

**Cardiothoracic Surgical Trials Network**  
**Cardiovascular Cell Therapy Research Network**

**LVAD THERAPY: EXPLORING THE EFFECT OF  
INTRAMYOCARDIAL INJECTION OF MESENCHYMAL PRECURSOR  
CELLS ON MYOCARDIAL FUNCTION**

**Protocol**



Sponsored By NHLBI, NINDS, and CIHR

CT Surgery Network Research Group  
In collaboration with  
Cardiovascular Cell Therapy Research Network

Data Coordinating Center  
InCHOIR  
Mount Sinai School of Medicine  
New York

July 2012

**CONFIDENTIAL**

NETWORKS FOR CARDIOTHORACIC SURGICAL TRIALS AND CARDIOVASCULAR CELL  
THERAPY RESEARCH

***CTSN Core Clinical Centers***

Cleveland Clinic Foundation (Eugene Blackstone, MD)  
 Columbia University Medical Center (Michael Argenziano, MD)  
 Duke University (Peter Smith, MD)  
 Emory University (John Puskas, MD)  
 Montefiore Medical Center - Albert Einstein College of Medicine (Robert Michler, MD)  
 Montreal Heart Institute (Louis Perrault, MD)  
 University of Pennsylvania (Michael Acker, MD)  
 University of Virginia Health Systems (Irving L. Kron, MD)

***CCTR***

Cleveland Clinic Foundation (Steven Ellis, MD)  
 Texas Heart Institute (James Willerson, MD)  
 University of Florida (Carl Pepine, MD)  
 University of Minnesota (Timothy Henry, MD)  
 Vanderbilt University (David Zhao, MD)

***CTSN Affiliated and Ancillary Clinical Centers***

Inova Heart & Vascular Institute (Alan M. Speir, MD)  
 The Ohio State University Medical Center (Chittor Sai Sudhakar, MD)

***CTSN Satellite Clinical Centers***

Baylor Research Institute (Michael Mack, MD)  
 Brigham and Women's Hospital (Fredrick Chen, MD, PhD)  
 Jewish Hospital and St Mary's Healthcare (Mark Slaughter, MD)  
 University of Maryland Medical Center (James Gammie, MD)  
 University of Southern California (Vaughn Starnes, MD)

***CCTR Satellite Clinical Centers***

Mayo Clinic College of Medicine (Andre Terzic, MD, PhD)  
 Metropolitan Cardiology – Mercy Hospital (Jeffrey Chambers, MD)  
 Pepin Heart Hospital and Patel Research Institute (Charles Lambert, MD)  
 University of Minnesota Medical Center, Fairview (Ganesh Raveendran, MD)  
 VA Medical Center – Houston (Biswajit Kar, MD)

***Data Coordinating Center***

International Center for Health Outcomes and Innovation Research, Mount Sinai School of Medicine (InCHOIR): Deborah D. Ascheim, MD; Annetine Gelijns, PhD; Ellen Moquete, RN; Alan J. Moskowitz, MD; Michael K. Parides, PhD; Emilia Bagiella, PhD

CCTR DCC: University of Texas Health Sciences Center: Lemuel Moyé, MD, PhD; Shreela Sharma, PhD

***Study Chair, Co-Chairs***

Timothy J. Gardner, MD (CTSN); Christiana Medical Center  
Patrick T. O’Gara, MD (CTSN); Brigham and Women’s Hospital  
Robert D. Simari, MD (CCTRN); Mayo Clinic

***Study Sponsors***

National Heart Lung and Blood Institute (Sonia I. Skarlatos, PhD; Marissa Miller, DVM MPH; David Gordon, MD, PhD; Wendy Taddei-Peters, PhD; Albert Lee, PhD; Ray Ebert, PhD; Neal Jeffries, PhD; Nancy Geller, PhD)  
National Institute of Neurological Diseases and Stroke (Claudia Moy, PhD)  
Canadian Institutes for Health Research (Ilana Gombos, PhD)  
Angioblast Systems, Inc.

***Protocol Development Committee***

Michael Acker, MD; U Penn (CTSN Co-chair)  
Carl Pepine, MD; U Florida (CCTRN Co-chair)  
Deborah D. Ascheim, MD; InCHOIR, Mount Sinai School of Medicine (CTSN DCC)  
Emma Birks, MD; Jewish Hospital/U. Louisville (CTSN)  
Annetine Gelijns, PhD; InCHOIR, Mount Sinai School of Medicine (CTSN DCC)  
Daniel Goldstein, MD; Montefiore/AECOM (CTSN)  
Gonzalo Gonzalez-Stawinski, MD; Cleveland Clinic Foundation (CTSN)  
Timothy Henry, MD; U. Minnesota (CCTRN)  
Neal Jeffries, PhD; NHLBI  
Carmelo Milano, MD; Duke (CTSN)  
Marissa Miller, DVM, MPH (NHLBI)  
Lem Moye, PhD (CCTRN DCC)  
Yoshifumi Naka, MD PhD; Columbia University Medical Center (CTSN)  
Michael Parides, PhD; InCHOIR, Mount Sinai School of Medicine (CTSN DCC)  
Sonia Skarlatos, PhD (NHLBI)  
Mark Slaughter, MD; Jewish Hospital/U. Louisville (CTSN)  
Wendy Taddei-Peters, PhD (NHLBI)  
Doris Taylor, PhD; Texas Heart Institute (CCTRN)  
James Willerson, MD; Texas Heart Institute (CCTRN)  
David Zhao, MD; Vanderbilt (CCTRN)

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**TABLE OF CHANGES**

<b>Rev</b>	<b>Section</b>	<b>Change</b>	<b>Reason</b>	<b>Page</b>
1.4	All	Changed footer to indicate Rev 1.4 and date of November 2011	Protocol Update	All
1.4	CTSN Satellite Clinical Centers	Removed several sites from list of CTSN Satellite Sites and corrected Dr. Chen's name	Protocol Update	2
1.4	Definitions, Acronyms & Abbreviations	Removed "Clinical Coordinating Center" from list	Internal consistency within protocol	8
1.4	Abstract	Corrected assessment windows	Internal consistency within protocol	10
1.4	Data Collection Schedule	Updated Data Collection Schedule	Internal consistency within protocol	11-12
1.4	Rationale for Selection of the Key Efficacy Endpoint	Clarification of endpoint timeline	Internal consistency within protocol	21
1.4	Endpoints	Corrected assessment windows	Internal consistency within protocol	22, 23
1.4	Key Efficacy Endpoints	Corrected Appendix III title	Internal consistency within protocol	22
1.4	Treatment Intervention	Clarified that the preparation may occur outside of operating room but must occur within a sterile field	Internal consistency within protocol	25
1.4	Treatment Intervention	Changed length of maximum thaw time from 60 to 75 minutes	For consistency with manufacturer's procedures	25-26
1.4	Secondary Endpoints	Corrected assessment windows	Internal consistency within protocol	27
1.4	Secondary Endpoints	Corrected Appendix III title	Internal consistency within protocol	27
1.4	Assessments During LVAD Wean	Corrected assessment windows	Internal consistency within protocol	28
1.4	6 Minute Walk	Added "15 minute"	Protocol Clarification	29
1.4	Ability to Tolerate Wean for 30 Minutes	Corrected Appendix III title	Internal consistency within protocol	29
1.4	Anti-HLA Antibody Sensitization	Removed IgM Class I and II	For consistency with core laboratory procedures	30
1.4	Tertiary Endpoints	Corrected assessment windows	Internal consistency within protocol	31-32
1.4	Procedure for Reporting Serious Adverse Events	Removed "(Sponsor's Representative)"	For clarity	33
1.4	Possible Effects of Cells on Fetus	Clarified that site reporting should be to the DCC	Protocol Update	35
1.4	Neurological Dysfunction	Removed Neurocognitive Testing	Internal consistency within protocol	39
1.4	Informed Consent	Added NINDS to list of sponsors	Internal consistency within protocol	43
1.4	Biospecimen Analysis	Removed specific test names	To avoid redundancy	44-45
1.4	Data Collection	Clarified which case report forms will capture each assessment	For consistency between protocol and electronic data capture system	45, 47-48, 50
1.4	Six Minute Walk	Corrected title of Appendix III	Internal consistency within protocol	50
1.4	Data Collection	Corrected assessment windows	Internal consistency within protocol	47-51
1.4	Monitoring	Added description of centralized monitoring processes	At the suggestion of FDA Guidance dated 8/24/11 regarding risk-based monitoring	53-54
1.4	Organization of the Study	Revised DCC and Angioblast sections to clarify regulatory reporting responsibilities	Protocol Update	58-59
1.4	Appendix I	Changed "sponsor" to "manufacturer"	For clarity	62
1.4	Appendix I	Changed length of maximum thaw time from 60 to 75 minutes	For consistency with manufacturer's procedures	62, 63

1.4	Appendix I	Changed 1ml to 5cc	Internal consistency within protocol	62
1.4	Appendix I	Changed “under refrigeration” to “frozen”	For consistency with manufacturer’s procedures	63
1.4	Appendix I	Removed “24 to”	For consistency with manufacturer’s procedures	63
1.4	Appendix I	Changed “sponsor” to “Lonza”	For clarity	63
1.4	Appendix II	Changed length of maximum thaw time from 60 to 75 minutes	For consistency with manufacturer’s procedures	64
1.4	Appendix V	Removed “in one of the course marker chairs”	For clarity	73
1.4	Appendix V	Corrected directions for six-minute walk	For clarity	74
1.4	Appendix VI	Removed details of sample collection, processing and handling	Text being moved to Manual of Procedures	75-83
1.4	Appendix VI	Corrected assessment windows	Internal consistency within protocol	81-82
1.4	Appendix VI	Updated Cell Surface Phenotyping section	For clarity	83
1.4	Appendix VII	Corrected assessment windows	Internal consistency within protocol	84
1.4	Appendix VII	Updated histocompatibility testing	For clarity	84
2.0	Protocol Development Committee	Changed lab location from University of Minnesota to Texas Heart Institute	Update to protocol to reflect change in laboratory location	3
2.0	Abbreviations	Removal of UMN	Internal consistency within protocol	9
2.0	Abstract	Clarification of time points when WMSI will be collected	Internal consistency within protocol	10
2.0	Assessments during LVAD Wean	Clarification of time points when WMSI will be collected	Internal consistency within protocol	22
2.0	Eligibility Criteria	Addition of footnotes	For clarity	24
2.0	Treatment Intervention	Changed needle gauge to 3/4 inch	To accommodate sites’ ability to obtain appropriate needle gauge	25
2.0	Echocardiographic Assessments of Myocardial Function	Clarification of time points when WMSI will be collected	Internal consistency within protocol	28
2.0	Cell Engraftment and Fate	Removal of UMN	Internal consistency within protocol	30
2.0	Immunologic Assessment	Clarification of process of obtaining immunologic assessment	For clarity	44
2.0	Biorepository Core Lab	Changed lab location from University of Minnesota to Texas Heart Institute	Update to protocol to reflect change in laboratory location	57
2.0	APPENDIX I	Changed syringe size to 10 or 15 ml	To accommodate sites’ ability to obtain appropriate syringe size	60, 61
2.0	APPENDIX II	Changed needle gauge to 3/4 inch	To accommodate sites’ ability to obtain appropriate needle gauge	62
2.0	APPENDIX VI	Changed lab location from University of Minnesota to Texas Heart Institute	Update to protocol to reflect change in laboratory location	73
3.0	All	Changed Footer to Reflect Rev 3.0 July 2012	Protocol Update	All
3.0	Staff Directory	Updated CTSN DCC	Staff Update	2
3.0	Data Collection Schedule	Changed dobutamine right heart catheterization to optional at 60 day time point, only to be conducted at centers where this is standard practice	At recommendation of steering committee, to minimize patient burden	13-14
3.0	Assessments during LVAD Wean	Removal of 6 month time point when WMSI will be collected	To remove non-essential assessments	23
3.0	LVAD Implant and Management	Added description of long-term LVAD management	For consistency with accepted clinical practice	27

3.0	Echocardiographic Assessments of Myocardial Function	Revision of sentence regarding echocardiographic measurements	For clarification and internal consistency within protocol	29
3.0	Echocardiographic Assessments of Myocardial Function	Removal of 6 month time point when WMSI will be collected	To remove non-essential assessments	29
3.0	Laboratory Assessment	Removed Eosinophils (%), Basophils (%), Monocytes (%)	To remove non-essential assessments	44
3.0	Laboratory Assessment	Removed Eosinophils (%), Basophils (%), Monocytes (%)	To remove non-essential assessments	47
3.0	Hemodynamics	Changed dobutamine right heart catheterization to optional at 60 day time point, only to be conducted at centers where this is standard practice	At recommendation of steering committee, to minimize patient burden	49
3.0	Echocardiography	Revision of sentence regarding when echo will be conducted on patients not tolerating wean	For clarification and internal consistency within protocol	49-50
3.0	Appendix I	Addition of supplies needed for study product preparation	For completeness of listing	62
3.0	Appendix I	Clarification on amount of air and needle size; administrative corrections	Updated in accordance with manufacturer's recommendations	63



## DEFINITIONS, ACRONYMS & ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMC	bone marrow cells
BTT	Bridge to transplant
C	centigrade
CABG	Coronary Artery Bypass Grafting
CAD	coronary artery disease
CALM	Center for Advanced Laboratory Medicine (Columbia University)
CCTRN	Cardiovascular Cell Therapy Research Network
CFR	Code of Federal Regulations
CHF	congestive heart failure
CI	cardiac index
CI	confidence interval
CO	cardiac output
CTSN	Cardiothoracic Surgical Trials Network
CUMC	Columbia University Medical Center
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
FDG	fluorodeoxyglucose
FMD	flow mediated dilation
GCP	Good Clinical Practice
GMP	Good Medical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HLA	Human leukocyte antigen
HPF	high power field
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
Ki67	cell proliferation marker
LV	left ventricle
LVAD	left ventricular assist device
LVEF	left ventricular ejection fraction
MBF	myocardial blood flow

MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MPC	mesenchymal precursor cells
NHLBI	National Heart, Lung and Blood Institute
PB	Peripheral blood
PET	Positron emission tomography
PRBC	packed red blood cells
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> ear Failure
RNA	ribonucleic acid
rpm	revolutions per minute
RV	right ventricle
RVSP	RV systolic pressure
SOP	Standard Operating Procedures
TR	Tricuspid regurgitation
TTE	Transthoracic echocardiogram
UF	University of Florida (Biospecimen Core Lab)
UNOS	United Network for Organ Sharing
VAD	ventricular assist device
VEGF	vascular endothelial cell growth factor
WMSI	Wall motion score index

## ABSTRACT

<b>Objectives</b>	<p>The <i>primary objective</i> of this exploratory trial is to provide evidence of the safety of direct myocardial injection of a single dose of MPCs in LVAD recipients.</p> <p>The <i>secondary objective</i> is to explore the efficacy of injecting MPCs into the native myocardium of LVAD recipients.</p>
<b>Study Design</b>	Prospective, multi-center, double-blind, randomized, single dose cohort, sham procedure controlled trial
<b>Target Population</b>	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)
<b>Rx arms</b>	<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"> <li>○ Group 1 (n=20): 25 million MPC (Revascor™)</li> <li>○ Group 2 (n=10): 50% Alpha-MEM/42.5% ProFreeze NAO Freezing Medium/7.5% DMSO (control)</li> </ul>
<b>Sample Size</b>	30 patients
<b>Duration</b>	All patients will be followed until cardiac transplantation (for bridge to transplant patients) or until 12 months post randomization, whichever comes first.
<b>1° Endpoints</b>	<p>The <i>primary safety endpoint</i> is the incidence of the following potential study-intervention related adverse events at 90 days post randomization:</p> <ul style="list-style-type: none"> <li>○ Infectious myocarditis</li> <li>○ Myocardial rupture</li> <li>○ Neoplasm</li> <li>○ Hypersensitivity reaction</li> <li>○ Immune sensitization</li> </ul>
<b>2° Endpoints</b>	<ul style="list-style-type: none"> <li>○ The <i>key efficacy endpoint</i> is functional status and ventricular function, while weaned from LVAD support, at 90 days post randomization.</li> <li>○ Echocardiographic assessments of the myocardial size and function by transthoracic echocardiography with LVAD at full support, and as tolerated at 1 and 5 minutes (limited echo) and 15 minutes following initiation of wean, including: <ul style="list-style-type: none"> <li>○ Left ventricular end-diastolic and end-systolic dimensions;</li> <li>○ Left ventricular fractional shortening;</li> <li>○ Regional wall motion score index (WMSI) at limited time points only;</li> <li>○ RV function (Qualitative: normal, mild, moderate, severe);</li> <li>○ RVSP from tricuspid regurgitation (TR) jet;</li> <li>○ Global and regional strain from speckle tracking</li> </ul> </li> <li>○ 6 Minute walk as tolerated at 20 (± 10) minutes following initiation of wean</li> <li>○ Ability to tolerate wean from LVAD support for 30 minutes without signs and symptoms of hypoperfusion (Note: 90 day time point is the key efficacy endpoint)</li> <li>○ Duration of ability to tolerate wean from LVAD support, without signs and symptoms of hypoperfusion</li> <li>○ Neurocognition at 90 days post randomization</li> <li>○ Incidence of study intervention-related adverse events</li> <li>○ Incidence of all serious adverse events</li> <li>○ Anti-HLA antibody sensitization while on LVAD support</li> <li>○ Cardiomyocyte regeneration at explant</li> </ul>

	<ul style="list-style-type: none"> <li>○ Cell Engraftment and Fate at explant</li> <li>○ Survival to cardiac transplantation</li> </ul>
<b>3° Endpoints</b>	<p>Phenotypic &amp; functional analyses will be performed on peripheral blood from each subject and on the lot(s) of Revascor™ administered as follows:</p> <ul style="list-style-type: none"> <li>○ Flow cytometry to phenotype the samples and quantify the following cell populations: Stro1+, CD3, CD11b, CD14, CD19, VEGFR2, CD31, CD34, CD45, CXCR4+, and CD133.</li> <li>○ Functional analysis of the delivered product will be performed using the CFU-F/MSC colony formation assay and of peripheral blood using EPC and MSC assays.</li> </ul> <p>Chemo/cytokine quantification of plasma derived from peripheral blood samples will be performed and include IFN-<math>\gamma</math>, IFN-<math>\alpha</math>, IP-10, Eotaxin, MIP-1<math>\alpha</math>, MIP-1<math>\beta</math>, RANTES, TNF-<math>\alpha</math>, MIG, IL-1RA, GM-CSF, IL-1<math>\beta</math>, IL-2, IL-4, IL-5, IL-6, IL-2R, MCP-1, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, VEGF, ANG-1, ANG-2, SDF-1, PDGF, and SCF.</p>
<b>Selected Inclusion Criteria</b>	<ol style="list-style-type: none"> <li>1. Admitted to the clinical center at the time of randomization</li> <li>2. Clinical indication and accepted candidate for implantation of an FDA approved implantable, non-pulsatile LVAD as a bridge-to-transplant or for destination therapy</li> </ol>
<b>Selected Exclusion Criteria</b>	<ol style="list-style-type: none"> <li>1. Planned percutaneous LVAD implantation;</li> <li>2. Anticipated requirement for biventricular mechanical support;</li> <li>3. Cardiothoracic surgery within 30 days prior to randomization;</li> <li>4. Myocardial infarction within 30 days prior to randomization;</li> <li>5. Prior cardiac transplantation, LV reduction surgery, or cardiomyoplasty</li> <li>6. Acute reversible cause of heart failure (e.g. myocarditis, profound hypothyroidism);</li> <li>7. Stroke within 30 days prior to randomization;</li> <li>8. Platelet count &lt; 100,000/ul within 24 hours prior to randomization;</li> <li>9. Active systemic infection within 48 hours prior to randomization;</li> <li>10. Presence of &gt; 10% anti-HLA antibody titers with known specificity to the MPC donor HLA antigens;</li> <li>11. A known hypersensitivity to dimethyl sulfoxide (DMSO), murine, and/or bovine products;</li> <li>12. History of cancer prior to screening (excluding basal cell carcinoma);</li> <li>13. Acute or chronic infectious disease, including but not limited to human immunodeficiency virus (HIV);</li> <li>14. Received investigational intervention within 30 days of randomization;</li> <li>15. Treatment and/or an incompleated follow-up treatment of any investigational cell based therapy within 6 months prior to randomization;</li> <li>16. Active participation in other research therapy for cardiovascular repair/regeneration;</li> <li>17. Prior recipient of stem precursor cell therapy for cardiac repair;</li> <li>18. Pregnant or breastfeeding at time of randomization.</li> </ol>

**DATA COLLECTION SCHEDULE**

Assessment	Pre-Implant	Randomization	LVAD Implant/ Intervention (INT)	12 hours post INT	1 Day post INT	7 Days post RAND	21 Days post RAND	30 Days post RAND	45 Days post RAND	60 Days post RAND	90 Days post RAND	Every 60 Days <sup>^</sup>	Month 6 post RAND	12 Months post RAND <sup>‡</sup>	Event Driven	End Study
<b>Window</b>				<b>± 4 hours</b>	<b>+1</b>	<b>± 3</b>	<b>± 3</b>	<b>± 3</b>	<b>± 7</b>	<b>± 7</b>	<b>± 14</b>	<b>± 14</b>	<b>± 14</b>	<b>± 14</b>		
<b>General</b>																
Informed Consent	x															
Pre-Screening Failure Form	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		
Immunotherapy Medication	x														x	
Laboratory Assessment	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		
Biospecimen: Phenotypic and Functional Analyses	x				x			x			x					x
Eligibility Evaluation	x															
Randomization & Treatment Assignment		x														
Hospitalization			x													x
Surgical Procedure			x													x
<b>Cardiac</b>																
Hemodynamics	x		x <sup>†</sup>					Δ		° , Δ	x, Δ	Δ				
Echocardiography	x							x		x	x	x		x		
Cardiac Histology <sup>#</sup>			x													x <sup>#</sup>
Six Minute Walk								x		x	x	x		x		
Intervention Injection Verification			x													
Wean Assessment								x		x	x	x		x		
<b>Neurological</b>																
NIH Stroke Scale <sup>**</sup>	x															x
Modified Rankin Scale <sup>**</sup>	x															x
Neurocognitive Testing	x										x					

**DATA COLLECTION SCHEDULE (continued)**

Assessment	Pre-Implant	Randomization	VAD Implant/ Intervention (INT)	12 hours post INT	1 Day post INT	7 Days post RAND	21 Days post RAND	30 Days post RAND	45 Days post RAND	60 Days post RAND	90 Days post RAND	Every 60 Days <sup>^</sup>	Month 6 post RAND	12 Months post RAND <sup>‡</sup>	Event Driven	End Study
Window				± 4 hours	+1	± 3	± 3	± 3	± 7	± 7	± 14	± 14	± 14	± 14		
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																X
End of Study/Investigator Statement																X

<sup>‡</sup> Final data collection

<sup>^</sup> Every 60 days (±7) thereafter until cardiac transplantation or until 12 months post randomization, whichever comes first.

