TRIAL OF ACE INHIBITION IN INFANTS

WITH SINGLE VENTRICLE

Original Date: 4-1-03

Amendment: 5-30-03

Amendment: 12-01-03

Amendment: 01-12-04

Amendment: 7-14-04

Amendment: 5-2-05

Amendment 4-13-07

Amendment 10-24-08

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

Pediatric Heart Network

Trial of ACE Inhibition in Infants with Single Ventricle

TABLE OF CONTENTS

TABLE OF CONTENTS, continued

TRIAL OF ACE INHIBITION IN INFANTS WITH SINGLE VENTRICLE

OVERVIEW (ABSTRACT)

Angiotensin converting enzyme (ACE) inhibitors are widely used in the treatment of infants with severe congestive heart failure to improve cardiac function and somatic growth. This multi-center, randomized, double-blind, placebo-controlled trial will evaluate the efficacy and safety of administering the ACE inhibitor enalapril to infants with a functional single ventricle.

Infants up to age 45 days with single ventricle physiology who have stable systemic and pulmonary blood flow will be eligible for inclusion in this study. After stratification by ventricular anatomy, neonates will be randomly assigned to receive enalapril or placebo and then followed for 14±1 months. Data will be collected at two time points: before palliative surgery at approximately age 6 months and at age 14±1 months (a minimum of 6 months post-palliative surgery for most infants). The primary aim of the study is to compare the effect of ACE inhibition to placebo on weight-for-age Z-score at age 14±1 months. Secondary aims include comparison of other measures of somatic growth (height- and head circumference-for-age Z-scores), signs and symptoms of congestive heart failure, developmental indices, and echocardiographic measures of ventricular mass, volume and function in the subjects in the two groups. The incidence of adverse events will also be compared between the treatment groups. Enrollment will continue through 2007 until the target of 230 subjects is met.

A. SPECIFIC AIMS

Growth impairment is common in infants and children with congenital heart disease [1], most often in the presence of congestive heart failure and/or cyanosis [2]. Growth failure is noted in many infants with single ventricle [3, 4], who manifest both cyanosis and heart failure, and persists after volume-unloading palliative surgery (the Glenn shunt). Whether this impairment is related to persistent or progressive abnormalities in cardiac structure and function is not known. All patients with single ventricle are exposed to chronic volume overload, which contributes to an inappropriate increase in myocardial mass and oxygen demand as well as abnormalities in ventricular systolic and diastolic function [5-7].

Angiotensin converting enzyme (ACE) inhibitors are widely used in the treatment of infants with severe congestive heart failure to improve cardiac function and somatic growth [8-11]. The ability of an ACE inhibitor to improve somatic growth in infants with single ventricle has not been previously studied. This multi-center, randomized, double-blind, placebo-controlled trial will evaluate the efficacy and safety of administering the ACE inhibitor enalapril to infants with single ventricle.

A.1 Primary Aim

To compare the effect of ACE inhibition therapy to that of placebo on somatic growth.

Hypothesis: Somatic growth will be greater in infants receiving ACE inhibition therapy compared to those receiving placebo.

Primary outcome:

- Weight-for-age Z-score determined at age 14±1 months

Secondary outcomes:

- Height-for-age, weight-for-height and head circumference-for-age Z-scores determined at age 14±1 months
- Weight-for-age, height-for-age, weight-for-height and head circumference-for-age Z-scores determined immediately before the Glenn shunt

A.2 Secondary Aim

To compare the effect of ACE inhibition therapy to that of placebo on the signs and symptoms of heart failure.

Hypothesis: ACE inhibition therapy will be associated with a lower heart failure class and lower levels of neurohormonal activation determined immediately before the Glenn shunt and at age 14±1 months.

Outcomes:

- Ross Heart Failure Class
- B-type natriuretic peptide (BNP) level

A.3 Secondary Aim

To compare the effect of ACE inhibition therapy to that of placebo on neurodevelopmental and functional status.

Hypothesis: ACE inhibition therapy will be associated with higher neurodevelopmental status determined at age 14±1 months.

Outcomes:

- Bayley Scales of Infant Development-II
- MacArthur Communicative Developmental Index
- Functional Status II-Revised Questionnaire

A.4 Secondary Aim

To compare the effect of ACE inhibition therapy to that of placebo on ventricular geometry, function, and atrioventricular valve regurgitation.

Hypothesis: ACE inhibition therapy will be associated with improved ventricular function and reduced ventricular remodeling, determined immediately before the Glenn shunt and at age 14±1 months.

Outcomes:

- Ventricular mass, volume, mass to volume ratio, ejection fraction and regurgitant volume measured by echocardiography
- Ventricular diastolic function assessed by echocardiographic techniques
- Ventricular filling pressure measured by catheterization immediately before the Glenn shunt
- Assessment of atrioventricular valve function by echocardiography

A.5 Secondary Aim

To determine the relationship between genetic polymorphisms linked to ventricular hypertrophy and the response to ACE inhibition therapy.

