Interdisciplinary Stem Cell Institute
University of Miami/ Miller School of Medicine and University of Maryland

Clinical Research Protocol

Study Title:	Autologous **C**ardiac Stem Cell Injection in Patients with **H**ypoplast**I**c **L**eft Heart Syn**D**rome: An Open Label Pilot Study. (CHILD Study)
Study Product:	Autologous c-kit positive cells
Indication:	Hypoplastic Left Heart Syndrome (HLHS)
FDA IND No.:	BB-IND #14699
Sponsor:	Joshua M. Hare, MD Telephone: (305) 243 – 5579
Protocol Version:	Version 8, dated March 23, 2021

Investigator Signature Page

Title: **Autologous Cardiac Stem Cell Injection in Patients with Hypoplastic Left Heart Syndrome: An Open Label Pilot Study.**

FDA IND Number: 14699

I have read the enclosed protocol. I will ensure the safety of the study subjects enrolled under my supervision, and will provide the sponsor with complete, accurate, and timely information on this study, as outlined in this Protocol. The signature below constitutes approval of this protocol and the attachments, and provides the required assurances that this trial will be conducted according to all stipulations of the protocol, including local legal and regulatory requirements, applicable US federal regulations and (ICF E6) guidelines. I shall hold strictly confidential all information pertaining to the study, and that this confidentiality requirement applies to all study staff at the site(s) and/or under my supervision.

_____ _____

Principal Investigator Signature **Date**

_____

Print Name for Principal Investigator

Table of Contents

List of Abbreviations And Definition of Terms

AE	Adverse Event
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
BDCPA	Bidirectional Cavopulmonary Anastomosis
BNP	Brain Natriuretic Peptide
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CHSS	Congenital Heart Surgeons' Society
CRCMP	Clinical Research Cell Manufacturing Program
DMSO	Dimethyl Sulfoxide
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
EDV	End-Diastolic Volume
EF	Ejection Fraction
ESR	Expedited Safety Report
ESV	End-Systolic Volume
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GVHD	Graft Versus Host Disease
HIPAA	Health Insurance Portability and Accountability Act
HLHS	Hypoplastic Left Heart Syndrome
HSA	Human Serum Albumin
HSC	Hematopoietic Stem Cell
ICD	Implantable Cardioverter-Defibrillator
IND	Investigational New Drug Application
IP	Investigational Product
IRB	Institutional Review Board
LOCF	Last-Observation Carried-Forward

LV	Left Ventricle
LVAD	Left Ventricular Assist Device
LVEDP	LV End Diastolic Pressure
LVEF	Left Ventricular Ejection Fraction
mBTS	modified Blalock-Taussig shunt
MMWR	Morbidity and Mortality Weekly Report
MRI	Magnetic Resonance Imaging
hMSC	Human Mesenchymal Stem Cell
NSF	Nephrogenic Systemic Fibrosis
NYHA	New York Heart Association
PBS	Phosphate Buffered Saline
PC MRI	Phase Contrast Magnetic Resonance Imaging
PIM	Percutaneous Intramyocardial
RV	Right Ventricle
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
SW	Stroke Work
TTE	Trans-thoracic echocardiogram
U.S.	United States

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SECTION 1

1. Introductory Statement

Title: Autologous **C**ardiac Stem Cell Injection in Patients with **H**ypoplast**I**c **L**eft Heart Syn**D**rome: An Open Label Pilot Study. (CHILD Study)

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Test Agent: Autologous c-kit-positive cells (c-kit$^+$ cells)

Population: Male or female neonates diagnosed with hypoplastic left heart syndrome (HLHS) undergoing Stage I Norwood operation.

Sample Size: A total of 32 subjects will be enrolled for this pilot study in a staged enrollment.

Treatment: All subjects with the diagnosis of hypoplastic left heart syndrome (HLHS) undergo a palliative reconstructive surgery performed in the first two weeks of life, termed the Norwood procedure. The Norwood procedure involves reconstruction of the aortic arch, resection of the atrial septum, and establishment of pulmonary blood flow through either a modified Blalock-Taussig shunt (mBTS) (a Gore-Tex tube from the innominate artery to the right pulmonary artery) or a Sano shunt (a Gore-Tex tube from the right ventricle to the right pulmonary artery). To initiate cardiopulmonary bypass for this surgery, the right atrial appendage is cannulated. Typically, the appendage is excised and discarded during this step. However, in our study, this excised right atrial appendage will be harvested and c-kit$^+$ cells will be isolated. The process and manufacturing of the c-kit$^+$ cells will be exactly as specified in the TAC-HFT II Study (IND# 14647, NCT00768066). TAC-HFT II was a Phase I/II, Randomized, Placebo-Controlled Study of the Safety and Efficacy of Transendocardial Injection of Autologous Human Cells (Mesenchymal or the combination of MSC and ckit+ in Patients with Chronic Ischemic Left Ventricular Dysfunction and Heart Failure Secondary to Myocardial Infarction trial of University of Miami under IND #14647. The patients will then proceed with the typical postoperative care. The second stage in the palliation of HLHS is a scheduled bidirectional cavopulmonary anastomosis, BDCPA) operation that

occurs between 4 to 6 months of age. The BDCPA operation is an anastomosis between the superior vena cava and the right pulmonary artery and removes the pulmonary shunt (the previously placed mBT or Sano shunt). The BDCPA operation can utilize the GLENN or Hemi-Fontan procedure. During this surgery when the patient is on cardiopulmonary bypass, we will deliver the entire dose of cells of 600 microliters previously harvested, isolated, and expanded autologous c-kit$^+$ cells in 6-10 intramyocardial injections. The intramyocardial injection of cells will take approximately 5-10 minutes, which is insignificant to the total bypass time. The remaining part of the BDCPA operation will proceed as anticipated. In this way, we will replicate the same administration route and cell dosing of these cells as the ELPIS trial, which is cross-referenced (IND# 16045, NCT02398604). ELPIS was a phase I, randomized, open label study of Allogeneic Human MEsenchymal Stem Cell (MSC) Injection in Patients with Hypoplastic Left Heart Syndrome: A Phase I/II Study (ELPIS).

In summary, we aim to overlay a novel cell therapeutic strategy on the two-stage surgical procedures that HLHS patients typically undergo in the first year of life: Stage I Norwood operation in the neonatal period and Stage II BDPCA operation at approximately 4-6 months of age. We will harvest endogenous c-kit$^+$ cells from right atrial appendage (RAA) tissue typically discarded during the patient's Stage I Norwood operation and then grow them under the exact conditions specified by the cross-referenced IND #14647. During the Stage II operation, the c-kit cells will be injected intramyocardially into the heart muscle. The c-kit+ cells have already been tested for their feasibility, safety, efficacy in adult humans with ischemic disease (cross-referenced IND #14647).

Duration: All subjects will be followed for 12 months post-treatment.

Objective: The objectives of this pilot study are to:

a. evaluate the feasibility and safety of intramyocardial injection of autologous c-kit$^+$ cells during the Stage II BDCPA operation
b. observe effects on clinical outcome including right ventricular myocardial function, severity of tricuspid regurgitation, incidence of serious adverse events, re-hospitalizations, changes in health status, the need for transplantation, or mortality.

Endpoints:

Primary: Consistent with the nature of this study, multiple assessments will be performed to evaluate the safety, feasibility, and efficacy of intramyocardial delivery of c-kit$^+$ cells in subjects with HLHS:

1. Safety will be assessed by monitoring the following major adverse cardiac events during the first 30 days post Stage II BDCPA operation:

- Greater than 30 seconds of sustained/symptomatic ventricular tachycardia requiring intervention with inotropic support or anti-arrhythmics
- Cardiogenic shock (i.e. tissue hypoperfusion presented by hypotension due to decreased cardiac output, determined by rising lactate levels (5x normal levels in first 24 hours, 2.5x normal levels in first 30 days)
- Unplanned cardiovascular operation due to right ventricular intramyocardial injection site bleeding in the first 5 days after Stage II BDCPA operation
- Need for new permanent pacemaker
- Stroke or embolic event to the brain determined by CT scan
- Death

2. Feasibility will be assessed by monitoring how many c-kit+ products can be manufactured and delivered to subjects and how many subjects can get baseline, 6-month and 12-month MRIs.

3. Efficacy will be assessed at 12 months post-Stage II BDPCA operation by change from baseline in right ventricular ejection fraction, right ventricular end-diastolic volume, right ventricular end-systolic volume, and tricuspid regurgitation measured by serial echocardiograms and MRI scans (baseline, 6 months and 12 months).

Secondary:

1. Incidence of mortality or need for transplantation after the Stage II BDCPA operation up to 12-month follow-up.

2. Incidence of the following after the Stage II BDCPA operation up to 12-month follow-up:
   i. All-cause mortality
   ii. Cardiovascular mortality
   iii. Need for transplantation
   iv. Hospitalization for heart failure
   v. Cardiovascular morbidity, including myocardial infarction, stroke, heart failure, or sustained/symptomatic arrythmias

3. Changes in somatic growth velocity (weight, length, head circumference) over 12 month follow-up.

4. Change in quality of life measured by the Infant Toddler Quality of Life Survey (ITQOL) at baseline, 6 and 12 months after Stage II BDCPA operation.

