patients are expected to undergo cardiac transplantation prior to the primary endpoint assessment, but if they do, they will be censored at the time of transplant. For patients who undergo cardiac transplantation, the incidence rate of successful weans will be calculated as the number of successful weans out of the number of weans performed over the time of follow-up until transplantation. The information fraction contributed by these patients to the primary outcome will be adjusted for the amount of follow-up time until transplantation.

A Bayesian approach based on the posterior distribution that active therapy is superior to control will be used to assess the strength of potential efficacy, should safety continue to be demonstrated. The procedure will be carried out as follows.



A binomial likelihood is specified for the primary efficacy outcome, the number of successful weans out of the three planned (or the number attempted). The reasons to have fewer than the planned number of weans include cardiac transplantation, patient refusal, or missed visit due to logistical obstacles. All patients considered too unstable to tolerate the wean attempt (e.g., patient in the ICU or on inotropic support) in the judgment of the primary heart failure cardiologist are considered a wean failure. The prior distribution for the success probability of the binomial likelihood is based on data from the control patients in the previous trial, where the rate of successful wean was found to be approximately 0.18. Independent gamma

priors with this mean and standard deviation of 0.10 are assumed for each group. The induced prior for the relative rate of success for active versus control therapy is shown in the figure. The prior is centered at one (consistent with no difference) with nearly all mass between 0.5 and 2.

Prior to any data collection, equipoise implies that the probability that active therapy is superior to control is 50%. Based on observed data, an increase in this probability to 80% or more, without safety concerns, will be considered a positive signal of efficacy for MPC therapy. Simulations indicate that our sample size of 159 patients provides a probability of 80% to detect such an efficacy signal if the true rate ratio is 1.5, with a false positive rate of 15%. These values are based on the estimated posterior distribution for the rate ratio, to be determined by simulation.

As enrollment is expected to be completed within 20 months, a formal interim analysis of efficacy is not planned (see below for continuous monitoring of safety). NHLBI will determine whether and how future development of MPC therapy continues within the NHLBI funded Network infrastructure based upon all efficacy results, safety outcomes, feasibility, and the state of the field as it relates to the scientific validity and integrity of a proposed future trial.

Analysis of secondary endpoints

Duration of time weaning is tolerated. The duration of time weaning is tolerated will be examined at each time point post randomization. Random effects models will be used to characterize changes over time in the duration that a patient tolerates weaning. Orthogonal contrasts with the Tukey's HSD correction will be used to compare the two groups at each point in time. An interaction term of time*treatment group will be used to determine the difference in the longitudinal course of duration of weaning between the two treatment groups. We will use an additional approach that separates the data into strata defined by the time of death or dropout. We will then estimate a separate linear model, including a treatment effect, for the data in each stratum. This method, known as pattern-mixture modeling is not sensitive to un-testable

assumptions about the dropout mechanism because it models the data directly in strata defined by dropout time. The method of Wu and Bailey is an instance of pattern-mixture modeling.²¹

Pattern of successful weans over time. The pattern of successful weans over both the 6 and 12 month follow-up periods will be explored using logistic regression with parameter estimation using generalized estimating equations.

Echocardiographic assessments. All of the echocardiographic measures (left and right ventricular dimensions, LVEF, regional wall motion score) will be described using both graphical and numerical summaries. Analysis of covariance will be used to estimate and assess differences between treatment arms in the change from baseline (defined as 30 day post randomization) to 12 months post randomization. Analyses incorporating all available follow-up data will be performed using random effects regression models. The primary aims of the random effects models will be to characterize the changes over time in the echocardiographic measures in each treatment group.

Six minute walk test. The 6MWT will be analyzed similarly to the duration of time weaning is tolerated.

Mortality. Time to death will be described by Kaplan-Meier curves and differences between randomization groups will also be assessed via the log-rank test. Patients who undergo transplantation will be censored at the time of transplant.

Safety and Adverse Events. Differences in the incidence of individual adverse events among randomization arms will be assessed using Poisson regression. Exact 95% confidence intervals (based on the Poisson distribution) for the risk ratios for individual adverse events will be computed. In addition, the proportion of patients who exhibit anti-murine and anti-bovine antibodies following study product administration will be compared among randomization arms by a chi-squared test of the equality of proportions.

Anti-HLA antibody sensitivity. The proportion of patients with anti-HLA antibody sensitivity while on LVAD support will be compared among randomization arms by a chi-squared test of the equality of proportions.

In addition to the analyses of clinical outcomes described above, explanted native hearts will be examined to quantify the extent of neovascularization, MPC cell engraftment, as well as histologic and gene regulation responses to cell implantation as detailed in the LVAD MPC II MOP. Confidence intervals will be used to compare treatment groups on these histological measures. Differences between groups in the prevalence of these measures will be quantified using relative risks and associated 95% confidence intervals.

Quality of life. Quality of life will be measured using the KCCQ and SF-12. We will employ two approaches to the analysis of quality of life. The first will be to base the analysis on longitudinal mixed effects models. These models would predict outcome from treatment group and time. The mixed modeling approach requires an assumption that patient dropout is ignorable in that the probability of dropping out at any time is related only to previously observed data items. Of course, this assumption may not hold, and moreover it is impossible to test robustly from the data

at hand. An alternative approach we will also use, not subject to this criticism, will be to separate the data into strata defined by the time of death or dropout. We will then estimate a separate linear model, including a treatment effect, for the data in each stratum. This method, known as pattern-mixture modeling is not sensitive to un-testable assumptions about the dropout mechanism because it models the data directly in strata defined by dropout time. The method of Wu and Bailey is an instance of pattern-mixture modeling.¹⁹

Neurocognitive Outcomes. Neurocognitive outcomes for each test will be standardized using the means and standard deviations observed in the overall sample and combined within cognitive domains using weights, which are being defined by the Neurocognitive Committee. Differences in the scores for each domain at 90 days and 12 months post-randomization will be compared between randomization arms based on an analysis of covariance that adjusts for important baseline values.

Hospitalization Length of Stay and Days in ICU. We will compare hospital length of stay and days spent in ICU between treatment groups using a Wilcoxon Rank-Sum Test.

Readmission. We will use Poisson regression models to compare the frequency of readmissions between groups at both 30 days and 12 months for any cause.

Hospital Readmission. Rates of all-cause hospitalizations, and rate of cardiovascular-and heart failure-specific hospitalizations, both within 30 days and within 12 months, will be compared between treatment groups using chi-square tests.

Tertiary Endpoints. Mixed effects models will be used to characterize levels of cytokines and chemokines over time. The association of cytokine and chemokine levels with engraftment of cells in native explanted hearts will be described graphically, and quantified by regression and correlation analysis.

Early Stopping Rules

Safety will be continuously monitored. The DCC will report all deaths and unexpected, as well as rare but expected serious adverse events to the NHLBI, who, in turn, will report to the DSMB chair. There are two components to the safety monitoring plan. The first focuses on rare events that are very likely to be related to the experimental treatment and not to the underlying heart failure. The second focuses on mortality which is not a rare event in this patient population. With respect to the rare events likely associated with experimental treatment, enrollment will be halted should any of the pre-specified events (infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, or immune sensitization syndrome) be observed. Stopping guidelines for mortality will use the same approach as that described to assess the presence of an efficacy signal using a very diffuse beta (2,8) prior for mortality for both groups, and will be initiated following the randomization of the 10th patient. We would propose to halt randomization if the probability that mortality on active therapy is increased compared to control exceeds 80%. The probability will be determined sequentially, after each mortality event. NHLBI, in conjunction with the DSMB's recommendations, will determine whether enrollment should be continued, suspended or terminated should any of the proposed stopping criteria be met.

Missing Data

Patients who require an LVAD are a highly dependent patient population and are extremely compliant with their care and follow-up. They receive extensive training in adverse event and alarm recognition prior to discharge, and they are called at least once a week when at home. Due to diligent patient and site monitoring, we anticipate that there will be few missing data points in this study. Imputation procedures will be used as appropriate.

ORGANIZATION OF THE STUDY

This section describes the overall study organization. The study is conducted by the Cardiothoracic Surgical Trials Network Core and Consortium clinical sites. The following committees and institutions will be involved in the administration of the study.

Event Adjudication Committee

The charge of the Event Adjudication Committee (EAC) is to review source documents and to adjudicate all adverse events, the severity and causality of the adverse events, and the causes of mortality. The individuals who will serve on the committee will be appointed by the DCC, and will be independent of the study intervention manufacturer (Mesoblast, Inc.) and the DCC. EAC members may be affiliated with the clinical centers but refrain from evaluating AEs at their site. The committee will consist of, at least, a cardiothoracic surgeon with LVAD experience, a heart failure cardiologist, a neurologist and an infectious disease expert. Additional experts in transplant immunology and cardiac pathology will be consultants to the committee as necessary. The EAC will meet every 6 months or as needed to adjudicate adverse events and outcomes data for each subject enrolled.

Data and Safety Monitoring Board (DSMB)

To meet the study's ethical responsibility to its subjects, an independent data safety monitoring board (DSMB) will monitor results during the study. The board consists of physicians, biostatisticians, ethicists and bioengineers, who have no formal involvement or conflict of interest with the subjects, manufacturer (Mesoblast, Inc.), investigators, the DCC or the clinical sites, and will be appointed by the NHLBI. The DSMB will act in a senior advisory capacity to the DCC and the NHLBI regarding data and safety matters throughout the duration of the study. In addition, the DSMB will review interim summary results of the accumulating data from the Event Adjudication Committee every 6 months. These data include adverse events (e.g., infection, bleeding, right heart failure) and mortality. They will communicate their findings directly with the DCC and the NHLBI. The clinical centers will have no contact with the members of DSMB and no voting member of the committee may participate in the study as an investigator.

Data Coordinating Center (DCC)

The university-based CTSN DCC (InCHOIR) will monitor interim data, and analyze the study's results in conjunction with the investigators and the sponsors. The CTSN DCC will also administrate the DSMB and EAC. The DCC holds the study-specific IND with the FDA and the study-specific CTA with Health Canada and will be responsible for reporting serious and unexpected adverse events due to the study product to the FDA according to 21 CFR 312.32 and ICH E2A guidelines and to Health Canada according to C.05.012(3)(c) and C.05.014. In addition, the DCC will be responsible for submitting the required progress reports to the FDA and Health

Canada. The DCC will provide Mesoblast with abridged versions of these reports to ensure Mesoblast remains blinded to the trial results and aggregate data for the duration of the trial.