<sup>†</sup> If **not** collected pre-implant then collect in the OR immediately prior to LVAD implantation

<sup>Δ</sup> Hemodynamics by right heart catheterization to be performed during LVAD wean *only at centers where this is standard practice for clinical LVAD wean monitoring*

<sup>°</sup> Dobutamine right heart catheterization is performed for all patients at 90 days. **The 60 day post-randomization time point is optional and may only be conducted at centers where it is standard clinical post-LVAD practice.**

<sup>\*\*</sup> Event driven within 72 hours of a neurological event, and at 30 (±10) and 60 (±10) days post neurological event

<sup>#</sup> At native heart explantation for cardiac transplantation or at autopsy (if applicable)

## OBJECTIVES

Intramyocardial injection of mesenchymal precursor cells (MPC) in patients with advanced heart failure who are treated with left ventricular assist device (LVAD) implantation may result in a renewable source of proliferating functional cardiomyocytes, as well as induce development of capillaries and larger size blood vessels to supply oxygen and nutrients to endogenous myocardium and newly-implanted cardiomyocytes, and release factors capable of paracrine signaling.

The Cardiothoracic Surgical Trials Network (CTSN) and Cardiovascular Cell Therapy Research Network (CCTR) jointly developed an evaluation plan for these MPCs in LVAD patients with advanced heart failure. This protocol describes an exploratory trial (stage 1), the *primary objective* of which is to provide evidence of safety of direct myocardial injection of a single dose (25 million) of MPCs in LVAD recipients. A *secondary objective* is to explore the efficacy of injecting MPCs into the native myocardium of LVAD recipients.

If safety is established and an efficacy signal is observed in this exploratory trial, then the investigators will design a follow-up trial (stage 2) based on an adaptive design. The next trial would randomize patients to active therapy at one of two doses (25 or 75 million MPCs) versus placebo, and based on a predetermined selection criterion, drop randomization to one of the dose arms as results accrue. Should this exploratory trial demonstrate safety but no signal of efficacy, then the subsequent trial would be based on a single dose of 75 million MPCs versus placebo.

## BACKGROUND AND RATIONALE

### LVADs and Stem Cells: a Novel Strategy for Myocardial Recovery

Congestive heart failure is a major public health problem with recent estimates indicating that end-stage heart failure affects over 60,000 patients in the US each year (1). Two-year mortality for these patients is approximately 70-80%, and their treatment options are extremely limited. Less than 3,000 cardiac transplants are available annually due to the limited supply of donor organs (2). Implantable left ventricular assist devices (LVADs), routinely used to support cardiac transplant patients who decompensate awaiting a donor heart, were approved by the FDA in 2002 for long-term support when cardiac transplantation is not an option (so-called destination therapy) (2). However, relatively few patients have availed themselves of this therapeutic option due to the high rate of associated adverse events, including bleeding, infection and thromboembolism (3, 4). Improvements in the device and its management have begun to decrease some of the risks of LVAD therapy. But these risks could be minimized further if the duration of support could be limited by myocardial recovery and explantation, and LVAD use would become a short-term therapeutic rescue.

Hemodynamic unloading and myocardial rest achieved through LVAD support alone has led to some recovery of cardiac function. Few patients, however, achieve sufficient recovery to warrant device explantation and those who do must still contend with ventricular dysfunction (5). The present consensus is that safe and successful LVAD explantation will only be achieved if the underlying condition affecting the native heart is ameliorated. Avenues to achieve this include reversing dysfunction of existing myocytes by revascularization of ischemic myocardium or

pharmacologic interventions including beta adrenergic agonists and blockers, or by repopulating myocardium with new cells via cell transplantation. It is the latter direction which we think holds the greatest promise and is the focus of this protocol.

### **Allogeneic Mesenchymal Precursor Cells**

Human bone marrow contains at least 2 different stem cell populations: *hematopoietic stem cells* that give rise to mature red and white blood cells, and *nonhematopoietic mesenchymal precursor cells* (MPCs) that give rise to stromal cells referred to as *mesenchymal stem cells* (MSCs) or bone marrow stromal stem cells. Adult stem cells can be derived from different parts of the body including bone marrow, blood and the brain. Stem cells have been shown to have the potential to induce therapeutic angiogenesis of ischemic tissues (6-12). Multi-potent MSCs can be induced, under appropriate culture conditions, to differentiate into lineages of diverse mesenchymal tissues, including bone, cartilage, fat, tendon, and both skeletal and cardiac muscle (13). MSCs have the potential for myocyte differentiation (14), and release cytokines and growth factors that stimulate endogenous repair mechanisms (15, 16). In this sense, cell therapy could potentiate endogenous mechanisms and the ability of the heart to heal itself by promoting regeneration and survival of natural stem cell niches.

Although clinical trials of autologous whole bone marrow for cardiac repair demonstrate promising results, many practical and mechanistic issues related to bone marrow harvest and cell preparation make this form of therapy a major obstacle. In contrast, mesenchymal stem cells possess properties that contribute to their ability to evade immunological detection and subsequent rejection and have been shown to function normally after preservation by freezing (17-19). Therefore, MSCs may serve as an allogeneic graft, thereby avoiding the need for bone marrow harvesting from prospective recipients, an extraordinary therapeutic advantage for this cell type.

Angioblast Systems, Inc. has developed a process to immunoselect and expand allogeneic MPCs from a single bone marrow donor. The MPCs (Revasacor™) are produced in a GMP manufacturing facility and cryopreserved until use. These cells are separated from the rest of the bone marrow mononuclear cell fraction by immunoselection using a GMP-grade monoclonal antibody specific for surface markers expressed on mesenchymal precursors. The cells are then cultured under GMP conditions in media supplemented with fetal bovine serum, grown to a master cell bank stage, and subsequently expanded through no more than twenty population doublings prior to lot release for use in humans. Throughout manufacturing, these cells are screened for a variety of endogenous and exogenous infectious and/or contaminating agents. In addition, both the master cell bank and final release lots are screened for neoplastic potential in FDA-compliant karyotypic and tumorigenicity assays. Angioblast Systems, Inc. has agreed to contribute its MPCs for this trial (see section on Study Administration). Further information on manufacturing and product characterization is presented in the Investigator's Brochure.

### **Improving Cardiac Function by Reversing Subsegmental Perfusion Abnormalities**

Use of stem cells for cardiac repair has been investigated for the potential to prevent cardiac remodeling in areas of ischemia, predominantly by the mechanism of neovascularization. However, the occurrence of subsegmental myocardial perfusion abnormalities in the absence of epicardial coronary artery disease is seen in patients with a wide spectrum of cardiovascular disorders, including hypertension and left ventricular hypertrophy, coronary atherosclerosis,



hypercholesterolemia, and non-ischemic left ventricular dysfunction. These patients have a limited coronary microvascular dilatory reserve which can be associated with the presence of myocardial ischemia. In a recent study, myocardial blood flow (MBF), as assessed by magnetic resonance imaging (MRI), was found to be significantly reduced in patients with heart failure due to non-ischemic idiopathic dilated cardiomyopathy (IDC) during exercise in comparison with healthy subjects (20). Moreover, a significant correlation has been demonstrated between MBF reserve and left ventricular ejection fraction, suggesting that the impaired coronary microcirculatory flow might play a direct etiological role in the heart failure in these patients. Similar studies using positron emission tomography (PET) in the IDC population have shown significantly impaired perfusion reserve and an exaggerated imbalance in right to left ventricular oxidative metabolism compared with normal controls, the effects of which appear to significantly contribute to the impaired exercise capacity in these patients (21). In addition, the impaired myocardial reserve in IDC patients does not seem to be associated with abnormalities of peripheral endothelial function, as measured by brachial artery flow mediated dilation (FMD) (21). This indicates a local etiology for the myocardial perfusion abnormalities in IDC, in contrast to the systemic defects in vascular tone associated with ischemic heart failure, such as abnormal peripheral FMD. Collectively, these observations indicate that local abnormalities in cardiomyocyte perfusion are a central feature in non-ischemic dilated cardiomyopathy, and that such abnormalities may be causally related to progressive loss of functional cardiac tissue and worsening heart failure. Since the mechanism of action of Angioblast Systems' MPCs includes both neovascularization and local release of paracrine factors, this suggests that MPC cellular therapy, particularly with local delivery, may improve the perfusion abnormalities characteristic of IDC as well as promote angiogenesis in ischemic regions. Consequently, patients with non-ischemic cardiomyopathy and ischemic cardiomyopathy will be included in the present clinical trial to evaluate whether adult stem cells can be used to increase the microvasculature in the myocardium of patients with advanced heart failure. The basis of the investigation will be to determine whether such neovascularization may improve cardiac function by reversing the subsegmental perfusion abnormalities inherent to both of these conditions.

The approach being proposed here to reverse myocardial remodeling is the use of allogeneic bone marrow-derived MSC to regenerate new cardiac myocytes (22-24). It is reasonable to anticipate that cellular therapies for congestive heart failure will need to address three interdependent processes: (a) a renewable source of proliferating functional cardiomyocytes, (b) development of a network of capillaries and larger size blood vessels to supply oxygen and nutrients to both the chronically ischemic, endogenous myocardium and to the newly-implanted cardiomyocytes, and (c) release of factors capable of paracrine signaling from the administered MPC. Each of these mechanisms may be relevant to the heart failure, LVAD bridge-to-transplant (BTT) or destination therapy (DT) patient population.

### **Pre-Clinical Studies**

Nagaya N, et al. demonstrated an improvement in cardiac function in a rat model of dilated cardiomyopathy, possibly through the induction of myogenesis, angiogenesis, as well as inhibition of myocardial fibrosis (25). They showed a significant increase in capillary density and decreased the collagen volume fraction in the myocardium of isogenic adult rats treated with MSCs versus vehicle. The cultured MSCs secreted large amounts of angiogenic, apoptotic, and mitogenic factors vascular endothelial growth factor, hepatocyte growth factor, adrenomedulin, and insulin-

like growth factor. Additionally, rats with acute myocarditis were treated with intravenous administration of MSCs and showed an improvement in inflammatory changes and cardiac function, suggesting an anti-inflammatory effect.

Angioblast has evaluated allogeneic MPCs in *in-vitro*, rodent and large animal (ovine) studies. These studies established the immunologic tolerance profile of Revascor™ in multiple models of lymphocyte cultures. Rodent studies established the principle of myocardial recovery via MPC induced arteriogenesis and myocyte regeneration and confirmed the early onset-of-action, limited survival of implanted cells, and sustained functional effect of a single application of cells. The ovine studies demonstrated the acute and chronic tolerance of multiple doses of MPCs (25-450 million) and established the cardiac and noncardiac safety profile in over 90 animals. Importantly, Angioblast has demonstrated that the delivery of Revascor™, by endomyocardial, intracoronary or epicardial delivery, in both acute and chronic ischemic (with heart failure) is well tolerated.

Overall, the administration of allogeneic MPCs at either low or high doses, delivered by either epicardial, intracoronary or transendocardial injection, resulted in significant early, and sustained cardiac functional recovery based on measurements of systolic function and LV chamber size. Arteriole proliferation was documented as early as 7 days and was sustained at the 2 month endpoint. Examination of ovine subjects 7 days following cell injection demonstrated that MPC administration did not impair the natural healing of the infarct scar region. However, early proliferation of arterioles, improvement in function, and increased perfusion were affected by the administration of Revascor™. The rapid onset of functional recovery (within 1 hour in many cases), together with exceedingly few implanted cells surviving at 48 hours in earlier studies (<1%) suggests that allogeneic MPCs may mediate their effects without long-term persistence in the ischemic or heart failure myocardium and without inducing any appreciable allogeneic immune response in the myocardium. The results of these studies (described in detail in the Investigator's Brochure) demonstrate the tolerance and functional effect of Revascor™ and provide the basis for the subsequent clinical studies in progress (acute myocardial infarction, chronic heart failure) and this proposed application.

### **Clinical Studies of Cell-based Myocardial Repair**

Cell-based myocardial repair has advanced from extensive pre-clinical studies in rodents and limited large non-primate animal investigations to early clinical trials throughout the world. Bone marrow derived stem cells are emerging as candidates likely to proceed to larger randomized efficacy based human trials (26). Previous cell research has focused on the use of stem cells to repair myocardial damage that is the result of infarction. Much of this research has focused on autologous myoblast transplantation. In a multicenter study, 97 subjects received injections of cultured autologous myoblasts (harvested from a skeletal muscle biopsy) into the epicardial surface and around the area of infarction during coronary artery bypass grafting (CABG) (27). After a 4-year follow-up period, successful implantation of skeletal myoblasts into coronary scar tissue was demonstrated in subjects with ischemic heart disease, as evidenced by increased fluorodeoxyglucose (FDG) on PET scan, area of tissue viability by MRI, or by the measurement of left ventricular ejection fraction (LVEF) via echocardiography. A significant safety concern was the development of arrhythmias due to engraftment of myoblasts with aberrant electrical conduction activity.

Intracoronary delivery of stem cells was investigated in a sentinel study (REPAIR-AMI) of 204 patients (28). Following successful reperfusion, acute myocardial infarction patients received either autologous bone marrow progenitor cells or placebo medium into the infarct artery. Improvement in left ventricular ejection fraction by angiography was observed in the cell treated subjects at 4 months and was statistically significant ( $5.5 \pm 7.3\%$  vs.  $3.0 \pm 6.5\%$ ,  $p=0.01$ ). Improvement was greatest for the subjects with pretreatment ejection fractions of 48.9% or less. One year major adverse coronary events (MACE) were also reduced in the cell treated compared to placebo control subjects. This study established clinical benefit and procedural tolerance from progenitor cell therapy.

Angioblast's MPCs have been evaluated in patients in two pilot trials of autologous cell therapy in Australia, and in ongoing trials in the United States of allogeneic cell therapy. In the two pilot trials in Australia, 16 patients received approximately 80-220 million autologous MPCs, manufactured under GMP conditions using the same SOPs as for the allogeneic cells. Ten patients were enrolled in an orthopedic trial treating non-union of long bones in the legs, and six patients with multivessel coronary artery disease, angina, and heart failure were enrolled in a trial where the MPCs were injected intramyocardially by NOGA catheter. All patients have been followed for a minimum of 6 and up to 12 months. No cell-related adverse events have occurred in any patient receiving autologous culture-expanded MPCs, either for orthopedic or cardiac applications.

In the ongoing U.S. trials, Angioblast's allogeneic MPCs are being implanted in the lumbar spine and cervical spine for patients needing spinal fusion (24 patients) and in patients suffering from heart failure 10-14 days after an acute myocardial infarction (2 patients). The 25 million allogeneic MPC dose has been well tolerated in these trials, with no cell-related adverse events seen. Total patient follow-up is between 2 and 24 months. In addition, Angioblast is evaluating endomyocardial catheter delivery of one of two doses of allogeneic MPCs in class II-IV heart failure patients. To date, 60 subjects have completed follow-up. Patients were randomized to 25 million MPCs, 75 million MPCs, or 150 million MPCs or control sham injections and no cell-related adverse events were reported. In particular, these patients with heart failure and acute myocardial ischemia demonstrated immune tolerance of allogeneic MPCs (Revascor™).

### **Allogeneic Trials**

Most cell therapy studies conducted to date lacked adequate power and randomization controls. Nevertheless, results demonstrate that bone marrow-derived cells can be safely delivered via an intracoronary, epicardial or transendocardial approach; and that this therapy shows signs of global improvement in cardiac function in patients following myocardial infarction, ischemic patients receiving CABG, and end-stage heart failure patients.

<b>Study Number</b>	<b>Product</b>	<b>Indication</b>	<b>Number of Subjects Receiving MPCs</b>	<b>Number of Control Subjects</b>
MSF 0106	NeoFuse™	Lumbar Spine Fusion	4	2
MSB-SF003	NeoFuse™	Lumbar Interbody Fusion	14	8
MSB-CF002	NeoFuse™	Anterior Cervical Fusion	6	3
EMT AB001	Revascor™	AMI	1	1
HF-AB002	Revascor™	Heart Failure	45	15
MSB-CAR001 (AUS study under TGA)	MSB-CAR001 (NeoFuse™/Plabebo)	Osteoarthritis	Blinded	Blinded
		<b>TOTAL</b>	<b>70</b>	<b>29</b>

This exploratory trial will investigate the use of LVAD in combination with Angioblast's allogeneic, MPC cell based therapy in patients with end-stage heart failure.

### **The Current Trial: Safety Considerations**

Safety will be determined by considering a spectrum of adverse events considered to be particularly meaningful by the clinical community in the target population of BTT and DT LVAD patients. These events are infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization.

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.

### **Concerns – Alloimmunization**

Similar to implantation of autologous whole bone marrow or the hematopoietic fraction of marrow, implantation of highly purified allogeneic MPC is not likely to induce allosensitization, and may even actively prevent this complication.

A dose range of Angioblast's allogeneic MPCs manufactured under identical conditions as contemplated in the present study (25 million) has been safely implanted in twenty patients in the setting of acute myocardial infarction, heart failure, or surgery for spinal fusion, without evidence of allosensitization or adverse events for up to twelve months of follow-up.

Furthermore, MPCs lack certain co-stimulatory molecules such as human leukocyte antigens (HLA) Class II, CD86, and CD40L, and consequently have the ability to both evade immune recognition and to *suppress a third party allogeneic cellular immune response* (29, 30). Indeed,

there is a growing, broad-based clinical experience using allogeneic mesenchymal stem cells for a wide range of immune-based diseases where their immunomodulatory properties make these cell types an attractive alternative to standard immunosuppression with a safe and effective profile for induction of a tolerogenic state (31). Follow-up of patients with hematopoietic bone marrow transplants where allogeneic mesenchymal stem cells were infused to treat graft-versus-host disease has demonstrated no allosensitization, as measured by lack of detection of alloantibodies (32). This was not due to a state of global anergy since these same patients were able to mount a clinically insignificant antibody response to fetal calf serum used in the culture medium for growth of the cells. The clinical effect appears to be due to the MPC ability to ameliorate the effector cell response in mixed lymphocyte culture as well as *in-vivo*. Additionally, MSCs are known to secrete anti-inflammatory cytokines which abrogate inflammatory responses, such as those present in graft-versus-host disease.

Therefore, if, following their implantation into the native heart, non-engrafted MPC were to disseminate and contact the LVAD surface, it is possible that these cells may prevent allosensitization in this at risk population in the setting of the LVAD procedure and its frequent sequelae necessitating transfusion of blood products. To assess the effects of MPC on the expected incidence of allosensitization in LVAD recipients, we will measure anti-HLA antibodies at regular intervals post-cell implantation in these patients and compare these parameters to sham injected LVAD controls.

### **The Current Trial: Efficacy Considerations**

Vascular network formation is the end result of a complex process that begins in the prenatal period with induction of vasculogenesis. Cells that can differentiate into endothelial and smooth muscle elements also exist in adult bone marrow (12, 33) and can induce vasculogenesis in ischemic tissues (7-9). Angioblast Systems, Inc. has identified a specific population of MPC derived from human adult bone marrow that has phenotypic and functional characteristics of vascular pericyte precursor cells, which provide the building blocks necessary for arteriogenesis. Since recent observations have suggested that a second compensatory response of viable cardiomyocytes is to proliferate and regenerate following injury (33, 34), it is theoretically possible that further increase in the infarct bed capillary network through regulated neovascularization could result in increased regenerative capacity of the heart leading to improvement in myocardial function. Thus, in addition to the safety and functional endpoints defined in this clinical protocol, the explanted heart obtained at transplantation for BTT patients offers an opportunity to study cell engraftment and fate, as well as histologic and gene regulation responses to cell implantation as secondary efficacy endpoints. It is because of these important secondary efficacy endpoints that the trial design includes a control group, who will receive sham intramyocardial injection (since needle injury has been shown to affect angiogenesis).

### **Rationale for Selection of the Key Efficacy Endpoint**

The field of mechanical circulatory support, particularly in the context of myocardial recovery and repair, remains nascent. It has become accepted clinical practice 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. This trial is designed primarily to assess the safety of intramyocardial injection of MPCs in LVAD recipients; however,

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 of patients. Standard cardiac imaging modalities are of limited use in this population because of the physical presence of the LVAD itself (e.g., nuclear imaging and MRI) and the physiologic state of off-loading the left ventricle (e.g., echocardiography and coronary angiography). Therefore, the most meaningful cardiac assessments in LVAD patients are those performed on little or no LVAD support. Accordingly, the ability to tolerate a wean from LVAD support for 30 minutes will be used to define a successful key efficacy outcome. The value of the ability to tolerate a wean as a predictor of long-term recovery has yet to be established within the field, yet it is generally accepted that those unable to tolerate a wean have persistent profound cardiac debilitation. In order to further evaluate the effects of MPCs on global and regional myocardial function as well as on functional capacity in this trial, additional physiologic and functional assessments will be performed at the time of the wean while off LVAD support. The most important of these assessments is the echocardiographic assessment of LVEF. Birks and colleagues have shown that LVEF, measured in patients during a wean from LVAD support, is an important predictor of myocardial recovery (35 and unpublished data). 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.

