Official Study Title: Phase IIb Study of Intramyocardial Injection of Autologous Umbilical Cord Blood Derived Mononuclear Cells During Stage II Surgical Repair of Right Ventricular Dependent Variants of Hypoplastic Left Heart Syndrome (AutoCell-S2)

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Phase IIb Study of Intramyocardial Injection of Autologous Umbilical Cord Blood Derived Mononuclear Cells during Stage II Surgical Repair of Hypoplastic Left Heart Syndrome (Auto Cell-II)

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PRINCIPAL INVESTIGATOR'S AGREEMENT PAGE

Study Title:	Phase IIb Study of Intramyocardial Injection of Autologous Umbilical Cord Blood Derived Mononuclear Cells during Stage II Surgical Repair of Hypoplastic Left Heart Syndrome (Auto Cell-II)
Protocol ID:	CSP-4401
Protocol Version:	3.0, 31 Aug 2020

By signing this form, I confirm:

I have read and understand the contents of this clinical protocol and will adhere to study requirements as presented.

I will conduct this study in accordance with this protocol, the applicable principles as described in the United States Code of Federal Regulations (CFR) 21 Parts 11, 50, 54, 56 and 312, and the International Conference on Harmonisation (ICH) Harmonised Guideline for Good Clinical Practice E6 (R2).

I will submit a copy of this protocol to the IRB of record for this study at this institution, and I will not conduct research under this protocol until written approval by the IRB has been received.

Neither my sub investigator(s) nor I, are members of the IRB of record for this study at this institution, *OR* my sub investigator(s) and/or I are members of the IRB of record for this institution but will not participate in the initial or continuing review of this protocol.

_____
Principal Investigator Name

_____ _____
Signature Date

The completed original of this Principal Investigator's Agreement Page shall be filed in your Site Regulatory Binder and a copy shall be sent to the Sponsor.

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CHD	Congenital Heart Defect/Disease
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
DMSO	Dimethyl sulfoxide
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic Case Report Form
EF	Ejection Fraction
FAC	Fractional Area Change
FDA	Food and Drug Administration
GCP	Good Clinical Practices
Hct	Hematocrit
HgB	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HLHS	Hypoplastic Left Heart Syndrome
IB	Investigator's Brochure
ICF	Informed Consent Form
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
MNC	Mononuclear Cells
MRI	Magnetic Resonance Imaging
NT-proBNP	N-terminal of the Brain Natriuretic Peptide
PHI	Protected Health Information
PI	Principal Investigator
PRA	Panel-Reactive Antibody
RBC	Red Blood Cell
RV/LV	Right/Left Ventricle
RVEF	Right Ventricular Ejection Fraction
SAE	Serious Adverse Event/Serious Adverse Experience
SUSAR	Serious and Unexpected Suspected Adverse Reaction
TAPSE	Tricuspid Annular Plane Systolic Excursion
TNC	Total Nucleated Cells
TSH	Thyroid Stimulating Hormone
TTE	Transthoracic Echocardiography
UCB	Umbilical Cord Blood

WBC	White Blood Cell

CLINICAL STUDY SUMMARY

Title	*Phase IIb Study of Intramyocardial Injection of Autologous Umbilical Cord Blood Derived Mononuclear Cells during Stage II Surgical Repair of Hypoplastic Left Heart Syndrome (AutoCell-II)*
Running Title	*Phase IIb Efficacy Study of UCB-MNC Intramyocardial Injection for HLHS and HLHS Variants*
Protocol Number	*CSP-4401*
Phase	*Phase IIb*
Study Design	*Prospective, Open label, Non-randomized, Observational Study*
Overall Study Duration	*6 years*
Subject Participation Duration	*Up to 4 years or until pre-operative work-up for Stage III surgical palliation has been completed or until cardiac transplant, whichever is first*
Single or Multi-Site	*Multi-Site*
Objectives	*This study will evaluate the efficacy and safety of UCB-MNC intramyocardial injections performed during Stage II surgical palliation by focusing on short and long-term cardiac function leading up to the pre-operative work-up for Stage III surgical palliation.*
Study Purpose	*Determine if the delivery of the autologous UCB-MNC product into the myocardium of the right ventricle of the heart at the time of Stage II surgical repair will provide a short-term improvement in cardiac function, while also providing a reduction in the cumulative days of hospitalization following Stage II surgical repair. Additionally, long-term improvement in cardiac function, growth, reaching Stage III surgical repair pre-op work-up and prolonging time to cardiac transplantation or death will be determined. Reaching developmental milestones and quality of life will also be monitored.*
Number of Subjects	*30-50 treated subjects receiving cell product, along with 30-50 subjects with standard surgical treatment (not receiving cell product).*
Study Population	*Subjects less than 13 months of age with HLHS or HLHS variant who are undergoing Stage II surgical palliation.*
Study Product, Dose, Route, Regimen	*The investigational product is autologous MNC derived from UCB at a concentration of 10-30 million TNC per mL. The investigational product will be delivered into the right myocardium via sub-epicardial injections of 0.1 mL per kg body weight to achieve the target dose of 1-3 million TNC per kg body weight.*
Time and Duration of Administration	*A single treatment will be administered intraoperatively upon the successful completion of the planned Stage II surgical repair.*

Reference therapy	*Control cohort of 30-50 subjects, enrolled in the control arm of this study, not receiving the cell product, which will be enrolled and followed using the inclusion/exclusion criteria and follow-up requirements outlined herein.*
Treatment Assignment	*When possible, prior to enrollment in this study, expecting parent(s) with the prenatal diagnosis of HLHS or HLHS variants will be offered enrollment in a separate protocol for the collection of UCB. The collected UCB will be used for UCB-MNC treatment described herein if the subject is enrolled in and meets all eligibility criteria for this open-label study, and if the UCB-MNC manufactured product is determined to be acceptable for release to the investigational site for clinical use. Subjects without frozen UCB-MNC product available or with frozen UCB-MNC that is NOT acceptable for clinical use, or that choose not to participate in the treatment arm may be enrolled in the control arm of this study.*
Primary and Secondary Short-Term Endpoints	*The primary short-term efficacy endpoint regarding the cell product and its delivery procedure is:* *Change in right ventricular cardiac function at 3 months post-Stage II surgery compared to baseline, measured by apical fractional area change (FAC), circumferential strain, and longitudinal strain all as determined by echocardiography.* *Secondary short-term safety and efficacy outcomes in order of their relevance and importance in this clinical study are:* *1) Change in right ventricular cardiac function between baseline and hospital discharge for Stage II surgery, measured by echocardiogram.* *2) Cumulative days of hospitalization per patient at 1 and 3-months post Stage II surgery.* *3) Change in weight, heart rate, and oxygen saturation at 3-months post Stage II surgery compared to baseline.*

Primary and Secondary Long-Term Endpoints	*The primary long-term efficacy endpoint regarding the cell product and its delivery procedure is:* *Change in right ventricular cardiac function at 12 months post-Stage II surgery compared to baseline, measured by echocardiogram.* *Secondary long-term safety and efficacy endpoints in their order of relevance and importance in this clinical study are:* *1) Change in weight every 6 months post-Stage II surgery compared to baseline.* *2) Change in heart failure medication taken every 6 months post-Stage II surgery compared to baseline.* *3) Change in arrhythmia medication taken every 6 months post-Stage II surgery compared to baseline.* *4) Success of subject being scheduled for Stage III surgical repair.* *5) Time (days) until listed for cardiac transplantation, or death following the 6-month follow-up.* *6) Change in right ventricular function at Stage III surgery pre-op work-up compared to baseline, obtained by echocardiogram and cardiac catheterization.*
Statistical Methodology	*Quantitative outcomes, like FAC, at specified time post-surgery, will be analyzed using analysis of covariance to estimate the difference in means between the cell-based therapy and control groups after adjusting for the baseline value (the measured value of the same variable, e.g. FAC, at the time of Stage II surgery). Categorical outcomes, like able to undergo Stage III palliation surgery, will be compared between groups using a chi-square test. Time-to event outcomes, like time until listing for cardiac transplant, will be compared using the Kaplan-Meier method and log-rank test. We will similarly do analyses of other variables based upon this patient subset. In secondary analyses we will also fit models which allow the treatment effect (mean difference between the cell-therapy group control groups) to depend on baseline cardiac function measures or other baseline values. This will be done by using the linear, logistic and Cox regression models as appropriate.*
Imaging Core Lab	*M. Yasir Qureshi, MBBS (Core Lab Director)* *Mayo Clinic Pediatric and Adolescent Medicine* *200 1st St SW* *Rochester, MN 55905*
Contract Manufacturing Organization	*ReGen Theranostics, Inc.* *3033 41st NW, Suite 200* *Rochester, MN 55091*

1 INTRODUCTION

This document is a protocol for a human research study involving subjects who require surgical palliation for HLHS and HLHS variants. This study aims to deliver an autologous cell-based product at the time of planned Stage II surgery and will be carried out in accordance with the applicable FDA regulations and this protocol. Based on the safety data from the initial 10 treated subjects in our Phase I safety study, we herein propose this Phase IIb efficacy study using autologous UCB as the source for MNC that will be delivered into the myocardium of the right ventricle. The objective of this study is to evaluate the short and long-term surrogate markers of safety and efficacy of autologous UCB-MNC intramyocardial injections. This add-on procedure is anticipated to pose little risk to the subject and has the potential to foster a new strategy that leverages the regenerative capacity of the pediatric heart during clinically mandated, multi-stage surgical repair.

This study will not be randomized as our preliminary data (summarized in 1.4) suggests that cell-based therapy may be beneficial to this high-risk patient population with little evidence to indicate there are any measurable safety concerns. This safety and efficacy data are consistent with our ongoing pre-clinical studies that are conducted using a double-blinded, randomized study design. Previously collected autologous umbilical cord blood in the UCB Collection Study will be used in the treatment arm of this open-label study. Controls will be screened using identical clinical inclusion/exclusion criteria that either do not have UCB collected as part of the UCB Collection Study, UCB-MNC product that is determined to be unacceptable for investigational use, or that choose to not participate in the treatment arm of this study.

This protocol is designed to evaluate the clinical outcomes following intramyocardial delivery of autologous UCB-MNC and is being conducted to investigate this product and its delivery procedure as a potential new therapy to strengthen the right ventricle of patients with HLHS or HLHS variants. The central hypothesis of this study is that the delivery of the autologous UCB-MNC product into the myocardium of the right ventricle of the heart at the time of Stage II surgical repair will provide a short-term improvement in cardiac function, reaching growth and developmental milestones, and quality of life, while also providing a reduction in the cumulative days of hospitalization following discharge for the Stage II surgical repair. It is also hypothesized that the autologous UCB-MNC product and its delivery procedure will ultimately provide long-term improvement in cardiac function, reaching growth and developmental milestones, reaching Stage III surgical repair pre-operative work-up, prolonging time to cardiac transplantation or death, and improving quality of life.

The safety and efficacy endpoints will be evaluated by the DSMB at least annually. This will determine the appropriateness of continued enrollment in both arms of this Phase IIb study. By demonstrating reasonable efficacy and safety of this product and its delivery procedure in this study, future definitive studies may be designed with the goal to establish a marketable regenerative therapy to treat this rare, life-threatening congenital heart disease as quickly as possible.

1.1 Background

HLHS, and HLHS variants that require single ventricle palliation, are severe forms of CHD with morphological right ventricles that consist of multiple obstructions to flow through the left heart and aorta, as well as hypoplasia (underdevelopment) of the left ventricle. This combination of defects requires the affected individual to undergo surgical palliation to create a functional "single ventricle" circulation [1]. Patients are diagnosed with HLHS or HLHS variants when hypoplasia of the mitral valve, left ventricle and/or aortic valve does not allow the left ventricle to support systemic/aortic circulation. Hypoplasia is defined based upon the aortic annular dimensions, and/or left ventricular cavity dimensions and volume. Patients with HLHS 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 HLHS but are not defining features of the diagnosis.

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. As clinical management has improved to decrease mortality to 10-20% [2], the majority of families in the United States are electing for surgical management rather than comfort care only, 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. The "single ventricle" approach requires the right ventricle to perform as the primary circulatory pump for the entire body. After initial surgery, 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 (i.e. protein losing enteropathy and congestive heart failure).