Clinical Sites and Investigators

The roles and responsibilities of the Investigators and Clinical Sites include, but are not limited to (a) assuring that the trial is conducted according to the Protocol and Operations Manual; (b) identifying, recruiting, and enrolling subjects; (c) obtaining informed consent from each subject and protecting their rights; (d) collecting and entering study data into the EDC, and following subjects through study completion; (e) collecting and filing source documentation; (f) ensuring that Form FDA 1572 or a Qualified Investigator Undertaking Form (for Canadian sites) is accurate and securely maintained with other essential clinical trial documents at the site with provided to the DCC; (g) assuring regular IRB/REB review; (h) maintaining communication with the DCC.

Core Labs

Echocardiography (Echo) Core Lab

All echocardiograms will be performed according to a standardized protocol (found in the LVAD MPC II MOP and Echo Core Lab Operation Manual for LVAD MPC II Trial) and will be centrally analyzed by the CTSN Echo Core Lab directed by Judy W. Hung, MD, located at the Massachusetts General Hospital, Boston, MA.

Biorepository Core Lab

The Biospecimen Core Laboratory will be responsible for storage of critical biomaterials (i.e., tissue samples, blood, and blood products); long-term integrity of these specimens (up to 10 years); management of immunologic, immunohistochemical, cellular, and molecular analyses of tissue and serum samples. The Biospecimen Core Lab will address an unmet clinical need in cardiovascular research to develop mechanistic understandings of the impact of cell therapy and will be located at the Texas Heart Institute and directed by Doris Taylor, PhD.

Neurocognitive Core Lab

The Neurocognitive Core Lab, located at Duke University is directed by Joseph Mathew, MD. The core lab will be responsible for training the clinical site personnel in administration of the specific tests. All neurocognitive tests will be scored centrally by the core lab.

Executive Steering Committee

The Network Steering Committee (with the assistance of the protocol development committee) will provide the overall scientific direction for the study. The responsibilities of the Steering Committee are to: (a) maintain contact with study investigators to ensure high quality data collection; (b) approve and implement major protocol changes in response to advice from the DSMB; (c) collaborate in data analysis, interpretation, and publication; (d) establish criteria for authorship on all manuscripts, publications and presentations that arise from the study.

Mesoblast, Inc.

Mesoblast, Inc. holds the IND for mesenchymal precursor cells and has provided a cross-reference letter allowing the FDA to review their IND files for the DCC-held study specific IND. Mesoblast has also filed a Drug Master File with Health Canada and provided a letter of access for the DCC CTA. Mesoblast will be responsible for labeling and supplying the study product/cryoprotective

media control to all participating sites. Mesoblast will receive regular reports from the DCC regarding adverse events for the study product group but will be blinded to all other data throughout the trial.

NIH

This trial is funded by the National Heart, Lung, and Blood Institute (NHLBI), National Institute of Neurologic Disorders and Stroke (NINDS), and the Canadian Institutes of Health Research (CIHR). The NHLBI has appointed an independent DSMB to provide oversight of this trial. NHLBI program officials will serve as members of the Steering Committee.

REFERENCES

1. Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W, Long JW, Ascheim DD, Tierney AR, Levitan RG, Watson JT, Meier P, Ronan NS, Shapiro PA, Lazar RM, Miller LW, Gupta L, Frazier OH, Desvigne-Nickens P, Oz MC, Poirier VL; Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) Study Group. Long-term use of a left ventricular assist device for end-stage heart failure. *N Engl J Med*. 2001;345:1435-43.
2. Slaughter MS, Rogers JG, Milano CA, Russell SD, Conte JV, Feldman D, Sun B, Tatrooles AJ, Delgado RM 3rd, Long JW, Wozniak TC, Ghumman W, Farrar DJ, Frazier OH; HeartMate II Investigators. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med*. 2009;361:2241-51.
3. Russo MJ, Hong KN, Davies RR, Chen JM, Sorabella RA, Ascheim DD, Williams MR, Gelijns AC, Stewart AS, Argenziano M, Naka Y. Posttransplant survival is not diminished in heart transplant recipients bridged with implantable left ventricular assist devices. *J Thorac Cardiovasc Surg*. 2009;138:1425-32.e1-3.
4. Miller LW, Pagani FD, Russell SD, John R, Boyle AJ, Aaronson KD, Conte JV, Naka Y, Mancini D, Delgado RM, MacGillivray TE, Farrar DJ, Frazier OH; HeartMate II Clinical Investigators. Use of continuous-flow device in patients awaiting heart transplantation. *N Engl J Med* 2007;357:885-96.
5. Ogletree-Hughes ML, Stull LB, Sweet WE, Smedira NG, McCarthy PM, Moravec CS. Mechanical unloading restores beta-adrenergic responsiveness and reverses receptor downregulation in the failing human heart. *Circulation*. 2001;104:881-6.
6. Thompson LO, Skrabal CA, Loebe M, Lafuente JA, Roberts RR, Akgul A, Jones V, Bruckner BA, Thohan V, Noon GP, Youker KA. Plasma neurohormone levels correlate with left ventricular functional and morphological improvement in LVAD patients. *J Surg Res* 2005;123:25-32.
7. Felkin LE, Lara-Pezzi EA, Hall JL, Birks EJ, Barton PJ. Reverse remodeling and recovery from heart failure are associated with complex patterns of gene expression. *J Cardiovasc Transl Res*. 2011;4:321-31.
8. Hall JL, Fermin DR, Birks EJ, Barton PJ, Slaughter M, Eckman P, Baba HA, Wohlschlaeger J, Miller LW. Clinical, molecular, and genomic changes in response to a left ventricular assist device. *J Am Coll Cardiol*. 2011;57:641-52.
9. Heerdt PM, Klotz S, Burkhoff D. Cardiomyopathic etiology and SERCA2a reverse remodeling during mechanical support of the failing human heart. *Anesth Analg*. 2006;102:32-7.
10. Birks EJ. Molecular changes after left ventricular assist device support for heart failure. *Circ Res*. 2013;113:777-91.
11. Birks EJ, George RS, Firouzi A, Wright G, Bahrami T, Yacoub MH, Khaghani A. Long-term outcomes of patients bridged to recovery versus patients bridged to transplantation. *J Thorac Cardiovasc Surg*. 2012;144:190-6.
12. Houtgraaf JH, de Jong R, Kazemi K, de Groot D, van der Spoel TI, Arslan F, Hoefler I, Pasterkamp G, Itescu S, Zijlstra F, Geleijnse ML, Serruys PW, Duckers HJ. Intracoronary infusion of allogeneic mesenchymal precursor cells directly after experimental acute myocardial infarction reduces infarct size, abrogates adverse remodeling, and improves cardiac function. *Circ Res*. 2013;113:153-66.
13. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB Jr, Reisman MA, Schaer GL, Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:2277-2286.
14. See F, Seki T, Psaltis PJ, Sondermeijer HP, Gronthos S, Zannettino AC, Govaert KM, Schuster MD, Kurlansky PA, Kelly DJ, Krum H, Itescu S. Therapeutic effects of human STRO-3-selected mesenchymal precursor cells and their soluble factors in experimental myocardial ischemia. *J Cell Mol Med*. 2011;15:2117-29.
15. Jellema RK, Wolfs TG, Lima Passos V, Zwanenburg A, Ophelders DR, Kuypers E, Hopman AH, Dudink J, Steinbusch HW, Andriessen P, Germeraad WT, Vanderlocht J, Kramer BW. Mesenchymal stem cells induce T-cell tolerance and protect the preterm brain after global hypoxia-ischemia. *PLoS One*. 2013;8:e73031.
16. Wang Y, Liu J, Xu C, Zhang W, Bai L, Li N, Liu Y, Wang Y, Su Y, Hu D. Bone marrow transplantation combined with mesenchymal stem cells induces immune tolerance without cytotoxic conditioning. *J Surg Res*. 2011;171:e123-31.
17. Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 2002;30(1):42-8.
18. Ascheim DD, Gelijns AC, Goldstein D, et. al. Mesenchymal Precursor Cells as Adjunctive Therapy in Recipients of Contemporary LVADs. *Circulation* (Conditionally accepted for publication)

19. Kramer DG, Trikalinos TA, Kent DM, Antonopoulos GV, Konstam MA, Udelson JE. Quantitative evaluation of drug or device effects on ventricular remodeling as predictors of therapeutic effects on mortality in patients with heart failure and reduced ejection fraction: a meta-analytic approach. *J Am Coll Cardiol*. 2010 Jul 27;56(5):392-406. doi: 10.1016/j.jacc.2010.05.011.
20. Opasich C, Pinna GD, Mazza A, Febo O, Riccardi PG, Capomolla S, Cobelli F, Tavazzi L. Reproducibility of the six-minute walking test in patients with chronic congestive heart failure: practical implications. *Am J Cardiol*. 1998 Jun 15;81(12):1497-500.
21. Wu MC, Bailey K. Analysing changes in the presence of informative right censoring caused by death and withdrawal. *Statistics in Medicine*. 1988;7:337-346.

APPENDIX I: Intramyocardial Injection Procedures

See the Study Product Technician Manual of Procedures for the list of supplies needed as well as detailed instructions on the procedures for preparing the study product for injection.

PREPARATION OF SYRINGES FOR STUDY PRODUCT INJECTION

Study Drug Injection Procedure

1. Four (4) sterile 1cc syringes must be prepared under sterile technique (see MOP), and labeled numbers 1-4.
2. Wrap the lower portion of the syringe with a wide steri-strip to obscure the lower barrel of the syringe and blind the operator to the nature of the contents (cell or placebo). Mark the plunger at least 1 cc above the top of the steri-strip with a nick or marker and thus allow tracking of amount injected.
3. Fill each syringe with 1ml of study product.
4. **Note: During any downtime or delay, each syringe should be gently hand rocked in order to prevent clumping**
5. The injection process should take place within 15 minutes and must take place no longer than 90 minutes following thawing of the MPCs.
6. A total of 16-20 intramyocardial injections of 0.2 mL (not to exceed a total of 4.0 mL) each will be performed. **Note: Cells thawed longer than 90 minutes will be discarded and the process will be continued with a new vial.**

INTRAMYOCARDIAL INJECTION PROCEDURE/DOCUMENTATION

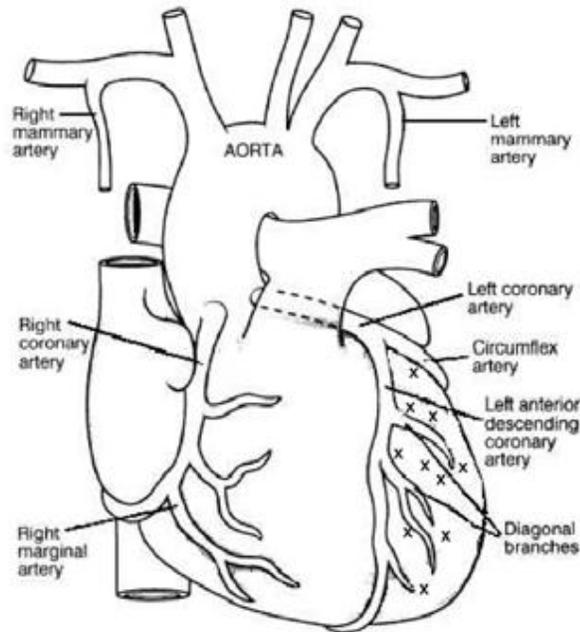
Consider injecting cells during or around the time of LV apical cannula guide implantation as the heart is positioned for maximal access to the entire left ventricle.