2. General Investigational Plan

A. Background

Surgical and Clinical Outcomes for HLHS

The epidemic of congestive heart failure (CHF) is a growing, worldwide concern and is expected to become worse. In adults, there are approximately 15 million people worldwide and 5 million patients in the United States who have CHF, with 400,000 new cases per year in the U.S. alone. In children, there is an equal trend of CHF with an increase in prevalence in the last decade. There were almost 3000 more CHF-related hospitalizations in children in 2006 than in 2000 with an increase in total hospital charges of over 1 billion dollars (1). This cost in children with CHF is less than but on the same scale as the monies spent for all cancer-related diagnoses ($2.24 billion) and myocardial infarction ($3.18 billion) combined. These numbers in children will only increase during the next decade as patients with congenital heart disease are now living longer than the number of congenital heart patients being born with congenital heart disease.

We have focused our efforts in a selected, homogenous population of children diagnosed with hypoplastic left heart syndrome. Prior to the development of palliative approaches for HLHS the lesion was universally lethal in infancy. With development over the last few decades of staged surgical treatments, outcomes of surgical palliation for HLHS have steadily improved. According to reports in the *MMWR* approximately 949 infants are estimated to be born each year with HLHS, and 318 die during the neonatal period for a death rate of approximately 33% (2). The cost of managing these patients is very high with typical hospitalizations for the neonatal first-stage surgery of $199,597 (2003 dollars). Despite surgical improvements the overall 5-year survival rate remains limited with reported survivals over 50%-60%, with cardiac transplantation remaining as the only alternative for patients with failing circulations (3;4).

As an increasing number of these patients survive the neonatal period there is a growing population of patients who present later in life with failure of the single ventricle. Maintenance of ventricular function for these patients is therefore very important for long term survival. All patients who require cardiac transplantation have a waiting list mortality of over 20%. In addition, even after cardiac transplantation, HLHS patients have a decreased survival rate when compared to other transplanted patients for other causes (5;6). Although the expenditure for heart transplantation exceeds that for other operations that are required for these HLHS patients, patients who fail these other operations for HLHS (see the following) due to ventricular dysfunction prove to be ultimately even more costly and have increased postoperative morbidity than heart transplantation by itself (7). Estimated charges for heart transplant in 2008 are $787,700 according to the Millman Reports. While this applies to all heart transplantations, the patients who undergo transplantation for HLHS are at an even higher risk, and constitute a costlier group. For the last three patients who underwent cardiac transplantation after surgical operations for HLHS at our institution the average hospital costs was

over $1,000,000. Interventions that improve ventricular function and avoid or defer transplantation in patients with HLHS are therefore urgently needed.

Patients with advanced heart failure represent about 10% of the total heart population, have a high short-term mortality, and consume tremendous resources. The cost of one year of care for a patient with advanced CHF is approximately $20,000; admission for cardiac transplantation including postoperative care averages $1,000,000 for HLHS patients; implantation of a left ventricular assist device (LVAD) averages $175,000. Expensive as these solutions are, they are in fact not available in sufficient supply. Cardiac transplantation is highly constrained by the donor shortage so that only approximately 1000 heart transplants per year are performed for the approximately 30,000 to 60,000 children and adults with end-stage CHF who might potentially benefit from this scarce resource.

Before consideration for transplant, patients with CHF are optimized with medical treatment. CHF in infants with HLHS is not related to typical coronary ischemia and thus revascularization is not applicable. Treatment such as extracorporeal membrane oxygenation (ECMO) or left ventricular assist device (LVAD) therapy are limited by the size of the patient and the duration of support on these devices. Furthermore, support of HLHS patients with these devices is technically challenging because they have only one ventricle.

Our hypothesis is that endogenous ckit+ cells can improve long-term performance in the right ventricle builds on surgical advances of the past thirty years, and is intended to address remaining obstacles to long-term cardiac function in HLHS patients. Since the early 1980s, a series of surgical interventions has been used to create a viable circulation in these infants, allowing the right ventricle to serve as the sole pump for systemic circulation. Currently, the strategy consists of three palliative operations: Stage I is the Norwood operation, Stage II is the BDCPA operation, and Stage III is the Fontan operation (4). The overall objective is to rely on the right ventricle to eject blood into the systemic circulation and for the systemic venous return to passively flow through the pulmonary circulation in the absence of a ventricular pumping chamber.

The goals of the Stage I Norwood operation are to reconstruct the aortic arch, create an atrial septal defect, and establish reliable pulmonary blood flow. With these modifications to the anatomy, the single right ventricle becomes responsible for pumping blood to both the body and lungs. Typically, pulmonary blood flow is achieved by either a modified Blalock-Taussig shunt (mBTS) which uses a polytetrafluoroethylene conduit to connect the subclavian artery to the pulmonary artery, or a Sano shunt which connects the right ventricle to the pulmonary artery using a similar polytetrafluoroethylene conduit. A prospective randomized study has been performed to compare the outcomes of the two shunt types (8). This study reported that the Sano shunt had better survival in the first year, but intermediate-term outcomes were not different for the two shunt types.

Cardiovascular surgeons continue to use both shunt variations pending long-term findings on whether one method is superior to the other.

Sometimes in high-risk HLHS patients who may have severe tricuspid regurgitation or severe right ventricle dysfunction, the Stage I Norwood operation is not recommended. Instead, the Stage I Hybrid operation is preferred which uses a median sternotomy approach for pulmonary artery banding, patent ductus arteriosus stenting, and an atrial septostomy. With these Hybrid patients, the Stage II operation occurs at 4-6 months of age and involves the BDCPA operation and the Norwood operation.

The Stage II operation, the BDCPA operation, is typically performed when the HLHS patient is 4 to 6 months of age. The procedure connects the superior vena cava to the right pulmonary artery to bypass the right ventricle and allow systemic blood to flow directly to the pulmonary circulation. The second part of the BDCPA operation includes ligation of the pulmonary shunt placed during the Norwood procedure. Finally, the Stage III Fontan operation, performed when the child is between 18 months to 3 years of age, directs inferior vena caval flow into the pulmonary artery, thus bypassing the heart.

The largest and most comprehensive outcome analysis of patients undergoing a Stage I Norwood operation was reported by the Congenital Heart Surgeons' Society in 2003 (9). Between 1994 and 2000 twenty-nine institutional members of CHSS enrolled 985 neonates who had either critical aortic stenosis or atresia. A total of 710 of the 985 patients underwent a Stage I Norwood operation. The survival was 76% at 1 month, 60% at 1 year, and 54% at 5 years. Numerous studies have determined the physiologic risk factors for poor perioperative outcome after Stage I, with conflicting results (10-14). Surprisingly in these studies, right ventricular (RV) function was not reported as a predictor of survival. This is an unexpected finding since in other congenital heart surgeries for other malformations poor ventricular function has been associated with adverse outcome after operative procedures (15). Two possible explanations may exist to explain these results: 1) previous analyses of RV function in the setting of HLHS have been limited to qualitative assessment of the right ventricle, and 2) other perioperative factors in the initial unstable hemodynamic state may overshadow the impact of RV function in the early post-stage I Norwood period.

A more accurate quantitative evaluation of the right ventricle has now been reported using the biplane pyramidal approximation method by echocardiography (ECHO) and phase contrast magnetic resonance imaging (PC MRI) (16-18). These newer imaging methods have demonstrated that the systemic right ventricle, working against the high-pressure systemic circulation, becomes dysfunctional, which may correlate with decreased survival. In fact, Altman and colleagues reported that a new qualitative assessment of the right ventricle using biplane pyramidal approximation method by ECHO was an operative predicator of survival after Norwood stage I (16;17). Their study revealed that patients with decreased

right ventricular function had a 35% survival rate when compared with 70% survival rate in patients with normal RV function during short-term follow-up. This study emphasizes the importance of RV function as it correlates with survival rates. Other studies using PC MRI have reported similar findings of decreased function of the right ventricle in HLHS. These studies suggest a critical need to improve RV function in HLHS patients. In fact, many HLHS survivors are now living longer and if they have right ventricular function, they may eventually require heart transplantation, the only remaining treatment option (19).Other HLHS patients die suddenly due to complications that rely on a systemic right ventricle (19). We propose that a cell-based therapy for HLHS patients may prevent RV dysfunction and therefore improve outcomes, reducing both mortality and the need for transplantation.

c-kit$^+$ cells

Preclinical Work

To verify the findings derived from a previously reported rat model on c-kit+ cells, experiments were conducted on pigs in a large model (21). In this study, myocardial ischemia was induced in Yorkshire pigs (n=21) by a 90 min coronary occlusion of the left anterior descending artery followed by reperfusion. At the same time of occlusion, the right atrial appendage was harvested for isolation and expansion of c-kit$^+$ cells. After four months, autologous ckit+ cells (n=11) or vehicle (n=10) were injected into the chronic infarct zone using a balloon catheter. At the time of treatment, left ventricle ejection fraction and LVEDP and LV dP/dt (Millar catheter) were similar in the two groups that implied the infarction zone was similar among the two groups. After one month, the ckit+ treated pigs demonstrated an increase in LV ejection fraction (51.7 +2.0% vs. 42.9 +2.3%. $P<0.01$) and an improvement in systolic thickening fraction in the infarcted LV wall relative to control pigs (31.7 +2.4% vs. 21.1+2.4% in controls, $P<0.01$). At the microscopic level, confocal microscopy showed clusters of small alpha-sacromeric actin positive cells expressing Ki67 were present in the scar of treated pigs, consistent with cardiac regeneration. This study showed that in a large animal model the ckit+ cells promoted cardiac regeneration in a chronic model of ischemia. Furthermore, these findings lay the groundwork for clinical trials of ckit+ cells in human patients requiring cardiac regeneration.

