PEDIATRIC HEART NETWORK

SINGLE VENTRICLE RECONSTRUCTION

EXTENSION STUDY

Date: February 12, 2008

Amendment: May 16, 2008

Amendment: October 20, 2008

Clarification: September 15, 2010

Amendment: January 21, 2011

Amendment: May 10, 2012

Amendment: July 3, 2013

Funded by the National Heart, Lung, and Blood Institute, NIH/DHHS

Prepared by: New England Research Institutes, Inc.
9 Galen Street, Watertown, MA 02472
Tel.: 617-923-7747 --- Fax: 617-673-9540
07/03/13

Pediatric Heart Network

Single Ventricle Reconstruction Extension Study

TABLE OF CONTENTS

OVERVIEW (ABSTRACT) **1**

A. SPECIFIC RESEARCH AIMS AND HYPOTHESES **3**

A.1 Primary Aim 3

A.2 Secondary Aim 3

A.3 Secondary Aim 4

A.4 Secondary Aim 4

A.5 Secondary Aim 4

A.6 Secondary Aim 5

A.7 Secondary Aim 5

B. BACKGROUND AND SIGNIFICANCE **5**

B.1 Surgical Intervention for Single Ventricle Physiology 5

B.2 The RV-to-PA Shunt (Sano Modification) 7

B.3 The Single Ventricle Reconstruction Trial 7

B.4 Rationale for Extension of the SVR Trial (SVR II) 8

B.4.1 RV Function 8

B.4.2 Incidence of Arrhythmias 9

B.4.3 Neurodevelopmental Outcome and Functional Status in HLHS 9

B.5 Rationale for Biologic Specimen Repository (“Biorepository”) 10

C. RESEARCH DESIGN AND METHODS **11**

C.1 Study Overview 11

Table of Contents (Continued)

C.2 Participants 12
C.2.1 Inclusion Criteria 12
C.2.2 Exclusion Criteria 12
C.2.3 Subject Availability 12
Figure 1. Study Flow 13
C.2.4 Recruitment Protocol 14
C.2.5 Human Subjects Considerations 15
C.3 Study Design 23
C.3.1 Blinding 23
C.3.2 Study Completion 23
C.3.3. Subject Withdrawal 23
C.4 Measurements 24
C.4.1 Schedule of Measurements 24
Table 1. Schedule of Measurements 24
C.4.2 Outcome Variables 25
Table 2. Schedule of Questionnaires on Neurodevelopment, Behavior, and QOL 31
C.5 Statistical Analysis 36
C.5.1 Primary Endpoint for This Study 36
C.5.2 Sample Size and Power Analysis 36
Table 3. Power to Detect an Absolute 12% Difference in Death/Transplant Rates at 6 years 37
Table 4. Power to Detect a Relative 42% Reduction in Death/Transplant Rates at 6 years 37
C.5.3 Analysis Plan ..... .39
Table 5. Minimum Mean Detectable Difference Δ between Groups 40
C.6 Data Management 45
C.6.1 Information Flow 45

Table of Contents (Continued)

C.7 Quality Control 45
C.7.1 Clinical Center Coordinator Training 46
C.7.2 Certification of Personnel 46
C.7.3 Data Monitoring/Site Visits 46
C.7.4. Quality Assurance Related to Neurodevelopment, Behavior, and Health Related Quality of Life Instruments 47

D. STUDY LIMITATIONS 47

E. REFERENCES 48

APPENDICES

Appendix A. Sample Informed Consent for Study 55

Appendix B. Sample Informed Consent for Subjects Who Underwent Cardiac Transplant Before Enrollment in the SVR Study 62

Appendix C. Sample Informed Consent for Biorepository 68

Appendix D. Echocardiography Technical Protocol 74

Appendix E. Holter Monitor Methods 84

ABSTRACT

Among critical congenital heart lesions, hypoplastic left heart syndrome (HLHS) and related single right ventricle (RV) anomalies are associated with the highest morbidity and mortality. The first stage in palliation for patients with these defects is the Norwood procedure. The essential components of the Norwood procedure include (1) an atrial septectomy, (2) anastomosis of the proximal pulmonary artery to the aorta with homograft augmentation of the aortic arch, and (3) establishment of a source of pulmonary blood flow, with either a modified Blalock-Taussig shunt (MBTS) or the right ventricle to pulmonary artery (RV-to-PA) shunt. First described in 2003, the RV-to-PA shunt has the theoretical advantage of reducing the aortic diastolic run-off and coronary arterial steal, but the right ventriculotomy could cause later RV dysfunction or arrhythmias. In May, 2005, the Pediatric Heart Network began a multi-center, randomized trial, the Single Ventricle Reconstruction (SVR) trial, comparing outcomes in subjects with HLHS or other single RV anomalies palliated using the Norwood procedure with either a MBTS or the RV-to-PA shunt. The primary outcome of this trial is freedom from death or cardiac transplantation by 12 months post-randomization. Secondary outcomes up to 14 months post-randomization include surgical morbidity, repeat cardiovascular intervention, right ventricular function, neurodevelopmental outcome and pulmonary arterial growth. The target enrollment is 554 subjects by mid-2008, leading to completion of the trial by late 2009.

The proposed extension of the SVR trial (i.e., SVR II) compares clinical outcomes and RV performance following the RV-to-PA shunt versus MBTS modifications of the Norwood procedure in subjects at 6 years post-randomization. We will collect data annually between ages 2 and 6 years, as well as before and after the Fontan surgery. Vital status and medical history will be ascertained annually until the last enrolled subject is 6 years old. If heart transplantation occurs, only vital status will be collected. Data will be obtained through medical record review, phone interview with the parent or guardian, electrocardiography (ECG), core laboratory analysis of echocardiographic images and Holter monitors, and completion of questionnaires regarding neurodevelopmental outcomes, behavior, health-related quality of life, and family functioning. The primary outcome variable in SVR II will be freedom from death or cardiac transplantation at 6 years post-randomization. All subjects who were randomized in the SVR trial will be included in analysis of this endpoint. Secondary outcome variables will include measures of RV function; clinical status including the incidence of heart failure and arrhythmias; the operative and post-operative course at the Fontan completion; and measures of neurodevelopmental outcome, behavior, health-related quality of life, and family function. The sample size for the primary outcome is expected to be 542

subjects assuming 2% loss to follow-up, and the available sample sizes for the secondary outcomes are expected to vary between 160-270 subjects depending on subjects available for the various outcome assessments. Subjects will be recruited from the spring of 2008 through late 2009, when the last enrolled subject in the SVR trial will be 14 months post-randomization and is approached for the SVRII study. The SVR II study will also collect biological specimens for storage in a central repository for future genetic and biomarker studies.

A. SPECIFIC RESEARCH AIMS AND HYPOTHESES

A.1 Primary Aim

To compare the effect of the modified Blalock-Taussig shunt (MBTS) to that of the right ventricular-to-pulmonary artery (RV-to-PA) shunt on the incidence of death or cardiac transplantation in children with hypoplastic left heart syndrome (HLHS) and other single RV anomalies undergoing the Norwood procedure.

Hypothesis: The incidence of death or cardiac transplant will be higher in the MBTS group compared to the RV-to-PA shunt group.

Primary outcome

- Freedom from death and cardiac transplantation at 6 years post-randomization.

Secondary Outcomes

- Freedom from death and cardiac transplantation, using all available follow-up data until the time the last enrolled subject is 6 years post-randomization.
- Conditional freedom from death or cardiac transplantation by 6 years if surviving >1 year post-randomization.
- Survival at 6 years post-randomization.
- Freedom from surgery or interventional catheterization (other than Fontan completion surgery) at the end of the study, using all available follow-up data until the last enrolled subject is 6 years post-randomization.
- Inability to complete Fontan surgery by 6 years post-randomization.

A.2 Secondary Aim

To compare the effect of the MBTS to that of the RV-to-PA shunt on direct and indirect measures of RV systolic and diastolic function.

Hypothesis: The RV-to-PA shunt will be associated with worse RV performance compared to the MBTS.

Secondary outcomes of greatest importance include:

- RV ejection fraction by echocardiogram pre-Fontan and at 6 years.
- RV fractional area change pre-Fontan and at 6 years post-randomization.
- Severity of tricuspid regurgitation by echocardiogram pre-Fontan and at 6 years.
- Heart Failure Class pre-Fontan and at 3, 4, 5, and 6 years.
- Somatic growth pre-Fontan and annually from ages 2-6 years.

A.3 Secondary Aim

To compare the effects of the MBTS to that of the RV-to-PA shunt on post-operative hospital course following the Fontan procedure.

Hypothesis: The RV-to-PA shunt will be associated with worse post-operative hospital course after the Fontan procedure.

Secondary outcome

- Length of hospital stay following Fontan procedure.

A.4 Secondary Aim

To compare the effect of the MBTS to that of the RV-to-PA shunt on the incidence of arrhythmias.

Hypothesis: The RV-to-PA shunt will be associated with a greater prevalence of ventricular arrhythmias, but will not differ from the MBTS in the prevalence of atrial arrhythmias.

Secondary outcomes

- Diagnosis of ventricular arrhythmias by 6 years.
- Diagnosis of atrial arrhythmias by 6 years.

A.5 Secondary Aim

To compare the effects of the MBTS to that of the RV-to-PA shunt on neurodevelopment, behavior, health-related quality of life, and family function.

Hypothesis: The RV-to-PA shunt will be associated with better measures of neurodevelopment, behavior, quality of life, and family function.

Secondary outcomes

- Ages & Stages Questionnaires (ASQ).
- Behavior Assessment System for Children, Second Edition (BASC-2).
- Pediatric Quality of Life Inventory (PedsQL).
- Impact on Family Scale.
- Functional Status II-R (FSII-R).
- Child Health Questionnaire Parent Form 50-Item (CHQ-PF50) Physical and Psychosocial Function Summary Scores.
- Vineland Adaptive Behavior Scales.

Neurodevelopmental questionnaires will be completed at ages 3, 4, 5, and 6 years, except for the Vineland and CHQ-PF50, which will be administered only at age 6 years, and the ASQ, which will be administered only at ages 3, 4, and 5 years.

A.6 Secondary Aim

To develop clinical-risk stratification models for the following classes of outcome: 1) transplant-free survival; 2) cardiac outcomes; and 3) neurodevelopmental outcomes. Potential correlates to be considered include sociodemographic factors, apo E genotype, medical history, earlier measures of cardiac and neurodevelopmental outcomes, and previous therapies or interventions, including but not limited to the randomized shunt type.

Hypothesis: Six-year outcomes of children with single ventricle palliated by the Norwood procedure will be associated with earlier events and evaluations.

Secondary specific aims will be to:

- Provide information about the association of events and interventions other than the randomized surgical procedures with later outcomes.
- Identify early cardiac and neurodevelopmental surrogate endpoints that might be useful in future studies of management strategies.
- Determine if correlates of long-term outcome vary according to patient subgroup factors.

A.7 Secondary Aim

To collect specimens from subjects and, when possible, parents of subjects with HLHS and other single RV anomalies for banking in a biologic specimen repository (biorepository).

Purpose: To establish a collection of biological material that will leverage the careful phenotyping of the SVR cohort by making samples available for future hypothesis-driven studies aimed at identifying molecular markers and genetic determinants of outcome in single ventricle lesions.

B. BACKGROUND AND SIGNIFICANCE

B.1 Surgical Intervention for Single Ventricle Physiology

Single ventricle physiology occurs in congenital heart lesions without two functioning ventricles. The most common form of single ventricle, occurring in approximately 60% of patients, is HLHS. Characterized by a small, nonfunctioning left ventricle, HLHS encompasses various combinations of aortic stenosis or atresia, mitral stenosis or atresia, and aortic arch hypoplasia or severe coarctation. HLHS was universally fatal in the first few months of life until the advent of the Norwood procedure in the late 1970's.[1]

The Norwood procedure allows the right ventricle to pump blood to the systemic circulation by disconnecting the branch pulmonary arteries from the main pulmonary artery and creating a

connection between the main pulmonary artery and the aorta. Blood flow to the lungs is re-established via a systemic to pulmonary artery shunt, historically a MBTS, a non-valved tube graft connecting the aorta to the pulmonary arteries. Although the Norwood procedure allowed early survival in many infants with HLHS, initial perioperative mortality was high and ranged from 30-40%, with one year survival in the range of 40-50%.[2] Survival statistics improved over the next decade with reports of 80% survival at 6 months of age and 60-70% survival at 1 year of age.[3] In patients whose Norwood procedure is performed using a MBTS, diastolic blood flow into the pulmonary artery lowers systemic diastolic blood pressure and has the potential to steal blood flow away from the coronary arteries. In addition, the presence of a systemic to pulmonary artery shunt produces an obligatory volume load on the single right ventricle. Mortality following the Norwood procedure in the recent era has remained in the 15-20% range, despite improvements in surgical techniques and in pre- and post-operative care.[4]

Following the Norwood procedure, two subsequent palliative surgeries are performed to reduce the excess volume load to the RV and to establish normal systemic oxygen saturation. The first of these procedures, a bidirectional Glenn (BDG) shunt or its variant, the hemi-Fontan, is performed most commonly between ages four to six months. The bidirectional Glenn shunt or hemi-Fontan procedure eliminates the systemic to pulmonary artery shunt and establishes a direct connection between the superior vena cava and the pulmonary artery to supply pulmonary blood flow. This allows systemic venous blood from the upper body to flow directly into the pulmonary artery, bypassing the RV. Mortality between the Norwood procedure and the second stage palliative procedure (inter-stage mortality) remains a significant cause of death in this population, with an incidence of 5-10%. Combined with a 3-5% mortality rate for the bidirectional Glenn shunt or hemi-Fontan, mortality within the first year of life in infants with HLHS is approximately 25-30%.[5]

Between ages 18 months and 4 years, the Fontan completion surgery is performed to baffle the inferior vena cava blood directly to the pulmonary artery. Following the Fontan completion surgery, the systemic venous blood flow returns directly to the pulmonary artery, bypassing the systemic right ventricle, and improving systemic oxygen saturation.

Unfortunately, some infants are not candidates for the bidirectional Glenn shunt or hemi-Fontan due to the development of severe right ventricular dysfunction or tricuspid insufficiency not amenable to valvuloplasty, and cardiac transplantation becomes the only surgical option.[6]

B.2 The RV-to-PA Shunt (Sano Modification)

In 2003, Sano *et al.* described a series of 19 infants who underwent a modified Norwood procedure using the RV-to-PA shunt instead of the MBTS.[7] Following this report, many small single center series reported improved survival with the use of the RV-to-PA shunt,[7-11] with others reporting no difference in one-year outcome.[8] In late 2004, Sano *et al.* reported the results of a multi-institutional study of 73 infants who underwent the RV-to-PA modification with 84% survival to hospital discharge.[12]

With the RV-to-PA shunt, pulmonary blood flow occurs only in systole, and the diastolic run-off associated with a MBTS is eliminated, potentially improving coronary blood flow and reducing pulmonary blood flow. Many deaths following the Norwood procedure are due to coronary artery insufficiency or pulmonary overcirculation. In addition, shunt thrombosis is an important cause of mortality in the inter-stage period.[13] Thus, the RV-to-PA shunt has the potential to decrease both early and mid-term mortality. On the other hand, the Norwood procedure with RV-to-PA shunt requires a right ventriculotomy, with potential risks of later right ventricular dysfunction or arrhythmia.

B.3 The Single Ventricle Reconstruction Trial

To compare the outcomes of the MBTS and RV-to-PA shunts in infants with single ventricle defect undergoing staged reconstruction, the Pediatric Heart Network (PHN) began enrolling subjects in a randomized, multi-center clinical trial, i.e., the Single Ventricle Reconstruction (SVR) trial, in May 2005. Inclusion criteria included: 1) diagnosis of hypoplastic left heart syndrome or related single, morphologic RV anomaly; 2) planned Norwood procedure; and 3) informed consent of parent(s) or legal guardian. Exclusion criteria included 1) single, morphologic left ventricle anomaly; 2) preoperative identification of anatomy rendering either a MBTS or a RV-to-PA shunt technically impossible; and 3) any major congenital abnormality (i.e., congenital diaphragmatic hernia, tracheoesophageal fistula) or acquired extracardiac disorder (e.g., meconium aspiration with need for high frequency ventilation, persistent renal failure requiring dialysis) that, in the opinion of the investigator, could *independently* affect the likelihood of the subject meeting the primary endpoint. The primary outcome of the trial is freedom from death or cardiac transplantation at 12 months post-randomization. The hypothesis is that the RV-to-PA shunt modification will demonstrate a lower incidence of death or cardiac transplantation than the conventional MBTS. Secondary outcomes up to 14 months post-randomization include surgical morbidity, repeat cardiovascular intervention, right ventricular function, neurodevelopmental outcome, and pulmonary arterial growth.

The target enrollment is 554 subjects by mid-2008, leading to completion of the trial by late 2009. Fifteen clinical centers (8 PHN and 7 auxiliary) are currently participating in the SVR trial.

B.4 Rationale for Extension of the SVR Trial (SVR II Study)

Mortality and morbidity in patients with single ventricle undergoing staged palliation continues beyond the 14 month endpoint in the SVR trial. The SVR II study will provide information on differences between the shunt groups in morbidity and mortality between the BDG and Fontan procedures and after the Fontan procedure, as well as differences in their rates of unsuitability for the Fontan procedure and of interim cardiac transplantation. Early mortality following the Fontan procedure, as high as 25% in the early era, is approximately 3-5% in the current era.[14-18] Non-randomized data indicate that the Fontan procedure has similar mortality in patients whose Norwood procedures were performed using the RV-to-PA shunt modification compared to the MBTS, but the small sample size limits statistical power.[12] No published reports compare morbidities following the Fontan procedure between the RV-to-PA shunt and MBTS groups. Fontan patients may develop atrial arrhythmias, thrombotic events, heart failure, chronic pleural effusions, protein-losing enteropathy, cirrhosis of the liver ("cardiac cirrhosis"), plastic bronchitis, and other problems related to chronically elevated venous pressures.[19] The rates of these morbidities should be compared between the shunt groups. Similarly, research is needed to explore whether the shunt groups differ in their rates of surgical or catheter-based reinterventions following the Fontan procedure; such interventions include pacemaker implantation, balloon angioplasty or stenting of the branch pulmonary arteries, fenestration closure or fenestration creation, Fontan revision or Fontan takedown.[19-22] In addition to collecting information about morbidities and procedures by medical history, the SVR II study will analyze echocardiograms for RV function, Holter monitors for arrhythmias, and neurodevelopmental questionnaires. The rationale for collection of these data is detailed below.

