Phase I safety study of autologous umbilical cord blood derived mononuclear cells during surgical Stage II palliation of Hypoplastic Left Heart Syndrome

Sponsor:  
Timothy J. Nelson, MD, PhD  
Mayo Clinic Transplant Center  
200 1st St SW  
Stabile 5-41  
Rochester, MN 55905  
507 538-7515

Investigator:  
Muhammad Y. Qureshi, MBBS  
Pediatric and Adolescent Medicine  
200 1st St SW  
Stabile 5-54  
Rochester, MN 55905  
507 244-3297

Protocol Number: (IRBe)  
12-008521

IND Number:  
15343

Initial version: [12-27-2012] Version (1.0)  
[02-26-2013] Version (2.0)  
[05-14-2013] Version (3.0)  
[08-13-2014] Version (4.0)  
[01-06-2015] Version (5.0)  
[06-03-2015] Version (6.0)
Table of Contents

STUDY SUMMARY ................................................................................................................................. 5

1 INTRODUCTION .................................................................................................................................. 6

   1.1 BACKGROUND .......................................................................................................................... 6
   1.2 INVESTIGATIONAL AGENT ..................................................................................................... 8
   1.3 PRECLINICAL DATA .................................................................................................................. 8
   1.4 CLINICAL DATA TO DATE ...................................................................................................... 9
   1.5 DOSE RATIONALE AND RISK/BENEFITS ............................................................................ 10

2 STUDY OBJECTIVES .......................................................................................................................... 11

3 STUDY DESIGN .................................................................................................................................... 12

   3.1 GENERAL DESIGN .................................................................................................................. 12
   3.2 PRIMARY STUDY ENDPOINTS ............................................................................................... 14
   3.3 SECONDARY STUDY ENDPOINTS ......................................................................................... 14

4 SUBJECT SELECTION ENROLLMENT AND WITHDRAWAL .................................................................. 14

   4.1 INCLUSION CRITERIA ............................................................................................................... 14
   4.2 EXCLUSION CRITERIA ............................................................................................................. 15
   4.3 SUBJECT RECRUITMENT, ENROLLMENT AND SCREENING ................................................ 15
   4.4 EARLY WITHDRAWAL OF SUBJECTS ...................................................................................... 16
      4.4.1 When and How to Withdraw Subjects ................................................................................ 16
      4.4.2 Data Collection and Follow-up for Withdrawn Subjects .................................................... 16

5 STUDY DRUG ....................................................................................................................................... 17

   5.1 DESCRIPTION, PREPARATION AND ADMINISTRATION OF STUDY DRUG ..................... 17
   5.2 TREATMENT REGIMEN ............................................................................................................ 17
   5.3 SUBJECT COMPLIANCE MONITORING .................................................................................. 17
   5.4 PRIOR AND CONCOMITANT THERAPY .................................................................................... 18
   5.5 PACKAGING ............................................................................................................................... 18
   5.6 RECEIVING, STORAGE, DISPENSING AND RETURN ............................................................. 18
      5.6.1 Receipt of Drug Supplies .................................................................................................... 18
      5.6.2 Storage ............................................................................................................................... 19
      5.6.3 Dispensing of Study Drug .................................................................................................. 19
      5.6.4 Return or Destruction of Study Drug ................................................................................ 19

6 STUDY PROCEDURES ......................................................................................................................... 20

   6.1 VISIT 1- UMBILICAL CORD BLOOD COLLECTION ............................................................ 25
   6.2 VISIT 2- CONSENTING TO PART B .......................................................................................... 25
   6.3 VISIT 3- PRE-PROCEDURE WORK-UP ..................................................................................... 25
   6.4 VISIT 4- STAGE II SURGICAL PROCEDURE .......................................................................... 26
   6.5 VISIT 5- HOSPITALIZATION ..................................................................................................... 26
   6.6 VISIT 6- HOSPITAL DISCHARGE .............................................................................................. 27
   6.7 VISIT 7- 1 MONTH FOLLOW-UP ............................................................................................... 27
   6.8 VISIT 8- 3 MONTH FOLLOW-UP ............................................................................................... 27
   6.9 VISIT 9- 6 MONTH FOLLOW-UP .............................................................................................. 28

7 STATISTICAL PLAN .............................................................................................................................. 29

   7.1 SAMPLE SIZE DETERMINATION .............................................................................................. 29
   7.2 STATISTICAL METHODS ............................................................................................................ 30
   7.3 SUBJECT POPULATION(S) FOR ANALYSIS .............................................................................. 31

8 SAFETY AND ADVERSE EVENTS ....................................................................................................... 32
8.1 DEFINITIONS .................................................................................................................. 32
8.2 RECORDING OF ADVERSE EVENTS ........................................................................... 34
8.3 REPORTING OF SERIOUS ADVERSE EVENTS AND UNANTICIPATED PROBLEMS ...... 34
  8.3.1 Site-Investigator reporting: Notifying the IRB ......................................................... 34
  8.3.2 Sponsor reporting: Notifying the FDA ..................................................................... 34
8.4 STOPPING RULES ......................................................................................................... 35
8.5 MEDICAL MONITORING ............................................................................................... 35
  8.5.1 Internal Data and Safety Monitoring Board ............................................................. 36
9 DATA HANDLING AND RECORD KEEPING .................................................................. 37
  9.1 CONFIDENTIALITY ...................................................................................................... 37
  9.2 SOURCE DOCUMENTS ................................................................................................. 37
  9.3 CASE REPORT FORMS ................................................................................................. 37
  9.4 RECORDS RETENTION ................................................................................................. 41
10 STUDY MONITORING, AUDITING, AND INSPECTING .................................................. 42
  10.1 STUDY MONITORING PLAN ...................................................................................... 42
  10.2 AUDITING AND INSPECTING ................................................................................... 42
11 ETHICAL CONSIDERATIONS ......................................................................................... 42
12 STUDY FINANCES .......................................................................................................... 44
  12.1 FUNDING SOURCE ...................................................................................................... 44
  12.2 CONFLICT OF INTEREST ......................................................................................... 44
13 PUBLICATION PLAN ...................................................................................................... 44
14 REFERENCES ................................................................................................................... 44
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse Event/Adverse Experience</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>CFSE</td>
<td>Carboxyfluorescein Succinimidyl Ester</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data and Safety Monitoring Board</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practice</td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
</tr>
<tr>
<td>HLHS</td>
<td>Hypoplastic Left Heart Syndrome</td>
</tr>
<tr>
<td>IB</td>
<td>Investigator’s Brochure</td>
</tr>
<tr>
<td>ILR</td>
<td>Implantable Loop Recorder</td>
</tr>
<tr>
<td>IND</td>
<td>Investigational New Drug Application</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>MNC</td>
<td>Mononuclear Cells</td>
</tr>
<tr>
<td>PHI</td>
<td>Protected Health Information</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>RV/LV</td>
<td>Right/Left Ventricle</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event/Serious Adverse Experience</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>UCB</td>
<td>Umbilical Cord Blood</td>
</tr>
</tbody>
</table>
## Study Summary

<table>
<thead>
<tr>
<th>Title</th>
<th>Phase I safety study of autologous umbilical cord blood derived mononuclear cells during surgical Stage II palliation of Hypoplastic Left Heart Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running Title</td>
<td>Safety of UCB-MNC Delivered into the Myocardium in HLHS Subjects</td>
</tr>
<tr>
<td>Protocol Number</td>
<td>12-008521</td>
</tr>
<tr>
<td>Phase</td>
<td>Phase I</td>
</tr>
<tr>
<td>Methodology</td>
<td>Open label</td>
</tr>
<tr>
<td>Overall Study Duration</td>
<td>2 years</td>
</tr>
<tr>
<td>Subject Participation Duration</td>
<td>6 months</td>
</tr>
<tr>
<td>Single or Multi-Site</td>
<td>Multi-Site</td>
</tr>
</tbody>
</table>

### Objectives

Determine the safety and feasibility of autologous mononuclear cells (MNC) collected from umbilical cord blood (UCB) and delivered into the myocardial of the right ventricle of children with HLHS. This add-on procedure is anticipated to pose little risk and has the potential to foster a new strategy for pediatric patients during clinically mandated surgery. This is an open-label study of autologous MNC and 6-month follow-up to document 1) systemic and cardiac adverse effects and 2) monitor changes in cardiac structure and function.

### Number of Subjects

10 Subjects

### Diagnosis and Main Inclusion Criteria

Subjects with HLHS who are 18 months of age or younger, who have successfully completed Stage I Norwood procedure without complications, and are scheduled to undergo Stage II surgical palliation (Glenn procedure) will be screened for study inclusion that includes previous collection and processing of umbilical cord blood (Part A- Umbilical cord blood release criteria).

