PROSPECTIVE, MULTI-CENTER, RANDOMIZED CLINICAL INVESTIGATION OF TRANSMEDICS® ORGAN CARE SYSTEM™ (OCS) FOR CARDIAC USE

Protocol CAR-05-2008

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CONFIDENTIAL – PROPRIETARY INFORMATION

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Investigator Agreement and Certification

PROSPECTIVE, MULTI-CENTER RANDOMIZED, CLINICAL INVESTIGATION OF TRANSMEDICS® ORGAN CARE SYSTEM™ (OCS) FOR CARDIAC USE

Protocol Version 1.6

I hereby agree to participate in this clinical investigation of the Organ Care System sponsored by TransMedics, Inc. (here in after "Study Sponsor"). I agree to conduct this investigation according to the requirements of the protocol provided by the Study Sponsor in accordance with applicable regulations from the U.S. Food and Drug Administration (FDA), with local regulations, and in accordance with the conditions imposed by the reviewing Institutional Review Board (IRB) or Ethics Committee (EC). I agree to supervise all use of the investigational devices and to ensure appropriate informed consent is obtained from all subjects prior to inclusion in this study.

I understand that this investigation will be monitored by the Study Sponsor and/or a designee employed by the Study Sponsor. This monitoring will involve periodic inspection of my investigational site and ongoing review of the data that are submitted by me to the Study Sponsor. I am also aware that I may be inspected by a representative of the FDA to verify compliance with applicable regulations related to clinical research on human subjects. I am aware that my contact for all matters related to this investigation is Leslie Rose at 978-552-0956.

I am aware that the Study Sponsor reserves the right to discontinue this investigation at any time.

My current curriculum vitae has been provided to the Study Sponsor along with the curriculum vitae of those physicians at this institution who will be using the investigational device or participating in this study as co-investigators under my supervision. This includes the extent and type of our relevant experience with pertinent dates and locations.

I certify that I have not been involved in an investigation that was terminated for noncompliance at the insistence of the Study Sponsor, the IRB or EC, or other regulatory authorities. I agree to provide the Study Sponsor sufficient, accurate financial disclosure information in accordance with US regulations 21 CFR Part 54.

I understand this study protocol and trial results are confidential, and I agree not to disclose any such information to any person other than a representative of the Study Sponsor or regulatory authorities without the prior written consent of the Study Sponsor.

Accepted by:

_____ _____

Principal Investigator Date

_____

Printed Name

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

AE	Adverse Event	**IRB**	Institutional Review Board
ALT	Alanine Transaminase	**ISHLT**	International Society for Heart and Lung Transplantations
AOP	Aortic Pressure	**ISO**	International Standards Organization
AST	Aspartate Transaminase	**K^+**	Potassium
BHCG	Beta Human Chorionic Gonadotropin	**LVH**	Left Ventricular Hypertrophy
BUN	Blood Urea Nitrogen	**MedDRA**	Medical Dictionary for Regulatory Activities
CEC	Clinical Event Committee	**Na^+**	Sodium
CF	Coronary Flow	**NYHA**	New York Heart Association
CFR	Code of Federal Regulations	**OCS**	Organ Care System
CO_2	Carbon Dioxide	**OR**	Operating Room
CMV	Cytomegalovirus	**PAP**	Pulmonary Artery Pressure
CRF	Case Report Form	**PCO_2**	Partial Pressure (Tension) of Carbon Dioxide
CV	Curriculum Vitae	**PCWP**	Pulmonary Capillary Wedge Pressure
CVA	Cerebrovascular Accident	**pH**	Hydrogen Ion Concentration
CVP	Central Venous Pressure	**PO2**	Partial Pressure (Tension) of Oxygen
DCF	Data Clarification Form	**PRCBC**	Packed Red Blood Cells
DMB	Data Monitoring Board	**PTLD**	Post-Transplant Lymphoproliferative Disorder
ECG	Electrocardiogram	**Rh**	Rhesus Factor in Blood
ECMO	Extracorporeal Membrane Oxygenation	**SAE**	Serious Adverse Event
FDA	Food and Drug Administration	**SVR**	Systemic Vascular Resistance
HCO_3	Bicarbonate	**TTE**	Transthoracic Echocardiogram
HCT	Hematocrit	**TIA**	Transient Ischemic Attack
IABP	Intraaortic Balloon Pump	**UNOS**	United Network for Organ Sharing
ICU	Intensive Care Unit	**VAD**	Ventricular Assist Device
INR	International Normalized Ratio (for Anticoagulant Monitoring)	**WBC**	White Blood Cell (count)

PROTOCOL SYNOPSIS

Protocol Title	Prospective, Multi-Center, Randomized Clinical Investigation of TransMedics, Inc.'s Organ Care System (OCS) for Cardiac Use
Intended Use of the Device Under Investigation	To preserve and monitor a donated heart during transportation for the eventual transplantation into a recipient.
Objective	To compare the safety and effectiveness of the OCS with the existing cold static cardioplegia standard of care for the preservation of donor hearts.
Study Design	A prospective, multi-center, two-armed randomized investigation.
Study Size	A maximum of 20 study sites and 128 male or female subjects who are primary heart transplant recipients, 64 in each arm.
Treatment Plan	Primary heart transplant recipients will be screened for study participation. Donor hearts will be evaluated for eligibility. Eligible donor hearts will be randomized and harvested and then preserved and transported on the Organ Care System or using cold static cardioplegia.
Primary Endpoint Effectiveness	30-day patient survival following transplantation with the originally transplanted heart and no mechanical circulatory assist device at Day 30. **Criterion for Success:** The proportion of subjects who achieve 30-day survival following transplantation with the originally transplanted heart and no mechanical circulatory assist device at the 30-day follow-up in the OCS group is established statistically as not being inferior to the proportion in the control group.
Secondary Endpoints	• Incidence of all cardiac related serious adverse events. • Incidence of biopsy proven ISHLT grade 2R (moderate) or 3R (severe) acute rejection on any of the surveillance endomycardial biopsies or clinically symptomatic rejection requiring augmentation of immunosuppressive therapy during the 30-day follow-up period. • Length of ICU stay.
Analysis populations	The primary analysis of the effectiveness endpoints will be based on the per protocol population; a secondary analysis will be based on the intent to treat population. As treated and completed treated analyses will also be conducted on the efficacy endpoints.
Analysis populations	Safety analyses will be based on the treated population.

Safety	
Safety Analyses	Safety will be analyzed principally by examination of the frequency of adverse events: • The incidence of all cardiac-related serious adverse events up to the 30-day follow-up will be examined. • The numbers and percentages of subjects experiencing at least one AE, at least one serious AE, at least one device-related AE, at least one unanticipated AE, and the number and percentage of deaths will all be tabulated by treatment group. • Also, the number of adverse events and the number and percent of subjects experiencing adverse events will be tabulated by system organ class and preferred term using MedDRA.
Study Duration	Subjects are followed for 30 days from the date of transplantation
Inclusion Criteria	**Donor Hearts** • < 60 years old • Mean arterial blood pressure > 60 mmHg at the time of final heart assessment • Satisfactory echocardiography assessment defined as: – Ejection fraction > 40% – Absence of severe segmental wall motion abnormalities – Absence of left ventricular hypertrophy (Inter Ventricular Septum (IVS) and Posterior Wall Thickness (PWT) < 1.3 cm) – Absence of valve abnormalities (trace to mild valvular regurgitation is acceptable) **Recipient Day of Transplant** • Registered male or female primary heart transplant candidate • ≥18 years old • Signed, written informed consent document and authorization to use and disclose protected health information
Exclusion Criteria	**Donor Hearts** • Abnormal coronary angiogram defined as > 50% stenosis, requiring coronary bypass • Donor-to-recipient body weight ratio of < 0.6 • Vasoactive medicinal support at time of final heart assessment including, but not limited to: – Dopamine > 10 ug/kg/min – Dobutamine > 10 ug/kg/min – Milrinone > 0.3 ug/kg/min – Epinephrine > 0.03 ug/kg/min – Norepinephrine > 0.03 ug/kg/min – Any bolus dose of the above prior to explants that would result in exceeding the above stated criteria

	• Presence of any exclusion criterion based on the standard practice of the investigational site Note: It is recommended that all vasoactive medications are lowered to minimal levels or completely weaned off prior to blood collection. **Recipient Day of Transplant** • > 4 previous sternotomies • Chronic renal failure as defined by chronic serum creatinine >3.0 mg/dL for more than 2 weeks and/or requiring hemodialysis (except for hemodialysis or hemofiltration for fluid overload) • Ventilator dependence at the time of transplant • Use of a ventricular assist device for > 30 days and the presence of any of the following: systemic sepsis, intracranial hemorrhage or heparin induced thrombocytopenia • Panel reactive antibodies > 40% with a positive prospective cross match and/or virtual cross match • Use of any investigational drug or device, other than OCS, during the study • Simultaneous transplant of non-heart allograft, except for concurrent kidney transplant
Study Sponsor	TransMedics, Inc. 200 Minuteman Road, Suite 302 Andover, MA 01810

1. Introduction

1.1 Name and Intended Use

The TransMedics Organ Care System is a portable organ perfusion and monitoring system intended to preserve a donated heart in a beating state during transport for the eventual transplantation into a recipient.

The Organ Care System (OCS) maintains organ viability by providing a controlled environment, continuously perfusing the donated heart with warm, oxygenated blood, supplemented with the TransMedics Cardiac Solution Set. The blood is collected from the donor and is continuously circulated to the organ in a closed circuit along with the Cardiac Solution Set.

The OCS preserves the heart and monitors the organ's perfusion parameters immediately after explantation from a donor and connection to the device, during transportation to the recipient site, and until disconnection from the device.

The OCS is intended for use only by qualified health care professionals specializing in organ transplants and trained in the use of the device.

1.2 Cardiac Transplantation

Cardiac transplantation has become a viable therapeutic option for patients with end-stage heart disease.[1] Significant progress has been made over the last 30 years in donor management, operative technique, immunosuppression regimens, and postoperative care. Despite these recent advances, primary graft dysfunction and acute heart failure continue to contribute significantly to postoperative morbidity and mortality after cardiac transplantation.[2] Although the etiology of these failures is likely multifactorial, suboptimal cardiac preservation and subsequent ischemia or reperfusion injury sustained by the heart play a role. New methods for donor heart preservation, which address these issues, may help to further advance cardiac transplantation.

OCS uses a combination of technological characteristics and incorporates a number of monitors to assess organ perfusion and preservation conditions, including flow rates, pressures and temperatures.

1.3 Prior Testing

The OCS has undergone extensive preclinical testing to demonstrate its safety, effectiveness and readiness for clinical use. Preclinical and animal studies demonstrate the OCS maintains and monitors a heart, and performs as intended. The Cardiac Disposable Set has been evaluated and tested in accordance with ISO-10993 "Biological Evaluation of Medical Devices," including evaluations for acute toxicity, irritation, sensitization, cytotoxicity, hemolysis, genotoxicity and pyrogenicity. These test results demonstrate the device and its materials are biocompatible and suitable for their intended use. The Cardiac Perfusion Set and the Cardiac Solution Set are sterilized using validated methods, and are appropriately packaged to maintain sterility.

The OCS also has undergone extensive preclinical testing, including electrical safety testing, electromagnetic compatibility testing, and validation and verification testing (which included an evaluation of the device's software). All tests and results demonstrate the OCS meets its

performance specifications and is safe and suitable for its intended clinical use. The OCS has also been tested in clinical settings in both Europe and the US. The most recent analysis of October 21, 2007 is presented in Section 14 of this document.

1.4 Purpose of the Clinical Study

The purpose of this study is to compare the safety and effectiveness of the OCS with the existing cold static cardioplegia standard of care for the preservation of donor hearts.

1.5 Size, Subject Follow-up and Duration of the Study

This study will be conducted at no more than 20 institutions and will include 128 subjects, 64 in each arm.

The number of subjects was determined by the statistical methods described in the statistical analysis described in Section 12, based on the assumption of 94% subject survival with no graft failure at the 30 day follow-up for the control, and at least a 95% subject survival with no graft failure at the 30 day follow-up for the OCS arm. The 94% estimate for the control is based upon results from the "Celsior™ study" where patient survival with the Celsior™ solution was 94%.[3] The unadjusted 30-day patient survival for heart transplant patients according the 2006 US Transplant Annual Report (SRTR, www.ustransplant.org) accessed on February 1, 2008 is 92.3%.

Subjects are followed for 30 days from the date of transplantation, unless they need to be followed to determine the resolution or stabilization of adverse events that occur within 30 days, to capture any newly occurring serious adverse event after 30 day follow-up and prior to discharge, to identify the length of hospital stay should the subject not be discharged $\leq$ 30 days, or to determine the time on ventilation if the subject was on a ventilator up to the 30-day follow-up.

All hearts preserved on the OCS or cold static cardioplegia are expected to be transplanted unless the OCS has malfunctioned, or sustained damage that adversely affects the heart, or if a surgeon based on his/her clinical judgment and to protect the enrolled patient's welfare and safety decided not to transplant a heart.

In those instances when a randomized subject does not receive a heart immediately subsequent to their randomization and procurement, the subject will be followed throughout the duration of the trial until he or she receives a heart, or is removed from the transplant waitlist. Any newly occurring serious adverse event will be collected during the follow-up period. However, since the subject would not have received a heart subsequent to randomization, none of the evaluations requiring interventions will be conducted, e.g., biopsies and echocardiograms, until the subject receives a transplanted heart according to their assigned randomization method. When this occurs, he or she will be followed for 30 days starting from the new transplant date and all information will be collected. Any organ that is not used regardless of the method of preservation will be sent to pathology for a complete histopathological evaluation (See Appendix D). All decisions not to transplant hearts will be reviewed by the Clinical Event Committee (CEC) and the Data Monitoring Board (DMB).

2. Study Rationale and Objectives

The OCS has been designed to improve organ preservation and overcome the limitations of current organ preservation techniques, such as time-dependent ischemia and reperfusion injuries.

The primary objective of this study is to compare the safety and effectiveness of the OCS with the existing cold static cardioplegia standard of care for the preservation of donor hearts. Specifically, the primary outcome measure is 30-day patient survival following transplantation with originally transplanted heart and no mechanical circulatory assist device at Day 30.

While seven-day outcomes are often used as the primary endpoint in studies of this nature, TransMedics believes it is more appropriate to use a 30-day survival endpoint. It is likely that severe ischemic damage will appear early postoperatively, i.e., in less than seven days, but less severe ischemic injuries may persist beyond seven days. Beyond thirty days, it is likely that other factors besides the quality of the transplanted organ will become more important to long-term outcomes; it is at this point that patients begin to be entered into long-term various immunosuppressive regimens (Fyfe et al, 1996; and, Naftel and Brown, 2000).[4]

3. Study Design

3.1 Type of Study

This is a two-armed, prospective, multi-center, randomized, open label investigation with subjects assigned to the standard cold static cardioplegia or the OCS.

3.2 Study Treatments

After screening and confirmation of eligibility, donor hearts will be preserved and transported using the OCS or according to the standard cold static preservation method of the participating institution. Eligible recipients will undergo heart transplantation according to standard practices at the investigational sites.

3.2.1 Standard of Care

The standard of care cold static cardioplegia is defined as preservation of an arrested donor heart in a static manner in an ice box using the cold preservation solution that is standard practice at the investigational site or commercially available. No machine perfusion devices are part of the standard of care. No heart and lung blocks are part of the control arm.