1. The intent is to inject study product intramyocardially, across as much of the left ventricular myocardium as possible.
2. Twenty (20) suggested locations are identified in the diagram below.
3. Injections should be made from an oblique angle into the mid-myocardium, with particular attention to avoid injection into the LV cavity.
4. Each 0.2ml should be injected slowly, over 5 seconds into each epicardial location, and the needle should then be held in place for an additional 5 seconds before removing.

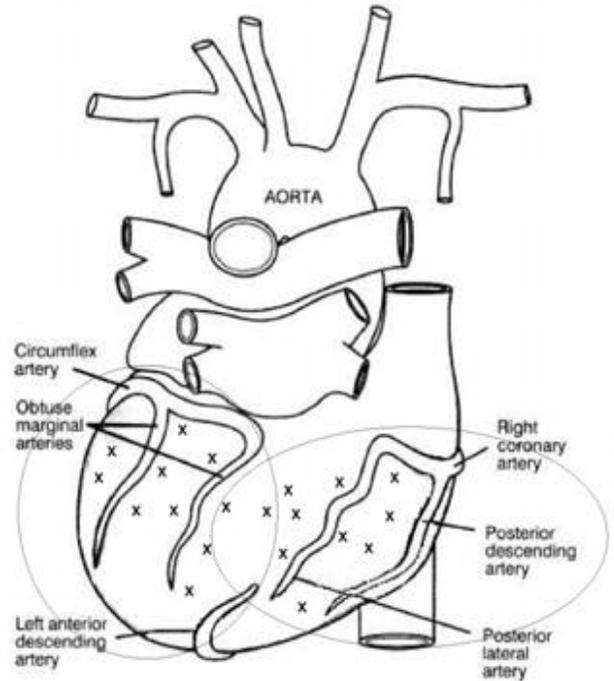
5. A total volume of 4.0ml will be delivered into the myocardium.

Suggested locations for intramyocardial injections:

Anterior View



Posterior View



6. Immediately following the treatment intervention the operating surgeon will document date and time of the intramyocardial injections, the number and locations of injection sites as well as the total volume implanted on the *Treatment Intervention Case Report Form (CRF)*.

The intramyocardial injection sites will be documented using the following table:

		Legend: AS Anterior Septum AW Anterior Wall LW Lateral Wall PW Posterior Wall IW Inferior Wall PS Posterior Septum	
Segment 1: <input type="checkbox"/>	# Injections given <input type="checkbox"/>	Segment 7: <input type="checkbox"/>	# Injections given <input type="checkbox"/>
Segment 2: <input type="checkbox"/>	# Injections given <input type="checkbox"/>	Segment 8: <input type="checkbox"/>	# Injections given <input type="checkbox"/>
Segment 3: <input type="checkbox"/>	# Injections given <input type="checkbox"/>	Segment 9: <input type="checkbox"/>	# Injections given <input type="checkbox"/>
Segment 4: <input type="checkbox"/>	# Injections given <input type="checkbox"/>	Segment 10: <input type="checkbox"/>	# Injections given <input type="checkbox"/>
Segment 5: <input type="checkbox"/>	# Injections given <input type="checkbox"/>	Segment 11: <input type="checkbox"/>	# Injections given <input type="checkbox"/>
Segment 6: <input type="checkbox"/>	# Injections given <input type="checkbox"/>	Segment 12: <input type="checkbox"/>	# Injections given <input type="checkbox"/>

7. The completed *Injection Verification* section of the *Treatment Intervention CRF* must be signed by the surgeon and uploaded into the EDC within 24 hours following treatment intervention. The original copy should be kept in the patient's source document binder.

STATISTICAL ANALYSIS PLAN

SAFETY & EFFICACY OF INTRAMYOCARDIAL INJECTION OF MESENCHYMAL PRECURSOR CELLS ON MYOCARDIAL FUNCTION IN LVAD RECIPIENTS (LVAD MPC II)



Sponsored By NHLBI, NINDS, and CIHR

CT Surgery Network Research Group

Data Coordinating Center
InCHOIR
Icahn School of Medicine at Mount Sinai
New York

August 2018

CONFIDENTIAL

Version 2

Table of Contents

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS.....	3
PURPOSE OF STATISTICAL ANALYTICAL PLAN (SAP)	4
1. INTRODUCTION.....	5
1.1 Study Objectives	5
1.1.1 Primary Objective	5
1.1.2 Secondary Objectives.....	5
1.2 Study Design.....	6
1.3 Study Duration and Time Points	6
1.4 Randomization and Masking.....	6
2. STUDY POPULATIONS	6
3. DEFINITIONS AND VARIABLES	7
3.1 Baseline Characteristics	7
4. EFFICACY ENDPOINTS	8
4.1 Primary Efficacy Endpoint.....	8
4.2 Secondary Efficacy Endpoints	9
4.2.1 Overall Survival and Survival to Cardiac Transplantation.....	10
4.2.2 Functional Status over 6 Months and over 12 Months Post Randomization.....	10
4.2.3 Physiologic Assessments	11
4.2.4 Quality of Life and Neurocognition	13
4.2.5 Index Hospitalization and Readmissions	13
4.3 Tertiary Endpoints.....	14
4.3.1 Cytokine Quantification.....	14
4.3.2 Angiotensin-converting enzyme-1 and Angiotensin-converting enzyme-2 Quantification	14
5. SAFETY ENDPOINTS	14
5.1 Primary Safety Endpoint.....	14
5.2 Secondary Safety Endpoints	15
5.2.1 Incidence and Rate of Non-Surgical GI Bleeding Events and/or Epistaxis by 6 Months Post Randomization	15
5.2.2 Serious & Study-Intervention Related Adverse Events	15
5.2.3 Anti-HLA Antibody Sensitization	16
6. STATISTICAL METHODOLOGY	16
6.1 Statistical and Analytical Issues.....	16
6.1.1 Statistical Methods.....	16
6.1.2 Handling of Dropouts and Missing Data	17
6.2 Patients Characteristics	18
6.2.1 Patients Disposition.....	18
6.2.2 Protocol Deviations and Violations	18
6.2.3 Demographic Characteristics	19
6.2.4 Prior and Concomitant Medication	19
6.2.5 Medical History	19
6.2.6 Pre-Implant Echocardiographic Parameters.....	19
6.3 Efficacy Analysis	19
6.3.1 Analysis of the Primary Efficacy Endpoint and Determination of Sample Size.....	19
6.3.2 Analysis of Secondary Efficacy Endpoints	20
6.4 Tertiary Endpoints.....	23
6.5 Subgroup Analyses	23
6.6 Safety Analysis.....	23
6.6.1 Incidence and Rate of Non-Surgical GI Bleeding Events and/or Epistaxis by 6 Months Post Randomization	23
6.6.2 Treatment-emergent Adverse Events (TEAEs)	24
6.6.2 Anti-HLA Antibody Sensitivity, Anti-Murine and Anti-Bovine Antibodies.....	24
6.7 Early Stopping Rules	24
6.8 Interim Analysis.....	25
7. REFERENCES.....	26

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

6MWT	Six Minute Walk Test
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BTT	Bridge to transplant
CI	Cardiac Index or Confidence Interval
CIHR	Canadian Institutes of Health Research
CMS	Centers for Medicare and Medicaid Services
CO	Cardiac Output
CRF	Case report form
CTSN	Cardiothoracic Surgical Trials Network
DCC	Data Coordinating Center
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
DT	Destination Therapy
EAC	Event Adjudication Committee
EDC	Electronic Data Capture System
FDA	Food and Drug Administration
HLA	Human Leukocyte Antigen
IgG	Immunoglobulin G
InCHOIR	International Center for Health Outcomes and Innovation Research
ITT	Intention To Treat
LDH	Lactate Dehydrogenase
LV	Left Ventricle
LVAD	Left Ventricular Assist Device
LVEDD	Left ventricular end-diastolic dimension(s)
LVEF	Left Ventricular Ejection Fraction
M	Million
MAR	Missing At Random
MNAR	Missing Not At Random
MCG	Medical College of Georgia
MPC	Mesenchymal Precursor Cells
NHLBI	National Heart, Lung and Blood Institute
NINDS	National Institute of Neurological Disorders and Stroke
QoL	Quality of Life
rpm	Revolutions Per Minute
RV	Right Ventricle
SAP	Statistical Analytical Plan
TEAE	Treatment-emergent Adverse Event
VAD	Ventricular Assist Device
WMSI	Wall Motion Score Index

PURPOSE OF STATISTICAL ANALYTICAL PLAN (SAP)

The purpose of this SAP is to outline the planned analyses to be completed for the LVAD MPC II trial. The analyses identified in this SAP will be included in abstracts and manuscripts reporting the results of the trial. Exploratory analyses not necessarily identified in this SAP may also be performed. Any post hoc, or unplanned, analyses not explicitly identified in this SAP will be clearly identified as such in any published papers from this study.

This SAP may be updated in response to additional developments, either within or outside the trial.

1. INTRODUCTION

Left ventricular assist devices (LVADs) have well-documented survival and quality of life benefits in patients with advanced heart failure both as a bridge to cardiac transplantation (BTT) and as a long-term therapy, so-called Destination Therapy (DT), in patients who are not transplant candidates.¹⁻⁴ Reports of improved myocardial function have motivated investigation of the use of LVADs as a bridge to recovery, but while most LVAD recipients do show some indications of reverse remodeling of the left ventricle (LV) as evidenced by salutary changes in ventricular structure, myocyte contractile strength⁵, normalization of extracellular matrix and tissue and circulating neurohormones⁶, and programs of gene expression,⁷⁻¹⁰ these improvements are rarely sufficient to allow removal of the device.¹¹ The disconnect between reverse remodeling and recovery of cardiac function, or “passive recovery”, have prompted our efforts to investigate “active recovery” to augment ventricular recovery with adjunctive therapies to LVAD support, such as the administration of stem cells at the time of LVAD implantation.