### Study Product, Dose, Route, Regimen

Autologous mononuclear cells derived from umbilical cord blood. Target dose is 3 million cells/kg body weight with range of 1.0-3.0 million cells/kg to be delivered into the myocardium via epicardial injections of 0.1 ml each with total volume of approximately 0.1 ml/kg.

### Duration of Administration

Single administration at the time of a planned Stage II surgical palliation (Glenn procedure).

### Reference therapy

Prospective case control cohort of 10 subjects (not receiving cell-based product) mirroring the clinical inclusion/exclusion criteria and follow-up protocol outlined herein.

### Statistical Methodology

The primary endpoints for safety parameters will be calculated in two groups using Fisher exact test to include a non-randomized observational cohort for comparison. The secondary endpoints for efficacy will be calculated according to covariance using ANCOVA from baseline throughout 6-month participation period comparing outcomes to the non-randomized observational cohort for comparison.
1 Introduction

This document is a protocol for a human research study involving subjects with Hypoplastic Left Heart Syndrome (HLHS) who require surgical palliation for this life-threatening congenital heart disease. This study aims to collect, process, and deliver a cell-based product at the time of planned Glenn procedure and will be carried out in accordance with the applicable United States government regulations and Mayo Clinic and Oklahoma University Children’s Hospital research policies and procedures. Based on our safety data from analogous pre-clinical studies, we herein propose a Phase I safety and feasibility study using autologous umbilical cord blood as the source for mononuclear cells (MNC) that will be delivered into the myocardium of the right ventricle at the time of a planned surgical procedure for individuals requiring surgical palliation for hypoplastic left heart syndrome. The objective of this study is to execute a Phase I study to determine the safety and feasibility of intramyocardial injections of autologous mononuclear cells (MNC) collected from umbilical cord blood (UCB) into the right ventricle of HLHS children undergoing a scheduled Glenn procedure. This add-on procedure is anticipated to pose little risk to the patient and has the potential to foster a new strategy that leverages the regenerative capacity of the pediatric heart during clinically mandated surgical palliation.

Overview of the purpose of this open label, Phase I safety study.
The proposed Phase I safety protocol is designed to evaluate autologous MNC derived from umbilical cord blood and is the critical next step to develop novel therapies to strengthen the right ventricle of patients with HLHS. The central hypothesis and outcome measure of the study is that autologous MNC delivery into the right ventricle of the heart at the time of planned surgery is safe and feasible. We will use this study to better understand the biological consequences of MNC delivery into right heart muscle of patients with HLHS. We will evaluate the electrical stability of the right heart muscle following MNC delivery, along with cardiac structure and function coupled with systemic toxicity of this local and focal surgical delivery. Demonstrating that this approach can be safely administered in a practical and feasible delivery system will establish the foundation for additional studies leading to the establishment of advanced new therapeutic modalities for congenital heart diseases.

1.1 Background

HLHS is a severe form of congenital heart disease that consists of multiple obstructions to flow through the left heart and aorta, as well as hypoplasia of the left ventricle. This combination requires the affected individual to undergo surgical palliation to create a functional “single ventricle” circulation [1]. For years, families were faced with making a choice between comfort care, multistage conventional surgical palliation or cardiac transplantation within the first days of life. Dramatic improvements in early survival after Norwood procedure (Stage I of the multistage surgical palliation approach) in the past decade have altered this situation. As surgical mortality is now 10-20% [2], the majority of families in the United States are electing for conventional surgical approach with the expectation that individual patients will have ~70% chance of achieving the final Fontan circulation by the age of 5 [3]. This therapeutic option requires several palliative surgeries designed to reconstruct the patient’s circulatory system into one that can be sustained by a single right ventricle. This involves a three-stage protocol starting within days of birth. Stage I of this process is the Norwood procedure involving the separation of the pulmonary
artery and joining it with the upper portion of the aorta to create a neoaorta. Stage II involves creation of a direct connection between the patient’s superior vena cava and the pulmonary arterial confluence (bidirectional Glenn anastomosis). Stage III is creation of a Fontan circulation with complete venous return from the body directed into the pulmonary artery. This “single ventricle” approach requires the right ventricle to perform as the only circulatory pump for the entire body. Initially, the right ventricle is presented with the obligatory volume overloads and chronic cyanosis. These insults, combined with the need for multiple open-heart surgeries and the effects of chronically increased right ventricular myocardial afterload, often lead to progressive ventricular dysfunction and potentially life threatening complications (protein losing enteropathy and congestive heart failure). Although results have improved, these patients are still at significant risk of requiring heart transplantation at any stage of the process. As a result, regenerative strategies are becoming increasingly important to minimize the risk of long-term ventricular failure in patients with HLHS. Thus, our goal is to re-engineer the native right ventricle of HLHS children with stem cell-based regenerative strategies. In this Phase I study, we aim to determine the safety and feasibility of intramyocardial injections of autologous mononuclear cells (MNC) collected from umbilical cord blood (UCB) from children with HLHS.

**Summary of our commitment to regenerative therapies for HLHS**- Children with HLHS are in need of regenerative applications to strengthen their right ventricle and sustain long-term pressure overload in order to minimize the need for cardiac transplantation. The re-purposing of autologous mononuclear cells as applied in adult cardiac regeneration is now feasible in the pediatric population of HLHS based on our standardized clinical practice at Mayo Clinic, standardized manufacturing of cell-based product within the Human Cell Therapy Laboratory at Mayo Clinic, and preclinical laboratory data supporting the product’s safety and efficacy profile in a long-term large animal study. The timing of the prenatal diagnosis allows subject identification and umbilical cord blood collection. The clinical necessity of open cardiac surgery allows the designed protocol herein to be an “add-on” procedure with minimal risks to the patient. The intensive clinical follow-up and advanced cardiac imaging required for these children enables a minimally disruptive research procedure to collect comprehensive clinical data in a customized electronic environment built using Medidata Rave. Patients born outside of Mayo Clinic who did not have umbilical cord blood collected prior to being transferred to Mayo Clinic will make up the non-randomized case control cohort to monitor safety and efficacy profiles. Collectively, our clinical experience in surgical management of HLHS, expertise with human cell therapy manufacturing protocols, and programmatic focus on regenerative solutions for HLHS provides our team at Mayo Clinic with a realistic vision of executing the Phase I safety study for HLHS that is grounded on previous experiences and aligned with the ongoing efforts in the broader field of cardiac regeneration.
1.2 Investigational Agent

The product is a Biologic that will be manufactured at Mayo Clinic under GMP compliant facilities and procedures. The manufactured product is autologous mononuclear cells derived from umbilical cord blood with pre-defined release criteria and without any ex vivo manipulations. Delivery vehicle contains a cryoprotectant solution with 10% dimethyl sulfoxide.

1.3 Preclinical Data

Preclinical data was generated specifically for this IND application upon constructive guidance from the FDA during our pre-IND meeting (Dec 2, 2011) and subsequent correspondences (Feb 23, 2011). The overall strategy was predicated on a two-tiered approach that utilized large animals for autologous cell testing and small animals for human cell testing. The large animal testing utilized a porcine model system for long-term safety monitoring using an autologous umbilical cord blood derived product that was analogous to the proposed clinical trial. This allowed validation of the entire collection, processing, and delivery components of the protocol in a large animal, clinical-grade protocol. The small animal testing utilized an immune-deficient rodent model system to determine the dose-dependent toxicology and efficacy of human-derived umbilical cord blood product as manufactured in the Human Cell Therapy Laboratory at Mayo Clinic. Therefore, the combination of autologous cells into a large animal system and human cells into a rodent model system was designed to comprehensively support the present clinical protocol through these complementary preclinical data sets. There are three preclinical studies that were specifically designed and executed to address A) long-term safety in an autologous large animal system, B) dose response and toxicology assessment, and C) potential benefit of human-derived manufactured product.