The key efficacy endpoint will be assessed at 90 days after randomization. Since the duration of LVAD support for the subgroup of BTT patients has become significantly longer than 90 days, the timing of the key efficacy assessment will ensure that a majority of BTT patients enrolled in this study will reach this endpoint prior to undergoing transplantation.

### **Rationale for Dose Selection**

The current protocol involves enrollment of LVAD recipients in a single dose cohort, who are randomized in a 2:1 fashion to intramyocardial injection of 25 million MPCs versus cryoprotective media alone (sham procedure). This dose has been chosen, as it did not show any evidence of toxicity in several dose-ranging studies of ovine models of acute and chronic myocardial ischemia. Furthermore, implantation of 25 million allogeneic MPCs in patients has not demonstrated any concerning side effects. The dose to be studied, 25 million cells, has been shown to be effective and well tolerated in the preclinical models.

### **STUDY DESIGN**

This is a prospective, multi-center, double-blind, randomized, single dose cohort, sham procedure controlled trial to define the safety and feasibility, and to explore the efficacy of intra-myocardial injection of mesenchymal precursor cells (Revascor<sup>TM</sup>) on functional status, myocardial function, cardiomyocyte regeneration, and neovascularization in patients with advanced heart failure, implanted with an FDA-approved LVAD as either a bridge to cardiac transplantation or for destination therapy. All patients will be followed until cardiac transplantation (for BTT patients) or until 12 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 Safety Endpoint

The primary safety endpoint of this study is the incidence of the following potential study-intervention related adverse events within 90 days post randomization: infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization.

### Secondary Endpoints

#### *Key Efficacy Endpoint*

The key efficacy endpoint of this study is functional status and ventricular function, while weaned from LVAD support, at 90 days post randomization. Functional status is defined by the ability to tolerate wean from LVAD support for 30 minutes without signs or symptoms of hypoperfusion, including, but not limited to symptoms of low output or signs of vascular congestion. See Appendix III (LVAD Wean Guidelines) for details. Ventricular function will be assessed by transthoracic echocardiogram (TTE) in those patients able to be weaned for 30 minutes from LVAD support.

#### *Assessments During LVAD Wean*

Additional secondary endpoints, for those patients who tolerate an LVAD wean, will be assessed during LVAD wean at 30 days ( $\pm 3$ ) and 60 days ( $\pm 7$ ) following randomization, and every 60 days ( $\pm 14$ ) thereafter following the 90 day key efficacy evaluation until cardiac transplantation or 12 months, whichever comes first. These endpoints include:

- Echocardiographic assessments of myocardial size and function by transthoracic echocardiography with the LVAD at full support, and as tolerated at 1 and 5 (limited echo), and 15 minutes following initiation of wean, including:
  - Left ventricular end-diastolic and end-systolic dimensions;
  - Left ventricular fractional shortening;
  - Regional wall motion score index (WMSI) (*Comprehensive post 6 minute walk echo at 90 days and 12 months only*);
  - RV function (Qualitative: normal, mild, moderate, severe);
  - RVSP from TR jet;
  - Global and regional strain from speckle tracking
- 6-Minute walk as tolerated at 20 ( $\pm 10$ ) minutes following initiation of wean;
- Ability to tolerate wean from LVAD support for 30 minutes without signs or symptoms of hypoperfusion (Note: 90-day time point is the key efficacy endpoint);
- Duration of ability to tolerate wean from LVAD support, without signs or symptoms of hypoperfusion;

#### *Assessments Unrelated to LVAD Wean*

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

- Incidence of study intervention-related adverse events (during the entire follow-up period);

- Incidence of all serious adverse events;
- Anti-HLA antibody sensitization while on LVAD support;
- Myocardial neovascularization at time of explant;
- Cardiomyocyte regeneration at explant;
- Cell engraftment and fate at explant;
- Neurocognitive outcomes at 90 days ( $\pm 14$ ) post randomization, but not during LVAD wear;
- Survival to cardiac transplantation

### **Tertiary Endpoints**

The following mechanistic endpoints will be assessed:

- Phenotypic & functional analyses will be performed on peripheral blood from each subject and on the lot(s) of Revascor™ administered as follows:
  - Flow cytometry will be performed to phenotype the samples and quantify the following cell populations: Stro1+, CD3, CD11b, CD14, CD19, VEGFR2, CD31, CD34, CD45, CXCR4+, and CD133.
  - Functional analysis of the delivered product will be performed using the CFU-F/MSC colony formation assay and of peripheral blood using EPC and MSC assays.
- Chemo/cytokine quantification of plasma derived from peripheral blood samples will be performed. Assessments include IFN- $\gamma$ , IFN- $\alpha$ , IP-10, Eotaxin, MIP-1 $\alpha$ , MIP-1 $\beta$ , RANTES, TNF- $\alpha$ , MIG, IL-1RA, GM-CSF, IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-2R, MCP-1, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, VEGF, ANG-1, ANG-2, SDF-1, PDGF, and SCF.

### **STUDY SUBJECTS**

A total of 30 patients with end-stage congestive heart failure who are scheduled to be implanted with an FDA-approved LVAD as a BTT or DT will be enrolled into Stage 1. We anticipate recruiting up to 3 patients/month and that enrollment will be completed within 10-12 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 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. Cardiothoracic surgery within 30 days prior to randomization;
4. Myocardial infarction within 30 days prior to randomization;
5. Prior cardiac transplantation, LV reduction surgery, or cardiomyoplasty;
6. Acute reversible cause of heart failure (e.g. myocarditis, profound hypothyroidism);
7. Stroke within 30 days prior to randomization;
8. Platelet count < 100,000/ul within 24 hours prior to randomization;
9. Active systemic infection within 48 hours prior to randomization;
10. Presence of > 10% anti-HLA antibody titers<sup>1</sup> with known specificity to the MPC donor HLA antigens<sup>2</sup>;
11. A known hypersensitivity to dimethyl sulfoxide (DMSO), murine, and/or bovine products;
12. History of cancer prior to screening (excluding basal cell carcinoma);
13. Acute or chronic infectious disease, including but not limited to human immunodeficiency virus (HIV);
14. Received investigational intervention within 30 days prior to randomization;
15. Treatment and/or an incompleting follow-up treatment of any investigational cell based therapy within 6 months prior to randomization;
16. Active participation in other research therapy for cardiovascular repair/regeneration;
17. Prior recipient of stem precursor cell therapy for cardiac repair;
18. Pregnant or breastfeeding at time of randomization.

### **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=20): 25 million MPC (Revascor™)

Group 2 (n=10): 50% Alpha-MEM/42.5% ProFreeze NAO Freezing Medium/7.5% DMSO  
(Control)

### **SAMPLE SIZE**

The primary purpose of this exploratory trial is to demonstrate the feasibility, assess the safety, and to obtain a preliminary estimate of the benefit of intra-myocardial injections of 25 million MPCs versus control on myocardial function in LVAD bridge-to-transplant or destination therapy

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<sup>1</sup> Documented by clinical site laboratory

<sup>2</sup> Documented by Core Lab

patients. In this trial, a total of up to 30 patients will be randomized in a 2:1 allocation to receive intramyocardial injection of 25 million MPC (Revascor™) or cryoprotective media alone (Control). Safety will be primarily assessed during this exploratory trial based on the presence of infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization. Mortality will also be monitored, with stopping criteria determined based on a sequential Bayesian approach. With respect to efficacy, the primary aim of this early phase trial is to assess whether the dose of MPCs studied demonstrates sufficient activity to warrant further examination of this dose, or if no signal of efficacy has been demonstrated, then a higher dose in larger, definitive trials.

## **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 (Cohort 1) 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 study investigators, coordinators or the patient. The syringes, once prepared will appear identical for both groups. Site personnel and patients, as well as the Data Coordinating Center (DCC) investigators (with the exception of one statistician), the Principal Investigator, and all core lab study personnel will be blinded to treatment assignment.

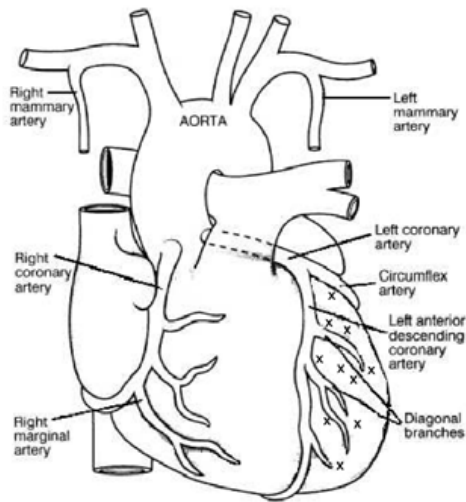
## **TREATMENT INTERVENTION**

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

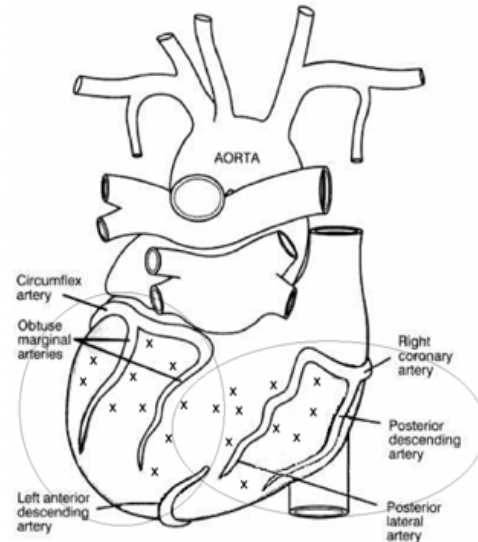
Four 5 cc syringes with 3/4 inch, 23 gauge needles will be prepared and maintained in the sterile field as detailed in Appendix II. Study product (Revascor™ or Control) will be thawed and prepared as detailed in Appendix I. The injection process should take place within 15 minutes, from time of first injection to last injection, and must take place no longer than 75 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 II. 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 Appendix I for detailed study product (Revascor™ or Control) handling and preparation. See Appendix II (Intramyocardial Injection Procedures) for detailed syringe preparation and injection procedures.

Of note, study product thawed longer than 75 minutes must be discarded and the process will be continued with a new cryobag.

**Figure 1.**  
**Anterior View**



**Posterior View**



## **LVAD IMPLANT AND MANAGEMENT**

Any permanent surgically implanted non-pulsatile **LVAD approved by the FDA for BTT or DT** for end-stage heart failure may be implanted at the discretion of the surgeon for patients enrolled in this trial. 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.

## **DEFINITION AND MEASUREMENT OF ENDPOINTS**

### **Primary Safety Endpoint**

The primary safety endpoint of this study is the incidence of the following potential study-intervention related adverse events within 90 days post randomization:

- Infectious myocarditis
- Myocardial rupture
- Neoplasm
- Hypersensitivity reaction
- Immune sensitization

The 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).

## Secondary Endpoints

### ***Key Efficacy Endpoint***

The key efficacy endpoint of this study is functional status and ventricular function, while weaned from LVAD support, at 90 days post randomization. Functional status is defined by the ability to tolerate wean from LVAD support for 30 minutes without signs or symptoms of hypoperfusion, including, but not limited to symptoms of low output or signs of vascular congestion. See Appendix III (LVAD Wean Guidelines) for details. Ventricular function, measured by LVEF obtained from LV volumes measured by Simpson's biplane, will be assessed by transthoracic echocardiogram (TTE) in those patients able to be weaned for 30 minutes from LVAD support. In those instances when LV volume cannot be measured, visual assessment will be used to determine LVEF. See Appendix IV (Echocardiography Procedures) for details of image acquisition and analysis protocol.

### ***LVAD Wean***

*The non-pulsatile LVAD Wean are adopted from the Harefield Hospital Protocol with permission of Dr. Emma Birks.*

See Appendix III for detailed LVAD weaning and early termination guidelines.

*Non-pulsatile LVAD (e.g., HM II, <sup>3</sup>HVAD<sup>TM</sup>) Weaning Guidelines:* Patients with an INR < 2.0 at the time of wean will receive a 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 Appendix III. Blood pressure measurements will be taken prior to wean and every 5 minutes thereafter throughout the wean.

### ***Early termination of LVAD Wean*** (See Appendix III)

The guidelines for early termination of device turn down during echocardiography and the six minute walk test dictate that the LVAD will be returned to full LVAD flow if the patient develops symptoms of low output or vascular congestion, including but not limited to the following:

- Light headedness
- Dyspnea
- Fatigue
- Chest pain
- Pulmonary edema

Based on the collective experience of its multi-center international collaborators, 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.

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<sup>3</sup> This device will only be used in this trial after FDA approval or clearance.

### ***Assessments During LVAD Wean***

Additional secondary endpoints will be assessed for those patients who tolerate an LVAD wean, at 30 days ( $\pm 3$ ) and 60 days ( $\pm 7$ ) post randomization, and every 60 days ( $\pm 14$ ) thereafter following the 90 day key efficacy evaluation, until cardiac transplantation or 12 months, whichever comes first. These endpoints include:

### ***Echocardiographic Assessments of Myocardial Function***

Echocardiographic parameters (Appendix IV) will be assessed at each time point at baseline with the LVAD at full flow prior to wean. Selected measurements will be repeated at 1, 5 and 15 minutes following successful wean from LVAD support (while LVAD flow remains weaned), and immediately following the six minute walk test, 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.

### ***Comprehensive Echo Assessments***

The echo imaging study prior to the wean will begin with the device on *full support* and include measurements of ventricular size, function and mass. The following echo assessments will be recorded (1) on full support, (2) at 15 minutes following initiation of wean, and (3) immediately following the 6 minute walk, as tolerated by the patient:

- Left ventricular (LV) end-diastolic and end-systolic dimensions
- LV fractional shortening
- LVEF by Simpson's Rule (when possible)
- LVEF by visual assessment
- Regional wall motion score index (WMSI) (*Comprehensive post 6 minute walk echo at 90 days and 12 months only*). 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.
- Right ventricular (RV) function (Qualitative: normal, mild, moderate, severe)
- RV systolic pressure (RVSP) from tricuspid regurgitation jet
- Global and regional strain from speckle tracking

### ***Limited Echo Assessments***

At 1 and 5 minutes following initiation of wean, the following limited measurements will be obtained:

- Left ventricular (LV) end-diastolic diameter
- LV end-systolic diameter
- LV ejection fraction (2D LV dimension measurements or visual assessment)
- LV fractional shortening will be obtained from the above measurements

**6 Minute Walk**

The total distance walked in six minutes will be assessed. For those who tolerate the LVAD wean, the 6 minute walk will be performed immediately following the 15 minute full echo assessment (at 20 (± 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 6 Minute Walk Test instructions are detailed in Appendix V.

The 6 Minute Walk will be performed immediately following the 15 minute echocardiogram if the BP remains stable. Patients will not receive any additional anticoagulation during the wean following the initial bolus (See Appendix III).

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

The ability to tolerate the LVAD wean without signs or symptoms of hypoperfusion including, but not limited to, symptoms of low output (e.g., light headedness, dyspnea, fatigue, chest pain) or signs of vascular congestion (e.g., pulmonary edema) will be assessed at each time point. See Appendix III for LVAD Wean Guidelines. The ability to tolerate LVAD wean for 30 minutes will be compared between groups. (Note: 90-day time point is the key efficacy endpoint).

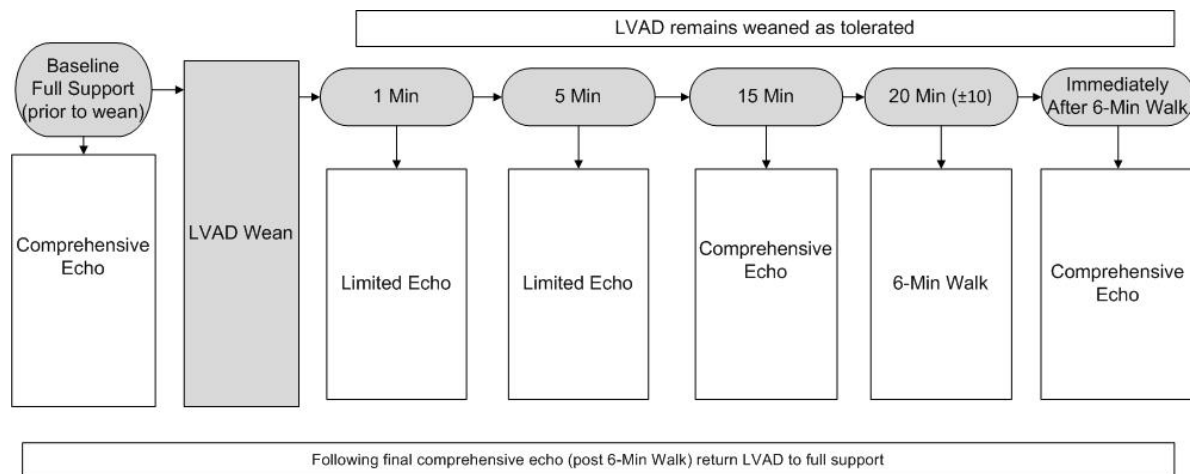
**Duration of Ability to Tolerate Wean**

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

- (a) Reaches 30 minutes off LVAD support, **and**
- (b) Completes the echo and 6 minute walk (if this takes longer than 30 minutes and the patient remains stable), **or**
- (c) Fails the wean due to signs or symptoms of hypoperfusion and low output (e.g., light headedness, dyspnea, fatigue, chest pain) or signs of vascular congestion (e.g., pulmonary edema).

These signs and symptoms will be assessed at each time point post intervention. See Appendix III for LVAD Wean Guidelines. The ability to tolerate LVAD wean at 1, 5, 15 and 30 minutes will be compared among groups.

**Timeline of Functional Assessments Completed During LVAD Wean:**



*The LVAD wean will continue until the patient (a) reaches 30 minutes off 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.*

#### ***Assessments Unrelated to LVAD Wean***

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

#### ***Serious & Study-Intervention Related Adverse Events***

The incidence and frequency of all anticipated and unanticipated serious adverse events will be determined. In addition, 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.

#### ***Anti-HLA Antibody Sensitization***

The incidence of anti-HLA antibody sensitization while on LVAD support 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, IgG Class II, will be assessed. All measurements will be performed by the Immunologic Core Lab (See Appendix VII).

#### ***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 analyzed in the Biospecimen Core Lab to evaluate for neovascularization, quantification of cardiomyocyte proliferation by immunohistochemistry or molecular analyses, general histology, and DNA analysis (See Appendix VI).

#### ***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. These analyses will be performed in the Biospecimen Core Laboratory. (Detailed in Appendix VI).

#### ***Neurocognition***

Neurocognition will be compared between treatment groups at 90 ( $\pm 14$ ) days post intervention, 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 (Appendix IX). 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 CTSN Neurocognition Committee.

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

### ***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.

### **Tertiary Endpoints**

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

### ***Phenotypic & Functional Analyses***

Functional analysis will be performed on the lot of Revascor™ administered during this trial as well as phenotypic and functional analysis on peripheral blood from each subject at baseline (prior to study intervention), and at days 1 (+1) post intervention and days 30 ( $\pm 3$ ), and 90 ( $\pm 14$ ) post randomization and study termination (or transplantation, whichever comes first).

Peripheral blood sample will be obtained and sent to the Biospecimen Core Laboratory where flow cytometry will be performed to phenotype the samples and quantify the following cell surface markers: Stro1+, CD3, CD11b, CD14, CD19, VEGFR2, CD31, CD34, CD45, CXCR4+, and CD133. Up to 42 cell subpopulations expressing single, double or triple markers will be quantified. (See Appendix VI) This information will be used to examine the relationship between Revascor™ administration and endogenous cell characteristics as well as the relationship between cell therapy outcomes and endogenous cell characteristics (e.g., cell type consistency, cell mobilization, and cytokine and nitric oxide production).

Peripheral blood sample will also be sent to the Biospecimen Core Laboratory to assess the colony forming capacity of peripheral MPCs. Functional analysis of the delivered product will be performed using the CFU-F/MSC colony formation assay and of peripheral blood using EPC and MSC assays. (Detailed in Appendix VI and Manual of Procedures).

### ***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 ( $\pm 3$ ), 30 ( $\pm 3$ ), 60 ( $\pm 7$ ), and 90 ( $\pm 14$ ) days post randomization, and at 6 and 12 months ( $\pm 14$  days) post randomization. Chemo/cytokine quantification of plasma derived from peripheral blood samples will be performed by Luminex multiplex assay and include IFN- $\gamma$ , IFN- $\alpha$ , IP-10, Eotaxin, MIP-1 $\alpha$ , MIP-1 $\beta$ , RANTES, TNF- $\alpha$ , MIG, IL-1RA, GM-CSF, IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-2R, MCP-1, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, VEGF, ANG-1, ANG-2, SDF-1, PDGF, and SCF.

## **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 the individual IRB as described below.

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. 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***

*These events include: infectious myocarditis, myocardial rupture, neoplasm, hypersensitivity reaction, and immune sensitization.* (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 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.

**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 can be explained by an alternative etiology commonly occurring in the population/individual, or
- The event is not a known reaction to the LVAD implant or investigational intervention but cannot be reasonably explained by an alternative etiology.