3.2.2 OCS

All donor hearts preserved and transported using the OCS will use the institution's standard of care or commercially available cardioplegia or preservative solutions, to arrest and cool the heart before and after preservation on the OCS.

4. Study Endpoints

4.1 Primary Study Endpoints and Criterion for Success

The primary study endpoint for effectiveness is 30-day patient survival following transplantation with the originally transplanted heart and no mechanical circulatory assist device at Day 30. The criterion for success is that the proportion of subjects who achieve 30-day survival following transplantation with the originally transplanted heart and no mechanical circulatory assist device at the 30-day follow-up in the OCS group is established statistically as not being inferior to the proportion in the control group.

4.2 Secondary Study Endpoints

The secondary study endpoints are as follows:

- Incidence of all cardiac related serious adverse events.
- Incidence of biopsy proven ISHLT grade 2R (moderate) or 3R (severe) acute rejection on any of the surveillance endomycardial biopsies or clinically symptomatic rejection requiring augmentation of immunosuppressive therapy during the 30-day follow-up period.
- Length of ICU stay.

5. Study Population

The study will enroll 128 subjects who are primary heart transplant recipients, 64 in each arm, at no more than 20 investigational sites.

5.1 Informed Consent

A written informed consent will be obtained from all subjects (or their guardian or legal representative) before any study-related procedures (including any pre-treatment procedures) are performed. Investigators may discuss the availability of the study and the possibility for entry with a potential subject without first obtaining consent. Informed consent will be obtained and documented prior to initiation of any procedures that are performed solely for the purpose of determining eligibility for research. Potential subjects who are initially found to be eligible for treatment within the context of the investigation will be evaluated again for eligibility just prior to transplantation. Potential subjects who are initially consented and screened but are found to be ineligible for enrollment as part of final eligibility evaluations; and, subjects who are eligible based on the first and second evaluations but for whom it is determined at the donor site that no matching or eligible donor is found, will not be considered enrolled or part of the "intent to treat" population.

The investigators have both an ethical and legal responsibility to ensure that each subject being considered for inclusion in this study is given a full explanation of the protocol. This will be documented on a written informed consent form that will be approved by the same Institutional Review Board (IRB) or Ethics Committee (EC) responsible for approval of the study. Each informed consent form will include the elements required by local regulations. The investigator

agrees to obtain approval from the Sponsor of any written informed consent form used in the study.

The IRB or EC-approved written informed consent form will be signed by the subject and the investigator (or investigator and IRB/EC-approved designee) obtaining consent. The subject will be given a copy of the signed informed consent form. The original will be kept on file by the investigator.

5.2 Donor Eligibility Criteria

5.2.1 Donor Inclusion Criteria

Donors are required to meet the inclusion criteria specified in Table 5-1.

Table 5-1: Inclusion Criteria for Donor Hearts	
1.	< 60 years old
2.	Mean arterial blood pressure > 60 mmHg at the time of final heart assessment
3.	Satisfactory echocardiography assessment* defined as: • Ejection fraction > 40% • Absence of severe segmental wall motion abnormalities • Absence of left ventricular hypertrophy (Inter Ventricular Septum (IVS) and Posterior Wall Thickness (PWT) < 1.3 cm) • Absence of valve abnormalities (trace to mild valvular regurgitation is acceptable)

*A copy of the echo media (DVD, CD, VHS) should be obtained from donor site if possible for all hearts considered eligible for the study.

5.2.2 Donor Exclusion Criteria

Donor hearts will be excluded if they meet any of the criteria specified in Table 5-2.

Table 5-2: Exclusion Criteria for Donor Hearts	
1.	Abnormal coronary angiogram defined as > 50% stenosis, requiring coronary bypass
2.	Donor-to-recipient body weight ratio of < 0.6
3.	Vasoactive medicinal support at time of final heart assessment including, but not limited to: • Dopamine > 10 ug/kg/min • Dobutamine > 10 ug/kg/min • Milrinone > 0.3 ug/kg/min • Epinephrine > 0.03 ug/kg/min • Norepinephrine > 0.03 ug/kg/min • Any bolus dose of the above prior to explants that would result in exceeding the above stated criteria Note: It is recommended that all vasoactive medications are lowered to minimal levels or completely

	weaned off prior to blood collection.
4.	Presence of any exclusion criterion based on the standard practice of the investigational site

5.3 Recipient Eligibility Criteria – Screening and Day of Transplant

5.3.1 Inclusion Criteria

Recipients are required to meet the following criteria specified in Table 5-3 at the time of screening and again on the day of transplant.

Table 5-3: Inclusion Criteria for Recipients – Screening & Day of Transplant	
1.	Registered male or female primary heart transplant candidate
2.	≥ 18 years old
3.	Signed, written informed consent document and authorization to use and disclose protected health information

5.3.2 Exclusion Criteria

Recipients will be excluded if they meet any of the criteria specified in Table 5-4 at the time of screening and again on the day of transplant.

Table 5-4: Exclusion Criteria for Recipients – Screening & Day of Transplant	
1.	> 4 previous sternotomies
2.	Chronic renal failure as defined by chronic serum creatinine >3.0 mg/dL for more than 2 weeks and/or requiring hemodialysis (except for hemodialysis or hemofiltration for fluid overload)
3.	Ventilator dependence at the time of transplant
4.	Use of a ventricular assist device for > 30 days and the presence of any of the following: systemic sepsis, intracranial hemorrhage or heparin-induced thrombocytopenia
5.	Panel reactive antibodies > 40% and positive prospective cross match and/or virtual cross match
6.	Use of any investigational drug or device, other than OCS, during the study
7.	Simultaneous transplant of non-heart allograft, except for concurrent kidney transplant

6. Device Description

The OCS consists of the following major components:

Organ Care System: The Organ Care System is the non-sterile, reusable portable enclosure that houses the permanent infusion and circulatory pumps, the batteries, the electronics, gas delivery devices, monitoring sub-systems, and wireless monitor. The wireless monitor allows the user to adjust various setting of the system and displays information.

Cardiac Perfusion Set: The Cardiac Perfusion Set is a sterile, single-use organ chamber and circuit. It consists of an organ chamber, tubing, connectors, blood reservoir and oxygenator, pump head (dome) for interface with the OCS circulatory pump, warmer, user-controlled valves, and integrated pressure, temperature, and heart rate sensors. It provides a closed circuit to perfuse the heart with oxygenated, warm blood supplemented with the Cardiac Solution Set to maintain and assess the organ and its preservation conditions during use.

Cardiac Solution Set: The Cardiac Solution Set is a sterile, single-use, solution set used to prime the Cardiac Perfusion Set and replenish necessary substrate throughout transport. It can be used in a variety of physical settings such as an operating room, ambulance, helicopter, airplane or sports utility vehicle. TransMedics representatives will perform device installation and servicing.

7. Schedule of Assessments

Table 7-1 provides an overview of the recipient and donor screening, baseline evaluations and follow-up. The schedule applies to both arms of the study, i.e., to both the OCS and standard of care arms.

Table 7-1 Study Overview

	Recipient	Donor	Donor Heart		Transplant Procedure							
	Screening	Screening	Pre-OCS/Heart Harvest	Heart Preservation and OCS Use	Transplant to day 6	Day 2	Day 7	Day 14	Day 21	Day 28	Discharge	Day 30
Informed Consent Form	X											
Demographics	X	X										
Medical/Surgical/Medication History	X	X										
Recipient/Donor Characteristics	X	X										
Laboratories	X	X										
Use of Cardiac/Respiratory Support	X	X			X		X	X	X	X	X	
Operative Details			X		X							
OCS Details			X	X								
Heart Function	X	X				X	X	X	X	X		
Angiogram (if available)[c]		X										
Echocardiogram		X					X			X		
Eligibility Assessment	X	X										
Pulmonary Renal Status	X											
EKG		X										
Assessment of Heart			X	X								
Randomization			X									
Endomyocardial Biopsy							X	X	X	X		
Pathology Slides for Central Read							X	X	X	X		

Transport and OCS Preservation Details				X								
Cold Ischemic/Warm Perfusion Time				X								
ICU/Hospital Stay					X		X	X	X	X		
Medications (related to SAEs only) Vasoactive Medications Immunosuppressant Medications					X		X	X	X	X	X	
Adverse Events (including all SAE's (cardiac and non-cardiac) and Rejection episodes					X		X	X	X	X	X	
Patient Completion Status												X

8. Pre-Operative Study Procedures

8.1 Recipient Pre-Transplant Preliminary Assessment

Investigators or their staff members will be responsible for obtaining a signed informed consent, the authorization to use and disclose protected health information, and conducting a preliminary assessment of the subject's eligibility for study participation.

The eligibility of the subjects will be reassessed and confirmed the day of the transplant.

8.2 Recipient Day of Transplant Screening Visit

The purpose of the day of transplant screening visit is to conduct a final assessment of whether the subject meets the eligibility criteria. The following information will be verified and recorded on the day of transplant:

- **Eligibility**: Investigator will review and confirm that the recipient meets all inclusion criteria and meets none of the exclusion criteria
- **Demographics/Characteristics**: The recipient's demographics, such as date of birth, gender, race, ethnicity, and weight and height, will be obtained. Also, date of consent, UNOS ID and panel reactive antibody % will be recorded. Other donor characteristics like blood type, RH factor will be collected as well.
- **Medical/Surgical History (Cardiac):** The recipient's cardiac medical history, including origin of heart failure e.g. coronary artery disease, heart valve disease, cardiomyopathy, congenital other. Investigator or coordinator will document if the recipient is on any type of mechanical support on the day of transplant e.g. IABP, VAD, and ECMO and will document date of insertion, if there is history of any sternotomies and the date of the last sternotomy and total number of sternotomies.

- **Pulmonary Status**: The investigator will verify if the patient has a COPD or not and will enter the values of the following pulmonary function tests (FEV, FEV/FVC, sO2, PO2, PCO2, PH, HCO3) and will indicate if the values were taken at room air or with oxygen supplementation.
- **Renal Status**: The investigator will verify if the patient is on any of the following renal replacement dialysis, hemofiltration, peritoneal dialysis. If the patient is on any of the above the most recent values for GFR, creatinine clearance, BUN and creatinine will be entered.
- **Right Heart Function**: This will include a review and collection of most recent measurements of heart function (systolic arterial blood pressure, diastolic arterial blood pressure, systolic PAP, diastolic PAP, pulmonary capillary wedge pressure (PCWP), transpulmonary gradient (mean PAP-PCWP). Also, if TPG is greater than 15 mmHg an assessment of irreversibility will be documented.

8.3 Donor Screening and Acceptance

Using the inclusion and exclusion criteria, the investigator will evaluate the donor and the quality and suitability of the heart for the study. The following evaluations will be conducted and recorded at this time:

- **UNOS ID**
- **Demographics**: Date of birth, gender, ethnicity, and race of the donor will be documented.
- **Donor Characteristics**: Blood type, height and weight, and Rh factor will be obtained.
- **Donor's Cause of Death**: All circumstances surrounding the donor's death will be collected including cause of death, and date and time of pronouncement of brain death. The occurrence and duration of any hypotensive episodes, and any cardiac arrest details will also be recorded including duration and whether it was witnessed or not.
- **Medical History**: Relevant medical history, including diabetes, hypertension, and other medical conditions, lifestyle factors (alcohol and tobacco use, history of drug abuse), for the donor will be obtained. Also the use of packed RBCs in the last 3 hours prior to explant will be documented.
- **Vasoactive Medications**: The use of vasoactive medications including type, dose and units at time of cross-clamp will be recorded.
- **Heart Function**: Systolic arterial BP, diastolic arterial BP, heart rate, temperature and central venous pressure will be obtained.
- **Angiogram**: Results will be collected if angiogram was requested by investigator or indicated according to guidelines. A copy of the source document will also be obtained.
- **Echocardiogram:** Ejection fraction, the presence or absence of wall motion and valvular abnormalities, and septal and posterior wall thickness will be obtained. A copy of source echo will be obtained if possible.

- **EKG**: Information from EKG will be collected and a copy of source document will be obtained.
- **Final visualization prior to acceptance in the chest**: The donor heart will be evaluated for any sluggish movement, enlargement and any gross abnormalities e.g. discoloration
- **Eligibility Assessment**: The donor will be evaluated to document whether the eligibility criteria are met.

8.4 Subject Identification and Randomization

Each transplant site will be assigned sealed randomization envelopes prepared by the statistical contractor employed by the Sponsor.

Subjects who are on the transplant waiting list will be identified and initially screened for the study eligibility. Subjects who are found eligible will be approached for consent. The study coordinator will maintain a log of all consented subjects. When a potential heart that initially meets the study criteria is available for a consented subject, a second evaluation for the consented subject will occur. If the subject is still eligible for the study, then the subject will be assigned a subject number and the randomization envelope with this number will be opened to reveal the treatment assignment. When the procurement team arrives at the donor site and upon final evaluation of the heart for acceptance into the study, then the investigators will set up the standard cold cardioplegia to arrest the heart.

If the patient was allocated to receive a heart on OCS, the investigator's team will set up the OCS for use, collect the donor's blood and will instrument the heart following the Instructions for Use provided. If the patient was allocated to receive a heart using the standard of care method, then the investigator's team will prepare the bags and ice.

The following donor assessments will be completed at this time:

- **Operative Details** (for both arms): Blood hematocrit levels should not drop below 25 prior to blood collection, date and time of aortic cross-clamp in the donor. Information with regards to the collection and types of other organs (e.g., lungs, liver, kidney, pancreas) harvested, if any, also will be documented.
- **Cardioplegia Details** (for both arms): Cardioplegia type and volume administered to the donor, and time aortic cross clamp was applied in the donor will be documented.
- **OCS Details**: Volume of blood collected from the donor, placement of blood collection cannula, date and time of instrumentation of heart on OCS, and the need for defibrillation will be documented.

8.5 Donor Heart Preservation and Transport/Recipient Skin Incision

Hearts preserved on the OCS should be maintained as follows:

- Mean coronary flow range: 300 – 900 mls/min
- Mean aortic pressure range: 40 mmHg – 90 mmHg
- Mean heart rate range: 50 - 100 BPM
- Arterial lactate < 5 mmol/L

The following information will be collected while the donor heart is preserved and transported:

- **Transportation Details** (for both arms): Location of donor site, and method of transportation
- **OCS Parameters Displays**: The OCS will record and store system performance parameters, including coronary flow, pump flow, aortic pressure, temperature, heart rate and oxygen saturation.
- **OCS Enabled Measurements**: Blood gases, electrolytes and chemistries will be collected: lactate, PCO2, PO2, and O2 sat, K+, HCO3, Ca++, Na+, Glucose, and pH, HCT from the arterial side, and lactate from the venous side The samples will be analyzed using a portable blood gas analyzer (I-Stat).
- **Lactate Level and other chemistries sampling scheme**: The organ transport team will collect samples from the donor and from the arterial and venous ports of the device and measure lactate levels using a portable blood gas analyzer (I-Stat) according to the following protocol:
    - One blood sample will be collected from the donor prior to cross clamp.
    - One blood sample will be collected after the donor's blood is added to the OCS circuit and prior to instrumentation of the donor's heart.
    - One arterial and one venous sample will be collected within 20 minutes of perfusing the heart on the OCS device.
    - At least, one additional arterial and venous sample will be collected before leaving the donor site.
    - The last lactate sample before departing from the donor hospital must be showing negative AV differential.
    - Samples will continue to be collected from the device at approximately hourly intervals and after any active CF adjustment or any other adjustments.