Hypothesis: The effect of ACE inhibition therapy on ventricular mass will be greater in infants with genetic polymorphisms involving the renin-angiotensin-aldosterone system (RAAS) compared to those without genetic polymorphisms involving the RAAS.

Outcome:

- Ventricular mass

A.6 Secondary Aim

To compare the incidence of adverse events occurring in subjects treated with ACE inhibition therapy to that in subjects receiving placebo

Hypothesis: ACE inhibition therapy will be associated with a similar rate of adverse events when compared to placebo.

Outcome:

- Incidence of adverse events including but not limited to an unexpected decrease in oxygen saturation, need for the Glenn shunt surgery to be performed at a younger age, elevated creatinine, hypotension, and increased length of hospital stay after the Glenn procedure.

B. BACKGROUND

B.1 Prior Literature/Studies

B.1.1 Single Ventricle: Definition and Surgical Approach

Defining the optimal therapeutic approach to the child with single ventricle physiology is one of the most challenging problems in pediatric cardiology. Although the incidence of single ventricle is only 1.25% in infants born with congenital heart disease [12], cardiac surgery for patients with single ventricle comprises up to 20% of total surgical volume in some centers. Anatomic variants of single ventricle are characterized by either absence or hypoplasia of the right or left ventricle, or incomplete ventricular differentiation leading to a single ventricle of indeterminate ventricular morphology.

The ultimate goal of palliative surgery for patients with single ventricle physiology is to establish the Fontan circulation by connecting the superior and inferior vena cavae directly to the pulmonary artery, allowing the systemic venous blood to bypass the single ventricle and flow directly to the lungs. Most commonly, three surgical procedures are performed within the first three years of life to achieve this result. The first procedure, which establishes stable systemic and pulmonary blood flows, is performed in the newborn period. As a result of this surgery, the single ventricle is exposed to an abnormally high circulatory volume load. The second surgery connects the superior vena cava directly to the pulmonary artery. There are a variety of names for the minor variations of this procedure including bi-directional Glenn shunt, hemi-Fontan, cavo-pulmonary anastomosis, and Kawashima; for the purpose of this protocol, the term Glenn shunt will be considered to include all of these variants. The Glenn shunt surgery is performed at about six months of age and only partially alleviates the increased volume load on the single ventricle. The timing of the Glenn shunt is often a matter of physician preference but is also dictated by the degree of cyanosis or heart failure. The third procedure, the modified Fontan operation, during which the inferior vena caval blood is directed into the pulmonary artery, is typically performed by two to three years of age. Prior to the completion of the Fontan, the single ventricle remains exposed to abnormal oxygenation and chronic volume overload which may result in an inappropriate increase in myocardial mass and oxygen demand and abnormalities in ventricular systolic and diastolic function [5, 7, 13].

B.1.2 Growth and Functional Status in the Infant with Single Ventricle

Growth Impairment in the Infant with Single Ventricle

Weight gain in infants with congenital heart disease is decreased compared to that in normal infants; the highest incidence of growth failure is seen in infants under age 3 months. Growth failure is manifested by impairment in both height and weight; weight gain is affected to a larger degree than height. In a study of infants with a variety of congenital heart defects, growth failure was most severe in infants with congestive heart failure and cyanosis [14]. In a study of infants with hypoplastic left heart syndrome, this appeared to be related in part to higher energy expenditure [15]. Nutritional management alone did not ensure adequate weight gain following the initial staged palliation [16]. Although the Glenn shunt may improve growth by reducing ventricular volume overload and increasing oxygen delivery, persistence of growth retardation after the Glenn shunt suggests that this surgical palliation is inadequate to restore normal growth. Preliminary data from Columbia University are shown in the first 3 rows of Table 1,

followed by published results [4, 17]. The data show the early onset of growth failure in these infants that persists well beyond infancy despite palliative surgery.

Table 1. Somatic Growth in Infants with Single Ventricle

Time	N	Mean Weight-for-Age Z-Score [SD]	Mean Height-for-Age Z-Score [SD]
Birth	36	-0.45 [1.35]	-0.10 [1.1]
Pre-Glenn [6.5 mo]	36	-1.71 [0.94]	-0.93 [1.41]
Post-Glenn [20.1 mo]	36	-1.48 [1.25]	-1.08 [1.77]
Post-Glenn [17]	65	-0.91 [0.99]	-0.89 [1.2]
Post-Glenn* [12.1 mo] [4]	55	-0.95 [1.11]	-1.1 [1.15]

*Growth data estimated from published figure

Neurodevelopmental Status in Infants with Single Ventricle

Infants who have undergone the staged Norwood procedure demonstrate intellectual, motor and behavioral impairment, and generally perform at lower levels than healthy control children [18, 19]. The etiology of these neurodevelopmental deficits appears to be multifactorial with medical, surgical and socio-demographic factors all contributing to outcome [20]. Miceli and colleagues reported a close correlation between the rate of medical complications (e.g. duration of ventilatory support, need for supplemental oxygen, intravenous or gavage feedings) and developmental outcomes at 4 and 13 months of age assessed using the Bayley mental and psychomotor scales in low birth weight, preterm infants [21]. Their data demonstrated that the medical complications infants experienced in the weeks and months after birth increase the risk of impaired development. These findings suggest that improvement in medical care has the potential to improve the neurodevelopmental outcome in infants with complex medical conditions. An improvement in functional outcome in infants following treatment with ACE inhibitors would provide a strong clinical rationale for their use in this population.