Most of the small animal or large animal studies have used direct intracoronary injection of the ckit+ cells. However, recently, a large animal study using cardiac stem cells demonstrated the feasibility, safety, and benefit of direct intramyocardial injection of cardiac stem cells into ischemic hearts (22). In these experiments, the cardiac stem cells used were a different type but very closely related to the c-kit$^+$ cells, called cardiosphere derived cells. These cardiosphere derived cells are similarly derived from the heart tissue and have similar regenerative abilities as the c-kit$^+$ cells. In fact, a phase I-II clinical study with these cardiosphere derived cells

is underway called the CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dysfunction) study. In this intramyocardial injection study, myocardial infarction was induced in female Yuctan mini-pigs by inflation of an angioplasty balloon in the mid-left anterior descending artery for two hours. After 4 weeks after infarction, c-kit$^+$ cells derived from endomyocardial biopsies were injected at a cell dose of (0.5 million cells/ site) into 20 peri-infarcted sites of the ischemic myocardium through a thoracotomy incision. The results showed that the ejection fraction was equivalent at baseline, but at 8 weeks was higher than the placebo in the stem cell treated group (placebo vs CDC, p=0.01). More importantly, the direct myocardial injection was extremely safe with only one reported (out of 660 injections) catheter related ventricular tachycardia, which was treated with electrical conversion. In the postoperative period, no morbidity or mortality was associated with the injection. This large animal study provides the necessary pre-clinical data to demonstrate the feasibility, dosage, and safety of injecting c-kit$^+$ cells directly into the myocardium using a 25-27gauge needle.

Clinical Work

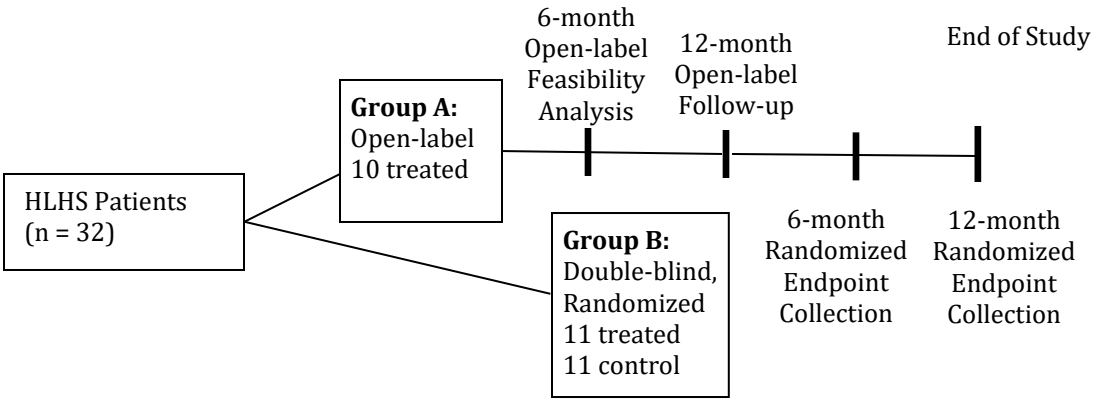
All of the previous (and ongoing) stem cell studies have been performed with adult ckit+ cells with ischemic coronary artery disease. Cardiomyopathy in children, however, are usually non-ischemic and therefore do not usually involve coronary artery disease. We have recently reported the largest and most systematic series, which characterized and tested the functionality of cardiac stem cells in congenital heart patients (23). In this study, 140 myocardial samples were collected from congenital heart patients, which included 10 HLHS patients. The major findings of our study are that resident ckit+ cells are present in the maturing postnatal human heart and are most abundant during the neonatal period with a steady decline with advancing age (neonates=9% versus children=3%). Regardless of age-dependent variations in resident ckit+ cells numbers or congenital cardiac diagnoses, we demonstrated the validity of our method to reproducibly isolate and expand cardiosphere derived cells from right atrial tissues. Finally, we showed that intramyocardial delivery of these cells into a nude rat triggered myocardial regeneration by differentiating into mature cardiomyocytes, smooth muscle cells, and endothelial cells. This histological evidence correlated with functional improvement by ECHO in the treated group with these cells by 15 percent in comparison with controls that received fibroblasts or media when measuring ventricular ejection function. More importantly, the neonatal derived cardiac stem cells from HLHS patients had the highest ability to regenerate the ischemic myocardium when compared to infant or adult derived cardiac stem cells. These data provide the important preclinical evidence of the existence and strong regenerative abilities of ckit+ cells within the hearts of HLHS patients. This study shows the feasibility of harvesting and expanding from HLHS patient's right atrial appendage tissue and the regenerative efficacy of these cells.

B. Clinical Protocol

B.1. Study Design

B.1.1. Basic Design Characteristics

We plan to enroll thirty-two patients with HLHS in a two staged enrollment process. In this study, a maximum of 21 patients will receive intramyocardial injection of c-kit$^+$ cells and 11 standard of care control patients with no injection. The enrollment of the patients will occur in two staged groups: Group A and Group B. Initially in Group A, 10 consecutive HLHS patients will be enrolled in the c-kit$^+$ cell treatment arm to determine feasibility and safety. Six months after the last patient is enrolled in Group A, the DSMB will conduct a review of all Group A patients in order to determine whether the methodology is feasible and safe, including the harvesting, processing, and administering of the c-kit$^+$ cells. Thereafter, Group B will enroll a total of 22 HLHS patients which will be randomized, double-blinded to the treatment and standard of care control arms in a 1:1 ratio, respectively, in order to have 11 c-kit$^+$ cell-treated patients and 11 control patients. Randomization will occur post-Norwood operation upon receipt of viable tissue sample at University of Miami Clinical Research Cell Manufacturing Program (CRCMP). The rationale underlying this two-staged enrollment is to initially test the feasibility and safety of this methodology of obtaining the c-kit $^+$ cells from the right atrial appendage with the Group A patients. The second Group B patients may provide the required numbers to potentially validate the efficacy of the trial. Eventually, a Phase II trial will provide the necessary numbers to provide an accurate assessment of efficacy of the c-kit$^+$ cells in HLHS patients.



B.1.2. Sample Size

Twenty-one (21) subjects will be enrolled in the treatment group and eleven (11) in the standard of care control group. The 10 Group A subjects are part of an open-label safety and feasibility analysis. The 22 Group B subjects will be randomized 1:1 to cell treatment and control to detect a difference of 6 RVEF units, given a standard deviation of 4, 89% power and type 1 error of 0.05. Twenty patients are needed with 10% added for possible lost to follow-up.

B.1.3. Dosage and Administration

The treated HLHS patients will receive up to 12,500 cells/kg. The terminology "up to" a certain dose indicates that if the number of the c-kit$^+$ cells is not sufficiently attainable from the cardiac specimen to the specified dose then the achievable dose will be delivered. A secondary analysis of subjects who achieve ≥80% of the assigned dose will be performed as part of the overall analysis plan. The decision to weight-adjust the dose of cells was made based on preclinical studies. The cells will be delivered through 6-10 intramyocardial injections of approximately 100uL per injection for total volume of 0.6 mL

B.1.4. Premature Withdrawal

For subjects withdrawn after receiving study product injection, all efforts will be made to encourage return for all assessments through the end of the study period, so that safety can continue to be monitored.

Because the data from the proposed trial may be used to design future phases of clinical trials, we put a high priority on collecting data that is complete and accurately describes the safety and efficacy of the intervention as described. Therefore, the goal is to have final MRI and other efficacy endpoint measurements on at least 20 Group B subjects during the randomized portion of trial. As such, Group B study treatment subjects who do not undergo the stem cell injection or do not get the Stage II BDCPA operation can diminish the goal sample size of 20. A maximum of six additional subjects can be randomized to replace this potential loss to achieve the target sample size of 20 evaluable subjects.

B.2. Criteria for Subject Enrollment

B.2.1. Inclusion Criteria

<u>For inclusion in the study, subjects must meet all of the inclusion criteria:</u>

1. Subjects with hypoplastic left heart syndrome (all types) requiring Stage I Norwood operation.

B.2.2. Exclusion Criteria

<u>Candidates will be excluded from the study if any of the following conditions are met:</u>

1. Subjects undergoing the Stage I Norwood operation who do not have HLHS.
2. Subjects requiring mechanical circulatory support immediately prior to Stage II BDCPA operation (within 5 days).

3. Parent or guardian unwilling or unable to comply with necessary follow-up(s).
4. Mother is serum positive for HIV 1/2, hepatitis BsAg or viremic hepatitis C and *Treponema pallidum*.
5. Subjects who are unsuitable for inclusion in the study in the opinion of the investigator(s).