***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 cannot be ruled out with certainty. The following characteristics will apply:

- The event occurs after exposure to the LVAD implant or investigational intervention, and
- There is a reasonable temporal relationship to the administration, but the event is not a known reaction to the LVAD implant or investigational intervention and could be explained by a commonly occurring alternative etiology, or
- In the absence of a reasonable temporal relationship, the event cannot 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. 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 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 Revascor™ 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 Revascor™ 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*

Revascor™ is 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 single 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 internal or external bleeding that results in death, the need for re-operation or hospitalization; or necessitates transfusion of red blood cells as follows:

Within any 24 hour period:

- 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)
- Any transfusion of PRBC within any 24 hour period *after 7 days following implant*

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., diminished LVAD flow, oliguria, pre-syncope or syncope) that requires hospitalization or requires a physician visit or occurs during a hospital stay.

Cardiac arrhythmias are classified as follows:

- Cardiac arrest
- Sustained ventricular arrhythmia requiring defibrillation or cardioversion.
- Sustained supraventricular arrhythmia requiring drug treatment or cardioversion.
- Cardiac conduction abnormalities or sustained bradycardia requiring permanent pacemaker placement

### ***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.

### ***Vasodilatory State***

Episode of vasodilation documented by hemodynamic evidence, without obvious underlying cause such as, but not limited to, sepsis or pharmacotherapy, lasting  $\geq 24$  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  $\leq 2$  hours post treatment intervention and lasting  $< 24$  hours, in the absence of clinical signs of concomitant infection.

#### ***Immune Sensitization***

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

### ***Device Malfunction***

Device malfunction denotes a failure of one or more of the components of the mechanical circulatory support device (MCS/D) system which either directly causes or would potentially induce a state of inadequate circulatory support (low cardiac output state) or death. The manufacturer must comment on device failure. A failure that was iatrogenic or recipient-induced will be classified as an Iatrogenic/Recipient-Induced Failure.

Device failure should be classified according to which components fail as follows:

- *Pump Failure* (blood contacting components of pump and any motor or other pump actuating mechanism that is housed with the blood contacting components)
- *Pump Thrombus* is suspected or confirmed to be present within the device or its conduits that result in or could potentially induce circulatory failure
- *Non-Pump Failure* (e.g. external pneumatic drive unit, electric power supply unit, batteries, controller, interconnect cable, compliance chamber)

### ***Hemolysis***

A plasma-free hemoglobin value that is greater than 40 mg/dl, in association with clinical signs associated with hemolysis (e.g. diminished LVAD flow, oliguria, pre-syncope or syncope) occurring after the first 72 hours post-implant. Hemolysis related to documented non-device related causes (e.g. transfusion or drug) is excluded from this definition.

***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 driveline 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.

***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).

### *Infectious Pericarditis*

Evidence of pericardial infection manifest by positive gram stain and/or cultures. May or may not be accompanied by signs or symptoms of acute pericarditis or systemic infection.

### ***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 at >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). The examining physician will distinguish between a Transient Ischemic Attack (TIA), which is fully reversible within 24 hours (and without evidence of infarction), and a stroke, which lasts longer than 24 hours (or less than 24 hours if there is evidence of infarction). 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 ( $\pm 10$  days) and 60 days ( $\pm 10$  days) post neurological event to document the presence and severity of neurological deficits. Each neurological event must be subcategorized as:

- *Transient Ischemic Attack*: Acute event that resolves completely within 24 hours with no evidence of infarction
- *Stroke*: Ischemic or Hemorrhagic Cerebrovascular Accident (CVA) event that persists beyond 24 hours, or less than 24 hours associated with infarction on an imaging study

- *Toxic Metabolic Encephalopathy*: A disorder of the brain function that arises from abnormal systemic metabolism or exogenous substances, altering awareness and/or consciousness, in which there is a non-focal neurological examination and a negative brain image
- *Other*

### ***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 dysfunction (e.g. central venous pressure [CVP] >18 mmHg with a cardiac index <2.0 L/min/m<sup>2</sup> in the absence of elevated left atrial/pulmonary capillary wedge pressure [>18mmHg], tamponade, ventricular arrhythmias, or pneumothorax) requiring RVAD implantation, or requiring inhaled nitric oxide or inotropic therapy for a duration of 7 consecutive days or more at any time after LVAD implantation.

### ***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 Event***

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 25 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 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. 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, Mount Sinai School of Medicine
- Completed Form FDA 1572 and curricula vitae
- IRB roster
- IRB approval, version and date for protocol and consent
- Clinical Center Laboratory Certification
- DCC site approval
- HIPAA compliance approval
- Dangerous Goods Certification Training
- NIH Stroke Scale Training Certification
- Signature Verification form
- Laboratory normals

**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, Good Clinical Practice Certificates and HIPAA certification 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 the institutional and NIH GCP 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). Conflict of interest statements will be updated as changes occur and no less than annually.

### **Patient Confidentiality**

Confidentiality of all patient records will be maintained according to HIPAA guidelines. Study Investigators, site Institutional Review Boards (IRBs), the DCC (InCHOIR), the Event Adjudication Committee (EAC), the NHLBI, Angioblast, Inc., 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 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.

## **SITE INITIATION**

IRB approval and the clinical study agreement between the clinical site and the DCC must be signed and executed prior to the site initiation. Additionally, the completed Form FDA 1572 and applicable CVs must be on file with the DCC prior to site initiation. A representative from the DCC will conduct a site initiation teleconference prior to enrollment of the first patient. The surgeon, cardiologist and coordinator will be required to attend the initiation. All other health care professionals who may be involved in the trial (e.g. neuropsychologists, engineers, social workers, in-patient staff) will be encouraged to attend.

## **DATA COLLECTION**

### **Screening/Pre-Implant Data Collection**

#### ***Pre-Screening Failure Form***

##### *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 in the Pre-screening Failure form in the EDC. The data collected is HIPAA compliant and does not include patient identifiers but does include screening quarter, screening year, age, gender and reason not eligible or not enrolled.

#### ***Informed Consent***

##### *Prior to screening data collection and all protocol defined procedures*

A signed informed consent form, which has been approved by the DCC and the individual IRBs, is required. The consent form must incorporate HIPAA clinical research authorization and Release of Medical Information that authorizes release of medical records to the trial investigators, monitors, sponsors (NIH, NINDS, CIHR and Angioblast, Inc.) and the DCC. The investigators or a designated 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, the first, middle, and last initial, date of birth, ethnic origin, and sex will be captured on the Demographics 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, weight and BSA), vital signs including temperature, and a cardiovascular, pulmonary, abdominal, and extremity exam.

**Medications**

*Within 7 days prior to randomization*

This form captures all medications that the patient received within 7 days prior to randomization.

**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
- Blood Type and Cross
- Chemistry Panel to include:  
Sodium (mM/L), Potassium (mM/L), HCO<sub>3</sub> or CO<sub>2</sub> (mM/L), Blood Urea Nitrogen (mg/dL), Creatinine (mg/dL), Total Bilirubin (mg/dL), Alanine Aminotransferase (ALT; U/L), Aspartate Aminotransferase (AST; U/L), Alkaline Phosphatase (IU/L), Albumin (g/dL) and Lactate dehydrogenase (LDH; U/L)
- Coagulation profile  
Prothrombin time (PT/sec), Partial Thromboplastin Time (PTT/sec), International Normalized Ratio (INR), Plasma free hemoglobin (PFH)
- CBC with differential and platelet count:  
White blood cell count (10<sup>3</sup>/mL), Red blood cell (10<sup>3</sup>/mL), Hemoglobin (g/dL), Hematocrit (%), Platelet count (10<sup>3</sup>/mL), Neutrophils (%), Lymphocytes (%)

**Biospecimen Analyses**

*Within 24 hours prior to randomization*

A baseline peripheral blood sample will be obtained. In addition to cells, plasma will be isolated, aliquoted and frozen within 1 hour of blood draw at the clinical site then shipped to the

Biospecimen Core Lab monthly for the following analyses (detailed in Appendix VI and the Manual of Procedures):

- Phenotypic analysis
- Cytokine quantification
- Colony forming ability of peripheral blood derived cells

In addition, an aliquot of MPCs from each lot administered will be sent from Angioblast Systems, Inc. to the Biospecimen Core Laboratory for function analyses. A minimum of 20 million cells will be sent from each lot administered.

### ***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). Baseline Immunologic assessment samples will be obtained within 24 hours prior to randomization.*

A screening anti-HLA antibody serum sample will be obtained and analyzed *at the clinical site*. Immune reactivity results for % IgG Class I, and IgG Class II will be recorded. Presence of > 10% anti-HLA antibody titers must trigger a comparison of recipient antibodies to the MPC donor HLA antigen profile performed by the Immunologic Core Laboratory. ***Known specificity to the MPC donor HLA antigens will exclude participation in this trial*** (See Eligibility Criteria section). A baseline anti-HLA antibody serum sample will be obtained for shipment and analysis at the Immunologic Core Lab (see Appendix VII). In addition, anti-murine and anti-bovine antibodies will be measured (Appendix VII). The form also records the specimen(s) for the Immunologic Assessments obtained as indicated and shipped to the Core Laboratory. If a specimen was not analyzed, the reason for the failure will be specified (e.g., reason specimen not obtained, issue with shipping, etc).

### ***Hemodynamics***

*Within 7 days prior to treatment intervention*

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<sub>S</sub>, PA<sub>D</sub>, PA<sub>M</sub>), pulmonary capillary wedge pressure (PCWP), transpulmonary gradient, cardiac output (CO), cardiac index (CI), pulmonary artery oxygen saturation (PAO<sub>2</sub> sat), and pulmonary vascular resistance (PVR measured in Wood Units) will be assessed by right heart catheterization.

### ***Echocardiography***

*Within 7 days prior to randomization*

A complete echocardiogram will be performed prior to LVAD implantation to assess ventricular size, function and regional wall motion (See Appendix IV). 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 shipped 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 (See Appendix VIII).

***Modified Rankin Scale***

*Within 7 days prior to randomization*

The Modified Rankin Scale will be performed prior to LVAD implant for baseline neurological assessment.

***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 Appendix IX)

***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.

***Randomization***

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

A representative from the DCC will be available to discuss any questions regarding patient eligibility. **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, on the schematic transverse sections of the heart. The diagram will be signed by the surgeon and faxed to the DCC. This form also captures the date and time of intramyocardial injections.

### ***Hospitalization***

*At initial 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 Appendix VI. 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; and 7(±3), 21 (±3), 45 (±7), 60 (±7), and 90 (± 14) days post randomization, and every 60 (± 14) days thereafter until cardiac transplantation (as applicable) or 12 months, whichever comes first*

- CBC with differential and platelets:  
White blood cell count ( $10^3/\text{mL}$ ), Red blood cell ( $10^3/\text{mL}$ ), Hemoglobin (g/dL), Hematocrit (%), Platelet count ( $10^3/\text{mL}$ ), Neutrophils (%), Lymphocytes (%)
- Chemistry Panel to include:  
Sodium (mM/L), Potassium (mM/L),  $\text{HCO}_3$  or  $\text{CO}_2$  (mM/L), Blood Urea Nitrogen (mg/dL), Creatinine (mg/dL), Total Bilirubin (mg/dL), Alanine Aminotransferase (ALT; U/L),

Aspartate Aminotransferase (AST; U/L), Alkaline Phosphatase (IU/L), Albumin (g/dL) and Lactate dehydrogenase (LDH; U/L)

- Coagulation profile: Prothrombin Time (PT/sec), Partial Thromboplastin Time (PTT/sec), International Normalized Ratio (INR), Plasma Free Hemoglobin (PFH)

### ***Biospecimen Analyses***

The following analyses will be performed at the time points specified below.

- Chemo- and Cytokine Quantification  
*1 (+1) day post intervention, 7 ( $\pm 3$ ), 30 ( $\pm 3$ ), 60 ( $\pm 7$ ), and 90 ( $\pm 14$ ) days, and 6 and 12 months ( $\pm 14$  days) post randomization*  
A peripheral blood sample will be obtained. Plasma will be isolated, aliquoted and frozen within 1 hour of blood draw at the clinical site then shipped to the Biospecimen Core Laboratory monthly for cytokine analyses (detailed in Manual of Procedures).
- Phenotypic Assessments  
*1 (+1) post intervention, 30( $\pm 3$ ), and 90 ( $\pm 14$ ) days post randomization and at study termination*  
A peripheral blood sample will be obtained and sent to the Biospecimen Core Laboratory for phenotypic analysis of the following cell populations: Stro1+, CD3, CD11b, CD14, CD19, VEGFR2, CD31, CD34, CD45, CXCR4+, and CD133. (detailed in Manual of Procedures).
- Colony Forming Capacity  
*1 (+1) post intervention, 30( $\pm 3$ ), and 90 ( $\pm 14$ ) days post randomization and at study termination*  
A peripheral blood sample will be obtained and sent to the Biospecimen Core Laboratory to assess the colony forming capacity of peripheral MPCs. (Detailed in Manual of Procedures).

The forms also record whether specimens for cytokines, cell phenotyping and function have been obtained and shipped to the Core Laboratory. 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 ( $\pm 3$ ) and 90 ( $\pm 14$ ), and at months 6 ( $\pm 14$  days) and 12 ( $\pm 14$  days) months post randomization*

An anti-HLA antibody serum sample will be obtained and sent to the Biospecimen Core Laboratory. Immune reactivity results for % IgG Class I and IgG Class II will be recorded. In addition, anti-murine and anti-bovine antibodies will be measured (See Appendix VII). The form also records whether specimens for the Immunologic Assessment have been obtained and shipped to the Core Laboratory. 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).



### ***Physical Examination***

*At days 1(+1) post intervention; days 7 ( $\pm 3$ ), 21 ( $\pm 3$ ), 45 ( $\pm 7$ ), 60 ( $\pm 7$ ), and 90 ( $\pm 14$  days) post randomization, and every 60 days ( $\pm 14$ ) thereafter until cardiac transplantation or 12 months, whichever comes first*

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

### ***Medications***

*At days 1(+1) post intervention; days 7( $\pm 3$ ), 21 ( $\pm 3$ ), 45 ( $\pm 7$ ), 60 ( $\pm 7$ ), and 90 ( $\pm 14$ ) post randomization, and every 60 days ( $\pm 14$ ) thereafter until cardiac transplantation or 12 months, whichever comes first*

These forms capture all medications that the patient is receiving at the time of data collection.

### ***Early Stopping Events***

*At LVAD implantation, 12 ( $\pm 4$ ) hours and days 1(+1) post intervention; days 7 ( $\pm 3$ ), 21 ( $\pm 3$ ), 30 ( $\pm 3$ ), 45 ( $\pm 7$ ), 60 ( $\pm 7$ ), and 90 ( $\pm 14$ ) post randomization, and every 60 days ( $\pm 14$ ) thereafter until cardiac transplantation or 12 months, 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.

### ***Hemodynamics***

*If not performed within 7 days prior to randomization, complete in the operating room immediately prior to LVAD implantation. Hemodynamics by right heart catheterization are to be performed during LVAD wean **only at centers where this is standard practice for clinical LVAD wean monitoring.** Dobutamine right heart catheterization is performed for **all patients at 90 ( $\pm 14$ ) days. The 60 ( $\pm 7$ ) day post-randomization dobutamine right heart catheterization is optional and may only be conducted at centers where it is standard clinical post-LVAD practice.*** Hemodynamics including central venous pressure (CVP), pulmonary artery pressures including systolic, diastolic and mean pulmonary artery pressures (P<sub>A<sub>S</sub></sub>, P<sub>A<sub>D</sub></sub>, P<sub>A<sub>M</sub></sub>), pulmonary capillary wedge pressure (PCWP), transpulmonary gradient, cardiac output (CO), cardiac index (CI), pulmonary artery oxygen saturation (PAO<sub>2</sub> sat), and pulmonary vascular resistance (PVR measured in Wood Units) will be assessed by right heart catheterization. A full hemodynamic assessment will be performed at baseline for each time point, and for the 90 day assessment, it will be repeated on Dobutamine infused at 10  $\mu\text{g}/\text{kg}/\text{min}$ .

## **LVAD Wean and Functional Assessment**

### ***Echocardiography***

*At 30 ( $\pm 3$ ), 60 ( $\pm 7$ ) and 90 days ( $\pm 14$ ) post randomization, and every 60 days ( $\pm 14$ ) thereafter until cardiac transplantation or 12 months, 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 Appendix III). The tests will be forwarded to the Echocardiography Core Lab for analysis (See Appendix IV). 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***

*At 30 ( $\pm 3$ ), 60 ( $\pm 7$ ) and 90 days ( $\pm 14$ ) post randomization, and every 60 days ( $\pm 14$ ) thereafter until cardiac transplantation or 12 months, whichever comes first*

The Six Minute Walk will be performed at 20 ( $\pm 10$ ) minutes following initiation of wean (immediately following the 15 minute echocardiogram), if the patient remains clinically stable (See Appendix III: LVAD Wean Guidelines). This form captures the distance in feet walked on a level hallway in six minutes (See Appendix V).

### ***Wean Assessment***

*At 30 ( $\pm 3$ ), 60 ( $\pm 7$ ) and 90 days ( $\pm 14$ ) post randomization, and every 60 days ( $\pm 14$ ) thereafter until cardiac transplantation or 12 months, 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 at 1 minute, 5 minutes and at 5 minute intervals thereafter following initiation of the LVAD wean (See Appendix III for LVAD Wean Guidelines).

The LVAD wean will continue until the patient:

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

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

**The functional assessments** (echocardiography, six minute walk test, and ability to tolerate wean) will be performed at 30 ( $\pm 3$ ), 60 days ( $\pm 7$ ), 90 days ( $\pm 14$ ) post randomization, and every 60 ( $\pm 14$ ) days thereafter until cardiac transplantation or 12 months, whichever comes first. Since the median duration of LVAD support until cardiac transplantation is approximately 100 days, a majority of patients will reach the primary endpoint assessment at 90 days post LVAD implantation and study intervention prior to undergoing transplantation.

### ***Neurocognitive Testing***

*At 90 days ( $\pm 14$ ) post randomization, but not during LVAD wean*

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. 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 Appendix IX)

## **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.

### ***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.

### ***NIH Stroke Scale***

*Event driven within 72 hours of a neurological event, and at 30 ( $\pm 10$ ) days and 60 ( $\pm 10$ ) days post neurological event*

The NIH Stroke Scale will be performed in the case of a neurological adverse event (See Appendix VIII).

### ***Modified Rankin Scale***

*Event driven within 72 hours of a neurological event, and at 30 ( $\pm 10$ ) days and 60 ( $\pm 10$ ) days post neurological event*

The Modified Rankin Scale will be performed in the case of a neurological adverse event.

### ***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 Appendix VI.

**Missed Visit**

*Event Driven*

If a patient is unable to return for follow-up before closure of a study visit window, a missed visit form must be completed.

**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 EDC application provides hierarchical user permission data entry, viewing, and reporting options. For optimum security, the system operates Secure Socket Layer (SSL) 128-bit encryption protocol over Virtual Private Networks. 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. 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: initials, date of birth, 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.
- Closeout visits will be conducted after the study has been completed at all sites.

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

In addition to demonstrating feasibility, two primary aims of the study are assessing the treatment's safety and determining its potential efficacy. 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 two measures; one measuring functional status and another measuring ventricular function. Functional status is determined 90 days post study randomization by the ability to tolerate weaning from LVAD support for 30 minutes without signs or symptoms of hypoperfusion, including, but not limited to symptoms of low output or signs of vascular congestion (See Appendix III LVAD Wean Guidelines for details). Patients able to be successfully weaned will be considered responders to therapy. Patients who expire prior to being assessed, or whose morbidity precludes assessment, will be considered treatment failures. Few patients are expected to undergo cardiac transplantation prior to the primary endpoint assessment, but if they do, they will be considered to be responders. Ventricular function will be assessed by LVEF based on transthoracic echocardiogram (TTE) in those patients able to be weaned for 30 minutes from LVAD support. The usefulness of LVEF in determining the potential efficacy of MPC therapy will depend on the overall proportion of patients able to tolerate weaning. If many patients are unable to tolerate weaning, then LVEF will play a limited role in the qualitative summary of the efficacy evidence. However, if nearly all patients are able to tolerate weaning, then LVEF will be the primary determinant of efficacy of the MPC therapy. A number of additional secondary analyses are planned and described below.

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. Let  $X_A$  and  $X_C$  be the observed number of successes in  $m_A$  and  $m_C$  patient observations, for active therapy and control respectively. We specify (assumed independent) non-informative  $beta(1,1)$  priors for the respective (true) success probabilities for each treatment  $\theta_A$  and  $\theta_C$ . We also specify, that  $X_A | \theta_A \sim binomial(\theta_A, m_A)$  and  $X_C | \theta_C \sim binomial(\theta_C, m_C)$ . It follows that the posterior distributions for  $\theta_A$  and  $\theta_C$  are  $\theta_A | X_A \sim$

$\text{beta}(1+X_A, 1+m_A-X_A)$  and  $\theta_C|X_C \sim \text{beta}(1+X_C, 1+m_C-X_C)$  respectively. The probability,  $P(\theta_A > \theta_C | \text{data})$ , is  $P(\theta_A > \theta_C | \text{data}) = \int P(\theta_C | Y_C) \int P(\theta_A | Y_A) d\theta_A d\theta_C$ ,

For example, prior to any data collection, equipoise implies that the probability that active therapy is superior to control is 50%. An increase in this probability to a level providing reasonable assurance of an efficacy signal (e.g., 75%), without safety concerns, would warrant additional study of MPC therapy. Simulations indicate that our total sample size of 30 patients will allow us to detect an approximate tripling of the odds that active therapy is superior (i.e., from 50% probability of active therapy's superiority, or 1:1 odds, to 75% or 3:1 odds) with probability 75% or more if the absolute probability of a successful outcome (ability to tolerate weaning) with active therapy is about 10-15% higher than for control. 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.