Recent pre-clinical and clinical evidence suggests that myocardial transplantation of allogeneic mesenchymal lineage cells, in particular, can enhance cardiac performance in settings of acute and chronic functional impairment.¹²⁻¹⁴ Unlike whole organ transplantation or many other allogeneic cell transplants, mesenchymal lineage cell transplants do not appear to cause rejection and instead may be associated with evidence of induced tolerance to the donor.^{15,16} Since our ultimate goal is the achievement of bridging to myocardial recovery and successful LVAD explantation, allosensitization, if it should occur, could adversely impact donor suitability in LVAD recipients who are transplant candidates.

To this end, the Cardiothoracic Surgical Trials Network (CTSN) recently conducted, in collaboration with the Cardiovascular Cell Therapy Research Network (CCTRN), an exploratory trial of a single low dose (25 million [M]) allogeneic mesenchymal precursor cells (MPCs; Mesoblast, Inc.) injected into the native myocardium at the time of LVAD implantation. The objectives of this trial were to provide evidence of immunological safety, provide preliminary data on clinically important serious adverse events, and explore the efficacy of the intervention. If safety were established, the pre-specified plan was to conduct a follow-up trial with a higher MPC dose administered in the same manner and timing as the pilot study.

Given that the exploratory trial did not demonstrate any safety concerns, the Network investigators decided to proceed with the current follow-up trial (LVAD MPC II) using the MPC dose of 150M in LVAD recipients. This document serves as the SAP for the LVAD MPC II trial.

1.1 Study Objectives

1.1.1 Primary Objective

The primary objective of this trial is to evaluate the safety and efficacy of injecting approximately 150 million allogeneic mesenchymal precursor cells (MPCs) into the native myocardium of LVAD recipients at the time of device implantation.

1.1.2 Secondary Objectives

The secondary objective of the trial is to explore the functional and physiologic effects of

intramyocardial injection of the 150M dose of MPCs in LVAD recipients at the time of device implantation.

1.2 Study Design

This is a prospective, multi-center, double-blind, randomized (2:1), single dose cohort, sham procedure-controlled trial to evaluate the safety and efficacy of injecting a dose of 150 M allogeneic MPCs into the native myocardium of LVAD recipients at the time of device implantation. A total of 159 patients with advanced heart failure who are scheduled to be implanted with an LVAD as a BTT or DT will be enrolled in this trial. FDA-approved LVADs will be utilized at US sites, and Health Canada-approved LVADs will be utilized at Canadian sites.

1.3 Study Duration and Time Points

All patients will be followed until cardiac transplantation (for bridge to transplant patients) or until 24 months post randomization, whichever comes first. Assessment of the study outcomes will be performed at 1 day, 7 days, 30 days, 60 days, 90 days, 4 months, 6 months, 9 months, and 12 months post randomization (or until transplant, whichever comes first). Safety will be assessed continuously throughout the 12-month follow-up period. A vital status follow-up assessment will be conducted at 24 months to document whether the patient is alive and to determine if the patient has undergone a cardiac transplant, LVAD explant, and/or LVAD replacement.

1.4 Randomization and Masking

Patients will be enrolled in a single dose cohort and randomized in a 2:1 allocation to receive either epicardial injection of study product (MPC) or cryoprotective media alone (Control) into the mid LV myocardial wall as add-on therapy, during LVAD implantation. Randomization will take place only after it has been determined that all study eligibility criteria have been met. LVAD implantation and study intervention must be administered within 24 hours following randomization.

This is a double-blind, sham procedure controlled trial. Site personnel and patients, as well as all core lab study personnel will be blinded to treatment assignment throughout the trial.

2. STUDY POPULATIONS

Two populations will be used for all summaries and analyses. Subgroup analyses will also be performed.

Intent-to-Treat (ITT) Population

The ITT population is defined as all randomized subjects, regardless of when they withdrew from the study or received the assigned treatment. The ITT population will be used to present all efficacy data (including the primary efficacy endpoint) by randomized treatment group. Subjects will be analyzed according to the treatment to which they were randomized, regardless of the treatment they actually received. Subjects with at least one assessment of the primary efficacy endpoint will be included in the ITT population.

Safety Population

The safety population, defined as all randomized subjects who received the study

treatment, will be used to present the safety summaries by actual treatment received. The Safety population will therefore be identical to the ITT population if all randomized subjects receive the assigned treatment.

Population Subgroups

Population subgroups will be defined for analysis purposes. These subgroups include patients who, at the time of LVAD implantation were categorized as Destination Therapy (DT) or Bridge to Transplant (BTT); patients who receive the HVAD or HeartMate II LVAD; and subgroups based on demographic variables (e.g. gender and age).

3. DEFINITIONS AND VARIABLES

3.1 Baseline Characteristics

Demographics

For all patients screened demographic variables will include age, racial category, sex, and education.

Medical History

Include information pertaining to the etiology of heart failure, the history of cardiovascular disease and other co-morbidities.

Physical Examination

Include anthropometric measures (height and weight), vital signs including temperature, and a cardiovascular, pulmonary, abdominal, and extremity exam.

Medications

All cardiovascular medications that the patient receives during the pre-implant period (within 7 days prior to randomization) through month 12 post randomization will be captured on the Medication Log in the electronic data capture (EDC) system. All non-cardiovascular medications will be captured on the Concomitant Medication case report form (CRF) in the EDC.

Immunotherapy Medication

All immunosuppressive medications/procedures that the patient received within 30 days prior to randomization will be recorded.

Laboratory Assessment

- Urine or serum beta HCG (IU/L) for women who have the potential to become pregnant
- Platelet count (10^3 /mL)
- Blood Type and Cross
- Chemistry Panel to include: Sodium (mM/L), Potassium (mM/L), Blood Urea Nitrogen (mg/dL), Creatinine (mg/dL), Alanine Aminotransferase (ALT; U/L), Aspartate Aminotransferase (AST; U/L)
- Hematology: White blood cell count (10^3 /mL), Hemoglobin (g/dL), Hematocrit (%)

Biospecimen Analyses

Peripheral blood chemokine and cytokine quantification.

Immunologic Assessment

Immune reactivity results for % IgG Class I and IgG Class II will be recorded. Presence of > 10% anti-HLA antibody titers triggers a comparison of recipient antibodies to the MPC donor HLA antigen profiles performed by the Immunologic Core Laboratory. A baseline peripheral blood sample will be obtained to measure anti-murine and anti-bovine antibodies.

Hemodynamics

Hemodynamics include central venous pressure (CVP), pulmonary artery pressures including systolic, diastolic and mean pulmonary artery pressures (P_{A_S}, P_{A_D}, P_{A_M}), pulmonary capillary wedge pressure (PCWP), transpulmonary gradient, cardiac output (CO), cardiac index (CI), pulmonary artery oxygen saturation (PAO₂ sat), and pulmonary vascular resistance (PVR measured in Wood Units) will be assessed by right heart catheterization.

Echocardiography

Echocardiogram will assess ventricular size, function and regional wall motion.

NIH Stroke Scale

The NIH Stroke Scale (NIHSS) is made up of 11 different elements each evaluating a specific ability. The score for each ability ranges between 0 and 4, 0 being normal functioning and 4 being completely impaired. The NIHSS score is calculated by adding the scores for each element. The total score ranges between 0 and 42 with the higher the score indicating more impairment.

Modified Rankin Scale

The Modified Rankin Scale ranges from 0 to 6, running from perfect health without symptoms to death.

Neurocognitive Testing

Cognitive performance prior to LVAD implant includes following battery of tests: Hopkins Verbal Learning Test; Trail making Form A and B; Digit Span; Digit Symbol; Medical College of Georgia (MCG) Complex Figures; Controlled Oral Word Association.

Quality of Life

Quality of life measures include the Kansas City Cardiomyopathy Questionnaire (KCCQ) and the Short Form 12 (SF12), which will be completed by the patient.

4. EFFICACY ENDPOINTS

4.1 Primary Efficacy Endpoint

The primary *efficacy* endpoint of this study is clinical functional status, defined by the number of successful temporary weans from LVAD support out of 3 planned assessments over the first 6 months post randomization. LVAD weans will be performed at 60 (± 7) days and at 4 and 6 months (± 14 days) (or until transplant, whichever comes first).

The wean begins when the patient reaches his/her low-speed LVAD flow (rpm) threshold (also called minimal support/time 0). Low-speed is defined as the lowest possible LVAD flow (rpm) the patient will tolerate for the wean assessment. The protocol recommends a target low-speed of 6,000 rpm for the HeartMate II and 1,800 rpm for the HVAD. Based on the patient, the cardiology investigators will use their discretion for the target low-speed for the wean assessment. A wean may terminate early if the cardiologist overseeing the wean determines that the patient has developed persistent signs and symptoms of low cardiac output and vascular congestion, in which case the LVAD will be returned to full LVAD flow. Signs and symptoms may include: light headedness, dyspnea, fatigue, chest pain, or pulmonary edema. A complete weaning assessment ends when measurements for the mean arterial pressure and heart rate are taken after the final comprehensive echocardiographic exam following the 6MWT. This occurs prior to increasing the pump speed back to the original pre-wean settings.

If, for any reason, the end of wean time is unknown, the time at the start of the weaning echocardiograms recorded on the CRF or the time recorded by the local core lab at the site closest to device turn up will be used. If that time is not available, the end of wean time may be extrapolated from sources for the 6MWT and times to when the mean arterial pressure and heart rate measurements were taken throughout the weaning.

A successful wean is defined as the ability to tolerate temporary weaning from LVAD support for 30 minutes without sustained symptoms of worsening heart failure. Patients whose LVAD is explanted for myocardial recovery and not replaced will be considered to have successful weans for all the assessments following pump explantation.

Wean failures are defined as follows:

- Inability to tolerate the temporary wean for 30 minutes; this includes patients who attempted the wean but were unable to reach minimal support and patients who achieved minimal support but were unable to tolerate the wean for 30 minutes;
- Death. Patients who die will be considered weaning failures for all assessments following their death;
- Patient too unstable, in the judgment of the primary heart failure cardiologist, to tolerate the wean attempt (e.g., in ICU, on inotropic support, having persistent heart failure symptoms despite LVAD support, suspicion or evidence of thrombus on baseline echo, aortic valve intervention, stroke within 30 days, evidence of hemolysis defined as LDH > 2.5x the upper limits of normal).