- **Additional OCS Information**: Any device malfunction, rate of solutions administration and any additives used will be recorded.
- **Skin Incision** (for both arms): The time of skin incision will occur when the procurement team is within a reasonable distance from the recipient hospital and it is to be determined according to the surgeon's standard practice. The timing will be captured.

8.6 Prior to Transplantation

All hearts preserved on the OCS or using cold static cardioplegia are expected to be transplanted unless the OCS has malfunctioned or sustained damage that adversely affects the heart or if the surgeon based on his/her clinical judgment and to protect the patient welfare and safety decided not to transplant the heart. Any decision to turn down hearts for transplants should be done with notification of Study PI and Site PI. Any organ that is not used will be sent to pathology for a complete histopathological evaluation. Please consult Appendix D, Section II for specimen

preparation techniques. Investigator will document the reason for not transplanting the heart, results of the examination, and intended recipient status.

OCS Details (removal of heart from the OCS): To allow for the surgical re-implantation of the donor heart into the recipient, the donor heart should be cooled and arrested by administering the institution's cold cardioplegia solution used for open heart surgery. At this time, the circulatory pump should be turned off. Then the donor heart will be removed from OCS and placed in a sterile bowl filled with cold cardioplegia solution. The following will be recorded: date and time of the termination of OCS perfusion, Cardioplegia fluid type, and volume infused to the donor heart and time of removal of cross clamp in the recipient after transplanting the donated heart.

9. Operative and Immediate Post-Operative Study Procedure

9.1 Removal of Cross Clamp

The time of removal of cross clamp in the recipient for both arms will be identified.

9.2 Transplant Details

Information concerning the transplant procedure such as name of surgeon, date of transplant, time of completion of procedure, skin incision time, and date and time of aortic cross clamp removal after transplant, number of cardiopulmonary bypass weaning attempts, time on bypass and time of weaning off bypass, heart function measurement, and inotropic support at the time heart function measurements are taken immediately prior to OR discharge and every 12 hours until the catheter is removed. The need for defibrillation, operative discharge (OR) time, intensive care unit (ICU) admission time, and any supplemental cardioplegia required will be recorded.

9.3 UNOS Unique Post-Transplant Patient Identifier

Identifier will be recorded.

9.4 Functional Assessments (for both arms):

9.4.1 Echocardiogram

Ejection fraction, wall motion and valvular abnormalities using echocardiogram will be recorded on Day 7 and Day 28 post transplant. For more details please see attached Echo protocol, Appendix D.

9.4.2 Use of Cardiac/Respiratory Support

Type and length of use of inotropic and ventilator support, ventricular assist devices (VAD), extracorporeal membrane oxygenation (ECMO), and intra-aortic balloon pump (IABP) will be recorded.

9.4.3 Heart Function

The following assessments of heart function will be recorded immediately prior to discharge from OR (after separation from the cardiopulmonary bypass), and then every 24 hours until the catheter is removed: systolic, diastolic, cardiac output, cardiac index, systemic vascular resistance, pulmonary artery pressures (PAP), pulmonary capillary wedge pressure (PCWP), central venous pressure, and transpulmonary gradient.

9.4.4 ICU Stay (for both arms)

Capture the date and time when clinical order for ICU discharge is written, and date and time of actual ICU discharge. Any readmissions will be recorded. If the patient's ICU stay is prolonged due to complications not related to the graft function or due to logistical issues this information will also be captured.

9.4.5 Medications (for both arms)

- Vasoactive and immunosuppressant medications will be recorded on the dedicated eCRF pages.
- All SAE related medications will be recorded on the dedicated eCRFs.

9.4.6 Adverse Events (for both arms)

Documentation of all new adverse events, serious adverse events, cardiac-related serious adverse events, or changes in previously reported events will be recorded.

9.5 Follow-up (Days 2, 7, 14, 21, 28 and Discharge)

The follow-up visits will be performed in +/- 3 days of the designated follow-up periods. The evaluations may be conducted over several days. In those instances when a randomized subject does not receive a heart subsequent to their randomization and procurement, the subject will be followed throughout the period of the study until they receive a heart or is removed from the transplant waitlist. Any newly occurring serious adverse event will be collected during the follow-up period. However, since those subjects would not have received a heart subsequent to procurement, none of the evaluations requiring interventions will be conducted e.g. biopsies, echocardiogram. When these subjects receive a transplanted heart according to their assigned randomization method, he or she will be followed for 30 days starting from the new transplant date and all information will be collected.

The following evaluations will be conducted at the designated follow-up periods:

9.5.1 Heart Function (Day 2, 7, 14, 21, 28 (when available))

Systolic arterial BP, diastolic arterial BP, heart rate, temperature, cardiac output, cardiac index, systemic vascular resistance, systolic PAP, diastolic PAP, pulmonary artery pressures (PAP), pulmonary capillary wedge pressure (PCWP) and central venous pressure (CVP) will be recorded. Transpulmonary gradient will be calculated as follows: mean PAP-PCWP. Vasoactive medications will also be recorded at time of each measurement.

9.5.2 Echocardiogram (Day 7 and Day 28)

2D measurements, doppler measurements, cardiac valves, ejection fraction, wall motion and valvular abnormalities will be recorded. For details, please see attached Echo Protocol (Appendix D).

9.5.3 Endomyocardial Biopsy (Day 7, 14, 21, and 28)

A biopsy sample will be collected for evaluation at the investigational sites. The ISHLT rejection grade will be evaluated according to the attached protocol (Appendix C). The presence of ischemic injury will also be evaluated by assessing the presence of vacuolization, coagulation necrosis and healing (inflammation) using a four-point scale (none, focal, multifocal and diffuse). The following definitions will be used:

- **Vacuolization**: The presence of clear spaces (vacuoles) within cardiac myocytes is a marker of sub-lethal ischemia. This finding usually is present in the subendocardial region of the heart, which is the region sampled by the bioptome. This finding can be seen in the first few biopsies after transplantation (up to 2-3 months), indicative of peritransplant-related sublethal ischemia, and is also seen late (years) after transplantation as a marker of graft vasculopathy. It can also be seen in the myocardium adjacent to a myocardial infarct that may have suffered sublethal ischemia but is not dead.
- **Coagulation Necrosis**: The histologic findings of coagulation necrosis in the myocardium include myocyte hypereosinophilia, contraction bands, absence of nuclei and loss of cell integrity. These changes represent irreversible, lethal injury (in contrast to vacuolization, which is reversible and sub-lethal).
- **Healing**: The presence of a mixed inflammatory infiltrate of moderate to low cellular density consisting of neutrophils, lymphocytes, macrophages and plasma cells is consistent with a healing injury. There also tends to be a background of cell and matrix debris. This is a different histologic pattern from cellular rejection, which is a dense, predominantly lymphocytic infiltrate.
- **Focal**: The presence of 1-2 small foci (involving groups of ~10 myocytes) within the biopsy sample.
- **Multifocal**: The presence of greater than 2 foci within the biopsy sample.
- **Diffuse**: Involvement of a significant proportion and involving a majority of the fragments of the biopsy sample.

Pathology slides from each biopsy will be made available for a central laboratory reading. Slides representative of the biopsy will be labeled with the subject's study ID and provided (on a loan basis) to the clinical monitor for central lab submission and reading. For details please see attached biopsy protocol (Appendix C).

9.5.4 Hospital Stay/ICU Readmission

Duration of hospital stay and any ICU or hospital re-admissions within the 30-day follow-up period will be recorded.

9.5.5 Mechanical Circulatory Support

Type and length of use of ventricular assist devices (LVAD, BiVAD, RVAD), intra-aortic balloon pump (IABP), and extracorporeal membrane oxygenation (ECMO) will be recorded.

9.5.6 Ventilator Support

The use of any ventilator support will be recorded.

9.5.7 Medications

- All SAE related medications will be recorded on the dedicated electronic Case Report Form (eCRF).
- Vasoactive and immunosuppressive medications will be recorded on the dedicated eCRF.
- Immunosupressive induction will be recorded on the dedicated eCRF.

9.5.8 Adverse Events

Documentation of all new adverse events, serious adverse events, and any changes in previously reported events will be recorded. For patients who are discharged beyond the 30 days, all new serious adverse events occurring after the 30-day period will be collected. If at Day 30 the study subject is still hospitalized, then any new serious adverse event occurring after Day 30 and before the discharge will be collected. The discharge date for each study subject in the study will be collected independent of the 30-day follow-up period. The discharge survival status of patients who are alive, but still hospitalized at Day 30 will be collected. All deaths occurring during the study 30-day period or prior to discharge will be collected.

9.5.9 Rejection

Each episode of rejection will be recorded on the rejection form. Rejection will be classified as biopsy proven or clinically presented only. Immunosupressive medications, including types, doses, trough doses, and all adjustments will also be recorded on this form.

10. Study Enrollment and Completion

The following will be used as guidelines for study enrollment and completion.

10.1 Screen Failures

Informed consent will be obtained prior to initiation of any procedures that are performed solely for the purpose of determining eligibility for research. Potential subjects who are found to be eligible for treatment within the context of the investigation will be evaluated again for eligibility just prior to transplantation. The second eligibility assessment is required because the potential subject's conditions may have changed since he or she signed the informed consent and the potential subject may no longer meet the eligibility criteria.

A "screen failure" is a potential subject from whom an informed consent is obtained but in whom treatment within the context of the investigation is not attempted because it is determined that the subject does not meet all of the eligibility criteria during the second evaluation, or to whom no matching and eligible heart has been found.

None of the screen failures will be counted towards the maximum total enrollment of 128 subjects, nor are they included in the "intent to treat" population. A study-specific identification number is not assigned to these subjects; therefore, the subject's initials and date of birth will be their identifier on the screening log with the reason for a screen failure identified. On rare occasions when a randomization envelope is opened and the heart is found to be not eligible for the study. The information will be documented and the study subjects will not be considered as part of the ITT population.

11. Evaluation of Adverse Events

11.1 Adverse Event Definition

Events that may have a deleterious effect on the patient are referred to as adverse events.

Adverse events are to be collected from the time a subject is prepared for transplant surgery until the completion of the 30-day follow-up evaluation. An adverse event will be followed until resolution or stabilization of the event.

If at Day 30 the study subject is still hospitalized, any new serious adverse event occurring after Day 30 and before the discharge will be collected. The discharge date for each study subject in the study will be collected independent of the 30-day follow up period. All deaths occurring during the study 30-day period or prior to discharge will be collected.

11.2 Serious Adverse Event (SAE)

An adverse event will be classified as serious if it meets any of the following criteria:

- Results in, leads to, or contributes to, a death;
- Is life-threatening;
- Results in permanent disability or incapacity (i.e., permanent impairment of a body function or permanent damage to a body structure);
- Requires in-patient hospitalization or prolongs hospitalization;
- Necessitates medical or surgical intervention to preclude a permanent disability or incapacity;
- Results in fetal distress, fetal death or a congenital anomaly/birth defect; or
- Meets the criteria for Serious as defined in Appendix B.

11.3 Recording and Reporting of Adverse Events

A listing of each adverse event to be reported is provided in Appendix B. This table includes:

- List of all adverse events that are to be reported to the Sponsor by the investigational site
- Definition of when each event is to be considered a Serious Adverse Event
- Determination of whether the event is considered to be cardiac-related

The description of the adverse event will include the date and time of onset, duration, severity, seriousness, suspected or known etiology, the relationship of the event to the investigational device, anticipated or not, and any treatment required. Specific treatments implemented in response to the adverse event will also be recorded.

Adverse events occurring during the course of the clinical study will be reported to TransMedics, Inc., and documented on the appropriate electronic case report forms. For all adverse events, the investigator is required to supply any additional data that may be deemed necessary by the Sponsor. This report will include an assessment of the severity and seriousness of the adverse event, its treatment and resolution, and its relationship to the investigational device.

Additional reporting procedures are required for serious adverse events, cardiac-related adverse events and unanticipated adverse device effects. These events will be reported to TransMedics, Inc., preferably within 24 hours of the time the investigator learns of the event but in no case later than 5 working days. Events will be recorded up to the 30-day follow-up or through hospital discharge if longer than 30 days. For a particular patient a different follow-up period may be specified by the Clinical Event Committee if required to protect patient safety.

11.3.1 Relationship to OCS or Standard of Care Preservation and Transportation

The investigator will assess the relationship of the adverse event to the investigational device or preservation and transportation with the standard of care. The relationship will be assessed using the following categories:

- **Definitely Related:** There is a reasonable causal and temporal relationship between treatment with the investigational device or preservation and transportation with the standard of care and the adverse event.
- **Probably Related**: It is more likely than not that there is a reasonable causal relationship between treatment with the investigational device or preservation and transportation with the standard of care and adverse event.
- **Possibly Related**: There is a reasonable relationship to the device treatment or preservation and transportation with the standard of care, but the causal relationship is unclear or lacking.
- **Not Likely Related**: There is a temporal relationship to the device treatment or preservation and transportation with the standard of care, but there is not a reasonable causal relationship between the study device and the event. For example, the adverse event occurs in a time relation to the device treatment, which makes relation improbable.
- **Unrelated**: There is no relationship between treatment with the investigational device or preservation and transportation with the standard of care and the adverse event.

11.3.2 Severity and Seriousness

Severity

The investigator will rate the severity of the adverse event using the following categories:

- **Mild**: The adverse event is transient and/or easily tolerated by the subject.
- **Moderate:** The adverse event causes the subject discomfort and interrupts the subject's usual activities.
- **Severe:** The adverse event causes considerable interference with the subject's usual activities.

Seriousness

The investigator will assess each adverse event for its seriousness using the definition above (Section 11.2). Please note the term "serious" adverse event is not synonymous with a "severe" adverse event, which may be used to describe the intensity of an event experienced by the subject.

11.3.3 Anticipated and Unanticipated Adverse Events

The investigator will assess each adverse event for whether it is anticipated or unanticipated. An unanticipated adverse event is defined as any adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device if that effect, problem or death was not previously identified in nature, severity or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

The following serious adverse events are associated with cardiac transplant procedures and have been documented within the first 30 days after heart transplant: acute rejection, arrhythmia, arterial non-CNS thromboembolism, need for aortic balloon pump (IABP) use greater than 8 hours post transplant, bleeding (major), cardiac tamponade, cardiogenic shock, death, fever, graft failure, heart failure, hemolysis, hepatic dysfunction, hypertension, hypotension, lymphoma, major infection, malignancy, myocardial infarction, multiple organ failure, neurological dysfunction, pericardial fluid collection, pneumonia, post-transplant lymphoproliferative disorder (PTLD), renal dysfunction, respiratory failure, venous thromboembolism event and wound dehiscense.[7,8]

11.3.4 Pre-Existing Conditions

Pre-existing diseases or conditions will not be reported as adverse events unless there has been a substantial increase in severity or frequency of the problem that cannot be attributed to the expected progression of the disease or condition.

11.3.5 Clinical and Laboratory Changes

The investigator will review the results of all clinical and laboratory tests as they become available. For each laboratory test result, the investigator will ascertain if this is an

abnormal (i.e., clinically significant) change from baseline for that individual subject. (This determination, however, does not necessarily need to be made the first time an abnormal value is observed. The investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests). If this laboratory value is determined to be an abnormal change from baseline for that subject, this will be considered an adverse event.