To the extent possible, we will attempt to characterize any effect of heart failure etiology (ischemic versus non-ischemic) and age on the relationship between the treatments and outcome. We will do so by adjusting the relationships between treatment and outcome by including age and etiology in the statistical models employed, and by employing subset analyses by etiology and age. Age categories will be chosen based on examination of observed age data, but prior to examination of outcome data.

#### *Analysis of additional secondary endpoints*

*Duration of time weaning is tolerated.* The duration of time weaning is tolerated will be examined at 30, 60, and 90 days post randomization, and at every 60 days after the 90 day assessment (i.e., at 150 days post randomization, at 210 days post randomization, etc.). The mean duration time between groups will be compared using one-way analysis of variance, or the non-parametric equivalent Kruskal-Wallis test if outcomes are observed to be highly skewed. We will compute two or three (as needed) pairwise 95% confidence intervals for the difference between each pair of group means. A formal comparison of the differences between pairs of means will be based on Tukey's HSD procedure.

*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 90 days post randomization. Pairwise differences between study arms will be quantified as 95% confidence intervals. Additional 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. Random effects models will also be used to characterize changes over time in both the total duration that a patient tolerates weaning, and in the proportion of patients able to tolerate weaning for thirty minutes.

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

*Mortality.* The proportion of patients surviving to cardiac transplantation among randomization groups will be compared by a chi-squared test of the equality of proportions. Time to death will be described by Kaplan-Meier curves and differences between randomization groups will also be assessed via the log-rank test.

*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 Appendices VI and VII. 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.

*Tertiary Endpoints.* Phenotypic and functional assessments of peripheral blood will be used to examine the relationship between cell therapy outcomes and cell characteristics e.g., cell type consistency, cytokine and nitric oxide production, and genome-wide expression profile. The influence of these variables on the endpoints of this study will be examined using the general linear model for continuous endpoints and logistic regression for dichotomous clinical outcome measures.

### **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) be observed. Stopping guidelines for mortality will use the same approach as that described to assess the presence of an efficacy signal. We would propose to halt randomization if, after 10 patients are randomized, the probability that mortality on active therapy is increased compared to control exceeds 80%. 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. Patients who die prior to 90 days, who are unable to attempt weaning due to severity of illness, and who are otherwise have a missing wean result will be considered non-responders in the intent-to-treat analysis.

Given the exploratory nature of this trial, the handling of missing LVEF data will be data dependent. The trial's small sample size likely precludes practical application of imputation procedures. In consultation with the trial's DSMB, we may perform additional analyses of LVEF employing imputation methods such as best case or worst-case scenarios, imputation models assuming informative (i.e., non-ignorable) missing data, rank-based comparisons, or randomization test approaches.

### **ORGANIZATION OF THE STUDY**

This section describes the overall study organization. The study is conducted as a collaboration between the Cardiothoracic Surgical Trials Network (including its Core, Ancillary and Satellite) clinical sites, the CCTRN (including its Core and Satellite) clinical sites, and selected C-SCCOR clinical sites that participated in the previous Cell Therapy LVAD trial. All sites were selected by NHLBI, in collaboration with NINDS. 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 (Angioblast Systems, Inc.), the DCC, the clinical centers and the investigators. 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 (Angioblast Systems, 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 collaborate with the CCTR DCC to coordinate this trial, monitor interim data, and analyze the study's results in conjunction with the investigators and the sponsors. The CTSN DCC will serve as the lead DCC, and will also administrate the DSMB and EAC. The DCC holds the study-specific IND under Deborah Ascheim, MD. The DCC 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. In addition, the DCC will be responsible for annual reports to the FDA and the final IND submission. The DCC will provide Angioblast with abridged versions of these reports to ensure Angioblast 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 is accurate and securely maintained with other essential clinical trial documents at the site with provided to the DCC; (g) assuring regular IRB review; (h) maintaining communication with the DCC.

### **Core Labs**

#### ***Echocardiography (Echo) Core Lab***

All echocardiograms will be performed according to a standardized protocol (Appendix IV) 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; phenotypic and functional analyses of cells and plasma 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<sup>4</sup> and directed by Doris Taylor, PhD, and at the University of Florida Stem Cell Laboratory by Christopher Cogle, MD.

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<sup>4</sup> The relocation of Dr. Taylor's lab from the University of Minnesota to the Texas Heart Institute is anticipated to occur on or around April 1, 2012.

### ***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.

### **Angioblast Systems, Inc**

Angioblast Systems, Inc. holds the IND for Revascor™ 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. Angioblast will be responsible for labeling and supplying the study product/cryoprotective media control to all participating sites. Angioblast 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.

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## APPENDIX I: Procedures for Study Product Preparation

### Supplies Needed:

- 1 study product contained within the metal rack and stored in a portable container with liquid nitrogen.
- 1 18-gauge Monoject spinal needle (supplied by manufacturer)
- 1 10 or 15ml sterile syringe
- 1 sterile female-female luer connector
- 4 BD 5cc luer-lock tip syringes
- 1 Ziploc plastic bag
- 70% alcohol wipes
- 4 Sterile drapes (small)
- Sterile gloves
- Steri-strips
- Sterile pen
- Water bath

### Product Preparation Procedure

***Note: Gloves should be worn when handling the study product at all times.***

Remove the study product transfer pack from the portable metal canister and place inside a zip-lock plastic bag.

1. Thaw the study product in a 37° C water bath filled with sterile water for no more than 4 minutes.
2. Gently shake the plastic bag until the study product is thawed, but for no more than 4 minutes and just before the last crystal of ice has fully melted.
3. Remove the study product transfer pack from the plastic zip-lock bag and inspect for any leaking or damage. ***Note: If damaged or leaks are found, the bag must be returned and another study product obtained immediately. Once thawed, start a timer to ensure that the product is injected within 75 minutes post-thaw.***
4. Set-up a sterile field using 3 sterile drapes and transfer the sterile needle, alcohol wipes and syringes to the sterile field. The use of a Biological laminar flow hood is acceptable if that is

the standard at your institution for working with human products. Always follow your institutional guidelines for handling the study product.

5. Remove the inner cannula from the 18-gauge spinal needle and attach the needle to the 10 or 15 cc syringe.
6. Twist and remove one of the capped ports on the study product bag which is wrapped in sterile drapes to maintain sterility of the person conducting the transfer procedure.
7. Swab the port with 70% alcohol.
8. Gently insert the spinal needle through the membrane within the bag port. *Note: The membrane will be firm, be careful not to puncture the product bag.*
9. Inject up to 10 ml of air into the bag. *Note: The air will assist in displacing the contents of the bag.*
10. Invert the bag with the ports facing up or at a 45-degree angle and withdraw the contents of the bag into the 10 or 15ml syringe. *Note: The bag contains 5 ml of study product. The ports should be facing up to avoid losing product within the other ports.*
11. Attach the 10 or 15ml syringe to the luer and to a sterile 5 cc luer lock syringe. Transfer 4 mls of the study product equally divided between the 4 5 cc syringes. Note: The total length of thaw to injection time will not exceed 75 minutes.
12. The study product bag should be then stored frozen for 48 hours. The bag may be discarded in the usual manner for disposal of biohazard waste according to the facility's SOP.
13. The metal canister must be saved and returned to Lonza with the shipping materials provided.

**Returned shipping instructions:**

1. The container has to be returned within 24 hours of receipt
2. All Liquid nitrogen must be evaporated before sending the container back
3. Place all the empty cassettes into the container and secure the lock
4. Use the pre printed FedEx labels or the following Fed Ex Account # 020063165
5. Call courier for pickup. Request Standard Overnight delivery service

**Shipping address:**

Lonza  
8830 Biggs Ford Road  
Walkersville, MD 21793  
Attention: Edit Druktenis, Sr. Project Manager  
301-898-7025

## APPENDIX II: Intramyocardial Injection Procedures

### PREPARATION OF SYRINGES FOR STUDY PRODUCT INJECTION

#### Study Drug Injection Procedure

1. Four (4) sterile 5cc syringes [e.g., Becton Dickinson] with a 3/4 inch 23 gauge needle must be prepared under sterile technique (see Appendix I), 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 75 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 75 minutes will be discarded and the process will be continued with a new cryobag.**

### INTRAMYOCARDIAL INJECTION PROCEDURE/DOCUMENTATION

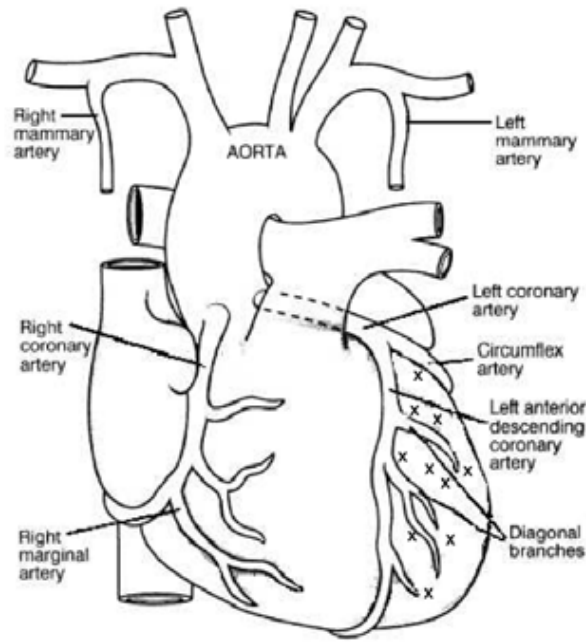
Consider injecting cells during or around the time of LV apical cannula guide implantation as the heart is positioned for maximal access to the entire left ventricle.

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.

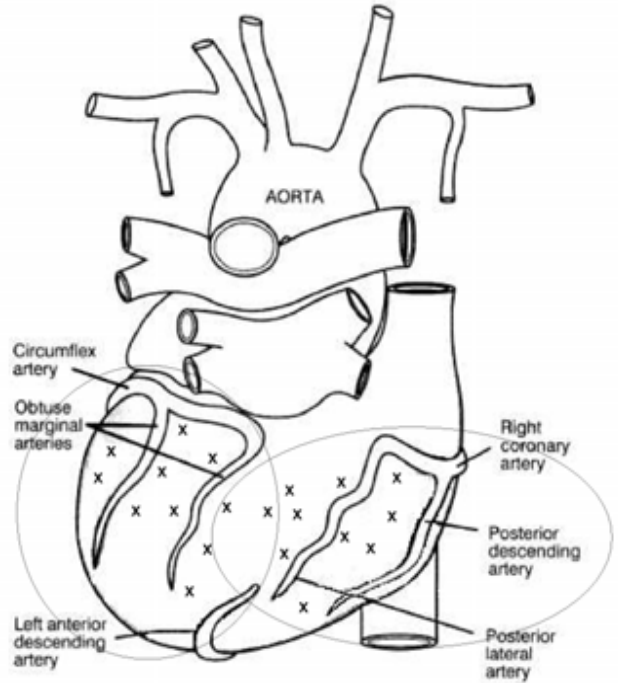


Suggested locations for intramyocardial injections:

**Anterior View**



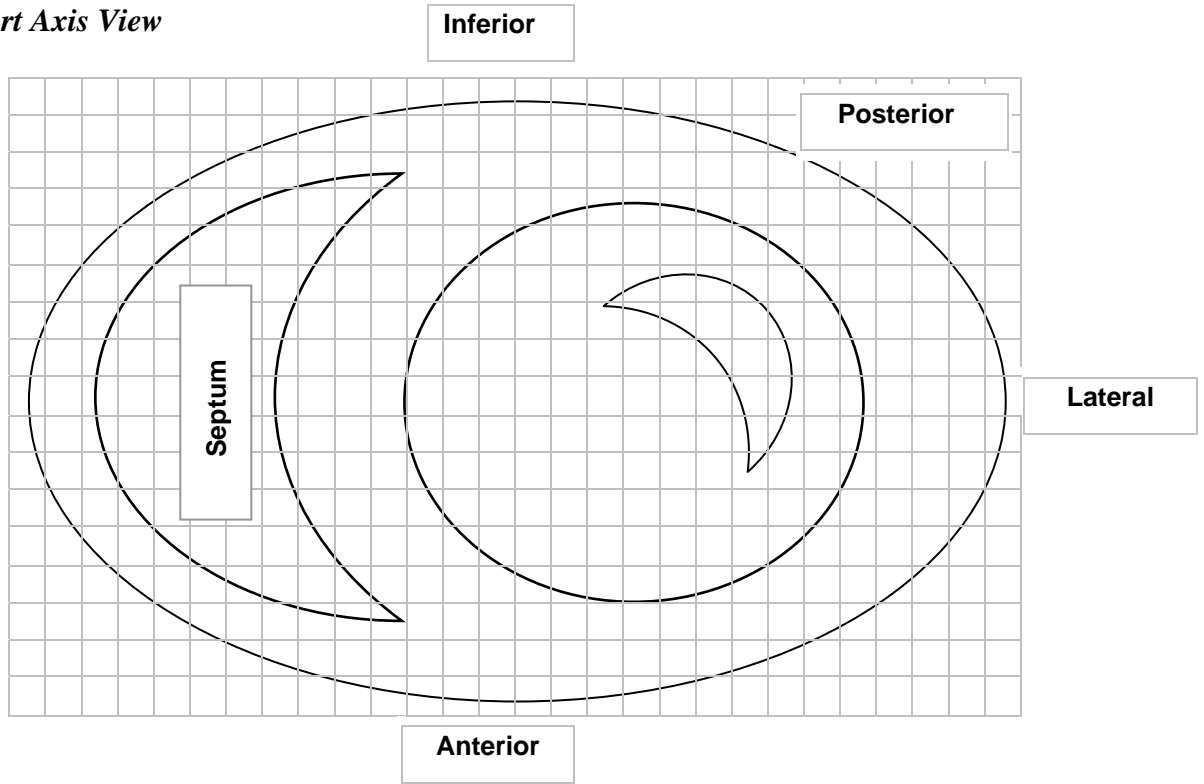
**Posterior View**



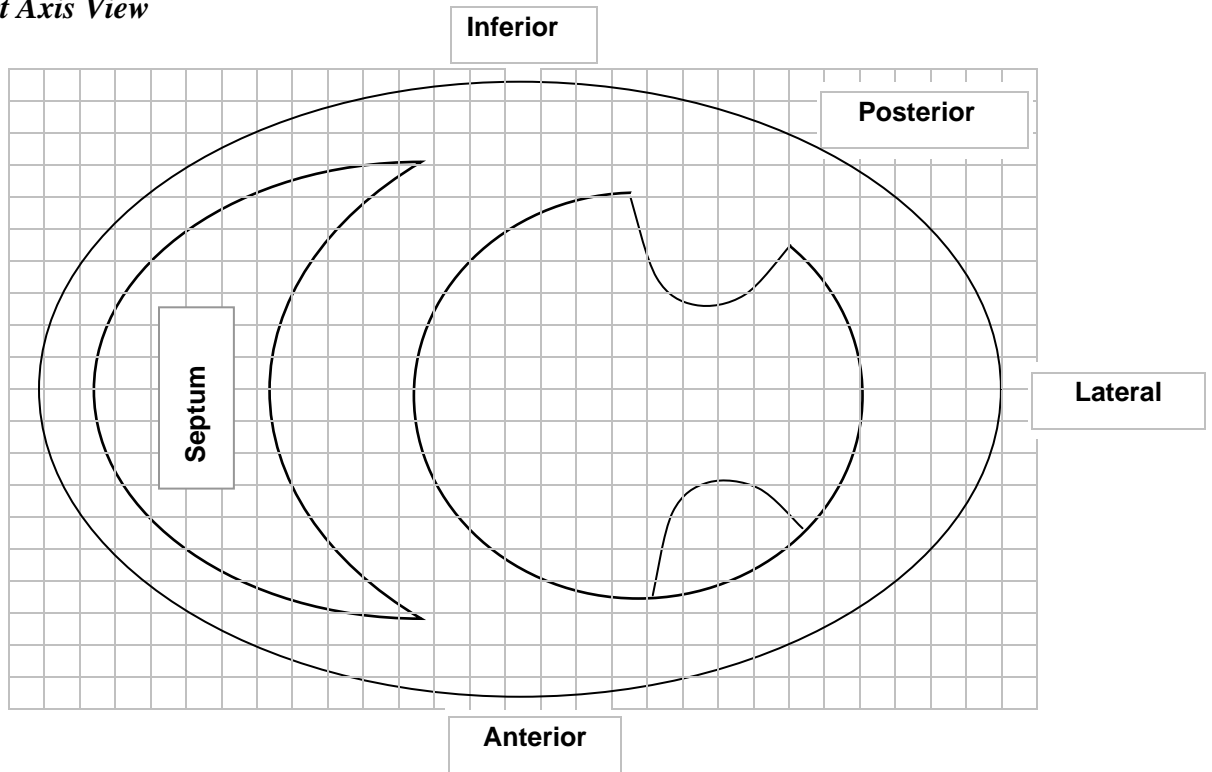
6. Immediately following the treatment intervention the operating surgeon will document date and time of the intramyocardial injections, the number and locations of injection sites as well as the total volume implanted on *Intervention Injection Verification Case Report Form (CRF)* which outlines transverse sections of the heart.
7. On the basal and mid short axis images the surgeon will check one box for each injection to indicate the intramyocardial injection sites (see basal and mid short axis views below).
8. The *Intervention Injection Verification CRF* must be signed by the surgeon and faxed to the DCC (fax: 212-731-7373) within 48 hours following VAD implantation. The original copy should be kept in the patient's source document binder.

Intramyocardial injections will be documented by marking X's on the following two images:

***Basal Short Axis View***



***Mid Short Axis View***



## APPENDIX III: LVAD Wean Guidelines

### Non-Pulsatile LVAD Weaning Guidelines

The non-pulsatile LVAD Wean and Early Termination Guidelines are adopted from the Harefield Hospital protocol, with permission of Dr. Emma Birks. The Patients on a non-pulsatile LVAD at Harefield Hospital have regular echocardiograms performed with the pump at a reduced speed in order to assess myocardial recovery. Over 90 such weans have been performed at Harefield Hospital in the UK without any complications to date.

### LVAD WEAN

- 1) Ensure that the INR is  $\geq 2.5$  within 48 hours of the day of testing or  $\geq 2.0$  on the day of testing before reducing device speed.
- 2) If INR  $< 2.0$  on the day of testing then administer 10,000 units of intravenous unfractionated heparin and allow it to circulate for at **least** 5 minutes before reducing the pump speed.
- 3) Connect the patient to the Power Base Unit (PBU) and record the pump speed at the beginning of the test. Ensure that spare kit is in close proximity and easily accessible.
- 4) After taking baseline blood pressure (systolic, diastolic and mean arterial pressure), heart rate and protocol-defined echocardiographic measurements, gradually turn the speed of the pump down as follows:
  - **HeartMate II**<sup>®</sup> (Thoratec Corporation, Pleasanton, CA): Incremental speed reductions of 1000 rpm to a target (“low speed”) of 6000 rpm
  - **<sup>5</sup>HVAD**<sup>®</sup> (HeartWare<sup>®</sup>, Framingham, MA): Incremental speed reductions of 200 rpm to a target (“low speed”) of 1,800 rpm
- 5) Perform regular vital sign measurements using an automated blood pressure cuff (e.g., Dynamat machine) as follows:
  - Baseline i.e. with device on full speed
  - 0 minutes of low speed
  - 5 minutes of low speed
  - 10 minutes of low speed
  - 15 minutes of low speed and every 5 minutes thereafter for duration of wean
- 6) The patient should be asked on regular basis about the following symptoms: shortness of breath, chest pain, blurred vision, dizziness, abdominal pain. (N.B. if patient develops chest pain perform a 12 lead ECG immediately)

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<sup>5</sup> This device will only be used in this trial after FDA approval or clearance.

- 7) If the patient tolerates the wean to 6000 rpm, and is stable at the completion of the 15 minute echo, the 6 minute walk should be performed. **Take patient’s spare batteries and second controller during the walk. Ensure the second controller is easily accessible.**
- 8) Monitor the patient for signs or symptoms detailed below.\*
- 9) Following completion of the functional assessments, immediately repeat the vital sign measurements and re-connect the patient to the PBU.
- 10) Once the final echocardiographic parameters are complete, increase the pump speed to the original pre-testing settings by increments of 1000 rpm.
- 11) **At the completion of the wean, ensure that both the main and the extended alarm resets are OFF and that both controllers are on the original settings.**

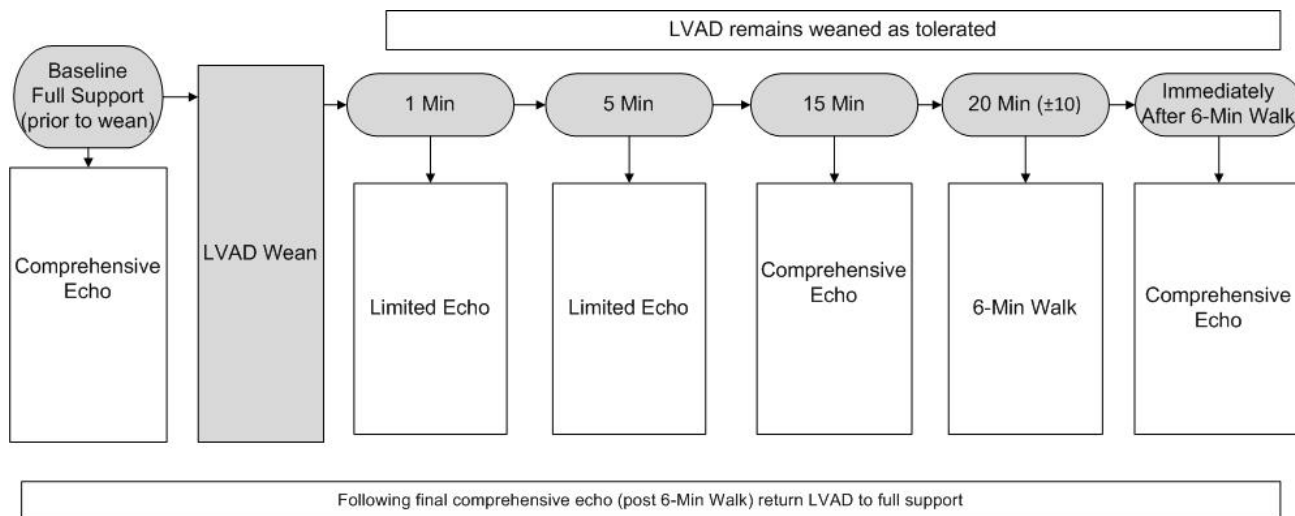
\* If the patient meets any of the stopping rules below, stop the test, disconnect the current controller (controller with reduced speed) and connect the second / spare controller immediately. Repeat hemodynamic (vital sign) measurements.