Central to the hypothesis of this study is the evidence that cardiac dysfunction is a life-long complication for these individuals. Specifically, the risk of morbidity and mortality does not significantly improve even for those individuals that are able to successfully achieve Stage III Fontan circulation. Evidence indicates that 40% of patients post-Stage III either require heart transplantation, major surgical revisions, or do not survive after 12 years [4]. Furthermore, right ventricular ejection fraction (RVEF) less than 40% as measured by echocardiogram after one year post-Stage II surgery was the strongest predictor of the nearly 5% of patients that died or require cardiac transplantation within this early time period [5]. Additionally, the most common shunt used today has improved 1-yr survival rates in the Single Ventricle Trial (RV to PA conduit) yet is now associated with a long-term decline in right ventricular function and a trend towards decreased transplant-free survival likely due to ventriculotomy required in the RV at Stage I [5]. Most concerning is the reality that hypoplastic left heart syndrome patients may have the highest risk of developing right heart failure throughout the course of their clinical care for this condition [6].

Moreover, the routine imaging by echocardiography is not able to detect early ventricular dysfunction in the single right ventricle, which may be detected with the use of magnetic

resonance imaging (MRI) in these patients [7, 8]. The right ventriculotomy also leads to an aneurysmal, dyskinetic segment in the right ventricular outflow tract, seen only by MRI which adversely affects overall right ventricular systolic function in these patients [9]. Finally, cardiac transplantation is not a perfect choice to manage these patients for multiple reasons that include 4x increased morbidity and mortality rate in the high-risk single ventricle patients and only a 63% survival after 3 years for transplanted single ventricle patients [10]. Long-term mechanical support is not a practical option for these patients.

Although outcomes have improved compared to the previous decades, these patients that are dependent on single morphological right ventricles are clearly still at significant risk of long-term morbidity and mortality. As a result, the development of regenerative therapies is becoming increasingly important to minimize the risk of long-term ventricular failure in patients with single ventricle circulatory systems. Thus, our goal is to strengthen and re-engineer the native right ventricle of CHD children using a stem cell-based regenerative therapy.

Children with single ventricle circulatory systems need regenerative applications to strengthen their right ventricle and sustain long-term pressure overload in order to minimize the need for cardiac transplantation. The current results from the first 10 subjects treated with the UCB-MNC product in our Phase I study provide the primary motivation to proceed with this Phase IIb study. The clinical necessity of open cardiac surgery allows the investigational UCB-MNC intramyocardial treatment described herein to be an “add-on” procedure with minimal risks to the subject.

Furthermore, studies conducted in Japan have been published that include a wide-spectrum of CHD patients using autologous cells derived from heart biopsy samples have demonstrated improvement in cardiac function as measured by cardiac MRI and echocardiogram with three years of follow-up. Despite the limitations of lack of randomization, heterogeneous patient population, wide range of ages, and different surgical approaches; these studies provide the best available data and suggest a measurable and meaningful improvement in treated patients [11].

1.2 Investigational Agent

The investigational product is autologous mononuclear cells (MNC) derived from umbilical cord blood (UCB) with minimal *ex vivo* manipulations. The delivery vehicle contains a cryoprotectant solution of 10% dimethyl sulfoxide.

1.3 Preclinical Data

Preclinical studies were conducted under this IND to comply with the recommendations received from the FDA during our pre-IND meeting (Dec 2, 2011) and subsequent correspondence (Feb 23, 2011). The pre-clinical investigations were predicated on a two-tiered approach that utilized large animals for autologous cell testing and small animals for human cell testing. The feasibility of the collection, manufacturing, and delivery components employed in prior pre-clinical and clinical studies has been demonstrated. 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 that was manufactured and administered in the same manner as

conducted for the Phase I study and will be conducted for this Phase II study. Pre-clinical safety and efficacy testing is ongoing in large animal studies to provide ongoing learning and optimization of various aspects of the clinical development for this product.

Preclinical Data Summary- Initially, three dedicated preclinical studies were designed and executed to address the specific questions required to determine the risk/benefit analysis for the proposed Phase I human study. The pre-clinical studies demonstrated that intramyocardial delivery of the umbilical cord blood-derived product does not cause any clinically significant adverse reactions throughout follow-up after intramyocardial injection of umbilical cord blood derived MNC compared to a 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 benefits of human umbilical cord blood-derived mononuclear cells as manufactured in the GMP facility at Mayo Clinic for these three pre-clinical studies were demonstrated in a pressure overloaded right ventricular animal model system as: improved cardiac structure, reversal of pathological gene expression profiles, and prevention of fibrotic changes upon histology analysis. Collectively, these original preclinical studies suggested a safe and effective dose of umbilical cord blood-derived mononuclear cells with intramyocardial delivery into the right ventricle [12].

Double-blinded, randomized, placebo-controlled studies are continuously performed in large animals using autologous UCB derived products at Mayo Clinic as part of product development. Ongoing pre-clinical studies have not identified any new or unexpected safety concerns of the product and delivery strategy. These ongoing studies continue to utilize a pulmonary artery banded piglet that aims to model the right ventricular stress of an age-matched patient undergoing Stage II surgical repair. The autologous UCB-MNC product is equivalent to our clinical product. The randomized and blinded studies have continued to demonstrate a favorable safety profile (lack of bleeding, arrhythmia, tumor formation, ventricular damage) of this product and the delivery procedure technique that is used within the Phase I clinical protocol [13]. A surrogate marker of efficacy that has recently demonstrated the most significant improvement has been the increased cardiac proliferation in the right ventricle following cell delivery (publication pending). Of note, improved ejection fraction in the right ventricle of this model has not been demonstrated in these preclinical studies as the source of cardiac stress is a surgically induced. Fixed narrowing of the outflow track of the pulmonary artery limits the ability to effectively measure change in cardiac function irrespective of any meaningful regenerative response to cell therapy. This permanent obstruction to outflow in these animal studies does not recapitulate the HLHS condition in humans and thus we have begun using this animal model system to characterize the mechanism of action at the molecular level. Of note, the increased cardiac proliferation of the right ventricle would indeed contribute to the increased cardiac mass needed for optimal functioning of the systemic right ventricle.

Thus, this product and product delivery procedure technique continues to demonstrate a favorable safety profile in the large animal model system without evidence of worsening arrhythmia, bleeding, cardiac dysfunction, or tumor formation at the time of and following cell

therapy. Furthermore, cardiac proliferation is directly attributable to cell therapy and this molecular mechanism is expected to contribute to long-term cardiac benefit.

1.4 Clinical Data to Date

The following is a summary of available data through May 17, 2018 for safety, feasibility, and efficacy from the first ten treated subjects enrolled in the Phase I study and the first three subjects enrolled and accrued in the concurrent observational control study.

Feasibility Experience in Phase I Study (IND 15343)

The clinical feasibility of this study design has been demonstrated without modifications to the original version of the Phase I protocol in the context of cell product manufacturing, method of delivering the cell product, or any changes recommended by the DSMB. As HLHS is rare, the inclusion and exclusion criteria were modified to achieve enough enrollment in a more reasonable time. The most significant change that improved feasibility was modifying the exclusion criterion pertaining to subjects being on mechanical cardio-pulmonary support such as ECMO. There were several patients that were pre-screened for the study that received ECMO support following Stage I surgery for reasons unrelated to cardiac dysfunction, and thus it was inappropriate to exclude those patients by assuming that they would be higher risk from a cardiac standpoint. The AE definition and reporting criteria were also modified after the second treated subject as the requirement to report all AEs for this subject population is challenging due to the frequent occurrence of expected events in this critically ill population, resulting from the Stage II standard of care procedure and the underlying HLHS congenital heart disease. Thus, the purpose of the modification to the AE definition and reporting criteria was to allow investigational sites to focus on the reporting of the more critical AEs given the clinical context of this unique subject population.

In the context of the feasibility of UCB collection and manufacturing, the study protocol and manufacturing SOPs ensure a high yield of available products to clinical sites for investigational cell therapy under this IND in subjects that participated in the UCB Collection Study. From the 98 consented patients that intended to undergo umbilical cord blood collection as participants in the Mayo Clinic UCB Collection Study, 80 patients had umbilical cord blood collected and received by the manufacturer. The 18% failure to collect/manufacture a UCB-derived product was most commonly related to mothers delivering early or mothers consenting too late, which affected the feasibility of the logistics. The majority of UCB collection failures have not been related to technical problems in the past 12 months as these have been effectively addressed with improvements in training of staff involved in UCB collections and manufacturing SOP refinements. The UCB collection failure rate will be further reduced with dedicated UCB training within the Phase IIb clinical sites. During the Phase I Study, on December 1, 2016, the product manufacturing was transitioned to a partnering CMO, ReGen Theranostics (Rochester, MN). Manufacturing of the last 40 products as of March 2018 at ReGen have resulted in 37 products available for release (92% success rate). The three products that were not made available for release were due to 1) a manufacturing error that was an unacceptable quality control issue, 2) a critical labeling issue between collection and manufacturing that was not able to be reasonably resolved, and 3) a low cell recovery linked to a low collection volume that

limited the manufacturing yield of that product. The manufacturing and UCB collection logistics have been refined such that they will be unlikely to limit enrollment and treatment in the Phase IIb study.

The feasibility of the Phase I study was most significantly compromised by the study inclusion and exclusion criteria. One in every six acceptable autologous products was administered during the Stage II surgery based largely on the narrow Phase I study eligibility criteria. The Phase IIb study has been designed to address the feasibility issue identified in Phase I by requiring fewer exclusion criteria for subject enrollment. It is anticipated that one out of every two subjects with manufactured autologous product will be eligible for enrollment in the treatment arm of this Phase IIb study. Therefore, treating up to fifty subjects under this Phase IIb study should be feasible within a 5-7 site clinical trial.

The feasibility of the Observational study that required prospective data collection for control subjects proved to be highly problematic as recruitment was limited to only three subjects in this time period. The primary limitation to recruitment was in part the bias for eligible subjects to choose to participate in the treatment study. Only a few subjects that met all eligibility criteria and had available UCB product decided to not participate in the Phase I cell-delivery study. Additionally, a significant number of subjects that did not have available autologous product and met all eligibility criteria of the Observational study were unwilling to participate in the Observational study that required multiple non-standard of care clinical visits with additional blood and imaging studies. Therefore, the Phase IIb study was designed with fewer non-standard of care clinical visits to address the feasibility of recruiting a proper control cohort.

Safety and Efficacy Experience in Phase I Study (IND 15343)
In the context of safety, adverse event reporting and data comparison across the small sample size has informed most changes between the Phase I and Phase II protocols, especially given the highly complex and individualized clinical care that these patients undergo. As previously reported to the FDA, the adverse event reporting criteria was modified in protocol amendments to allow investigational sites to focus on the reporting of the more clinically relevant AEs given the clinical context of this unique subject population. We have utilized this Phase II protocol for reporting the current Phase I study clinical data for all treated subjects as of the May 17, 2018 cut-off date. There were no SAEs classified with a “definite,” “probable,” or “possible” relationship to the product or product delivery. The Sponsor has adjudicated all SAEs and the DSMB has systematically reviewed all these SAEs. Of these 27 SAEs, one treated subject experienced 15 SAEs and another treated subject experienced 7 SAEs over the course of a complicated, multi-factorial sequence of events, which were all classified as not related to the product or the product delivery. Of note, there was a single non-serious AE that was classified as having a “definite” relationship to the product delivery. This was due to slight bleeding at the site of one injection during cell delivery that was controlled by the placement of a suture. This first-in-child study has been achieved without definitive safety concerns in terms of events such as arrhythmias, bleeding, cardiac damage, or tumor formation that were considered potential risks of the cell product and its delivery procedure (see Section 1.5).

The majority of SAEs reported were experienced by the first subject. This subject ultimately died and thus put the clinical trial on "hold" while the case was reviewed by DSMB and FDA. Upon DSMB review, the cause of death was determined to be directly related to surgical complications following an elective gastrointestinal surgery 3 month after cell delivery. The death was determined to not be attributable to the cell product or delivery. DSMB review concluded that there was not a need for protocol modifications and FDA removed the clinical hold. The unexpected and unfortunate clinical course of the first HLHS subject treated in the Phase I study triggered a later protocol modification to change the AE reporting criteria as described above. Subsequent subjects have been treated and followed without additional clinical holds or reporting of any serious adverse events that were classified with a "possible," "probable," or "definite" relationship to the cell product or delivery. The nine subsequently treated subjects are currently surviving or completed the study, with one subject having ongoing issues with portal vein thrombosis that has required hospitalizations and surgeries without resolution to date. These complications are attributed to underlying disease processes and are not related to the product or product delivery.