Overall preclinical data summary- Three dedicated preclinical studies were designed and executed to address the specific questions required to determine the risk/benefit analysis for the proposed human study. The pre-clinical studies concluded that intramyocardial delivery of the umbilical cord blood-derived product does not cause increased risk to the right ventricle of the juvenile pig heart throughout a three-month follow-up. There was no evidence of predictable risk for intramyocardial injection of umbilical cord blood derived MNC compared to placebo control cohort as measured by cardiovascular surrogate markers as well as clinical morbidity and mortality. The safety of the dose was confirmed in this large animal model in parallel to the confirmed safety profile of even higher doses as tested in a rodent model system. Finally, the benefit of human umbilical cord blood-derived mononuclear cells as manufactured in the GMP facility at Mayo Clinic was demonstrated in a pressure overloaded right ventricular model system with improved cardiac structure, reversal of pathological gene expression profiles, and prevention of fibrotic changes upon histology analysis. Collectively, these preclinical studies indicate a safe and effective dose of umbilical cord blood-derived mononuclear cells with intramyocardial delivery into the right ventricle.
1.4 Clinical Data to Date

There is no available clinical data to date that uses umbilical cord blood-derived mononuclear cells delivered into the myocardium for the purpose of heart regeneration or intramyocardial delivery of a cell-based product in the pediatric setting. Therefore, preclinical data as noted previously was extensively studied to provide the evidence to justify the risk/benefit for the specific clinical protocol proposed herein for the “add-on” application during required HLHS surgical palliation. Despite the lack of comparable clinical data, we do have experience with a single emergency use application at the Mayo Clinic of bone marrow derived mononuclear cells delivered in a high-risk patient with end-stage heart failure secondary to HLHS. This experience was based on our clinical expertise, preclinical efforts, and published case reports in pediatric heart disease using similar cells (bone marrow derived mononuclear cells) delivered into the coronary circulation that indicated feasibility, safety, and importantly therapeutic benefit. Furthermore, adult applications focused on the regenerative potential of similar cells (bone marrow derived mononuclear cells) in left ventricle heart disease have been extensively documented and include well-documented experience with intramyocardial delivery strategies.
1.5 Dose Rationale and Risk/Benefits

The dose of the test article is 1-3 million cells per kg body weight divided in multiple injections of 0.1 ml from a frozen concentrate of 30 million cells/ml. The dose of our cell-based product was determined according to clinical experience in adult clinical trials and our pre-clinical safety monitoring. (Thus a 6 kg child would receive 18 million cells in 0.6 ml volume of a frozen stock of 30 million cells/ml in 6 injections of 0.1 ml to the right ventricle.)

Dose comparison- Clinical trials have used a range of $10^6$-$10^8$ total MNC delivered in chronic ischemic hearts in divided doses of 0.2 to 0.5 ml per injection with a total volume of 2-20 ml of solution. (Thus an analogous example based on our dose for a 70 kg adult would be 210 million cells in 7 ml of 30 million cells/ml in 14 injections of 0.5 ml each.) Therefore, we aimed to keep the concentration of cells comparable to the adult experience recognizing that total volume of injected cells, volume of each injection, and total dose of cells were equally important considerations.

Delivery approach- The rationale for intramyocardial delivery through the epicardial route for this cell-based product was chosen based on minimizing the risk of cell-delivery for HLHS patients. Because these patients are required to undergo open chest cardiac surgery, the epicardial surface is routinely exposed and intramyocardial delivery at the time of surgery as an “add-on” was determined to be less invasive and less risky compared to intracoronary infusion that would require a separate catheter-based procedure. Intramyocardial injections were monitored in both small and large animal models to determine feasibility, safety, and efficacy of delivery as noted in preclinical data. The frozen stock approach, with consistent concentrations, enabled the study team to control the reproducibility of the product delivery. Children undergoing this stage of surgery will be less than 10 kg and thus will only require a maximum of 1.0 ml of frozen solution. The ability to collect and process this concentration of human cell-based product from umbilical cord blood was reproducibly confirmed in preclinical studies in the Human Cell Therapy Laboratory. The ability to “thaw and deliver” reduced the risk of product calculation errors and mixing errors at the bedside and streamlined the process from frozen stock to patient. The amount of dimethyl sulfoxide (DMSO) delivered in small volume stocks is considerably lower than in established clinical applications such as bone marrow derived stem cells for hematopoietic applications.

Dose/delivery toxicity experimentations- We empirically tested for toxicity in an intramyocardial dose escalation study using preclinical models to determine potential risk of tumor formation, arrhythmia, and cardiac structure/function as discussed in the preclinical data presented in three independent experimental protocols. Specifically, we completed a double-blinded, randomized study using autologous umbilical cord blood derived MNC in a size-matched porcine cohort for long-term safety studies using the defined dosage of 3 million cells per kg of body weight and a delivery scheme based on intramyocardial delivery during open chest surgery.
Risks of intramyocardial delivery- As stated in the consent form, the risks of the intramyocardial injections are:

- Arrhythmias (irregular heartbeats) that could require medication or pacemaker
- Damage to the heart muscle if injections compromise coronary blood flow
- Local inflammation that could provoke myocarditis (inflammation of the heart muscle)
- Elevated temperature and white blood count (leukocytosis) for 2-3 days following cell injection
- Changes in blood glucose levels that could require medications
- Bleeding from the injection sites leading to pericardial effusion

2 Study Objectives

The primary purpose of this Phase I clinical study using an add-on intramyocardial delivery of MNC into patients with HLHS requiring stage II reconstruction is to determine the safety/feasibility of autologous MNC delivery. We propose to study 10 patients with HLHS scheduled for Stage II surgical palliation. Umbilical cord blood will be isolated at the time of birth, MNC processed, and stored. We propose to employ a single target dose according to body weight for all 10 patients, and monitor safety profile following the procedure for 6 months.

Primary Objective

Determine the safety and feasibility of autologous umbilical cord blood-derived mononuclear cells delivered into the myocardium of the right ventricle during planned Stage II surgical procedure for individuals with HLHS. Determine the incidence and severity of adverse events focused on cardiovascular structure and function that may be attributable to the intramyocardial delivery of cell-based product.

Secondary Objective

Estimate the potential for cardiovascular benefit of autologous umbilical cord blood-derived mononuclear cells using a non-randomized prospectively collected case-control cohort in a parallel non-interventional observation study that is designed with analogous clinical inclusion/exclusion criteria. (ClinicalTrials.gov Identifier: NCT01708863)
3 Study Design

3.1 General Design

This study is an open label Phase I trial to determine the safety and feasibility of umbilical cord blood-derived mononuclear cells delivered into the right ventricular myocardium of subjects with HLHS at the time of a planned Stage II surgical palliation. Subjects will be screened at outpatient clinic visits and interested qualified subjects will be consented and offered participation in this trial. The investigational plan includes a two-step process. Upon prenatal diagnosis of HLHS, expecting parents will be offered enrollment and consented for the collection of umbilical cord blood for the explicit purpose of research and development of cardiac regenerative protocols according to ongoing Mayo Clinic study (IRB 11-007176, Part A). This part of the protocol will not require active participation by the University of Oklahoma as the family will be responsible for contacting Mayo Clinic to arrange cord blood collection and processing prior to birth. **IF release criteria are met for the autologous cell-based product that has previously been collected and processed AND the infant requires Stage II palliative reconstructive surgery, THEN** the family may be consented for the clinical trial intended to determine the safety of MNC transplantation during planned surgical procedure of Stage II reconstruction. This two-step consenting process will help ensure the focus is on the clinical scenario facing the families at that time while minimizing the distraction of future possibilities and unnecessary speculation. Once consent has been obtained and Stage II surgery is planned, preoperative values will be established and a local selection committee will review subjects prior to planned surgery to confirm inclusion and exclusion criteria. Following Stage II surgery and cell-based product delivery, subjects will be followed for 6-months according to a predetermined schedule that includes regular imaging studies along with electrophysiology and laboratory studies.

Given this study is first-in-child for cell-based regeneration via an intramyocardial delivery, we will require a minimum of 3-month follow-up data to be collected prior to cell-based delivery with the second infant, likewise for the third infant to be enrolled. This will allow interim analysis of any adverse events to be reviewed prior to subsequent subject participation beyond the hard stopping rules as outlined in this protocol for the first three subjects enrolled.
The overall study participant flow chart for Part B with cell-therapy is as shown below. Autologous umbilical cord blood (Part A) is required prior to consenting to the open label cell-delivery protocol. Furthermore, if umbilical cord blood release criteria are not achieved, then subjects may still be eligible for the concurrent observational study that does not require cell-based delivery. (IRB# 12-002887)
3.2 Primary Study Endpoints

The primary endpoint is safety and feasibility with the objective to monitor and document 1) adverse cardiac events including death, sustained/symptomatic ventricular arrhythmias, heart failure, myocardial infarction, infections, and unexpected cardiovascular procedures within 6 months following cellular transplantation and 2) percentage of individuals that meet all release criteria in Part A for umbilical cord blood collection and the percentage of consented individuals that have cells delivered in Part B.

3.3 Secondary Study Endpoints

The secondary endpoints are cardiac structure and function before and after 6-month follow-up from the time of clinically planned Stage II surgery. Given the small cohort size, the trend of cardiac function will be compared to a non-randomized case control cohort that will be prospectively collected according to analogous inclusion criteria in a parallel observational study and differences will be used to determine power calculations for potential future studies. The variables measured will be primarily right ventricle ejection fraction from magnetic resonance imaging (MRI) at the end of study and changes in right ventricle ejection fraction using echocardiography from baseline to 1, 3, and 6-month follow-up. The secondary measures will be the change in right ventricle tricuspid annular plane systolic excursion (TAPSE) measurements from baseline to 1, 3, and 6-months along with fractional area change of the right ventricle as determined by echocardiography.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Eligibility for this study (Part B):
Both genders with HLHS requiring planned Stage II palliative surgery that meet the following inclusion and exclusion criteria.