**EARLY TERMINATION GUIDELINES FOR LVAD WEAN**

LVAD will be returned to full LVAD flow if the patient develops any of the following at any time during the weaning process:

- Symptoms of low output (e.g., light headedness, dyspnea, fatigue, chest pain)
- Vascular congestion (e.g., pulmonary edema)

**TIMELINE OF FUNCTIONAL ASSESSMENTS PERFORMED DURING WEAN**



## APPENDIX IV: Echocardiography Procedures

### General Considerations

- For the echocardiogram, the patient will be positioned in left lateral recumbency or in the position that permits optimal imaging. It is strongly recommended that a customized echocardiography bed be used with a standard cut-out mattress to expose the cardiac apex.
- With digital archiving, 5 cardiac cycles are requested for two-dimensional and color Doppler imaging. At least 3 cardiac cycles are required for spectral pulsed wave (PW) and continuous wave (CW) Doppler. For patients in atrial fibrillation, a minimum of 2 captures of 5 consecutive cardiac cycles are required.
- Unless otherwise specified, depth should be adjusted to maximize the image while including all necessary structures.
- All images should be acquired with quiet breathing or suspended breathing as necessary to limit motion artifact.
- Frame rate may be adjusted to optimize image quality, but for speckle tracking, it should be between 50 and 80 Hz,
- Harmonic imaging should be employed to optimize visualization of endocardial borders.
- Color gain will be adjusted to eliminate random color in areas without flow.
- All PW, CW Doppler and m-mode recordings should be performed at a sweep speed of 100 mm/sec.
- Color Doppler Nyquist limits will be adjusted to the range of 0.5 – 0.7 m/sec, unless otherwise specified.
- An electrocardiographic signal will be obtained before initiating the study. Care should be taken to optimize the ECG quality (R wave > 1 cm).
- A single study will be digitally recorded and copied in DICOM format onto CD/DVD for later analysis.
- Contrast will be used for endocardial border delineation when less than 2/3rds of the endocardium can be visualized on the harmonic image. Contrast can also be employed for enhancement of the tricuspid regurgitant flow signals. During the contrast imaging, the mechanical index (MI) should be adjusted according to manufacturer recommendations.
- Additional images not specified in the protocol should be obtained at the discretion of the sonographer/physician.

## Comprehensive Two-dimensional and Doppler Echocardiography Protocol

\*Five consecutive cardiac cycles will be recorded for each view.

- Parasternal Long-Axis View
  - 2D
  - 2D zoom of aortic valve
  - color Doppler of mitral and aortic valves
  
- RV inflow view
  - 2D
  - Color Doppler across tricuspid valve, angle so that proximal jet is captured and include all of right atrium in color sector to capture distal jet
  - TR jet velocity by continuous wave Doppler
  
- Parasternal Short-Axis Views (at aortic valve, mitral valve, papillary muscles and apical levels; include RV in views at all levels) (3-5 beats at each level), obtained at a frame rate of 50-80 Hz
  
- Apical Four-Chamber View
  - 2D
  - Color Doppler across mitral and tricuspid valves; angle so that proximal jet is captured and include all of atrium in color sector to capture distal jet
  - If moderate > greater MR by qualitative screen: Zoom on proximal MR jet (depth of 8 cm or less); shift baseline down so that Nyquist number on bottom scale is between 30-40 cm/sec and capture digital clip
  - Continuous wave Doppler across mitral and tricuspid valves for MR and TR peak jet velocities
  - Pulsed Doppler of MV and TV inflow at leaflet tips
  - Tissue pulse wave Doppler at basal lateral LV, septal and RV lateral walls,
  - Tissue velocity imaging of LV and RV myocardial walls
  - Color M-Mode flow propagation through the mitral valve (color acquisition to stretch from mid-LA to near the LV apex, with the baseline shifted so that the forward aliasing velocity is about 2/3 of the maximal transmitral pulsed Doppler velocity)
  - Acquisition with depth adjusted to focus on the LV and RV (separately if heart is too big to allow both to be optimally imaged in a single view) with the frame rate 50-80 frames/sec
  
- Apical Five-Chamber View
  - 2D
  - Color Doppler across mitral and aortic valves
  - pulsed and continuous wave Doppler of LV outflow tract/aortic valve
  
- Apical Two-Chamber View
  - 2D

- Color Doppler angle so that proximal jet is captured and include all of atrium in color sector to capture distal jet
  - Tissue velocity imaging of LV myocardial walls
  - Continuous wave Doppler across mitral valve for MR peak jet velocities-if envelope not obtainable in apical 4 chamber
  - Acquisition with depth adjusted to focus on the LV with the frame rate 50-80 frames/sec
- Apical Long-Axis View
    - 2D
    - color Doppler across mitral and aortic valves; for mitral include entire left atrium in color sector,
    - pulsed and continuous wave Doppler of LV outflow tract/aortic valve
    - tissue velocity imaging of LV myocardial walls
    - Continuous wave Doppler across mitral valve for MR peak jet velocities-if envelope not obtainable in other apical views
    - Acquisition with depth adjusted to focus on the LV with the frame rate 50-80 frames/sec

**Abbreviated Echo protocol for: 1 and 5 minute time periods**

- Parasternal Long-Axis View (2D)
- Parasternal Short-Axis Sweep-**10 beats** (across aortic valve, mitral valve, papillary muscles and apical levels; include RV in views at all levels)
- Apical Four-Chamber View (2D, color Doppler across both mitral and aortic valves) at frame rate of 50-80 Hz
- Apical Two-Chamber View (2D, color Doppler (across both mitral and aortic valves) at frame rate of 50-80 Hz
- Apical Long-Chamber View (2D, color Doppler (across both mitral and aortic valves) at frame rate of 50-80 Hz

The above abbreviated protocol will be selectively obtained as required by the study protocol.

**The following parameters will be measured when required by the study protocol:**

- Measurements of left ventricular end-diastolic (LVEDD) and end-systolic (LVSD) diameters and wall thicknesses (Interventricular Septum Thickness (IVS), Posterior Wall (PS)) will be measured according to the recommendations of the American Society of Echocardiography.
- Left ventricular fractional shortening (FS) will be calculated as follows:
 
$$FS = (LVEDD - LVSD) / LVEDD$$

- LV volumes (LVED volume and LVES volume) will be calculated using the biplane Simpson's method (summation of disks), replaced by modified Simpson's method in case one of the perpendicular apical views (four- and two- chamber view) is not of adequate quality.
  - if Simpson's method not possible due to poor LV endocardial resolution, LVEF will be assessed visually.
- LV ejection fraction will be calculated from LV volumes as follows:  
$$\text{LVEF} = 100 * (\text{LVDD volume} - \text{LVES volume}) / \text{LVDD volume}$$
- 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.
- Global and regional strain will be measured by speckle tracking from the high frame rate apical and short axis images.
- Tricuspid regurgitant jet velocity will be measured. TV gradient will then be calculated using the modified Bernoulli equation ( $4V^2$ ) on the obtained velocity.
- RV function (Qualitative: normal, mild, moderate, severe).
- Qualitative assessment of MR, TR and AR (mild, moderate, severe).

#### **Limited Echo Assessments**

At 1 and 5 minutes following initiation of wean, the following limited measurements will be obtained:

- Left ventricular (LV) end-diastolic diameter
- LV end-systolic diameter
- LV ejection fraction (2D LV dimension measurements or visual assessment)

All echocardiograms will be analyzed by the Echocardiology Core Lab, directed by Dr. Judy Hung and Massachusetts General Hospital.



## **APPENDIX V: Six-Minute Walk Test Instructions**

The walking test will be conducted in an enclosed corridor (preferably free of distractions) on a course that is 60 feet long (Guyatt, 1985<sup>6</sup>). The corridor will be divided into 5-foot sections using a method unnoticeable to the patient. Chairs will be placed at either end of the 60-foot course markers. The distance covered during the preceding walk test will not be revealed to the patient during the study.

Before the test, the patient will sit quietly for 10 minutes. These instructions will be read verbatim to the patient:

**THE PURPOSE OF THIS TEST IS TO FIND OUT HOW FAR YOU CAN WALK IN SIX-MINUTES. YOU WILL START FROM THIS POINT AND FOLLOW THE HALLWAY TO THE CHAIR AT THE END, THEN TURN AROUND AND WALK BACK. WHEN YOU ARRIVE BACK AT THE STARTING POINT, YOU WILL GO BACK AND FORTH AGAIN. YOU WILL GO BACK AND FORTH AS MANY TIMES AS YOU CAN IN THE SIX-MINUTE PERIOD. IF YOU NEED TO, YOU MAY STOP AND REST. JUST REMAIN WHERE YOU ARE UNTIL YOU CAN GO ON AGAIN. HOWEVER, THE MOST IMPORTANT THING ABOUT THE TEST IS THAT YOU COVER AS MUCH GROUND AS YOU POSSIBLY CAN DURING THE SIX MINUTES. I WILL TELL YOU THE TIME, AND I WILL LET YOU KNOW WHEN THE SIX MINUTES ARE UP. WHEN I SAY STOP, PLEASE STAND RIGHT WHERE YOU ARE.**

**DO YOU HAVE ANY QUESTIONS ABOUT THE TEST?**

**PLEASE EXPLAIN TO ME WHAT YOU ARE GOING TO DO.**

Repeat the entire instructions if the patient does not seem to understand.

Repeat the sentence:

**THE MOST IMPORTANT THING ABOUT THE TEST IS THAT YOU COVER AS MUCH GROUND AS YOU POSSIBLY CAN DURING THE SIX MINUTES.**

**ARE YOU READY?**

**START WHEN I SAY "GO"**

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<sup>6</sup> Kirshner, B, Guyatt, G. (1985) A methodological framework for assessing health indices. *Journal of Chronic Diseases* 38: 27–36.

During the test, the walking pace of the patient should not be influenced. The test supervisor must walk behind the patient - not walk with, rush up behind, or rush past the patient.

While walking, the patient will be encouraged every 30 seconds with the following phrases:

0-3 minutes:

**THAT'S IT; YOU'VE GOT THE IDEA.  
YOU'RE DOING WELL.  
KEEP IT UP NOW.**

3-6 minutes:

**REMEMBER, AS FAR AS YOU CAN GO.  
WE'LL WANT YOU TO GO AS FAR AS YOU POSSIBLY CAN.  
THAT'S IT; KEEP WORKING AT IT.  
COME ON; KEEP GOING.**

The patient should be spoken to only at the 30-second encouragements and no response should be made to the patient's questions about the time and distance elapsed. If the patient is not concentrating on the walking, the patient can be reminded at a 30-second mark:

**THIS IS A WALKING TEST. TALKING WILL UTILIZE YOUR ENERGY RESERVE AND INTERFERE WITH YOUR PERFORMANCE.**

Encouragement phrases can be repeated as needed. For example, if the patient is slowing down and expresses that he/she wants to stop, say:

**REMEMBER, IF YOU NEED TO, YOU MAY REST. JUST REMAIN WHERE YOU ARE UNTIL YOU CAN GO ON AGAIN.**

If necessary, the patient may rest in a course marker chair although he/she should not be encouraged to do so.

The patient will be told the time elapsed at 2 and 4 minutes, i.e.:

**YOU HAVE COMPLETED 2 MINUTES  
and  
YOU HAVE COMPLETED 4 MINUTES**

At the end of the test, the patient should not move from where he/she was told to "STOP" until the distance walked (measured to the nearest foot) has been recorded. The patient will then be directed back to the table for their echocardiographic assessment and observed for at least 10 minutes.

Record the DISTANCE WALKED during the six-minute test.

## APPENDIX VI: Immunohistochemical, Cellular and Molecular Assessments

### 1. Biospecimen Core Laboratory

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; phenotypic and functional analyses of cells and plasma samples. The Biospecimen Core Lab will be operated by:

Doris Taylor, PhD  
Texas Heart Institute  
Houston, TX 77030

Christopher Cogle, MD  
Program in Stem Cell Biology and Regenerative Medicine  
University of Florida  
Gainesville, FL 32610

### 2. Tissue Specimens

As part of the Immunohistochemical, Cellular and Molecular Assessments, myocardial core samples will be obtained at the time of the LVAD implantation. Subsequently, at the time of explantation (e.g., for cardiac transplantation, autopsy, or for any other indication), every native heart will be harvested and tissue sections will be divided into the four chambers and septum. Two representative areas of each chamber and two segments of the septum will be collected. Samples will be processed and stored appropriately for histopathology, immunohistochemical analyses of neovascularization and cardiomyocyte proliferation and PCR-based detection of donor cell engraftment, as described in the Manual of Procedures.

### 3. Cellular Specimens

In brief, the Biospecimen Core Lab will receive peripheral blood (PB) and MPC specimens and perform the following assays: (1) cytokine analysis on PB plasma, (2) phenotypic analyses on cells (PB mononuclear cells (MNCs), and MPCs) by flow cytometry, and (3) functional CFU-F colony assays (PB MNCs and MPCs). In addition, the Biospecimen Core Lab will receive serum and tissue samples for subsequent analysis in-house or distribution to the appropriate processing laboratories.

#### a. Mesenchymal Progenitor Cell (Revascor™) Product

An aliquot of MPCs from each lot administered will be sent to the Biospecimen Core Laboratory for function analyses. A minimum of 20 million cells will be sent from each lot administered.

#### b. Peripheral blood

##### Plasma for Chemo- and Cytokine Analyses

Peripheral blood (PB) for plasma analysis will be obtained from each subject prior to LVAD procedure, at day 1 (+1) post intervention, and at days 7 ( $\pm 3$ ), 30 ( $\pm 3$ ), 60 ( $\pm 7$ ), and 90 ( $\pm 14$ ) days and 6 and 12 months post randomization. One BD purple top tube (6 mL) will be collected at the site for analysis of cytokines. The purple top tubes will be

processed immediately at the site (centrifuged to achieve separation of plasma), plasma and buffy coat will be transferred to separate cryovials supplied by the core laboratory and flash frozen at -80 degrees C. These samples will be shipped once a month on dry ice to the Biospecimen Core laboratory for cytokine analysis. SOPs and supplies for shipping will be provided by the core laboratory.

Plasma separated by centrifugation will be analyzed by Luminex multiplex assay to quantify cytokines.

#### Cell Surface Phenotyping

At pre-implant (within 24 hours LVAD implant), and days 1 (+1) post intervention, 30 ( $\pm 3$ ), and 90 ( $\pm 14$ ) post randomization, one BD purple top (10 mL) tube will be collected and sent overnight with a cold pack to the Biospecimen Core Laboratory for further processing and analyses. Shipping materials will be supplied by the Core Laboratory. Core lab SOPS will be included in the Manual of Procedures. SOPs and shipping materials will be supplied by the Core Laboratory.

#### Functional CFU-F Colony Assays

A sample of injected MPCs will be supplied by Angioblast. Peripheral blood will be collected pre-implant (within 24 hours of LVAD implant), at days 1 (+1) post intervention, and at days 30 ( $\pm 3$ ), and 90 ( $\pm 14$ ) post randomization. One BD purple top (10 mL) tube will be collected and sent overnight with a cold pack to the Biospecimen Core Lab.

## **APPENDIX VII: Immunologic Assessments**

### **Immunologic Assessments**

Since LVAD implantation is associated with prominent B-cell activation and heightened antibody production, we intend to screen study patients at risk for sensitization before transplantation and to identify the presence, isotype, and specificity of anti-HLA antibodies that portend heightened risk for adverse post transplant outcomes. Injection of stem cells into the native heart may fuel this process further or potentially reduce sensitization due to secretion of local immune downregulating cytokines. In addition to measuring anti-HLA antibody production, anti-bovine and anti-murine antibody titers will be measured over time.

### **Tests to be Performed**

Serum samples will be collected for Immune Testing (anti-HLA antibodies), anti-murine and anti-bovine antibodies. Specimens will be collected for all tests according to the following schedule: Pre-implant, days 30 ( $\pm$  3days) and 90 ( $\pm$ 14 days), and months 6 & 12 ( $\pm$ 14 days) post randomization.

### **Measurement of anti-HLA Antibodies**

The objective of this part of the study is to determine whether implantation of a given bone marrow-derived cell type into the native heart affects the development of B cell hyperreactivity in LVAD recipients. Serum specimens will be obtained from all patients.

Histocompatibility testing, including HLA typing, anti-HLA antibody and HLA antibody specificity studies, will be performed. If the PRA (percent reactive antigens) of either class I or class II HLA antibodies are  $>10\%$  then specificity analysis will be performed. This specificity test is to measure the presence of antibodies to HLA antigens including MPC donor specific HLA antigens. Specificity titration study (where specific HLA antibodies have been identified) or a PRA Titration Study (to determine the titer of HLA non-donor specific) antibodies will also be performed on samples with either class I or class II HLA antibodies are  $>10\%$ .

### **Measurement of Anti-Murine and Anti-Bovine Antibodies**

The MPC product, Revascor™, is derived from an allogeneic, human bone marrow donor. Bone marrow cells are immuno-selected utilizing a proprietary IgG murine derived, monoclonal antibody directed at stromal antigen targets. Immuno-selected bone marrow cells are ex-vivo expanded in cell culture media containing fetal bovine serum under GMP conditions. Following trypsin and collagenase harvest, cells are cryopreserved in DMSO containing cryoprotectant until use. The goal of testing for anti-murine and anti-bovine antibodies is to initiate immunological studies for the detection of sensitization to xenogenic components in the manufacturing process of the MPC product, Revascor, such components in the murine sourced monoclonal antibody and fetal bovine serum. A surveillance sampling plan is proposed for the detection of antibody development to these sources. Samples will be derived according to the study's schedule of assessment from clinical patients in this current proposed study. Anti-Murine and anti-bovine antibodies will be measured by IBT Laboratories (11274 Renner Blvd, Lenexa, KS, 66219).

## APPENDIX VIII: NIH Stroke Scale Administration Guidelines

The NIH Stroke Scale (NIHSS) is a standardized neurological examination intended to describe the neurological deficits found in large groups of stroke patients participating in treatment trials. The instructions reflect primary concern for reproducibility. The purpose of this form is to collect data representing the baseline stroke status of each participant and the stroke status at different exam time frames of the trial. Please Note: The NIH Stroke Scale must be administered by a Stroke Neurologist or trained site coordinator. The coordinator and the neurologist must be trained and certified in the NIH Stroke Scale.

This is also part of the neurological exam conducted for suspected stroke during follow-up.

Date and time of form completion. Record the date (dd/mm/yyyy) and time (24-hr clock) the form was completed.

**Directions:** Indicate one box for each category. If any item is left untested, a detailed explanation must be clearly written on the form in the comment section.

### Level of Consciousness

Three items are used to assess the patient's level of consciousness. It is vital that the items be asked in a standardized manner, as illustrated in the Stroke Scale training tape. Responses must be graded based on what the patient does first. Do not give credit if the patient corrects himself/herself and do not give any clues or coaching.

#### 1a. Level of Consciousness (LOC)

Ask the patient two or three general questions about the circumstances of the admission. Also, prior to beginning the scale, it is assumed that the examiner will have queried the patient informally about the medical history. Based on the answers, score the patient using the 4-point scale on the Stroke Scale form. Remember not to coach. A score of 3 is reserved for the severely impaired patient who makes, at best, reflex posturing movements in response to repeated painful stimuli. If it is difficult to choose between a score of 1 or 2, continue to question the patient about historical items until you feel comfortable in assessing level of consciousness.

#### 1b. LOC Questions

Ask the patient "how old are you now" and wait for a response. Then ask "what month is it now" or "what month are we in now". Count the number of incorrect answers and do not give credit for being "close". Patients who cannot speak are allowed to write. Do not give a list of possible responses from which to choose the correct answer. This may coach the patient. Only the initial answer is graded. This item is never marked "untestable". (Note: On Certification Tape #1 an intubated patient was given a series of responses from which to choose, but the score for this patient would still be 1.) Deeply comatose (1a=3) patients are given a 2.

#### 1c. LOC Commands

Say to the patient "open your eyes...now close your eyes" and then "Make a fist...now open your hand". Use the non-paretic limb. If amputation or other physical impediment prevents the response, use another suitable one step command. The priming phrase is not scored, and these are used only to set the eyes or hand in a testable position. That is, the patient may be asked first to open the eyes if they are closed when you begin the test. Scoring is done on the second phrase "close your eyes".

Count the number of incorrect responses and give credit if an unequivocal attempt is made to perform the operative task, but is not completed due to weakness, pain or other obstruction. Only the first attempt is scored and the questions should be asked only once.

## **2. Gaze**

The purpose of this item is to observe and score horizontal eye movements. To this end, use voluntary or reflexive stimuli and record a score of 1 if there is an abnormal finding in one or both eyes. A score of 2 is reserved for forced eye deviation that cannot be overcome by the oculocephalic maneuver. Do not do caloric testing. In aphasic or confused patients it is helpful to establish eye contact and probe about the bed. This item is an exception to the rules of using the first observable response and not coaching. In the patient who fails voluntary gaze, the oculocephalic maneuver, eye fixation, and tracking with the examiner's face, are used to provide stronger testing stimuli.