B.3 Sequence of Measurements and Evaluation

B.3.1. Subject Introduction and Informed Consent

Prior to the Stage I Norwood operation, the subject's guardians will be given a complete explanation of the purpose of the study and evaluations to be performed which are in addition to those of standard of care. The Institutional Review Board (IRB) approved informed consent document will be reviewed with the guardians and any questions answered at that time.

The consent document (and the discussion with the guardians) will clearly outline the following:

1) the subject's guardians do not have to consent to this research for their child to receive the planned surgeries,
2) if the guardians want the subject to participate, there is a need for tissue sample collection to take place at the time of the Stage I Norwood operation (to enable study product to be manufactured) and consent is needed for this, if the subject is randomized to the standard of care group (i.e. no cells to be delivered) then the tissue will be discarded at the manufacturing facility.
3) there is a potential that the subject could be deemed ineligible for study participation prior to the Stage II BDCPA operation; for example, due to changes in health status, and
4) what a change in participation status would mean for any acquired tissue if the subject is discontinued from the protocol prior to the Stage II BDCPA operation.

Should the guardians consent to the study, a copy of the signed consent form will be provided to them at that time.

Prior to the Stage II BDCPA operation, the subject's guardians will be contacted to schedule appointments for research-related testing needed for determining continued eligibility. Before any testing is initiated, the consent form will be re-reviewed with the guardians to affirm their continued willingness to allow their child to participate. This re-review will be documented in the subject's chart. If the guardians are no longer interested in participation, the subject will be withdrawn from the study, and this will be noted in the subject's chart.

B.3.2. Screening Phase Assessments

The Screening Phase starts at enrollment and completes at the initiation of the harvest during the Stage I Norwood operation. The screening phase may last between 2 – 3 weeks, and will not alter the patient's pre-surgical duration.

The following evaluations will be performed 21 days or less before Stage I Norwood operation (including right atrial tissue collection). These evaluations will include the following, which are routinely obtained for Stage I Norwood HLHS patients:

a. Demographic data, including date of birth, gestational age, length, weight, gender, and race.
b. Physical exam with vitals (length, weight, head circumference, oxygen saturation, heart rate, respiratory rate and blood pressure).
c. Laboratory Assessments:
    1. Clinical chemistry: BUN, creatinine, sodium, potassium, chloride, glucose, calcium, bilirubin (total), total protein, albumin alanine aminotransferase (ALT), aspartate aminotransferase (AST), B-type Natriuretic Peptide (BNP).
    2. Hematology: complete blood count (CBC) with differential and platelet count
d. Assessment of mother's infectious disease status for HIV 1/2, hepatitis BsAg or viremic hepatitis C and syphilis (*Treponema pallidum)*.
e. 12-lead electrocardiogram (ECG)
f. Concomitant medication history

The investigator may use clinical judgment when determining the clinical significance of laboratory parameter findings throughout the study.

During the screening phase, and prior to the tissue collection phase, the condition of the subject will be verified and key safety parameters will be assessed by the PI and study team to confirm feasibility of operation, including:

- Physical Examination
- Standard of Care pre-procedural labs
- ECHO results to determine valve status
- Tricuspid insufficiency
- Restrictive septum
- Poor RV function.

B.3.3. Tissue Collection Phase

B.3.3.a. Tissue Collection Procedures

The cell processing and manufacturing will be performed exactly as specified in the TAC-HFT II (A Phase I/II, Randomized, Placebo-Controlled Study of the Safety and Efficacy of Transendocardial Injection of Autologous Human Cells (Mesenchymal or the combination of MSC and Cardiac Stem Cells) in Patients

With Chronic Ischemic Left Ventricular Dysfunction and Heart Failure Secondary to Myocardial Infarction) trial of University of Miami (IND #14647). As specified, the tissues will be sent and processed at the University of Miami Clinical Research Cell Manufacturing Program (CRCMP), Interdisciplinary Stem Cell Institute.

Prior to tissue collection the investigators must confirm that Mother is serum negative for HIV 1/2, hepatitis BsAg and negative for viremia of hepatitis C and *Treponema pallidum* and transmit that information to the CRCMP.

All HLHS patients will undergo the Stage 1 Norwood operation, which is the standard operation for all HLHS patients. If the HLHS patients are considered high risk for the Norwood operation, which includes moderate or more tricuspid regurgitation, moderate or more right ventricular function, or a significant neurological complication, then we would not proceed with the Norwood operation but would proceed with the Hybrid operation. The Hybrid operation consists of pulmonary artery banding, ductus arteriosus stenting, and atrial septostomy, which is approached through a median sternotomy.

During the Norwood operation, the HLHS patient will be placed on cardiopulmonary bypass in the usual standard fashion, which involves cannulating the right atrium. The right atrial tissue is then discarded. However, in our study this excised tissue will be harvested. If the Hybrid operation is performed, the right atrial appendage will be harvested during the open heart procedure. The ELPIS protocol IND # 16045 will be cross-referenced to this protocol and will follow what is described below:

- Right atrial tissue (averaging 1 g or less in weight) will be harvested under sterile conditions in the operating room at the participating clinical centers from patients undergoing HLHS surgery procedures.
- Each time a right atrial sample is taken, the patient's study ID/ACROSTIC will be used for identification at the University of Miami CRCMP, Interdisciplinary Stem Cell Institute and participating clinical centersto refer to a given sample. These data will be kept and recorded in the electronic data capture system. The culture medium and all medium used during RAA and ckit+ cells ex-vivo processing is devoid of any antimicrobials or antifungals.
- Within the sterile field, the right arterial tissue samples will be placed into sterile cryovials containing Ham's F-12 medium supplemented with 10% Fetal Bovine Serum (FBS), 100 ng/ml recombinant human basic fibroblastic factor, L-glutathione, and 5 mU/ml human erythropoietin, which has been tested for sterility using aerobic, anaerobic, and fungal cultures. This media will be provided by CRCMP.
- Shipping Procedures - Tissue will be enclosed in a rigid shipping container with temperature insulating properties. Cold temperature packs will be used to maintain the shipping temperature between 2°C to

10°C. The rigid shipping container will include a document on the inside of the container and a label on the outside of the container according to Health Resources and Services Administration (HRSA) guidelines for organ transport policies and procedures. Shipping container will be accompanied by appropriate documentation including the "Chain of Custody form" with subject and sample unique identifiers, and a temperature logger. The vials containing the tissue will be shipped overnight using FEDEX to CRCMP (see cell processing manual for more detail).

B.3.3.b. Post Norwood Monitoring Phase (Standard of Care)

Following the completion of the Stage I Norwood operation and up to the Stage II BDCPA operation, all patients will be followed by both parent(s) and faculty per institutional standard of care. There will be no research-related procedures or data collected during this phase until the baseline visit and eligibility assessment.

B.3.3.c. Baseline Assessment

Prior to admission for the Stage II BDCPA operation, all subjects will undergo baseline testing and study eligibility determination as described below:

a. Complete medical history including any co-morbidities such as central nervous system, renal, gastrointestinal and genetic abnormalities,
b. Physical exam and vital signs (length, weight, head circumference, oxygen saturation heart rate, respiratory rate, blood pressure, and Modified Ross Score for heart failure classification (see Appendix A))
c. 12 lead Electrocardiogram (ECG)
d. Complete Trans-thoracic echocardiogram (TTE)
e. Cardiac Magnetic Resonance Imaging (cMRI)
f. A complete right and left cardiac catheterization, which is routinely performed before the Stage II BDCPA operation, if clinically indicated.
g. Laboratory Assessments:
    1. Clinical chemistry: BUN, creatinine, sodium, potassium, chloride, glucose, calcium, bilirubin (total), total protein, albumin alanine aminotransferase (ALT), aspartate aminotransferase (AST), B-type Natriuretic Peptide (BNP).
    2. Hematology: complete blood count (CBC) with differential and platelet count
h. Infant and Toddler Quality of Life (ITQOL) survey
i. Collection of Blood for Biomarker analysis of circulating pro-inflammatory markers (see MOP for blood collection and shipping instructions to CRCMP)

The research-only assessments listed above are the cMRI, ITQOL survey, and biomarker blood sample tests thus AE monitoring will begin when the first of these is performed.

B.3.3.d. Study Product Injection

The Stage II BDCPA operation occurs between 4 to 6 months of age. For Group B subjects randomized to study treatment, during this surgery when the patient is on cardiopulmonary bypass, the previously harvested c-kit^{+} cells will be injected into the right ventricle directly intramyocardially using a 25-27gauge needle syringe at the completion of the repair but before separating from cardiopulmonary bypass. The entire dose of the cells, up to 12,500/kg patient weight in 600 microliters, will be divided and delivered in 6-10 intramyocardial injections. The intramyocardial injection of cells will take approximately 5-10 minutes, which is insignificant to the total bypass time. The remaining part of the operation will proceed as anticipated.

B.3.3.e. Investigational Final Product Preparation

The harvested c-kit+ cells will undergo quality control testing and review by CRCMP laboratory director and quality assurance manager prior to release for clinical use. When the Stage II BDCPA operation is scheduled for subjects randomized to receive study product, upon request from the clinical site, product is shipped cryopreserved from CRCMP to the local cell processing laboratory in a validated vapor phase cryoshipper maintained at temperatures < -120 ^{o}C with a temperature monitoring device. Upon arrival, product is inspected to ensure that product is in good condition and the temperature data will be verified before transferring to the secured vapor phase storage freezer at the site. In the event that the temperature deviates from the specified shipping temperature, the product will not be used. Local cell processing laboratory notify CRCMP. The incident will be investigated with corrective and preventive actions implemented following the CRCMP standard operating procedures. CRCMP will prepare a new shipment of the product.