Missing weans due to other reasons (besides death) including cardiac transplantation, patient refusal, missed visit, or study dropout (withdrawal, lost to follow-up) will not be counted as a failure and will not be imputed. Details on how missing values on the primary and secondary endpoints will be handled and the sensitivity analyses for missing data that will be performed are provided in Section 6.1.2

This endpoint will be analyzed using a binary goal attainment variable (30 minute wean achieved [yes/no]) at each time point.

4.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints are measured by 6 and 12 months post randomization. The following secondary endpoints will be analyzed.

4.2.1 Overall Survival and Survival to Cardiac Transplantation

Overall survival and survival to cardiac transplant from randomization will be assessed over 12 months. For the overall survival analysis, the endpoint is death from any cause at 12 months (+14 days). Patients who receive a transplant will be censored at the time of transplant. Patients who withdraw consent or are lost to follow-up will be censored at the date of early termination. For patients who are lost to follow-up, the early termination date will be the date of last known proof of life defined as whichever chronological date that is last of any available dates such as: contact documented in the medical record, including encounters with other services, correspondence with study team, or in-person visit. Patients who are alive at 12 months and have not exited the study early for any reason will be censored at the time the 12 month window closes.

A second mortality endpoint is death from any cause at 24 months (+30 days). Patients who are alive at 24 months and have not exited the study early for any reason will be censored at the time of last data collection for the 24 months vital status assessment.

For survival to cardiac transplant, the endpoint of interest is cardiac transplantation at 12 months (+14 days) and 24 months (+30 days). Follow-up time for patients who withdraw consent, drop out of study, or are alive without experiencing cardiac transplant and death will be defined similarly as the overall survival endpoint.

4.2.2 Functional Status over 6 Months and over 12 Months Post Randomization

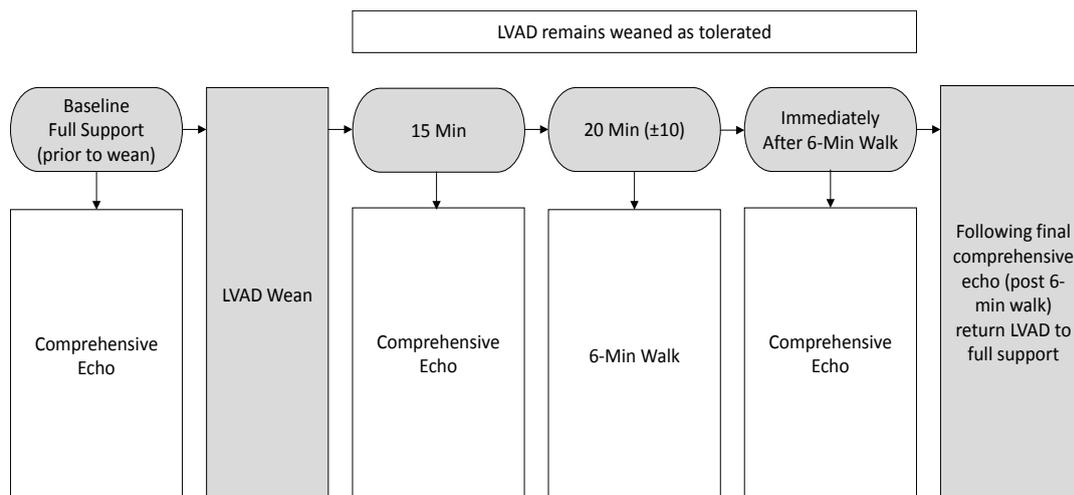
Weaning and 6-Minute Walk will be assessed at 60 (± 7) days, 4, 6 months (± 14 days) (or until transplant, whichever comes first).

- **Ability to Tolerate Wean from LVAD Support for 30 Minutes**

The ability to tolerate the temporary wean from full LVAD support to target low flow for at least 30 minutes will be assessed at 60 days and at 4, 6 and 12 months. Wean successes and failures are defined similarly as the primary efficacy endpoint and will be analyzed using a binary goal attainment variable (30 minute wean achieved [yes/no]) at each time point.

- **Duration of Ability to Tolerate Wean**

(a) The duration of ability to tolerate wean from LVAD support will begin at the time the patient reaches his/her low-speed LVAD flow (rpm) threshold and will be measured until resumption of LVAD at full support. The duration of ability to tolerate the wean will be measured for all patients who attempt the wean regardless of whether they successfully complete the wean.



Patients will receive a value of 0 for their duration of time weaning if they meet any of the following criteria:

- Wean attempted but minimal support not reached
- Wean not attempted because patient too unstable in the judgment of the primary heart failure cardiologist to tolerate wean attempt

• Six Minute Walk Test

The total distance walked in six minutes will be assessed. After maintaining the patient at minimal support for 15 minutes, the patient will undergo a comprehensive echocardiogram if stable and asymptomatic. For those patients stable and asymptomatic at the completion of the echo, the 6MWT will be performed (about 20 minutes into the wean) while the patient is at his/her low-speed LVAD flow threshold. The distance is measured (in feet), regardless of the number of times stopped or the rate of ambulation.

In most cases, patients who tolerate the wean for 30 minutes will undergo the 6MWT. However, since the 6MWT begins immediately after the 15 minute comprehensive echo, it may be performed by patients who are weaned for less than 30 minutes. The distance walked for these patients will still be included in the 6MWT endpoint.

Patients will receive a value of 0 for the total distance walked in six minutes if they meet any of the following criteria:

- Wean attempted, minimal support reached, and 6MWT not performed
- Wean attempted, minimal support not reached, and 6MWT not performed
- Wean not attempted because patient too unstable in the judgment of the primary heart failure cardiologist to tolerate wean attempt

4.2.3 Physiologic Assessments

• Echocardiographic Assessments of Myocardial Function

Echocardiographic parameters will be assessed at each wean assessment time point at (a) LVAD on full support prior to wean, (b) 15 minutes following initiation of wean from

LVAD support (while LVAD flow remains weaned), and (c) immediately following the 6MWT, as tolerated by the patient.

The following echo assessments will be recorded and/or calculated at each time point before and after the LVAD wean as per protocol:

- Left ventricular end-diastolic dimensions (LVEDD) and left ventricular end-systolic dimensions (LVESD)
- LV fractional shortening
- LVEF by Simpson's Rule (when possible).
- LVEF by visual assessment
- LV regional wall motion score index (WMSI; comprehensive post 6MWT echo at 6 and 12 months). WMSI assessment will be performed using the American Society of Echocardiography scoring system, modified to exclude the 4 apical segments because of the presence of the LVAD cannula in the LV apex. Therefore, 12 segments (basal and mid-ventricular segments) will be utilized instead of 16.
- LV mass by the formula of Devereux (only full support, comprehensive echo study)
- RV function (normal, mild, moderate, severe) by Integrative Assessment
- RV chamber size (normal, dilated [mild, moderate, severe])

- **LV Chamber Remodeling**

Echocardiographic assessments of LV chamber remodeling will be performed while the LVAD is at full support. The baseline echocardiographic evaluation will be defined as the LVEDD minor axis assessment prior to LVAD implantation. The percent change from baseline in LV end-diastolic minor axis dimension (LVEDD) will be determined.

The following secondary endpoints will be assessed in ALL patients, regardless of the ability to tolerate the temporary wean from LVAD support:

- **Neovascularization and Cardiomyocyte Proliferation**

Myocardium from the apical core removed at LVAD implant and cardiac samples taken at the time of explant for cardiac transplantation or any other indication, if applicable, will be categorized with regard to region, including anterior, anterolateral, lateral, inferior, midventricular and basilar specimens. All samples will be evaluated for neovascularization, quantification of cardiomyocyte proliferation by immunohistochemistry or molecular analyses, general histology, and DNA analysis.

- **Cell Engraftment and Fate**

Myocardium from multiple samples taken at the time of explant for cardiac transplantation or any other indication, if applicable, will be evaluated for cell engraftment. Relative number of donor cells in the recipient heart will be estimated by real time PCR method measuring copy numbers of donor HLA-DRB alleles different from the recipient in the myocardial samples.

- **Immunologic Assessment**

Immune reactivity results for % IgG Class I and IgG Class II will be recorded. Presence of >10% anti-HLA antibody titers triggers a comparison of recipient antibodies to the MPC donor HLA antigen profiles performed by the Immunologic Core Laboratory.

A peripheral blood sample will be obtained to measure anti-murine and anti-bovine antibodies.

4.2.4 Quality of Life and Neurocognition

Quality of life (QoL) data will be collected and evaluated as a secondary endpoint. Quality of life will be assessed with the KCCQ, a widely used tool in heart failure populations, and the SF12, a widely used overall health status measure. QoL will be compared between treatment groups at baseline, 6 and 12 months (± 14 days) post randomization.

The KCCQ is a 15-item questionnaire that assesses how heart failure affects a patient's life. Ten summary scores can be calculated within the KCCQ including but not limited to the Overall Summary score, Clinical Summary score, and Total Symptom score. These 3 scores range from 0 to 100, with high higher scores indicating better quality of life, fewer symptoms, and physical limitations associated with heart failure.

The SF 12 is a 12-item survey that assesses a patient's view about their health. The Physical and Mental Health Composite Scale scores can be derived from the SF-12. These composite scores are normed as T-scores (mean=50, SD=10) where a higher score indicates a better health state.

A neurocognition battery will be administered at baseline and at 90 (± 14) days and 12 months (± 14 days) post randomization, but not during LVAD wean. Cognitive performance will be assessed using the following battery of tests: Hopkins Verbal Learning Test; Trailmaking Tests A and B; MCG Complex Figures; Digit Span; Digit Symbol Substitution Test; and Controlled Oral Word Association. Neurocognitive testing will be administered by clinical site personnel who have been trained and certified for test administration by the Neurocognitive Core lab personnel.

4.2.5 Index Hospitalization and Readmissions

All readmissions ≥ 24 hours will be considered. Readmissions due to heart transplantation or LVAD explantation for myocardial recovery will be excluded.

- **Total Number of Days Alive and out of the Hospital**

Total number of days alive out of the hospital will be calculated as the total number of days, from the day of randomization to 12 months (+14 days) post randomization during which the patient is not in the hospital. For patients who die, receive a heart transplant, or drop out of study (withdrawal, lost to follow-up) before month 12, the total number of days alive out of the hospital will be calculated as the total number of days, from the day of randomization to death, transplant, or study discontinuation during which the patient is not in the hospital.

- **Length of Index Hospitalization**

Overall post-operative length of stay for the index hospitalization will be measured (and broken down by days spent in the ICU versus days spent on telemetry/step down unit and regular floors). In addition, discharge location will be captured.