Efficacy data from the Phase I study is difficult to assess as, by design, the inclusion criteria of this Phase I study was limited to the healthiest patient population. Of note, there was a single subject that had reduced RVEF (35%) at the time of Stage II surgery that provided the most sensitive clinical setting to determine if there could be measurable cardiac function improvement in this time period using echocardiography. This subject demonstrated improvement to 50% at 3-months follow-up which suggests a significant improvement in cardiac function beyond what would be normally expected at this stage. More specifically the summarized data through May 17, 2018 that includes 10 treated subjects and 3 accrued control subjects demonstrates that there was no difference in RVEF between the two groups at baseline (p-value 0.27) as both groups had normal cardiac function above 50%. At hospital discharge, treated subjects had a higher mean RVEF (53.8%) compared to control subjects (42.3%) with a p-value of 0.02. At the 1-month follow-up, the treated subjects maintained a significantly higher mean RVEF (55.1%) compared to control subjects (43.9%) with a p-value of 0.05. At the 3-month follow-up, mean RVEF in treated subjects (two pending data points) was not significantly different than the control subjects as improvement in the control subjects trended towards baseline (mean RVEF of 56.3% in the treated group vs 49.9% in the control group, p-value 0.11).

Comparing the treated vs. control cohorts at the time of hospital discharge highlights the biggest difference in mean RVEF. This data suggests that RVEF following Stage II surgery is acutely decreased in the untreated control patient population due to the nature of open chest cardiac surgery. The Phase I clinical data suggests that there may be a ~10% difference in RVEF at the time of hospital discharge between the treated and control cohorts. This normal decline in cardiac function from baseline to hospital discharge has been confirmed in retrospective chart reviews in an equivalent time period for patients at Mayo Clinic that would have met the eligibility criteria of the Phase I protocol. With no measurable decrease in cardiac function from baseline to hospital discharge for the initial 10 treated patients in Phase I, this data suggests that this cell product may have acute cardioprotective and regenerative mechanisms.

The subjects in the Phase I study will continue to be followed according to the existing Phase I protocol. New subjects, both treatment and control, will be enrolled in the Phase IIb study while enrollment in the Phase I and Observational studies are simultaneously discontinued. Enrollment closure in the Phase I and Observational studies will be closed at each investigational site at the time that corresponding site is approved to enroll in the Phase IIb study.

1.5 Dose Rationale and Risks/Benefits (Treatment arm only)

The target dose of the investigational agent is 1-3 million TNC per kg body weight divided into multiple injections of 0.1 mL from a thawed product containing 10-30 million TNC cells per mL. The dose of this cell-based product was originally determined according to clinical experience in adult clinical trials, our pre-clinical safety studies, and now our Phase I clinical study.

Dose Comparison- Other clinical trials have used a range of 10^6-10^8 TNC delivered in chronic ischemic adult 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- Intramyocardial injections of UCB-MNC and subsequent follow-up was conducted in both small and large animal models to determine feasibility, safety, and efficacy of cell delivery. The rationale for intramyocardial delivery through direct injections from the epicardial surface for this cell-based product was chosen based on minimizing the risk of cell delivery for single ventricle 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 which would require a separate catheter-based procedure and its associated ionizing radiation.

Children undergoing Stage II surgery will most likely be less than 10 kg and thus will require not more than 1.0 mL of frozen product to achieve the full target dose. The ability to collect and process this concentration of human cell-based product from umbilical cord blood was determined to be reproducible in preclinical studies and in our Phase I clinical study. The ability to "thaw and deliver" without requiring individual dosing of the cell product is meant to reduce the risk of product calculation errors and mixing errors at the bedside. 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 preclinical study to determine potential risk of tumor formation, arrhythmias, and decrease in cardiac structure/function due to possible damage to blood supply. We also completed a double-blinded, randomized study using autologous umbilical cord blood derived MNC in a size-matched porcine cohort for long-term safety using the same dosage of 3

million cells per kg of body weight and intramyocardial delivery procedure technique as used in the Phase I study. These potential risks are based on literature review and expert experience and not informed based on any specific data obtained in our studies. No clinically significant adverse reactions occurred in these preclinical studies, as described in Section 1.3 above.

Additionally, the Phase I clinical study, up to the time of the data cut-off, did not result in any SAEs that were classified as having a "definite," "probable," or "possible" relationship to the cell therapy product or to its delivery procedure.

Risks of Autologous UCB-MNC Product and Intramyocardial Delivery Procedure-
The clinical experience up to the time of data cut off has been reported in Section 1.4. The clinical experience has highlighted a single AE with minor bleeding that was definitively related to product delivery. The theoretical risks (in addition to risk of bleeding) that will continue to be closely monitored for in this protocol include:

- Mild ventricular tachycardia and/or other mild ventricular arrhythmias
- Mild myocarditis
- Mild pericarditis
- Mild to moderate myocardial infarction
- Mild to moderate pericardial effusion
- Mild to moderate fever for up to 3 days following cell delivery
- Mild to moderate bleeding at the injection site

Benefits of Autologous UCB-MNC Intramyocardial Delivery- The potential benefits of autologous UCB-MNC are derived from pre-clinical studies and preliminary follow-up data from the current Phase I clinical study, suggesting that benefits of the UCB-MNC and its delivery procedure may improve and/or sustain cardiac function within the first 3-months after Stage II surgery.

2 STUDY OBJECTIVES

The purpose of this Phase IIb clinical study is to evaluate the efficacy and safety of autologous UCB-MNC intramyocardial injections into the single, morphological right ventricle of subjects with HLHS or HLHS variants requiring Stage II surgical repair. Thirty to fifty treated subjects and thirty to fifty control subjects will be accrued. Subjects accrued in the treatment arm (Arm A) of this study will be treated with a single dose following successful completion of Stage II surgical repair. Subjects accrued in the control arm (Arm B) of this study will be followed according to the same protocol requirements as the treatment arm but will not receive cell therapy. In the treatment and control arms, surrogate markers of cardiac function, growth, success in reaching Stage III surgical repair pre-operative work-up, time to cardiac transplantation or death, and cumulative days of hospitalization will be recorded and evaluated following the completion of Stage II surgery for up to four years, or until the pre-operative work-up for the Stage III surgical repair has been completed or until cardiac transplant, whichever is first. Development and quality of life as measured by questionnaires will also be monitored. Follow-up surrogate markers and the other variables noted above that are recorded for this study will be compared to baseline and by study arm. The surrogate markers and other variables are described in Section 3 of this protocol.

The objective is to evaluate the short-term (up through 3 months post-Stage II surgical repair) cardiac function, growth, and cumulative days of hospitalization following discharge for the Stage II surgical repair in the treatment and control arms, adjusting for baseline.

This evaluation will also include long-term (6 months and up to 4 years post-Stage II surgical repair or until the pre-operative work-up for the Stage III surgical repair has been completed or until cardiac transplant), cardiac function, growth, ability to reach Stage III surgical repair pre-operative work-up and time to cardiac transplantation or death in the treatment and control arms, adjusting for baseline.

As part of a sub-group analysis, the images collected at 3-months post-Stage II surgery will provide a stand-alone analysis to test the hypothesis that MRI provides a more sensitive measure of sub-clinical cardiac dysfunction, pulmonary artery size, and patency of the surgical anastomoses. It is anticipated that nearly 10% of HLHS and HLHS variant patients may have actionable findings detected by MRI at 3 months post-Stage II surgery that would have been otherwise undetected with a standard of care echocardiogram. This sub-group analysis could provide the evidence to establish a new standard of care following Stage II surgery.

3 STUDY DESIGN

3.1 General Design

This Phase IIb study is a multicenter, prospective, open-label, non-randomized, observational study designed to evaluate the efficacy and safety of UCB-MNC delivered into the right ventricular myocardium of subjects with HLHS or HLHS variants with right ventricular dependent single ventricular CHD at the time of a planned Stage II surgical repair. This will be achieved by comparing the data collected in the treatment group (Arm A) to the equivalent data collected in the control group (Arm B), adjusting for baseline.

The concurrent control arm (Arm B) must meet identical study eligibility criteria as the treatment arm (Arm A), except for the requirements related to UCB-MNC product acceptability and DMSO sensitivity. The control arm will undergo the same planned Stage II surgical repair without the investigational cell delivery add-on procedure and will follow-up post-surgery in the same manner as the treatment arm (see Section 6 of this protocol).

3.1.1 Treatment Assignment

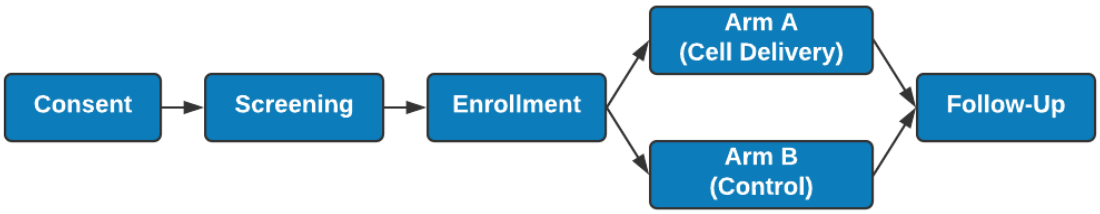
When possible, prior to enrollment in this study, expecting parent(s) with prenatal diagnosis of HLHS or HLHS variants will be offered enrollment in a separate, ongoing Umbilical Cord Blood Collection Study for the collection of UCB. The parent(s) and/or legal guardian(s) may be consented to allow their child to participate in the treatment group (Arm A) of this clinical study if the patient requires Stage II surgical repair, if the autologous UCB-MNC manufactured product is determined to be acceptable for release to the investigational site for clinical use, and if the subject meets all other eligibility criteria for this study. Patients without frozen UCB-MNC product or without UCB-MNC product determined to be acceptable for clinical use in this study may be consented to the control arm of this clinical study if the subject meets all non-treatment-only eligibility criteria for this study.

3.1.2 Subject Evaluation

Once consent has been obtained, subjects will enter the screening phase where a pre-operative baseline work-up will be performed as outlined in Section 6 of this protocol. Following the pre-operative work-up, Stage II surgical repair, and final eligibility confirmation according to the inclusion and exclusion criteria listed in Section 4 of this protocol, the subject will be enrolled on to the trial. Following Stage II surgical repair hospitalization, subjects will enter active follow-up. This includes evaluation in-clinic at 3 and 12-months post-Stage II surgical repair, which will include the collection of imaging data. Phone follow-ups will be conducted at 1 month and 6 months post-Stage II surgery and conducted every 6 months following the 12-month in-clinic visit up to pre-operative work-up for the Stage III Fontan palliative surgery, cardiac transplant or to 4 years post-Stage II surgery, whichever is first.

3.1.3 Study Schema

The overall study participant flow chart for this study is shown below.



3.2 Short Term Endpoints

The primary short-term efficacy endpoint regarding the cell product and its delivery procedure is:

> Change in right ventricular cardiac function at 3 months post-Stage II surgery compared to baseline, measured by *apical fractional area change, circumferential strain, and longitudinal strain on echocardiogram*.

Secondary short-term efficacy and safety endpoints in order of their relevance and importance in this clinical study are:

1) Change in cardiac function between baseline and hospital discharge for Stage II surgery, measured by echocardiogram.
2) Cumulative days of hospitalization per patient at 1 and 3-months post Stage II surgery.
3) Change in weight, heart rate, and oxygen saturation at 3-months post Stage II surgery compared to baseline.

Additional variables may be assessed in an exploratory manner.

3.3 Long Term Endpoints

The primary long-term efficacy endpoint regarding the cell product and its delivery procedure is:

> Change in right ventricular cardiac function at 12 months post-Stage II surgery compared to baseline, measured by echocardiogram.

Secondary long-term efficacy safety endpoints in their order of relevance and importance in this clinical study are:

1) Change in weight every 6 months post-Stage II surgery compared to baseline.
2) Change in heart failure medication taken every 6 months post-Stage II surgery compared to baseline.
3) Change in arrhythmia medication taken every 6 months post-Stage II surgery compared to baseline.

4) Success of subject being scheduled for Stage III surgical repair.
5) Time (days) until listed for cardiac transplantation, or death following the 6-month follow-up.
6) Change in right ventricular function at Stage III surgery pre-op work-up compared to baseline, obtained by echocardiogram, cardiac MRI, and cardiac catheterization.

Additional variables may be assessed in an exploratory manner.

3.4 Ancillary MRI Imaging Analysis:

The cardiac MRI images that are obtained to evaluate the secondary endpoint of this study provide an opportunity to analyze the utility of cardiac MRI vs. echocardiography. The baseline cardiac MRI images will be compared to the 3-month cardiac MRI images in both the cell treatment and control arms of this study to evaluate the secondary endpoint. The hypothesis that MRI is a more sensitive measure of sub-clinical cardiac dysfunction, patency of the surgical anastomoses, and pulmonary artery size compared to echocardiogram will be evaluated within this study by examining the available MRI and echocardiography imaging datasets obtained.