12. Statistical Analysis

12.1 General

Continuous variables will be summarized using descriptive statistics, specifically the mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized using frequencies and percentages. All statistical tests will be performed at the 0.05 significance level.

12.2 Analysis Populations

12.2.1 Per Protocol population

The per protocol population will consist of all randomized subjects who are transplanted and have no major protocol violations. **The major protocol violations that will exclude a subject from this population are the following:**

- Ineligible for the study according to the recipient inclusion and exclusion criteria
- Ineligible for the study according to the donor heart inclusion and exclusion criteria

In analyses based on the per protocol population, subjects will be analyzed as randomized.

12.2.2 Intent-to-treat Population

The intent-to-treat (ITT) population will consist of all randomized subjects for whom it is determined at the donor site that there is a matching and eligible heart. In analyses based on the ITT population, subjects will be analyzed as randomized.

12.2.3 Treated Population

The treated population will consist of all randomized subjects who receive a donor heart transported either by the OCS or the standard of care subsequent to randomization. In analyses based on the treated population, subjects will be analyzed as treated. For any patient who receives a heart transported in part on OCS and in part on standard of care, the subject will be analyzed according to the initial treatment.

12.2.4 Completed Treated Population

The completed treated population will consist of all subjects in the treated population who complete the study. In analyses based on the completed treated population, subjects will be analyzed as treated.

12.2.5 OCS Heart Population

The OCS heart population will consist of all hearts that are transported by the OCS.

12.2.6 Safety Population

The safety population will consist of all subjects who receive a heart transported by either OCS or standard of care. In analyses based on the safety population, subjects will be analyzed as treated (preservation method of transplanted heart). For any subject who receives a heart transported in part on OCS and in part on standard of care, the subject will be analyzed according to the initial treatment.

12.3 Effectiveness

12.3.1 Primary effectiveness endpoint

The primary hypothesis for this study is that the OCS treatment is non-inferior to the standard of care treatment with respect to the primary effectiveness endpoint, 30-day patient survival following transplantation with originally transplanted heart and no mechanical circulatory assist device at day 30. The primary hypothesis statement is as follows:

$$H0: \pi OCS < \pi SOC - \delta \text{ and}$$

$$H1: \pi OCS \geq \pi SOC - \delta,$$

where πOCS and πSOC are the proportions of subjects surviving at Day 30 following transplantation with originally transplanted heart and no mechanical circulatory assist device at day 30 with the OCS and standard of care treatments, respectively, and δ is the noninferiority margin which is here taken to be 0.10. If non-inferiority is demonstrated, a corresponding (two-sided) test of superiority will be performed.

The primary effectiveness endpoint will be analyzed by calculating for each treatment group the sample proportion of subjects surviving to Day 30 following transplantation with originally transplanted heart and no mechanical circulatory assist device at day 30 and an exact 95% confidence interval for the corresponding population proportion. Also, the 95% upper confidence limit based on the normal approximation will be calculated for the difference between the two population proportions (πSOC – πOCS). An upper confidence limit less than or equal to $\delta = 0.10$ will result in rejection of the null hypothesis (H0) in favor of the alternate hypothesis (H1) and achievement of the success criterion for the primary effectiveness endpoint.

This endpoint will be analyzed using the per protocol population, the intent-to-treat population, the treated population, and the completed treated population. The per protocol analysis will be considered the primary analysis.

12.3.2 Secondary endpoints

For incidence of biopsy proven ISHLT grade 2R (moderate) or 3R (severe) acute rejection on any of the surveillance endomycardial biopsies or clinically symptomatic

rejection requiring augmentation of immunosuppressive therapy during the 30 day follow-up period, the hypotheses are as follows:

$$H0: \tau OCS > \tau SOC + \delta \text{ and}$$

$$H1: \tau OCS \leq \tau SOC + \delta,$$

where τOCS and τSOC are the proportions of patients with such rejection for the Organ Care System and standard of care groups, respectively, and δ is the non-inferiority margin which is here taken to be 0.10. If non-inferiority is demonstrated for this endpoint, a corresponding (two-sided) test of superiority will be performed. This endpoint will be analyzed in a manner analogous to the primary endpoint using the treated population.

For length of ICU stay, the hypotheses are as follows:

$$H0: \lambda OCS > \lambda SOC + \delta \text{ and}$$

$$H1: \lambda OCS \leq \lambda SOC + \delta,$$

where λOCS and λSOC are the median lengths of ICU stay for the Organ Care System and standard of care groups, respectively, and δ is the non-inferiority margin which is here taken to be 12 hours. (The choice of 12 hours was selected as being approximately 10% of the mean ICU time for both the control arm (mean = 126 hours) and the Celsior arm (mean = 113 hours) in a study by Vega et al. (2001). If non-inferiority is demonstrated for length of hospital stay, a corresponding (two-sided) test of superiority will be performed. This endpoint will be analyzed using Wilcoxon rank sum tests and the treated and the completed treated population.

12.4 Safety

Safety will be analyzed principally by examination of the frequency of adverse events. In particular, the incidence of cardiac-related serious adverse events will be analyzed as a secondary endpoint of the study.

For the incidence of cardiac-related serious adverse events up to the 30-day follow-up after transplantation, the hypotheses are as follows:

$$H0: \tau OCS > \tau SOC + \delta \text{ and}$$

$$H1: \tau OCS \leq \tau SOC + \delta,$$

where τOCS and τSOC are the cardiac-related serious adverse event incidence rates (i.e. the proportions of patients experiencing at least one cardiac-related serious adverse event for the Organ Care System and standard of care groups, respectively, and δ is the non-inferiority margin which is here taken to be 0.10. If non-inferiority is demonstrated, a corresponding (two-sided) test of superiority will be performed.

This endpoint will be analyzed by calculating for each treatment group the sample proportion of subjects experiencing at least one cardiac-related serious adverse event and an exact 95% confidence interval for the corresponding population proportion. Also, the 95% upper confidence limit based on the normal approximation will be calculated for the difference between the two population proportions ($\tau OCS - \tau SOC$). An upper confidence limit less than

or equal to $\delta = 0.10$ will result in rejection of the null hypothesis (H0) in favor of the alternate hypothesis (H1).

In addition, the numbers and percentages of subjects experiencing at least one AE, at least one severe AE, at least one device-related AE, at least one unanticipated AE, and at least one serious AE, and the number and percentage of deaths will all be tabulated by treatment. Also, the number of adverse events and the number and percent of subjects experiencing adverse events will be tabulated by system organ class and preferred term using MedDRA. A similar analysis will be performed for serious AEs. The number and percent of subjects experiencing AEs will also be tabulated by system organ class and preferred term and the relationship of the adverse event to the device (focusing on differentiating adverse events deemed definitely and probably related to the device). Similar analyses will be performed by the severity of the adverse event.

Safety analyses will be conducted principally based on the safety population. In the analyses of AEs for this population, for subjects who were not transplanted with the initial heart but were transplanted with a second heart, any AEs occurring from the beginning of subject preparation for transplantation with the initial heart to the beginning of subject preparation for transplantation with the second heart would be excluded. However, such AEs, as well as AEs occurring in patients not included in the safety population, will be summarized separately.

12.5 Multiplicity

An adjustment for multiplicity among the secondary endpoints will be made using the Holm Stepwise Test. The secondary analyses will be conducted only if the primary analysis achieves statistical significance.

12.6 Sample Size Determination

The sample size for this study was determined, in part, based on the primary effectiveness endpoint.

The sample size calculations were based on the following assumptions:

- Alpha = 0.05
- Power = 80%
- $\delta = 0.10$
- 1:1 allocation
- πTreatment = 0.95, πControl = 0.94

The sample size per group (n) was calculated for the use of the Z-test based on the normal approximation to the binomial distribution. Based on the above assumptions, the minimum required sample size per treatment group is 54 subjects for a total of 108 subjects. In order to have a sufficient sample size in the OCS group to improve the probability of detecting frequently occurring types of adverse events, the sample size has been increased to 64 subjects per group, for a total of 128 subjects.

A sample size of 64 provides the probabilities shown in Table 12-1 of detecting various types of adverse events. A given type of adverse event is considered to be detected if there is at

least one occurrence of such an event in the study. Thus, for example, a given type of adverse event that has a probability of occurrence of 5% has a 96.2% probability of being detected in the study.

Table 12-1: Probability of Detecting a Type of Adverse Event	
Probability of Occurrence (%)	**Probability of Detecting (%)**
2	72.6
3	85.8
4	92.7
5	96.2
10	99.9
15	>99.9

13. Risk Analysis

This clinical study has been designed to ensure that the benefits and knowledge gained from the study outweigh the potential risks to the subjects. The subjects are adults undergoing primary heart transplants.

13.1 Potential Risks

The potential risks to subjects from participation in this clinical study are similar to those for patients undergoing heart transplantation. The potential risks associated with heart transplant procedures include post-operative complications, such as heart rejection, graft vessel disease (an expression of chronic rejection), infection, abnormal kidney function, diabetes, high level of cholesterol, high blood pressure, cancer and neurological complications.

The potential risk associated with the investigational device and the preservation and transportation with the standard of care is the failure to preserve the heart. As with any medical device, there is always a risk of extremely rare or previously unknown side effects developing from the treatment.

Serious adverse events associated with cardiac transplant procedures are listed in Section 11.4.3.

A summary of the literature regarding anticipated adverse events for cardiac transplant procedure follows.

Table 13-1 presents adverse events that occurred in 10% of patients in each treatment group in a clinical study comparing one cold preservation solution (Celsior™) with conventional

solutions (Control). Celsior™ is the only cold storage solution cleared for marketing in the U.S. for flushing and cold storage of donor hearts.[6]

Table 13-1: Adverse Events in a Cold Storage Preservation Trial		
Body System Individual Adverse Event	**Celsior (n=64)**	**Control (n=67)**
Cardiac System	42(66%)	46 (69%)
Rejection	20 (31%)	25 (37%)
Cardiovascular Disorder	7 (11%)	9 (13%)
Atrial Fibrillation	9 (14%)	5 (7%)
Hypotension	7(11%)	5 (7%)
Arrhythmia	7 (11%)	3 (4%)
Cardiovascular System	13 (20%)	10 (15%)
Hypertension	9 (14%)	5 (7%)
Body as a Whole	23 (36%)	18 (27%)
Fever	7 (11%)	3 (4%)
Respiratory System	16 (25%)	18 (27%)
Metabolic and Nutritional	13 (20%)	17 (25%)
Nervous System	11 (17%)	14 (21%)
Urogenital System	17 (27%)	12 (18%)
Kidney Function Abnormal	7 (11%)	5 (7%)
Digestive System	12 (19%)	12 (18%)
Hematological and Lymphatic Systems	15 (23%)	14(21%)
Coagulation Disorder	6 (9%)	8 (12%)

Information on the known clinical experience with the OCS system, including a summary of adverse events is found in Section 14.

13.2 Manner in Which the Potential Risks Have Been Minimized

The Sponsor has relied upon a number of different means, including the device design, risk analysis and management process, preclinical testing, and the clinical protocol itself, to minimize the risks to subjects and to protect their safety and welfare.

The Sponsor has conducted extensive preclinical testing of the OCS to demonstrate its safety, effectiveness and readiness for clinical use. The OCS has undergone extensive preclinical and animal studies to demonstrate the device performs as intended. These studies strongly indicate that the OCS maintains heart viability by providing a controlled environment and monitoring its function. The Cardiac Disposable Set has been tested for biocompatibility to minimize the risk of adverse tissue reactions. These test results demonstrate the device and its materials are biocompatible and suitable for their intended use. The Cardiac Solution Set is composed of nutrients (amino acids, sugars and other compounds) with an established history

of safe use. The Cardiac Disposable Set and Cardiac Solution Set are provided sterile and for single use to minimize the risk of infection.

The OCS has also undergone extensive design verification and validation testing to optimize its performance and minimize the risks associated with its use. Preclinical studies demonstrate the device performs as intended and meets its performance specifications including the circulatory pump, the oxygenator, and other functions. The software has undergone extensive testing to demonstrate it and its safety functions and alarms perform as intended.

The clinical protocol also incorporates several procedures to minimize the risks to subjects and to ensure the benefits of the clinical study outweigh its potential risks. Subjects will be monitored before, during and after the operative procedure to help ensure their safety. The investigators are members of transplant teams who have extensive experience with cardiac transplants and who will be trained to use the OCS to further minimize risk. Subjects in the study will undergo frequent visits and routine monitoring to help detect any abnormal changes and to provide appropriate treatment as necessary.

The study will be monitored to ensure the identification, documentation and analysis of adverse events, compliance with the protocol, and ethical requirements are in place for conducting research to protect the safety and well-being of all subjects.

13.3 Potential Benefits

The OCS achieves the primary goals of organ preservation by being designed to maintain and support the donor heart to minimize damage and ischemic reperfusion injuries. It is anticipated that minimizing the time-dependent ischemic and reperfusion injuries may result in improved immediate benefits to patients. Subjects may have the potential of receiving hearts that are in a better physiological condition when transplanted. It is also hoped that this technology will ultimately allow greater flexibility in preservation times by minimizing the onset of time-dependent ischemia-reperfusion injuries, thereby facilitating an optimal donor/recipient matching process and geographical distribution of organs.

The OCS incorporates various monitors to assess the heart during transportation. These technological features allow the transplantation team to monitor the heart during transportation and immediately before transplantation into the recipient. In contrast today, the evaluation of donor hearts is limited to the donor site. These monitoring capabilities ultimately may allow physicians to better evaluate the quality and suitability of donor organs.

13.4 Risk Benefits Ratio

This risk analysis demonstrates that the anticipated benefits of this clinical study outweigh its anticipated risks, and that the safety and welfare of subjects will be protected during the course of this investigation.

14. Clinical Experience

The purpose of this clinical experience section is to provide information on patients who were transplanted with hearts that were instrumented on the OCS device. The clinical information is compiled from two European studies (PROTECT and PROTECT II) and the U.S. feasibility study (PROCEED). This section includes information on all the subjects enrolled in the studies as of the data cut-off date of October 21, 2007. The PROTECT study was a 20 patient European feasibility study initiated in January 2006 and was completed in February 2007. The device received its CE mark in September 2006. Patient enrollment in Europe continued in a post-marketing study, known as PROTECT II, utilizing a similar trial protocol and case report forms. The European clinical study PROTECT was conducted at four centers in the UK and Germany. The PROTECT II study was expanded into sites in Austria and Italy as well. The U.S. PROCEED study was initiated in April 2007 under IDE G060127. As of October 21, 2007, 35 subjects were enrolled in the European PROTECT family of studies and 10 subjects were enrolled in the PROCEED study. Subjects in all studies underwent cardiac transplantation and were expected to participate in the study for 30 days following surgery. The primary end point was 7-day survival following transplantation. Secondary endpoints included 7- and 30-day graft survival and 30-day patient survival. Due to the ongoing nature of studies, the information presented below is based on a data cut-off date of October 21st, 2007, which is not related to any specific study completion date.

The following provides a summary of the outcomes of this combined patient population.