B.4.1 RV Function

The preservation of RV function is a critically important consideration in assessing the effectiveness of any surgical intervention performed in children with a single RV. Right ventricular dysfunction may compromise short-term outcome following the Fontan procedure and may increase long-term morbidity and mortality; RV dysfunction could be caused by either coronary ischemia after the Norwood with MBTS, or by the right ventriculotomy that is performed with the RV-to-PA shunt modification. Few published studies have evaluated the late impact of the RV-to-PA shunt, compared to the MBTS, on RV function. Echocardiographic studies performed early after the RV-

to-PA shunt have demonstrated improved RV fractional area change, basal free wall longitudinal strain, and strain rate compared to the MBTS.[23] However, qualitative echocardiographic assessment of RV function at a mean follow-up interval of 29 months, prior to stage II and Fontan palliation, has suggested decreased function in the cohort who received the RV-to-PA shunt compared to the MBTS[24] Decreased RV hypertrophy prior to the bidirectional Glenn shunt has been reported following the RV-to-PA shunt. On the other hand, an invasive study by Tanoue *et al*.[25] compared catheter-based determinations of end-systolic elastance before and after the bidirectional Glenn and Fontan procedure in patients following the RV-to-PA and MBTS; a decrease in end-systolic elastance, suggesting RV dysfunction, was seen in the RV-to-PA subjects compared to the MBTS.[16] These preliminary studies emphasize the need for further evaluation of the effects of the RV-to-PA shunt versus the MBTS modifications of the Norwood procedure on RV function following the Fontan procedure.

B.4.2 Incidence of Arrhythmias

The proximal RV-to-PA shunt is connected to the systemic right ventricle via a ventriculotomy. Ventricular arrhythmias and sudden death have been reported late following right ventriculotomy in children undergoing repair of tetralogy of Fallot.[26] The potential for increased ventricular arrhythmias from right ventriculotomy among patients who underwent the Norwood procedure with RV-to-PA shunt is balanced by the potential for greater early RV myocardial ischemia among patients whose Norwood procedure was performed with MBTS. Further studies are required to assess whether the MBTS or RV-to-PA shunt is associated with greater risk for later ventricular arrhythmias.

B.4.3 Neurodevelopmental Outcome and Functional Status in HLHS

Published studies have consistently demonstrated that children with HLHS and other single RV anomalies have a high risk of neurodevelopmental and behavioral abnormalities. [27-34, 33, 35-38] In the ongoing SVR trial, we are comparing the MBTS and RV-to-PA shunt groups with respect to neurodevelopment and functional status at age 14 months. Although we are meeting the objectives of our original study, neurodevelopment testing at age one year has low sensitivity and low positive predictive value for later intelligence and achievement in patients who have undergone infant heart surgery.[39] Completion of questionnaires on neurodevelopment and quality of life up to 6 years after randomization will enhance the predictive validity of the assessments and elucidate subjects' strengths and weaknesses. Compliance should remain high because of the appeal to families of such assessments as the children approach and enter kindergarten.

An additional goal of the SVR II study is to identify risk factors for adverse neurodevelopmental outcome other than the randomized shunt techniques. Many factors contribute to adverse neurodevelopment in children with HLHS and other single RV anomalies, including patient characteristics, genetic factors, operative and perioperative events around the Norwood procedure, risk factors related to subsequent operations, and general cardiac morbidities, such as cyanosis and congestive heart failure.[27-29]

Wide practice variation exists across centers and states in their use of particular interventions; i.e., infants with critical congenital heart disease of similar status are treated differently. For example, centers vary widely in prescribing early intervention programs and home monitoring. With the use of propensity scores to minimize selection bias related to provision of such interventions, data collected in SVR II could provide a basis for designing future, more definitive studies.

The SVR trial has assembled the largest cohort to date of children with single RV anomalies. Inferences regarding neurodevelopmental outcome in almost all previous studies have been handicapped by relatively small sample size and limited power for multivariable analysis; the largest recent study on outcomes in HLHS patients included only 88 patients.[29] Furthermore, published information regarding neurodevelopment in children with HLHS is limited to evaluating children at a single point in time or as part of a cross-sectional study. Subjects in the SVR trial have been enrolled prospectively from a broad sample of North American centers. In summary, the cohort in the SVR Trial provides an unparalleled opportunity to study the subject characteristics (including genetic factors), perioperative factors, and therapies that predict longer-term neurodevelopmental, behavioral, quality of life and family outcomes in a well-characterized population at high risk of neurodevelopmental morbidity.

B.5 Rationale for Biologic Specimen Repository ("Biorepository")

The SVR II study will collect biological specimens to bank in an established biorepository as a resource for future, hypothesis-driven studies. This is a unique and timely opportunity to leverage the detailed characterization of cardiac anatomy and clinical outcomes already available in this population of children with single ventricle lesions. The specific scientific questions addressed and the approaches used will necessarily be determined by the investigators who propose studies using the specimens. Therefore, the intent is to ensure maximal flexibility for future studies. Accordingly, when possible, blood will be drawn, processed, and stored in such a way as to permit both genetic studies and studies of serum biomarkers. The intent is to obtain blood from study subjects and

parents (i.e., “trios”). When obtaining blood is not possible, however, saliva will be obtained so that at least genetic information can be stored for future studies.

Genetic factors are expected to play a critical role not only in the underlying etiology of congenital heart disease but also in the determination of sub-phenotypes such as the presence of intact atrial septum in HLHS. Furthermore, genotype will almost certainly influence long-term cardiovascular and other clinical outcomes in these patients. For example, neonatal susceptibility to cerebral or cardiac ischemia reperfusion injury in response to surgery or hemodynamic insults may be influenced by genetic polymorphisms in the pathways that regulate inflammation, thrombosis, vascular reactivity, and oxidative stress.[40] Recent research has begun to identify biomarkers from serum and tissue that might permit risk stratification or predict outcomes in children with heart disease, but information regarding biomarkers is limited in the single ventricle population. A biorepository of subject and parent DNA and serum will be a key resource to help elucidate determinants of cardiac, neurodevelopmental, and other outcomes in this high-risk population.

C. RESEARCH DESIGN AND METHODS

C.1 Study Overview

The proposed SVR II study extends follow-up of subjects originally enrolled in the SVR trial, a multi-center, randomized prospective trial of the RV-to-PA shunt vs. MBTS in subjects undergoing the Norwood procedure (Figure 1). Data will be collected annually from ages two through six years post-randomization, as well as immediately before and after the Fontan surgery. Vital status and medical history will be ascertained annually until the last enrolled subject is 6 years old. If heart transplantation occurs, only vital status will be collected. Sources of data will include medical record review, phone interview with the parent or guardian, echocardiograms (Core Lab), Holter monitors (Core Lab) and ECGs, and questionnaires on neurodevelopmental outcomes, behavior, health related quality of life, and family functioning. The primary outcome variable in the SVR II study will be freedom from death or cardiac transplantation at 6 years post-randomization. All subjects who were enrolled in the SVR trial be included in analysis of this endpoint. Secondary outcome variables will include measures of RV function; clinical status including the incidence of heart failure and arrhythmias; the operative and post-operative course at the Fontan completion; and measures of neurodevelopmental outcome, behavior, health-related quality of life, and family function. The sample size for analysis of the primary outcome is expected to be 542 subjects, assuming 2% loss to follow-up for vital and transplant status. The sample size for the secondary

outcomes is expected to vary between 160-270 subjects depending upon the numbers of subjects available for the various outcome assessments. Subjects will be recruited from the spring of 2008 through late 2009, when the last enrolled subject in the SVR trial will be 14 months post-randomization and is approached for the SVRII study. The SVR II study will also include a biologic specimen repository.

C.2 Participants

C.2.1 Inclusion criteria

Randomized subject in the Single Ventricle Reconstruction Trial

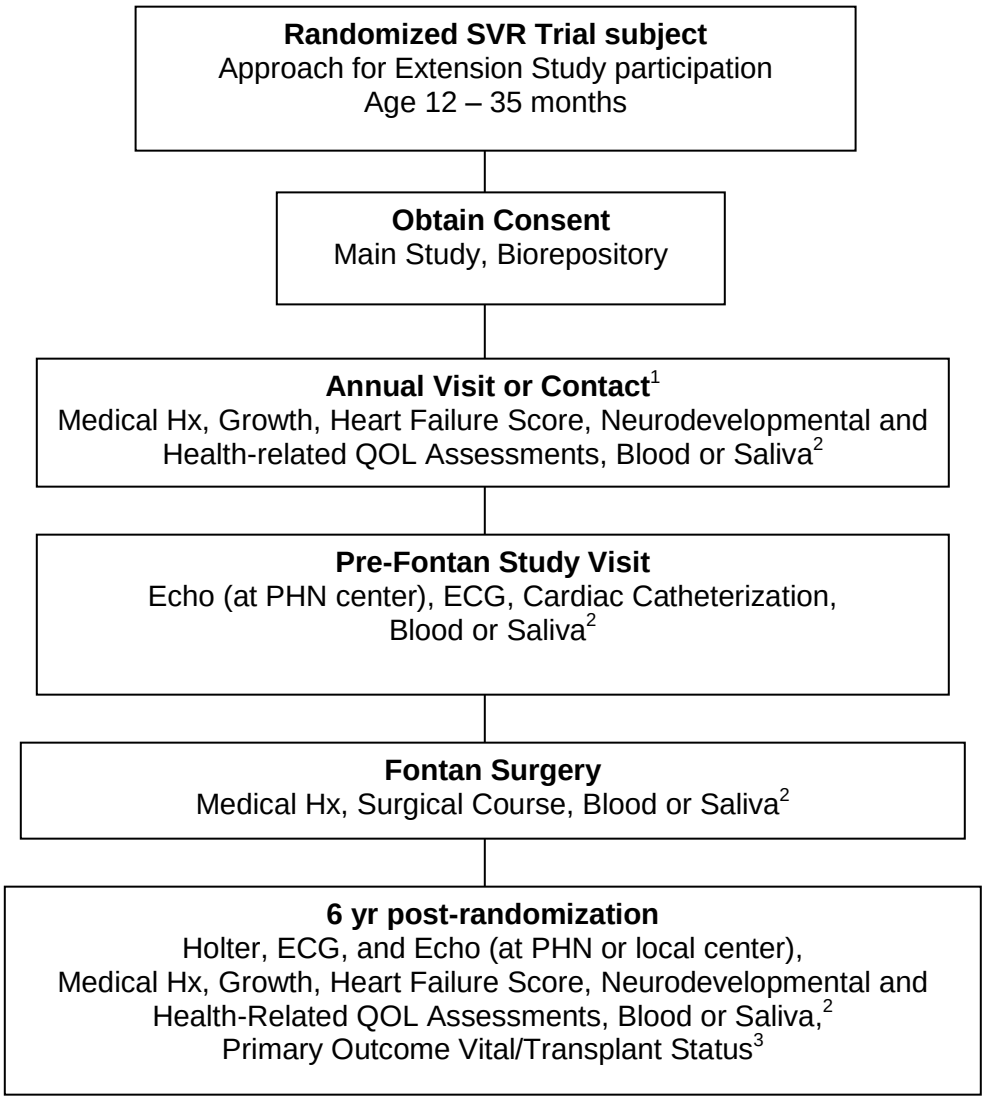
C.2.2 Exclusion criteria

No subjects enrolled in the original SVR Trial will be excluded from analysis of the primary endpoint.

C.2.3 Subject availability

The target enrollment for the original SVR trial is 554 subjects, 277 in each treatment arm. The proportion of subjects experiencing an adverse outcome (death or transplantation) prior to 12 months post-randomization is predicted to be 28% in the MBTS group and 16% in the RV-to-PA group. At the end of the SVR trial, the hypothesized one-year transplant-free survival rates will yield a total of 432 subjects (199 in the MBTS group and 233 in the RV-to-PA group) alive with single ventricle physiology at age one year. The current SVR trial informed consent form includes permission for annual medical record review, and it is expected that the primary endpoint of death or cardiac transplantation will be available in virtually all (98%) of the subjects at 6 years post-randomization. A survey of the PHN sites suggests that we will be able to collect data for most secondary endpoints on approximately 50% of subjects originally randomized in the SVR trial. Thus, we estimate that approximately 277 subjects will be available for the majority of secondary outcomes. Pre-Fontan echocardiograms will be analyzed for only those subjects who return to a PHN center, limiting the number of subjects available for this particular secondary endpoint. Based on a survey of the sites, 200-250 echocardiograms are anticipated. Potential reasons for unavailability of other secondary outcomes at age 6 years include death or cardiac transplantation; the subject is not followed at a PHN center and the local cardiologist doesn't send echocardiograms; the subject consents but doesn't complete or return neurodevelopmental questionnaires or Holter monitor; or the subject's family declines participation or is lost to follow-up.

Figure 1. Study Flow



[1] At 2 years, only vital status and medical history are obtained.

[2] Blood or saliva may be obtained for the biorepository at any visit, but patients generally will not supply these materials more than once during the study period. Some of these subjects are also in the Infant with Single Ventricle Trial and may have already provided adequate blood for genotyping.

[3] All subjects will be followed for vital/ transplant status until the last enrolled subject is 6 years post-randomization, i.e., up to 9 years post-randomization for the earliest enrolled SVR trial subjects.

C.2.4 Recruitment protocol

The principal investigator at each clinical center, his or her designees, and the study coordinator will have responsibility for subject recruitment. Many families will be expecting to hear about a follow-up study through continued contact with the study coordinator and research team. The study coordinators and study investigators requesting informed consent for participation in the follow-up study will have a high degree of familiarity and rapport with families in the SVR trial, and this should optimize recruitment of subjects from the SVR trial into the proposed extension study. Data on all screened subjects will be entered on a screening form, regardless of the decision to participate in the study, in order to define the eligible study population. The consent process will require separate forms and signatures indicating consent for participation in the follow-up study and in the biorepository. Assent will not be required because all subjects are <6 years old.

The principal investigator or his or her authorized research staff members will obtain informed consent for participation in the follow-up study at the time of patients' in-person 14-month visits. For patients who have already had their 14-month visit, informed consent will be obtained verbally via phone, followed by return mailing of the signed consent form. The specific process will depend upon the guidelines of each site's Institutional Review Board. The following description is a general guideline.

A brief letter describing the follow-up study, copies of the consent form, and a postage paid, self-addressed op-out postcard, will be sent to the family of the potential study participant three to six months prior to the potential participant's third birthday. The letter will be double-sided with English on one side and Spanish on the other. Study personnel will wait two weeks following the mailing so that families have ample time to decline participation with the op-out postcard.

1. After two weeks, if the opt-out card is not received back from the family, we will contact the family directly.

2. During the phone call, once the parent/guardian has a copy of the consent in front of him or her, each section on the informed consent form will be read verbatim by the researcher. After each numbered section or individual paragraph is read, we will ask the parent/guardian if he/she has any questions. The PI/research staff member will ask questions to gauge comprehension, and will respond to parent/guardian questions and concerns.

The PI or PI-authorized staff member will document the entire informed consent process for each person in a memo or related study document. This documentation will be particularly important for those who decline participation.

3. After all questions are answered and the PI/research staff feels confident each parent/guardian understands the study, he or she will ask each parent/guardian to sign and date the consent form (we will flag or highlight the correct signature line), and mail back the signed consent copy. Each signor will be asked to keep the other copy of the consent for his or her files.
4. Once the consent form signed by the parent/guardian is received at the study site, the PI/research staff member who explained the study will sign the appropriate signature line with the current date (not the date on which he or she spoke to the parent/guardian). The PI/PI authorized signor will specify to whom the study was explained, the date on which verbal consent was obtained, and the date on which the signed consent form was received within the PI/PI authorized signor section.

e.g. "*Discussed with [person] via telephone on [insert date], and received sign consent form on [insert date].*"

We will ensure that all signatures and dates were accurately documented. Any errors will be noted in a note or memo. If necessary because of mistakes, re-consent of the participant/parent/guardian may be performed.

C.2.5 Human subjects considerations

The characteristics of the subject sample, sources of research material, and recruitment protocol are specified in Sections C.2.1-C.2.4. Appendices A, B, and C, respectively, include sample consent forms for participation of subjects who 1) are participating in the main study and have not had cardiac transplantation; 2) have had cardiac transplantation by the time of enrollment in the SVR II study (ascertainment only of vital status); and 3) are participating in the biorepository. Measurements made in this study will be recorded on study forms. Data collection will be performed by telephone interviews with parents or legal guardians, medical record review, echocardiogram, Holter monitor, ECG, and parental self-completion of questionnaires on neurodevelopment, behavior, quality of life, and family impact. The pre-Fontan echocardiogram will be analyzed by the Core Laboratory only in subjects returning to a PHN site for Fontan completion.

Data concerning the Fontan operation and hospital course will be obtained by medical record review regardless of where the subject's Fontan operation is performed (i.e., PHN or local center).

C.2.5.a Potential risks

1. We will contact the subject's cardiologist first to be sure that the subject is alive. There is a tiny chance that the cardiologist might not have been informed about a subject's death and that we will cause distress by contacting parents of an expired subject not known to have died.

There is some inconvenience and burden of completing questionnaires and some families may feel uncomfortable answering questions. Approximately 60-90 minutes will be required for completion of all instruments at the 3, 4, and 5 year assessments. The 6 year assessment, including completion of the CHQ-PF50 and Vineland, will require approximately 2 hours. However, subjects' families will be under little time pressure to complete the questionnaires. Study coordinators will be available to answer questions and respond to concerns of families.

All testing that is not part of routine care will be performed free of charge. Subjects and their families will be reimbursed for costs associated with participating in the protocol that would not have occurred as part of routine clinical care.

The echocardiograms and electrocardiograms are part of routine care of infants and children with a single right ventricle. Subjects will be sedated for the echocardiograms if clinically indicated according to practice guidelines at the individual center. There are no risks associated with echocardiography or electrocardiography.

The 24-hour Holter monitor at 6 years post-randomization will be performed for research purposes. The only risk from the Holter monitor may be brief skin irritation from the patches placed on the skin.

2. Protection of confidentiality: Investigators of this research will take all reasonable measures to protect the confidentiality of the medical records of subjects and their families.
    a. Information on study subjects and families obtained during this research will be maintained confidentially by the research staff. The risk of breach of subject confidentiality will be minimized by storage of all study materials in a locked, secure location accessible only to study investigators. All research related

information will be maintained in a system completely separate from the hospital's medical record system. The informed consent form states that subject data will be made available to the Data Coordinating Center (DCC), NIH/NHLBI, Institutional Review Board (IRB) and Data and Safety Monitoring Board (DSMB) if necessary for study safety and quality control.

b. The subject's name and any other identifying information will not appear in any presentation or publication resulting from this study.