Days prior to the product injection, the principal investigator will send a request for product with date, time and location of the operation to local cell processing laboratory. On the day of the operation, local cell processing laboratory will prepare the product and perform quality control testing. The endotoxin assay is done according to the standard operating procedures (SOPs) provided by CRCMP. The gram stain and sterility tests are performed following the local cell processing laboratory protocols.

The product thawing process is performed asepticaly under sterile condition of the biolocical safety cabinet (BSC). Product is thawed in a waterbath at 37 ^{o}C. After the product is thawed, in the BSC, the product is transferred from a vial to a sterile conical containing 5-10 mL. The product vial is rinsed with thaw media containing Plasma-Lyte A supplemented with 1% of 25% Human Serum Albumin (HSA) and transferred to the same sterile conical and the media volume brought up to 45mL. The conical containing the product is centrifuged at 500g for 5 minutes at 4 ^{o}C, break set at low. This is the first wash. Supernatant is removed and leaves approximately 2mL to resuspend the pellet. A 60 microliter x 2 for cell count using

crystal violet and viability using trypan blue. The media volume is brought up to 45mL and the tube is centrifuged at 500g for 5 minutes at 4 °C, break set at low. This is the second wash. Sterility and endotoxin samples are collected from supernatant and small drops of supernatant are used to prepare glass slides for gram stain. Sample tubes and slides are labelled with product unique ID, patient acrostic and ID, type of test to be performed and date. Cell dose adjustment is calculated and prepared to obtain 12,500/kg in 0.6mL total volume concentration. Sterile field is set up in the BSC to prepare final product. Cell dose is loaded in a sterile cryovial and place in a sterile plastic bag, seal and place in another sterile plastic bag before removing from the BSC. Product is transport in a validated vessel that maintains the temperature at 1-15 °C. A temperature monitoring device is used to monitor transport temperature.

B.3.3.f. Post-treatment (Stage II BDCPA operation) Assessments

All subjects will be cared for according to institutional standard postoperative protocols, which included standard of care post-surgery assessments. Research-related study visits will occur at Day 5 (± 3 days), Week 4 (30 days ± 7 days), Month 6 (180 days ± 30 days) and Month 12 (365 days ± 30 days) (see Table 1). Data from standard of care visits can be used for study visits if collected within visit window. If a patient remains hospitalized at the planned visit date, the assessment will be performed as an inpatient.

The following assessments will be collected post-Stage II BDCPA operation (Day 0) visits as part of standard of care:

a. Physical exam and vital signs (length, weight, head circumference, oxygen saturation, heart rate, respiratory rate, blood pressure, and Modified Ross Score for heart failure classification (see Appendix A))
b. 12 lead electrocardiogram
c. Complete TTE (Day 5, Month 6, and Month 12)
d. Laboratory assessments at 5 days, 6 months, and 12 months:
    1. Clinical chemistry: BUN, creatinine, sodium, potassium, chloride, glucose, calcium, bilirubin (total), total protein, albumin alanine aminotransferase (ALT), aspartate aminotransferase (AST), B-type Natriuretic Peptide (BNP).
    2. Hematology: complete blood count (CBC) with differential and platelet count
e. 24-hour Telemetry (Day 5)
f. AE monitoring

The following research-related assessments will be collected post-Stage II BDCPA operation (Day 0):

a. cMRI scan (Month 6 and Month 12)
b. ITQOL survey (Month 6 and Month 12)

c. Biomarker blood collection (Day 5, Month 6, and Month 12) (See MOP for collection and shipping instructions)

Table 1

Test/Procedure	Pre-Norwood	Pre-BDCPA	BDCPA	Post-BDCPA			
		Baseline[1]	Day 0	Day 5 ± 3 days	Week 4 30 days ± 7 days	Month 6 180 days ± 30 days	Month 12 365 days ± 30 days
Informed Consent	X						
Demographics	X						
Medical History		X					
Physical Exam[2]	X	X		X	X	X	X
Lab Assessments[3]	X	X		X		X	X
Infectious disease testing[4]	X						
ECG	X	X		X	X	X	X
ITQOL survey		X				X	X
Trans-thoracic Echo (TTE)[5]		X		X		X	X
MRI[5]		X				X	X
Cardiac Cath[6]		X					
Randomization[7]	X						
Study Product Injection (SPI)			X				
Telemetry				X			
Biomarker blood collection		X		X		X	X
Adverse Event Monitoring		X	X	X	X	X	X

1 – Baseline testing should preferably occur within 30 days of Stage II BDCPA operation (Day 0), however can occur up to 90 days before

2 – Physical exams to include: vitals, Modified Ross score for heart failure classification and current medication usage

3 –Labs to include: CBC with differential BUN, creatinine, electrolytes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (total), albumin, glucose, calcium, total protein, B-type natriuretic peptide (BNP).

4 – Infectious disease assessment will be conducted through mother's testing during pregnancy or through blood tests during screening phase for HIV, hepatitis BsAg or viremic hepatitis C and syphilis (*Treponema pallidum*).

5 – Will be analyzed by CHoA MRI Core lab and Lurie Children's Hospital Echo Core lab

6 – If clinically indicated

7 – Randomization will occur post-Norwood operation upon receipt of viable tissue sample at University of Miami Clinical Research Cell Manufacturing Program (CRCMP)

C. Statistical Methods

C.1. Statistical and Analytical Plan

A statistical analysis plan (SAP) will be prepared and finalized prior to database lock. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives.

A data review will be conducted prior to database lock. This review will assess the accuracy and completeness of the study database and appropriateness of the planned statistical methods.

C.2. Analysis Sets

Analysis populations are defined as the following:

Safety Analysis Set (SAS): All subjects in the Group A lead-in group and the Group B randomized group. The primary and secondary safety/feasibility analyses will be performed on the SAS population.

Efficacy Analysis Set (EAS): All subjects who are randomized and will be analyzed for intention-to-treat according to the treatment group to which they were assigned. Primary efficacy and secondary safety and efficacy analyses will be performed on the EAS population.

Safety and feasibility analysis will be based on the Safety Analysis Set (SAS). The analyses of bioactivity will be based on the Efficacy Analysis Set (EAS), also known as the intention-to-treat population.

C.3. Safety Analysis

Safety will be evaluated based on the adverse events (AE) profile of the subjects. AEs will be classified with regard to severity, duration and frequency of the event. AE data will be listed individually, and described by body system, and preferred terms within a body system.

Each occurrence of an AE (based on preferred terminology) will be reported separately for a given subject, and its relationship to the study drug will be assessed independent of whether the event occurred previously. If two or more AE's are reported as a unit, the individual terms will be reported as separate events. If a subject experiences more than one episode of a particular coded adverse event, the subject will be counted only once by the maximum intensity of the episode (i.e. once per unique preferred term per subject).

A Data Safety Monitoring Board (DSMB) will review all safety data in a timely fashion. The DSMB will consist of an independent group of clinicians (a minimum of three). A pediatric cardiologist specializing in heart failure will chair the DSMB,

which will include an additional pediatric specialist, and a biostatistician. Serious adverse events will be reviewed by the DSMB as the events occur. Semi-annual meetings of the DSMB will focus on a review of frequency and severity of all adverse events as well as demographic and bioactivity data. The DSMB will closely monitor the safety of the subjects participating in this study, and they will make a decision to stop the study if, in their judgment, safety concerns arise.

C.4. Analysis of Demographics and Other Baseline Characteristics

Baseline characteristics collected and selective data will be summarized for each subject. Such variables include: 1) demographic variables, 2) type of HLHS, 3) physical examination, 4) laboratory data, 5) echocardiography variables, 6) cardiac magnetic resonance imaging variables. Descriptive statistics (e.g., number of subjects, mean, SD, median, minimum, and maximum) will be generated for continuous demographic variables and baseline variables (e.g., age, length, weight, head circumference and BMI) for all subjects. The number and percentage of subjects in each class of categorical demographic variables and baseline variables (e.g., gender, ethnicity, and race) will be tabulated. Chi-square test (for categorical variables) and student t-test (for continuous variable) will be used to evaluate the difference between the treated and non-treated subjects. Individual subject demographic and baseline characteristic data will be listed.

C.5. Analysis of Bioactivity

Analyses of bioactivity will be exploratory in nature in order to aid in endpoints, time points, and sample size determination for subsequent larger trials, if appropriate. We will need to look at the totality of data at the end of this study and use best clinical/statistical judgment in developing a larger study design. As an example, we may see improvement in right ventricular function in treated subjects. Given the objective and predictive nature of this endpoint, and in the absence of safety concerns we could make a decision to proceed with a larger study based on this finding alone. Alternatively, if treated subjects exhibited improved tricuspid regurgitation associated with reductions in symptoms or pleural effusion, this constellation of findings might justify proceeding with additional studies. Regarding the primary endpoint for a Phase I/II study, we will need to look at the outcomes from the proposed study as well as the outcomes from similar studies and the evolving consensus of the research community regarding the importance and predictive nature of specific endpoints in this patient population. To account for patient body surface area, RVESV and RVEDV indices are reported.