Specifically, paired cardiac MRI and echocardiograms collected at baseline and 3-months post Stage II surgery will be interpreted by the imaging core laboratory. Each site's images also will be available to their respective site investigators, without core lab interpretation for the purposes of adverse event reporting and clinical decisions. Site investigators will determine if the 3-month follow up cardiac MRI provides additional actionable insight beyond the echocardiogram images alone. The expectation is that additional detail related to cardiac function, performance of the conduit, and/or size of the pulmonary arteries that was missed completely or sub-optimally imaged with echocardiograms only. Actionable findings determined by the site investigator based on the availability of research MRI studies may lead to closer patient monitoring, medication changes, stenting procedures or even surgical corrections that could possibly improve the long-term outcome, and thus offer a potential benefit to subjects in both the treatment and control arms of this study. These actionable findings based on MRI studies at the sites will be documented in the study CRFs by surveying site investigators. The size and completeness of this dataset could define a new standard of care for post Stage II surgical evaluation that would not be possible without these MRI vs Echo images. Additional questions and data analysis may be performed by the imaging core to refine and validate site investigator finds as it relates to 3-month post-Stage II surgical MRI vs Echo studies, and de-identified MRI and echo images that are uploaded for analysis by the imaging core may be used for future research or shared with other researchers without additional informed consent.

4 SUBJECT ELIGIBILITY ASSESSMENTS, ENROLLMENT, AND WITHDRAWING SUBJECTS

4.1 Eligibility for This Study:

Both sexes with HLHS or HLHS variants with right ventricular dependent single ventricular CHD requiring planned Stage II surgical repair that meet the following inclusion and exclusion criteria are eligible to participate in this study. Inclusion and exclusion requirements may be specific to the treatment arm only and will be specified as such in parentheses.

4.1.1 Inclusion Criteria

1. Diagnosis of HLHS or HLHS variant with single right ventricular dependent CHD having undergone Stage I surgical repair and undergoing Stage II surgical repair.
2. Less than 13 months of age at time of Stage II surgical repair.
3. Previous participation in the UCB collection protocol with autologous UCB-MNC product that is acceptable for use (treatment arm only).

4.1.2 Exclusion Criteria

1. History of DMSO reaction (treatment arm only).
2. Parent(s) and/or legal guardian(s) unwilling to have their child participate or unwilling to follow the study procedures.
3. Severe chronic diseases at the discretion of the treating physician.
4. Extensive extra-cardiac syndromic features.
5. Known history of cancer.
6. Any of the following complications of his/her congenital heart disease:
    a. Any condition requiring urgent, or unplanned interventional procedure within 15 days prior to Stage II surgical repair, unless complete and full cardiac recovery is documented by site investigator
    b. Severe pulmonary hypertension (reported in the medical record as >70% systemic pressure)
    c. Other clinical concerns as documented by a site investigator that would predict (more likely to happen than not to happen) a risk of severe complications or very poor outcome, not directly related to the stem cell product or its injection procedure, during or after Stage II surgical repair.

4.2 Consent/Screening

Potential subjects for the treatment arm (Arm A) of this study will be pre-screened from the Umbilical Cord Blood collection study. Parent(s) and/or legal guardian(s) of a child with HLHS or HLHS variant with single right ventricular dependent CHD requiring Stage II surgical repair, whose UCB-MNC has been documented to be acceptable for release to the investigational site, and whose child appears to meet the other study eligibility criteria (Section 4.1) during pre-screening may be consented to allow their child to participate in the treatment arm of this study.

Potential subjects for the control arm (Arm B) will be pre-screened during cardiology visits when Stage II surgery is being scheduled at the investigational site. Otherwise, potential subjects for the control arm will be pre-screened from the Umbilical Cord Blood collection study when their product is documented to not be acceptable for release to the investigational site. Parent(s) and/or legal guardian(s) of a child with HLHS or HLHS variant with single right ventricular dependent single ventricular CHD requiring Stage II palliative reconstructive surgery and whose child appears to meet all other study eligibility criteria (Section 4.1) during pre-screening may be consented to allow their child to participate in the control arm of this study.

Consent will occur after the parent(s) and/or legal guardian(s) have discussed the study with the study staff, a site investigator has pre-screened subject eligibility, and the parent(s) and/or legal guardian(s) have been provided adequate time and information to decide if they would like their child to participate. If both parent(s) and/or legal guardian(s) do not sign informed consent, the reason for only one parent or legal guardian signing should be documented at the time of informed consent. Consent must occur before any study procedures are completed, however standard of care procedures done within 30 days prior to Stage II surgery may be used rather than repeating procedures to collect study data. The original signed informed consent will be filed in the subject file and a copy will be given to the parent(s) and/or legal guardian(s). The informed consent process will be documented and provided in the subject file.

All subjects will enter screening phase at the time of informed consent.

4.3 Enrollment/Procedural Timeout

Enrollment in either arm of this study will occur at the Procedural Timeout. The Procedural Timeout will be conducted in the operating room, by a trained site investigator, following the completion of the Stage II repair. During the Procedural Timeout, a site investigator will confirm all inclusion and exclusion criteria have been met per Section 4.1 of this protocol and document his/her decision to enroll the subject. If the site investigator determines that the subject, in either arm, fails to meet all inclusion and exclusion criteria, the subject will be declared a screen failure (see Section 4.5) and exited from the study. A subject should not be enrolled in either arm of the study if the subject has surgical complications during the Stage II repair that result in, or could be reasonably expected to result in, significantly decreased cardiac function.

For the treatment group, if a subject is determined to be eligible to enroll, the Procedural Timeout will also include confirmation that the subject's identity matches the patient identifiers on the investigational product label, confirmation of the number of injection sites and product volume to be delivered, and confirmation that there aren't any anticipated complications or concerns.

4.4 Time of Treatment (Treatment Arm Only*)*

For subjects enrolled in the treatment arm, thawing of the product and the cell delivery procedure will occur immediately following the Procedural Timeout. A subject is considered treated at the time that cell delivery injections begin. If only a partial cell delivery occurs, the subject will still be considered treated and will be followed as planned according to the procedures described in

Section 6 of this protocol. The cell delivery procedure will be entered in the eCRF and the reason(s) for failing to deliver a full dose, if applicable, will also be documented in the eCRF.

4.5 Screen Failures

Subjects will be considered screen failures if they fail to meet one or more of the study eligibility criteria. Screen failures will be tracked and monitored to facilitate in assessing the feasibility of the overall study. Screen failures will be documented in the eCRF. Screen failures do not count toward the study enrollment cap of 50 subjects in each arm.

4.6 Discontinuation and Withdrawal Subjects

A subject will be considered a lost to follow-up subject after three documented attempts (the third attempt will be a certified letter) have resulted in failure to contact the parent(s) and/or legal guardian(s).

For the treatment arm, subjects will be discontinued from the study if there is a compromise to the cell product (see Section 5.4.2) following subject enrollment.

Parent(s) and/or legal guardian(s) may decide to withdraw their child from the study at any time during participation in the study. If the parent(s) and/or legal guardian(s) request that their child be withdrawn from the study, the Sponsor will utilize data collected up to that time point but will discontinue future study follow-up assessments. Survival status data will be collected through search of public records by the enrolling site. If a subject has discontinued from active participation but not from the study, standard of care echocardiograms, MRIs, cardiac catheterizations, ECGs, laboratory tests, weight/vitals, and new or worsening conditions that are normally collected for this study will be collected from the subject's medical records, if the parent(s) and/or legal guardian(s) have previously consented to this additional collection of standards of care data. This data is important to ensure there are no safety issues and to monitor for evidence of efficacy in subjects that have decided to withdraw from active participation in the study. Withdrawn subjects and subjects that discontinue from active participation but not from the study will be documented in the applicable eCRF. Documentation must be provided in the subject study chart for any study withdrawal or discontinuation.

4.7 Prior and Concomitant Therapy

Concomitant medical therapies required for the medical management of HLHS or HLHS variants are expected. Concomitant therapies may include additional surgery, devices, and complex pharmacological management. All medically necessary options are permitted. However, concomitant research studies that are not standard medical care are not permitted during participation in this study, including the follow-up period. These restrictions 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 exclude studies that are minimally invasive diagnostics and non-interventional observational studies that require the collection of blood, tissue, or non-radiation-based imaging such as echocardiography.

Medical history and concomitant medications will be recorded on a concomitant medication log and on the applicable eCRFs starting at the time of consent through hospital admission for Stage II surgery. Changes in concomitant medications will also be recorded on the concomitant medication log and applicable eCRFs throughout the subject's participation in the study starting at the time of hospital discharge following Stage II surgery. Medication changes will not be recorded in the eCRFs (and are not required on the concomitant medication log) for the Stage II surgery and post-operative hospital stay or for any other surgery, hospitalization, or procedure that a subject undergoes. If change(s) in medication(s) are a result of new or worsening condition(s) that occur after the time of enrollment, the new or worsening condition(s) will be recorded as adverse events per Section 8 of this protocol.

5 STUDY DRUG (TREATMENT ARM ONLY)

5.1 Description of Study Drug

The autologous cell-based product is collected under the UCB Collection protocol. The autologous product is manufactured at ReGen Theranostics, Inc. (Rochester, MN) according to the procedures described in IND 15343.

UCB is collected at birth and shipped to ReGen Theranostics, Inc for manufacturing. The UCB mononuclear cells (MNC) are isolated from the cord blood and frozen in cryovials containing approximately 1.5 mL of cell product, with a target concentration of 10-30 million total nucleated cells (TNC) per mL. The UCB-MNC product is tested for sterility and endotoxin and is characterized for TNC concentration and mononuclear cell content as described in IND 15343. Test results are reviewed against the pre-specified release criteria, and products that meet all release criteria are made available for distribution. Non-conforming products (products that don't meet pre-specified release criteria or have documented quality issues) are individually reviewed by the Sponsor to determine the risk of using product in this study. Non-conforming products can only be made available for distribution only if the risk is deemed acceptable by the Sponsor and documentation is sent to the site investigator(s) prior to enrolling the patient in this study to notify the investigator(s) of the specific details.

5.2 Packaging and Storage

The product will be labeled according to manufacturer's procedures at the time of processing. It will be stored under GMP conditions at the manufacturing facility until it is transported by the Sponsor or Sponsor delegate to the site. There is no storage of the product at the investigational site.

5.3 Treatment Regimen/Dose

Subjects in the treatment arm of this study will receive a single treatment with a target dose of 1-3 million TNC per kilogram of body weight of the UCB-MNC product. The product, mononuclear cells (MNC) derived from umbilical cord blood (UCB) at a concentration of 10-30 million TNC per mL, will be administered via direct epicardial injections of 0.1 mL per injection. To achieve the target dose, subjects will receive one injection of 0.1 mL of autologous product for every 1 kg of bodyweight. The number of injections will be determined prior to the start of the surgical case and will be based on the body weight (kg) of the subject obtained at the pre-operative baseline visit, rounded down to the nearest integer. (For example, a 6.9 kg child would receive six 0.1mL injections.)

5.4 Product Request, Administration, Accountability, and Return/Destruction

5.4.1 Product Request

After consent of the subject, the site investigator will request the investigational product for the designated subject from the Sponsor. The Sponsor will review the request and confirm the subject identity prior to submitting a request for distribution to the manufacturer. Product will be

transported in a dry shipper to the investigational site prior to cell delivery by the Sponsor or Sponsor delegate.

5.4.2 Product Administration

Prior to thawing the cells, the identity of both the product and the subject will be confirmed by the study team at the time of enrollment. Following enrollment, trained and delegated team member(s) will thaw the investigational product within the surgical suite. Once thawed, the pre-calculated volume (see Section 5.3) of UCB-MNC product will be loaded into a 1 mL syringe fitted with a 27-gauge butterfly needle and handed off to a site investigator for the cell delivery procedure. The product delivery into the heart tissue should begin within 30 minutes from the beginning of product thawing.

The cell delivery will be performed with injections directly through the epicardial surface of the myocardium, within the free wall of the right ventricle, avoiding epicardial vessels. Approximately 0.1mL 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. Direct visualization will ensure the cell-based product is entering the myocardial wall and not entering into the ventricular lumen or coronary vessels or being displaced backwards through the injection site upon removal of the needle. The injection sites will be spaced approximately 1 cm apart from each other across the free wall of the right ventricle. Injection sites will be documented on a diagram to record the approximate location of injection sites relative to the coronary vasculature.