3. For subjects participating in the biorepository: Subjects will sign a single informed consent form for future studies using DNA or sera in the biorepository, i.e., we do not expect subjects to sign additional consent forms for research using biorepository materials. Therefore, we have provided a detailed list of potential risks.
    a. Collection of blood or saliva for the biorepository is performed only for research purposes, and families will indicate their consent for this part of the research study separately (i.e., one can participate in other aspects of the study without consenting to the biorepository).
    b. Risks associated with a blood draw are minor discomfort, bleeding, bruising, and lightheadedness. When possible, we will draw blood for the biorepository at the time of a clinically indicated procedure so that the subject may not need to have blood drawn only for research purposes.
    c. There are no risks or discomforts associated with providing a saliva sample.
    d. For those participating in the biorepository, biological specimens (DNA and sera) will be assigned a repository identification number without other identifying information. All research related information is maintained in a system completely separate from the hospital's medical record system. Informed consent will state that subject data will be made available to the DCC, NIH/NHLBI, IRB and DSMB if necessary for assessment of study safety.
    e. To help us protect the privacy of subjects participating in the biorepository, we will obtain a Certificate of Confidentiality from the National Institutes of Health (NIH). With this Certificate, the researchers of this study cannot be forced to disclose information that may identify a subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The Certificate cannot be used to resist a request for information from the United States government when it is used for evaluating federally funded study projects or for information that must be disclosed to meet the

requirements of the Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent a subject or his/her family from voluntarily releasing information about the subject's involvement in this research. If an insurer, employer, or other person obtains a subject's or family's written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

f. Information from DNA analyses and clinical studies or medical records may be placed into a central data repository in the future, such as the National Center for Biotechnology Information (NCBI) repository. The purpose of a central data repository is to help researchers work together to find out more information about ways in which genes affect disease. The NCBI or a similar repository makes data accessible through the Internet. The repository has two databases, open access and controlled access. The open access database is available to anyone on the Internet and includes DNA sequence traces that are not linked to medical or personal information. The controlled access database includes de-identified medical information and more detailed analyses of de-identified samples that are made available to researchers with IRB approval to conduct human genetic studies and who have received approval from an NIH Data Access Committee.

g. The results of future tests on biological specimens will not be released to the subject/family. At the end of the study, the results of the genetic testing may be published for all the subjects as a group, but it will not be possible to provide results for an individual subject and medical management will not be changed based on individual results. There is a reasonable possibility that no findings will result from this research effort. If findings are detected, it may be years before any utility of these findings are realized. Further, if samples are "annonymized" prior to release to other investigators for research, it may not be possible to trace the results back to the subject.

The research using samples and data may result in inventions or discoveries that could create new tests and medicines that may have commercial value. Although subjects and their families will not receive any compensation now or in the future for their samples or data, income that may be derived from future research or sales of the grouped data will be used to support biomedical research.

C.2.5.b Potential benefits

1. It is possible that the Holter monitors obtained for research purposes may disclose an arrhythmia of importance to the subject's management. Results will be provided to the subject's cardiologist, and the subject's family will be informed about this information transfer.
2. Evaluation of neurodevelopmental outcome and functional status may provide valuable information that would not otherwise be available. If abnormalities are detected, this information may lead to early intervention measures designed to maximize developmental potential.
3. Currently, there is no known direct benefit from the participation of the subject and family in the biorepository. However, we hope that DNA and serum donation will help investigators to learn more about the relationship between genetic factors or biomarkers and longer-term cardiac and neurodevelopmental outcomes. This information may help physicians provide better answers to families' questions regarding causes, risk, and recurrence risks. It may also provide clues to future interventions and/or treatments.
4. An indirect benefit may also come from the awareness that the results of this study may serve to help improve the care of children with similar problems in the future. Families may derive a sense of altruism, accomplishment, and contribution to furthering understanding of the problem through their participation.

C.2.5.c Risk/benefit ratio and importance of information to be obtained

1. The risk/benefit ratio is favorable for this study. The baseline risk is minimal because there are no therapeutic interventions. In addition, although an individual subject may not benefit from participation, the results of this study will make important contributions to the design of an optimal management algorithm for infants with single ventricle. Extending the length of follow-up of the SVR study up to six years to obtain the intermediate incidence of death or cardiac transplantation is crucial to evaluating the potential survival benefit of the RV-to-PA connection.
2. Neurodevelopment and functional status have never been studied in such a large population of infants with single ventricle, or in a longitudinal fashion, and are important correlates to the primary endpoint.
3. The assessments of RV function and the incidence of arrhythmias address important long-term concerns regarding the potential negative effects of a ventriculotomy on the single right ventricle following the RV-to-PA shunt.

4. Data generated from this study will be unique in terms of the breadth and depth of the guidance that can be provided to parents and medical care providers of children with univentricular hearts who have undergone the Norwood procedure.

C.2.5.d Data and safety monitoring plan

The data and safety monitoring plan for the SVR II study will follow standard PHN monitoring principles. Oversight of data and safety for all PHN studies is provided by the PHN's, NHLBI-appointed independent DSMB. The DSMB, which meets at least biannually, is composed of experts in pediatric cardiology, congenital heart surgery, biostatistics and study design, and ethics, as well as a lay member. The DSMB will monitor patient screening, study accrual, number of subjects undergoing each of the different types of evaluations in the protocol, adverse events, data quality, and protocol violations on a regular basis, and it will make recommendations about study conduct to the Director, NHLBI. A summary of study recommendations following each DSMB meeting is posted on the secure PHN Web site. As the SVR II study does not involve any interventions, an early stopping rule for efficacy is not indicated (see Section C.5.3.d).

In addition to the DSMB, local IRBs are also responsible for the safe conduct of research at each study site. Participation in the SVR II study cannot begin at a clinical center until the local IRB has approved the protocol. Per NHLBI policy, the consent form from each site, once approved, is reviewed again centrally to ensure that no changes inconsistent with the Office of Human Research Protections policy of study design have occurred.

After each DSMB meeting, a summary report of adverse events will be prepared within 30 days and will be distributed by NHLBI staff to each principal investigator and study coordinator with instructions that each principal investigator forward the summary report to their local IRB. The summary report will contain the following information:

- A statement that a DSMB review of outcome data, adverse events, and information relating to study performance across all centers took place on a given date.
- A statement as to whether or not the frequency of adverse events exceeded what was expected and indicated in the informed consent.
- A statement that a review of recent literature relevant to the research took place.
- The DSMB's recommendation with respect to progress or need for modification of the protocol or informed consent. If the DSMB recommends changes to the protocols or informed consent, the rationale for such changes and any relevant data will be provided.

- A statement that if safety concerns are identified, the NHLBI Program Official will communicate these promptly to the investigators.

C.2.5.e Adverse events

A major component of safety monitoring is ascertainment and reporting of adverse events. The approach to these activities for the SVR II study is summarized in the sections below.

C.2.5.e.1 Definition

An adverse event is any untoward medical occurrence experienced by a study subject. An event can be any unfavorable and unintended sign, symptom, laboratory abnormality, or disease associated with study participation.

C.2.5.e.2 Classification

Monitoring of adverse events in a therapeutic trial requires that they be classified as to seriousness, expectedness, and potential relationship to the study intervention, which then drives the reporting process. In this observational study, adverse events should be rare, and monitoring should be commensurate with risk, which is minimal. The only procedures being conducted solely for research are the Holter monitor, completion of questionnaires, and, with separate consent, participation in the biorepository. These testing procedures are well established in children and are associated with a very low risk of adverse events. In this study, adverse events will be recorded only for the duration of study-specific testing and 24 hours afterwards.

A serious adverse event is one that:
(a) Results in death,
(b) Is life-threatening,
(c) Requires inpatient hospitalization or prolongation of existing hospitalization,
(d) Results in persistent or significant disability/incapacity, or
(e) Is a congenital anomaly/birth defect in the offspring of a participant.

An event definitely or probably related to the study procedure is one that follows a reasonable temporal sequence from the time of study testing, and cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

C.2.5.e.3 Data collection procedures for adverse events

Events occurring within 24 hours of study-mandated testing will be recorded by the clinical center study coordinator, including the date and time of occurrence, severity, duration, treatment prescribed, and resolution. If classified as serious, this event information will be forwarded electronically within three working days to the DCC (see C.2.5.e.4).

C.2.5.e.4 Reporting procedures

Serious adverse events related to the study procedures would all be unexpected, but if any occur, they will be reported to the DCC within 3 working days of learning of the event. The DCC will report any such events to the NHLBI, and the PHN Medical Monitor as soon as possible, but no later than 7 calendar days after first knowledge of the event, followed by a complete report within 15 calendar days. All other adverse events occurring during the time period specified for this study will be reported quarterly to the DCC. The DCC will report these events at least twice a year to NHLBI and the DSMB. The site investigator or designee will report all serious adverse events to the local IRB according to local IRB policies.

C.2.5.f Women and minority inclusion in clinical research

HLHS and other single right ventricle anomalies are congenital defects requiring surgery early in life. The incidence is somewhat higher in males than females. All eligible subjects are enrolled in the main SVR Trial without regard to gender, race, or ethnicity. The distributions of subjects according to gender, race, and ethnicity reflect both the epidemiology of single right ventricle anomalies and the referral patterns to participating PHN centers. All subjects in the SVR II study will be children.

C.3 Study Design

The SVR II study is a prospective follow-up study of an existing cohort of children with HLHS and other single RV anomalies enrolled in early infancy in a randomized clinical trial of Norwood procedure with MBTS versus RV-to-PA shunt.

C.3.1 Blinding

The ongoing SVR trial has an unblinded design with respect to knowledge of treatment assignment. Family members and the majority of personnel caring for the subjects will know which shunt an individual subject has had. The exceptions will be that Holter monitors and echocardiograms will be interpreted in the core laboratories by personnel who will not be aware of the original shunt assignment. Neurodevelopmental questionnaires will be scored objectively.

C.3.2 Study completion

Subjects will be considered to have completed the primary endpoint of the study if their vital status with respect to survival and cardiac transplantation is known at age 6 years. All subjects thought to be alive will continue to be followed by telephone contact for vital/ transplant status and medical history until the last subject has reached the SVR II study endpoint at six years post-randomization (only vital status will be collected after cardiac transplantation), and the primary findings of the study will be reported at that time. However, we hope to continue to follow this unique cohort beyond the SVR II study period. To provide continuity of follow-up during application for PHN or other funding for the next grant period (i.e., "SVR III"), the consent form is written to permit annual ascertainment of vital status and medical history of each subject to age 11 years (see Section C.2.5.a.3).

C.3.3 Subject withdrawal

Subjects may withdraw from participation in the study at any time. The reason for withdrawal and the circumstances of withdrawal will be documented for all subjects withdrawn from the study. Every effort will be made to obtain permission to continue to follow subjects to obtain vital/transplant status for the primary outcome variable at age 6 years.

C.4 Measurements

C.4.1 Schedule of measurements

Follow-up data for SVR II analyses will be obtained annually from 2 years post-randomization until the last enrolled subject is 6 years post-randomization, as well as in the period encompassing the Fontan procedure. The data to be obtained are summarized in Table 1.

Table 1. Schedule of Measurements

	2 Years	3 Years	Pre-Fontan	Post-Fontan	4 Years	5 Years	6 Years[6, 7]
Death or cardiac transplantation	X	X		X	X	X	X
Medical history	X	X		X	X	X	X
Echocardiogram (centrally read)			X[1,2]				X[1]
Heart Failure Class		X			X	X	X
Growth measurements	X	X			X	X	X
Cardiac catheterization			X[1]				
Electrocardiogram			X[1]				X[1]
24 hour Holter monitor							X[5]
Neurodevelopmental Questionnaires[3]		X			X	X	X
Biorepository[4]		X	X	X	X	X	X
Feasibility of Future Research	X[8]						

[1] Data to be collected if performed for clinical indication

[2] For pre-Fontan studies, echocardiograms will be submitted to Core Lab only if performed at a PHN site.

[3] Neurodevelopmental and related questionnaires will be completed at ages 3, 4, 5, and 6 years, except for the Vineland – II and CHQ-PF50, which will be administered only at age 6 years. The ASQ will not be administered at age 6 years.

[4] Blood or saliva may be collected at any study visit, but will typically be collected only once.

[5] Holter monitors are performed on all study subjects and analyzed in the Holter Core Laboratory.

[6] For reporting of main results of SVR II, all subjects will be followed until the last enrolled subject is 6 years post-randomization.

[7] To provide continuity of follow-up into the next study period (i.e., "SVR III"), the consent form is written to allow for annual contact for ascertainment of vital status and medical history of subjects to age 11 years.

[8] A survey to assess the feasibility of continued follow-up (i.e., "SVR III") will be completed once for each enrolled subject at any study visit.

C.4.1.a Data collection

Information will be collected annually by phone and mail on vital status, medical history, feeding information, objective cardiac status (e.g. measures of myocardial and valve function by echocardiogram, arrhythmias by Holter monitor) and neurodevelopment by questionnaires, as outlined in Table 1. At 6 years post-randomization, in addition to transplantation-free survival, we will also analyze the echocardiogram and electrocardiogram most recently performed for clinical indications, a 24-hour Holter monitor, and neurodevelopmental and related questionnaires, including the CHQ-PF50 and Vineland.

Echocardiograms and the Holter monitors will be analyzed in core laboratories blinded to original shunt assignment.

Data obtained at the pre-Fontan study visit will include a clinically-indicated electrocardiogram, echocardiogram and, in most cases, a cardiac catheterization. The pre-Fontan echocardiogram will be submitted for blinded review by the Echocardiographic Core Laboratory only if performed at a PHN site. The Fontan surgery will be scheduled according to local clinical practice. Following discharge from the hospital after the Fontan procedure, a medical history form that includes details of the post-operative course will be completed for each subject, regardless of where the Fontan is performed.

C.4.1.b Windows for Data Collection

- Routine data collection will occur annually from 2 to 5 years post-randomization, with a window of ± 3 months. Study time points post-randomization are essentially equivalent to subject age, since average age at randomization is 5 days.
- End of Study data collection will occur at age 6 years ± 6 months, with the exception of vital/transplant status for the primary endpoint, which must be known at a minimum of 6 years, the window for neurodevelopmental questionnaires, which will occur at age 6 to 6.5 years, and the window for the echocardiogram, which will occur at age 6 years ± 1 year.
- Pre-Fontan visit data will be included if within six months of the planned Fontan surgery.
- Post-Fontan data collection will include 30 days after surgery or until the first hospital discharge, whichever is longer.

C.4.2 Outcome Variables

- Primary outcome variable
    - Death or cardiac transplantation: The occurrence of death or transplantation will be ascertained through data collected at the time of planned study visits, phone contact, and review of medical records of the primary cardiologist, surgeon and study site. The cause of death will also be collected for secondary analyses. For the rare subject who might be lost to follow-up, we will search the Social Security or National Death Index.

- Secondary outcome variables
    - Medical History
        - Need for cardiovascular interventions (surgical or catheter-based)

- Inability to complete Fontan procedure by 6 years post-randomization
- Permanent pacemaker
- Arrhythmia requiring medication or intervention
- Thrombosis
- Stroke
- Seizure
- Protein losing enteropathy
- Cirrhosis
- Plastic bronchitis
- Other complications
- Medications
- Developmental history

- Post-Fontan hospitalization
  - Length of hospital stay
  - Complications: use of ECMO, open chest, cardiac arrest, stroke
  - Need for cardiovascular intervention during Fontan hospitalization (reoperation or transcatheter intervention)
  - Permanent pacemaker placement
- RV systolic and diastolic function assessed by echocardiogram
  - RV ejection fraction indexed to age (z-score)
  - RV fractional area change
  - Degree of tricuspid and neoaortic insufficiency
  - Largest neoaortic diameter
- Ross Heart Failure Class annually from 3 to 6 years post-randomization
- Growth measurements before Fontan and annually from 2 through 6 years post-randomization
  - Weight-for-age z-score
  - Height-for-age z-score
  - Weight-for-height z-score
- Rhythm disturbances
  - Incidence of ventricular or atrial arrhythmias by history, electrocardiogram, or 24-hour Holter monitor up to 6 years post-randomization

- Neurodevelopment and health-related quality of life (see Table 2 below for schedule of questionnaire completion)
    - Ages & Stages Questionnaires: (ASQ)
    - Behavior Assessment System for Children, Second Edition (BASC-2)
    - Pediatric Quality of Life Inventory (PedsQL)
    - Impact on Family Scale
    - Functional Status II-R (FSII-R)
    - Child Health Questionnaire-50 (CHQ-PF50)
    - Vineland Adaptive Behavior Scales, Second Edition (Vineland – II)

C.4.2.a Medical History

At each annual time point, the nurse coordinator will obtain a medical history from the parent or legal guardian, and a review of the medical chart will be conducted. Details regarding complications, medication use, feeding history and the occurrence of cardiovascular interventions (surgical or catheter-based) will be recorded. If necessary, we will obtain the medical record(s) from non-PHN centers providing care. At the post-Fontan time point, the medical records for the post-operative Fontan hospitalization will also be reviewed for operative course (e.g., type of Fontan performed; durations of total support, cross-clamping, circulatory arrest, and regional cerebral perfusion; first and lowest hematocrits on bypass, lowest temperature on bypass), post-operative complications, the need for postoperative cardiovascular interventions (e.g., catheterization or reoperation), total length of hospital stay, and readmission to the hospital within 30 days of Fontan surgery. To be able to assess gene-environment interactions in patients participating in the biorepository, for whom genetic testing may be conducted in the future, additional medical information that is not routinely available through medical records will be obtained from the family at enrollment and at subsequent visits. This includes a detailed pedigree and family history, environmental exposures, obstetric history, and neurodevelopmental history.

C.4.2.b Echocardiographic Measures of RV function (Appendix D)

Echocardiography is the standard imaging modality used to monitor cardiac function in the age group being studied. Echocardiograms will be analyzed for measures of RV function, degree of tricuspid and neoaortic regurgitation, and largest neoaortic diameter (Appendix D). Echocardiographic images in the SVR II study will be analyzed at two time points: pre-Fontan (within 6 months of surgery) and at 6 years post-randomization. The pre-Fontan

echocardiogram will be obtained from the PHN clinical sites only, as part of the expected clinical assessment prior to surgery and will be acquired according to optimal clinical standards which are expected to meet technical echo SVR II protocol guidelines. The post-Fontan echocardiogram will be performed by the cardiologist providing long-term care for the child (whether at a PHN site or another location) as part of routine clinical management of a patient with Fontan physiology; the timing of this study within the window of 6 years ± 1 year post-randomization will be at the discretion of the cardiologist. The SVR II study echo protocol will be provided to the local cardiologist to suggest methods for obtaining optimal echocardiographic data for clinical care in this complex population. The SVR II Echocardiographic Core Laboratory will analyze relevant data from the pre-Fontan echocardiogram when it is performed at PHN centers, as well as all echocardiograms at the 6-year time point regardless of where the study is performed. The SVR trial and SVR II study (which have the same echocardiographic core laboratory) will thus provide longitudinal assessment of RV function from the time of enrollment prior to the Norwood operation through Fontan palliation.