Of the total of 45 subjects who signed informed consent and received hearts preserved on the OCS, and were therefore enrolled in the studies, the eligibility criteria for the donor and recipient were met in 41 instances. The 4 remaining subjects received hearts that were preserved on the OCS but either the donor or recipient eligibility criteria were not met. As of the cut-off date, 1 subject had not reached the 7-day follow-up period and 2 additional subjects had not reached the 30-day follow-up period. In addition, data were still being collected on 5 "as treated" and 4 "per protocol" subjects who had passed the 30-day follow-up period. This summary includes information on all the enrolled subjects that were monitored and for whom information has been collected and entered in the database using an "as treated" analysis (all patients treated with hearts that were instrumented on the device) as the primary analysis and "per protocol" as the secondary analysis.

Table 14-1: Summary of Subject Enrollment

	As-Treated	Per Protocol
Subjects consented and implanted	45	41
7-day follow-up period elapsed as of 10/21/07	44	40
30-day follow-up period elapsed as of 10/21/07	42	38
30-day follow-up period elapsed, but data not yet entered	5	4
Subjects with all data available for analysis	37	34

In summary, while we present in the text the most salient survival information for all 45 "as treated" and 41 "per protocol" subjects, several tables summarize information from patients having completed the 30-day study period (n = 37 for "as treated", and n = 34 for "per protocol").

Table 14-2 and Table 14-3 below present the demographics of the donors and recipients respectively.

Table 14-2: Demographic and Baseline Characteristics of the Donor		
Donor Characteristic	**As Treated Number of Donors (N=37)**	**Per Protocol Number of Donors (N=34)**
Age (yrs) [mean±SD]	42 ± 9.82	41.4 ± 9.93
Female [n (%)]	9 (24.3%)	8 (23.5%)
Male [n (%)]	28 (75.7%)	26 (76.5%)
Race [n (%)]		
Black	3 (8.1%)	3 (8.8%)
Hispanic	1 (2.7%)	1 (2.9%)
White	33 (89.2%)	30 (88.2%)
Weight (kg) [mean±SD]	76.4 ±14.16	76.8 ± 14.59
Height (cm) [mean±SD]	174.9 ± 7.99	175.1 ± 8.14
Blood Type [n (%)]		
A	12 (32.4%)	12 (35.3%)
AB	2 (5.4%)	2 (5.9%)
B	4 (10.8%)	4 (11.8%)
O	19 (51.4%)	16 (47.1%)
Rh Factor [n (%)]		
Negative	9 (24.3%)	8 (23.5%)
Positive	28 (75.7%)	26 (76.5%)

Table 14-3: Demographic and Baseline Characteristics of the Recipients		
Recipient Characteristic	**As Treated Number of Recipients (N=37)**	**Per Protocol Number of Recipients (N=34)**
Age (yrs) [mean±SD]	49.5 ± 13.61	49.2 ± 13.24
Female [n (%)]	12 (32.4%)	11 (32.4%)
Male [n (%)]	25 (67.6%)	23 (67.6%)
Race [n (%)]		
Asian	2 (5.4%)	2 (5.9%)
Black	3 (8.1%)	3 (8.8%)
White	32 (86.5%)	29 (85.3%)
Weight (kg) [mean±SD]	78.7 ± 16.49	79.8 ± 16.77
Height (cm) [mean±SD]	168.4 ± 27.34	168.6 ± 28.45
Blood Type [n (%)]		
A	13 (35.1%)	13 (38.2)
AB	5 (13.5%)	5 (14.7)
B	4 (10.8%)	4 (11.8)
O	15 (40.5%)	12 (35.3)
RH Factor: Negative	8 (21.6%)	7 (20.6%)
RH Factor: Positive	29 (78.4%)	27(79.4%)
NYHA Class [n (%)]		
II	2 (5.4%)	2 (5.9%)
III	16 (43.2%)	15 (44.1%)
IV	18 (48.6%)	16 (47.1%)
Unknown	1 (2.7%)	1 (2.9%)

Table 14-4 presents the data for both 7-day and 30-day patient and graft survival using the "as treated" approach and the "per protocol" approach. The Unadjusted 30-day patient survival for heart

transplant patients according the 2006 US Transplant Annual Report (SRTR, www.ustransplant.org) accessed on February 1st 2008 is 92.3%.

Table 14-4: Patient Survival & Graft Survival		
Patient Survival at Day 7	**As Treated OCS, N= 44** (1 subject transplanted < 7 days)	**Per Protocol OCS, N=40** (1 subject transplanted < 7 days)
Yes	41 (93.2%)	39 (97.5%)
No	3 (6.8%)	1 (2.5%)
Graft Survival at Day 7	**As Treated OCS, N= 44** (1 subject transplanted <7 days)	**Per Protocol OCS, N=40** (1 subject transplanted <7 days)
Yes	41 (93.2%)	39 (97.5%)
No	3 (6.8%)	1 (2.5%)
Patient Survival at Day 30	**As Treated OCS, N= 42** (3 subject transplanted <30 days)	**OCS, N =38** (3 subject transplanted <30 days)
Yes	38 (90.5)	37 (97.4%)
No	4 (9.5)	1 (2.6%)
Graft Survival at Day 30	**As Treated OCS, N= 42** (3 subject transplanted <30 days)	**OCS, N =38** (3 subject transplanted <30 days)
Yes	39 (88.6%)	37 (97.4%)
No	5 (11.36%)	1 (2.6%)

There have been a total of five deaths reported in the 45 subjects enrolled in the combined studies. A summary of these cases is shown in Table 14-5 below.

Table 14-5: Summary of Deaths in Entire Study Population				
Subject ID	**Protocol eligibility requirements**	**Days post transplant**	**Cause of Death**	**Relationship to Device**
PROCEED 001-004	Yes	2	Multi-organ failure resulting from primary allograft (heart)	Possible
PROTECT 002-001	No	8	Pre-existing pulmonary hypertension	None
PROTECT 001-002	No	>30 days	Failure of 2nd graft	None reported
PROTECT II 002-002	No	7	Multiple organ failure	None
PROTECT II 006-005	No	<7 days	Ruptured right pulmonary artery	None reported

Of the 5 total deaths reported in these studies, 3 occurred on or before Day 7. One occurred between Day 7 and Day 30, and one occurred after the 30-day follow-up period. As of the analysis date, the remaining 40 subjects are alive. Four of the five deaths occurred in subjects that did not meet the protocol eligibility criteria. These numbers reflect a misuse of device, in that either the donor heart and/or the recipient did not meet protocol eligibility criteria.

Table 14-6 below describes the ineligibility criteria for each subject:

Table 14-6: Summary of the Protocol Eligibility for Enrolled Subjects (N=45)	
Met All Protocol Eligibility Criteria	**Number of Subject Pairs**
Yes	41
No	4
Reasons for Not Meeting All Protocol Eligibility Criteria	
Recipient: Chronic Pulmonary Hypertension and advanced age Donor: Advanced Donor Age	1
Donor: Received bolus dose of noradrenaline during heart retrieval and blood collection	1
Recipient: Rare High Risk Condition (Restrictive Cardiomyopathy)	1
Recipient: Re-transplant	1

Table 14-7 provides a summary of relevant operative details:

Table 14-7: Summary of the Operative Details for the Recipients Who Received Hearts Preserved and Transported Using OCS		
Operative Details	**As Treated Number of Recipients (N=37)**	**Per Protocol Number of Recipients (N=34)**
Cardiac Pacing Needed following Transplant [n(%)] No Yes	 9 (24.3%) 28 (75.7%)	 8 (23.5%) 26 (76.5%)
Defibrillation Needed Following Transplant [n(%)] No Yes	 29 (78.4%) 8 (21.6%)	 26 (76.5%) 8 (23.5%)
Number of Attempts to Wean from Cardiopulmonary Bypass [n(%)] 1 2 3 Unknown	 31 (83.8%) 1 (2.7%) 1 (2.7%) 4 (10.8%)	 29 (85.3%) 1 (2.9%) 0 (0%) 4 (11.8%)

Table 14-8 summarizes the adverse events by organ system class utilizing the MeDRA coding conventions the analysis is presented for the as treated and per protocol populations.

Table 14-8: Number of subjects reporting one or more Serious Adverse Events							
MedDRA (System Organ Class) / (Preferred Term)	**As Treated**				**Per Protocol**		
	# subjects reporting (n=37)	**Device Relatedness**			**# subjects reporting (n=34)**	**Device Relatedness**	
		Possible	**Unrelated**	**Unknown**		**Possible**	**Unrelated**
Any SAE	15 (40.5%)	6 (40%)	8 (53%)	1 (6.7%)	13 (38%)	6 (17.6%)	7 (20.6%)
Cardiac Disorders	5 (13.5%)	2 (40%)	3 (60%)	0	4 (11.8%)	2 (5.9%)	2 (5.9%)
Gastrointestinal Disorders	2 (5.4%)	0	2 (100%)	0	2 (5.9%)	0 (0%)	2 (5.9%)
General Disorders	4 (10.8%)	1 (25%)	3 (75%)	0	3 (8.8%)	1 (2.9%)	2 (5.9%)
Immune Disorders	5 (13.5%)	2 (40%)	2 (40%)	1 (20%)	4 (11.8%)	2 (5.9%)	2 (5.9%)
Infections	1 (2.7%)	0	1 (100%)	0	1 (2.9%)	0 (0%)	1 (2.9%)
Procedural Complications	1 (2.7%)	0	1 (100%)	0	1 (2.9%)	0 (0%)	1 (2.9%)

Investigations	2 (5.4%)	0	2 (100%)	0	2 (5.9%)	0 (0%)	2 (5.9%)
Renal Disorders	1 (2.7%)	0	1 (100%)	0	1 (2.9%)	0 (0%)	1 (2.9%)
Respiratory Disorders	4 (10.8%)	1 (25%)	3 (75%)	0	4 (11.8%)	1 (2.9%)	3 (8.8%)
Skin	1 (2.7%)	1 (100%)	0	0	1 (2.9%)	1 (2.9%)	0 (0%)
Surgical and Medical Procedures	1 (2.7%)	1 (100%)	0	0	1 (2.9%)	1 (2.9%)	0 (0%)
Vascular Disorders	3 (8.1%)	0	3 (100%)	0	3 (8.8%)	0 (0%)	3 (8.8%)

The reported adverse events are expected in nature and frequency following heart transplantation. The investigators have not assigned any of the events as definitely or probably device-related. For comparison, Vega *et al* reported a serious adverse event rate of 38% in the Celsior group and 46% in the control group, and that 66% of subject in the Celsior group and 69% in the control group experienced at least one cardiac related adverse event (2001).

15. Device Management

15.1 Packaging and Labeling

The investigational device will be provided to the investigator(s) by the Sponsor. The Cardiac Disposable Set and accessories and the Cardiac Solution Set will be supplied sterile and are intended and labeled for single use.

The Organ Care System and its components will be clearly labeled as an investigational device. The labeling provides instructions for use for the device. A copy of the Instructions for Use will be provided to each investigational site.

15.2 Storage

The investigational devices will be stored in a secure place. Access should be strictly limited to the investigators and their designees. Neither the investigators nor any designees may provide the investigational device to any subject not participating in this study. Special storage instructions for the components are described below.

The OCS and its components (the Cardiac Disposable Set and Cardiac Solution Set) should be stored at ambient temperatures (15-35°C), and ambient humidity (10-90%). The Cardiac Solution Set should not be frozen.

15.3 Accountability

The investigator or designee will maintain an inventory record of investigational devices received, used for treatment, otherwise discarded, and returned to the Sponsor to assure FDA and the Sponsor that the investigational device will not be dispensed to any person who is not a subject under the terms and conditions set forth in this protocol.

15.4 Device Complaints and Malfunctions

The investigator will inform the Sponsor of any complaints or malfunctions during the course of the study. The Sponsor will investigate all device complaints and malfunctions.

16. Regulatory and Ethical Requirements

This clinical study will be conducted in accordance with the significant risk provision of the US FDA Investigational Device Exemptions Regulation (21 CFR Part 812); the Protection of Human Subjects Regulation (Informed Consent; 21 CFR Part 50); the Institutional Review Boards Regulation (21 CFR Part 56); and, the Financial Disclosure by Clinical Investigators Regulation (21 CFR Part 54), EN ISO 14155, and with all applicable local regulations.

16.1 Informed Consent

Informed consent will be obtained from all subjects prior to study participation.

16.2 Institutional Review Board

Prior to initiation of any study procedures, the protocol, informed consent and device labeling will be submitted to an IRB or EC for review and approval. In addition, any amendments to the protocol or informed consent form will be reviewed and approved (if necessary) by the IRB. The Sponsor must receive a letter documenting the IRB or EC approval at the clinical site prior to the initiation of the study.

17. Records and Reports Management

This investigational study will follow the investigator record keeping and reporting requirements specified in US FDA regulations 21 CFR §812.140(a) and 812.150(a). These requirements are summarized below.

17.1 Investigator Records

Prior to participation in the investigation, the investigator will provide the following documentation to the Sponsor:

- Investigator Agreement, signed by the investigator, which lists any physicians who will be involved in conducting the investigation under the direction of the primary investigator;
- A copy of the primary investigator's curriculum vitae (CV) as well as copies of CVs for any co-investigators;
- A letter signed by the chairperson of the IRB or EC indicating that the IRB has reviewed and approved this protocol; and,
- A copy of the IRB or EC approved informed consent document.

During the study, investigators will be required to maintain on file the following accurate, complete and current records relating to this study as described in US FDA regulation 21 CFR §812.140. A summary of these records is described below:

- All correspondence and required reports which pertain to the study;

- Records of receipt, use or disposition of the investigational device, including the type and quantity of the device; the dates of receipt; the lot number; the names of all persons who received, used or disposed of each device; and why and how many units of the device have been returned to the Sponsor, repaired, or otherwise disposed;
- Records of each subject's case history and exposure to the device;
- Signed and dated consent forms;
- Relevant observations, including records concerning adverse events, condition of each subject upon entering and results of diagnostic tests;
- Electronic case report forms;
- Protocol and amendments;
- Subject recruiting materials; and,
- Investigator curricula vitae.

US FDA regulations require all investigators participating in investigational device studies to maintain detailed clinical records during the investigation and for a period of at least 2 years after the latter of the following two dates:

1. The date on which the investigation is terminated or completed; or,
2. The date the records are no longer required for purposes of supporting a marketing application to US FDA.

The investigator will not dispose of any records relevant to this study without (1) written permission from the Sponsor and (2) providing an opportunity for the Sponsor to collect such records. The investigator will take responsibility for maintaining adequate and accurate electronic or hard copy source documents of all observations and data generated during this study, including any data clarification forms (DCFs) received from the Sponsor. Such documentation is subject to inspection by the Sponsor and the US FDA.

17.2 Investigator Reports

Investigators will be required to prepare and submit to the Sponsor the following complete, accurate, and timely reports on this investigation when necessary. These reports, which follow, include all of those described in US FDA regulation 21 CFR §812.150(a), and some additional reports requested by the Sponsor:

- The investigator will notify the Sponsor of a subject death occurring during the investigation as soon as possible, preferably within 24 hours of learning of the subject's death, but in no event later than 48 hours. The investigator will also notify the Sponsor immediately of a serious adverse event, preferably within 24 hours of learning of the serious adverse event, but in no event later than 5 working days.
- The investigator will notify the Sponsor of any unanticipated adverse device effects preferably within 24 hours after the investigator first learns of the effect, but in no event later than 5 working days. The investigator will notify its reviewing IRB or EC of any

unanticipated adverse device effects as soon as possible, but no later than 10 working days after the investigator first learns of the effect.