## **3. Visual Fields**

Visual fields are tested exactly as demonstrated in the training video. Use finger counting or movement to confrontation and evaluate upper and lower quadrants separately. A score of 3 is reserved for blindness from any cause, including cortical blindness. A score of 2 is reserved for a complete hemianopia, and any partial visual field defect, including quadrant anopia, scores a 1.

## **4. Facial Movement (Facial Paresis)**

Ask the patient "Show me your teeth ...now raise your eyebrows ...now close your eyes tightly". Assess the response to noxious stimulation in the aphasic or confused patient. A useful approach to scoring may be as follows: score a 2 for any clear cut upper motor neuron facial palsy. Normal function must be clearly demonstrated to obtain the score of 0. Anything in between, including flattened nasolabial fold, is scored a 1. The severely obtunded or comatose patient; patients with bilateral paresis, patients with unilateral lower motor neuron facial weakness would receive a score of 3.

## **5. Motor Arm-Right**

Perform the test for weakness as illustrated in the video. When testing arms, palm must be down. Count out loud to the patient, until the limb actually hits the bed or other support. The score of 3 is reserved for the patient who exhibits no strength whatsoever, but does minimally move the limb on command when it is resting on the bed. The basic patient may understand what you are 'testing' if you use the non-paretic limb first. Do not test both limbs simultaneously. Be watchful for an initial dip of the limb when released. Only score abnormal if there is a drift after the dip. Do not coach the patient verbally. Count out loud in strong voice and indicate count using your fingers in full view of the patient. Begin counting the instant you release the limb. (Note that on some of the video illustrated patients, the examiners erroneously delay seconds before beginning to count).

## **6. Motor Arm-Left**

See explanation of 5.

## **7. Motor Leg-Right**

Perform the test for weakness as illustrated in the video. When testing motor leg the patient must be in the supine position to fully standardize the effect of gravity. Count out loud to the patient, until the

limb actually hits the bed or other support. The score of 3 is reserved for the patient who exhibits no strength whatsoever, but does minimally move the limb on command when it is resting on the bed. The aphasic patient may understand what you are testing if you use the non paretic limb first. Do not test both limbs simultaneously. Be watchful for an initial dip of the limb when released. Only score abnormal if there is a drift after the dip. Do not coach the patient verbally. Count out loud in strong voice and indicate count using your fingers in full view of the patient. Begin counting the instant you release the limb. (Note that on some of the video illustrated patients, the examiners erroneously delay seconds before beginning to count).

### **8. Motor Leg-Left**

See explanation of 7.

### **9. Limb ataxia**

Ataxia must be clearly present out of proportion to any weakness. Using the fingernose-finger and the heel-test, count the number of ataxic limbs, up to a maximum of two. The aphasic patient will often perform the test normally if first the limb is passively moved by the examiner. Otherwise the item is scored 0 for absent ataxia. If the weak patient suffers mild ataxia, and you cannot be certain that it is out of proportion to the weakness, give a score of 0. Remember this is scored positive only when ataxia is present. If the item is scored 00' or 09', skip to Item 12.

Please indicate presence of ataxia in arms and legs.

### **10. Sensory**

Do not test limb extremities, i.e., hands and feet when testing sensation because an unrelated neuropathy may be present. Do not test through clothing.

### **11. Best Language**

It is anticipated that most examiners will be ready to score this item based on information obtained during the history taking and the eight prior items. The attached picture and naming sheet therefore should be used to confirm your impression. It is common to find unexpected difficulties when the formal testing is done, and therefore every patient must be tested with the picture, naming sheet, and sentences. The score of 3 is reserved for the globally mute or comatose patient. NEW aphasia would score a 1. To choose between a score of 1 or 2 use all the provided materials; it is anticipated that a patient who missed more than two thirds of the naming objects and sentences or who followed only very few and simple one step commands would score a two. This item is an exception to the rule that the first response is used, since several different tools are used to assess language.

### **12. Dysarthria**

Use the attached word list in all patients and do not tell the patient that you are testing clarity of speech. It is common to find slurring of one or more words in patients one might otherwise score as normal. The score of 0 is reserved for patients who read all words without any slurring. Aphasic patients and patients who do not read may be scored based on listening to the speech that they do produce or by asking them to repeat the words after you read them out loud. The score of 2 is reserved for the patient who cannot be understood in any meaningful way, or who is mute. On this question, normal speech must be identified to score a 0, so the unresponsive patient receives the score of 2.



### **13. Extinction and Inattention (formerly Neglect)**

Place the hand in position exactly as shown in the training video. Fingers may be spread or together, The score of 0 is given only if the fingers maintain full extension of five seconds. The score of 2 is reserved for the hand that has no strength at all. Any change from the fully extended posture within five seconds scores a 1. Note: This item is open to significant variation among examiners, and all neurologists have slightly different methods of assessing neglect. Therefore, to the extent possible, test only double simultaneous stimulation to visual and tactile stimuli and score 2 if one side extinguishes to both modalities, a 1 if only to one modality. If the patient does not extinguish, but does show other well developed evidence of neglect, score a 1.

Total Score: Please provide the total score for the subject as determined by the 11 categories of questions. Do not include scores of "9" in total.

**APPENDIX IX: Neurocognitive Testing****HOPKINS VERBAL LEARNING TEST TRIAL INSTRUCTIONS****Trial 1**

Say the following:

**I am going to read a list of words to you. Listen carefully, because when I'm through, I'd like you to tell me as many of the words as you can remember. You can tell them to me in any order. Are you ready?**

- Repeat or paraphrase the instructions if necessary
- Read the words at the rate of approximately one word every 2 seconds
- If the individual does not spontaneously begin reporting words after the last word is read, say the following:

**OK. Now tell me as many of those words as you can remember**

Record the responses verbatim (including repetitions and intrusions) in the Trial 1 column. When the individual indicates no more words can be recalled, proceed to Trial 2.

**Trial 2**

Say the following:

**Now we are going to try it again. I am going to read the same list of words to you. Listen carefully, and tell me as many of the words as you can remember, in any order, including all the words you told me the first time.**

Use the same procedure as in Trial 1 to record the responses in the column for Trial 2. Then proceed to Trial 3.

**Trial 3**

Say the following:

**I am going to read the list one more time. As before, I'd like you to tell me as many of the words as you can remember, in any order, including all the words you've already told me.**

Record the responses in the column for Trial 3 using the same procedure as in the previous trials.

NOTE: *Do not tell the respondent that recall of the words will be tested later.*

### **Delayed Recall Trial Instructions**

After the 20 –25 minute delay, say the following:

**Do you remember that list of words you tried to learn before?**

If the response is “No,” remind the individual that you read the list three times and that he or she was asked to recall the words each time. Say the following:

**Tell me as many of those words as you can remember.**

### **Delayed Recognition Trial Instructions**

The delayed recognition (forced choice) trial is administered immediately after the Delayed Recall trial. Say the following:

**Now I am going to read a longer list of words to you. Some of them are words from the original list, and some are not. After I read each word, I'd like you to say “Yes” if it was on the original list or “No if it was not.**

Read the words of the Delayed Recognition trial list in numerical order. Allow the individual as much time as needed to respond. You may use the prompt, **“Was horse on the list? Yes or no?”** The individual must give you a response for every word. If the individual is not sure, ask for a guess.

## TRAIL MAKING TEST INSTRUCTIONS

### Part A:

Give the subject a pencil and the test page and say: *"On this page are some numbers." Point to some numbers. "Begin at number 1" Point to number 1. "and draw a line from 1 to 2, " Point to number 2. "2 to 3," Point to 3. "3 to 4," Point to 4. "and so on, in order, until you reach the end." Point to the circle marked "end". "Draw the lines as fast as you can. Ready ----- Begin!"* If the subject completes the sample item correctly demonstrating his/her understanding say: *"Good! Let's try the next one."* Turn the paper over and give Part A of the test. If the person makes a mistake on sample A, point out the error and explain it.

The following explanations of mistakes serve as illustrations.

1. "You started with the wrong circle. This is where you start (point to number one)"
2. "You skipped this circle (point to the circle the subject omitted). You should go from number 1 (point) to 2 (point), to 3 (point), and so on, until you reach the circle marked "end" (point)."

If the subject cannot complete Sample A, take his/her hand and guide the pencil, using the eraser end, through the trail. Then say: *"Now you try it."*

Return the pencil to the subject with the point down and say: *"Remember, begin at number 1 (point) and draw a line from 1 to 2 (point to 2), 2 to 3 (point to 3), 3 to 4 (point to 4) and so on, in order, until you reach the circle marked "end" (point). Do not skip around, but go from one number to the next in the proper order. Remember to work as fast as you can. Ready --- Begin!"*

If the subject succeeds this time proceed to Part A. If the subject still has difficulty, repeat the above procedure until the task is completed successfully or it becomes evident that the subject cannot do the task.

After the subject has completed Sample A, turn the paper over to Part A and say: *"On the page are numbers. Do this the same way. Begin at number 1 (point 1) and draw a line from 1 to 2 (point to 2), 2 to 3 (point to 3), 3 to 4 (point to 4), and so on, in order, until you reach the end (point). Remember to work as fast as you can. Ready ---Begin!"*

Using a stopwatch, start timing as soon as the instruction is given to begin. The examiner must watch the subject closely in order to catch any errors as soon as they are made. If the subject makes an error, call it to his/her attention immediately, return the subject's pencil to the last correct circle, and continue the test from that point. Do not stop timing while correcting the subject's error.

After the subject completes Part A, take the test sheet and record the time in seconds. Errors contribute to evaluation of performance principally by increasing the total performance time.

**Trails (Part B):**

Next, tell the patient: *"That's fine. Now we'll try another one."* Place the sample side of Part B on the table in front of the subject, in the same position as the sheet for Part A was placed. Point to the sample and say:

*"On this page are some numbers and letters. Begin at 1 (point) and draw a line from 1 to A" (Point to A) "A to 2," (Point to 2), "2 to B" (point to B), "B to 3" (point to 3), "3 to C" (point to C), "and so on, in order, until you reach the end" (point to the circle marked "end").*

Then say: *"Remember, first you have a number" (point to 1), "then a letter" (point to A), "then a number" (point to 2), "then a letter" (point to B), "and so on. Draw the lines as fast as you can. Ready--- Begin!"*

If the subject completes the sample B correctly say: *"Good! Let's try the next one."* Proceed immediately to Part B. If the subject makes a mistake on sample B, point out the error and explain why it is incorrect. The following explanations of mistakes serve as illustrations:

1. *"You started with the wrong circle. This is where you start (point to number 1)"*
2. *"You skipped this circle" (point to the circle the subject omitted). "You should go from 1" (point to 1) "to A" (point to A), "A to 2" (point to 2), "2 to B" (point to B), "B to 3" (point to 3) "and so on until you reach the circle marked 'end'. (point)*

If the subject cannot complete Sample B, take his/her hand and guide the pencil, using the eraser end, through the circles. Then say: *"Now you try it. Remember, you begin at number 1" (point) "and draw a line from 1 to A" (point to A), "A to 2" (point to 2), "2 to B" (point to B), "B to 3" (point to 3), "and so on until you reach the circle marked "end" (point). "Ready --- Begin!"*

If the subject succeeds this time, go on to Part B. If not repeat the procedure until the task is performed successfully or it becomes evident that the subject cannot do the task.

After the subject has completed the sample, turn the paper over to Part B and say:

*“On this page are both numbers and letters. Do this the same way. Begin at number 1”* (point to 1) *“and draw a line from 1 to A”* (point to A), *“A to 2”* (point to 2), *“2 to B”* (point to B), *“B to 3”* (point to 3), *“3 to C”* (point to C), *“and so on, in order, until you reach the end”* (point to the circle marked "end"). *“Remember, first you have a number”* (point to 1), *“then a letter”* (point to A), *“then a number”* (point to 2), *“then a letter”* (point to B), *“and so on. Do not skip around, but go from one circle to the next in the proper order. Draw the lines as fast as you can. Ready ---Begin!”*

Using the stopwatch, start timing as soon as the subject is told to begin. Remember to be alert for mistakes. If the subject makes an error, point it out immediately, return the subject to the last correct circle, and continue the test from that point. Do not stop timing.

After the subject completes Part B, take the test sheet and record the time in seconds. Errors contribute to the evaluation of the performance principally by increasing the total performance time.

### **Scoring**

Part A and Part B are scored separately. The score for each part is the number of seconds required to complete the task.

## **DIGIT SPAN INSTRUCTIONS**

### **Digit Span** (Wechsler Adult Intelligence Scale - Third Edition)

#### Administration Rules:

Administer Digits Backward even if participant scores a 0 on Digits Forward.

Read digits at a rate of 1 per second in a loud, even voice, dropping the tone of your voice at the end of the string of digits, as if you were ending a sentence.

Write down the numbers that the participant says, in the order he/she repeats them. Do not let the participant know whether or not the responses are correct.

The participant is allowed to change his/her response. If the participant changes the response on one of the items, write 'participant changed mind' next to the correction.

Digits Forward: State to the participant:

***“I am going to say some numbers. Listen carefully, and when I stop, say them right after me.”***

Digits Backward: State to the participant:

***“Now I am going to say some numbers, and this time when I stop I want you to say them backward. For example, if I say 7-1-9 what would you say?”***

If participant says 9-1-7, say ***“That’s right.”*** and continue with test

If participant is incorrect, say ***“No, you would say 9-1-7. I said 7-1-9, so to say it backward, you would say 9-1-7. Now try these numbers. Remember, you are to say them backward. 3-4-8.”*** Do not provide any assistance on this example or any of the items.

Whether or not the participant responds correct (i.e., 8 – 4 – 3), proceed to Trial 1 of Item 1.

#### Scoring:

Each item is scored 0, 1, or 2 points as follows:

- 2 points if the participant passes both trials
- 1 point if the participant passes only one trial
- 0 points if the participant fails both trials

#### Discontinuation Rule:

Digits Forward and Digits Backward

Discontinue test when participant obtains a trial score of 0 on both trials of any item.

## DIGIT SYMBOL SUBSTITUTION TEST INSTRUCTIONS

### DIGIT SYMBOL SUBSTITUTION TEST (Wechsler Adult Intelligence Scale - Third Edition)

- A smooth drawing surface must be provided. If the table has a rough surface, the Record Form should be placed on a clipboard, a piece of cardboard, or another flat surface.
- To introduce the subtest, say:  
**In this section, I'm going to ask you to copy some symbols.**
- If examinees ask what they should do if they make a mistake, encourage them to continue to work as fast as they can. However, do not discourage examinees from making spontaneous corrections unless they do so repeatedly and it impedes their performance.
- If, after completing a row, an examinee to start at the beginning of the row and not to skip any.

#### Item Instructions

Turn to the Digit Symbol-Coding page. Hand the examinee a pencil without an eraser, point to the key above the test items, and say:

**Look at these boxes. Notice that each has a number in the upper part and a special mark in the lower part. Each number has its own mark.**

Point to 1 and its mark in the key, then 2 and its mark. Then point to the seven squares located to the left of the heavy black line and say:

**Now look down here where the squares have numbers in the top part but the squares at the bottom are empty. In each of the empty squares, put the mark that should go there. Like this:**

Point to the first Sample Item, then point back to the key to show its corresponding mark, and say:

**Here is a 2; the 2 has this mark. So I put it in this empty square, like this:**

Write in the symbol. Point to the second Sample Item and say:

**Here is a 1; the 1 has this mark (point to the second Sample Item, then to the mark below the 1 in the key), so I put it in this square.**

Write in the symbol. Point to the third Sample Item and say:

**This number is a 3; the 3 has this mark (point to the third square and to the mark below the 3 in the key). So I put in the square (write in the symbol).**

After marking the first three Sample Items, say:

**Now you fill in the squares up to this heavy line.**



If the examinee makes an error on any of the Sample Items, correct the error immediately and review the use of the key. Continue to provide help if needed. Do not proceed with the subtest until the examinee clearly understands the task. When the examinee completes a Sample Item correctly, offer encouragement by saying **Yes** or **Right**. When all the Sample Items have been completed, say:  
**Now you know how to do them. When I tell you to start, you do the rest of them.**  
Point to the first square to the right of the heavy line and say:

**Begin here and fill in as many squares as you can, one after the other without skipping any. Keep working until I tell you to stop. Work as quickly as you can without making any mistakes.**

Sweep across the first row with your finger and say:

**When you finish this line, go on to this one.**

Point to first square in the second row. Then point to the heavy black line and say:  
**Go ahead.**  
**\*\*\*Begin timing.**

If the examinee omits an item or starts to do only one type (e.g., only the 1's), say:

**Do them in order. Don't skip any.**

Point to the first item omitted and say:

**Do this one next.**

Provide no further assistance except to remind the examinee to continue until instructed to stop. At the end of 120 seconds, say: **Stop**

**MEDICAL COLLEGE OF GEORGIA (MCG) COMPLEX FIGURES TEST  
INSTRUCTIONS**

MCG Complex Figures (A compendium of neuropsychological tests (3<sup>rd</sup> Edition); Strauss E, Sherman EMS, Spreen O. New York, USA: Oxford University Press, 2006: 1216

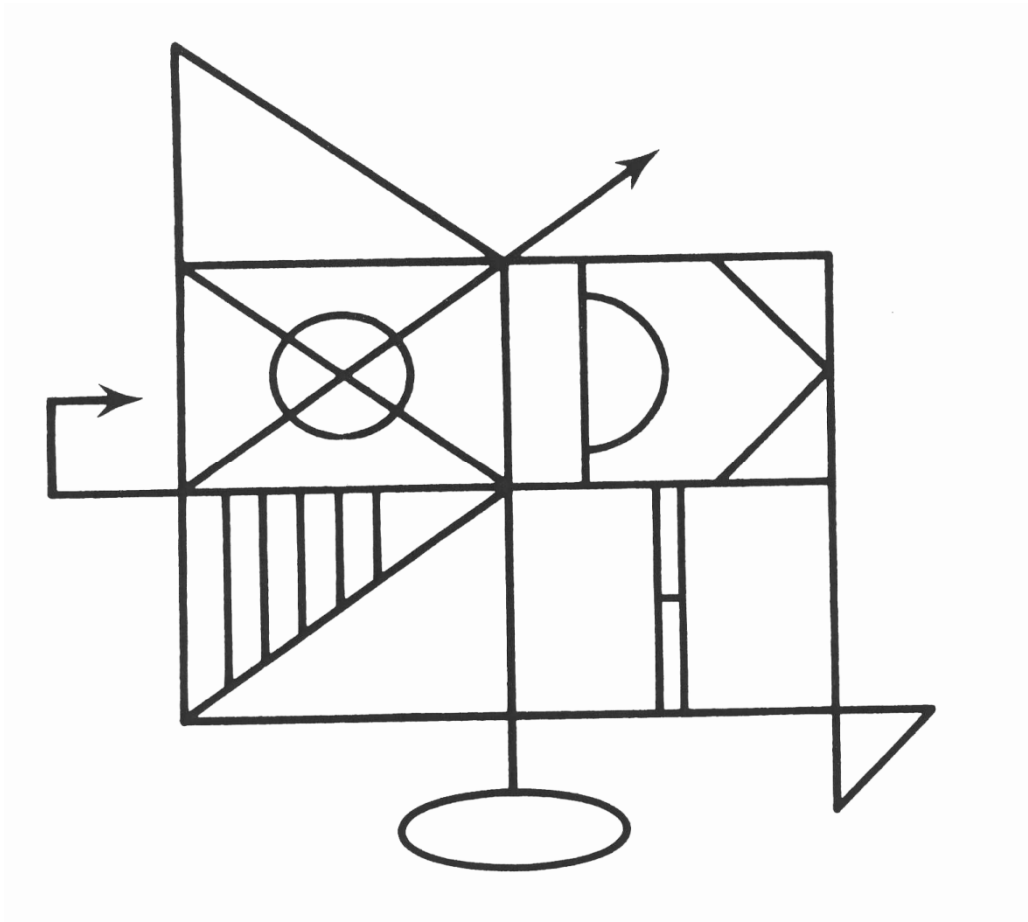
Present figure to participant and ask participant to replicate it as precisely as possible on an 8.5 in. by 11 in. sheet of paper. Once completed, remove the figure. Ask the participant to reproduce the figure following a 3 minute delay (immediate recall) and a 30 minute delay (delayed recall). There are no time limits for all figural reproductions.