Echocardiographic outcome measures include:

1) RV volumes and ejection fraction: Two-dimensional echocardiographic measurements of RV volume and RV rejection fraction will be made by the core laboratory. Volumes will be calculated by the biplane pyramidal algorithm currently used in the main SVR trial, in order to compare to data obtained in the original trial. Because this technique requires subxiphoid imaging, which is not consistently available in older children, an additional technique for RV volume analysis will be obtained from RV short and long axis imaging using a biplane modified Simpson's rule; it is anticipated that this will increase the yield for RV volume assessment for the cohort. These methods are described in Appendix D, which includes information on *desired* image acquisition as well a description of core lab analysis methodology.
2) Fractional Area Change: Although volume data are most desirable and consistently applied to analysis of left ventricular function, the RV is more difficult to assess by two-dimensional echo because its shape does not conform to geometric modeling. Right ventricular area and percent area change have been used to assess RV global function [23] and are almost universally available and easily measured from standard two-dimensional echo imaging. RV fractional area change has been acquired in the first

phase of the SVR trial (SVR I) and will likely provide the highest yield assessment of serial RV function in the SVR II study.

3) Spectral Doppler imaging of the tricuspid regurgitant jet will be used to measure RV dP/dt as a gauge of ventricular function.
4) Doppler tissue imaging will be used to identify changes in myocardial velocities at the tricuspid annular ring as an additional measure of RV systolic and diastolic function.
5) Tricuspid and neoaortic insufficiency can adversely affect ventricular function and cardiac output; they will be assessed quantitatively from color Doppler interrogation of the regurgitant jet *vena contracta* from orthogonal views to calculate regurgitant orifice size.
6) Cardiac output can be assessed non-invasively as the product of the velocity time integral of the spectral Doppler envelope across the neoaortic valve and the neoaortic annulus orifice area in patients without significant contribution to cardiac output from the left ventricle. This calculation will be excluded for those who have antegrade flow across the native aortic valve.
7) Largest neoaortic diameter will be measured and is hypothesized to be larger in the MBTS group, in whom all RV output was directed into the neoaorta after initial palliation, compared to the RV-to-PA group, in whom systemic and pulmonary outputs were distributed directly from the right ventricle.

All measures will be adjusted for variation in body surface area or age to allow comparisons among subjects of differing sizes. The height and weight will be obtained at the time of each echocardiogram and body surface area will be calculated. The echocardiogram will be forwarded via the DCC to the Echocardiographic Core Laboratory for review.

C.4.2.c Heart Failure Class

The Ross Heart Failure Class for infants and children is comparable to the New York Heart Association (NYHA) heart failure class for adults.[42,43] Increasing Ross Heart Failure Class is associated with higher plasma norepinephrine levels and down-regulation of the beta-receptor density on cells in infants and children with congestive heart failure secondary to a large left-to-right shunt.[42,43] Four classes are defined by a composite assessment of respiratory effort, feeding difficulties and growth.

Ross' Classification of Congestive Heart Failure:

Class I: No limitations or symptoms

Class II: Mild tachypnea and/or diaphoresis with feeds in infants; dyspnea on exercise in older children. No growth failure.

Class III: Marked tachypnea and/or diaphoresis with feeds or exertion and prolonged feeding time with growth failure

Class IV: Symptomatic at rest with tachypnea, retractions, grunting or diaphoresis

The Ross Heart Failure Class will be determined by medical history and growth measurements.

C.4.2.d Growth measurements and feeding history

Weight and height measurements will be obtained from the medical record. These measurements will be translated into corresponding z-scores using data available from the National Center for Health Statistics of the Centers for Disease Control. The study medical history/interview form will also include information about feeding practices.

C.4.2.e Rhythm disturbances

The electrocardiogram performed pre-Fontan and at age 6 years for clinical purposes will be reviewed by the site investigator. We will record the atrioventricular conduction pattern, conduction intervals, including the presence of conduction delay, and the occurrence of arrhythmias. A 24-hour Holter monitor will be performed at 6 years post-randomization to document the occurrence of arrhythmias and heart rate variability patterns. Holter monitors will be mailed to families, mailed back to clinical centers, and then downloaded to a Holter Core Lab for blind interpretation. In addition to the 24-hour Holter monitors performed for research purposes at 6 years post-randomization, we will record the following information for each subject: 1) arrhythmia-specific procedures done at the time of the Fontan procedure; 2) the reasons and class indication (i.e., I, IIa, IIb, or III) for implantation of pacemakers; and 3) findings documented in reports of pre-Fontan Holter monitors that are obtained for clinical care. The presence of a scar in the site of the previous right ventriculotomy in patients who underwent their Norwood with an RV-to-PA shunt may lead to a right bundle branch block pattern and also may serve as a focus for ventricular arrhythmias. The protocol for core laboratory interpretation of Holter monitors is included in Appendix E.

C.4.2.f Neurodevelopment, Behavior, Health Related Quality of Life, and Impact on Family

Neurodevelopment, behavior, and quality of life are among the most important outcomes for children with HLHS and other single RV anomalies. A combination of seven instruments using parental report techniques will be used to assess these areas. Serial measurements with these instruments will be performed at 3, 4, 5, and 6 years post-randomization, though only a portion of the questionnaires will be completed at each of these time points (Table 2). We will mail questionnaires to families at the start of the window for the targeted age. In some cases, the study coordinator may be able to interview a parent at the time of the telephone contact to help complete an instrument. Approximately 60-90 minutes will be required for completion of all instruments at the 3, 4, and 5 year assessments. The 6 year assessment will require approximately 2 hours, including completion of the CHQ-PF50 and Vineland – II. All instruments will be mailed back to the individual study center, where the instruments will be reviewed and de-identified before being sent for data entry to the DCC.

Table 2. Schedule of Questionnaires on Neurodevelopment, Behavior, and Quality of Life

	3 Years	4 Years	5 Years	6 Years
ASQ (15 min)	X	X	X	
Vineland – II (60 min)				X
BASC-2 (15 min)	X	X	X	X
Peds-QL (15 min)	X	X	X	X
CHQ-PF50 (15 min)				X
FSII(R) (15 min)	X	X	X	X
Impact on Family (15 min)	X	X	X	X

C.4.2.f.1 Neurodevelopment assessment tools

For this study, neurodevelopmental level will be assessed using the Ages & Stages Questionnaires (ASQ) and the Vineland Adaptive Behavior Scales.

The ASQ will be used to measure developmental levels at 3, 4, and 5 years post-randomization. At each of these time points, five areas will be assessed: a) communication, b) gross motor, c) fine motor, d) problem solving, and e) personal social. The ASQ instruments are each composed of 30 items written at the 4th-6th grade level and will require 10-20 minutes for completion. Illustrations are included to facilitate parent understanding.

Each item is scored depending upon whether the child performs the item consistently (10 points), sometimes (5 points) or not yet (0 points). Scores for each area are then summed.

Area scores can be analyzed as continuous variables based on the normative means and standard deviations defined for each area at the age being tested.[44] In addition, based on the published means and standard deviations, threshold cut-offs have been established for each tested area to define if a child's score is outside the normal range. Preliminary scoring of the ASQ will be performed at each study center. If a study participant is found to be outside the normal range for one or more areas, the parent(s) and the pediatrician or pediatric cardiologist will be notified.

The ASQ instruments have been validated with repeated testing with test–test reliability of 94% (SEM 0.10)[44] and have been validated with comparison to standard infant childhood tests including the Bayley Scales of Infant Development and the McCarthy Scales of Children's Abilities.[45, 46] The ASQ is simple to complete and is particularly useful in identifying significant delays; thus, the ASQ should provide an excellent measure of development in this high risk group.

Because the ASQ cannot be administered at age 6 years, the Vineland Adaptive Behavior Scales, Second Edition (Vineland – II) will be used to measure developmental level at 6 years post-randomization. This instrument assesses three domains of development by parental report: a) communication skills, b) skills of daily living, and c) socialization. Information from these three domains is also combined to obtain the adaptive behavior composite score. Completion of the Vineland will be performed using the parent/caregiver rating form. Completion of this instrument by a parent or caregiver will require approximately 60 minutes.

C.4.2.f.2 Behavior Assessment System for Children, Second Edition (BASC-2)

The BASC-II is a parent report measure of behavioral symptoms for children that is used both to measure adaptive and abnormal behaviors present in the home and at school. It includes 14 subscales which compose 4 composite scores: Externalizing Problems, Internalizing Problems, Behavioral Symptoms Index, and Adaptive Skills. The BASC-2 is an excellent tool for identifying attention deficit disorder. This tool has been used serially to assess behavior in other groups of children with chronic illness.[47] The BASC-2 Serial will be completed at 3, 4, 5, and 6 years post-randomization. As with the ASQ, the BASC-2 will be mailed or provided in clinic at the start of the window for the target age. Completion of this instrument by a parent or guardian will require only 10-20 minutes.

Following completion of the BASC-2, the instrument will be returned to the participant's specific center for de-identification. Scoring will be performed at the DCC. Each of the four composites will be assessed based on the standard population mean of 50 points and a standard deviation of 10 points. Scores outside of the normal range will be reported to the study center using the participant identification number. The center investigator will contact the family and the pediatric cardiologist or pediatrician regarding scores outside of the normal range.

C.4.2.f.3 Quality of Life Assessments

Quality of life measurement will be performed using both the Pediatric Quality of Life Inventory (Peds-QL) and the Child Health Questionnaire-Parent Form 50 (CHQ-PF50).

The Peds-QL will be used to measure health related quality of life at 3, 4, 5, and 6 years post-randomization. This is a parent report instrument designed to assess quality of life in both healthy and acute or chronically ill children.[48, 49] The Peds-QL system provides a general quality of life assessment which addresses physical functioning, emotional functioning, social functioning, and school functioning for children 2 to 18 years old. In addition, a cardiac disease specific module assesses issues of quality of life specific to children with cardiac disease. This system provides instruments for 2-4 year olds, and for 5-7 year olds. At 3 and 4 years, the 2-4 year old instruments will be used. At 5 and 6 years, the 5-7 year old instruments will be used. Each of the instruments includes 23 items. Together, the general Peds-QL and the cardiac-specific module will require 15 minutes to complete.

The CHQ-PF50 will be completed at 6 years post-randomization. This instrument is valid for assessment of quality of life measurements, including physical and psychosocial well-being, for children age five years and older. The Parent Report form includes 50 items that are used to assess 14 concepts (physical functioning, physical role or social limitations, general health, bodily discomfort, parental impact, emotional role or social limitations, self esteem, mental health, general behavior, family, and change in health. This instrument will require approximately 15-20 minutes for completion. The CHQ-PF50 has been used to assess quality of life outcomes for children following the Fontan operation in a previous PHN study; use of the CHQ-PF50 in the SVR II study will allow us to compare outcomes with those in this older group of Fontan patients, as well as with published CHQ-PF50 outcomes in other diagnostic groups.[34, 50, 51]

C.4.2.f.4 Functional Health Status

Functional health status will be assessed at 3, 4, 5, and 6 years with the Functional Status II(R) [FSII(R)]. This instrument is a parent self-report questionnaire that can be used to assess health status in children with chronic health conditions. The FSII(R) assesses the degree of daily age-appropriate function and is being used to assess functional status at the 14 month visit in the SVR Trial. The instrument provides a Total Score with common elements across all ages, in addition to age-specific domain scores. Serial measurements will elucidate tracking of functional health status in this high-risk group of children.

C.4.2.f.5 Impact on family

Caring for a child with chronic illness may impact families in a variety of ways. The Impact on Family is a self report tool which provides information for four specific areas: a) Financial, b) Familial Social, c) Personal Strain, d) Mastery-Positive Effects. To assess the overall impact on families of caring for a child with single ventricle, parents will be asked to complete the Impact on Family when the study participant is 3, 4, 5, and 6 years post-randomization. Completion of this instrument will require approximately 15 minutes. After completion of this instrument, the questionnaire will be mailed to the study center for de-identification prior to return to the DCC.

C.4.2.g Biologic Specimen Repository (Biorepository)

A suitable facility for storage of samples will be selected through the PHN's standard competitive process for core laboratories. Samples will be acquired from subjects, and both parents when possible, in accordance with Best Practices for Biospecimen Resources.[53] Biorepository data will be linked with the SVR II electronic database, which will include subject demographics; cardiac phenotype, interventions, and outcomes; neurodevelopmental outcomes; and family pedigree and medical history.

C.4.2.g.1 Blood or saliva specimen acquisition

Blood specimens will be drawn by the clinical site nurse or physician in conjunction with routine blood draws and procedures where possible. No more than 5 ml per 5 lb body weight up to a maximum of 20 ml will be drawn. Blood is preferred and will be obtained if at all possible. However, if this is not possible, and saliva collection is feasible, 2-4 ml of saliva will be collected in saliva kits. This can be done during hospital visits or by mail. If either

type of sample is unusable for technical reasons, a repeat sample may be requested at a convenient time for the family.

In the SVR trial, we obtained genomic DNA from a buccal swab for subjects whose parents consented to this genetics component of the study. DNA not used in this aim has been banked at the SVR DNA Core Laboratory. Any remaining DNA that is suitable for future genetic studies will become part of the SVR II biorepository if the family agreed to future study in the SVR trial consent form.

In the SVR II study, we will attempt to obtain blood from all subjects and their parents, regardless of whether a buccal swab was obtained previously. Ideally, we will obtain sufficient blood to acquire a serum sample as well as to permit extraction and storage of DNA. Some subjects who will be in SVR II study were also in the PHN Infant Single Ventricle study and may have had blood drawn for genotyping. If the consent permits, and blood or DNA is available, we will attempt to use this material rather than require another specimen collection. Banked serum will be used for the study of potential molecular biomarkers of disease and outcome.

C.4.2.g.2 Specimen processing and shipment

Following acquisition, specimens for this study will be processed as necessary at the local clinical site, then packaged for shipping to the central biorepository. For each shipment sent, clinical sites will complete a shipping notification form, shipping log and a specimen information form.

C.4.2.g.3 Biorepository procedure

The biorepository and the DCC will follow standard procedures for ensuring appropriate identification, processing, aliquotting, and storage of specimens. Specimens will be tracked in ADEPT and in the repository database.

C.4.2.g.4 Biospecimen Committee

A Biospecimen Committee will be formed to develop and oversee procedures for reviewing applications for use of samples, and to explore options for external collaborations and funding sources. The Advisory Committee membership will be determined by the PHN Executive Committee and will include PHN investigators, a representative of NHBLI, and *ad hoc* members with appropriate expertise in fields relevant to the biorepository, such as

genetics or bioinformatics. The Biospecimen Committee will ensure compliance with best practices for repositories as published by the International Society for Biological and Environmental Repositories[52] and the National Cancer Institute's Office of Biorepositories and Biospecimen Research (OBBR)[53].

C.5 Statistical Analysis

C.5.1 Primary Endpoint for this Study

This protocol will extend the follow-up of the SVR trial subjects to a minimum age of 6 years. The primary hypothesis for this study is that the RV-to-PA shunt will continue to have a lower incidence of the combined endpoint of death or cardiac transplantation at 6 years post-randomization compared to the MBTS.

C.5.2 Sample Size and Power Analysis

C.5.2.a Primary endpoint

The primary endpoint event rates (death or transplant by one year) assumed for the main trial were 28% vs. 16% for the MBTS and RV-to-PA shunt groups, respectively, which, with 271 subjects per arm (prior to inflation for interim looks at the primary endpoint), provided 80% power using a two-sided test with Type I error rate of 0.05 and assuming a blinded crossover rate based on data accrued two years after trial initiation (rate not specified here). This hypothesized difference was 12% in absolute size and a 0.43 relative reduction in death or transplant for the RV-to-PA group relative to the MBTS group. The sample size calculation was based on a smaller rate difference than 12%, resulting from calculation of weighted rates that incorporate study crossover, which cannot be reported here since crossover is a trial outcome.

It is now assumed that the 6-year event rate will have an absolute 5% increase, to 33% for the MBTS group, and that 6-year status will be obtained on 98% of subjects (265 per arm). Because the main trial is not yet complete and interim findings are blinded, the true event rate is unknown; and scenarios 5% and 10% below and above the estimated rate of 33% are also shown in Tables 3 and 4. Table 3 shows that if the MBTS event rate at 6 years does not exceed 28%, there is at least 79% power to detect a 12% absolute difference. If the MBTS event rate at 6 years is $\geq$33%, power is below 70%. It should be noted that the lower power in these scenarios is due to the fact that the 12% absolute rate difference

results in much smaller relative reductions in the event rate (0.28 to 0.36 relative reduction instead of 0.42). Table 4 displays scenarios that all reflect a 0.43 relative reduction from the range of assumed MBTS 6-year event rates. There is ≥ 79% power to detect a 0.43 relative reduction in all scenarios shown except the extreme setting where the MBTS event rate at 6 years is only 23%.

Table 3. Power to Detect an Absolute 12% Difference in Death/Transplant Rates at 6 years with 265 subjects per arm using a Two-Sided Test and Type I error of 0.05 with assumption of blinded crossover rate

p_1^*	p_2^*	% Reduction	Power
.43	.31	.28	66%
.38	.26	.32	69%
.33	.21	.36	73%
.28	.16	.43	79%
.23	.11	.52	86%

*Rates shown are prior to dilution imposed by blinded crossover rates;
power calculation is based on rates that result in a smaller %reduction than shown.
p_1 and p_2 are the 6-year death/transplant rates in MBTS and RV-to-PA arms, respectively.
%Reduction shown is $[(p_1-p_2)/p1]x100$.

Table 4. Power to Detect a Relative 42% Reduction in Death/Transplant Rates at 6 years with 265 subjects per arm using a Two-Sided Test and Type I error of 0.05 with assumption of blinded crossover rate

p_1^*	p_2^*	Rate Difference	Power
.43	.25	.18	96%
.38	.22	.16	92%
.33	.19	.14	87%
.28	.16	.12	79%
.23	.13	.10	68%

*Rates shown are prior to dilution imposed by blinded crossover rates;
power calculation is based on rates that result in a smaller rate difference than shown.
p_1 and p_2 are the 6-year death/transplant rates in MBTS and RV-to-PA arms, respectively.
Rate Difference is p_1-p_2.

C.5.2.b Secondary endpoints

From a survey conducted at all the participating SVR trial sites, it is estimated that 50% of randomized subjects will be alive and able to be contacted at age 6 years for secondary endpoint data collection. For power calculations, instead of using hypothesized death rates to estimate subject availability, we therefore assume 271 subjects (half total target N for the original trial prior to inflation for interim looks) might have secondary endpoint data.

Assuming that the treatment difference is as hypothesized above, the MBTS:RV-to-PA shunt ratio will be approximately 0.85 (e.g., 125 MBTS and 146 RV-to-PA shunt subjects). Some secondary outcomes such as parent-report questionnaires can be completed even if the family does not return to the PHN center. Other secondary endpoints, such as RV ejection fraction, may have a smaller sample size due to subjects not returning to the PHN center (pre-Fontan), or due to technical limitations in obtaining RV volumes. Therefore, scenarios of 40% (n=217) and 30% (n=163) of randomized subjects are also considered in power calculations for secondary endpoints (Table 5).