Injections will be halted upon complications such as excessive bleeding, ventricular fibrillation, sustained arrhythmias, low blood pressure requiring pharmacological support, or any other concerns of atypical physiology from anyone on the surgical team; the event(s) will be recorded as adverse event(s) on the applicable eCRF. Total volume delivered will be recorded for all partial (less than the planned cell delivery volume) and complete cell deliveries. If a partial cell delivery has occurred, subjects will continue to be followed as planned according to this protocol. Partial cell deliveries along with the specific rationale will be documented on the applicable eCRF but are not considered to be a protocol deviation.

The investigational product will NOT be used if there is any significant exposure to an unsterile field or potential compromise to the integrity of the cells, the container leaking, a failure to maintain the cells as a frozen stock prior to intentional thawing or greater than 30 minutes from the time product is started to be thawed to the beginning of injections. If a redundant back-up vial is not available in the surgical suite to replace the compromised vial of cells, then the cell delivery procedure will be cancelled.

The Sponsor and the site investigator (cardiac surgeon) performing the cell delivery procedure will be notified immediately of any discrepancies, damage or unusable product that is received prior to commencing the delivery of cells.

5.4.3 Product Accountability

Product accountability will be maintained by site personnel and will include documentation of investigational product receipt, administration of the product, and final product disposition. Any discrepancies in product accountability documentation will be investigated and documented. Additional details of the cell delivery procedure, such as the actual product volume and number of injection sites will also be recorded and entered into the applicable eCRF and within the subject's study file.

5.4.4 Return or Destruction of Study Product

Any remaining thawed product will be destroyed or discarded on site per hospital procedures. The final disposition of all unused product will be documented.

Frozen product, which remains under the continuous custody of Sponsor-delegated team member(s), may be returned to the manufacturer by the Sponsor's delegate(s).

6 STUDY PROCEDURES

Tests/procedures will be ordered per the schedule and requirements listed in this section of this protocol. All assessments for eligibility and adverse events must be performed by a study trained and delegated investigator. Investigator assessment of eligibility is performed at time of enrollment and must be specifically documented within the subject's study file.

6.1 Consent/Screening

During routine clinical follow-up after Stage I surgery, as Stage II surgery is being planned, the parent(s) and/or legal guardian(s) will be approached by study staff to consider enrollment of their child in this study. See Section 4.2 for additional details pertaining to the pre-screening process. Potential subjects must be pre-screened prior to beginning the consenting process. Information about the study will be provided to the parent(s) and/or legal guardian(s), and adequate time for questions will be allowed prior to signing the consent form. The IRB-approved, study-specific Informed Consent Form and HIPAA Authorization will be signed by both parents, unless one parent and/or legal guardian is not reasonably available. See Section 3.1.1 of this protocol for treatment group assignment.

The following data will be collected *after* consent has been obtained and the data will be entered in the applicable eCRFs and the subject's study file:

6.1.1 Baseline/Pre-Op:

- Medical History
- Concomitant Medications
- Physical Exam (including weight)
- Vital Signs (temperature, heart rate, blood pressure, oxygen saturation)
- Cardiac Markers (CRP, High Sensitivity Troponin T, NT-proBNP)
- CBC w/ Differential (RBC, WBC, Hemoglobin, Hematocrit, Platelets, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils)
- Liver/Renal Function (ALT, ALP, AST, total bilirubin, albumin, total protein, BUN, creatinine)
- TSH
- Panel-Reactive Antibody (PRA) Test (see Appendix D)
- 12 lead ECG
- Cardiac MRI
- Echo (TTE)
- Cardiac Cath (when clinically available)
- Questionnaire Completion

Consent must occur before any study procedures are completed, however standard of care procedures done within 30 days prior to Stage II surgery may be used rather than repeating procedures to collect study data. Standard of care cardiac MRI data may be used if done within 60 days prior to Stage II surgery.

6.1.2 Study Timeout (Treatment Group Only):

Within five days prior to Stage II surgery, the site investigator (cardiac surgeon) performing the cell delivery procedure must complete the Study Timeout form. The UCB-MNC product volume and number of injection sites, based on the pre-operative baseline visit weight, will be calculated and documented. See Section 5.3 for instructions pertaining to dosing calculations. The site investigator should also document assignment of responsibility to all site personnel involved on the day of procedure. The completed Study Timeout form must be available at the time of surgery to ensure the calculated volume of investigational product is prepared for investigational product administration.

6.1.3 Stage II Surgery:

- Changes in Medical History
- Changes in Concomitant Medications
- Physical Exam (including weight)
- Vital Signs (temperature, heart rate, blood pressure, oxygen saturation)

Any new findings/abnormalities identified prior to enrollment will continue to be recorded as medical history unless considered related to a study-related test or procedure.

6.2 Enrollment

6.2.1 Enrollment/Cell Delivery:

- Procedural Timeout (after completion of Stage II surgery)
- Assessment of Eligibility/Enrollment
- AE Assessment
- Cell delivery (treatment group only)
- Telemetry
- High Sensitivity Troponin T at 3 hour +/- 30 min and 6 hour +/- 30 min after enrollment

A site investigator will assess subject eligibility up to the time of enrollment. If there are new test results or conditions identified up to the time of enrollment that causes a subject to no longer be eligible for participation in this study, the subject will be considered a screen failure.

Adverse events will be recorded in the eCRF and subject's study binder following enrollment to document new or worsening symptoms.

During the hospitalization (starting on Day 0), subjects will be monitored with continuous telemetry from enrollment through discharge (or up to 30 days post-surgery if the subject remains hospitalized). The date and time and the type of any arrhythmias that occur via telemetry that are determined to be clinically significant by a study cardiologist will be recorded in the appropriate eCRF and within the subject's study file.

6.2.2 Post- Op Hospital Stay:

- AE Assessment
- Physical Exam
- Vital Signs (temperature, heart rate, blood pressure, oxygen saturation)
- Telemetry

The tests/procedures included in the bulleted list above will be performed daily during the hospitalization. Any tests/procedures that are performed, but not listed above, will be for the treatment of the subject according to post-surgical standard of care.

Adverse events will be recorded in the eCRF and subject's study binder following enrollment to document new or worsening symptoms throughout the hospitalization.

6.2.3 Hospital Discharge:

- Changes in Concomitant Medications
- AE Assessment
- Physical Exam (including weight)
- Vital Signs (temperature, heart rate, blood pressure, oxygen saturation)
- Cardiac Markers (CRP, High Sensitivity Troponin T, NT-pro BNP)
- CBC w/ Differential (RBC, WBC, Hemoglobin, Hematocrit, Platelets, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils)
- Liver/Renal Function (ALT, ALP, AST, total bilirubin, albumin, total protein, BUN, creatinine)
- TSH
- 12 Lead ECG
- Echo (TTE)

At the time of discharge from the hospital, the above tests/procedures will be conducted. The laboratory tests, 12-lead ECG, and echo should be completed within 3 days of the final discharge date. However, the laboratory tests, 12-lead ECG, and echo don't have to be repeated if the discharge date changes unexpectedly due to non-cardiac related issues.

6.3 Follow-up

6.3.1 1 Month Phone Visit:

- Changes in Concomitant Medications
- AE Assessment

6.3.2 3 and 12 Month In-Clinic Visits:

- Changes in Concomitant Medications
- AE Assessment
- Physical Exam (including weight)
- Vital Signs (heart rate, blood pressure, oxygen saturation)

- Cardiac Markers (CRP, High Sensitivity Troponin T, NT-proBNP)
- CBC w/ Differential (RBC, WBC, Hemoglobin, Hematocrit, Platelets, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils)
- Liver/Renal Function (ALT, ALP, AST, total bilirubin, albumin, total protein, BUN, creatinine)
- TSH
- Panel-Reactive Antibody (PRA) Test (see Appendix D)
- 12 Lead ECG
- Echo (TTE)
- Questionnaire Completion (12 month visit only)

If a subject is unable to safely attend the 3 month and/or 12 month in-clinic follow-up visit(s), the phone follow-up procedures as described in this protocol will replace the in-clinic visits. Medical records will be obtained to provide evidence of the condition that precludes a subject from safely attending the 3 and/or 12 month in-clinic follow-up visit and local medical doctor notes, if available, will be collected.

6.3.3 6 Month and Bi-Annual Phone Visits:

- Changes in Concomitant Medications
- AE Assessment
- Questionnaire Completion (to be completed annually following Stage II surgery)
- Weight

At 6 and 18 months post-Stage II surgery, and every 6 months thereafter for up to 4 years (or until the pre-operative Stage III work-up or cardiac transplant), a phone follow-up with the parent(s) and/or legal guardian(s) will include the collection of the above data. In addition to specific questioning by phone follow-up, medical records should be used for documentation of changes in concomitant medications, AE assessment and subject weight.

6.3.4 Pre-Op Stage III Workup Visit:

- Changes in Concomitant Medications
- AE Assessment
- Physical Exam (including weight)
- Vital Signs (heart rate, blood pressure, oxygen saturation)
- 12 Lead ECG
- Echo (TTE)
- Cardiac Cath (when clinically available)

When the Stage III surgery is scheduled, the date of the Stage III pre-operative work-up and the following standard of care work-up data will be collected from the subject's medical records. If data is not available, the lack of this data will not be considered a protocol deviation. The goal of collecting this data for this study is to evaluate cardiac function that is normally obtained through

standard of care pre-operative testing for the Stage III surgery, without requiring any additional research-only tests/procedures at this time point.

6.4 Questionnaires

The ITQOL: Infant Toddler Quality of Life Questionnaires and the ASQ-3: Ages and Stages Questionnaires will be administered to participants at baseline and at 12, 24, 36 and 48 months post Stage II surgery for the duration of a subject's participation in the study. Questionnaires should be completed or returned during scheduled in-clinic visits when possible. For non-clinic follow-up visits, questionnaires should be mailed to participants for completion with a self-addressed return envelope. If participants do not return the questionnaires, site staff should administer questionnaires via phone call. This should be documented with in the subject's study binder.

6.5 Schedule of Study Tests/Procedures

The below schedule will be followed to complete the study test and procedure requirements.

	Schedule of Study Tests and Procedures									
	Screening			Enrollment/Treatment			Follow Up			
	Consent[1]	Baseline/ Pre-Op Workup	Stage II Surgery	Enrollment/ Cell Delivery	Post-Op Hospital Stay[3]	Hospital Discharge	1-Mo. Phone Visit	3- and 12-Mo. In-Clinic Visit	6-Mo. And Bi-Annual Phone Visit[5]	Pre-Op Stage III Workup[6,16]
Visit Window	No more than -30 days	No more than -10 days[1,2,15]	Day 0	Day 0	Day 0 through discharge	No more than 3 days prior[4]	+/- 7 days	+/- 14 days[15]	+/- 14 days	No more than 30 days prior
Consent[1]	X									
Assessment of Eligibility [7]				X						
Enrollment[7]				X						
Stage II Surgery			X							
Cell Delivery (Arm A only)				X						
Medical History[8]	X	X	X							
Concomitant Medications [9]	X	X	X			X	X	X	X	X
AE Assessment[8]				X	X	X	X	X	X	X
Physical Exam[10]		X	X		X	X		X		X
Vital Signs[11]		X	X		X	X		X		X
Cardiac Markers[12]		X		X		X		X		
CBC w/ Differential[13]		X				X		X		
Liver/Renal Function[14]		X				X		X		
TSH		X				X		X		
PRA test		X						X		
12 Lead ECG		X				X		X		X
Cardiac MRI[15]		X								
Echo (TTE)		X				X		X		X
Cardiac Cath.[16]		X								X
Telemetry[3]				X	X					
Questionnaires[17]		X						X	X	
Study Timeout[18] (Arm A Only)		X								
Procedural Timeout[19]				X						

1 Consent must be obtained prior to commencing any study-related tests/procedures, however standard of care procedures done within 30 days prior to Stage II surgery may be used rather than repeating procedures to collect study data. The subject must appear to meet all study eligibility criteria prior to beginning the consenting process. If the site investigator subsequently determines that the subject is not eligible for the study, the subject will be declared a screen failure and exited from the study.

2 If Stage II surgery is rescheduled, Baseline/Pre-Op tests and procedures do not need to be repeated unless the rescheduled surgery will occur more than 30 days after the baseline testing was initially performed.

3 Tests/procedures will be performed daily during the hospitalization starting on Day 0. Continuous telemetry is not required on day of hospital discharge.