Inclusion Criteria:
- Individuals with autologous cord blood product enrolled, consented and collected under IRB #11-007176, (Part A) that met all release criteria according to that protocol.
- Individuals with HLHS undergoing planned Stage II palliative surgeries.
- Ages up to 18 months are eligible if written informed consent can be obtained from both parents (unless one parent is not reasonably available) and/or legal guardians.
4.2 Exclusion Criteria

Exclusion Criteria for Part B:

- Individuals with UCB collected under consent and processed according to IRB #11-007176, not meeting specified release criteria
- History of DMSO reaction for either the infant or mother
- Individuals with families unwilling to participate.
- Individuals with severe chronic diseases, extra-cardiac syndromes, or cancer.
- Infants with the following conditions within 15 days prior to the date of the Stage II Glenn surgery:
  - Cardiogenic shock
  - Pulmonary hypertension requiring chronic medical therapy (e.g. supplemental oxygen, vasodilator)
  - Arrhythmia that required medication for control
  - Any documented infection requiring treatment with IV antibiotics, and/or current infection being treated with antibiotics
- Infants with the following complications of their congenital heart disease:
  - Any cardiac condition requiring urgent, or unplanned procedure 15 days prior to Stage II Glenn surgery
  - Tricuspid repair and/or aortic arch repair at the time of Stage II Glenn surgery
- Length of hospitalization of more than 60 days for Stage I Norwood procedure, unless cardiac function is normal within 10 days prior to Glenn Surgery
  - Chylothorax requiring dietary modifications
  - Seizure or neurological injury
  - Severe tricuspid regurgitation prior to Stage II Glenn surgery
  - History of extracorporeal membrane oxygenator (ECMO) support, unless cardiac function is normal within 10 days prior to Glenn Surgery.

4.3 Subject Recruitment, Enrollment and Screening

Potential subjects with specimens meeting release criteria from Part A will have their case reviewed by the Site Investigator and study team as the Glenn procedure is planned according to the primary clinical service. Additionally, a Pediatric Cardiologist or Surgeon not associated with the research study will independently review the clinical case and document a clinical note to determine the appropriateness of inclusion in the study. These potential subjects will then meet with study coordinators that will provide adequate time and information to determine if subjects/families are interested in participation. All subjects will be reviewed case-by-case at a final selection committee prior to planned Stage II surgery to ensure all inclusion and exclusion criteria for both Part A and Part B are documented and communication between manufacturing and clinical teams is confirmed.
4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects may withdraw from this study for many reasons given the extended time period between screening and Stage II surgery and the two-part consenting process. Part A, subjects may be enrolled with cord blood collected and may be excluded from Part B due to:
- failure of meeting release criteria
- unexpected morbidity and/or clinical complications prior to Stage II surgery.
These failures to enroll in Part B will be tracked and monitored to determine the feasibility of the overall study.

Subjects may be withdrawn from the study prior to cell delivery for any safety concern from the sponsor or the surgical team at the Study Time-Out (within 3 days prior to surgery) or throughout the time until procedural time-out (occurring in the operating room immediately prior to the injection). This may be due to:

- lack of pre-procedural work-up
- evolving clinical concerns such as bleeding or infections
- unexpected disease progression with need for additional surgeries
- unexpected complications during the surgery prior to cell delivery

Subjects and families may also decide to withdraw consent at any point prior to actual delivery of cell-based product. Notifying the study or site principle investigators is required to ensure a withdrawal request is communicated timely and appropriately. A withdrawal will have no impact on the study subject or data interpretation.

If the subject/family requests withdrawal from the study after cell-transplantation, then we will attempt to utilize data collected up to that time point, and discontinue future follow-up plans. Follow-up would be according to standard clinical care only at that point.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Subjects that have withdrawn from the study at any point will be approached to obtain permission to collect 6-month follow-up data during standard clinical follow-up. This data is important to ensure there are no safety issues in subjects that have decided to withdraw from the study for any reason. We will make every reasonable attempt to collect as much data as possible from clinical records without requesting additional studies for the purposes of this research.
5 Study Drug

5.1 Description, Preparation and Administration of Study Drug

The autologous cell-based product, collected under IRB# 11-007176 will be manufactured at Mayo Clinic Human Cell Therapy Laboratory according to procedures as documented in the FDA approved IND 15,343 that describes umbilical cord blood collection, mononuclear cell preparation, and the release criteria required for the manufactured product to be released to the clinical team.

For this study, the autologous cell-based product will be transported to the clinical setting as a frozen product that will be thawed by trained clinical study team members at the point of care upon confirmation of identification without further manipulation according to clinical protocol and delivery procedure.

The autologous cell-based product will be loaded into the syringe delivery system by trained clinical study team members as described in the clinical protocol and delivery procedure according to sterile technique with date, time, and volume recorded on data entry forms.

The autologous cell-based product will be injected into the right ventricular tissue by cardiac surgeon and trained clinical study team members following the clinical protocol and delivery procedure with date, time, and volume recorded on data entry forms.

5.2 Treatment Regimen

Cell-based product will be thawed and dose calculated based on body weight between the range of 1 to 3 million cells per kg. Product will be administered via direct epicardial injections of 0.1 ml per injection to achieve target dose of cells. (Example: cell concentration of frozen product is 30 million cells/ml and the body weight is 10 kg, then 10 kg X 3 million cells = 30 million cells to be delivered in 10 injections of 0.1 ml volume.) All subjects will be assigned to the open label study. This is the only arm of the study. There will be no randomization to alternative treatment arms for this safety and feasibility study.

5.3 Subject Compliance Monitoring

The study team will monitor and track all consented subjects from the time of HLHS diagnosis and umbilical cord blood collection at birth through 6-month post-study follow-up using a dedicated, and customized database built on Medidata Rave platform. Upon cell delivery, the main issue will be to ensure compliance to clinical follow-up for all pre-determined studies. The clinical information and follow-up will be captured using this customized online Medidata Rave database as it has been designed and built to be compliant with IRB and FDA regulations.
5.4 Prior and Concomitant Therapy

Concomitant medical therapies required for medical management of HLHS at the Stage II surgical palliation are expected. This may include intensive clinical care teams, additional surgery, devices, and complex pharmacological management that could be necessary given the complexity of the clinical situations. All medically necessary options are permitted. However, concomitant research studies that are not standard medical care are not permitted during the 6-month follow-up period to avoid confounding interactions with the cell-based safety study.

These restrictions will include any pharmacological-based studies or treatment protocol where a study agent is administered to the subject, including radiation exposure that is not clinically necessary such as x-rays or CT scans. These restrictions will exclude studies that are laboratory-based diagnostics that require the collection of blood, tissue, or non-radiation based imaging such as echocardiography.

5.5 Packaging

The cell-based product produced at Mayo Clinic Human Cell Therapy Laboratory will be labeled according to standardized procedures in the laboratory at the time of initial processing that includes name, birth data, product type, cell concentration, and disclaimer of investigational use only. A 2.0 ml vial (plus a second vial if available as a redundant backup vial) containing the frozen product will be transported from the Mayo Clinic Human Cell Therapy Laboratory in a labeled box by the manufacturing team.

5.6 Receiving, Storage, Dispensing and Return

5.6.1 Receipt of Drug Supplies

The frozen product will be transported from the Mayo Clinic Human Cell Therapy Laboratory prior to planned surgery. The product will be shipped in a dry shipper before the planned surgery and frozen in liquid nitrogen vapor for no more than 6 hours on dry ice before the planned surgical procedure. Custodial domain will be transferred from the manufacturing team to the clinical team and documented throughout the process according to Medidata Rave data entry by the trained clinical team. As the product is thawed for delivery, the identity will be confirmed and recorded in Medidata Rave by the trained clinical team. The Investigator will be notified immediately of any discrepancies, damage, or unusable product that are received. If a replacement product is not immediately available, then the study will be discontinued due to product failure and documented as an adverse event.
5.6.2 Storage

The storage conditions are according to standard procedures within the Mayo Clinic Human Cell Therapy Laboratory.

5.6.3 Dispensing of Study Drug

Regular study product reconciliation will be performed to document autologous product assigned, product dispensed, product returns, and product remaining following the Stage II surgical procedure. This reconciliation will be logged within the Medidata Rave system according to trained clinical team members during the planned surgical procedure. Any product remaining after the Stage II surgery could be due to unused product based on excess cells or failure to deliver product due to unexpected complications during the surgery or other feasibility issues. This unused product will be transported or shipped on dry ice back to the Mayo Clinic Human Cell Therapy Laboratory to be used for research purposes only and will not be used for clinical studies.