Minimum Detectable Mean Difference for Secondary Endpoints

- CHQ-P50 and BASC-2: Based on data from the PHN Fontan Cross-Sectional Study restricted to subjects with HLHS under age 9 years, the standard deviations of the CHQ Physical and Psychosocial summary scores are 12.0 and 10.4, respectively. Table 5 demonstrates that there is 85% power to detect a mean group difference of 3.7 to 4.3 points even if only 40% of all randomized subjects contribute data. Given that normative data from the CHQ suggest that 5 to 10 point differences represent true disease effects, detection of a 4 point difference is appropriate for differences between shunt types within a cohort with congenital heart disease. Mean detectable differences for the BASC-2 are expected to be similar to that for the CHQ summary scores, since the standard deviation in healthy children is 10 and therefore may be 10-12 in a sample of children with heart disease.

- FSII(R): Based on data from the SVR Trial subjects at 14 months, the standard deviation of the FSII(R) Total Score is 9 and differences of 3-4 points are detectable with 85% power for all three sample size scenarios. This half standard deviation mean difference is typically considered clinically relevant.

- RV function

  Based on data from the PHN Fontan Cross-Sectional Study restricted to subjects with HLHS under age 9 years, the standard deviations of (total) ejection fraction z-score and RV ejection fraction (raw %) are 2.4 and 12, respectively. It is likely that the highest sample size scenario will not be realized for echocardiographic results. Assuming 217 or 163 subjects with RV ejection fraction, Table 5 indicates that there will be 80% power to detect a 0.9 to 1.0 mean difference in RV ejection fraction z-score, and 5% absolute mean difference in raw RV ejection fraction between shunt types.

Based on data from the SVR Trial subjects at 14 months, the standard deviation of RV fractional area change is 7 and a mean absolute difference of 3% is detectable with 85% power for the two lower sample size scenarios. Therefore, there is good power for this endpoint, as a half standard deviation mean difference is typically considered clinically relevant.

It should also be noted that this relatively large sample will also provide reasonable power (>70%) to detect significant subgroup interactions with shunt type, assuming that the subgroups are of roughly equal size and subgroup differences are of at least one-half of a standard deviation. Finally, the neurodevelopmental instruments will be administered annually, and this sample will provide good power to detect shunt type by time interactions using longitudinal modeling.

C.5.3 Analysis Plan

C.5.3.a Primary analysis of the primary endpoint (Primary Aim A.1)

All primary analyses will be performed on an intention-to-treat basis. However, as in the original SVR Trial protocol, if a subject undergoes no palliation at all, then the subject will be excluded from primary analysis if adjudication demonstrates that the reason for not undergoing surgery was independent of shunt assignment. The proportion of MBTS vs. RV-to-PA subjects experiencing the primary clinical composite endpoint of death or transplantation at age 6 years will be compared using a Wald test comparing the pointwise estimates of 6 year transplant-free survival from the estimated Kaplan-Meier survival curves, regardless of the actual treatment received. This analysis will produce nearly the same result as a Fisher exact test comparing the two proportions, but will accommodate the very small number of subjects who may be lost to follow-up before 6 years by incorporating their

Table 5. Minimum Mean Detectable Difference Δ between Groups using a Two-Sided level α=0.05 Two-Sample t-test and MBTS: RV-to-PA group size ratio of 0.85

Standard Deviation	Total N	Δ detectable with Power: 85%	80%	75%
2.4	271	0.88	0.82	0.77
	217	0.98	0.92	0.86
	163	1.13	1.06	0.99
7	271	2.56	2.39	2.25
	217	2.86	2.67	2.52

	163	3.30	3.08	2.90
9	271	3.29	3.08	2.89
	217	3.68	3.44	3.23
	163	4.24	3.97	3.73
10.4	271	3.89	3.56	3.35
	217	4.25	3.97	3.74
	163	4.90	4.58	4.31
12	271	4.39	4.10	3.86
	217	4.90	4.59	4.31
	163	5.65	5.29	4.97

partial follow-up. A comparison of the composite rate of death or transplantation at 3 years, an age by which most subjects will have undergone the Fontan procedure, will also occur as an interim analysis (see Section C.5.3.d), in order to provide timely dissemination of results to the field prior to 2014. In addition to comparison of specific rates, the overall comparison between the two groups using the Kaplan-Meier method for estimation and the log-rank test will also be performed to determine if time to death or transplantation is different between the two surgical strategies, even if no difference is observed in the overall comparison of proportions at 6 years post-randomization. The Kaplan-Meier analysis will include a maximum of 9 years follow-up (for the first subject randomized into the SVR Trial) and a mean potential follow-up time of 7.5 years per subject. To identify differences in time to death/transplant according to time since randomization, weighted log rank statistics will also be used for comparison of the transplant-free survival curves by shunt type. For example, a Gehan-Wilcoxon test will have greater power to detect treatment differences that occur early after surgery than will a log rank test. Cox proportional hazards modeling can also be used to compare the event-free distributions by shunt type; a time-dependent treatment indicator will be used to alter the proportional hazards assumption and to determine if the size of the treatment difference varies by time.

C.5.3.b Secondary analysis of the primary endpoint

In addition to the intention-to-treat analysis, secondary analysis of the primary endpoint will be performed

1) by comparison of the groups according to the treatment actually received (non-intention-to-treat) and
2) by comparison of groups after the exclusion of any subjects randomized who were found after randomization to be trial ineligible at the time of enrollment. It is expected that the most common reason for trial ineligibility following enrollment will be due to anatomic

findings that are discovered during the operation that precluded performing either the RV-to-PA shunt or the MBTS. If for some reason a subject did not undergo surgery at all, the subject will be excluded from the secondary analysis as well. This secondary analysis will provide important information regarding treatment efficacy.

C.5.3.c Analyses of secondary endpoints

All primary analyses of secondary endpoints will also be performed according to the intention-to-treat principle as described in Section C.5.3.a. Secondary analysis of secondary endpoints will be performed as described for the primary study endpoint in Section C.5.3.b. Methods for analysis of secondary endpoints will be determined by the specific secondary endpoint.

All analyses of the secondary endpoints that are not time-to-event variables are conditional: if the subject dies or is transplanted before age 6 years, the analyses will be based on the non-transplanted survivors. Sensitivity analyses may also be conducted to assess the impact of loss due to death and transplant on observed differences (or lack thereof) by shunt type, by imputing a last-observed vs. 'worst-case' value. This approach would mitigate the potential finding that one type of shunt may have a higher associated mortality, leading to a healthier cohort of survivors, demonstrated by better cardiac and neurodevelopmental status than the opposite shunt. However, for purposes of management and targeting interventions to address post-Fontan status, the results of the conditional analysis (i.e., based only on survivors) may be preferable.

Primary Aim A.1: Time-to-event outcome measures other than death and transplant include freedom from surgery and interventional cardiac catheterization procedures. This outcome can be analyzed independently, or a competing risks analysis of death vs. transplant vs. surgery or interventional catheterization procedures may be conducted to obtain unbiased cumulative incidence rate estimates of these events. For the 'landmark' analysis of death/transplant conditional on survival to one year, the methods employed will be as described in Section C.5.3.a.

Secondary Aim A.2: For continuous, reasonably normally distributed endpoints such as RV ejection fraction, RV fractional area change, and growth z-scores, Student's t-test will be used to compare group means from the pre-Fontan and age 6 year echocardiograms. If the distribution of any of these outcomes is highly skewed, a Wilcoxon rank sum test or a t-test

will be applied to transformed data. A Mantel-Haenzel test for linear trend and a Fisher exact test (to detect potential nonlinear differences in the distributions) will be used to compare the distributions of tricuspid regurgitation grade and Ross heart failure class by shunt type at each time point (pre-Fontan, 6 years). Because growth measures and heart failure class will be assessed four time points, longitudinal regression models will be used to examine treatment differences over time in these outcomes.

Secondary Aim A.3: The distributions of time to hospital discharge post-Fontan will be compared using the Kaplan-Meier method. By definition, any subject who does not undergo the Fontan procedure will be excluded from analysis of this endpoint. As a secondary analytic approach, the distributions of length of stay following the Fontan procedure for the two treatment groups will be compared using a t-test after log transformation of this outcome, as length of stay is known to be positively skewed.

Secondary Aim A.4: The 6-year rates of ventricular and atrial arrhythmias by treatment group will be compared using a Fisher exact test. These outcomes may also be analyzed using time-to-event methodology, although the diagnoses made as a result of protocol-mandated Holter monitoring at six years may document the existence of an arrhythmia that was present earlier.

Secondary Aim A.5: A nonparametric test will be used to analyze discrete data, such as the domain or summary scores of neurodevelopmental tests that increase in multiple-point increments (e.g. Ages and Stages). The primary analysis of neurodevelopmental scores will include data from instruments acquired within the validated windows (described in the study MOO). For the neurodevelopmental instruments with more than two time points available, longitudinal mixed models will be employed we will test for the presence of a shunt type by time interaction. However, we recognize that the interpretation of these analyses will be conditional and based on only those subjects who have the data for the multiple time-points. Test scores will also be compared against normative values, regardless of treatment group.

Secondary Aim A.6: This aim involves the development of risk stratification models for late outcome in this cohort. This study will be well equipped to develop such models given the long follow-up and wealth of carefully collected sociodemographic, genetic (e.g., ApoE), therapeutic, and clinical data from the time of birth onward. For a dichotomous endpoint of death/transplant at 6 years, logistic regression will be used. For analysis of all available

follow-up, Cox proportional hazards modeling will be employed to analyze long-term death/ transplant, with the use of stratified modeling for factors that are not of interest as correlates and impose differing baseline hazards. Time-dependent covariates will be used to accommodate any factors that are of predictive interest but do not meet the proportional hazards assumption. Models will also be developed for other study outcomes measured at 6 years, such as neurodevelopmental or behavioral summary score, or measures of cardiac function. These models will utilize linear regression. The impact of selected therapies, such as early intervention programs or interstage home monitoring, on late outcome can also be assessed by the model, but need to account for potential selection biases. Propensity score modeling can first be utilized to predict the likelihood of receiving early intervention based on a multivariate set of factors. The propensity score for each subject will then be included (or used as a stratification variable after classification into quantiles) in the time-to-event or logistic regression modeling of death/transplant to ensure that causal inferences made regarding the impact of early intervention are not biased. Interaction terms will be used in modeling to assess whether certain patient subgroups have differing sets of significant risk factors associated with long-term outcome. Also of note, the risk stratification models will use patient variables from birth through age 14 months; the models that incorporate 14 month findings (i.e., neurodevelopmental test scores or RV ejection fraction) will by definition include only subjects who have survived without undergoing transplant by age 14 months. Lastly, it may be of interest to explore mechanistic hypotheses by developing models that use outcomes and identify correlates of study measurements that all obtained at 6 years.

C.5.3.d Interim monitoring

Efficacy: The last SVR Trial subject is expected to enroll and be randomized to an MBTS or RV-to-PA shunt in mid 2008. Enrollment in the SVR II study will occur between early 2008 and 2010. Given the follow-up nature of this study, an early stopping procedure for efficacy is not indicated. However, the data will be analyzed when the last enrolled subject has completed the 3-year contact, in order to disseminate outcome information on the long-term relative efficacy of the MBTS and RV-to-PA shunt. After these results are reported, follow-up will continue and final results of the SVR II study will be reported when the last subject enrolled has completed the 6 year contact. It should be noted that the primary endpoint of the original SVR Trial was death or transplantation by one year post-randomization. All other endpoints based on later follow-up data, including but not limited to the 3-year and 6-

year endpoints, are secondary. There will be no adjustment to the p-value for the treatment comparisons of secondary endpoints. It is possible that the results of the 1, 3, and 6-year treatment comparisons may vary with respect to which shunt appears efficacious. The one-year comparison will reflect whether the SVR Trial yielded a positive result with respect to the original primary hypothesis for this investigation. However, the comparisons of longer-term outcome are also of interest and the findings have different clinical implications for management of this patient population.

Safety: The nature of the study tests included in this proposal make it unlikely that the DSMB will stop the study for safety concerns. The DSMB reports will include summaries of accrual, subjects characteristics, adverse events, frequency of protocol violations, data quality, primary and secondary endpoints and other information as requested or arise as a result of unanticipated problems that arise during the study.

C.5.3.e Subgroup Analyses

To determine if the effect of the RV-to-PA shunt differs from the MBTS across subgroups, separate comparisons by shunt type will be made of the primary and secondary outcomes within the following subgroups:

- Birth weight: < 2500 g vs. ≥ 2500 g
- Pre-Norwood tricuspid regurgitation: Proximal jet width < 2.5 mm vs. ≥ 2.5 mm determined by core laboratory echocardiographic analysis
- Type of cerebral perfusion during Stage I palliation: Deep hypothermic circulatory arrest vs. regional cerebral perfusion. If a subject was administered both types of cerebral perfusion during surgery, the subject will be classified as having undergone deep hypothermic circulatory arrest.
- Experience of the surgeon: Average number of Norwood procedures performed on randomized subjects per year (as a continuous variable), and classified as ≤ 5, 5-10, 11-15, >15 procedures per year.
- Center volume: Average number of Norwood procedures performed on randomized subjects per year (as a continuous variable), and classified as ≤ 10, 11-25, 26-40, >40 per year.

C.6 Data Management

An Electronic Data Capture (EDC) system will be used for the study that is designed to support reliable and secure data entry for clinical research purposes. The system also provides seamless integration of electronic Case Report Forms (eCRF), implementation of protocol amendments, and SAS and XML study data exports.

C.6.1 Data Entry

Data can be entered directly from multiple study sites via a fully validated and 21 CFR Part 11 compliant, secure Web application and stored centrally. A configurable sample-based double data entry system is available. Data are entered by subject study identification number; names will not be linked with subject data in the database. Study sites will maintain records in secure areas linking the subject name with the identification number assigned for the study. Study sites will have full access to their own data and be able to view these data remotely. Study staff will not be able to view subject data associated with other sites.

C.6.2 Data Validation and Monitoring

Integrated into the data entry system are real time validations, including both inter- and intra-instrument data checks. Inconsistent or questionable values are flagged during entry, and an edit report is automatically generated to the data entry client. These edit reports provide the information necessary to investigate any data entry errors or resolved questions regarding out-of-range or questionable values. Second-level query tracking allows monitors and data managers real time access to unresolved queries as well as the date and time of query generation and resolution.

C.6.3 Data Security and Integrity

All data changes are written to an audit trail. The audit trail identifies the data item by table, column and key field. The entry includes the user, date and time, as well as the old value and new value. Both subject related data as well as trial configuration data are written to the audit trail. Data are saved at regular intervals during data entry to prevent loss of information in the event of a disruption of the Internet connection. In the unlikely event of a major disruption, a backup connection allows full access to the Data Management System (DMS).

Several levels of security are employed to ensure privacy and integrity of the study data, including the following:

- Study access requires use of assigned user names and passwords.
- Individual roles and access levels are assigned by the study data manager.
- Passwords are changed regularly.
- Web-based entry uses secure socket layer (SSL) data encryption.
- Data will not be stored on laptop computers.

C.7 Quality Control

This section describes the quality control program that will be implemented as part of the study to ensure standard implementation of the protocol, protocol compliance, and data integrity. The DCC will develop and update the Manual of Operations in collaboration with study investigators and Core Laboratory directors. The manual will serve as both training and reference manuals and will be accessible on PHN administrative website.

C.7.1 Clinical Center Coordinator Training

The DCC Project Director, in collaboration with the study PI will provide central training of clinical center staff in the areas of protocol implementation, data collection and management, collection and handling of imaging studies and biologic specimens, medical records abstraction, and quality control expectations. Training slides will be prepared that reflect clearly and succinctly the learning goals for clinical coordinators and represent the skills and protocol components required to collect quality data. Training follow-up will be completed through conference calls and site visits.

C.7.2 Certification of Personnel

Echocardiography personnel at each center will undergo sessions on standardization of technique as recommended by the Core Laboratories. All echocardiograms will be read in a Core Laboratory. Quality evaluations by the echo core lab will only be recorded for the pre-Fontan echo due to the fact that these will be acquired at PHN clinical centers only. The 6 year echo will also undergo quality evaluation by the core lab, but because these will be acquired and obtained from both PHN and non-PHN clinical centers, the assessments from the echos obtained at non-PHN centers will not be binding indicators of center performance or protocol compliance. Placement of Holter monitors is a standard clinical procedure that is not operator dependent. Therefore, we do not believe that standardization sessions for local center personnel are needed.

C.7.3 Data Monitoring/Site Visits

Each clinical site will be visited at least once by representatives from the DCC and the NHLBI during the overall SVR Trial and Extension study period. The primary roles of the site visit team will be to evaluate general protocol compliance and adherence to IRB requirements, review site data files for correct filing of copies of consent forms and study forms, audit a random sample of records to assess data integrity, and identify and resolve general problems with study progress. At each site visit, the site monitor will review procedures, observe form completion and data entry (where applicable), and assess adherence to protocols and flow. A random sample of medical records will be reviewed in order to determine whether reporting of data has been accurate and complete. Follow-up actions by the site coordinator or investigator and a schedule for completion will be identified at each site visit. An evaluation checklist will be completed at each site visit for inclusion in a Site Visit Report to the investigators. New staff will be trained and existing staff will be retrained, if necessary. Site coordinators will be expected to provide materials and answer questions prior to and during these visits.

After completion of the SVR Trial, data monitoring for the Extension Study will be conducted remotely by the DCC. Each study site will undergo remote monitoring at least once during the course of the Extension Study period. A data audit will be conducted by comparing data entered in study forms to data in source documents for a random sample of study subjects. To conduct the audit, sites will be asked to submit copies of the CRFs and related appropriately de-identified source documents to the DCC. Any resulting discrepancies will be forwarded to the study team for resolution. All findings will be presented in writing to the study team in a Summary Report. The Summary Report will also be provided to the NHLBI Program Office.

C.7.4. Quality Assurance Related to Neurodevelopment, Behavior, and Health Related Quality of Life Instruments

Each of the instruments in the schedule of measurement is validated as a parental report tool. The study Manual of Operations will include a section dedicated to test administration, including but not limited to contacts to be made with parents regarding each instrument, encouragement of the same parent to complete instruments serially, and standardized scripts to guide parents through instruments when required (i.e. parent with limited reading skills). Training via conference call including review of this manual will be provided for study coordinators prior to study initiation.

D. Study Limitations

- Some secondary outcome findings are conditional on the characteristics of the cohort who returned to the study centers, whose representativeness of all children with HLHS is unknown.
- This study may be underpowered to detect differences by shunt type in secondary endpoints.
- The original study design did not include a control group. A normal control group is not necessary to evaluate the primary hypothesis of the trial, involving a comparison transplantation-free survival of children with single ventricle palliated with a Norwood procedure with MBTS vs. RV-to- PA conduit.
- The current extension study involves neurodevelopmental testing. We believe that the nationally-representative standardization samples for the neurodevelopmental, behavioral and quality of life questionnaires used in this study provide an unbiased source of information about the expected neuropsychological status and quality of life of young children with single ventricle who undergo staged palliation.

E. References

(1) Norwood WI, Lang P, Casteneda AR, Campbell DN. Experience with operations for hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg* 1981;82(4):511-9.