- The investigator will notify the Sponsor of the withdrawal of IRB or EC approval as soon as possible, but no later than 5 working days after the investigator first learns of the withdrawal.
- The investigator will provide current progress reports to the Sponsor and reviewing IRB or EC at regular intervals but at least on an annual basis.
- The investigator will notify the Sponsor and reviewing IRB or EC of any deviation from the investigational plan to protect the life and physical well-being of a subject in an emergency as soon as possible, but no later than 5 working days after the emergency occurred.
- The investigator will notify the Sponsor and reviewing IRB or EC that an informed consent was not obtained from a subject as soon as possible, but no later than 5 working days after such an occurrence.
- The investigator will provide a final summary report within 3 months after termination or completion of the study to the reviewing IRB or EC. The site study completion report may serve as the study completion for the Sponsor.
- The investigator will provide any other information upon the request of an IRB, EC, US FDA, or the Sponsor.

17.3 Data Collection

During each subject's visit to the clinic, an investigator participating in the study will record progress notes to document all significant observations. In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes as described above.

For transmission to the Sponsor, information from the study progress notes and other source documents will be promptly entered into the electronic Case Report Forms (eCRFs).

17.4 Source Documents

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents will include, but are not limited to, progress notes, electronic data, computer printouts, screening logs, and recorded data from automated instruments. All source documents pertaining to this study will be maintained by the investigators and made available for inspection by authorized persons.

18. Clinical Monitoring

18.1 Monitoring

The Sponsor has ethical, legal and scientific obligations to carefully follow this study in a detailed and orderly manner in accordance with established research principles and FDA

regulations. As part of a concerted effort to fulfill these obligations, the Sponsor's monitors will visit the center during the study in addition to maintaining frequent telephone and written communication.

The following guidelines are provided to describe the Sponsor's procedures for monitoring the clinical studies. If the investigator is not complying with the signed Investigator Agreement, the protocol, or any condition of the study, e.g., incomplete data forms, the Sponsor has the right to terminate the investigator's participation in the study. The Sponsor is responsible for selecting study monitors qualified by training and experience to conduct monitoring of the trial and for ensuring the quality of the study monitoring visits by the monitor.

The Sponsor's general monitoring procedures for investigational studies are described below.

18.2 Pre-Study Monitoring Procedures

18.2.1 Selection of Monitors

All monitors will be qualified by education, training, and experience.

18.2.2 Qualification Visits

The Sponsor will conduct an on-site Qualification Visit in order to assess the suitability of the investigative site to participate in the clinical trial. The Sponsor will evaluate:

- Investigator and staff qualifications
- Facilities, equipment and storage availability
- Monitoring requirements

18.2.3 Initiation Visits

A monitor will be responsible for determining and documenting that each investigator clearly understands and accepts the responsibilities and obligations incurred in conducting a clinical study. The monitor will ensure prior to study initiation that the investigator:

- Understands the requirements for a well-controlled study.
- Understands the nature of the clinical protocol.
- Understands his/her reporting obligations.
- Understands the requirements for device accountability.
- Understands and accepts the obligations to obtain informed consent.
- Understands and accepts the obligation to obtain IRB or EC review and approval of the clinical investigation before it is initiated and to ensure continuing review of the study by the IRB or EC, and to keep the Sponsor informed of all IRB or EC actions concerning the study.
- Understands and accepts the requirements regarding financial disclosure of clinical investigations, US FDA regulation 21 CFR Part 54.

- Has adequate facilities and access to an adequate number of suitable subjects to conduct the investigation.
- Has the required documentation on file, including IRB or EC approval and a signed investigator agreement.

18.3 Periodic Monitoring Visits

Monitoring visits will be conducted. The monitor should visit each site frequently to ensure the following:

- Facilities continue to be adequate and acceptable.
- The protocol is being properly followed.
- The IRB or EC has approved or been notified of any protocol changes.
- Accurate, complete and current records are being maintained, and the information recorded and submitted to the Sponsor is representative of the subject's record and other supporting documentation.
- Accurate, complete and timely adverse event reports are being submitted to the Sponsor.
- Informed consent has been obtained.
- The reason for a subject's withdrawal from the study has been documented.
- Reports are being submitted to the IRB or EC, and to the Sponsor.
- The appropriate staff is carrying out study activities.

The investigator or designee will, upon request, provide to the Sponsor or US FDA the necessary study records for a thorough review of the study's progress. These records include, but are not limited to, case report forms and original documents and records such as hospital and clinic charts, consent forms, and operative reports.

18.4 Frequency of Monitoring Visits

The frequency of monitoring visits will be determined on the basis of several factors, including the duration of the study, number of subjects enrolled, number of investigators/sites, complexity of the study, and number of outstanding issues from previous visits.

18.5 Study Completion Visit

All routine monitoring functions will be performed prior to the study termination visit; the study termination visit may be combined with a monitoring visit. The following tasks will be completed at the last visit by the monitor:

- Ensure that all forms have been sent to the Sponsor.
- Remind the investigator of the obligation to retain the records.

18.6 Reports of Monitoring Visits

Monitoring reports will be completed for all visits. Reports will include the date of the visit, list of study personnel present, and a summary of the findings.

18.7 Additional Auditing

Regulatory authorities worldwide may also audit the investigator during or after the study. The investigator will contact the Sponsor immediately if this occurs, and will fully cooperate with the audits conducted at a reasonable time in a reasonable manner.

18.8 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of a subject requires a protocol deviation, based on the judgment of the investigator (or a responsible, appropriately trained professional designated by the investigator). If the deviation from the protocol is necessary to protect the life and physical well-being of a subject in an emergency, such protocol deviations will be reported to the Sponsor and the reviewing IRB or EC as soon as possible, but no later than 5 working days after the emergency occurred. In the event of a significant deviation from the protocol due to an accident or mistake, the investigator or designee will contact the Sponsor at the earliest possible time by telephone to discuss the deviation and its impact on the study and subject continuation in the study. These discussions will be documented by the investigator and the Sponsor, and reviewed by the monitor.

18.9 Clinical Events Committee

The Sponsor will utilize an independent Clinical Events Committee (CEC) to provide individual adverse event adjudication for the study. It is anticipated that the CEC will meet with the Sponsor on a quarterly basis or as needed depending on the rate of patient accrual. The primary responsibilities of the CEC are to:

- Review adverse events that occur over the course of the study and the subsequent classification of these adverse events as related to the device or procedure.
- Review donor and recipient treatment group eligibility issues, including investigator decision's to not use a heart based on OCS assessments.
- Evaluate possible protocol deviations.
- Provide oversight for issues affecting general patient welfare.
- Provide recommendations to extend the length of follow-up past 30 days post transplant for a subject experiencing an adverse event.

18.10 Data Monitoring Board

An independent Data Monitoring Board (DMB) will be established by the Sponsor to periodically assess the progress of the trial, the safety data and the primary efficacy and safety endpoints. The DMB will make recommendations to the Sponsor regarding continuation, modification or termination of the clinical study.

The DMB will review all data submitted to them and may request additional information to assist in their decision process. They will attend scheduled meetings and issue written minutes of their meetings; furthermore, the appointed Chair will be responsible for issuing final written decisions.

18.11 Investigator Training

Device training will be provided to all participating investigators prior to patient enrollment in the study. Device training will include the following:

- A didactic session, where the investigators will be introduced to the device and its use model through presentations and videos
- Hands-on experience

The investigators will perform animal procedures to train on the device use model phases as follows:

- Device set up, which includes system check, installation and priming of the Cardiac Disposable Set.
- Heart instrumentation on the device, which includes cannulation of the donor heart and subsequent instrumentation of the heart on the device. It also includes adjustment of different parameters such as gas flow rate, temperature and blood flow rate of the blood and solution perfusate as recommended in the Instructions for Use Manual.
- Cessation of heart perfusion and removal from the device by using cardioplegic solution and then terminating the circulatory pump.

19. Confidentiality

All information generated in this study will be considered highly confidential and must not be disclosed to any persons not directly concerned with the study without written prior permission from the Sponsor. Authorized regulatory officials and Sponsor personnel (or their representatives) will be allowed full access to inspect and copy the records. All investigational devices, subject bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the Sponsor.

Subjects will be identified by initials and unique subject numbers in the eCRFs. If necessary, their full names may be made known to the Sponsor, a regulatory agency, or other authorized officials.

20. Amendment Policy

The investigator will not make any changes to this protocol without prior written consent from the Sponsor and subsequent approval by the IRB or EC, except if the deviation from the protocol is necessary to protect the life and physical well-being of a subject in an emergency. Such protocol

deviations will be reported to the Sponsor and the reviewing IRB or EC as soon as possible, but no later than 5 working days after the emergency occurred.

Any permanent change to the protocol, whether it is an overall change or a change for specific study center(s), will be handled as a protocol amendment. Any amendment to the protocol that appears indicated as the study progresses will be fully discussed by the investigator(s) and the Sponsor. If agreement is reached regarding the need for an amendment, it will be written by the Sponsor. The written amendment will be submitted to the chairman of the IRB or EC identified with the responsibility for reviewing amendments. Except for "administrative letters," investigators will await IRB or EC approval of protocol amendments before implementing the change(s). Administrative letters are defined to have no effect on the validity of the data or information resulting from the completion of the approved protocol, or the relationship of likely patient risk to benefit relied upon to approve the protocol; the scientific soundness of the investigational plan or protocol; and, the right, safety or welfare of the human subjects involved in the investigation.

When, in the judgment of the chairman of the local IRB or EC, the investigators and/or the Sponsor, the amendment to the protocol substantially alters the study design and/or increases the potential risk to the subject, the currently approved written informed consent form will require similar modification. In such cases, repeat informed consent will be obtained from subjects enrolled in the study before continued participation.

21. Study Sites and Investigators

This clinical study will be conducted at a maximum of 20 sites in U.S. and Europe. All sites and investigators will have experience with heart transplants and will undergo training in the use of the OCS.

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Appendix A: List of Required Laboratories

Donor and Recipient Required Laboratories

Serum Chemistry	Hematology
Glucose	Hct
HCO_3	WBC
Lactate	Hgb
Total Calcium	Platelet Count
Sodium	INR
Potassium	
Chloride	
Creatinine	
BUN/Urea	
Magnesium	
ALT	
AST	
GGT	
Billirubin	
BHCG	
pH	
PO_2	
PCO_2	
O2 Sat	

Appendix B: Adverse Events

*If event does not meet listed definition, report as an AE (not an SAE)

AE Term	Definition for Serious*	Cardiac Related
Acute Rejection	An endomyocardial biopsy finding of ISHLT 3R or higher grading	Yes
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 (this excludes neurological events): 1) Standard clinical and laboratory testing 2) Operative findings 3) Autopsy findings	No
Balloon pump (IABP)	Any use of an aortic balloon pump required to correct arrhythmia post-transplant for greater than eight (8) hours. The use of an IABP for less than 8 hours is anticipated as standard practice in some institutions.	Yes
Bleeding (Major)	Any episode of internal or external bleeding that results in death, the need for re-operation or hospitalization, or necessitates transfusion of packed red blood cells (pRBCs) as follows: 1) During the first 7 days post transplant: > 2 units of pRBCs within any 24 hour period 2) After 7 days post transplant: Any transfusion of pRBCs	No
Cardiac Arrhythmia	Any documented arrhythmia that results in clinical compromise (e.g., oliguria, presyncope or syncope, tachycardia, bradycardia) that requires hospitalization or occurs during the hospital stay post transplant. Cardiac arrhythmias are classified as 1 or 2 types: 1) Sustained ventricular arrhythmia requiring defibrillation or cardioversion 2) Sustained supraventricular arrhythmia requiring drug treatment or cardioversion	Yes

Cardiogenic Shock	Decreased cardiac output and evidence of tissue hypoxia SvO2 < 50% or lactic acidosis) in the presence of adequate intravascular volume. Specifically, the hemodynamic criteria for cardiogenic shock are: 1) Sustained hypotension (systolic blood pressure < 80 mmHg for at least 60 min) and 2) A reduced cardiac index (<2.0 L/min/m2) in the presence of elevated pulmonary capillary occlusion pressure (>18 mmHg). 3) Also any newly installed Intra aortic balloon pump to come off bypass.	Yes
Graft Failure	Primary or nonspecific severe acute heart dysfunction necessitating the sustained use of a mechanical support device (VAD or ECMO), listing for transplant or re-transplant.	Yes
Heart Failure	Right Heart Failure: Symptoms and signs of persistent right ventricular dysfunction [central venous pressure (CVP) > 18 mmHg with a cardiac index < 2.0 L/min/m2 in the absence of elevated left atrial/pulmonary capillary wedge pressure (greater than 18 mmHg), tamponade, ventricular arrhythmias or pneumothorax] requiring either RVAD implantation or inotropic therapy beyond 7 days or what is typical for inotropic management at the site. Left Heart Failure: Symptoms and signs of persistent left ventricular dysfunction (left atrial pressure > 18 mmHg with a cardiac index < 2.0 L.min/m2) in the absence of hemo-pericardium, pneumo-pericardium, hemothorax or pneumothorax, requiring either LVAD implantation or Inotropic therapy, 7 days or more after transplant.	Yes
Hemolysis	A plasma-free hemoglobin value that is greater than 40 mg/dl, in association with clinical signs associated with hemolysis (e.g., anemia, low hematocrit, hyperbilirubinemia) occurring after the first 72 hours post-transplant.	No
Hepatic Dysfunction	An increase in any two of the three following hepatic laboratory values: (total bilirubin, aspartate aminotransferase/AST and alanine aminotranferease/ALT) to a level greater than five times the upper limit of normal for the hospital, sustained for 14 days post-transplant (or if hepatic dysfunction is the primary cause of death).	No
Hypertension	New onset blood pressure elevation greater than or equal to 140 mmHg systolic or 90 mmHg diastolic.	Yes
Hypotension	Systolic less than 90 mmHG or diastolic less than 60 mmhg leading to fainting.	Yes

Major Infection	A clinical infection (focal or systemic) that is treated by anti-microbial agents (non-prophylactic). This category includes, but is not limited to cytomegalovirus (CMV), pneumonia, septicemia, myocarditis, pericarditis). A positive culture from the infected site or organ should be present unless strong clinical evidence indicates the need for treatment despite negative cultures. A positive CMV test is necessary for the CMV diagnosis; otherwise a negative CMV denotes a non-CMV infection. X-ray diagnosis of pneumonia may be used instead of positive culture results.	No
Malignancy	Cancerous cells that usually have the ability to spread, invade, and destroy tissue. Presence of which is confirmed through standard diagnostic methods for that particular malignancy (e.g., histopathological confirmation). This definition excludes non-melanoma skin cancer.	No
Multi-Organ Failure	The failure of two or more systems (e.g., the cardiovascular and renal systems). See definitions for heart failure, hepatic dysfunction, renal dysfunction, and respiratory failure.	No
Myocardial Infarction	Peri-Operative Myocardial Infarction: The clinical suspicion of myocardial infarction together with CK-MB or Troponin > 10 times the local hospital upper limits or normal, found within 7 days following transplant together with ECG and/or echo cardiocardiogram findings consistent with acute myocardial infarction. Non-Perioperative Myocardial Infarction: The presence at > 7 days post-transplant of the following criteria: 1) ECG with a pattern or changes consistent with myocardial infarction; and 2) Troponin or CK (measured by standard clinical pathology/laboratory medicine methods) greater than the normal range for the local hospital with positive MB fraction (≥ 3% total CK). This should be accompanied by a new regional LV or RV wall motion abnormality on a myocardial imaging study.	Yes
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). Each neurological event must be subcategorized as: 1) Transient Ischemic Attack (acute event that resolves completely within 24 hours with no evidence of infarction) 2) Ischemic or hemorrhagic cerebrovascular accident (CVA) event that persists beyond 24 hours or less than 24 hours associated with infarction on an imaging study.	No