5.6.4 Return or Destruction of Study Drug

At the completion of the Stage II surgical procedure, there will be a final reconciliation of product shipped, product dispensed, product returns, and product remaining. This reconciliation will be logged on the product reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study product. Remaining thawed product will be destroyed on site and documented in the study files; otherwise, frozen product will be returned for non-clinical purposes only to Mayo Clinic Human Cell Therapy Laboratory.
6 Study Procedures

To execute this Phase I study, there are four major events that are required. Study subjects and families will need to participate in 1) umbilical cord blood collection (Part A, IRB 11-007176) as a Mayo Clinic study, 2) Stage II baseline analysis prior to surgery, 3) autologous cell-based product delivery at the time of Stage II surgery, 4) a multi-visit 6-month follow-up schedule, and 5) phone contact every 6-months to document any major changes in overall health for two years following cell delivery. These study procedures are organized into two phases for consenting purposes, Part A and Part B.

1) Umbilical cord blood release criteria
The umbilical cord blood collection procedure is described in standard procedures and also serves as the procedure to ensure proper training of collection personnel. This process includes procedures on kit production, labeling, checklists, collection forms, and transportation procedures to the laboratory. The umbilical cord blood collection is approved by Mayo Clinic Institutional IRB (11-007176) to allow for consenting and collection procedures. Trained study personnel will document release criteria in Medidata Rave following standard procedures using data provided by the manufacturing team and clinical serology test results recorded in the electronic medical record.

2) Stage II baseline analysis prior to surgery
The baseline analysis is completed by a standardized procedure and documents how individual studies are ordered. These studies are required to be completed within 10 days prior to planned Stage II surgery and will be used for the Study Time-Out that will be scheduled within 3-days prior to Stage II surgery. The Study Time-Out will ensure proper review of individual cases with manufacturing and clinical teams. The Study Investigator within Medidata Rave will complete the inclusion/exclusion criteria and the Study Time-Out form, which is required for the study to be able to go forward in the electronic environment. This multi-disciplinary team meeting will ensure real-time and just-in-time communication immediately prior to planned procedure. This procedural requirement will also document logistical plans and assign responsibility to individuals for the day of procedure.

3) Autologous cell-based product delivery
The autologous cell-based product delivery procedure is described in a standard procedure and documents how the product will be handled at the bedside to be thawed, loaded into the delivery device, and injected into the right ventricle myocardium as an add-on during the standard Stage II surgery. Trained clinical personnel will be responsible for receiving the product, preparing the sample, delivering the product with the surgical team, and entering data into Medidata Rave to document the delivery procedure. Trained study personnel will monitor laboratory data, clinical notes, and clinical testing according to standardized procedures using Rave data entry templates to document all adverse events according to standardized procedures. Clinical cardiologists will be assigned the responsibility of collecting and entering echocardiography and cardiac electrophysiology data sets throughout the hospitalization with the assistance of trained study personnel.
4) Multi-visit 6-month follow-up
The follow-up clinical and laboratory data will be collected according to the attached procedure in conjunction with standard clinical cardiology and cardiac surgery follow-up. Studies will be ordered and data collected according to a pre-determined schedule that is organized according to the customized database Medidata Rave. The data will be entered into the Medidata Rave system by trained study coordinators and personnel. Case report forms and adverse events will be recorded by trained study personnel Rave electronic environment, printed, and stored in subject specific binders. The system and procedures to complete these steps includes standardized procedures to inform the study investigator and trigger appropriate reporting requirements to both the IRB and FDA.

5) Phone contact every 6-months for two years following cell delivery
Study coordinators will document any major changes in overall health status by completing the scheduled phone follow-up. This will ensure that long-term adverse events are not missed beyond the active participation period of 6-months. This will also be used for exploratory endpoints that may suggest benefit and/or risk of the cell-based procedure for two years following cell delivery. The phone follow-up will be completed after two years following cell delivery, unexpected transplant, or loss to follow-up. This phase of the protocol will not require adverse event reporting as it will be difficult to ascribe association with cell-based delivery to remote events.

Overview of Specific Study Procedures: Patients will be evaluated for eligibility, and the study team will obtain written informed consent. Patients are diagnosed with HLHS when hypoplasia (underdevelopment) of the mitral valve, left ventricle and/or aortic valve does not allow the left ventricle to support systemic/aortic circulation. When surgery is undertaken, these patients are treated with a functional single ventricle protocol (Norwood operation followed by clinically planned conversion to a modified Fontan circulation). Hypoplasia is defined based upon the aortic annular dimensions, and/or left ventricular cavity dimensions and volume. Patients with hypoplastic left heart syndrome have valves and chamber dimensions more than two standard deviations below the expected newborn mean value (Z scores < 2.0). Ascending aorta and aortic arch hypoplasia with coarctation often coexist with hypoplastic left heart syndrome but are not a defining feature of the diagnosis. Families with prenatal diagnosis of HLHS will be consented for collection and clinical-grade processing of umbilical cord blood into a mononuclear cell (MNC) preparation (Part A). The collection will be done at the time of infant delivery for the specific purpose of a potential cardiac regenerative application and research studies. The autologous MNC will be stored under GMP conditions and upon meeting all release criteria will be available for autologous cell-therapy protocols.

Infants who have successfully completed Stage I surgical palliation, and have MNC stored that have met all release criteria, will be eligible for this safety study to determine if autologous mononuclear cells are safe and feasible in pediatric populations with palliated congenital heart disease. Families will be consented for participation in this study for cell-based delivery at the time of the planned, non-emergent Stage II surgery. Upon consent for the add-on cellular transplantation at Stage II surgery (Part B), stored cells will be immediately on standby to be coordinated with the planned surgical procedure. Patients at the time of planned Stage II surgery will then receive a target dose of 3 million cells/kg body weight of mononuclear stem cells via intramyocardial injection. The acceptable range of cell dose will include values as low as 1
million cells/kg if cells are a limiting reagent due to unexpected complications with collection or processing.

During this study, the tests and procedures that would normally be done prior to, during, and following the Stage II surgery (Glenn procedure) will be completed. Any of the following tests and procedures that are not ordered as part of the regular clinical care, will be ordered for research purposes only. The tests and procedures for research only are a 24-hour Holter monitor, an MRI (optional), and blood tests for cardiac markers and NT-Pro-BNP. Blood samples drawn for the research study will be coordinated with the clinical blood draws to avoid any duplication or unnecessary sampling.

**Cardiac delivery:** Upon completion of successful surgical reconstruction at Stage II as part of planned clinical care, the surgical team will decide to proceed with the cellular transplantation upon determination that the patient’s heart is devoid of sustained arrhythmias, stable blood pressure and heart rate, and no complications are anticipated in the recovery phase. Anticoagulation, as required for the surgical procedure, will be reversed prior to initiation of cellular transplantation. Cells will be thawed by placing the vial in the hands of a trained team member for 4-5 minutes in the surgical suite as soon as the surgical team decides to proceed with the cellular transplantation and not before. To confirm the identity of the autologous cells, a member of the investigative study team will verbally communicate to the surgical team leader as the custody is transferred to the surgical team. The surgical team will perform a “procedural timeout” to re-confirm the identity, volume, and injection sites with the investigative team member present in the surgical suite. The pre-calculated volume and cell concentration of the cell preparation will be delivered to the surgical field with 1 ml syringes containing a 27-gauge needle. Any significant exposure to an unsterile field or potential compromise to the integrity of the cells, such as sample being dropped on the floor, container leaking, or failure to maintain the cells as a frozen stock prior to thawing, will require a reportable event, and discard of sample. If redundant aliquots are not available in the surgical suite, then a stop to the planned delivery will be documented and communicated to the family, sponsor, IRB, and FDA. With confirmation of the planned procedure, cell delivery will be performed directly into the epicardial surface of the myocardium within the free wall of the right ventricle avoiding epicardial vessels following procedures as executed in the corresponding porcine safety study (HLHS-UCB-001). Approximately 100 µl will be delivered per injection site over 5-10 seconds followed by 20 seconds of rest prior to needle withdrawal to maximize cell retention within the myocardium (0.1 ml/kg delivered in no more than 15 injection sites). Direct visualization will ensure the cell-based product is entering into the myocardial wall and not entering into the ventricular lumen, coronary vessels, or being displaced backwards through the injection site upon removal of the needle. The injection sites will be spaced at minimum 1 cm apart from each other across the free wall of the right ventricle while avoiding visible coronary vessels. Injection sites will be recorded with a video camera and documented with a diagram recording relative location of coronary vasculature for future reference if needed in response to unexpected adverse event. Injections will be halted upon complications such as excessive bleeding, ventricular fibrillation or any sustained arrhythmias, low blood pressure requiring pharmacological support, or any other concerns of atypical physiology from anyone on the surgical team. If partial delivery is achieved before stopping the full dose as determined by the clinical team, then exact dose will be recorded as such and the event will be reported to the sponsor, IRB and FDA as an adverse event.
Patients that have completed the Glenn or Stage II surgery and have successfully received cell delivery will be followed clinically according to the following procedures and schedule.

The table below demonstrates the timing of each component to ensure the collection of autologous umbilical cord blood, processing, delivery, and clinical follow-up according to a defined schedule.