(2) Pigott JD, Murphy JD, Barber G, Norwood WI. Palliative reconstructive surgery for hypoplastic left heart syndrome. *Ann Thorac Surg* 1988;45(2):122-8.

(3) Iannettoni MD, Bove EL, Mosca RS et al. Improving results with first-stage palliation for hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg* 1994;107(3):934-40.

(4) Daebritz SH, Nollert GD, Zurakowski D et al. Results of Norwood stage I operation: comparison of hypoplastic left heart syndrome with other malformations. *J Thorac Cardiovasc Surg* 2000;119(2):358-67.

(5) Mahle WT, Spray TL, Gaynor JW, Clark BJ, III. Unexpected death after reconstructive surgery for hypoplastic left heart syndrome. *Ann Thorac Surg* 2001;71(1):61-5.

(6) Jayakumar KA, Addonizio LJ, Kichuk-Chrisant MR et al. Cardiac transplantation after the Fontan or Glenn procedure. *J Am Coll Cardiol* 2004;44(10):2065-72.

(7) Sano S, Ishino K, Kawada M et al. Right ventricle-pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg* 2003;126(2):504-9.

(8) Pearl JM. Right ventricular-pulmonary artery connection in stage 1 palliation of hypoplastic left heart syndrome. *J Thorac Cardiovasc Surg* 2003;126(5):1268-70.

(9) Pizarro C, Norwood WI. Right ventricle to pulmonary artery conduit has a favorable impact on postoperative physiology after Stage I Norwood: preliminary results. *Eur J Card Thorac Surg* 2003;23(6):991-5.

(10) Pizarro C, Mroczek T, Malec E, Norwood WI. Right Ventricle to Pulmonary Artery Conduit Reduces Interim Mortality After Stage 1 Norwood for Hypoplastic Left Heart Syndrome. A*nn Thorac Surg* 2004;78(6):1959-64.

(11) McGuirk SP, Griselli M, Stumper O et al. Staged surgical management of hypoplastic left heart syndrome: a single-institution 12-year experience. *Heart* 2005;Epub ahead of print.

(12) Sano S, Ishino K, Kado H et al. Outcome of Right Ventricle-to-Pulmonary Artery Shunt in First-Stage Palliation of Hypoplastic Left Heart Syndrome: A Multi-Institutional Study. A*nn Thorac Surg* 2004;78(6):1951-8.

(13) Bartram U, Grunenfelder J, Van Praagh R. Causes of death after the modified Norwood procedure: a study of 122 postmortem cases. *Ann Thorac Surg* 1997;64(6):1795-802.

(14) Lee JR, Choi JS, Kang CH, Bae EJ, Kim YJ, Rho JR. Surgical results of patients with a functional single ventricle. *Eur J Cardiothorac Surg* 2003;24(5):716-22.

(15) Jacobs ML, Norwood WI. Fontan operation: influence of modifications on morbidity and mortality. *Ann Thorac Surg* 1994;58(4):945-51.

(16) Nakano T, Kado H, Ishikawa S et al. Midterm surgical results of total cavopulmonary connection: clinical advantages of the extracardiac conduit method. *J Thorac Cardiovasc Surg* 2004;127(3):730-7.

(17) Kumar SP, Rubinstein CS, simsic JM, Taylor AB, Saul JP, Bradley SM. Lateral tunnel versus extracardiac conduit Fontan procedure: a concurrent comparison. *Ann Thorac Surg* 2003;76(5):1389-96.

(18) McGuirk SP, Winlaw DS, Langley SM et al. The impact of ventricular morphology on midterm outcome following completion total cavopulmonary connection. *Eur J Cardiothorac Surgery* 2003;24(1):37-46.

(19) Alphonso N, Baghai M, Sundar P, Tulloh R, Austin C, Anderson D. Intermediate-term outcome following the fontan operation: a survival, functional and risk-factor analysis. *Eur J Cardiothorac Surg* 2005.

(20) Hosein RB, Clarke AJ, McGuirk SP et al. Factors influencing early and late outcome following the Fontan procedure in the current era. The 'Two Commandments'? *Eur J Cardiothorac Surg* 2007;31(3):344-52.

(21) Griselli M, McGuirk SP, Ofoe V et al. Fate of pulmonary arteries following Norwood Procedure. *Eur J Cardiothorac Surg* 2006;30(6):930-5.

(22) Petko M, Myung RJ, Wernovsky G et al. Surgical reinterventions following the Fontan procedure. *Eur J Cardiothorac Surg* 2003;24(2):255-9.

(23) Hughes ML, Shekerdemian LS, Brizard CP, Penny DJ. Improved early ventricular performance with a right ventricle to pulmonary artery conduit in stage 1 palliation for hypoplastic left heart syndrome: evidence from strain Doppler echocardiography. *Heart* 2004;90(2):191-4.

(24) Ballweg JA, Dominguez TE, Ravishankar C et al. A contemporary comparison of the effect of shunt type in hypoplastic left heart syndrome on the hemodynamics and outcome at stage 2 reconstruction. *J Thorac Cardiovasc Surg* 2007 August;134(2):297-303.

(25) Tanoue Y, Kado H, Shiokawa Y, Fusazaki N, Ishikawa S. Midterm ventricular performance after Norwood procedure with right ventricular-pulmonary artery conduit. *Ann Thorac Surg* 2004 December;78(6):1965-71.

(26) Dietl CA, Cazzaniga ME, Dubner SJ, Perez-Balino NA, Torres AR, Favaloro RG. Life-threatening arrhythmias and RV dysfunction after surgical repair of tetralogy of Fallot. Comparison between transventricular and transatrial approaches. *Circulation* 1994;90(5):II7-II12.

(27) Goldberg CS, Bove EL, Devaney EJ et al. A randomized clinical trial of regional cerebral perfusion versus deep hypothermic circulatory arrest: outcomes for infants with functional single ventricle. *J Thorac Cardiovasc Surg* 2007 April;133(4):880-7.

(28) Visconti KJ, Rimmer D, Gauvreau K et al. Regional low-flow perfusion versus circulatory arrest in neonates: one-year neurodevelopmental outcome. *Ann Thorac Surg* 2006 December;82(6):2207-11.

(29) Tabbutt S, NOrd AS JG, Bernbaum J et al. Neurodevelopmental outcome following stage palliation for hypoplastic left heart syndrome. *Pediatrics.* In press 2007.

(30) Mahle WT, Visconti KJ, Freier MC et al. Relationship of surgical approach to neurodevelopmental outcomes in hypoplastic left heart syndrome. *Pediatrics* 2006 January;117(1):e90-e97.

(31) Hoffman GM, Mussatto KA, Brosig CL et al. Systemic venous oxygen saturation after the Norwood procedure and childhood neurodevelopmental outcome. *J Thorac Cardiovasc Surg* 2005 October;130(4):1094-100.

(32) Ikle L, Hale K, Fashaw L, Boucek M, Rosenberg AA. Developmental outcome of patients with hypoplastic left heart syndrome treated with heart transplantation. *J Pediatr* 2003;142(1):20-5.

(33) Goldberg CS, Schwartz EM, Brunberg JA et al. Neurodevelopmental outcome of patients after the Fontan operation: A comparison between children with hypoplastic left heart syndrome and other functional single ventricle lesions. *J Pediatr* 2000;137(5):646-52.

(34) McCrindle BW, Williams RV, Mitchell PD et al. Relationship of patient and medical characteristics to health status in children and adolescents after the Fontan procedure. *Circulation* 2006 February 28;113(8):1123-9.

(35) Forbess JM, Visconti KJ, Bellinger DC, Jonas RA. Neurodevelopmental Outcomes in Children After the Fontan Operation. *Circulation* 2001;104(90001):127I.

(36) Mahle WT. Neurologic and cognitive outcomes in children with congenital heart disease. *Curr Opin Pediatr* 2001;13(5):482-6.

(37) Uzark K, Lincoln A, Lamberti JJ, Mainwaring RD, Spicer RL, Moore JW. Neurodevelopmental outcomes in children with Fontan repair of functional single ventricle. *Pediatr* 1998;101(4 Pt 1):630-3.

(38) Kern JH, Hinton VJ, Nereo NE, Hayes CJ, Gersony WM. Early developmental outcome after the Norwood procedure for hypoplastic left heart syndrome. *Pediatr* 1998;102(5):1148-52.

(39) McGrath E, Wypij D, Rappaport LA, Newburger JW, Bellinger DC. Prediction of IQ and Achievement at Age 8 Years From Neurodevelopmental Status at Age 1 Year in Children With D-Transposition of the Great Arteries. *Pediatr* 2004;114(5):e572-e576.

(40) Podgoreanu MV, Schwinn DA. New paradigms in cardiovascular medicine: emerging technologies and practices: perioperative genomics. *J Am Coll Cardiol* 2005 December 6;46(11):1965-77.

(41) Hinton RB, Jr., Martin LJ, Tabangin ME, Mazwi ML, Cripe LH, Benson DW. Hypoplastic left heart syndrome is heritable. *J Am Coll Cardiol* 2007 October 16;50(16):1590-5.

(42) Ross RD, Daniels SR, Schwartz DC, Hannon DW, Shukla R, Kaplan S. Plasma norepinephrine levels in infants and children with congestive heart failure. *Am J Cardiol* 1987;59:911-4.

(43) Wu JR, Chang HR, Huang TY, Chiang CH, Chen SS. Reduction in lymphocyte beta-adrenergic receptor density in infants and children with heart failure secondary to congenital heart disease. *Am J Cardiol* 1996;77(2):170-4.

(44) Squires J, Potter L, Bricker D. *The ASQ Users Guide for the Ages and Stages Questionnaires" A Parent Completed Child Monitoring System*. 2nd Edition ed. Baltimore, Maryland: Paul H. Brookes Publishing Company; 2004.

(45) Skellern CY, Rogers Y, O'Callaghan MJ. A parent-completed developmental questionnaire: follow up of ex-premature infants. *J Paediatr Child Health* 2001 April;37(2):125-9.

(46) Klamer A, Lando A, Pinborg A, Greisen G. Ages and Stages Questionnaire used to measure cognitive deficit in children born extremely preterm. *Acta Paediatr* 2005 September;94(9):1327-9.

(47) Carpentieri SC, Meyer EA, Delaney BL et al. Psychosocial and behavioral functioning among pediatric brain tumor survivors. *J Neurooncol* 2003 July;63(3):279-87.

(48) Varni JW, Burwinkle TM, Rapoff MA, Kamps JL, Olson N. The PedsQL in pediatric asthma: reliability and validity of the Pediatric Quality of Life Inventory generic core scales and asthma module. *J Behav Med* 2004 June;27(3):297-318.

(49) Varni JW, Seid M, Knight TS, Uzark K, Szer IS. The PedsQL 4.0 Generic Core Scales: sensitivity, responsiveness, and impact on clinical decision-making. *J Behav Med* 2002 April;25(2):175-93.

(50) Dunbar-Masterson C, Wypij D, Bellinger DC et al. General health status of children with D-transposition of the great arteries after the arterial switch operation. *Circulation* 2001 September 18;104(12 Suppl 1):I138-I142.

(51) Culbert EL, Ashburn DA, Cullen-Dean G et al. Quality of life of children after repair of transposition of the great arteries. *Circulation* 2003 August 19;108(7):857-62.

(52) ISBER Best Practices for Repositories. March 2005 ed. *Cell Preservation Technology* (CPT); 2005.

(53) Best Practices for Biospecimen Research. *National Cancer Institute*, National Institutes of Health, US Department of Health and Human Services; 2007.

PEDIATRIC HEART NETWORK

SINGLE VENTRICLE RECONSTRUCTION

EXTENSION STUDY

Date: February 12, 2008

Amendment: May 16, 2008

Amendment: October 20, 2008

APPENDICES

Appendix A. Sample Informed Consent for Study 57

Appendix B. Sample Informed Consent for Subjects Who Underwent Cardiac Transplant Before Enrollment in the SVR Study 64

Appendix C. Sample Informed Consent for Biorepository 68

Appendix D. Echocardiography Technical Protocol 76

Appendix E. Holter Monitor Methods 83

APPENDIX A

SAMPLE INFORMED CONSENT FOR STUDY

For Subjects Without Cardiac Transplant Before Enrollment in the SVR II Study

Sample Informed Consent for Study

Pediatric Heart Network

Study Title: Outcomes after Single Ventricle Reconstruction in Children with Hypoplastic Left Heart Syndrome and Other Single Right Ventricle Anomalies (SVR II)

IRB Study Number:

Why is this research study being done?
Your baby was enrolled in the Single Ventricle Reconstruction or SVR study that looked at how infants with single ventricle heart defects did after surgery. Your child got one of two kinds of shunts; a modified Blalock-Taussig shunt (MBTS) or right ventricle to pulmonary artery shunt (RV-to-PA shunt) at the first stage of surgery (Norwood operation).

Now we would like to learn if children (2-6 years of age) do better with one of the two shunts (RV-to-PA or MBTS) in the years after surgery. Also we want to see how other medical and surgical factors affect children's health and how they develop.

About XXX patients will be studied at <*site/institution*>. This study is being done at all Pediatric Heart Network sites as well as other sites in the United States. We plan to enroll at least XXX patients from all of the sites. This study is funded by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health (NIH). Parts of Dr. <*site PI*> and his/her research team's salaries are being paid by this grant.

Why are you being asked to participate in this research study?
You have been asked to allow your child to join this study because your child was in the SVR study and we would like to continue to track your child's health and progress. In order for your child to participate in this study you must sign this consent form.

How long will your child be in the study and what will happen during the study?
For this study, we will collect the following information until your child reaches age 6.

1. **Medical History Review:** We will look at your child's medical chart and contact you every year starting when your child reaches 2 years of age until your child is 11 years old. We will ask about surgeries, tests or times that your child was in the hospital. We will also ask if your child is taking medicine or getting services like physical or speech therapy. We will also ask when the third stage of surgery, the Fontan operation, is planned. We will then collect hospital data when that occurs. You may be asked to sign a form allowing us to get data from other places that your child goes to for care.

2. **Echocardiogram (Echo):** We will collect data about your child's heart function from echos that your cardiologist ordered as part of regular clinical care at two time points. The first time point is before your child has the third staged surgery or Fontan operation. The second time point is when your child has an echo ordered by your doctor around age 6 years (any time between 5 and 7 years of age).

If your child has an echo at a place different than this hospital, we will ask your child's cardiologist for a copy. We will look at these records to help us understand how patients' hearts are doing years after the first stage of surgery. We will not do any echos as part of this research but will simply use the data from echos done as part of your child's routine care.

3. **Electrocardiogram (ECG):** We will obtain a copy of your child's ECG that is usually done before the Fontan procedure and again at around 6 years of age. It will provide data on heart rate and rhythm. We will get this data from your child's medical record. The ECG will have been done as part of your child's regular clinical care and not for research purposes.

4. **Holter Monitor:** When your child is 6.0 years of age we will want to measure your child's heart rate and rhythm with a Holter monitor. A Holter monitor makes a record over a 24-hour period.

   We will give you the monitor or mail it to your home. Your child will wear patches on the skin; the patches will be connected to a small box or recorder. Your child will be able to have regular daily activity during this time. After a 24 hour recording, you will mail the Holter monitor back to the study site using a pre-paid envelope. The results will be shared with your child's cardiologist. You will be told of any important findings.

5. **Neurodevelopmental Questionnaires:** As part of the study we will ask you to fill out surveys when your child is 3, 4, 5 and 6 years of age. The study coordinator will explain the forms to you and how to fill them out. You may ask questions about how to complete the forms but we cannot help you decide how to answer. The surveys include the following:

   a. *Ages and Stages Questionnaires ® (ASQ):* You will be asked questions about your child's physical abilities and communication.
   b. *Behavior Assessment System for Children, BASC-II:* You will be asked questions about your child's behavior at home and school.
   c. *Pediatric Quality of life Inventory (PedsQL):* You will be asked questions about quality of life for your family and child.
   d. *Impact of Family Scale:* You will be asked questions about how your family is affected by your child's heart problems and treatments.
   e. *Functional Status II R:* You will be asked questions about your child's health and abilities.
   f. *Child Health Questionnaire-50 (CHQ-50)*: You will be asked questions about your child's physical and emotional well-being.
   g. *Vineland Adaptive Behavior Scales:* You will be asked questions about how your child is doing in three areas: communication, skills of daily living and getting along with others.

   Some surveys may be done over the phone and some will be mailed to you to fill out and return. The surveys mailed at 3, 4, and 5 years will take about 60 to 90 minutes to complete. The surveys at 6 years will take about 2 hours to complete. The last two surveys on the list (the CHQ-50 and Vineland Adaptive Behavior Scales) will only be done at age 6.

The following table shows the study tests and surveys that will be done and when.

SCHEDULE OF STUDY PROCEDURES				
	Age 3 years	**Age 4 years**	**Age 5 years**	**Age 6 years[3]**
Phone call	**X**	**X**	**X**	**X**
Medical record review	**X**	**X**	**X**	**X**
Echocardiogram (Echo)				**X[1]**
Electrocardiogram (ECG)				**X[1]**
Holter monitor				**X**
Questionnaires[2]	**X**	**X**	**X**	**X**

[1] Done only if your cardiologist orders the test for your child's clinical care
[2] Questionnaires will ask about your child's development.
[3] We will contact you each year and review your child's medical records until your child is 11 years old.

You will be done with all tests and surveys when your child turns 6 years old. But it is our hope to continue to follow the patients who are in this study for years to come, through childhood and into the teen years. When you agree to join this study the nurse or doctor will continue to contact you once each year by a brief telephone call or letter, until your child is 11 years of age. We will ask about how your child is doing, if this information is not available in the medical record, and we will describe any further follow-up studies. You are **not** committed to entering any other studies.

What are the risks and discomforts of the research study?
Your child may have minor skin irritation from the patches placed on the skin during the Holter monitor (a 24-hour EKG). Wearing the small monitor for 24 hours can be inconvenient.

You may find it inconvenient to complete the surveys or to talk with the study nurses by phone. We will make sure that you will have plenty of time to fill out the forms. Also, you do not have to answer any questions that make you feel uneasy. Study nurses will be willing to answer questions or provide support as you complete the forms.

Are there benefits to taking part in the research study? It is possible that the Holter monitor done for research purposes may show an abnormal heart rhythm. If this occurs, we will contact your child's cardiologist with the results and your family will be told.

The surveys will provide valuable details about how your child develops, behaves and functions, which might not otherwise be known. If problems are found, early help can be suggested for your child.

Your child may not benefit from being in this study. But the information we learn from this study may help improve the care of other children who have also been born with a heart defect.

What other choices are there?

You do not have to be in this study. If you do not want your child to join this study, your child will receive normal medical care by his or her doctors.

What are your rights as a participant?

Your participation in this study is completely **voluntary**. You should not feel any pressure to join. If you do not want to be included it will not affect the care your family receives here at <*institution*>.

If you agree to be in the study, you may leave at any time. This will not affect your regular care or cause you to lose any benefits that you would normally have. If you do decide to withdraw, it is important that you contact Dr <*site PI*> and let him/her know. If you do leave the study, no new data will be collected for study purposes.