Bioactivity endpoints are changes from baseline in:

1. RVEF - continuous variable, with improvement indicated by an increase;
2. RVESVI and RVEDVI - continuous, with improvement indicated by a decrease;

3. Tricuspid regurgitation- continuous, with improvement indicated by a decrease

Every effort will be made to obtain all the data from all the enrolled patients in the study. If not, two analytical approaches will be used to account for missing data: multiple imputations and available data. The primary analysis will be based on the available data. Multiple imputations will be used as a sensitivity analysis.

All endpoints will be summarized by treatment group. Summary on continuous variables will include the following descriptive statistics: mean, median, maximum, minimum, standard deviation, and the 95% confidence interval.

For each bioactivity endpoint, the difference (in change from baseline) between treatment arms will be estimated with the 95% confidence interval. The main bioactivity endpoints of interests will be the changes from baseline at 12 months.

Analysis of bioactivity endpoints

1. Student t-test will be used to assess the difference between the treatments in the change from baseline for continuous outcomes; also, analysis of covariance (ANCOVA) with adjustment for baseline values will be used to assess treatment effect.
2. Wilcoxon rank sum test will be used to assess the difference in treatment effect in the change from baseline for continuous outcomes if normality assumption does not hold.
3. Chi-square test or Fisher's exact test will be used to assess difference between treatments in incidence of categorical outcomes.
4. Logistic regression with baseline covariates will be used to assess treatment effect for categorical outcomes with adjustment for baseline covariates if appropriate.
5. No adjustment will be made for multiple comparisons.

C.6. Determination of Sample Size

A total of twenty-two subjects will be randomized to two groups (eleven control and eleven treated). In our statistical plan we have allowed for a 10% attrition rate, with one subject per group for the bioactivity analysis.

A sample of 20 participants (10 in the treatment group and 10 in the control) provides 89% power with an alpha of 0.05 to detect a 6 unit change in RVEF (with a SD of 4 units) between the stage II operation (baseline) and 12 months later as measured by cMRI

We performed a power analysis based on our pilot study and the PERSEUS trial published in the administration of cardiosphere derived cells in HLHS patients. The sample size for comparing means from two independent samples of a continuous variable is as follows:

$$n_C = \frac{2 * \left(Z_{1-\propto/2} + Z_{1-\beta}\right)^2 \sigma^2}{\delta^2}$$

In a 1:1 randomization, $n_A = n_C$, where n_A = number in the active groups and n_C = number in the control group $\delta = \mu_1 - \mu_2$ (difference of 2 means) and σ^2 is common variance. Total sample size is $2n_C$.

$Z_{1-\alpha/2}$ for an α of .05 for a 2-sided test is 1.96, Z1-β for power of .89 is 1.21. The primary analysis will consist of one comparison – cKit versus control. We assume that will have a 6 unit change in RVEF with a SD of 4 units based on data from the PERSEUS trial.[1]

Analyses of bioactivity will be exploratory in nature in order to aid in selection of dosage, endpoints, time points, and sample size determination for subsequent larger trials.

C.7. Heart Tissue Analysis

In the event of a heart transplant or left-ventricular device insertion during the post-treatment phase, the heart or tissue will be retrieved and analyzed. Parents/guardians of participants will give/deny permission for this process at time of enrollment in trial. Consent to this analysis is optional and does not impact the participant's ability to take part in the trial if the parents/guardians decline to consent to tissue donation. If tissue is collected, the sites of cell injection will be studied, along with a detailed gross and light microscopic cardiac examination.

D. General Procedures

D.1. Adverse Events

AEs will be classified with regard to severity, relationship to study, duration, and seriousness.

AE data will be listed individually and described by system organ class (SOC) and preferred terms using the MedDRA hierarchy. Each occurrence of an AE will be reported separately for a given subject, and its relationship to the study therapy will be assessed independent of whether the event occurred previously. If two or more AEs are reported as a unit, the individual terms will be reported as separate events.

AEs will be listed by subject and summarized by and within SOC. Listings and tables will be provided for study-emergent adverse events (defined for an individual subject as an event not present prior to beginning study therapy, or, if present prior to beginning study therapy, an event which increases in intensity, is considered related to the study therapy, or becomes serious during the treatment of follow-up

phases of the study). Separate listings and/or tables will also be provided for adverse events by maximum intensity, drug-related adverse events, serious adverse events, and adverse events resulting in discontinuation of study drug and deaths.

D.2. Procedures for Handling Adverse Events and Positive Microbial Post Product Administration

The following definition of adverse event will be used for this study: Any untoward medical occurrence in a subject for whom investigational product (IP) was administered, and not necessarily appearing to have a causal relationship to the IP. An adverse event can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

It is the responsibility of the investigator or co-investigator(s) to perform periodic and special assessments for AEs. The investigator and clinical staff will note all AEs from time of consent re-review at baseline testing, when research-related procedures begin.

The Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, is used for detailed descriptions of Severity Grades. The CTCAE schema is classified by body system and event using the MedDRA hierarchy and provides descriptions of events that qualifies under each severity rating. The following table contains general descriptions of Adverse Event Severity Grades.

Please note: Grade 1 (Mild) AE/SARs are not entered in the electronic CRF in the database.

Severity Grade	Description
1	Mild. Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
2	Moderate. Minimal, local, or non-invasive intervention indicated or limiting age-appropriate instrumental activities of daily living (i.e. preparing meals, shopping for groceries/clothes, managing money, using
3	Severe or medically significant but not immediately life-threatening. Hospitalization or prolongation of hospitalization indicated OR disabling OR limiting self-care (e.g. bathing, dressing, feeding self, using toilet, taking medications, and not bedridden etc.)
4	Life-threatening consequences; urgent intervention indicated.
5	Death. Death related to adverse event.

All clinical complaints volunteered by, or elicited from, the subject's parents/guardians during the study period will be recorded on the appropriate page

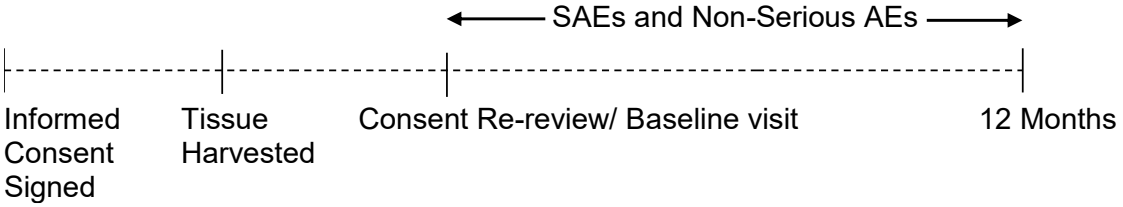
of the eCRF for the study phase indicated. If any AE occurs, the subject will receive appropriate treatment and medical supervision. In addition, the investigator will record the AE on the appropriate page of the eCRF. All AEs judged to be clinically significant will be followed until resolution. Serious AEs will require special reporting.

D.2.1. Reporting of Serious Adverse Events

Serious Adverse Event (SAE) is an untoward medical occurrence that may occur. The following regulatory criteria qualify as an adverse event as serious:

Death of a subject:	An event resulting in the death of a subject.
Life-threatening:	In the opinion of the investigator, an event that would have resulted in immediate death if medical intervention had not been initiated. This does not include an event that would have been fatal if it had occurred in a more severe form.
Hospitalization:	An event resulting in formal inpatient admission of the subject to the hospital. Visits to the emergency room or outpatient facility do not constitute hospitalization for the purpose of this definition.
Prolongation of Hospitalization:	An event that prolongs the subject's stay in the hospital. By definition, this is a different event from the event that resulted in the hospitalization.
Persistent or Significant Disability/Incapacity:	An event that substantially interferes with the subject's usual daily activities of living. This category is not intended to include events of relatively minor medical significance such as minor trauma, diarrhea, nausea, etc.
Important Medical Event:	Although not resulting in death or hospitalization, an important medical event should be considered a serious adverse event if, based on medical judgment, it significantly jeopardizes the subject and/or requires medical or surgical intervention to prevent one of the other serious outcomes from occurring.

D.2.2. Collection Period for Adverse Events



The causality category chosen for an AE or SAE that occurs during the time interval between the signing of the informed consent and study product injection should reflect whether the event was thought to be “possibly related” or “not related” to any protocol mandated procedures during this interval.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition described previously. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction). The event itself, however, may be of relatively minor medical significance. This is not the same as “serious,” which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The terms “serious,” “unexpected,” and “expected” are regulatory definitions. Therefore, if a subject experiences any of the reactions described as expected, the only way this reaction can be unexpected is if it is either more severe than usually seen, or if the incidence of an event (e.g., hypertension) at a specific center is higher than usually expected. Similarly, if a subject with a concomitant heart condition suffers a myocardial infarction, this must be noted as unexpected, even if it is recognized as an expected event for that subject.

D.2.3. Reporting Procedures

The DCC medical monitor will assess each serious adverse event (SAE) to determine if the SAE qualifies as an expedited report according to FDA regulations and ICH GCP guidelines. Expedited reports will be submitted to the appropriate regulatory authorities including the governing IRB.