<table>
<thead>
<tr>
<th>Study Activity</th>
<th>Wk-2</th>
<th>Wk-1</th>
<th>days-3</th>
<th>day 0</th>
<th>Wk 1</th>
<th>Wk 2</th>
<th>Wk 4</th>
<th>Wk 12</th>
<th>Wk 24</th>
<th>Off Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent Part B</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-procedure studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Time-Out</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cell delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>POD hospitalization (daily VS, telemetry)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>1- month follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3- month follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>6- month follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

The pregnant mother expecting a child with HLHS will be consented to Part A (umbilical cord blood collection and processing for mononuclear cells to be used for cardiac regenerative protocols, IRB 11-007176). The mother will also consent to blood collection for serology testing according to Umbilical Cord Blood Collection protocol.
6.1 Visit 1- Umbilical Cord Blood Collection

This visit will be determined by the delivery of the child with HLHS according to IRB Protocol 11-007176.

- Release criteria achieved

The action following this visit will be dependent on achieving all release criteria.

6.2 Visit 2- Consenting to Part B

During routine clinical follow-up as Stage II surgery is being planned typically between 3-8 months of age (up to 18 months), subject and family will be approached by study coordinators to consider consenting for Part B of the protocol that includes autologous cell-based product injected into the myocardium at the time of Stage II surgery. Information will be provided to family and adequate time allowed for questions prior to signing of Part B consent. Of note, the first three subjects enrolled will be staggered by 3-months to allow sufficient time for interim analysis of adverse events due to the delivery and thus enable protocol modifications if needed to ensure adequate safety monitoring of this first-in-child regenerative protocol.

- Consent to Part B protocol by both parents, unless one parent is not reasonably available

The action following this visit will be dependent on meeting all inclusion criteria as determined by trained study personnel to begin pre-procedure work-up within 10 days prior to Stage II surgery.

6.3 Visit 3- Pre-procedure work-up

Within 10 days of the planned Stage II surgery, subject will undergo pre-procedural testing to document baseline condition with the following studies (listed below) that will be ordered as part of the research study and be recorded in Medidata Rave to document completion of studies.

- Clinical exam
- Laboratory tests including cardiac markers (CK-MB, C-reactive protein (CRP), troponin T), complete blood count with differential (RBC, WBC, hemoglobin, hematocrit, and platelets), blood chemistry (Na, K, Ca, phosphorus, glucose, chloride, bicarbonate, NT-Pro-BNP), liver and renal function tests (ALT, ALP, AST, total bilirubin, albumin, total protein, BUN, creatinine (peds), PT, aPTT)
- Cardiac function using 12-lead ECG
- 24-hr Holter
- Transthoracic echocardiography

The action following this visit will be dependent on the outcome of testing and study-timeout that includes manufacturing and clinical teams and final documentation by study investigator (3-days prior to surgery).
6.4 Visit 4- Stage II surgical procedure

The day of the planned Stage II surgery will not require any additional studies beyond the standard surgical care. Data will be collected during surgery to document the procedure (e.g., cardiopulmonary bypass start and end times). Pre- and post-bypass echocardiography (transesophageal) data will be obtained. Cell-based product preparation will be performed within the operating room and documented by trained clinical personnel, which includes time of thaw, volume of preparation, volume of delivery, and number of injection sites.

- Cell-based product received, delivered, and procedure recorded by trained clinical personnel
- Biomarker panel starting within 3-5 hours post delivery
- Glucose and temperature monitoring every 2 hours for the first 48 hours post delivery

*The action following this visit will be dependent on the outcome of the clinical care as documented by laboratory data collection, clinical notes, and planned or unplanned clinical testing.*

6.5 Visit 5- Hospitalization

During hospitalization, subjects will be monitored with continuous telemetry throughout the postoperative recovery period and arrhythmias captured by monitoring alarms will be recorded as date, time, and type. Blood glucose levels will be monitored for 48 hours after the procedure. Standard clinical examinations and daily vital signs (heart rate, blood pressure, respiratory rate, temperature) will be recorded into Medidata Rave by trained clinical team. Additional studies such as echocardiography will be performed according to clinical necessity along with 12-lead ECG, 24-hr Holter monitor, and clinical examination to document signs and symptoms of heart failure. Adverse events will be monitored according to clinical records documenting new problems throughout the hospitalization. Blood chemistry will be monitored as clinically mandated.

- Daily review by study team to record vital signs, clinical changes in medical record, and telemetry alarms

*The action following this visit will be dependent on the outcome of the clinical care as documented by laboratory data collection, clinical notes, and planned or unplanned clinical testing.*
6.6 Visit 6- Hospital Discharge

At the time of discharge from the hospital, additional studies will be completed beyond the daily follow-up data that are collected.

- Laboratory tests including cardiac markers (CK-MB, C-reactive protein (CRP), troponin T), complete blood count with differential (RBC, WBC, hemoglobin, hematocrit, and platelets), blood chemistry (Na, K, Ca, phosphorus, glucose, chloride, bicarbonate, NT-Pro-BNP), liver and renal function tests (ALT, ALP, AST, total bilirubin, albumin, total protein, BUN, creatinine (peds), PT, aPTT)
- Cardiac function using 12-lead ECG
- Transthoracic echocardiography

The action following this visit will be dependent on the outcome of the clinical care as documented by laboratory data collection, clinical notes, and planned or unplanned clinical testing.

6.7 Visit 7- 1 month follow-up

At the time of 1 month follow-up, a standard clinical evaluation will be accompanied by cardiac structure and function analysis including:

- Echocardiography (heart size and function)
- Electrophysiology using 12-lead ECG
- 24 hr. Holter monitor

The action following this visit will be dependent on the outcome of the clinical care as documented by laboratory data collection, clinical notes, and planned or unplanned clinical testing.

6.8 Visit 8- 3 month follow-up

At the time of 3 month follow-up, a standard clinical evaluation will be accompanied by cardiac structure and function analysis including:

- Echocardiography (heart size and function)
- Electrophysiology using 12-lead ECG
- 24 hr. Holter monitor

The action following this visit will be dependent on the outcome of the clinical care as documented by laboratory data collection, clinical notes, and planned or unplanned clinical testing.
6.9 Visit 9- 6 month follow-up

At the time of 6 month follow-up, a standard clinical evaluation will be accompanied by cardiac structure and function analysis including:

- Laboratory tests including cardiac markers (CK-MB, C-reactive protein (CRP), troponin T), complete blood count with differential (RBC, WBC, hemoglobin, hematocrit, and platelets), blood chemistry (Na, K, Ca, phosphorus, glucose, chloride, bicarbonate, NT-Pro-BNP), liver and renal function tests (ALT, ALP, AST, total bilirubin, albumin, total protein, BUN, creatinine (peds), PT, aPTT)
- Echocardiography (heart size and function)
- Electrophysiology using 12-lead ECG
- 24 hr. Holter monitor
- (MRI will be an optional study as this will require additional sedation for children of this age.)

The action following this visit will be dependent on the outcome of the clinical care as documented by laboratory data collection, clinical notes, and planned or unplanned clinical testing. Successful completion of follow-up will conclude the subject’s participation in this study for the purposes of adverse event reporting. However, subjects will continue to have phone follow-up every 6-months for two years following cell delivery to allow for surveillance monitoring of individuals.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Pre-op</th>
<th>Operative</th>
<th>Hospital (Daily)</th>
<th>Discharge</th>
<th>1-month</th>
<th>3-month</th>
<th>6-month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital signs (VS):</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Cardiac markers ¹:</td>
<td>x</td>
<td>* x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC w/differential ²:</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Chemistry ³:</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver/Renal function ⁴:</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telemetry</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 lead ECG</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echo (TTE)</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>24 hour Holter monitor</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical exam</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

¹: CK-MB, C-reactive protein (CRP), Troponin T
²: RBC, WBC, HgbB, Hct, Platelets
³: Na, K, Ca, Phosphorus, Glucose, Chloride, Bicarbonate, NT-Pro-BNP
⁴: ALT, ALP, AST, Total Bilirubin, Albumin, Total protein, BUN, Creatinine, PT, aPTT

* Cardiac markers to be drawn 3-5 hours post-op
  (*Biomarker panel only - Troponin T, 3hr, 6hr)

Glucose and temperature will be monitored Q2H for the first 48 hrs. post surgery
7 Statistical Plan

7.1 Sample Size Determination

The primary endpoint of this Phase I study is safety and feasibility with the objective to monitor and document 1) adverse cardiac events including death, sustained/symptomatic ventricular arrhythmias, heart failure, myocardial infarction, infections, and unexpected cardiovascular procedures within 6 months following cellular transplantation and 2) the percentage of the individuals with collected umbilical cord blood that meet all release criteria in Part A, and the percentage of individuals with collected umbilical cord blood that have cells delivered in Part B.