If your child receives a heart transplant, we will continue to follow him/her once a year, but we will not ask you to complete any study tests or surveys.

The investigator or research sponsor (NHLBI) may decide to stop your child from taking part in this study if the study is stopped. The sponsor may stop the study at any time.

A signed copy of this consent form will be given to you for your records.

How will your child's information be kept confidential and private?

We will do everything we can to keep your child's medical and research data private. <site/institution> and/or <site PI> will do the following things to maintain your child's privacy:

- Study records that identify your child will be kept private as required by law. There are laws (Federal Privacy Regulations) to protect your privacy. Your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records sent outside of <site/institution> except when required by law or as described in this consent.
- Your child will be given a study identification (ID) number. All study records will be labeled with this number and not your child's name or other personal data. The files that link the ID number to your child will be kept in a locked, secure area that only the study team can get to.
- The tapes or disks with your child's echocardiogram studies will be sent to New England Research Institutes (NERI), the Pediatric Heart Network Data Coordinating Center. These will be sent to labs outside of <site/institution> for reading. These tapes or discs may have your child's name on them and will be kept in locked files at these labs. Names will not be recorded in any other records kept outside of <site/institution>.
- Data gathered during this study and medical records may be checked and verified by staff of the NIH, <site/institution> Institutional Review Board, or NERI. All medical records from this site or from other institutions that have personal identifiers will be treated as private and will be shared only with these agencies, or as required by law.
- The results of this study may be published for all the subjects as a group but will not identify your child individually.

- To help us protect the privacy of subjects, a *Certificate of Confidentiality* from the National Institutes of Health (NIH) has been obtained. With this *Certificate*, the researchers of this study cannot be forced to give out any data that may identify a subject, even by a court order, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings.

  There are some times when the *Certificate* cannot be used to protect privacy:

  - If the United States government asks for the information to evaluate federally funded study projects
  - When information is needed to meet requirements of the Food and Drug Administration (FDA)

  Also, a *Certificate of Confidentiality* does not prevent you from releasing information about your child's involvement in this study. We will give data that we collect for the study to any insurer, employer, or other person if you give your written consent for us to release that information.

Will it cost you anything to be in this study?

There will be no extra costs to you when your child joins this study. Tests needed only for the study and not part of regular care will be provided free of charge. You must pay for all other costs related to your child's normal medical care such as hospital stays, surgery, drugs, lab tests, and doctor's fees which are thought to be standard medical care for patients with your child's condition.

Will you be paid to join this study?

The study investigators will pay for <insert center language here related to parking, travel, meals, stipend etc>.

What happens if you believe your child is injured during this study?

Immediate necessary medical care is available at <site/institution> in the event that your child is injured as a result of being in this research study. However, there is no promise by <site/institution>., Dr. <site PI> or your <site> physicians to repay costs or give free medical care to you in the event of a study-related injury. Further information concerning this and your rights as a research subject can be obtained from the <site/institution> Institutional Review Board (IRB) Office at: <phone number>. (Or insert institutional language)

Who do you call if you have questions about this study?

If you have questions about this study, you should contact:

<Site PI>, MD **Telephone Number:** **<telephone number>**

Pager Number: **<pager number>**

If you have questions or want more information about the Pediatric Heart Network or about being in a study, you may go to www.PediatricHeartNetwork.org. You will also find information about this study on the website.

If you have questions concerning your rights as a subject in this study, you should contact: <Site> Institutional Review Board (IRB) Office at:

Telephone Number: **<phone number>**

CONSENT:
"The purpose of this study, what will happen, risks and benefits have been explained to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I have been told who to contact if I have more questions. I have read this consent form and agree for my child to be in this study. I know that I may withdraw permission at any time. I have been told that I will be given a signed copy of this consent form."

_____ _____
Signature of Parent/Guardian Date

_____ _____
Signature of person obtaining consent Date

APPENDIX B

SAMPLE INFORMED CONSENT

For Subjects Who Underwent Cardiac Transplant Before Enrollment in the SVR II Study

Sample Informed Consent for Subjects with Cardiac Transplant

Pediatric Heart Network

Study Title: Outcomes after Single Ventricle Reconstruction in Children with Hypoplastic Left Heart Syndrome and Other Single Right Ventricle Anomalies (SVR II)

Heart transplant patients

IRB Study Number:

Why is this research study being done?

Your baby was enrolled in the Single Ventricle Reconstruction or "SVR" study, which looked at how infants with single ventricle heart defects did after surgery. We are now asking for your continued participation in a follow-up study of the outcomes in childhood. Since the time of the original surgery, your child has received a heart transplant. We would like to include children who have had heart transplants in our analyses of whether the type of shunt performed during the Norwood procedure influences how children do in the years after surgery.

About XXX patients will be studied at <*site/institution*>. This study is being done at all Pediatric Heart Network sites as well as other sites in the United States. We plan to enroll at least XXX patients from all of the sites. This study is funded by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health (NIH). Parts of Dr. <*site PI*> and his/her research team's salaries are being paid by this grant.

Why are you being asked to participate in this research study?

You have been asked to allow your child to join this study because your child was in the SVR study and we would like to continue to track your child's health and progress. In order for your child to participate in this study you must sign this consent form.

How long will your child be in the study and what will happen during the study?

The nurse or doctor will continue to contact you once each year by a brief telephone call or letter, until your child is 11 years of age. We will ask about how your child is doing, and we will describe any further follow-up studies. You are **not** committed to entering any other studies.

What are the risks and discomforts of the research study?

You may find it inconvenient to talk with the study nurses by phone.

Are there benefits to taking part in the research study?

Your child may not benefit from being in this study. But the information we learn from this study may help improve the care of other children who have also been born with a heart defect.

What other choices are there?

You do not have to be in this study. If you do not want your child to join this study, your child will receive normal medical care by his or her doctors.

What are your rights as a participant?

Your participation in this study is completely **voluntary**. You should not feel any pressure to join. If you do not want to be included it will not affect the care your family receives here at <*institution*>.

If you agree to be in the study, you may leave at any time. This will not affect your regular care or cause you to lose any benefits that you would normally have. If you do decide to withdraw, it is important that you contact Dr <*site PI*> and let him/her know. If you do leave the study, no new data will be collected for study purposes.

The investigator or research sponsor (NHLBI) may decide to stop your child from taking part in this study if the study is stopped. The sponsor may stop the study at any time.

A signed copy of this consent form will be given to you for your records.

How will your child's information be kept confidential and private?

We will do everything we can to keep your child's medical and research data private. <site/institution> and/or <site PI> will do the following things to maintain your child's privacy:

- Study records that identify your child will be kept private as required by law. There are laws (Federal Privacy Regulations) to protect your privacy. Your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records sent outside of <site/institution> except when required by law or as described in this consent.
- Your child will be given a study identification (ID) number. All study records will be labeled with this number and not your child's name or other personal data. The files that link the ID number to your child will be kept in a locked, secure area that only the study team can get to.
- Data gathered during this study and medical records may be checked and verified by staff of the NIH, <site/institution> Institutional Review Board, or NERI. All medical records from this site or from other institutions that have personal identifiers will be treated as private and will be shared only with these agencies, or as required by law.
- The results of this study may be published for all the subjects as a group but will not identify your child individually.
- To help us protect the privacy of subjects, a *Certificate of Confidentiality* from the National Institutes of Health (NIH) has been obtained. With this *Certificate*, the researchers of this study cannot be forced to give out any data that may identify a subject, even by a court order, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings.

  There are some times when the *Certificate* cannot be used to protect privacy:

  - If the United States government asks for the information to evaluate federally funded study projects
  - When information is needed to meet requirements of the Food and Drug Administration (FDA)

  Also, a *Certificate of Confidentiality* does not prevent you from releasing information about your child's involvement in this study. We will give data that we collect for the study to any

insurer, employer, or other person if you give your written consent for us to release that information.

Will it cost you anything to be in this study?
There will be no extra costs to you when your child joins this study. You must pay for all other costs related to your child's normal medical care such as hospital stays, surgery, drugs, lab tests, and doctor's fees which are thought to be standard medical care for patients with your child's condition.

Will you be paid to join this study?
You will not receive any compensation for participation in the study.

What happens if you believe your child is injured during this study?
Since your child is not undergoing any tests or procedures for this study, we do not anticipate that your child could be injured in any way. Further information concerning this and your rights as a research subject can be obtained from the <site/institution> Institutional Review Board (IRB) Office at: <phone number>. (Or insert institutional language)

Who do you call if you have questions about this study?
If you have questions about this study, you should contact:

<Site PI>, MD **Telephone Number:** **<telephone number>**

Pager Number: **<pager number>**

If you have questions or want more information about the Pediatric Heart Network or about being in a study, you may go to www.PediatricHeartNetwork.org. You will also find information about this study on the website.

If you have questions concerning your rights as a subject in this study, you should contact: <Site> Institutional Review Board (IRB) Office at:

Telephone Number: **<phone number>**

CONSENT:
"The purpose of this study, what will happen, risks and benefits have been explained to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I have been told who to contact if I have more questions. I have read this consent form and agree for my child to be in this study. I know that I may withdraw permission at any time. I have been told that I will be given a signed copy of this consent form."

_____ _____
Signature of Parent/Guardian Date

_____ _____
Signature of person obtaining consent Date

APPENDIX C

SAMPLE INFORMED CONSENT FOR BIOREPOSITORY

Informed Consent Template for Biorepository

Pediatric Heart Network

Study Title: Biologic Specimen Repository for Hypoplastic Left Heart Syndrome and other Single Right Ventricle Anomalies

IRB Study Number:

Why is this research study being done?

After your baby was born, he or she was enrolled in the Single Ventricle Reconstruction or SVR study. That study looked at how infants with single ventricle heart defects did after surgery. In this study we want to collect biological samples (blood or saliva) to study biomarkers and genes that might be related to the type of heart problem that your child has.

First, we want to find out whether biomarkers can tell us how well a child's heart is working. Biomarkers (blood proteins, chemicals or markers) in the blood can tell doctors how your child's body is working. These can be measured and can tell us about normal body functions, the body's response to illness, or the body's response to interventions, like surgery or medicines.

We also want to find out which genes cause heart defects in children and how genes can affect a child's health. Genes are made of DNA and instruct our body on how to develop, grow, and repair itself. If we know about these genes, it may help us find ways to prevent these defects in the future. Or we might find better ways to treat children with heart disease so they can live longer and healthier lives.

For this study, we want to collect either blood or saliva from your child and both biological parents, if possible. These samples will not be collected from parents who are not blood relatives. Obtaining samples from many family members may help researchers to find out the meaning of any genetic changes that are found. These samples will be stored in a repository, also called a biobank, for future research.

About XXX patients will be studied at <*site/institution*>. This study is being done at all Pediatric Heart Network sites as well as additional sites in the United States. We plan to enroll at least XXX patients from all of the sites. This study is funded by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health (NIH). Parts of Dr. <*site PI*> and his/her research team's salaries are being paid by this grant.

Why are you being asked to participate in this research study?

Because your child has a heart defect, you are being asked both to allow your child to participate in this study and for you as biological parent(s) to participate in this study so that we can collect blood and/or saliva and store it for future research on heart defects. Your child and you can participate in this study even if you decide not to participate in the SVR II follow-up study. If you decide not to allow your child to participate in this study, you will not be asked to participate as his/her parent(s).

What will happen during the study?

If you decide to allow your child to participate in this study and if you agree to participate in this study, participation will consist of providing blood or saliva samples and completing an interview.

1. Blood or saliva sample: We will collect a small amount of blood or saliva. Blood samples can be collected at any time, and we will try to collect blood from your child when it is being drawn for other reasons. The total amount of blood drawn for this testing will be up to 20 mls (4 teaspoons). For children who weigh less than 20 pounds, we will obtain no more than 5 ml (1 teaspoon) per 5 pounds of body weight.

   If you do not wish to have your or your child's blood taken, we will collect a saliva sample, which will be used for the genetic studies. Saliva can be taken at an in-person visit or we can mail you a saliva kit and you can obtain the samples yourself and return them to us. Saliva samples provide much less DNA than blood samples and this may prevent researchers from using your sample for all studies. In addition, saliva samples cannot be used to check blood proteins or chemicals. For these reasons we prefer to take a blood sample from your child and you, except under unusual circumstances.

   Sometimes the sample is damaged and if this happens, you or your child may be asked to donate blood or saliva again.

   The test results for these samples will not be added to your child's or your medical records.

2. Interview: If you are the biological parent, we will ask about your family's health history to see if there are possible links between genes and the environment. We will ask about your close and more distant family members. We may ask about ages, pregnancy and health histories, ethnicity, place of birth, and possible risk factors for heart disease. You may have a family member who has passed away. We may ask why the person died as well as the age and time of death. Please feel free to share only the facts that you feel comfortable sharing. Let us know if there are people or issues you do not want to discuss.

   If you/your child receive medical care outside of (PHN institution), we may ask for your consent to get medical records from other places.

   Any information about your or your family's medical history will be used only for research and will not be added to your child's or your medical records.

The time required for the interview and to obtain the blood or saliva sample should be about one hour.

What are the risks and discomforts of the research study?

1. Blood or saliva sample: For a blood sample, you or your child may have minor soreness and/or bruising. You or your child may feel lightheaded, and although rare, bleeding might continue at the site of the needle stick. We will try to draw blood at the same time that your child needs to have blood drawn for health reasons. There are no risks or discomforts connected with providing a saliva sample.

2. Genetic tests: The results of the tests will not be given to you. We use a research lab, not a clinical lab, with certified procedures for reporting results. This is a study to identify genes and biomarkers that might be involved in heart disease and its effects in children. We will not understand the meaning of most of the differences in this information until there is more research in the future. Some people in genetic studies feel anxious if they think they might have a gene that puts them at risk or that may be passed on to children. If you have

these feelings at any time during the study, you may contact us and we will arrange for you to speak with a genetics counselor.

You should also know that social and economic problems could result when genetic information is released. For example, the tests done in this study might find a defective gene that puts you or a relative at risk for a genetic disorder. You or your family could be affected if this genetic information is given to the wrong source like an insurance company or employer. The test results will not be added to your child's or your medical records. If the test results were released and became part of your medical records, you might be denied future health or life insurance coverage. For these reasons we will take several steps to keep all data private and confidential. Please see the section on Confidentiality below, where we describe how we will protect facts about you or your child with a *Certificate of Confidentiality* from the National Institutes of Health (NIH).

3. Biobank: When you participate in a study that stores biological samples in a biobank for future studies, it is different than being in a specific study. The key difference is that you will not know in which study or in how many studies that the samples are used. We do not know how long the specimens will be in the biobank. Researchers will study the data for many years, and it is not possible to know when the studies will be done. This might make you feel uneasy.

   In the future, information from your genetic tests and other study data might be placed in a Federal data repository, such as the National Center for Biotechnology Information (NCBI) repository. The purpose of this data repository is to help qualified researchers work together to learn more about ways in which genes affect disease. Further procedures (described below) will be taken to safeguard your privacy before the information is provided to the Federal repository.

Are there benefits to taking part in the research study?

There is no known direct benefit to you or your child from participating in this study or providing blood or saliva samples for the biobank. However, the sample that you give may help researchers to learn more about the link between genetic and other factors and long-term outcomes of children with heart defects. It may help doctors to talk with families better about the causes of heart defects and the chance of having other children with heart defects. It is very possible that no findings will result from this research.

The samples that you and/or your child provide may be used to develop new medical tests or treatments. It is possible that the researchers, hospitals, or companies sponsoring the research might benefit financially if the tests or treatments can be patented or commercialized (sold). There are no plans to provide you with payments or royalties if these discoveries are marketed (sold) or licensed. Although subjects and their families will not receive any compensation now or in the future for their samples or data, income that may be derived from future research or sales of the grouped data will be used to support biomedical research.

What other choices are there?

You may choose not to provide permission for a blood or saliva sample to be collected from your child. If you do not want to allow a sample to be collected from your child, your child may still remain in the SVR II follow-up study, if your child is enrolled. Your child can participate in this study and one or both parents might choose not to give a sample for this study.

How will information be kept confidential and private?

We are very concerned about keeping data secure and private in this genetic study. While we cannot fully guarantee the privacy of this information, <site/institution> and/or <site PI> will do the following things to maintain your privacy:

- Study records that identify you or your child will be kept private as required by law. There are laws (Federal Privacy Regulations) to protect your privacy. You and your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records sent outside of <site/institution> except when required by law.
- Blood and saliva samples will be labeled only with a sample identification (ID) number. The labels will not include your name or any other information that would identify you. The files with the ID numbers that link the sample to you or your child will be kept in a locked, secure area that only the study team can get to. Any health information that might identify you or your child will not be available to any person or group other than the investigators of this study except in the case of study safety. In the case of safety, patient data will only be given to the Data Coordinating Center (New England Research Institutes or NERI), <site/institution> Institutional Review Board, and NIH/NHLBI if needed.
- If the results of the genetic tests and other study data are placed in a Federal data repository, any information that could identify you or your child will be removed and the information will be labeled with a new number that is different from your study number and cannot be linked back to you.
- Data gathered during this study and medical records may be checked and verified by staff of the NIH, <site/institution> Institutional Review Board, or NERI. All medical records from this site and other institutions that contain personal data will be treated as private and will be shared only with these agencies, or as required by law.
- The results of this study may be published for all the subjects as a group, but any report will not identify you or your child individually.
- To help us protect the privacy of subjects, a *Certificate of Confidentiality* from the National Institutes of Health (NIH) has been obtained. With this *Certificate*, the researchers of this study cannot be forced to give out any data that may identify a subject, even by a court order, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings.

  There are some times when the *Certificate* cannot be used to protect privacy:

  - If the United States government asks for the information to evaluate federally funded study projects
  - When information is needed to meet requirements of the Food and Drug Administration (FDA).

  You should understand that a Certificate of Confidentiality Certificate does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research study. Note however, that if an insurer or employer learns about your participation, and obtains your consent to receive research information, the investigator may not use the Certificate of Confidentiality to withhold this information. This means that you and your family must also actively protect your own privacy.

Will it cost you anything to be in this study?
There will be no extra costs to you when your child joins this study. Tests needed only for the study and not part of regular medical care will be provided free of charge. You must pay for all other costs related to your child's normal medical care such as hospital stays, surgery, drugs, lab tests, and physician fees which are thought to be standard medical care for patients with your child's condition.

Will you be paid to join this study?
The study investigators will pay for <insert center language here related to parking, travel, meals, stipend etc>.

What happens if you believe your child is injured during this study?
Immediate necessary medical care is available at <site/institution> in the event that your child is injured as a result of being in this research study. However, there is no promise by <site/institution>., Dr. <site PI> or your <site> physicians to repay costs or give free medical care to you in the event of a study-related injury. Further information concerning this and your rights as a research subject can be obtained from the <site/institution> Institutional Review Board (IRB) Office at: <phone number>. (Or insert institutional language)

What are your rights as a participant?
Your participation in this study is completely **voluntary**. You should not feel any pressure to participate. If you do not want to participate, it will not affect the care you or your family receives here at <*institution*>.