- **Time to First Readmission**

The timing of the first hospital readmission over the 12 months of study will be calculated as the time from index hospitalization discharge to the date of the first hospital readmission for all patients who were discharged alive.

- **Readmission Rates**

The rate of hospital readmissions post index hospitalization as well as the total number of days alive and out of the hospital will be considered over the 12 months (+14 days) study period. Readmissions due to heart transplantation or LVAD explantation for myocardial recovery will be excluded. Readmission rates will be calculated as the ratio of number of hospital readmissions over the number of days alive out of hospital.

- **Hospital Resource Use**

Inpatient claims will be obtained through UB-04/92 hospital billing data for clinical sites located in the United States. Cost data will be obtained from Vizient or from the site directly. Hospital costs will be calculated by converting the reported charges to costs using institution and cost-center specific Cost-to-Charge Ratios (CCRs) obtained from annual CMS cost reports. In addition to index hospitalization costs, costs associated with subsequent readmissions will be included in the study. Patients will also be asked at each follow-up visit if they have been hospitalized at another hospital and if yes for how long. Outpatient costs incurred at non-Network and non-US hospitals will not be captured.

4.3 Tertiary Endpoints

4.3.1 Cytokine Quantification

Plasma samples will be processed at the site at baseline (prior to study intervention), at day 1 (+1) post intervention, and at days 7 (± 2), 30 (± 3), 60 (± 7), and 120 (± 14) days post randomization, and at 6 and 12 months (± 14 days) post randomization. Chemo/cytokine quantification of plasma derived from peripheral blood samples will be performed by Luminex multiplex assay and include TNF- α , IL-1, IL-6, IL-10, IL-17, TGF- β , RANTES, SCF and SDF-1.

4.3.2 Angiotensin-1 and Angiotensin-2 Quantification

Because angiotensin-1 and -2 play important roles in vascular development and maturation of normal stable angiogenesis, these analytes will also be measured in peripheral blood plasma collected at the following timepoints: at baseline (prior to study intervention), at day 1 (+1) post intervention, and at days 7 (± 2), 30 (± 3), 60 (± 7), and 120 (± 14) days post randomization, and at 6 and 12 months (± 14 days) post randomization.

5. SAFETY ENDPOINTS

The following additional endpoints will be assessed for all patients (including those who do not tolerate LVAD wean):

5.1 Primary Safety Endpoint

The primary safety endpoint of this study is the incidence of potential study intervention-related adverse events, defined as

- (a) infectious myocarditis,
- (b) myocardial rupture,

- (c) neoplasm,
- (d) hypersensitivity reaction, and
- (e) immune sensitization syndrome

Infectious Myocarditis: Evidence of myocardial infection manifest by positive myocardial gram stain and/or cultures, with or without signs or symptoms of systemic infection. May be accompanied by new acute inflammatory process on histological examination (if absent on histological examination of the apical core at the time of LVAD implantation).

Myocardial Rupture: The presence of myocardial rupture as evidenced by direct visualization (intra-operative or at autopsy) or by standard diagnostic modalities including, but not limited to echocardiography or ventriculography, with or without hemodynamic instability.

Neoplasm: Any new uncontrolled growth of aberrant cells.

Hypersensitivity reaction: Clinical syndrome including but not limited to fever, leukocytosis, or rash with onset ≤ 2 hours post treatment intervention and lasting < 24 hours, in the absence of clinical signs of concomitant infection.

Immune sensitization syndrome: Clinical syndrome including but not limited to fever, leukocytosis, rash or arthralgias with onset ≥ 7 days post treatment intervention and subsequent detection of anti-HLA antibodies against the donor cells detected ≤ 30 days following onset of syndrome, in the absence of clinical signs of concomitant infection.

5.2 Secondary Safety Endpoints

5.2.1 Incidence and Rate of Non-Surgical GI Bleeding Events and/or Epistaxis by 6 Months Post Randomization

Evidence from the LVAD I study involving 30 patients suggests that treatment with MPC may reduce GI bleeding events and/or epistaxis by 6 months after randomization. Therefore, particular emphasis will be placed on the analysis of these events.

Serious GI bleeding and/or epistaxis events whose source is documented as either suspected or confirmed and occur prior to 6 months (+14 days) will be defined using the INTERMACS definition for mucosal bleeding. Based on the INTERMACS criteria, a non-surgical major mucosal bleeding event that occurs >7 days after initial LVAD implantation is defined as a bleeding event that results in death, re-operation, re-hospitalization or prolonged hospitalization and/or transfusion of red blood cells. The latter is quantified as the number of units given per 24 hour period. Bleeding due to trauma (e.g. falls) will be excluded.

5.2.2 Serious & Study-Intervention Related Adverse Events

The incidence and frequency of all anticipated and unanticipated serious adverse events that occur prior to 12 months (+14 days) post injection of MPC or cryoprotective media alone, will be determined. Moreover, the incidence of all adverse events classified by the clinical site investigator or independent Event Adjudication Committee as “possibly” or “probably” study-intervention related (to either the study product or to the

intramyocardial injection procedure itself) will be determined.

5.2.3 Anti-HLA Antibody Sensitization

Anti-HLA antibody serum sample will be collected at days 30 (± 3) and 90 (± 14), and at months 6 and 12 (± 14 days). Immune reactivity results for % IgG Class I and IgG Class II, will be assessed. If the PRA (percent reactive antigens) of either class I or class II HLA antibodies are $>10\%$, specificity analysis will be performed. The specificity test is to measure the presence of antibodies to HLA antigens including MPC donor specific HLA antigens.

6. STATISTICAL METHODOLOGY

6.1 Statistical and Analytical Issues

6.1.1 Statistical Methods

All data will be presented by treatment group as well as for the whole sample. Subgroup analyses will be performed as described in section 6.5.

For all baseline, demographic and efficacy variables, data will be summarized and analyzed by randomized treatment group as per ITT. Safety variables will be summarized by actual treatment received.

In summary tables of continuous variables, the following descriptive statistics will be used: minimum and maximum, mean, median, 95% confidence interval (CI), standard deviation (SD) and standard error (SE).

In summary tables of non-normally distributed variables data will be presented using the minimum, maximum, median and lower and upper quartiles.

In summary tables of categorical variables, counts and percentages will be used. The denominator for each percentage will be the number of patients within the randomization group unless otherwise specified.

Rates of events will be calculated as the ratio of the total number of events recorded over a period of time over the total patient/time (months or days). Total patient/time will be calculated by summing the time (in months or days) that patients were at risk for a specific event from the time they were randomized in the study. For patients who die, are transplanted, or drop out of study (withdrawal, lost to follow-up) before they experience an event, their patient/time will be calculated as the time from randomization to death, transplant, or study discontinuation. Rates and 95% confidence intervals will be reported.

For any variable measured at different points in time, change from baseline will be calculated as the difference between the value of the variable at a specific point in time (e.g. 90 days) minus the baseline value.

Relative change from baseline will be calculated as the value of a parameter at a specific point in time minus the baseline value of the parameter divided by the baseline value of

the parameter.

Percent change will be calculated as the relative change multiplied by 100.

All hypothesis testing will be carried out at the 0.05 (2-sided) significance level, unless otherwise specified. There will be no formal correction of the Type I error rate for multiple testing of statistical hypotheses.

P-values will be rounded to three decimal places. P-values less than 0.001 will be reported as <0.001 in tables. P-values greater than 0.999 will be reported as >0.999.

Should any of the statistical methods proposed prove unsuitable during data analysis, more appropriate methods will be used. These include data transformation (for example to a logarithmic scale) to satisfy model assumptions such as normally distributed residuals with constant variance, or the application of non-parametric techniques.

Additional ad-hoc analyses may be conducted as deemed appropriate.

All statistical analysis will be performed using SAS V9.4 and R V3.4.3 or higher.

6.1.2 Handling of Dropouts and Missing Data

Missing baseline values that are needed to compute absolute, relative, or percent change from baseline will be imputed using mean imputation.¹⁷ The missing values of a variable will be replaced with the observed sample mean of that variable. Mean imputation is appropriate because baseline variables are independent of randomization assignment.

Patients who require an LVAD are a highly dependent patient population and are extremely compliant with their care and follow-up. They receive extensive training in adverse event and alarm recognition prior to discharge, and they are called at least once a week when at home. Due to diligent patient and site monitoring, we anticipate that there will be few missing data points in this study.

- **Missing Values for the Primary Outcome**

Missing outcome values on the primary endpoint will not be imputed. For the primary efficacy endpoint, missing data can be the result of patient receiving cardiac transplantation, patient refusal, missed visit, or study dropout (withdrawal, lost to follow-up). Patients who receive a heart transplant will be censored at the time of transplant and the number of successful weans out of the number of possible wean assessments up to the time of transplant will be considered in the analysis. Intermittent missing data due to patient refusal or missed visits are assumed to be missing at random (MAR) and the number of successful weans out of the number of possible wean assessments will be used for analysis. Study dropout is also assumed to be MAR and the number of successful weans out of the number of possible wean assessments up to the time of dropout will be computed. As described in Section 4.1 patients who do not attempt the wean because of death or physician contraindication will be considered as failures.

- **Missing Values for the Secondary Efficacy Outcomes**

Missing outcome values on secondary endpoints will not be imputed. Analytic rules

have been developed in Section 4.2.2 for missing wean duration and/or 6MWT due to patient's morbidity that precludes evaluation or patient's inability to complete the entire wean assessment. Echo parameters related to LVEDD chamber remodeling are measured while the LVAD is at full support and therefore, do not depend on the ability to perform the wean. Since longitudinal models will be used for the analysis of wean duration, 6MWT, and echo parameters over time (see Section 6.3.2), intermittent missing data under MAR are allowed. To accommodate for missingness due to death, transplant or study drop-out (withdrawal, loss to follow-up) pattern-mixture analyses will be used for longitudinal models. Values of the duration of weaning that are missing because the patient was not able to reach minimal support or did not perform the wean because of cardiology discretion will be assigned a value of zero.

- **Missing Values for All Other Outcomes**

Consideration for missing data for secondary and tertiary outcomes will be handled similarly to what described above.

- **Sensitivity Analysis**

Sensitivity analysis will be conducted to investigate possible violations of the missing at random assumption. Using a pattern-mixture model approach for MNAR¹⁸ (missing not at random), we will impute the missing data under several MNAR scenarios. Specifically, we will use delta adjustments after several multiple imputation models in which the imputed values will be modified (e.g. shifted by a constant) to reflect a better or worse score for specific patients (e.g. patients who do not perform the 6MWT because of refusal or withdrawal of consent will receive a worse score). The consistency of results across these different models will indicate the robustness of the assumptions.