The secondary endpoints are cardiac structure and function before and after 6-month follow-up from the time of clinically planned Stage II surgery. The variables measured will be primarily right ventricle ejection fraction from MRI at the end of study and changes in right ventricle ejection fraction using echocardiography from baseline to 1, 3, and 6-month follow-up. The secondary measures will be the change in right ventricle TAPSE measurements from baseline to 1, 3, and 6-months along with fractional area change of the right ventricle as determined by echocardiography.

Given the small cohort size (n=10), the trend of cardiac function will be compared not only to a non-randomized case control cohort (n=10) (that will be prospectively collected according to analogous inclusion criteria in a parallel observational study) but also described on its own for potential comparison to historical or published values. Further, information gained from this study will be used to determine power calculations for potential future studies.

Safety - With the proposed sample size, this study has limited power to differentiate the two groups. A Fisher's exact test with a 0.05 two-sided significance level will have 80% power to detect the difference between a group proportion of 0.001 and a group proportion of 0.59 when the sample size in each group is 10. For comparison of one group against a supposed historical frequency though may provide greater assurance. An exact binomial test with a nominal 0.05 two-sided significance level will have 80% power to detect the difference between the null hypothesis proportion of 0.001 and the alternative proportion of 0.15 when the sample size is 10. Thus this study will have reasonable power to distinguish common rates for adverse events from very rare rates. This will be inferred if at least 1 adverse event has occurred. If 1 event occurs in a sample of size 10, than the exact 95% confidence interval will be used (0.0025, 0.445).

Therefore, the sample size is sufficient to identify common adverse events while allowing a preliminary comparison with a control group.

Efficacy - A sample size of 10 in each group will have 80% power to detect an effect size of 1.325 using a two-group t-test with a 0.050 two-sided significance level. In particular when analyzing ejection fraction, this study is designed to be able to identity a difference between the groups 1.325 times the within group standard deviation. Therefore, the sample size is sufficient to provide preliminary comparison of the treated group with a control.
7.2 Statistical Methods

Descriptive Statistics

Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for the treatment groups.

Handling of Missing Data

Missing data will be addressed using last observation carry forward (LOCF) where appropriate. Where LOCF is not appropriate missing data will be left as missing. Heavy tailed distributions and outliers will be addressed by considering transformations to allow approximate normality of the means. Data will be reviewed for outliers before analysis and values may be checked against source data.

Multiplicity

For both the primary hypothesis, which concerns safety, and the secondary hypothesis, which concerns efficacy, there will be one primary (or first) analysis and thus no correction will be made for multiple comparisons. A sequence of outcomes of interest will be described for each of the safety and efficacy analyses, and significance will be assessed in the order of these sequences. Therefore, testing involves closed testing procedures that protect the probability of falsely rejecting the null hypothesis. If a test fails to be significant, then further tests will be calculated in an exploratory rather than inferential manner.

Primary Hypothesis: Intramyocardial injection of umbilical cord blood-derived MNC will not cause adverse events denoted by systemic toxicity, worsening of cardiac structure/function, or new cardiac arrhythmias.

The primary analysis is focused on the safety of intramyocardial deliver of the manufactured cell-based product in pediatric hearts at the time of planned surgical palliation for HLHS. The incidence and severity of prospectively observed and recorded events as defined in this study will be compared to analogous parameters observed and recorded in a parallel observational cohort. Given the small sample size of this Phase I study, we will be looking primarily at common adverse events when determining and comparing incidences.

The primary analysis will compare frequency of patients with an adverse event indicating systemic toxicity, worsening of cardiac structure/function, or new cardiac arrhythmia between the two groups using a Fisher exact test. A confidence interval for the difference between groups will also be given. Secondly, the frequencies will be described per group and exact confidence intervals provided. Further specific adverse event categories in the order for consideration are:

- worsening of cardiac structure/function, or new cardiac arrhythmia
- worsening of cardiac structure/function,
- new cardiac arrhythmia
- systemic toxicity

These event data will be analyzed analogous to the primary endpoint. Additionally all adverse events will be described for the two groups by body system.
Secondary Hypothesis 1: Intramyocardial injection of umbilical cord blood-derived MNC will improve right ventricular ejection fraction at 6-months compared to case control subjects as collected in a parallel observational cohort.

The secondary analysis is focused on the potential benefit of intramyocardial delivery of the manufactured cell-based product in pediatric hearts at the time of planned surgical palliation for HLHS. The change from baseline to 1, 3, and 6-month follow-up in right ventricular ejection fraction is the primary variable along with secondary variables of change in right ventricle TAPSE and fractional area change. Comparisons will be with the observational cohort.

The first analysis of the efficacy outcome will compare ejection fraction between the treated and control group at 6 months using an analysis of covariance (ANCOVA) including baseline ejection fraction as a covariate. A confidence interval will also be described. Further specific echocardiography outcomes in the order for analysis, still using ANCOVA, are:

- change in TAPSE at 6 month
- change in Fractional Area Change at 6 month
- RVEF at 3 and 1 month
- change in TAPSE at 3 and 1 month
- change in Fractional Area Change at 3 and 1 month

Other measured parameters derived from echocardiography and MRI will be analyzed in an exploratory fashion.

Interim Analysis

The study will have a Data Safety Monitoring Board (DSMB) as part of the data safety-monitoring plan with responsibilities to review all stopping rules with interim analysis to determine if any serious adverse effect is attributable to the test article and/or if protocol changes are required. The DSMB will meet every 6 months independent of any new patients enrolled or AEs. The DSMB will confer in the case of any new SAE and the primary statistician for this study will support interim analysis. All reports will be submitted to the Mayo Clinic IRB at least with 6-month intervals.

7.3 Subject Population(s) for Analysis

Only consented patients will be used in analysis. Safety analysis will be based upon all patients who receive any cell delivery (partial or complete) in the treatment group and all patients in the control group. Efficacy analysis will be based upon all patients who completed cell delivery and follow-up in the treatment group and all patients who completed follow-up in the control group.
8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- **Serious**: Serious problems or events that result in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**

- **Unanticipated**: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**

- **Related**: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. cell-based biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol, may be problems/events that in the opinion of the sponsor and/or investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, will be regarded as **non-serious adverse events**.
Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as 30 days following the injection of study product at the time of Stage II surgery. Although we will continue to follow the subject for 6 months after delivery of the product, it will become difficult to ascribe causal relationships between the product and adverse event beyond the 30-day study treatment follow-up.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the Investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the Investigator will instruct each subject to report, to the Investigator, any subsequent event(s) that the subject, or the subject’s personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality will be documented as an adverse event if the laboratory values are repeatedly abnormal, do not resolve within 7 days back to baseline, and do not have a clinically suitable explanation in the context of other management conditions such as mechanical support, ventilator support, medically induced conditions, or nutritional/intake complications.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgeries are reported as an adverse event in the following circumstances:
  • Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
  • Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
  • Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.
8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) as defined in our customized Medidata Rave electronic environment according to defined procedures (51011). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results will be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event in Medidata Rave. The site-investigator will be responsible for evaluation of the event and determining the necessary follow-up and reporting according to local IRB requirements. The site-investigator will also report all adverse events to the sponsor.

8.3.1 Site-Investigator reporting: Notifying the IRB

The site-investigator will report to the local IRB any adverse events according to IRB Policy and Procedures. According to Mayo IRB Policy, any serious adverse event (SAE), which the site-investigator has determined to be an UPIRTSO must be reported to the Mayo IRB within 5 working days after the investigator first learns of the problem/event.

The site investigator at OU will report all adverse events to OU IRB within 5 working days after the site investigator first learns of the problem/event. The site investigators will also notify the Sponsor of the study at this time.

The Sponsor will review all adverse event reports to determine if specific reports need to be made to the FDA. The Investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the respective IRB. The IRB at Mayo Clinic will also receive 6-month progress reports independent of adverse event reporting.

8.3.2 Sponsor reporting: Notifying the FDA

The Sponsor will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be
reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the Sponsor’s initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the Sponsor’s initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the Sponsor’s initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor’s initial receipt of the information about the event.

8.4 Stopping Rules

This study is primarily a safety study and therefore will be put on hold with the IRB and FDA under the following unanticipated situations:

- Any death within 30 days following cell delivery at the Stage II surgical procedure.
- Any unanticipated cardiovascular surgery requiring open chest procedure within 30 days following cell delivery at the Stage II surgical procedure.
- Any life-threatening condition that develops within 30 days following cell delivery at the Stage II surgical procedure.

These stopping rules will require full DSMB review of the case and provide a written summary of the conclusions to the IRB and FDA. The DSMB will need to either conclude that the SAE was not reasonable caused by the study product or delivery design or recommend protocol changes prior to resuming the study. Pending review by both the IRB and FDA, the study may not come off clinical hold.