If you agree to be in the study, you may stop participating in the study at any time. This will not affect your regular care or cause you to lose any benefits that you would normally have. If you do decide to withdraw, it is important that you contact Dr <*site PI*> and let him/her know. If you want to stop participating in the study, you will not be asked to provide future blood or saliva samples. Samples that you have provided will remain in the biobank and may still be used for testing, if you agree. You will have the choice to have the study identification number removed from your sample and/or your child's sample and leave the samples in the biobank for future research or you may ask to have the remaining samples destroyed. Once your samples are placed in a federal repository, they cannot be identified as yours and therefore cannot be removed.

The investigator or research sponsor (NHLBI) may decide to stop your child from taking part in this study if the study is stopped. The sponsor may stop the study at any time.

A signed copy of this consent form will be given to you for your records. You are not giving up any of your or your child's rights by signing this form. Even after you have signed this form, you may change your mind at any time.

Who do you call if you have questions about this study?
If you have questions about this study, you should contact:

<Site PI>, MD Telephone Number: <telephone number>

Pager Number: <pager number>

If you have questions or want more information about the Pediatric Heart Network or about being in a study, you may go to www.PediatricHeartNetwork.org. You will also find information about this study on the website.

If you have questions concerning your rights as a subject in this study, you should contact: <Site> Institutional Review Board (IRB) Office at:
Telephone Number: <phone number>

CONSENT FOR CHILD'S PARTICIPATION:

The purpose of this study, what will happen, risks and benefits have been explained to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I have been told whom to contact if I have more questions. I have read this consent form and agree to allow my child to be in this study. I know that I may withdraw permission at any time. I have been told that I will be given a signed copy of this consent form.

Please check one of the following:

_____ I agree to allow my child's blood or saliva sample to be studied for any disease, health condition or risk factor in the future.

_____ I agree to allow my child's blood or saliva sample to be studied only for heart defects, heart disease, or risk factors for them.

_____ _____
Signature of Parent/Legal Guardian Date

***CONSENT FOR PARENT'S PARTICIPATION**:*

PARENT #1

The purpose of this study, what will happen, risks and benefits have been explained to me. I have been allowed to ask questions and my questions have been answered to my satisfaction. I have been told whom to contact if I have more questions. I have read this consent form and agree to participate in this study. I know that I may withdraw permission at any time. I have been told that I will be given a signed copy of this consent form.

Please check one of the following:

_____ I agree to allow my blood or saliva sample to be studied for any disease, health condition or risk factor in the future.

____ I agree to allow my blood or saliva sample to be studied only for heart defects, heart disease, or risk factors for them.

_____ _____

Signature of Parent Date

PARENT #2

Please check one of the following:

_____ I agree to allow my blood or saliva sample to be studied for any disease, health condition or risk factor in the future.

____ I agree to allow my blood or saliva sample to be studied only for heart defects, heart disease, or risk factors for them.

_____ _____

Signature of Parent Date

INVESTIGATOR:

I have fully explained to the parent(s) the nature and purpose of this study and the risks involved in participation. I have answered all questions to the best of my ability. I have given a copy of the consent form to the parent(s).

_____ _____

Signature of person obtaining consent Date

APPENDIX D

ECHOCARDIOGRAPHY TECHNICAL PROTOCOL

Echocardiography Technical Protocol

Study Equipment

1. Echocardiographic imaging system equipped with transthoracic transducers appropriate to subject size.

2. Studies must be recorded in DICOM format with embedded calibration information if possible, as this facilitates review and accuracy of core lab measures. Minimal clip length for dynamic imaging is 3 beats; minimal clip length for spectral Doppler information is 5 beats. Recordings do not need to be deidentified prior to transfer to the DCC or Echo Core Lab.

Timing of Studies

Two echocardiograms per subject will be evaluated by the Core Laboratory. All studies will be performed according to the acquisition protocol below and obtained prior to Fontan surgery (within 6 months of the surgical date) and after Fontan palliation at 6 years of age (± 1 year). The pre-Fontan study will be performed at the PHN clinical site where the surgery will be performed as part of the routine pre-operative evaluation. The post-Fontan study will be performed by the cardiologist providing long-term care for the child as part of routine clinical management of a patient with Fontan physiology; the timing of this study within the window of 5 –7 years will be at the discretion of the cardiologist.

Study Acquisition

1. Height and weight: Subject length in centimeters and weight in kilograms will be measured at the time of echocardiography (1).

2. Sedation may be required to obtain high quality echocardiographic images, but will only be performed as indicated for the clinical care of the patient. Sedation will be performed according to the Sedation Policy at each study center (2).

3. Two-dimensional echocardiography: In addition to complete orthogonal sweeps from subxiphoid, apical, parasternal, and suprasternal notch windows, the following specific information pertinent to derivation of the indices of systolic and diastolic ventricular function, tricuspid and neoaortic valve size and function, and neoaortic arch size and patency will be acquired.:

A. *Two-dimensional recording of the right ventricular short axis:* The short axis coronal image will be obtained at the position of the largest short-axis cross-sectional area in a plane parallel to the plane of the tricuspid valve and orthogonal to the long-axis of the right ventricle from subxiphoid or parasternal windows.

B. *Two-dimensional recording of the right ventricular long axis:* The long axis image will be recorded in the transverse plane transecting both atrioventricular valves (if both are present) and intersecting the true apex of the right ventricle from apical windows.

C. *Two-dimensional recording of the right ventricular inflow-outflow view.* The image of the para-sagittal plane intersecting the apex of the right ventricle, the tricuspid valve, and the neoaortic valve will be recorded from subxiphoid, apical, or parasternal windows.

D. *Two-dimensional recording and measurement of the neoaortic annulus:* Orthogonal parasternal long-axis and transverse apical or subxiphoid images of the neoaortic root will be recorded with zoom mode activated to maximize resolution of the neoaortic annulus.

E. *Two-dimensional recording and measurement of the tricuspid annulus:* Orthogonal parasternal long-axis and transverse apical images of the tricuspid annulus will be recorded with zoom mode activated to maximize resolution of the tricuspid annulus.

F. *Color Doppler assessment of the severity of tricuspid valve regurgitation:* In subjects with tricuspid valve regurgitation, color Doppler images of the proximal jet width including the *vena contracta* are to be recorded from orthogonal apical transverse and parasternal long-axis views (3,4) using the maximal Nyquist limit of the transducer.

G. *Color Doppler assessment of the neoaortic valve:* In subjects with neoaortic valve regurgitation, color Doppler images of the proximal jet width including the *vena contracta* are to be recorded from orthogonal parasternal long-axis and transverse apical or subxiphoid images of the neoaortic valve (3,4) using the maximal Nyquist limit of the transducer.

H. *Spectral Doppler recording of the tricuspid regurgitant jet:* 2D color Doppler mode is used to align the spectral Doppler sample parallel with the regurgitant jet. Gain should be adjusted to provide the sharpest Doppler envelope, particularly of the accelerating velocity between 1 and 3 meters/second, with sweep speed recorded at 150 mm/s.

I. *Spectral Doppler recording of the tricuspid valve inflow pattern:* 2D color Doppler mode is used to direct spectral Doppler recording of the tricuspid inflow from the apical 4 chamber window. The Doppler cursor should be placed at the tips of the tricuspid leaflets with the maximal sweep speed .

J. *Spectral Doppler recording of the pulmonary vein inflow pattern:* 2D color Doppler mode is used to direct spectral Doppler recording of the right upper pulmonary venous inflow from the apical 4 chamber window. The Doppler cursor should be placed within the vein before it empties into the left atrium with sweep speed recording at 150 mm/s

K. *Spectral Doppler recording of the neoaortic outflow: :* 2D color Doppler mode is used to direct spectral Doppler recording of the neoaortic outflow across the valve from the view that best aligns the Doppler cursor parallel to flow (parasternal, apical, or subxiphoid). The Doppler cursor should be placed within the valve orifice so that valve clicks are displayed on the tracing with the sweep speed recording at 150 mm/s

L. *Spectral tissue Doppler recording of the tricuspid valve annular velocities:* 2D imaging is used to align the Doppler cursor parallel to longitudinal motion of the right ventricular free wall transecting the plane of the tricuspid valve from the apical 4 chamber window at the cardiac crux. The pulsed sample volume is positioned within the myocardium just proximal to the valve lateral (non-septal) annular junction and adjusted until the sample volume remains within myocardium throughout the cardiac cycle. Tissue Doppler mode is activated with the sweep speed recording at 150 mm/s.

M. *Spectral Doppler assessment of the neoaortic arch:* Using color Doppler guidance, the maximum peak Doppler gradient will be recorded through the narrowest segment of the arch by continuous wave Doppler from the suprasternal window.

N. *Assessment of ascending neoaorta size:* The diameter of the ascending neoaorta at its maximum dimension will be obtained by two-dimensional recording from a parasternal or suprasternal window.

Core Laboratory Data Processing and Analysis

1. Centers will transfer DICOM-compatible digital images directly to CDROM or DVD (one study per disk). The disks are to be labeled on the exterior with labels supplied by the DCC and transferred to the Data Coordinating Center semi-monthly. Subject identification data does not need to be masked on screen.

All studies will receive a study identification number and will be tracked by the DCC and Echo Core Lab using this number; studies will be transferred from the DCC to the Echo Core Lab for analysis via overnight express mail.

2. Measurements will be performed on a microcomputer-based workstation custom programmed for electronic caliper overlay of captured digital images for recording.

The following measured and derived parameters will be obtained:

Ventricular size and function: End-diastolic (frame at which atrioventricular valve closure occurs) and end-systolic (frame preceding atrioventricular valve opening) endocardial borders of the right ventricle excluding the papillary muscles from the apical 4 chamber window will be used to compute RV areas and % area fraction. RV volumes will be calculated from these areas and RV long axis length obtained from the subxiphoid window using the biplane pyramid algorithm (2/3 RV area x RV long axis length) as per the original SVR protocol. In addition, endocardial borders of the ventricle on long and short axis imaging will be used to compute volumes using a modified Simpson's rule algorithm (5) to provide end-diastolic and end-systolic volumes and ejection fraction. The shape of the ventricle(s) is quantified as eccentricity from the end-diastolic long axis dimension (L) and short axis area (A) as Eccentricity = [L2 - (4A/L)2]0.5/L [2]. RV dP/dt will be calculated by dividing the difference in the pressures corresponding to 3 and 1 m/s (using the simplified Bernoulli equation) or 32 mm Hg by the time interval between 1 and 3 m/s velocities as $dP/dt = [4(3)^2–4(1)^2]/time$ (6).

Right Ventricular Doppler Stroke Volume. The Doppler-derived right-ventricular stroke volume will be calculated as the neo-aortic time-velocity integral times the neoaortic valve annulus area.

Tissue Doppler indices of systolic and diastolic function: Tissue Doppler velocities for both systolic longitudinal contraction (annular displacement toward the apex) and for diastolic recoil (annular displacement away from the apex) will be averaged from at least three consecutive cardiac cycles. The isovolumic acceleration will be measured from the lateral annulus recording. The isovolumic contraction and relaxation times and the ejection time will be recorded from the lateral annulus recording and the Tei index will be calculated as (8): isovolumic contraction time + isovolumic relaxation time/ejection time.

Doppler indices of diastolic function: Numerous derived variables have been reported from these tracings, but those that are considered of most interest are the ratio of peak early velocity (E_P) to

peak atrial velocity (A_P), the early deceleration time, and the duration of the atrial contraction-related retrograde pulmonary vein Doppler signal (9).

Neoaortic arch size and patency: The maximal neoaortic anterior-posterior diameter as measured from leading edge to leading edge for ascending neoaortic arch imaging. The peak gradient across the narrowest segment of the aortic arch will be determined from continuous wave Doppler interrogation using the modified Bernoulli equation.

Tricuspid and neoaortic valve size and regurgitation: The diameter of the tricuspid valve annulus will be measured from leading edge to leading edge using orthogonal views (parasternal long axis and apical). The diameter of the neoaortic valve annulus will be measured from leading edge to leading edge using orthogonal views (parasternal long axis, apical, and/or subxiphoid). The proximal jet width will be measured directly from orthogonal color Doppler images of the vena contracta, and the area of the regurgitant orifice will be calculated from these diameters using the formula for an ellipse=3.14 x (diameter/2) x (diameter/2)

References

(1) Haycock G, Schwartz G, Wisotsky D. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. J Pediatr 1978; 93:62-66.

(2) Gross J, Bailey P, Connis R, Cote C, Davis F, Epstein B, Gilbertson L, Nickinovich D, Zerwas J, Zuccaro G. Practice guidelines for sedation and analgesia by non-anesthesiologist. Anesthesiology 2002;96:1004-10017.

(3) Roberts BJ, Grayburn PA. Color flow imaging of the vena contracta in mitral regurgitation: technical considerations. J Am Soc Echocardiog, 2003;16(9):1002-1006 and

(4) Enriquez-Sarano M, et al Effective mitral regurgitant orifice area: clinical use and pitfalls of the proximal isovelocity surface area method. J Am Coll Cardiol, 1995.2(3):703-709.].Fischl S, Gorlin R, Herman M. Cardiac shape and function in aortic valve disease: physiologic and clinical implications. Am J Cardiol1977;39:170-176.

(5) Matitiau A, Perez-Atayde A, Sanders S, Sluysmans T, Parness I, Spevak P, Colan S. Infantile dilated cardiomyopathy: Relation of outcome to left ventricular mechanics, hemodynamics, and histology at the time of presentation. Circulation 1994;90:1310-1318.

(6) Michelfelder EC, Vermilion RP, Ludomirsky A, Beekman RH, Lloyd TR. Comparison of simultaneous Doppler- and catheter-derived right ventricular dP/dt in hypoplastic left heart syndrome. Am J Cardiol. 1996;77(2):212-214.

APPENDIX E

HOLTER MONITOR METHODS

Holter Monitor Methods

Application and Data Acquisition Protocol

Each subject will undergo 24-hour ambulatory ECG monitoring (Holter monitoring) using a PHN network digital Holter from Forest Medical. Data analysis will consists of measuring several parameters of cardiac rhythm and rate, as well as an analysis of long and short-term heart rate variability (HRV).

The system will be placed on the subject by their parents or caretakers, with the assistance of instructions, or by trained staff if they prefer to have the Holter placed during a clinical visit at the study center in the study time window. The patient and family will be instructed to record daily events in a diary and mark in the recorder any major changes in activity as well as any symptoms. Subjects will be instructed to undertake a range of activities that are "normal" for them. A minimum of 12 hours usable recording will be required for inclusion in the analysis of heart rhythm and daytime heart rate. However, a minimum of 16 hours which includes both sleep and daytime activities will be required for full analysis of heart rate and HRV.

Holter monitors will be returned to the clinical center, downloaded to the computer and an automatic analysis performed. The digital files which contain the automated analysis and the full disclosure will be forwarded by either CD or FTP to NERI where the files will be archived to CD's and transferred to the Holter core laboratory.

Core Laboratory Analysis Protocol

Rate

Average (mean), maximum and minimum heart rate will be assessed for the entire recording, daytime hours (10AM-6PM) and night time hours (11PM-7AM). In addition, *5 minute averages* will be obtained as mean, minimum and maximum value for each consecutive 5-minute epoch (maximum 288 epochs).

Holter Rhythm

The predominant rhythm will be classified as normal sinus, atrial non-sinus, or junctional, defined as the rhythm that accounts for more than 50% of the beats in the recording. Significant non-predominating atrial or junctional rhythms, which account for more than 10% of the beats will also be quantified, and shorter segments will simply be noted.

The presence of atrial and ventricular ectopy will be classified as isolated, couplet, non-sustained tachycardia (4-30 beats), or sustained tachycardia (> 30 beats). Each category will have modifiers of rare, occasional, frequent or incessant.

Heart Rate Variability (HRV)*(1,2)*
Long and short term HRV will be characterized in both the time and frequency domains and performed as follows:

Time Domain Analysis
The following will be calculated using the R-R intervals involving 2 normal beats for each subject's predominant rhythm for the entire recording and all 5 minute segments:
Standard deviation (SD) to yield
SDNN – SD or full recording
SDANN – SD of 5 minute average heart rates
SDNN index - average of all 5 min SD's
pNN50 – percent of successive RR intervals with a change of more than 50 msec
Root mean square of successive differences

Frequency Domain Analysis

1. ***Long Term Spectrum*** - A single 262,144 point power spectrum will be calculated on the entire 24 hour recording by simply squaring the magnitude of the Fast Fourier Transform of the HR time series. The power spectrum will be displayed with logarithmic smoothing in over frequency. The power in the following frequency bands will be calculated by integrating the point spectrum: a 0.00003-0.0033 Hz, 0.033-0.4 Hz, 0.04-0.15 Hz and 0.15-1.00 Hz (ultra low frequency (ULF), very low frequency (VLF), low frequency (LF), high frequency (HF), respectively). The fractional power in each band will also be computed as a percent of the total power (variance) from 0.00003-1.0 Hz. The spectral slope on a log-log scale, and the Y-axis intercept at 10^{-4} Hz will be examined as variables which may vary independently from the total power (= the area under the spectrum) and each other.

2. ***Short-Term Spectra*** - Spectra will be calculated on the consecutive 5 minute data segments in the entire 24 hour record. They will be quantified by computing for each 5 minute segment the total spectral area (TOT= the variance (SD^2) of HR), and the variance of the spectral powers in the frequency bands which best differentiate sympathetic and parasympathetic effects - 0.01-0.15 Hz (low frequency, LF), and 0.15-1.0 Hz (high

3. frequency, RHF). In addition, the LF/HF ratio, and a sympathetic/parasympathetic balance parameter (BAL), defined as (LF-HF)/(LF+HF) will be computed for each segment.

4. ***Short-Term Averages*** - The 24 hour recording will be further quantified by computing the average and SD of the 5 minute TOT, LF, HF, LF/HF ratio and balance parameters during the following periods:

a) entire recording,

b) 1 hour segment with the lowest mean HR to represent comparative sleep states.

This frequency domain analysis will yield numerous frequency specific variability measures which will allow an assessment of cardiac autonomic activity over the course of a day including:

a) Parasympathetic HR modulation assessed using: *TOT* and *HF;*

b) Sympathetic HR modulation assessed using: *LF* and the *LF/HF* ratio;

c) Sympathetic/Parasympathetic balance assessed using: *BAL*, +1 *sympathetic, -1 parasympathetic.*

References

(1) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 93: 1043-1065, 1996.

(2) Berntson GG, Bigger JTJ, Eckberg DL, Grossman P, Kaufmann PG, Malik, Nagaraja HN, Porges SW, Saul JP, Stone PH and Van der Molen MW. Heart rate variability: origins, methods, and interpretive caveats. [Review] [220 refs]. *Psychophysiology* 34: 623-648, 1997.