D.2.4. Management of Positive Microbial Post Product Administration

In the event that microbial test results become positive following injection of the cellular product the following actions will be taken:

a. All positive microbial test results will be reported immediately to local cell processing laboratory director, the principal investigator, laboratory medical director, study sponsor and the attending physician (if not the same as principal investigator). .
b. The subject's parents or legal guardian is notified immediately by telephone to inform him/her of the result. Three attempts at telephone contact will be made. If unsuccessful, a registered letter will be sent to the subject's home, with instructions to contact the principal investigator or study coordinator for additional follow up requirements.
c. Each subject's parents or legal guardian will be interviewed about symptoms of infection, including; lassitude, cough, coryza, conjunctivitis, rash, dysuria, and diarrhea. All subjects will be instructed to report to the hospital immediately for blood work, sputum and urine cultures, throat and nasal swab, and a complete examination and consultation with an infectious disease specialist. Subject is monitored by his/her physician based on the patient's symptomology (fever, chills or other indicators suggestive of infection) and started on broad spectrum antibiotics if the organism identification results are not yet available. The subject will remain in the study and be monitored for clinical signs of infection. The local research team will report in the EDC any resultant adverse events per protocol. When organism ID is available, the antibiotics are selected based on these results. The subject will remain in the study and be continuously monitored for clinical signs of infection.
d. Additional microbiological testing will be performed to identify the microbial organism, once available, those results will be reported to local cell processing laboratory director, laboratory medical director, study sponsor and the attending physician (if not the same as principal investigator). Results of organism identified will be reviewed by the principal investigator in consultation with an infectious disease specialist and decisions regarding treatment and repeat testing will be based on these consultations. A decision regarding the use of antimicrobial agents will be based on the recommendations of the infectious disease consultant and the clinical judgment of the investigator.
e. A full investigation of the incident will be performed to determine the potential root cause of contamination following the cell processing laboratory protocols (CPL). Corrective actions and preventive plan will be implemented to prevent a recurrence of the event.
f. The clinical research program to report the incident related to administration of positive microorganism to the IRB regardless of patient adverse event. Upon IRB review, the incident will be reported to the FDA

if recommended. Reporting of the incident will follow 21 CFR 312. 31 and 312.32.

g. In the event that subject exhibits the symptom in association with the microbial contamination that falls into the serious adverse event (SAE) grade 4 or 5 (life threatening or fatal), the event will be reported through Medwatch form and information be submitted in an **IND safety report** within 7 calendar days of learning of the event, under SAE grade 3 (non-life threatening or non-fatal) report will be submitted through Medwatch form and information be submitted in an **IND safety report** within 15 days of learning of the event. Local cell processing laboratory will provide the investigation of the root cause and corrective/preventive actions will be reported to the study sponsor to be submitted to the FDA as soon as it becomes available.

In the event of no adverse event, the positive sterility test results, the investigation of the cause and corrective/preventive actions will be reported, in an **information amendment** and will be submitted to the FDA within 30 calendar days.

D.3. Expected Adverse Events

Because the subjects in this study have, by definition, a serious congenital heart defect, they may experience adverse events that are related to the natural history or standard of surgical care of their defect including death. These subjects also frequently have other cardiovascular disease involving the cerebrovascular, renal, and coronary arteries; all of which may become clinically active at any time in such subject.

In addition, adverse events that are specified in the labeling of the various components of this intervention, in this protocol, investigator's brochure, or in the informed consent are deemed anticipated for the purpose of reporting requirements.

D.4. Potential Risks

During the Stage I Norwood operation, there is no additional risk to the HLHS patient in conjunction with collection of the right atrial appendage tissue. To initiate cardiopulmonary bypass for this surgery, the right atrial appendage is cannulated. Typically, the right atrial appendage is excised and discarded during this step of the operation. However, in our study, this excised right atrial appendage will be collected and c-kit^{+} cells will be isolated. So, no additional tissue from the heart will be required and thus no potential risks to the function of the heart or to the patient will occur.

D.4.1. Potential Risks of Cell Selection

Potential risks related to cell processing include the risk of infusion of bacterially contaminated cells that may cause infection. There is the potential for infusion of

foreign proteins that are residual process components, which may evoke an immune response.

D.4.2. Potential Risks of Cell Therapy Delivery

As with any new drug, an allergic reaction could occur. This could result in hives, rash, difficulty breathing or collapse of the lung and breathing systems. These reactions are usually reversible but could lead to permanent disability or death. Other rare but possible risks include infection, respiratory distress or failure, pulmonary edema, renal or hepatic failure. As with any investigational drug, administration of autologous c-kit-positive cells could cause currently unknown side effects that might be permanently disabling or fatal, including tumor formation or arrhythmias.

The c-kit$^+$ cells will be delivered through intramyocardial injection. Risks associated with intramyocardial injection into cardiac tissue include possible sustained rapid heart rates and ventricular arrhythmias, ectopic tissue formation, cardiac perforation, infections and abnormal laboratory values. Additional risks associated with the delivery are minimized due to the nature of the study agent, namely autologous stem cells. It is possible that a small amount of cells will enter the systemic circulation of the heart rather than the myocardium, although since these are autologous cells there should be no sequelae of such an event.

D.4.3. Potential Risks of MRI Study

The risks associated with the MRI study with gadolinium include anesthetic risks, anaphylactoid reaction, arrhythmia, vasodilation, and nephrogenic systemic fibrosis. At Children's Memorial Hospital, the University of Maryland, a study was conducted that has shown no complications with the MRI study in congenital heart patients during the last nine years. This protocol is mentioned below.

The most serious complication is nephrogenic systemic fibrosis, or NSF, with administration of gadolinium in adult patients with decreased renal function. In a study by Prince, et al., none of 74,124 adult patients developed NSF after administration of single dose of gadolinium (0.1 mmol/kg), but 15 of 8997 (0.017%) patients developed NSF after the administration of high dose (≥ 0.2 mmol/kg) (24). Of 265 patients undergoing chronic hemodialysis, 1 (0.4%) developed NSF (all received high-dose gadolinium); and of 131 patients in acute renal failure, 11 (8.4%) developed NSF (24). Therefore, NSF results from a high dose of the gadolinium administered or if the patient has decreased renal function. At Children's Memorial Hospital, we use Gadavist: Gadopentetate dimeglumine 469.01 mg/ml for our gadolinium-based contrast agent at a dose of 0.2mmol/kg that is required for delayed enhancement to assess for myocardial fibrosis. To assess for renal toxicity, patients are screened by creatinine if they are less than one year of age and Glomerular Filtration Rate (GFR) if greater than one year of age. If the creatinine level is <0.64 mg/dl for infants less than one year of age, we use Gadavist at a dose of 0.2 mmol/ kg. If the creatinine level is >0.64 mg./dl, then

Gadavist is contraindicated and not given. In patients greater than one year of age, if the GFR is > 60 ml/min/1.73 m^2, the patient is cleared to receive the normal dose. Between 30-60 will need consent from the parents and a half dose of gadolinium (0.05 mmol/kg) was used, which is not ideal and the quality of images are sometimes not conclusive. If the GFR is < 30, then gadolinium administration is contraindicated. By basing the administration of gadolinium on these strict criteria, we have not had any complications with the MRI study at Children's Memorial Hospital.

The basis of these criteria for dosing gadolinium is due to several reasons. We now base it on the creatinine level, which is due to several reasons for infants less than one year of age. First, we have had no complications with the 0.2 mmol/kg dosing for GBCA. Secondly, the creatinine levels are more stable after 2 months of age up to one year of age. The creatinine levels reflect more of the intrinsic function and does not reflect muscle mass. Finally, GFR calculated values for patients less than one year of age may not reflect the actual kidney function but in fact the immaturity of the nephrons, which may in fact be normal and have no intrinsic disease processes. Furthermore, GFR may be given a true estimate of kidney function in this very young patient population. Taken together, we will base the GBCA dosing at 0.2 mmol/kg up to creatinine level of 0.64 mg/dl for patients less than one year of age and GFR for patients greater than one year of age.

Other complications are mostly theoretical and have not been seen in our practice. The mortality of MRI is directly related to whether a metallic object is within the body of the patient. Every patient will undergo routine screening for metallic implants. Since we will be operating on and following these patients, we will be aware of metallic objects in the patients. Another risk is general anesthesia, which is less than < 0.1%. Safety efficacy of gadolinium have not been established in children < 2 years of age or for use in magnetic resonance angiography. Side effects include anaphylactoid reaction, arrhythmia, vasodilation, and wheezing and are reported as less than < 1%.

D.5. Risk-Benefit Assessment

The potential benefit in this clinical study is augmentation of right ventricular function that is of sufficient magnitude to reduce tricuspid regurgitation, improve symptoms, and decrease pleural effusions. Since the right atrial tissue used in this study is typically discarded, there will be no consequence to the heart function. In the absence of cell therapy, deleterious consequences, which may occur as part of the natural history of the subject's cardiac disease, might include persistent cardiac dysfunction, progressive loss of tissue integrity, and/or death.