8.5 Medical Monitoring

It is the responsibility of the site-investigators to oversee the safety of this study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 “Study Monitoring, Auditing, and Inspecting”). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

The first three subjects will be staggered to have at least 3 months follow-up between cell delivery for the first three subjects. This will allow sufficient time to track for adverse events prior to delivery of subsequent patients. There will be a report submitted to the FDA upon completing 3-month follow-up for the third subject and include 6-month follow-up on the second
subject and 9-month follow-up on the first subject in order to request increasing the enrollment schedule. This data will be reviewed by the DSMB and submitted to the respective IRB as well as the FDA

8.5.1 Internal Data and Safety Monitoring Board

The DSMB or committee for this open label Phase I study will be organized beyond the requirements of the IRB and FDA to provide critical review and summary of events leading to a clinical hold as noted above. The Sponsor will be responsible for FDA reporting of all SAE and will not require DSMB involvement in ordinary reporting requirements. However, the Sponsor may request special review in cases of particular interest. The primary statistician involved in the study will be responsible for providing the DSMB with details regarding recruitment, enrollment, withdrawal, adverse events, clinical information collected, and any other information upon the request of the DSMB. Although this study is an open labeled study, there will be a parallel case-control observational study that will provide comparative data. The primary statistician will be the same person for these two studies and allow comparative analysis, as the inclusion/exclusion criteria are analogous as well as the data entry systems and study coordinators.

The DSMB will be comprised of four individuals and require three of the four at each meeting. The board will comprise of clinicians informed about cardiovascular diseases and informed about the current status of stem cell clinical trials. The primary statistician, data quality assurance reviewer, and designated personnel from the Mayo Clinic Office of Research Regulatory Support will support the DSMB and assist the Sponsor in reporting requirements.
9 Data Handling and Record Keeping

9.1 Confidentiality
Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents
Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects’ diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms
The study case report form (CRF) is the primary data collection instrument for this study and will primarily be completed in the customized Medidata Rave database.
The above screen shot of the Rave system highlights the multiple folders on the left-hand side that are rolled out in real-time as a study participant progress through each phase of the study. This enables a systematic approach to ensure all steps are followed and data is collected in the appropriate time. Additionally, this “home-page” of the study allows adverse events to be recorded using the “Add Event” function at any time-point of the study.
The above example of an electronic case report form (CRF) illustrates the user login, title, data, time, and edit checks as denoted by green check that indicates the values entered fulfill data constrains for cardiac enzymes levels. Values can be edited, but will have person, data, and time to track changes accordingly.

All data requested on the CRF will be recorded. All missing data must be explained. The Medidata Rave system has built-in edit checks that require trained personnel to enter data prior to saving forms. The user-defined system tracks all data entry and edit changes with person, data, and time. If a space on the CRF is left blank because the procedure was not done or the question was not asked, we will write “N/D”. If the item is not applicable to the individual case, we will write “N/A”. In the case where data is recorded on hard copies, all entries should be printed legibly in black ink. If any entry error has been made on a paper document, to correct such an error, we will draw a single straight line through the incorrect entry and enter the correct data above it. All such changes will be initialed and dated. We will not erase or use “white-out” for errors. For clarification of illegible or uncertain entries, we will print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, we will neatly include the details to justify the correction.
Data Management

Clinical Trial Management Systems (CTMS) is the Mayo Clinic Research Committee-endorsed institutional resource for clinical data management. CTMS is a robust institutional effort initiated in 2010 to address emerging changes within the data and statistical coordinating centers affiliated with NCI-funded cooperative groups. In 2010, NCI selected Medidata Rave (http://www.mdsol.com/) as the required data collection tool for all cooperative studies. To capitalize on Mayo Clinic and the NCI’s investment in Medidata Rave, Mayo Clinic formalized a three-tier data management infrastructure with the Medidata Rave product as the premier system.

Medidata Rave is a product for multi-center clinical trials conducted under 21 CFR Part 11 requirements. This web-based system provides ease of use coupled with an integrated randomization module (Medidata Balance™), custom reporting, robust data validation routines, and straightforward integration with SAS.

• Electronic Data Capture:
Medidata Rave allows for data collection. During the course of the data entry into Medidata Rave, the system provides real-time within-case report form (CRF) and inter-CRF data consistency verification. Medidata Rave is flexible in nature so that all data can be entered even if “required” fields and or other consistency checks requirements are not satisfied. The system uses an internal “flagging” or “query” system to distinguish the valid from the invalid data thereby ensuring compliance with the FDA guidance document “Computerized Systems Used in Clinical Trials.” All data discrepancy issues are tracked and audited by the system to ensure the highest quality data is available for analysis and study reporting.

Contained within the CTMS initiative at Mayo Clinic is a diverse set of administrative and technical personnel to support the development and implementation of clinical trials in Medidata Rave. While the time necessary to program Rave’s electronic case report forms (eCRFs) has been directly budgeted, the CTMS initiative supports protocol independent activities such as software/server maintenance, data standards, institutional system integrations, SAS data, and training of study personnel through institutional resources.

The dedicated VPN connection between Mayo Clinic and Medidata provides the conduit for data connectivity. Clinical trial data hosted in Medidata is accessible when needed for SAS using the SAS OnDemand Connection, in combination with Mayo Clinic’s SAS Pipeline program, which creates a common and direct combination of the metadata (labels, formats, etc.) and data (raw values) into SAS datasets on a scheduled (nightly) basis. This process removes the need to separately label and format the entire clinical trial database separately in SAS.
Data Processing

The study will be organized within the Medidata Rave system that only enables subsequent data entry forms upon appropriate completion of previous forms. This system further ensures that trained and qualified individuals are entering data that is appropriate for them at the appropriate time. Upon data verification, data can be exported to SAS databases used for statistical analysis and reporting. As this is not a blinded study, all data will be available to study personnel throughout the study.

Data Security and Confidentiality

The customized Medidata Rave system will ensure data integrity and quality assurance according to user tracking and log of data entry and editing required for detailed audit trail. The electronic system also enables systematic procedures for de-identification of data for IRB and FDA reports.

Data Quality Assurance

The Medidata Rave system will provide the primary CRFs for data entry throughout the study. Edit checks and open queries have been customized throughout the study to ensure a systematic and real-time quality assurance plan. If queries remain open after data entry, then an individual responsible for data quality assurance will be able to track and monitor all fields that remain open or data that is outside of the acceptable ranges. It will be the responsibility of the quality assurance reviewer to engage the appropriate study personnel to update the data fields accordingly.

Data Clarification Process

The quality assurance reviewer will notify the study personnel that has inappropriately entered data or incompletely entered data. The study personnel will be requested to update information according to primary data source. If satisfactory resolution is not possible, then quality assurance reviewer will notify the study investigator to provide any further clarification.

9.4 Records Retention

The Investigator will maintain records and essential documents related to the conduct of this study at the Mayo Clinic site. The Site Investigator will be responsible to maintain these records at the OU site. These will include subject case histories and regulatory documents.

The Investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the product; or, if a marketing application is not submitted or approved for the product, until 2 years after shipment and delivery of the product for investigational use is discontinued and the FDA has been so notified. OR

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan
The investigator will allocate adequate time for such monitoring activities. The investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide clinical monitoring for the trial as a service for the investigator, in addition to other entities. Clinical trial monitoring involves review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the protection of human research subjects. This will assist investigators in complying with Food and Drug Administration regulations.

A continuing review (CR) will be submitted every six months to the Mayo Clinic IRB with the input and guidance from the DSMB. This report will include documentation of any subjects that have been omitted or dropped from further study consideration. The CR will also include documentation of any subjects who participated in Protocol A (umbilical cord blood collection), but did not receive cell injection treatment for Protocol B. In addition, a signed statement from the Director of the Human Cellular Therapy Laboratory will be included in each CR to attest that GMP standards are being adhered to.

Data and Safety Monitoring Plan (DSMP) will be managed by the DSMB with the primary responsibility of reviewing all indications for clinical holds to the IRB and FDA. The sponsor, working with the quality assurance reviewer and personnel from the Office of Research Regulatory Support, will be responsible for reporting SAE to the FDA.

10.2 Auditing and Inspecting
The investigator will permit study-related monitoring, audits, and inspections by the IRB, the monitor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations
This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.
All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject’s legally authorized representative, and the individual obtaining the informed consent.
12 Study Finances

12.1 Funding Source

This study is financed by Mayo Clinic on behalf of the Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study investigator prior to participation in this study. This process is managed by a mandatory Conflict of Interest review with the Mayo Clinic policy required for IRB approval.

13 Publication Plan

The investigator of this study will have the responsibility working with co-investigative team to identify authorship and release of all information for the purposes of publication of data internally and externally to Mayo Clinic. The Phase I study will be registered on ClinicalTrials.gov prior to subject recruitment and results will be posted to ClinicalTrials.gov within 12 months of final data collection for the primary outcome.

14 References