4 Laboratory tests, 12-lead ECG, and echo should be completed within 3 days of the discharge date. However, the laboratory tests, 12-lead ECG, and echo don't have to be repeated if the discharge date changes unexpectedly due to non-cardiac related issues.

5	To be conducted at 6 months post-Stage II surgery and every 6 months following the 12 month in-clinic visit for up to 4 years or until pre-operative Stage III work up or cardiac transplant.
6	Data to be collected from standard of care Stage III surgery pre-op work up in subject's medical records.
7	A subject will be considered enrolled on the study after completion of the procedural time-out.
8	Collection of medical history to occur up to enrollment. AE assessments to begin immediately following enrollment.
9	During Stage II Surgery hospital stay, changes in concomitant medications will not be recorded.
10	Includes weight, except for during post-op hospital stay
11	Includes temperature, heart rate, blood pressure and oxygen saturation. Temperature not required at 3 or 12-month in clinic visit.
12	Includes the following tests: High Sensitivity Troponin T, CRP, and NT-proBNP. On day 0, only the cardiac biomarker panel (High Sensitivity Troponin T) will be collected at 3 hr ± 30 min and 6 hr ± 30 min after enrollment.
13	Includes the following tests: RBC, WBC, Hemoglobin, Hematocrit, Platelets, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils
14	Includes the following tests: ALT, ALP, AST, Total Bilirubin, Albumin, Total Protein, BUN, and Creatinine.
15	Cardiac MRI will only be required at baseline. It is not required at 3 month visit or 12-month follow up. Baseline cardiac MRI should occur within 60 days prior to Stage II surgery.
16	When clinically available.
17	Questionnaires will be administered to participants at baseline and at 12, 24, 36 and 48 months post Stage II surgery for the duration of a subject's participation in the study.
18	Treatment arm only. Must occur within 5 days prior to cell delivery (see Sect. 6.1.2).
19	Required for both treatment and control arms (see Sect. 4.3).

7 STATISTICAL ANALYSIS PLAN

Sections 3.2 and 3.3 of this protocol provide a full description of the study endpoints.

7.1 Sample Size Determination

Sample size calculations were performed using alpha = 0.05 and assume equal variance across study arms. With 50 subjects per arm we will have 80% power to detect an effect size as small as 0.57 in difference in cardiac function measures. This is built on the assumption all subjects from both arms have complete data at baseline and 3-month follow-up visits. In the event of attrition or incomplete data in 40% of participants, equal across study arms, the study has 80% power to detect an effect size as small as 0.74 in difference in cardiac function measures.

7.2 Subject Population(s) for Analysis

Only consented and eligible subjects will be used in analysis. Safety analysis will be based upon all subjects who undergo surgery and receive any volume of cell delivery (partial or full) and have follow-up data in the cell-therapy group and all subjects who have follow-up data in the control group. Efficacy analysis will be based upon all subjects who receive full cell delivery and have follow-up data in the cell-therapy group and all subjects who have follow-up data in the control group.

7.3 Statistical Methods

Descriptive Statistics

Descriptive statistics (e.g., mean standard deviation, median, minimum, and maximum for numerical data and counts and percent for categorical data) will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables will be described separately for the cell-based therapy and control 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.

Lost to follow up

Follow up times for subjects who are in study at the time of analysis will be censored in time-to event analysis, as with time until listing for cardiac transplant. Since the outcome regarding time to listing includes death as part of a compound outcome, there will be no censoring for death. In case of lost to follow up before time of analysis subjects will be censored, unless the investigators feel the subject could have been indicated for transplant in which case they will be included as such in the analysis.

Multiplicity

For both the primary analyses of short-term outcomes and the secondary analysis of long-term analyses, there will be one primary (or first) analysis and thus no correction will be made for multiple comparisons. A sequence of outcomes of interest is also given (Sections 3.2 and 3.3) for each of the short and long-term outcomes, and significance will be assessed in the order of these sequences. Therefore, no adjustment is made for multiple testing.

7.4 Analysis

7.4.1 Interim Analysis

This study has 5 planned safety and efficacy interim analysis planned. An interim analysis will be performed according to statistical analysis plan on all enrolled subjects after 10, 20, 30, 40 and 50 subjects have been treated and completed the 3-month follow-up visit. Results will be reviewed by the DSMB.

7.4.2 Final Analysis

The final analysis of the study will be completed no later than after 50 subjects have completed both Treatment (Arm A) and Control (Arm B) of the study. However, the Sponsor may determine according to interim analysis results that both safety and efficacy endpoints have been achieved for the Phase IIb study. The Sponsor can make this determination at any time following the first interim analysis timepoint.

7.4.3 Short Term Efficacy Analysis

Primary Outcome

The primary analysis of the primary outcome apical fractional area change 3 months post Stage II palliative surgery will be done using analysis of covariance (ANCOVA), including a model term for the difference in means between the cell-based therapy and control groups, as well as a model term for apical fractional area change at time of surgery to account for subject baseline condition. In secondary analyses of the primary outcome we will, using interaction terms, fit a model allowing the treatment effect (mean difference between the cell-therapy and control groups) to depend and baseline fractional area change.

Secondary outcomes

The other short-term efficacy outcomes will be analyzed similarly to the primary outcome.

7.4.4 Long Term Efficacy Analysis

Primary Outcome

The analysis of the primary long-term efficacy outcome, apical fractional area change 12 months post-surgery, will be analyzed like the primary outcome of short-term efficacy, regarding both primary and secondary analyses.

Secondary Outcomes

Quantitative long-term outcomes will be analyzed like the primary long-term efficacy outcome of apical fractional area change. Yes/no outcome variables like “able to undergo Stage III palliative surgery” will first be analyzed by describing percent by group and the groups

compared using a chi-square test. We will analyze time-to-event outcomes, like time to listing for cardiac transplant or death, first describing naïve rates with the Kaplan-Meier method and then using Cox regression models including terms for treatment group and baseline cardiac function. If the primary analysis suggests dependency of treatment on cardiac function, we will also repeat these analyses considering cardiac function as an effect modifier by including interaction terms between group and baseline cardiac function.

7.4.5 Safety Analysis

Adverse Events (AEs) will be described by count and frequency, by body system and severity and groups compared using the chi-square test.

The long-term endpoint regarding arrhythmia events and arrhythmia medication will be analyzed regarding safety. Additional adverse events which will be separated out for analyses of their own will be occurrences of bleeding, to such an extent they would be regarded as AEs, decreased cardiac function, and tumor formation.

7.4.6 Center Variability

Treatment effect could vary due to different patient mixes between the treating hospitals. We will investigate, at least for the primary endpoints and first secondary endpoints for short and long-term outcomes, the possible dependence of treatment effect on treatment hospital. First, we will describe treatment effects without the use of any baseline covariates, and then we will include hospital effects in our models used in the original analyses. We will perform further exploratory analyses as indicated by the data.

8 SAFETY AND ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product or protocol specified procedure, whether considered related to the product or protocol specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporarily associated with the use of the Sponsor's product, is also an adverse event. Sponsor's product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by the Sponsor for human use.

8.1.2 Serious Adverse Event

A serious adverse event is any adverse event occurring during any use of Sponsor's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is an important medical event based upon appropriate medical judgement; it may jeopardize the subject, or it may require intervention to prevent one of the other outcomes listed in the above definitions.

8.1.3 Suspected Adverse Reaction

Any adverse event for which there is a reasonable possibility that the cell therapy product or cell delivery procedure caused the adverse event will be considered a suspected adverse reaction.

8.1.4 Unexpected Adverse Event

An adverse event is considered unexpected if it is not listed in the Investigator's Brochure or the study protocol, or if it has occurred at a greater severity or specificity than is listed in the Investigator's Brochure or the study protocol.

8.1.5 Serious and Unexpected Suspected Adverse Reaction (SUSAR)

An adverse event is considered a SUSAR if it is serious AND unexpected AND is a suspected adverse reaction per the definitions above.

8.2 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization, or results in in-patient (≥ 24 hours) surgery (e.g., pacemaker generator replacement) will be recorded as a serious adverse event.

8.3 Medical History/Preexisting Conditions

Medical history will be recorded once consent in obtained. Any new findings/abnormalities identified prior to enrollment will continue to be recorded as medical history.

A preexisting condition is one that is present prior to enrollment. A preexisting condition will be recorded as an adverse event only if the frequency, severity and/or the character of the condition worsens after enrollment and the event meets the definition of an adverse event as described in this protocol.

8.4 Evaluating Adverse Events

At each contact with the subject, the study team must seek information on new or worsening adverse events by specific questioning and, as appropriate, by examination. Information on any adverse event reported to the study team by the parent(s) and/or legal guardian(s), the subject's physician, or identified in medical records will be recorded by the site. A study investigator who is a qualified and trained physician will evaluate all adverse events for:

- Severity (see Section 8.5)
- Causality (see Section 8.6)
- Seriousness (see Section 8.1.2)

Any adverse event which changes severity over the course of a given episode will have each change of severity recorded on the adverse event case report forms/worksheets.

For adverse events that occur at an outside facility, medical records will be requested from that outside facility and filed in the subject file to provide source documentation of the event(s).

8.5 Severity

Severity of AEs should be assessed using the following categories and using the following definitions as general guidelines:

- Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention indicated
- Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- Severe: Severe or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization indicated; disabling
- Life-threatening: Life-threatening consequences; urgent intervention indicated
- Death: death related to AE

8.6 Causality

Causality of AEs should be assessed using the following relatedness categories:

- Definitely related: the event is *clearly* related the cell therapy product or its delivery procedure
- Probably related: the event is *likely* related the cell therapy product or its delivery procedure
- Possibly related: the event *may be* related the cell therapy product or its delivery procedure
- Unlikely related: the event is *doubtfully* related the cell therapy product or its delivery procedure
- Not Related: the event is *clearly not* related the cell therapy product or its delivery procedure

Adverse events that are unrelated to the cell therapy product or its delivery procedure, but are related to study-required tests and/or procedures, must also be specifically assessed.

8.7 Unresolved Adverse Event

All unresolved adverse events will be followed by a site investigator until the events are resolved or stabilized, the subject is lost to follow-up, or the adverse event is otherwise explained.

8.8 Recording Adverse Events

For the purpose of adverse event recording for this study, investigational sites will document and record in the eCRF in Medidata Rave any adverse event, according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, which meets any of the following criteria:

- It is a serious adverse event
- It is classified by a site investigator as possibly, probably or definitely related to the study drug or its delivery procedure
- It is classified by a site investigator as moderate, severe or life-threatening according to CTCAE version 5.0

Serious adverse events are to be reported to the Sponsor in the AE eCRF in Medidata Rave within 24 hours of site notification of the event. If medical records require review to confirm the details of a serious adverse event, updates will be made accordingly to the serious adverse event report upon medical record review.

The outcome of each adverse event will be recorded in the eCRF.

The Sponsor will be the final adjudicator of adverse events.

8.9 Sponsor Responsibility for Reporting Adverse Events

Adverse events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations, i.e., per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.

8.10 Expected Events

The following are events that may occur as a result of receiving the cell product:

- Mild ventricular tachycardia and/or other mild ventricular arrhythmias
- Mild myocarditis
- Mild pericarditis
- Mild to moderate myocardial infarction
- Mild to moderate pericardial effusion(s)
- Mild to moderate fever for up to 3 days following cell delivery
- Mild to moderate bleeding at the injection site

There are no long-term adverse effects associated with an MRI. There are some potential side effects associated with the general anesthesia required to complete the MRI examination. Most of these are very mild and will go away shortly after the anesthesia care is stopped. The mild side effects include nausea, dizziness, and headache. Other potential risks include, but are not limited to, adverse medication reactions (allergies, etc.), abnormal heart rhythms, low blood pressure or impaired breathing. These more serious problems are very rare, affecting less than 1 in 2000 children.

The risks associated with a Transthoracic Echocardiogram (TTE) include the possibility of mild discomfort associated with placement of the ultrasound transducer on the chest wall while acquiring the echo images. The risks associated with a TTE using moderate (conscious) sedation include possible prolonged sedation, nausea, vomiting and/or a slight risk of allergic reaction. There are no long-term adverse effects associated with the echocardiogram itself.

Repeated or lengthy use of general anesthetic and sedation drugs during surgeries or procedures in children younger than 3 years may affect their brain development.

Intravenous access catheters may be placed as part of sedation/anesthesia for the echoes and MRIs. Placing an IV may cause some pain and bleeding or bruising at the spot where the needle enters the body. Rarely, it may cause fainting. The longer an IV catheter is left in place, the more common it is for redness or infection to develop.