D.6. Data Safety Monitoring Board (DSMB)

A Data Safety Monitoring Board (DSMB) will review all safety data in a timely fashion. The DSMB will consist of an independent group of clinicians (a minimum of three) including two pediatric cardiologists or surgeons and statistician that are

not actively recruiting subjects for this trial. Serious adverse events will be reviewed by the DSMB as the events occur. Semi-annual meetings of the DSMB will focus on a unblinded review of frequency and severity of all adverse events as well as demographic and bioactivity data. Ad hoc meetings also may be held for any safety concerns during the study. Based upon review of the data, the DSMB will make recommendations to continue, modify, or stop the study if it appears that the subject's safety is at risk. Further responsibilities and details of the DSMB will be included in the DSMB Charter.

D.7. Stopping Guidelines

The sponsor will review all SAE reports. If conditions arise during the study indicating that the enrollment should be halted, this action may be taken by the sponsor and the DSMB.
The IRB and FDA will be notified of any cessation of enrollment. The IRB and FDA will review the safety data that led to the suspension of enrollment and make a determination regarding continuation of the study.

D.7.1. Subject Discontinuation

Individual subjects may withdraw from the study at any time, however, all efforts will be made to encourage return for all assessments through the end of the study period, so that safety can continue to be monitored. The investigator will provide a written report on the appropriate eCRF page describing the reason for discontinuation. If a subject withdraws or is discontinued from the study before completion, every effort will be made to complete the assessments scheduled during the post-treatment phase to monitor safety.

D.8. Data Quality Assurance

CRF workbook data will be entered into a validated electronic clinical database using the physical source copies of the study documents. Computerized data checks and manual review checks will be implemented to check for discrepancies. An electronic audit trail system will be used to track all changes in the database subsequent to the reconciliation of the data.

D.9. Data Collection and Processing

Case report forms (CRFs) will be completed for each study visit, and adverse events. All CRFs will then be entered into the electronic data capture (EDC) system managed by the DCC. Errors on CRFs will be corrected by making a single strike-out through the erroneous data and writing the correct data legibly above, beside, or below the erroneous data so that the original entry is not obscured. Each correction must be initialed and dated by the person making the correction. Errors on eCRFs will be made through the correction process in the EDC system.

The completed, original CRF will be retained in the investigator's study files. A copy of the original CRF will be reviewed by the clinical monitor and a copy may be requested to be sent to the sponsor and/or monitor for review.

Before the CRFs are finalized, the clinical monitor may request copies of the CRFs for preliminary medical review. Electronic data queries will then be generated and provided to the study personnel. The study personnel will take appropriate action based on the queries, after which the clinical monitor will collect the original, completed CRFs.

D.10. Human Subjects

The data and records from the conduct of this trial will be obtained from the individual subject's guardians who will voluntarily agree to enroll in this study. We will obtain informed consent for study participation prior to study enrollment and administration of intervention. In all cases the guidelines of the governing Institutional Review Board (IRB) will be followed. The principal investigator or his/her designee will explain the study's purpose, methods, risks, and potential benefits to each subject's guardian. Reviewing the written Informed Parent Consent Form and answering any questions will be part of the informed consent process. This process includes describing all of the testing, including laboratory and diagnostics pre-treatment to the follow-up evaluations that are required for each enrollee.

Recruitment of subjects will be entirely voluntary. Guardians of all eligible subjects will be invited to participate in this study. The principal investigator or his/her designee will obtain consent after a complete description of the study. Consent will be documented in writing by having the subject's legal guardian sign a written informed parent consent form approved by the Institutional Review Board.

In the event of an adverse event, subjects will have ready access to the study coordinator and principal investigator for the appropriate intervention. All adverse events will be reported in accordance with existing guidelines of IRBs and the FDA.

D.11. Subject Confidentiality

Assigning each subject a study number and acrostic will protect subject confidentiality. This number will be the only identification used in any communication or report. Accordingly, subject confidentiality and anonymity will be protected at all times according to institutional policies, IRB and HIPAA regulations.

E. Ethics

E.1. Independent Ethics Committee/Institutional Review Board

The investigator must submit this protocol, informed parent consent form, and subject information to an Institutional Review Board (IRB). Prior to the beginning of the study, the investigator must obtain written IRB approval of the protocol, consent form, and subject information. The investigator may not initiate the study until IRB approval has been obtained. The Investigator has the responsibility to notify the IRB if the study is either amended or terminated. Emergency changes to protect the life of the subject do not require prior approval, but must be reported to the IRB immediately.

Written informed consent must be obtained from the participant's legal guardian prior to their participation in the study after the IRB approval of the study protocol and parent consent form.

E.2. Ethical Conduct of the Study

The study will be conducted under the following conditions:

1. Declaration of Helsinki
2. ICH Guidelines for Good Clinical Practice (GCP)
3. GCP/International Conference on Harmonization (ICH) guidelines

E.3. Subject Information, Confidentiality and Consent

All subject information, medical records, and laboratory data will be kept confidential. Information and data may be discussed, analyzed, and reported. However, ID numbers will identify the subject on the case report forms and the subject's identity will be kept confidential.

Once the entire study has been explained and understood, the subject's legal guardian will sign and date the IRB approved informed parent consent form.

F. Study Administration Structure

F.1. Investigator Responsibilities

F.1.1. Investigator Agreement

The investigator must sign FDA Form 1572, the Investigator Agreement, certifying that he/she will conduct the investigation in accordance with U.S. Federal Investigational New Drug regulations (21 CFR 312). The investigator is responsible for ensuring that the investigation is conducted in accordance with the investigational plan, and all IND and relevant FDA regulations for the protection of subjects' rights, safety, and welfare while under the investigator's care.

The investigator must notify the sponsor and the reviewing IRB of any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency. This notice shall be given as soon as possible but no later than five working days after such deviation occurred.

F.1.2. Institutional Review Board (IRB) Approval

Under the Code of Federal Regulations, 21 CFR §50, an appropriate Institutional Review Board, or like assembly, must review this protocol, the Informed Consent Form, and any other supporting study documents which impact subject safety, prior to study initiation.

The Institutional Review Board must review the protocol, and any amendments or revisions, and the Informed Consent Form at least annually over the course of this study. The study cannot continue until the IRB has given written renewal approval, signed by the board chairperson or authorized personnel, and the sponsor has on file a copy of the renewal approval letter and the approved Informed Consent Form.

F.1.3. Informed Consent

The investigator is required to obtain in writing a legally effective informed consent from the study subject's parents/guardian prior to the subject's participation in the investigation. No human being as a subject can participate in this study without such consent. The Informed Consent Form must be approved by the site's Institutional Review Board (IRB). The investigator shall seek such consent only under circumstances that provide the prospective subject's guardian sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject's parents/guardian shall be in language understandable to the subject's parents/guardian. Sufficient time must be available for the subject's guardian to have all questions answered.

In addition to the written explanation in the Informed Consent Form, a verbal explanation will be given by the investigator or his/her designee. The investigator or his/her designee will further explain the reason for the study, the risk to the subject of participating in the study as well as the potential benefits and alternative treatments the subject would receive should he/she choose not to participate in the study. The consent process will also be documented in the subject's research chart.

F.1.4. Records Maintenance

F.1.4.a. Administrative and Regulatory Files

The administrative and regulatory files include the following:

- IRB membership list / address;
- Original application to IRB, original approval letter - signed and dated;
- Additional submissions with amendments or changes and all approval letters
- Informed Consent original approval letter, signed and dated;
- All subsequent renewal application, and renewal approvals;
- Reports, at least annually, to the IRB.

F.1.4.b. Investigator Information

Including but not limited to the following:

- Investigator Curriculum Vitae;
- FDA Form 1571
- FDA Form 1572 - Investigator Agreement(s) - signed / dated;
- Monitoring records - including a sign-in log listing date, duration, and purpose of visit

F.1.4.c. Documentation of Adverse Events, Protocol Deviation, Failure to Obtain Informed Consent, Subject Withdrawal

Documentation regarding any of the following:

- Adverse events or drug complications;
- Deviations from the protocol;
- Failure to obtain informed consent prior to subject participation;
- Subject withdrawal from the study.

F.1.4.d. Data Corrections

All corrections to the data, either on a case report form or the medical record, will be:

- lined through so that the original entry can still be viewed
- initialed and dated by the individual making the correction
- corrected legibly
- reason for the correction will be noted only in the event that it is a “subjective” change (e.g., change from mild to moderate); otherwise changes should be supported by source document

Please note that whiteout or other corrective fluids will not be used at any time on the forms.

SECTION 2

Literature Cited in Research Plan

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Appendix A: Modified Ross Score for Heart Failure Classification

The Modified Ross Score for heart failure classification (25) will be used to assess heart failure during physical exams in the CHILD trial. It will be assessed at baseline and follow-up visits (Day 5, Week 4, Month 6, Month 12)

	Score (points)		
	0	**1**	**2**
History			
Diaphoresis	Head only	Head and body during exercise	Head and body at rest
Tachypnea	Rare	Several times	Frequent
Physical examination			
Breathing	Normal	Retractions	Dyspnea
Respiratory rate (respirations/min)			
0-1 y	<50	50-60	>60
1-6 y	<35	35-45	>45
7-10 y	<25	25-35	>35
11-14 y	<18	18-28	>28
Heart rate (beats/min)			
0-1 y	<160	160-170	>170
1-6 y	<105	105-115	>115
7-10 y	<90	90-100	>100
11-14 y	<80	80-90	>90
Hepatomegaly (liver edge from right costal margin)	<2 cm	2-3 cm	>3 cm