**SCORING:**

Consider each of the eighteen units separately. Appraise accuracy of each unit and relative position within the thole of the design. For each unit count as follows:

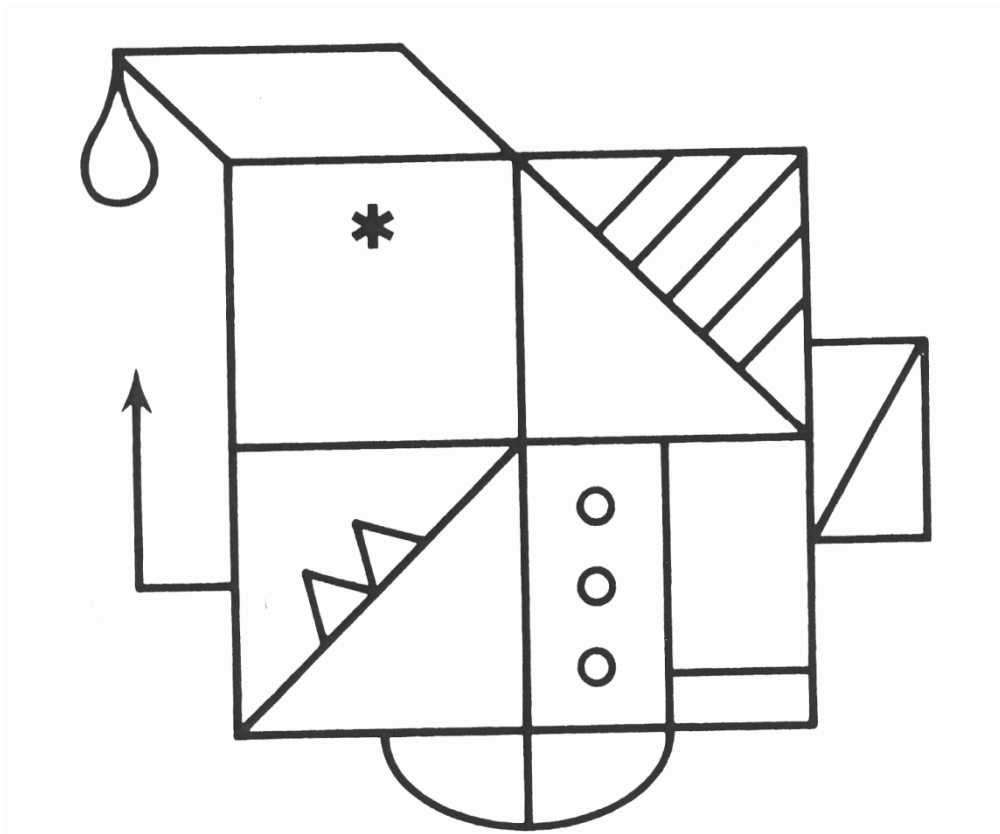
Correct, placed properly	2 points
Correct, placed poorly	1 point
Distorted or incomplete but recognizable, placed properly	1 point
Distorted or incomplete but recognizable, placed poorly	1/2 point
Absent or not recognizable	0 points
Maximum total points	36 points

FIGURE 1:



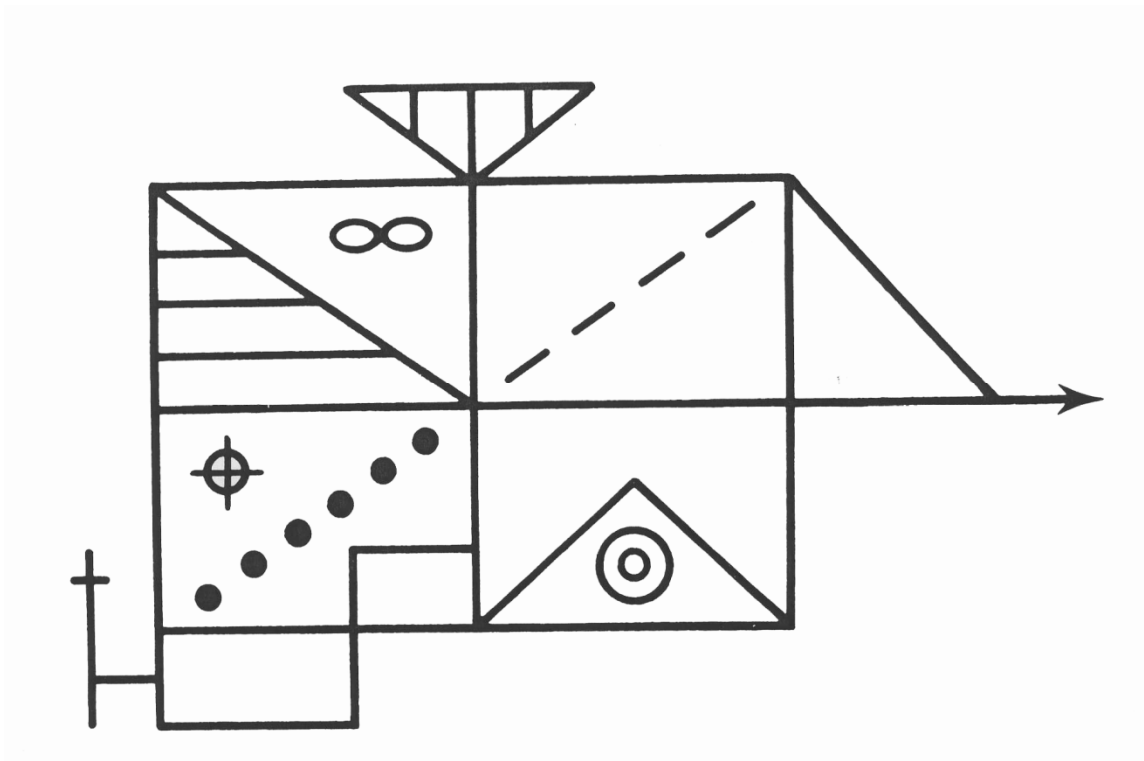
1. Large rectangle
2. Vertical midline of 1
3. Horizontal midline of 1
4. Small triangle on right corner of 1
5. Oval and attaching line at the bottom of 1
6. Bent arrow to the left of 1
7. Triangle above left upper quadrant of 1
8. Tilted arrow at top of 1
9. Diagonal in upper left quadrant of 1
10. Second diagonal in upper left quadrant of 1
11. Circle in upper left quadrant of 1
12. Diagonal in lower left quadrant of 1
13. Five vertical lines extending above 12
14. Vertical lines and horizontal connection ("H") in lower right quadrant of 1
15. Vertical line in right upper quadrant of 1
16. Semicircle attached to the right of 15
17. Diagonal line at upper right corner of 1
18. Diagonal line extending from 17 to 3

**FIGURE 2:**



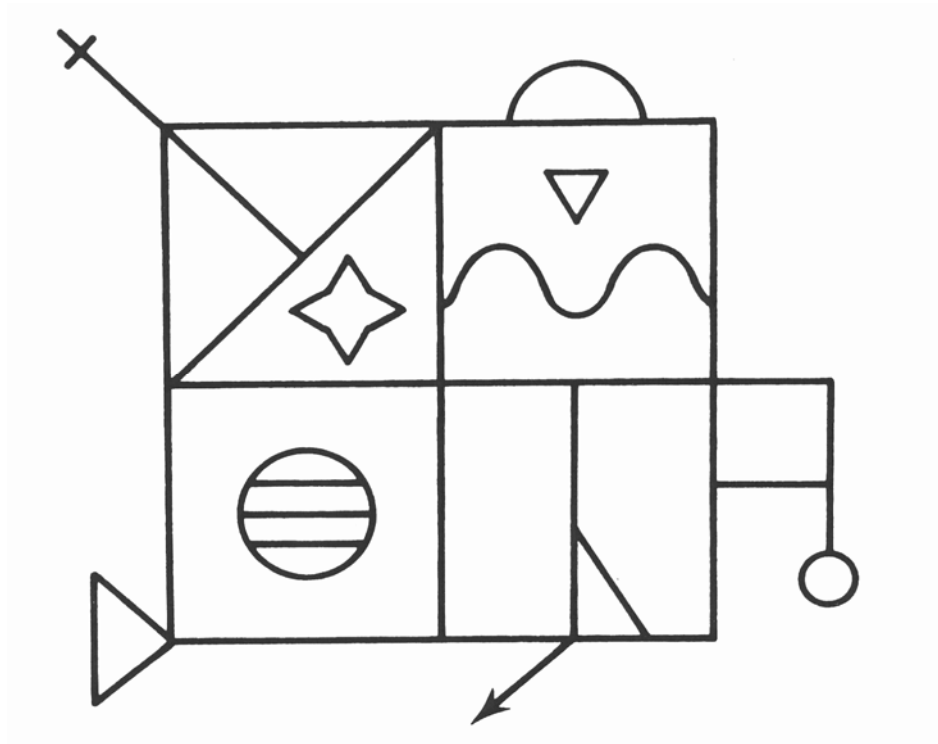
1. Large square
2. Vertical midline for 1
3. Horizontal midline for 1
4. Asterisk in the upper left quadrant of 1
5. Diagonal in the lower left quadrant of 1
6. Two triangles attached to 5
7. Three circles in the lower right quadrant of 1
8. Vertical midline in the lower right quadrant of 1
9. Horizontal line to the right of 8
10. Diagonal line in the upper right quadrant of 1
11. Five diagonal lines perpendicular to 10
12. Small rectangle to the right of 1
13. Diagonal line in 12
14. Semicircle at the base of 1
15. Vertical line in 14
16. Angled arrow to the left of 1
17. Parallelogram above 1
18. Teardrop attached to 17

FIGURE 3:



1. Large rectangle
2. Vertical midline of 1
3. Horizontal midline of 1
4. Diagonal line in left upper quadrant of 1
5. Three horizontal lines extending to 4
6. Infinity sign in left upper quadrant of 1
7. Circle and cross in lower left quadrant of 1
8. Six diagonal dots in lower left quadrant of 1
9. Small rectangle in lower left quadrant of 1
10. Small rectangle extending from bottom of 1
11. Cross attached to 10
12. Right angle in lower right quadrant of 1
13. Two concentric circles placed under 12
14. Four dashed lines in upper right quadrant of 1
15. Triangle atop 1
16. Three vertical lines in 15
17. Triangle to the right of 1
18. Arrow attached to the right of 17

**FIGURE 4:**



1. Large square
2. Vertical midline of 1
3. Horizontal midline of 1
4. Rectangle to the right of 1
5. Circle with stem attached to 4
6. Angled arrow at bottom of 1
7. Small triangle outside lower left corner of 1
8. Cross outside of upper left corner of 1
9. Semicircle on top of 1
10. Diagonal line in the upper left quadrant of 1
11. Perpendicular line to 10
12. Star in the upper left quadrant of 1
13. Circle in the lower left quadrant of 1
14. Three horizontal lines inside of 13
15. Small triangle in upper right quadrant of 1
16. Sine wave in upper right quadrant of 1
17. Vertical midline of the lower right quadrant
18. Diagonal line extending to right of 17

## CONTROLLED ORAL WORD ASSOCIATION (COWA)

### Description

This is an oral fluency test in which the subject is required to make verbal associations to different letters of the alphabet by saying all the words which he or she can think of beginning with a given letter. Three letters of progressively increasing associative difficulty are presented successively as stimuli. The difficulty level of each letter was defined in terms of the relative frequency of words beginning with that letter found in standard dictionaries of the English language.

*Form A:* The letter S (frequency rank =1) is used to demonstrate the test to the patient. The first letter in the test is C (frequency rank =2). The second letter is F (frequency rank = 10). The third letter is L (frequency rank =14). This form has been standardized for clinical use.

*Form B:* The letter S is used to demonstrate the test. The first letter in the test is P (frequency rank =3). The second letter is R (frequency rank =9). The third letter is W (frequency rank =16). This form has not been independently standardized but its correlation with Form A has been assessed. The correlation coefficient between Forms A and B in a sample of 54 normal subjects, who were given both forms in counterbalanced order, was .82. Mean scores for Forms A and B were 36.9 and 38.1 respectively, the difference between the means being non-significant.

### Administration

*Instructions:* "I AM GOING TO SAY A LETTER OF THE ALPHABET AND I WANT YOU TO SAY AS QUICKLY AS YOU CAN ALL THE WORDS THAT YOU CAN THINK OF WHICH BEGIN WITH THAT LETTER. YOU MAY SAY ANY WORDS AT ALL, EXCEPT PROPER NAMES SUCH AS THE NAMES OF PEOPLE OR PLACES. SO YOU WOULD NOT SAY *ROCHESTER* OR *ROBERT*. ALSO DO NOT USE THE SAME WORD AGAIN WITH A DIFFERENT ENDING, SUCH AS *EAT* AND *EATING*. FOR EXAMPLE OF I SAY S, YOU WOULD SAY *SON, SIT, SHOE* OR *SLOW*. CAN YOU THINK OF OTHER WORDS BEGINNING WITH THE LETTER S?"

Wait for the subject to give a word. If successful, indicate that he or she is performing correctly and ask for another word beginning with the letter S. If he or she gives a second appropriate word, indicate that the subject is performing correctly and proceed to the test itself. If an inappropriate word is given on either occasion, correct him or her and repeat the instructions. If the subject then succeeds, proceed to the test. If he or she fails to respond, repeat the instructions. If it becomes clear that the subject does not understand the instructions or cannot associate, terminate the procedure.

If the subject has succeeded in giving two appropriate words beginning with the demonstration letter, say, "THAT IS FINE. NOW I AM GOING TO GIVE YOU ANOTHER LETTER AND AGAIN YOU SAY ALL THE WORDS BEGINNING WITH THAT LETTER THATN YOU CAN THINK OF. REMEMBER, NO NAMES OR PLACES, JUST ORDINARY WORDS. ALSO, IF YOU SHOULD DRAW A BLANK, I WANT YOU TO KEEP ON TRYING UNTIL THE TIME LIMIT IS UP. YOU WILL HAVE ONE MINUTE FOR EACH ONE. THE FIRST LETTER IS C."

*Allow one minute.* If the subject discontinues before the end of the time period, encourage him or her to try to find more words. If silent for 15 seconds, repeat the basic instruction and the letter. Not extension on the time limit is made in the event that the instruction is repeated in the course of the association.

Continue the test with the letters F and L, allowing one minute for each. If the patient produces one or more questionable responses (e.g. *frank, ford*, which could represent a proper name), the associations should simply be recorded and he or she should not be interrupted. However, at the end of the one minute period of association, the patient should be asked what he or she meant by the responses.

**Recording and Scoring**

The Record Sheet provides numbered lines on which the subject's responses can be entered. If the speed of word production is too fast to permit verbatim recording, a "+" should be entered to indicate a correct response. However, all incorrect responses should be recorded verbatim.

The instructions include a specific prohibition against giving different forms of the same word. Hence, inflections of the same word (e.g., *eat-eating; eat-ate; mouse-mice; eat-eats; loose-loosely; eat-eaten*) are not admissible responses. Subjects often give both a verb and the substantive derived from the verb or adjective (e.g., *fun-funny; sad-sadness*). These are not admissible responses. On the other hand, if the substantive refers to a specific object (e.g., *clap-clapper; foot-footstool; hang-hanger*) it would be counted as an admissible response.

Repetition of a word having more than one meaning (e.g., *foot; can; hand*) is acceptable if the subject definitely indicates the alternative meaning. Slang terms are admissible if in general use. Foreign words (e.g., *passé; lasagna; pasta; Lebensraum*) are admissible if they can be considered part of the English lexicon, the criterion being their listing in a standard English dictionary.

The total number of acceptable responses for the three letters constitutes the patient's raw score on the test.



## **APPENDIX X: Anti-HLA Antibody Desensitization Guidelines**

*(Developed by Columbia University Medical Center Transplantation Immunology Service)*

### **Rationale**

Certain individuals who are awaiting organ transplantation have high levels of circulating anti-HLA antibodies, as measured by routine panel reactive antibody (PRA) testing. The precise etiology of these antibodies is not always clear; however blood transfusions, multiparity, prior organ transplantation, and ventricular assist devices may place the patient at increased risk for sensitization. To minimize the risk of acute humoral rejection of a transplanted heart, potential cardiac allograft recipients at Columbia Presbyterian Medical Center (CPMC) who have elevated PRA levels are presently tested for the presence of donor-specific antibodies prior to receiving a particular organ. A positive cross-match precludes the potential recipient from receiving the organ. Very frequently, sensitized patients undergo multiple unsuccessful donor-specific crossmatches until a negative cross match is identified. Therefore, the presence of anti-HLA antibodies in an individual's circulation leads to a prolongation in the waiting period for cardiac transplantation.

Unfortunately, a negative cross match alone does not eliminate post transplantation immunologic complications. Firstly, the presence of elevated levels of preformed circulating anti-HLA antibodies prior to cardiac transplantation has been reported by several centers to be a risk factor for increased mortality caused by acute and chronic rejection, and for significantly decreased long-term graft survival (1,2). Secondly, there is a theoretical risk that patients with high pre-transplant levels of non donor-specific anti-HLA antibodies may be more likely to develop donor-specific antibodies post-transplantation and consequently to be at risk for acute humoral rejection of the allograft. A third complication appears to be specific for retransplantation candidates. The onset of accelerated coronary artery disease (CAD) in cardiac transplant recipients has been associated with the development post-transplant of both donor-specific and non-specific anti-HLA antibodies (3-5). Since these antibodies appear to precede the development of CAD, and may be causative in the pathogenesis of the lesions, it is quite possible that their presence prior to retransplantation may portend an even poorer outcome in the second graft than in the first. For these reasons, an immunosuppressive regimen is instituted to deplete circulating anti-HLA antibodies pre-transplant in order to increase the likelihood of a negative cross match, and continued immunosuppressive therapy post-transplant to prevent the development of anti-HLA antibodies, thereby attempting to decrease the incidence of acute and chronic rejection, and of accelerated coronary artery disease.

This guideline uses a combination of IVIg, Cytoxan and plasmapheresis on cardiac transplant outcome of highly sensitized individuals. IVIg has been used extensively in antibody-mediated autoimmune disorders, and its effect may relate to interruption of idiotypic-antiidiotypic networks. IVIg can prevent the binding of anti-HLA antibodies to their target cells and inhibit complement-mediated cytotoxicity by these antibodies (6). In alloimmunized patients awaiting kidney transplantation a single course of IVIg was accompanied by a significant and prolonged decrease of anti-HLA antibodies in 50% of treated patients, enabling transplantation to proceed (7).

Cytosan has been used for many years for the treatment of diseases of disordered immunity, in particular those associated with autoantibody formation such as systemic lupus erythematosus. This agent is particularly effective in vitro against B cells, and inhibits both IgG and IgM antibody

production by these cells. Since its mode of action involves anti-mitotic mechanisms, it is most effective when used against B cells which are proliferating highly. Plasmapheresis removes circulating antibodies. While this leads to rapid antibody depletion, the effect is temporary since removal of IgG antibodies also removes inhibitory feedback controls and, paradoxically, stimulates B cell clones to proliferate and secrete more IgG. This effect has been used by various investigators to develop a method of delivering Cytoxan at a time when B cells are maximally proliferating, typically 7-10 days after depletion of circulating IgG antibodies by plasmapheresis. This type of immunosuppression is termed “synchronization”.

### **Procedures**

Patients with a clinical indication for anti-HLA desensitization should receive 1-2g IVIg/kg in four divided daily doses. Anti-HLA levels should then be measured on a weekly basis. Patients whose anti-HLA antibody levels are unchanged after completion of multiple courses of the IVIg may be considered to undergo 2-3 weekly treatments of plasmapheresis. After 7 days, patients should receive an intravenous (IV) infusion of Cytoxan at 0.5-1g/m<sup>2</sup> total body mass. This should be accompanied by IV Mesna at mg/mg of Cytoxan to prevent hemorrhagic cystitis. Patients should be adequately hydrated and given prophylaxis for nausea. Following IV Cytoxan administration, complete blood cell counts (CBC) with differentials should be monitored closely until the nadir of leukocyte depletion is observed, then weekly-biweekly thereafter. The dose of Cytoxan should be adjusted according to the individual response to the previous course. Throughout this period, patient sera should be cross-matched with lymphocytes of appropriate potential donors, and patients may be transplanted if the results are negative. Serum creatinine levels should be obtained prior to daily IVIg administration.

IVIg and cyclophosphamide infusions should be repeated every 3 weeks awaiting transplantation.

Following cardiac transplantation all patients should receive monthly IV Cytoxan at 0.5-1g/m<sup>2</sup> in addition to Cyclosporine and Prednisone at dosage schedules used in other transplant recipients. It is anticipated that during the 6-month period post-transplantation most patients will be weaned off Cytoxan. Mycophenolate mofetil should be substituted for Cytoxan at this time

### **Drugs**

Side-effects of IVIg may include renal insufficiency, headaches, chills and fevers, and symptoms of serum sickness such as arthralgias or rashes. These are generally transient, and can be managed by using IVIg preparations with no or low dose sucrose content, and premedications with anti-inflammatory agents.

Cytoxan is a non-specific immunosuppressive agent, causing suppression of all elements of the immune system. Since the objective is to obtain selective B cell suppression, Cytoxan dosage will be carefully tailored to prevent myelosuppression. Cytoxan metabolites are excreted in the urine. These may be toxic to the bladder and can cause hemorrhagic cystitis and bladder neoplasia. All patients may be referred for cystoscopic visualization of the bladder every six months. Bladder complications are significantly reduced by giving Cytoxan as a bolus infusion rather than daily oral doses. In addition, use of Mesna together with Cytoxan binds up the metabolites and decreases bladder complications. Other potential side effects of Cytoxan include decreased fertility and increased long-term risk of certain cancers. It is important to note that in long-term studies of

patients with lupus nephritis treated with intravenous pulses of Cytoxan at dosages similar to those proposed in this guideline, no increased incidence of malignancy or hemorrhagic cystitis was observed (mean follow-up periods 48-52 months, mean of 9 monthly Cytoxan pulses) (8,9).

Side-effects of plasmapheresis may include lightheadedness, transient mild hypotension, chills and fevers. There may be some bleeding or bruising at the needle insertion site, however no coagulation abnormalities should occur since clotting factors will be adequately replaced during each treatment. If patients have poor venous access, an indwelling catheter may have to be placed in the internal jugular vein.

### **Clinical Parameters**

Hematologic exclusionary criteria for all patients will include Hb <8.0 g/dl, WBC <2000/mm<sup>3</sup>, and platelets <20,000.

Hemodynamic exclusionary criteria for consideration of plasmapheresis:

- Systolic BP <90mm Hg
- Cardiac index <2.0 l/min/m<sup>2</sup>
- Ejection fraction <20%
- Dopamine >15 mcg/kg
- Dobutamine >10 mcg/kg

Therapies may be held or interrupted in the presence of active infection, and re-considered within two-four weeks of having completed treatment.

### **Risks and benefits**

For risks, please refer to the section headed as drugs.

It is anticipated that use of the treatments outlined in this guideline will lead to successful depletion of circulating anti-HLA antibodies pre and post cardiac transplantation. This should shorten the pre-transplant waiting period for a sensitized patient by increasing the chances of a successful donor-specific cross match. In addition, reduction of circulating anti-HLA antibodies may decrease the frequency or severity of post-transplant rejection episodes and increase long-term survival.

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