There may be data privacy risks pertaining to the collection of patient data for any study, including this study.

8.11 Stopping Rules

Enrollment and cell therapy treatments in the treatment arm of the study will be put on hold with the IRBs and FDA under the following unanticipated situations:

- Any death within 30 days following cell therapy that is classified by either the Sponsor or investigator as definitely related to the product or the product delivery procedure.
- Any non-elective cardiovascular surgery requiring open chest procedure within 30 days following cell therapy that is performed because of an adverse event that is classified by either the Sponsor or investigator as definitely related to the product or the product delivery procedure.
- Any life-threatening condition that develops within 30 days following cell therapy that is classified by either the Sponsor or investigator as definitely related to the product or the product delivery procedure.
- Any other pattern of safety concerns that are identified by the Sponsor or DSMB that are unexpected and require clarification prior to treating additional subjects.

Events meeting these stopping rules will require full DSMB review. The DSMB will evaluate causality of the event and make recommendations to the Sponsor, who will forward to the FDA and to the sites to send to their respective IRBs prior to resuming enrollment/treatments in the treatment arm of the study or prior to terminating the study. The study will remain on hold to enrollment and cell therapy treatments in the treatment arm until approval to resume enrollment/treatments in the treatment arm of the study is granted by FDA.

8.12 Medical Monitoring

It is the responsibility of the site investigators to oversee the safety of this study at each site. This safety monitoring will include careful assessment and appropriate recording and reporting of adverse events as noted above. There will be a medical monitor who will also oversee this study along with review of safety information by the DSMB.

8.13 Data and Safety Monitoring Board

The DSMB for this study will act in an advisory capacity to the Sponsor to evaluate the overall conduct and progress of the study, evaluate study outcomes, and review safety information. For each meeting, the DSMB will provide a written summary of the topics discussed, individual findings, overall safety assessments, voting decisions, and recommendations to the Sponsor. If the DSMB recommends suspending or terminating all or part of the study or recommends substantially modifying the design or conduct of the study, the DSMB will notify the Sponsor who will then notify the site investigators and FDA. Sites will be required to also notify their respective IRBs.

As part of the DSMB's responsibilities, the Board will provide critical review of adverse events that meet the stopping rules. The DSMB will evaluate causality of the event and make recommendations to the Sponsor who will forward to the FDA and sites to send to their respective IRBs prior to resuming enrollment/treatments in the treatment arm of the study or prior to terminating the study.

The Sponsor will be responsible for FDA reporting of all events according to the required IND Safety Reporting timelines, formats, and requirements and will not require DSMB involvement

in ordinary reporting requirements. However, the Sponsor may request special DSMB review in cases of interest, such as serious suspected adverse reactions. The DSMB may make recommendations to ensure adequate subject safety.

A study statistician or data manger will prepare summary tables for the DSMB pertaining to baseline characteristics/demographic representation, enrollment, accrual, screen failures and withdrawals, adverse events, protocol deviations, and any other information upon the request of the DSMB.

The DSMB will be comprised of four individuals and will require three of the four at each meeting. The Board will be comprised of a non-study biostatistician and clinicians with relevant clinical and scientific knowledge of the disease and of the investigational therapy.

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

If a subject revokes authorization to collect or use PHI, the site 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's eCRFs are the primary data collection instrument for this study and will be completed in the study-specific, 21 CFR Part 11 compliant, Medidata Rave database. When source worksheets/documents are used, the data will be transcribed from the worksheet/document to the appropriate eCRF. Otherwise, data will be obtained from medical records for data entry. Only trained study personnel will enter study data into the database. Data will be entered within 2 weeks from the time of data collection, unless not reasonably available due to lengthy data analysis process within this time period. In such cases, data will be entered as soon as possible.

9.4 Data Quality Assurance

The Medidata Rave system will provide the eCRFs for data entry throughout the study. Edit checks have been customized to facilitate in ensuring compliance with the protocol and systematic and real-time data quality assurance. Routine review of open queries will be performed by the Sponsor or a representative of the Sponsor. It will be the responsibility of the

Sponsor or Sponsor's representative to engage the appropriate study personnel to update the data fields accordingly and ensure that all open queries are resolved in a timely manner.

9.5 Data Clarification (Query) Process

If there are eCRF data discrepancies related to a programed edit check, data review or monitoring, queries will be generated, and the study team should make the appropriate corrections within 10 business days.

9.6 Protocol Deviations

Deviations from the outlined protocol will be reported to the Sponsor on the Protocol Deviation eCRF and will be reported to the site IRB by the site per its policy. Deviations that occur emergently to protect the safety/well-being of a study subjects will be recorded on the Protocol Deviation eCRF and reported to the Sponsor within 24 hours of site notification of occurrence and to the respective IRB as per its policy. Protocol deviations will be reported by the Sponsor to FDA in the final study report.

9.7 Records Retention

9.7.1 Sponsor

The Sponsor and designated personnel will maintain records and essential documents related to the conduct of this study at the Sponsor's site in the study's Regulatory Binder. These include correspondence between Sponsor and sites, IRBs, DSMB, contract research organizations, monitors and FDA.

9.7.2 Sites

The site investigator and designated personnel will maintain records and essential documents related to the conduct of the study at the investigational site in the study's Regulatory Binder. These include subject case histories, regulatory documents and correspondence between the site and the Sponsor, IRB, contract research organizations, monitors, and FDA.

9.7.3 Sponsor and Sites

All study records and reports will be retained by all parties according to the more stringent requirement below:

1. A period of 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 the investigation is discontinued and the FDA has been so notified.
2. As outlined per the site's policy or procedure.

10 STUDY MONITORING, AUDITING, AND INSPECTING

10.1 Study Monitoring

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support, a Contract Research Organization (CRO) or other qualified Sponsor employee(s) will provide clinical monitoring for the trial. Clinical trial monitoring involves review of the study data and generation of manual queries to identify and resolve data discrepancies and ensure the validity and integrity of the data along with the protection of human research subjects. This will assist site investigators in complying with FDA regulations.

The site investigators will allocate adequate time for monitoring activities. The site investigators will also ensure 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.

10.2 Auditing and Inspecting

The site 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 site investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as a site 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, Good Clinical Practice, and institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local IRB, in agreement with local legal prescriptions, for formal approval of the study. The study-specific informed consent form (ICF) will be submitted with the protocol for review and approval by the IRB. The decision of the IRB concerning approval of the study will be made in writing to the site investigator before commencement of this study at the investigational site.

The parent(s)/legal guardian(s) of all subjects in this study will be provided with the study-specific, IRB-approved ICF describing this study and providing sufficient information for parent(s)/legal guardian(s) to make an informed decision about participation in this study. The formal consent of the subject/parent(s)/legal guardian(s), using the IRB-approved consent form, must be obtained before the subject undergoes any study procedure. The consent form must be signed by both of the subject's parent(s) and/or legal guardian(s) (unless one parent or legal guardian is not reasonably available).

The subject's parent(s)/legal guardian(s) should be given a copy of the signed ICF, and the site shall retain the original copy of the signed and dated ICF. The site shall also document and retain the informed consenting process (and the re-consenting process if applicable).

12 STUDY FINANCES

12.1 Funding Source

This study is financed by 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 (e.g. patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must disclose this conflict of interest to the Sponsor and local IRB. Any changes in financial disclosure information must be disclosed throughout the study and for a period of 1 year following the completion of the study.

13 PUBLICATION PLAN

The study Sponsor, in collaboration with the investigative team, will have the responsibility of identifying authorship and release of all information for the purposes of publication of data.

This study will be registered on ClinicalTrials.gov and results will be posted to ClinicalTrials.gov.

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APPENDIX A: PRODUCT HANDLING

This appendix is to serve as a guide to product preparation and administration for UCB-MNC intramyocardial injections. Trained and delegated Sponsor delegates or trained and delegated site staff may perform thawing or loading activities. Site staff, including a study-trained and delegated cardiac surgeon, will perform product administration.

Product Description

The investigational product is frozen in cryovials containing approximately 1.5mL of cell product with a target concentration of 10-30 million total nucleated cells (TNC) per mL. Each 1.5mL aliquot is sufficient for a single treatment. The number of available aliquots per subject is variable and dependent on the initial cord blood collection.

Thawing Procedure

Prior to product thaw, ensure sterile workspace is assembled. Confirm the cell delivery supply kit is intact and within expiration.

Following the Procedural Timeout (see Section 4.3) and enrollment into the treatment arm of the study, commence product thaw. **NOTE:** The product delivery into the heart tissue should begin within 30 minutes from the beginning of product thawing.

1. Document the temperature of the dry shipper prior to removal of product.
2. Remove product vial from dry shipper and secondary containment.
    a. Record date and time product removed from dry shipper (start of thaw).
    b. Confirm product identity matches patient identity.
3. Thaw product vial.
    a. Place vial into Sponsor provided controlled rate thaw device until thaw cycle is complete.
    b. If device is unavailable, thaw in a gloved hand, approximately 3-5 minutes.
4. Upon completion of thaw, invert vial several times to ensure mixing of the contents.
5. Use five (5) to six (6) alcohol wipes to cleanse the outside of the vial and cap.
6. Slightly loosen cap.
7. Place vial into rack within the sterile workspace. Remove cap from the thawed vial.

Cell Delivery Device Loading Procedure

Preparation of the cell delivery device will occur within the operating room.

Assemble the sterile workspace. Cover an appropriately sized area with sterile drapes. Place the following supplies into the sterile workspace.

- two (2) 1mL syringes
- two (2) 18 G (1 ½ in.) needles attached to the 1mL syringes
- one (1) 27 G (1/2 in.) needle with 8 in. tubing
- one (1) cryovial rack

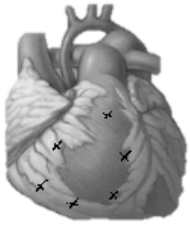
Once the product vial is placed into sterile workspace, proceed with loading of the cell delivery device as described below.

1. Slowly draw up product into the first 1mL syringe to the volume designated on the subjects Study Timeout form, plus an additional 0.1mL. Carefully expel all air and remove the 18 G needle and set aside in sterile workspace.
2. Draw remaining product into the second 1mL syringe, carefully expel all air and remove the 18 G needle.
3. Place the 27 G needle/tubing on the second syringe and expel product to fill tubing and needle with product.
4. Remove the 27 G needle/tubing from the second syringe, ensure needle/tubing remains air-free.
5. Attached first 1ml syringe onto product filled 27 G needling/tubing. Ensure syringe and needle/tubing remains air-free.
6. Expel any volume exceeding the volume designated on the subjects Study Timeout form. Document the final volume loaded into the delivery syringe.
7. Completion of product preparation will yield a loaded cell delivery device which consists of a 1mL syringe with attached 27-guage butterfly needle containing sufficient product volume to treat the designated subject as defined in the Procedural Timeout.

Administration Procedure:

Product administration will be completed by trained and delegated site staff.

Once the site investigator obtains the loaded cell delivery device, this procedure can be used as a guidance for administering the investigational product.



1. A site investigator identifies a target circle on the right ventricle, avoiding epicardial vessels. The number of injection sites equals the number of kg body weight of the subject (rounded down to the nearest integer). Injections are to be placed in a circular pattern approximately 1cm apart from each other across the free wall of the right ventricle. Each injection will be approximately 0.1mL. (Example: a 6.9kg child receives 6 injections, 0.1 mL each, evenly spread around the perimeter of the target circle, for a total delivered volume of 0.6 mL cell product).
2. A site investigator (cardiac surgeon) inserts the 27 G needle pointing outward in relation to the target circle at an angle of 20-30 degrees to the horizontal of the surface of the heart. The needle should be placed within the myocardium just below the epicardial surface and avoiding the ventricular lumen.
3. A trained supporting study staff member slowly depresses the syringe to administer approximately 0.1 mL per injection site, taking 5-10 seconds to complete a single injection while the surgeon maintains position of the needle in the tissue. The surgeon

will wait approximately 20 additional seconds prior to needle withdrawal to maximize cell retention within the myocardium.

4. Both the site investigator and supporting study staff member will repeat the outward pointing injections moving around the perimeter of the target circle to deliver the full dose. **NOTE:** Injections will be halted upon complications such as excessive bleeding, ventricular fibrillation or any sustained arrhythmias, blood pressure drops requiring pharmacological support, or any other concerns of atypical physiology from site investigators or the surgical team.
5. A site investigator will verbalize the completion of cell delivery.
6. As appropriate during the cell delivery, study personnel will record the necessary data points as defined in the cell delivery worksheet.
7. The site investigator confirms lack of bleeding, arrhythmias, hemodynamic stability prior to proceeding with the closure of the chest and/or confirming the end of the procedure.