Pericardial Effusion	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 and those without signs of tamponade.	Yes
Pleural Effusion	Collection of fluid within the pleural space as visualized by chest x-ray with the following characteristics: Exudative and not decreasing output within 10 days post-transplant, or protein content not decreasing, or lymphocyte proportion not increasing, or empyema, or requiring thoracentesis.	No
Pneumothorax	Prolonged collection of air in the pleural space as noted by chest x-ray at > 14 days post-transplant or requiring chest tube (tube thoracostomy) placement.	No
Pulmonary Hypertension	Diagnosis requires the presence of pulmonary hypertension with two other conditions. Pulmonary artery occlusion pressure (PAOP or PCWP) must be less than 15 mmHg (2000 Pa) and pulmonary vascular resistance (PVR) must be greater than 3 Wood units (240 dyn•s•cm-5 or 2.4 mN•s•cm-5). Diagnosis should be made through a right side catheter.	No
Renal Dysfunction	Acute Renal Dysfunction: Abnormal kidney function requiring dialysis (including hemofiltration) in patients who did not require this procedure prior to transplant , or a rise in serum creatinine of greater than 3 times baseline or greater than 5 mg/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 sustained for at least 30 days.	No
Respiratory Failure	Impairment of respiratory function requiring reintubation, tracheostomy or the inability to discontinue ventilatory support within 4 days (96 hours) post transplant. This excludes intubation for re-operation or temporary intubation for diagnostic or therapeutic procedures.	No
Valve Disease	Any disease process (e.g.: insufficiency or stenosis) involving one or more of valves of the heart (mitral, aortic, pulmonary and tricuspid). The disease will be considered serious if the valvular abnormality was diagnosed as moderate to severe in an echo reading.	Yes
Venous Thromboembolism Event	Evidence of venous thromboembolic event (e.g., deep vein thrombosis, pulmonary embolism) by standard clinical and laboratory testing.	No
Wound Dehiscence	Disruption of the deep surfaces of a surgical incision, excluding infections etiology, and requiring surgical repair.	No

Appendix C : Myocardial Biopsy

I- Endomyocardial Biopsy Slides

GENERAL PRINCIPLES:

All endomyocardial biopsy samples obtained according to this protocol will be read initially by the local center pathologist in order to guide the clinical management of the patient. The slides will be reviewed at a later date by the pathology central reviewers.

BIOPSY SCHEDULE:

Endomyocardial biopsies to monitor allograft rejection and other post-transplant findings, including ischemic injury, will be performed as follows:

- 7 days post-transplant
- 14 days post transplant
- 21 days post-transplant
- 28 days post transplant

Additional biopsies may be performed when clinically indicated at the investigator's discretion.

SPECIMEN PROCUREMENT AND ADEQUACY:

Biopsy samples should be obtained from the right ventricular apical septum. Biopsy samples should be handled with care and not be pierced by a needle or crushed with forceps when removing them from the bioptome and/or specimen container. Because many of the pathologic processes being assessed may be focal, a minimum of 4 samples of myocardium should be obtained and tissue samples should not be divided after they are procured because this practice results in less representative sampling.

SPECIMEN PROCESSING:

Biopsy specimens should be fixed in 10% neutral buffered formalin, paraffin embedded and sectioned at 6 microns thickness. All tissue fragments received from a biopsy procedure should be processed in a single cassette and paraffin block. A minimum of 3 hematoxylin and eosin (H & E) stained slides (minimum 3-4 sections per slide) are required. Additional sections and/or stains may be prepared according to local practices.

INTERPRETATION OF ENDOMYOCARDIAL BIOPSIES BY LOCAL PATHOLOGISTS:

All H & E sections will be examined by the local pathologist. An evaluable endomyocardial biopsy sample consists of at least 50% myocardium, excluding previous biopsy site, scar, adipose tissue, or

blood clot. Rejection will be graded according to the International Society for Heart and Lung Transplantation (ISHLT) Revised Working Formulation.

ISHLT 2004 Grading of Acute Cellular Rejection		
Term	**Grade**	**Comments**
No Rejection	0R	
"Mild" Rejection	1R	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
"Moderate" Rejection	2R	Two or more foci of infiltrate with associated myocyte damage
"Severe" Rejection	3R	Diffuse infiltrate with multifocal damage ± edema ± hemorrhage ± vasculitis

REVIEW OF SLIDES BY PATHOLOGY CONSULTANTS:

All H & E sections should be mailed as a group to TransMedics accompanied by identifying information or Clinical Affairs may also collect the biopsy slides during monitoring visits. TransMedics will forward slides to the pathology consultants for review, after which the slides will be returned to TransMedics for redistribution to the local pathologists.

Mailing Address:

TransMedics, Inc.
Attn: Clinical Affairs
200 Minuteman Road, Suite 302
Andover, MA 01810

Ensure each slide is appropriately labeled. Store slides in containers provided by TransMedics, Inc. Label the slide container with the label provided.

Label all slides as follows:

- Site Number- Patient Number
- Date (DD-MM-YYYY)
- Biopsy time point (Day 7, Day 14, Day 21, and Day 28)

In the event of a study patient's death, the local center is encouraged to secure permission and perform an autopsy. The local pathologist performs and reports the autopsy according to local protocol. The heart should be saved until review of the slides by the pathology consultants is complete. The slides of the heart should be sent to TransMedics for review by the pathology consultants.

II- Whole Heart Biopsy

OVERVIEW

Hearts that are not transplanted after randomization and procurement from the donor's chest should have a formal pathologic evaluation to determine the nature and extent of disease. Optimal pathologic examination of the specimen requires adequate fixation of the specimen as soon as practically possible.

MATERIALS & EQUIPMENT

1. Refrigerator or ice-water bath
2. Plastic specimen bag large enough to accommodate heart
3. Plastic specimen container large enough to accommodate heart (~1 L)
4. 10% neutral buffered formalin, enough to submerge heart in container (~500ml)
5. *Large knife or equivalent (e.g., TissueTek AccuEdge trimming blade and holder)*
6. Sharpie permanent marker or equivalent
7. 4x4 gauze pads or equivalent

PROCEDURE

1. If formalin is not immediately available at the donor institution, the heart should be kept cool until it can be placed in formalin. The heart can be refrigerated at 4°C, or be kept in a plastic bag submerged in an ice-water bath. Do not freeze the heart. It is anticipated that the heart will be able to be fixed in formalin within 12-24 hours.
2. When formalin is available, label the specimen container with the appropriate information and fill with ~500ml of 10% neutral buffered formalin. Please enter the following information: PROCEED II Study, your site #, date of explant and contact information.
3. Make a transverse cross-section through the ventricles near the apex (see Figures 1 & 2 below). This will allow formalin to adequately enter the ventricular cavities.
4. Make small incisions in the left atrium and right atrium.
5. Place the entire heart (including the apical section) into the formalin and submerge to adequately remove any air from the chambers.
6. Cover with gauze pads to ensure coverage of the heart (which may tend to float if there is significant epicardial fat) under the surface of the formalin.
7. The heart, now immersed in formalin, should be stored at room temperature until shipment.
8. Ship heart to core pathology lab at the following address:

   Robert Padera, M.D., Ph.D.
   Department of Pathology
   Brigham and Women's Hospital
   75 Francis St.
   Boston, MA 02115
   (617) 525-6792
9. Guidelines for shipping formalin can be found at:
   http://www.fedex.com/us/services/options/hazmat/ and it t is anticipated that the pathology

department at the institution will have adequate knowledge of these procedures including labeling and packing required for hazardous shipments.

10. **Please use TransMedics FedEx account #122381501, ground transportation and signature required requested. Also, please inform TransMedics of the tracking # immediately prior to shipment.**

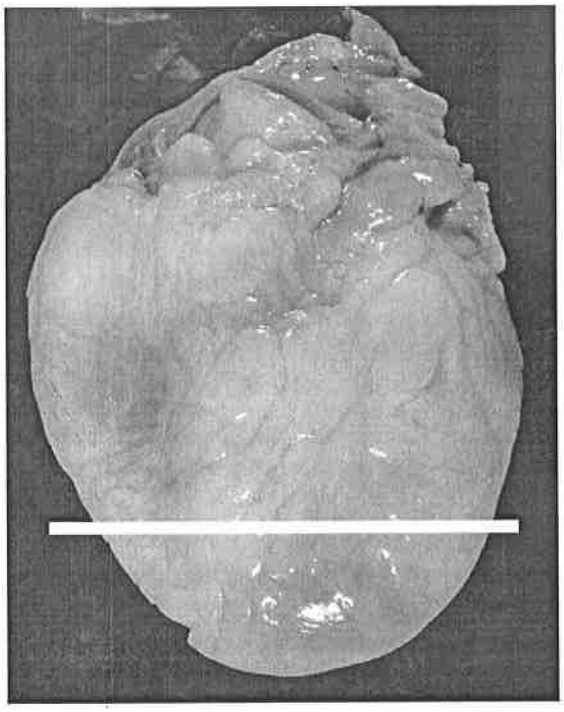


Figure 1 - Take transverse apical section as indicated by white line.

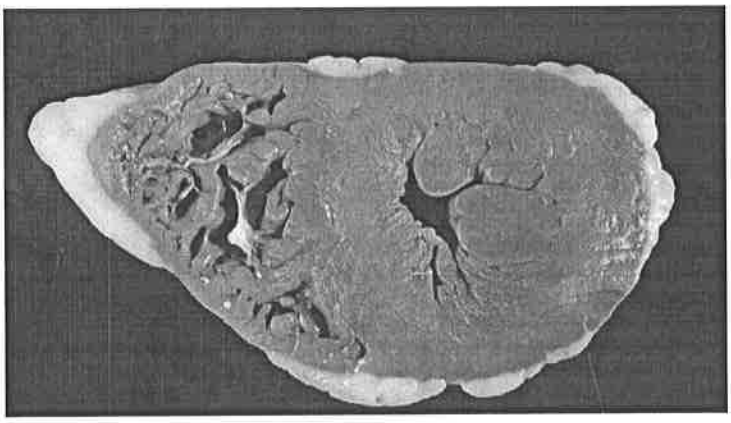


Figure 2 - The resultant apical section will look like this.

Appendix D : Echo Protocol

Version: 1.0 February 2009

The information contained in this manual is the confidential and proprietary information of TransMedics, Inc. It is to be used solely for the purposes of conducting the clinical trial in accordance with the clinical trial agreement.

ECHOCARDIOGRAM MANUAL

Purpose

The purpose of this document is to provide sites participating in the PROCEED II clinical trial with an instruction manual for obtaining high quality Echocardiograms, as well as to provide operational instructions on sending the Echo images to TransMedics™, Inc. (hereinafter referred to as "TransMedics").

Scope

The Echocardiogram CD-ROM or DVD process is to be performed by those trained to conduct the procedure and certified by TransMedics.

Process

- Only qualified individuals at the site will perform the echocardiogram
- The qualification procedure is detailed in Section 6 of this Manual
- The site will utilize their standard procedures for initial interpretation of Echo results in order to guide the clinical management of the patient.
- The Echo reports generated by the site will be included as source documents in the recipient binder.
- A digital copy (CD-ROM/ DVD) of the study echo in addition to a copy of the echo report will be collected and transferred to TransMedics Clinical Affairs.

Responsibility

- The Clinical Site is responsible for ensuring that each echo is copied on CD-ROM or DVD in DICOM format and packaged and shipped to TransMedics Clinical Affairs.
- All patient unique identifiers should be removed prior to CD capture.

Schedule

The protocol specifies that echocardiograms will be obtained for each patient at the following time points:

- 7 Days Post Transplant.
- 28 Days Post Transplant

Additional Echocardiograms may be performed when clinically indicated at the principal investigator's discretion.

Echo Procedure

6.1 Sonographer's Training and Certification

- Sonographers are required to submit a sample echo with all view requirements and quality as discussed with TransMedics clinical affairs and outlined in this manual.
- Each sample echo should be performed on a healthy volunteer.
- The sample echo should be labeled and submitted with a sonographer's certification form provided by TransMedics labeled as TEST + subject initials.
- TransMedics echo support will review the sample echo and determine whether the quality is acceptable or not.
- If the echo is acceptable, the sonographer is then certified for the PROCEED II trial and notified via E-mail which will include a copy of the certificate.
- If the echo is not acceptable, the sonographer will be required to submit a second sample echo. If a sonographer cannot be certified based on two sample echos, TransMedics will contact the site to discuss a further training option.

A site should only submit patient echoes performed by a TransMedics certified sonographer. Only patient echos performed by a TransMedics certified sonographer should be accepted for analysis.

6.2 Conducting Patient Echocardiograms

- It is strongly recommended that all echocardiograms are performed for a patient by the same sonographer, on the same type of machine with the same settings of 2D gain and depth, whenever possible.
- Contrast agent for LV opacification should be considered only if it is the standard of care of the participating site and when the endocardium of more than 2 segments of same left ventricular (LV) wall are poorly defined.
- If decision is taken to use contrast agents according to FDA approved recommendation, it should be used after all required 2D, color and Doppler images are acquired.
- If contrast echo is performed on day 7 post transplant, it should be used with day 28 echo of the same subject.

A. Echocardiographic Equipment

- Generally, a 3.5 MHz transducer is recommended for obtaining images. Occasionally, patients with poor quality images will require a lower frequency transducer (i.e., 2.5 MHz), while some patients may be imaged with a higher frequency transducer (5 MHz). In general, use the highest possible frequency that allows adequate penetration for endocardial border definition.

- **At least three cardiac cycles (heart beats)** from each view must be recorded as long as subject has regular sinus rhythm, otherwise not less than 5 cardiac cycles should be captured for both 2D and Doppler views.

- Tissue harmonic imaging should be used, unless this worsens endocardial border definition. Whether or not tissue harmonic imaging will be used is up to the discretion of the sonographer and investigator.

B. Instructions for Echocardiographic Recording

- Sites are required to send a digital copy (CD-ROM or DVD) of each study subject to TransMedics. Echos are recorded and transferred on CD or DVD. **THE CD MUST BE SAVED IN DICOM FORMAT.**

- The echo must be blinded with the patient's name and other patient's identifiers (medical record number, etc.) must not be visible on the echo or written on the CD/DVD.

- Each CD/DVD must contain the site number, subject's ID number, subject's initials, and echo time point (day 7 or day 28).

C. Patient Preparation

- The patient's blood pressure should be taken just prior or at the time of echo and recorded on the echo tracking form.(digital BP measurements are accepted)

- For TTE the patient should be placed in the left lateral decubitus position unless this position is not possible.

- Patient should be connected to 3 electrocardiographic leads at time of echo with a good quality ECG display (QRS complex is clearly identified) on the echocardiographic monitor.

- **THE ECG TRACING MUST BE CLEARLY VISIBLE ON CAPTURED CLIPS.**

- Echocardiograms should be obtained in a manner that is most consistent with good-quality patient care. Needless to say, patient care issues including patient comfort should always supersede research interests. Indeed, patient cooperation and comfort are extraordinarily important in obtaining the highest quality echocardiographic examination.