6.2 Patients Characteristics

6.2.1 Patients Disposition

The subject disposition table will summarize patients' characteristics by randomization group and overall.

- The number (%) of patients randomized
- The number (%) of patients withdrawn or lost to follow-up by month 12
- The number (%) of patients who received a transplant by month 12
- The number (%) of patients who completed the study at month 24
- The number (%) of patients in the ITT population
- The number (%) of patients in the Safety population

The number (%) of subjects who complete and withdraw/lost to follow-up from the study and the primary reason for withdrawal will be summarized by randomization group and overall for all subjects.

The percentages will be calculated based on the total number of patients randomized in each randomization group.

6.2.2 Protocol Deviations and Violations

Protocol deviations and violations are defined as deviations from the procedures outlined in the protocol. There is no "Per Protocol" population defined for this study. All statistical analyses and summaries will be conducted on an intent-to-treat basis for the

efficacy endpoints and on an as-treated basis for the safety endpoints.

6.2.3 Demographic Characteristics

Demographic and baseline characteristics data including age, gender, race, ethnicity, indication for LVAD (DT, BTT), device type (HeartMate II, HVAD) will be summarized using summary statistics for continuous variables or by group frequencies and percentages for categorical variables, as appropriate.

6.2.4 Prior and Concomitant Medication

The number (%) of patients receiving cardiovascular medications prior to or at the time of randomization will be reported and categorized by treatment group and medication type.

6.2.5 Medical History

Information pertaining to the etiology of heart failure, NYHA and Intermacs classification, the history of cardiovascular disease and other co-morbidities, and cardiovascular procedures will be summarized.

The number of patient with specific conditions in their medical history will be summarized using frequencies and percentages.

6.2.6 Pre-Implant Echocardiographic Parameters

Pre-implant echocardiographic parameters such as left ventricular ejection fraction will be presented with summary statistics for continuous variables.

6.3 Efficacy Analysis

6.3.1 Analysis of the Primary Efficacy Endpoint and Determination of Sample Size

A Bayesian approach based on the probability that active therapy is superior to control will be used to assess the strength of potential efficacy, should safety continue to be demonstrated. Simulations will be conducted to estimate this probability. The procedure will be carried out as follows: a binomial likelihood is specified for the primary efficacy outcome of the number of successful weans out of the three planned. The prior distribution for the success probability of the binomial likelihood is based on data from the control patients in the LVAD MPC I trial, where the rate of successful wean was found to be approximately 0.18. Independent gamma priors with this mean and standard deviation of 0.10 are assumed for each group. Under the null hypothesis of no difference between the two groups, the simulated prior distribution of the success rate ratio is centered at one with nearly all mass between 0.5 and 2. Before any data collection, equipoise implies that the probability that active therapy is superior to control is 50%. Based on observed data, an increase in this probability to 80% or more, without safety concerns, will be considered a positive signal of efficacy for MPC therapy. Simulations indicate that a sample size of 159 patients provides a probability of 80% to detect such an efficacy signal if the true rate ratio is 1.5, with a false positive rate of 15%.

An additional analysis based on a frequentist approach will also be conducted on the primary outcome as a sensitivity analysis. Negative binomial regression will be used to compare the number of successful weans in the first 6 months after randomization between the MPC and the control group and will include an offset term to adjust for the total number of possible wean assessments. The model accounts for between-subject heterogeneity, and accommodates over-dispersed data. If there are excess zeros due to

failure to wean that cannot be satisfactorily accounted for in the negative binomial model, a zero-inflated negative binomial regression will be considered.

6.3.2 Analysis of Secondary Efficacy Endpoints

All secondary efficacy analyses will be performed using the ITT population, unless otherwise specified.

- **Overall Survival and Survival to Cardiac Transplantation**

Time to death will be calculated as the number of days between randomization and death due to any cause at 12 (+14 days) and 24 (+30 days) months. Patients who are alive at the respective time points will be censored. Patients who undergo cardiac transplantation will be censored at the time of transplant. Patients who drop out (withdraw, lost to follow-up) will be censored at the time of study discontinuation.

Survival at 12 and 24 months will be described by Kaplan-Meier curves and differences between randomization groups will be assessed via the log-rank test.

If the number of events permits, additional analyses will be performed for cardiovascular and non-cardiovascular cause of death and for LVAD related and non-LVAD related cause of death.

Time to cardiac transplantation by 12 or 24 months post randomization will be calculated as the number of days between randomization and transplant. Patients who do not receive a transplant by month 12 or month 24 will be censored. Patients who drop out (withdraw, lost to follow-up) will be censored at the time of study discontinuation. Death before transplant will be considered a competing risk in the calculation of the cumulative incidence curves of time to cardiac transplantation.

Time to cardiac transplantation by 12 and 24 months will be described by cumulative incidence curves and differences between randomization groups will be assessed via Gray's test.¹⁹

- **Functional Status by 6 Months and 12 Months Post Randomization**

Ability to Tolerate Wean from LVAD Support for 30 Minutes

The pattern of successful weans over 6 months and 12 months follow-up will be explored using logistic regression with parameter estimation using generalized estimating equations.

Duration of Ability to Tolerate Wean

The duration of time weaning is tolerated will be examined at each time point post randomization. Random effects pattern mixture models will be used to characterize changes over time in the duration that a patient tolerates weaning.

This approach is not sensitive to untestable assumptions about missing data mechanism because it separates the data into strata based on the missing patterns and estimates a separate linear model, including a treatment effect, for data in each stratum. An interaction term of treatment-by-time will be used to determine the difference in the longitudinal course of duration of weaning between the two randomization groups in each stratum. The model will, at a minimum, include a random subject effect. If a random slope on time is needed, an unstructured

covariance matrix among the random effects will be used and independence among the residuals will be assumed. The model makes assumptions regarding the distribution (mean and correlation structure) of the random effects and the residuals which will all require checking.

The method of Wu and Bailey²⁰ is an instance of pattern-mixture modeling.

If there are excess zeros, a two-part mixed effects pattern-mixture model will be considered. A pattern-mixture binary mixed model will be used to analyze the zero part of weaning duration and a pattern-mixture Gaussian (or other appropriate link function) mixed model for the non-zero response.

Six Minute Walk Test

The 6MWT will be analyzed similarly to the duration of time weaning is tolerated using random effects pattern mixture models. An interaction term of treatment-by-time will be used to determine the difference in the longitudinal course of the 6MWT between the two randomization groups.

- **Physiological Assessments**

At each wean assessment, echo parameters are measured 3 times – prior to wean, 15 minutes after wean started, and after the 6MWT. Separate analyses will be conducted for each of these endpoints. All of the echocardiographic measures will be described using both graphical and numerical summaries. We will use random effect models similar to the ones described for the analysis of the secondary efficacy endpoints to analyze the echo parameters collected before the wean. The primary aims of the random effects models will be to characterize the changes over 12 months in the echocardiographic measures in each treatment group. For the echo parameters collected during and at the end of the weaning we will restrict the analysis to descriptive measures and their 95% confidence intervals.

LV performance and LV chamber remodeling

The percent change from baseline in LV end-diastolic minor axis dimension (LVEDD) will be measured from the echocardiogram performed when the LVAD is at full support before the wean. Similar to the duration of wean and 6MWT endpoints, changes in echo parameters over time will be analyzed using random effects pattern mixture models with an interaction term of treatment-by-time group to determine the difference in the longitudinal course of LVEDD between the two randomization groups.

- **Index Hospitalization and Readmissions**

Total Number of Days Alive and out of the Hospital

Total number of days alive and out of the hospital during the first 6 months (+14 days) post randomization will be analyzed using negative binomial regression models in which the randomization arm will be the independent variable of interest and the total days alive as an offset.

Hospitalization Length of Stay and Days in ICU of the Index Hospitalization

We will compare post-operative hospital length of stay and days spent in ICU of the index hospitalization between randomization groups using a Wilcoxon Rank-Sum Test. Patients who die in the hospital before discharge will be assigned the worst rank

according to the time of death (with patients dying earlier having the worse rank).

Time to First Readmission

Time to first readmission by 12 months post randomization will be calculated as the number of days between time of discharge from index hospitalization and first readmission. Patients who are not re-admitted by month 12 will be censored. Patients who drop out (withdraw, lost to follow-up) or are transplanted without being re-admitted will be censored at the time of study discontinuation. Death post discharge from the index hospitalization will be considered a competing risk in the calculation of the cumulative incidence curves of time to cardiac transplantation. Time to cardiac first readmission by 12 months will be described by cumulative incidence curves and differences between randomization groups will be assessed via Gray's test.

Readmission Rates

Rates of all-cause hospitalizations, and rate of cardiovascular-specific hospitalizations, both within 30 days and 12 months (+14 days), will be compared between randomization groups using negative binomial regression models.

Hospital Resource Use

Hospital resource utilization or hospital costs will be calculated by converting charges to costs using institution specific Ratio-of-Cost-to-Charges (RCCs). Costs associated with the index hospitalization and readmissions at 12 months will be totaled for each patient and compared between treatment groups by Student's t-test after log transformation. Independent predictors of total cost, including randomization group, baseline factors, operative factors and postoperative events occurring during the index hospitalization, will be determined by multivariate regression analysis with the appropriate distribution and link function based on the modified Park test.

• **Quality of Life and Neurocognition**

Quality of Life

Quality of life will be measured using the KCCQ and SF-12 at baseline and at 6 and 12 months post randomization. These patient-reported outcomes will be analyzed using random effect pattern mixture models.

Neurocognitive Outcomes

Neurocognitive outcomes for each test will be standardized using the means and standard deviations observed in the overall sample and combined within cognitive domains using weights. Differences in the scores for each domain at 90 days and 12 months post-randomization will be compared between randomization arms based on an analysis of covariance that adjusts for important baseline values.

Results from each test will be normalized to a Z-score based on the baseline means and standard deviations for the whole sample. Function of 4 cognitive domains (verbal learning and memory, visual learning and memory, executive functioning, and visuospatial/constructional functioning) will be measured by averaging the Z-scores of tests that correspond to each domain.

The impact of MPC treatment over time will be assessed using linear mixed effects models with random intercepts. In these models, the standardized neurocognitive scores assessed at each time point will be used as the dependent variable, treatment,

assessment time and their interaction will be the primary predictors of interest. Models will also be adjusted for age and education level. We will also analyze the proportion of patients with neurocognitive decline defined as a change from baseline to the 1-year assessment of 0.5, or greater, SD in 2 or more domains. For this analysis we will use logistic models with the proportion of patients with neurocognitive decline at 1 year as the outcome and treatment assignment as the primary predictor. Models will also be adjusted for age and education level.