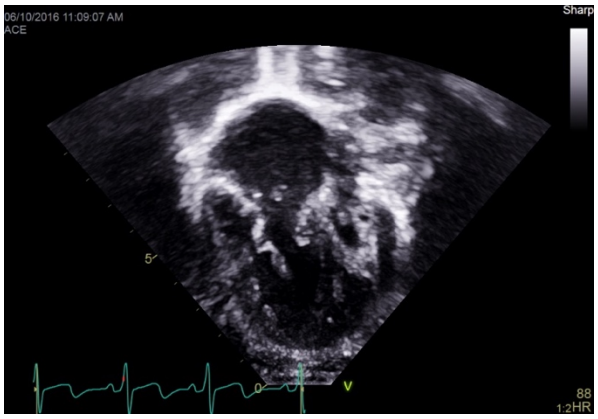
APPENDIX B: ECHOCARDIOGRAPHY

This appendix is to serve as a guide to acquire and interpret echocardiographic images at baseline, hospital discharge, 3 and 12 months after Stage II surgery, and before Stage III surgery.

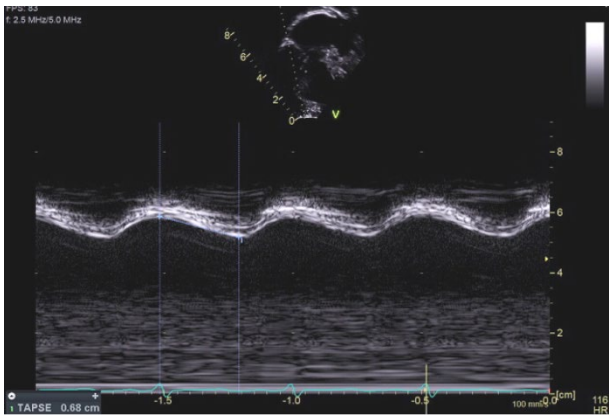
Image Acquisition

Images can be acquired by sites' echocardiographic laboratory standards. In addition to a clinically standard examination, the following technical details must be observed during image acquisition.

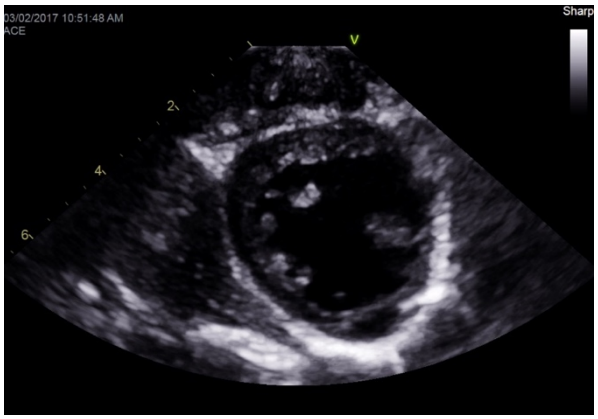
1. The following views should be at least 3 beats long and must be acquired without a sweep.
    a. Apical "4-chamber" view should be acquired in a plane central to atrioventricular valves with probe positioned over the right ventricular apex (Figure). If the apical view has suboptimal acoustic window, a corresponding subcostal view should be attempted.



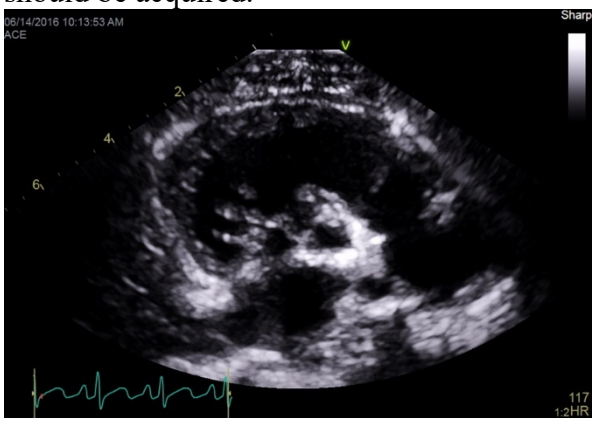
    b. M-mode across tricuspid valve lateral annulus should be acquired from the apical view after zooming into the tricuspid valve lateral annulus (Figure).



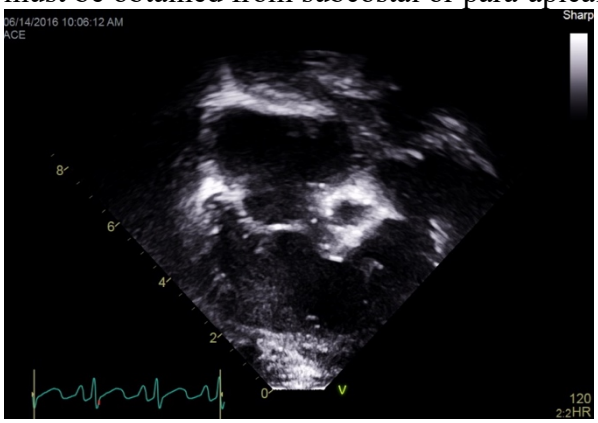
c. Parasternal short axis view should be acquired at the base, mid ventricle, and apex (Figure). If parasternal window is suboptimal, the corresponding views must be obtained from the subcostal window.



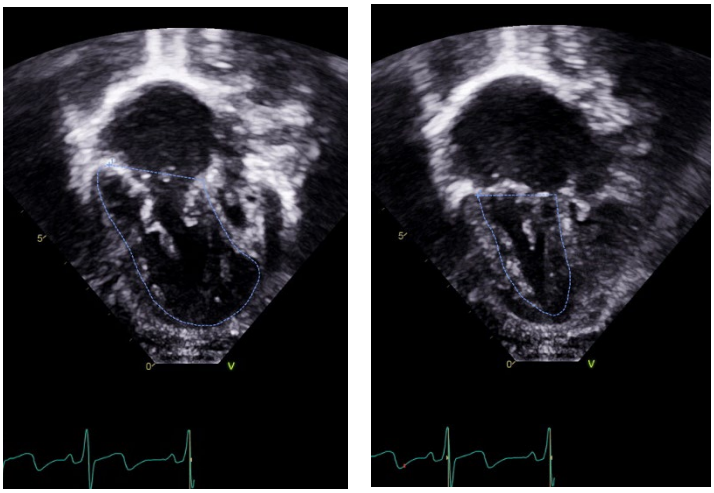
d. Right ventricular inflow and outflow view from the parasternal position (Figure) should be acquired.



If the parasternal window is suboptimal, the corresponding inflow-outflow view must be obtained from subcostal or para-apical positions (Figure).



2. Right ventricular end-diastolic and end-systolic areas should be traced in the apical "4-chamber" view to allow calculation of fractional area change. Tracing of the endocardial borders should be along the blood-tissue interface, while including all small trabeculations in the blood volume (Figure). The end-diastolic frame is the frame showing initial coaptation of the tricuspid valve leaflets or the frame with the largest ventricular dimension. The end-systolic frame is the frame preceding initial early diastolic tricuspid valve opening or the frame with the smallest ventricular dimension.



3. Time gain compensation and overall two-dimensional gain should be adjusted to be able to visualize all myocardial walls adequately to assess ventricular function and regional wall motion abnormalities.
4. Color Doppler assessment of all cardiac valves (even when atretic) must be done in multiple planes (apical, parasternal, subcostal).

Image Recording

Images will be recorded in DICOM digital format.

Anonymization of the Images

Anonymization of all images will be performed by sites prior to transfer. This process includes removal of any personal information and subject study number by DICOM and pixel anonymization. New subject identifiers will be provided to sites through a third-party vendor to keep the core lab blinded.

Transfer of Images

Anonymized images will be electronically transferred to the Sponsor-delegated Imaging Core in DICOM digital format with new subject identifiers provided to sites by a third-party vendor.

APPENDIX C: CARDIAC MAGNETIC RESONANCE IMAGING

This appendix is to serve as a guide to acquire and interpret cMRI images conducted at baseline.

Patient Preparation

- Magnetic resonance safety screening by MR personnel.
- Measure and document subject height (cm) and weight (kg) if not already performed during this visit.
- Obtain intravenous access, if not already performed, preferably in upper extremities. May need one cannula in each arm if patient has bilateral SVC/Glenn.
- General anesthesia is given.
- Transfer subject to the MR scanner.
- Attach ECG leads for cardiac gating (ensure that gating is good once in the scanner)

Image Acquisition:

Images can be acquired by site's radiology standards. In addition to a clinically standard examination, the following technical details should be observed during image acquisition. The only required sequences for this protocol are a stack of cine images covering the entire heart in short axis orientation at all timepoints, and MR angiogram (with or without contrast). All other sequences are optional. Additional sequences can be acquired as clinically needed.

1. Stack of gated steady state free precession (SSFP) or equivalent images (e.g. FIESTA, True FISP, FFE, GRE, SPGR etc.) in the axial plane covering the entire heart from aortic arch to the bottom of the heart.
2. Gated SSFP or equivalent images in 4 chamber planes.
3. Stack of gated SSFP or equivalent images in short axis plane covering the entire heart.
4. MR Angiogram to cover all major blood vessels (native and neo-aorta, aortic arch, SVC, pulmonary arteries, and pulmonary veins). This can be done either by using gadolinium-based contrast, or without contrast utilizing non-contrast MRA sequences (e.g., 3DSSFP).
5. Delayed enhancement images in short and long axis (axial or 4Ch) planes if gadolinium-based contrast is used.

Imaging Plane Prescription and Parameters Selection

- Target in-plane resolution is 1-2 mm. Select FOV and matrix accordingly.
- Slice thickness: 6-7 mm. No gap.
- Reconstructed phases per RR interval: 20-30. Keep the same throughout the study.
- If using ASSET or other parallel imaging, select low acceleration factor, such as 1.2 X.
- RV is typically dilated in these patients. Ensure that the basal area of the right ventricle at the lateral tricuspid valve annulus, RVOT and apex are fully covered in axial and short axis stacks.
- Use Cine IR to guide inversion time for delayed images.

Post-Acquisition Measurements

- Use short axis SSFP or equivalent stack for RV volumes.
- Use rounded contours along the most definite endocardial border.
- Include the trabeculations (not the papillary muscle) in the blood volume.
- Use cross reference to know the extent of ventricular volumes.

Image Recording

Images will be recorded in DICOM digital format.

Anonymization of the Images

Anonymization of all images will be performed by sites prior to transfer. This process includes removal of any personal information and subject study number by DICOM and pixel anonymization. New subject identifiers will be provided to sites through a third-party vendor to keep the core lab blinded.

Transfer of Images

Anonymized images will be electronically transferred to the Sponsor-delegated Imaging Core in DICOM digital format with new subject identifiers provided to sites by a third-party vendor.

APPENDIX D: PANEL-REACTIVE ANTIBODY TEST

Whole blood samples for panel-reactive antibody (PRA) blood tests should be collected at baseline and at the 3- and 12-month post Stage II surgery visits.

It is the responsibility of the primary investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens are trained and certified as required by national regulations and they ship materials in accordance with all current regulations relating to the handling and shipping of hazardous goods.

Collection and Storage:

1. Collect 1-3 mL (1 mL is the minimum required amount) of whole blood into red top Vacutainer tube.
2. Complete the tube label, and affix to tube.
3. After the whole blood collection, keep at room temperature until ready to ship. Do not freeze tubes or specimens.
4. Samples collected Monday – Thursday should be shipped day of collection. Samples collected Friday-Sunday should be shipped the following Monday. If unable to ship day of collection, samples should be stored at ambient temperature until ready to ship.

Packaging and Shipping:

1. Place labeled tube in plastic bag containing the absorbent material. Seal the bag.
2. Pack sample in shipping box provided.
3. Include copy of the completed lab requisition form. File original copy of lab requisition form in subject's study file.
4. Use return shipping label provided to ship sample. Contact shipping courier to schedule shipment.

Site-Provided Supplies:

- Plastic Red Top Vacutainer (no anti-coagulant or serum separator gel)

Sponsor-Provided Supplies*

- Tube labels (must include: protocol number, subject ID, visit ID, date and time of collection)
- Shipping materials (plastic bag containing absorbent material, shipping box, return label, and lab requisition form)

*Please contact Sponsor to order more tube labels and shipping materials as needed.