D. Echo Time Points

The study protocol requires two echocardiographic examinations for each subject:

- 7 days post transplant
- 28 days post transplant

E. Echo Views

The Echo protocol requires the following views:

Table 1 - Summary of required TTE views

Parasternal Long Axis View (PLAX)	☐ 2D of antroseptal and posterior LV walls. ☐ 2D with special attention to aorta and left atrium. ☐ Color Doppler across mitral and aortic valves.
Parasternal Short Axis View (SAX)	☐ 2D Mid-papillary muscle level and mitral valve. ☐ M-mode across and perpendicular to LV papillary muscle level (perpendicular to antroseptal and infero posterior walls).
Parasternal Short Axis View at the base. (SAX-base)	☐ 2D Aorta-LA-RVOT. ☐ Color Doppler across pulmonary valve and RVOT. ☐ PW Doppler across RVOT and pulmonary Valve. (Sample vol.3-5mm)
Apical Four-chamber View (4CH) If right ventricle is foreshortened, zoomed 4CH for RV and RA is required by narrowing sector width and acquire on higher frame rate (60-90 fps)	☐ 2D Left ventricle and whole left atrium. ☐ 2D Right ventricle free wall motion. ☐ Color Doppler across mitral valve. ☐ Color Doppler across tricuspid valve ☐ PW Doppler of mitral inflow at mitral valve leaflet tips. (Sample volume 1-2mm) ☐ TR velocity by continuous Wave Doppler ☐ CW Doppler of mitral valve. ☐ PW-TDI septal mitral annulus* ☐ PW-TDI lateral mitral annulus*
Apical 5 chamber View	☐ LV outflow tract and Aortic pulsed wave (PW) Doppler. (Sample vol.3-5mm below and above aortic Valve, respectively) ☐ Color Doppler across the LVOT and Aorta.

Apical 2 chamber View (2CH)	☐ 2D of anterior and inferior walls of LV.
Apical long-axis View (3CH)	☐ 2D of antroseptal and posterior wall. ☐ LV outflow tract and Aortic pulsed wave (PW). ☐ Color Doppler across the LVOT and Aorta.
Right Ventricle View (Zoomed 4 CH on RV) Only if RV is foreshortened in apical 4CH view	☐ 2D Right ventricle free wall motion (frame rate 60-90fps) ☐ Color Doppler for tricuspid regurgitation ☐ TR velocity by continuous wave Doppler

All echocardiographic images for this study must be optimized. The exact process by which optimization will be attained will be left to the discretion of the sonographer and the individual echocardiographic investigator. Additional views/assessments may be necessary depending on individual patient's pathology and echogenicity.

F. Detailed Examination

The protocol includes conventional 2D echocardiographic imaging and tissue Doppler imaging.

- Three heart cycles must be recorded for every view for patients with regular heart rate, otherwise five heart cycles are required for irregular rhythm.
- For patients in sinus rhythm avoid recording premature beats.
- All Doppler and tissue Doppler profiles are to be recorded during end-expiration at a sweep speed of 100mm/s.
- It is essential to obtain a high quality ECG-signal, as this is required for the timing of myocardial events and loop averaging (cine compound).

The following describes the views to be stored for each patient and the corresponding TTE parameters to be assessed.

A) Parasternal Views:

1-Parasternal Long Axis View: (PLAX)

In the ideal echocardiographic "window" for the long axis, the proximal interventricular septum is horizontal and continuous with the aortic root. The anterior and the posterior mitral valve leaflets, and the right and noncoronary aortic valve leaflets are visible. **Care must be taken to acquire the true LV long axis and not to artifactually truncate it (see figure 1).**

2D images should be optimized and at least three cardiac cycles must be digitally captured for patients in sinus rhythm.

- PLAX view is the main view used to assess LV dimensions, septal and posterior wall thickness.

- Color Doppler across the mitral and aortic valves required to assess valve's regurgitation in this view as well.

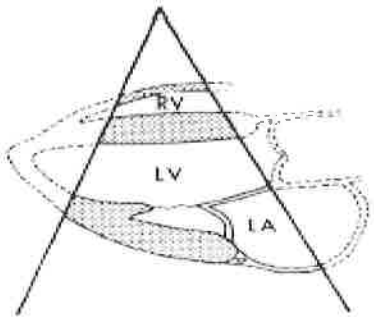


Figure 1

2) Parasternal Short Axis Views of LV: (SAX)

- The parasternal short axis views will be obtained and recorded at the mid-papillary muscle level.
- Apply M-mode across and perpendicular to the scanned short axis view of LV at the level of the papillary muscle to measure left ventricular dimensions (in case PLAX view is truncated)
- Segmental wall motion (SWM) of mid segments of LV can be assessed in this view in addition to the apical views.
- SAX at the level of mitral valve should be considered **only** if basal segments in apical views are poorly defined to assess SWM of basal segments of LV.

3) Parasternal Short Axis View of the Base: (SAX-base)

This view is obtained by superior angulation of the probe from the parasternal short axis of the LV, until the aorta as well as the right ventricular out flow tract (RVOT) and the pulmonary valve are visualized.

Both color and pulse wave (PW) Doppler are required across the pulmonary valve and RVOT in this view.

B) Apical Views:

Three apical views will be obtained: the apical four-chamber, apical two-chamber views, and apical long-axis. In all apical views, please be careful to maximize LV length and not truncate the true long axis.

1) Apical four-chamber view:

- The entire LV endocardium must be within the scanned sector in both end-diastole and end-systole. The most difficult areas to visualize the endocardium are usually the apex and the lateral LV free wall; please pay attention to these areas.
- Special attention should be paid to properly align the image to avoid any foreshortening of the scanned chamber.
- In addition to 2D imaging, the following Doppler examination will be required from this view:

- ***i) Color and spectral Doppler of mitral inflow:*** The mitral inflow velocity curve will be recorded from the apical four-chamber view with the pulsed and continuous -wave Doppler sample volume positioned at the tips of the mitral leaflets during quiet respiration for 30 seconds (Sample volume of 1-2 mm is recommended).
- For all Doppler recordings, care should be taken to align the pulsed Doppler signal parallel to the presumed direction of flow.
  - Set the scale to 80 cm/sec and record at 50 mm/sec and 100 mm/sec sweep speeds for at least five cardiac cycles.
  - The faster speed is essential for more accurate measurements. Reset scale if necessary to optimize the flow signal.
  - Avoid acquiring Doppler trace without including the scale of the Doppler trace.

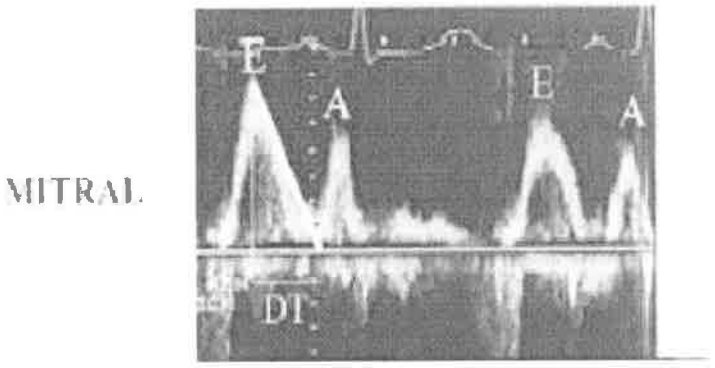
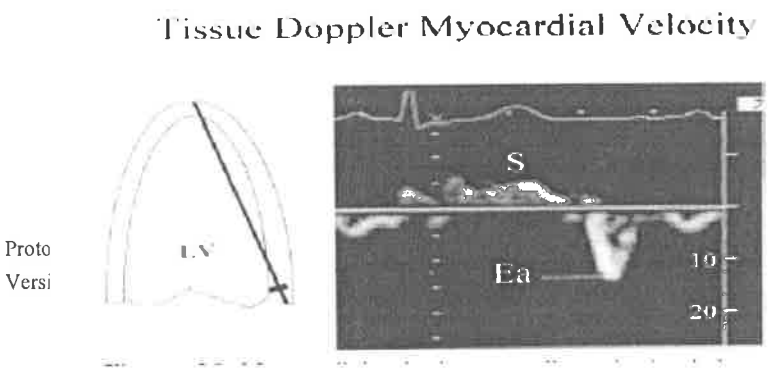


Figure 2

ii) TDI (Tissue Doppler Imaging) spectral of mitral annulus:

Adjust the image to orient the motion of the lateral wall as parallel to the cursor as possible. For optimal recording of tissue velocity, both gains and filter settings should be set low. Initiate 2D color DTI and position the sample volume at the point of the mitral-annulus at end-systole (**See Figure 3 below).**



Proto
Versi

Figure 3

- The sample volume (3-4 mm) should be placed at the junction of the LV wall with the mitral annulus of the septal and lateral myocardial segments from the four-chamber view. Hence, the sample volume is best set at the center of the region of the myocardium to be studied. Switch to PW spectral DTI and set the scale to 20 cm/sec with a sweep speed of 100 mm/sec.
- Before collecting data, the pulsed Doppler velocity range should be set to ensure velocity aliasing does not occur. Normal myocardial velocities at rest seldom exceed 20cm/sec and a velocity range of +/- 20 cm/sec is usually appropriate.
- For hearts from young donors, this setting may have to be increased, as their peak velocities may be much higher.
- Once a clear pattern is obtained, record at least 5 beats, during quiet respiration (or preferably during breath holding at end-expiration).

iii) Color and continuous wave Doppler across mitral valve.

- Color Doppler across the mitral valve is required to assess valve regurgitation with a recommended Nyquist limit of (50-60 cm/s) and color Doppler gain set at lowest gain to avoid aliasing.

- Continuous wave (CW) Doppler is recommended to be color Doppler guided at time of acquisition of mitral valve flow.

2) Apical two-chamber view:

- Obtain the apical two-chamber view in which the scan plane transects the anterior LV wall on the left and neither RV nor the LV outflow tracts are visualized.
- As mentioned earlier, special attention should be paid to properly align the image. Avoid any foreshortening of left atrium or left ventricle.

3) Apical long-axis view:

- Standard 2D imaging of the apical long-axis view should be obtained. Images should be adjusted to optimize myocardial resolution.
- In this view, record LV outflow tract pulse wave Doppler. The Doppler sample volume (3-5 mm) should be positioned about 5 mm proximal to the aortic valve. Recordings should be performed at 100 mm/s over five cardiac cycles.

- Color flow Doppler across the mitral valve and aortic valve are to be recorded with the Nyquist limit being set between 50-60 cm/sec.

4) Right ventricle (RV) from apical 4-chamber view:

- Record a four-chamber view with specific attention to imaging the right ventricle and acquire the widest diameter of the (RV). Narrow the image to increase frame rates (60-90 fps) by zooming on RV as much as possible but not on expense of shore shortening of the maximal RV length.

- Color Doppler across the tricuspid valve is required to assess valve regurgitation with a recommended Nyquist limit of (50-60 cm/s) and Color Doppler gain optimized to avoid aliasing (recommended<50%).

- Continuous wave Doppler is recommended to be color Doppler guided at time of acquisition of Tricuspid valve flow making sure to document the highest velocity.

Instructions on How to Properly Label and Send Study Echos to TransMedics

It is critical that echos are properly labeled and submitted to TransMedics. The sections below describe the steps involved in submitting echos.

A. Contact Information

We encourage you to ask any questions you may have. TransMedics Echo support is available to assist sites regarding the procedures for performing and submitting echos as well as to answer any administrative or technical questions. If you have any questions, please contact **Amira Hassanein,** at the following.

Telephone: 1-978-552-0972
Hours: 8:30 am – 5:30 pm EST/EDT
E-mail: ahassanein@transmedics.com

B. Label Media

The media case and the media must be separately labeled with the study identifiers (the Echo, media case, and media must be blinded).

- Affix a square Echo label on the case:
  All lines must be completely filled in as follows.
  (☐☐ / ☐☐☐ / ☐☐☐) [Site/subject/initials]

- Fill in the required information on the label provided by TransMedics. Place the label on the CD or DVD ROM.

C. Advance Fax Echo Tracking Form (ETF)

The completed Echo Tracking Form must be **faxed in advance** to TransMedics at the time of courier shipment.

Please Fax ETF to: 1-978-552-0977 (no cover sheet is necessary)

D. Ship Echo and Echo Tracking Form

Sample echo and study echos (with sonographer certification and echo tracking forms respectively) should be **shipped after being copied in DICOM format on CD ROM or DVD to:**

Shipping Address: TransMedics, Inc.

TransMedics, Inc.
Attn: Clinical Affairs
200 Minuteman Road, Suite 302
Andover, MA 01810

There must be an echo tracking form with each CD-ROM or DVD. Please send all echos to TransMedics within one week of the examination, if not sooner.

Thank you in advance for following these administrative procedures to facilitate echo processing for the **PROCEED II** study.

Brigham & Women's Hospital
Site 008

10/10/11	# patients on transplant list	# patients eligible for trial (Fit inclusion and exclusion criteria)	Consented
Status 1A Patients	1	1	1
Status 1B Patients	7	7	5
Status 2 Patients	0	0	0

10/11/11	#
All heart transplant activities last week	X
All heart donors offers last week	X

Brigham & Women's Hospital
Site 008

Patient Code	Age	VAD? (Yes/N0)	Status (1A, 1B, 2)	Location (Home/Hospital)	Meets Criteria? (Yes/No)	If no, specify the reason	Surgeon	Cardiologist	Patient approached for consent? (Yes/No)	Dates of Discussion	Consented? (Yes/No)	If no, why?	Randomized? (Yes/No)	Date of Transplant	Notes
1	50														Tx
2	68														Tx
3	54	Yes	7	Home	Yes		Dr Couper	Dr Givertz	No		No	Off List			
4	62														Died 05/08/11
5	26														Tx
6	39														Tx
7	62														Tx
8	51	Yes	1AB	Hospital	Yes		Dr Couper	Dr Mudge	Yes	07/26/11 & 08/30	Yes			9-Oct-11	No CTSurgeon available to explant LVAD
9	42														Tx
10	51	Yes	7	Home	Yes		DrCouper	Dr Givertz	Yes	26-Jul-11	No	Off List			SAH
11	68														Tx

Highlight Rows As Follows:

Any patient consented and eligible for trial

Any patient consented but not currently eligible for trial

Any patient consented but transplanted off study

12	[illegible]	Yes	1B	Home	Yes		[illegible]	[illegible]	Yes	[illegible]	Yes				
13	[illegible]	Yes	1B	Home	Yes		[illegible]	[illegible]	Yes	[illegible]	Yes				
14	[illegible]	Yes	1B	Home	Yes		[illegible]	[illegible]	Yes	[illegible]	Yes				
15	[illegible]	Yes	1B	Home	Yes		[illegible]	[illegible]	Yes	[illegible]	Yes				
16	[illegible]	Yes	1B	Home	Yes		[illegible]	[illegible]	Yes	[illegible]	Yes				
17	42														Tx
18	62	No	1B	Home	Yes		Dr Couper	Dr Stevenson	No	Will see	No				
19	[illegible]	[illegible]	1A	Home	Yes		[illegible]	Dr Givertz	Yes	8/30/11	Yes				
20	65	Yes	1B	Hospital	Yes		Dr Couper	Dr. Givertz	No	Will see	No				