6.4 Tertiary Endpoints

Mixed effects models will be used to characterize levels of cytokines and chemokines over time. The association of cytokine and chemokine levels with engraftment of cells in native explanted hearts will be described graphically, and quantified by regression and correlation analysis.

6.5 Subgroup Analyses

Stratified analyses will be conducted, if the sample size permits, to explore the effect of the MPC treatment in particular subgroups of patients. These analyses will be stratified by LVAD indication (DT or BTT). Since the randomization was not stratified according to this factor, there is no assurance that the randomization groups will be balanced within these strata. An assessment of the available sample size within each stratum and reasonable balance of the treatment assignment will be conducted before performing the subgroup analyses. Strata that contain less than 20% of the sample size and/or in which the number of patients assigned to a specific group is less than 10 will not be considered.

6.6 Safety Analysis

6.6.1 Incidence and Rate of Non-Surgical GI Bleeding Events and/or Epistaxis by 6 Months Post Randomization

Evidence from the LVAD I study involving 30 patients suggest that treatment with MPC may reduce GI bleeding events and/or epistaxis by 6 months after randomization. Therefore, particular emphasis will be placed on the analysis of these events.

A time to first serious GI bleeding and/or epistaxis analysis will be conducted to compare the two randomization groups. Time to first bleeding event over 6 months (+14 days) will be calculated as the time from randomization to the first episode of bleeding. Time to first bleeding event will be described by cumulative incidence curves and differences between randomization groups will be assessed via Gray's test.¹⁸ Death before bleeding will be considered a competing risk in the calculation of the cumulative incidence curves of time to first bleeding event. Patients who do not experience bleeding by the end of the 6 months period will be censored. For patients who undergo cardiac transplantation prior to a bleeding event, censoring will be at the time of transplant. Similarly, patients who drop out without having a bleeding event will be censored at the time of study discontinuation.

The rate of serious GI bleeding events and/or epistaxis in each group will be calculated as the ratio of the total number of bleeding events and the total patient/months. A negative binomial model will be used to compare the rate of bleeding between the MPC and the control group.

Should the two groups be different with respect of baseline factors, such as type of LVAD implanted (Heartmate II or HVAD), the LVAD indication (DT or BTT), or history of GI bleeding, negative binomial models will be fitted to examine the effect of these factors on bleeding. Interaction terms may also be added to these models depending on the frequency of the outcome variable in the subgroups.

6.6.2 Treatment-emergent Adverse Events (TEAEs)

All adverse events in this study will be analyzed from the time of injection. All safety analysis will be based on the safety population. Subjects who were randomized, but never been administered with the study drug (either MPC or control) will be excluded from the safety analysis.

AEs will be mapped to the MedDRA coding system. The incidence of TEAEs and serious TEAEs will be summarized by System Organ Class, MedDRA Preferred Term and treatment.

Separate tables will be presented for all TEAEs, TEAEs by severity, serious TEAEs, study intervention-related TEAEs, LVAD related TEAEs and TEAEs leading to death.

The number and percentage of patients with a TEAE or serious TEAE will be presented. A patient will be counted only once in the incidence count for a specific MedDRA Preferred Term, although a MedDRA Preferred Term might be recorded more than once for a particular patient.

The number and rate of each TEAE will be presented. Differences in the incidence of individual TEAEs will be assessed using negative binomial regression with the treatment groups as the predictor.

6.6.2 Anti-HLA Antibody Sensitivity, Anti-Murine and Anti-Bovine Antibodies

The proportion of patients with anti-HLA antibody sensitivity while on LVAD support will be reported along with 95% confidence intervals by treatment group. In addition, the proportion of patients who exhibit anti-murine and anti-bovine antibodies following study product administration will be reported similarly. The analysis will be based on all available samples at each time point. Missing data will not be imputed. In addition to the analyses of clinical outcomes described above, explanted native hearts will be examined to quantify the extent of neovascularization, MPC cell engraftment, as well as histologic and gene regulation responses to cell implantation. Confidence intervals will be constructed and differences between treatment groups in the prevalence of these measures will be summarized.

6.7 Early Stopping Rules

Safety will be continuously monitored. The DCC will report all deaths and unexpected adverse events, as well as rare but expected serious adverse events to the NHLBI, who, in turn, will report to the DSMB chair. There are two components to the safety monitoring plan. The first focuses on rare events that are very likely to be related to the experimental treatment and not to the underlying heart failure. The second focuses on mortality which is not a rare event in this patient population. With respect to the rare events likely associated with experimental treatment, enrollment will be halted should any of the pre-specified events (infectious myocarditis,

myocardial rupture, neoplasm, hypersensitivity reaction, or immune sensitization syndrome) be observed. Stopping guidelines for mortality will use the same approach as that described to assess the presence of an efficacy signal using a very diffuse beta (2, 8) prior for mortality for both groups, and will be initiated following the randomization of the 10th patient. We would propose to halt randomization if the probability that mortality on active therapy is increased compared to control exceeds 80%. The probability will be determined sequentially, after each mortality event. NHLBI, in conjunction with the DSMB's recommendations, will determine whether enrollment should be continued, suspended or terminated should any of the proposed stopping criteria be met.

6.8 Interim Analysis

There is no planned interim analysis for this study.

7. REFERENCES

1. Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W, Long JW, Ascheim DD, Tierney AR, Levitan RG, Watson JT, Meier P, Ronan NS, Shapiro PA, Lazar RM, Miller LW, Gupta L, Frazier OH, Desvigne-Nickens P, Oz MC, Poirier VL; Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) Study Group. Long-term use of a left ventricular assist device for end-stage heart failure. *N Engl J Med.* 2001;345:1435-43.
2. Slaughter MS, Rogers JG, Milano CA, Russell SD, Conte JV, Feldman D, Sun B, Tatroles AJ, Delgado RM III, Long JW, Wozniak TC, Ghumman W, Farrar DJ, Frazier OH; HeartMate II Investigators. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med.* 2009;361:2241-51.
3. Russo MJ, Hong KN, Davies RR, Chen JM, Sorabella RA, Ascheim DD, Williams MR, Gelijns AC, Stewart AS, Argenziano M, Naka Y. Posttransplant survival is not diminished in heart transplant recipients bridged with implantable left ventricular assist devices. *J Thorac Cardiovasc Surg.* 2009;138:1425-32.e1-3.
4. Miller LW, Pagani FD, Russell SD, John R, Boyle AJ, Aaronson KD, Conte JV, Naka Y, Mancini D, Delgado RM, MacGillivray TE, Farrar DJ, Frazier OH; HeartMate II Clinical Investigators. Use of continuous-flow device in patients awaiting heart transplantation. *N Engl J Med* 2007;357:885-96.
5. Ogletree-Hughes ML, Stull LB, Sweet WE, Smedira NG, McCarthy PM, Moravec CS. Mechanical unloading restores beta-adrenergic responsiveness and reverses receptor downregulation in the failing human heart. *Circulation.* 2001;104:881-6.
6. Thompson LO, Skrabal CA, Loebe M, Lafuente JA, Roberts RR, Akgul A, Jones V, Bruckner BA, Thohan V, Noon GP, Youker KA. Plasma neurohormone levels correlate with left ventricular functional and morphological improvement in LVAD patients. *J Surg Res* 2005;123:25-32.
7. Felkin LE, Lara-Pezzi EA, Hall JL, Birks EJ, Barton PJ. Reverse remodeling and recovery from heart failure are associated with complex patterns of gene expression. *J Cardiovasc Transl Res.* 2011;4:321-31.
8. Hall JL, Fermin DR, Birks EJ, Barton PJ, Slaughter M, Eckman P, Baba HA, Wohlschlaeger J, Miller LW. Clinical, molecular, and genomic changes in response to a left ventricular assist device. *J Am Coll Cardiol.* 2011;57:641-52.
9. Heerdt PM, Klotz S, Burkhoff D. Cardiomyopathic etiology and SERCA2a reverse remodeling during mechanical support of the failing human heart. *Anesth Analg.* 2006;102:32-7.
10. Birks EJ. Molecular changes after left ventricular assist device support for heart failure. *Circ Res.* 2013;113:777-91.
11. Birks EJ, George RS, Firouzi A, Wright G, Bahrami T, Yacoub MH, Khaghani A. Long-term outcomes of patients bridged to recovery versus patients bridged to transplantation. *J Thorac Cardiovasc Surg.* 2012;144:190-6.
12. Houtgraaf JH, de Jong R, Kazemi K, de Groot D, van der Spoel TI, Arslan F, Hoefler I, Pasterkamp G, Itescu S, Zijlstra F, Geleijnse ML, Serruys PW, Duckers HJ. Intracoronary infusion of allogeneic mesenchymal precursor cells directly after experimental acute myocardial infarction reduces infarct size, abrogates adverse remodeling, and improves cardiac function. *Circ Res.* 2013;113:153-66.
13. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB Jr,

- Reisman MA, Schaer GL, Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:2277–2286.
14. See F, Seki T, Psaltis PJ, Sondermeijer HP, Gronthos S, Zannettino AC, Govaert KM, Schuster MD, Kurlansky PA, Kelly DJ, Krum H, Itescu S. Therapeutic effects of human STRO-3-selected mesenchymal precursor cells and their soluble factors in experimental myocardial ischemia. *J Cell Mol Med*. 2011;15:2117-29.
 15. Jellema RK, Wolfs TG, Lima Passos V, Zwanenburg A, Ophelders DR, Kuypers E, Hopman AH, Dudink J, Steinbusch HW, Andriessen P, Germeraad WT, Vanderlocht J, Kramer BW. Mesenchymal stem cells induce Tcell tolerance and protect the preterm brain after global hypoxia-ischemia. *PLoS One*. 2013;8:e73031.
 16. Wang Y, Liu J, Xu C, Zhang W, Bai L, Li N, Liu Y, Wang Y, Su Y, Hu D. Bone marrow transplantation combined with mesenchymal stem cells induces immune tolerance without cytotoxic conditioning. *J Surg Res*. 2011;171:e123-31.
 17. White I and Thompson SG. Adjusting for partially missing baseline measurements in randomized trials, *Statistics in Medicine*. 2004;24: 993 – 1007.
 18. Carpenter J and Kenward M. *Multiple Imputation and its Application* 1st Edition, 2013. John Wiley & Sons.
 19. Gray, R. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. *The Annals of Statistics*. 1988;16, 1141–1154.
 20. Wu MC, Bailey K. Analysing changes in the presence of informative right censoring caused by death and withdrawal. *Statistics in Medicine*. 1988;7:337–346.