The Bayley Scales of Infant Development (BSID-II) is a standardized instrument to measure psychomotor and mental development [22-24] that has been widely used in the assessment of infants with chronic medical conditions. Marked abnormalities in BSID-II in early infancy correlate with performance in later life. This is important in infants with single ventricle since

they demonstrate not only early abnormalities in development, but they also have a lower intellectual performance at school-age compared to the general population [25]. Since ACE inhibitors may improve cardiac status in these infants, this may be reflected in improved motor and overall functional status as measured using the BSID-II. Preliminary data from Children's Hospital Boston obtained in 20 infants (mean age 18 months) with hypoplastic left heart syndrome demonstrated significant abnormalities in the BSID-II Psychomotor Development and Mental Development Index Scores; mean±SD of 74±15 and 85±14, respectively (unpublished data); BSID-II scores in the normal population for both indexes are 100±15.

B.1.3 Cardiac Remodeling in the Single Ventricle

B.1.3.a Ventricular Mass and Volume in the Single Ventricle

Ventricular mass is significantly increased in patients undergoing palliative surgery for single left ventricle [26-28], and increased mass correlates negatively with outcome [26, 29, 30]. The increase in mass often persists despite the Glenn shunt and may contribute to late systolic and diastolic ventricular dysfunction [27, 28]. Although ventricular hypertrophy in response to a volume load on the single ventricle may be compensatory, the persistence of hypertrophy despite volume unloading and the association with poor function and outcome suggest that the hypertrophy is maladaptive and pathologic.

B.1.3.b Role of the Renin-Angiotensin System in Ventricular Remodeling

Persistence of increased mass despite volume unloading surgery suggests that factors other than volume load may contribute to hypertrophy of the single ventricle. The role of neurohormonal activation in cardiac remodeling and dysfunction is well-defined. A mechanical load on the heart increases local angiotensin II production. Angiotensin II, via the angiotensin 1 receptor, promotes cellular phenotypic changes, cell growth, and apoptosis in cardiac myocytes, fibroblasts and vascular endothelial and smooth muscle cells, leading to cardiac hypertrophy, remodeling and heart failure [31, 32]. There is increasing evidence of neurohormonal activation in patients with congenital heart disease at all ages despite surgical correction or palliation [33, 34-36]. The plasma concentrations of several neurohormones, including angiotensin II and BNP, are increased in patients who have undergone the Glenn or Fontan procedures [34, 37].

The infant ventricle appears to be particularly susceptible to ventricular hypertrophy and remodeling in response to abnormal loading conditions. Experimental animal studies demonstrate enhanced myocardial remodeling in response to volume overload in the neonatal period and a greater dependence of the infant ventricle on the cardiac renin-angiotensin system for increases in ventricular size and muscle mass [38, 39].

B.1.3.c Role of Genetic Polymorphisms in Ventricular Response to Load

The degree of ventricular hypertrophy and the response to therapy may be modified by the genotype of the individual. Polymorphisms in the genes of the RAAS including ACE, angiotensinogen (AGT), and angiotensin II receptor type 1 gene (AT1R) have been shown to influence the degree of hypertrophy in many studies, although not consistently [40-43]. The variability of results has been explained in part by the failure to examine the compounding influence of RAAS polymorphisms as a whole. Ortlepp and colleagues [44] evaluated 26 members of a family who were carriers for a mutation in a myosin-binding protein which is associated with hypertrophic cardiomyopathy. Despite the presence of the mutation, only nine of these 26 gene carriers had the phenotype of hypertrophic cardiomyopathy. Each family member was evaluated for the presence of a polymorphism in five RAAS genes (ACE, AGT, AT1R, aldosterone synthase, chymase) and an individual with one or more polymorphisms was considered to have a “pro-left ventricular hypertrophy (LVH)” or “risk enhancing” genotype. The investigators found that LV mass and septal thickness were significantly greater in family members with one or more polymorphisms in the RAAS genes; a direct correlation was present between the number of polymorphisms and the degree of LVH. This study provides evidence that the evaluation of compound polymorphisms may better explain phenotypic variation in ventricular hypertrophy than the analysis of single polymorphisms alone [44]. A similar influence of compound polymorphisms on LVH was found in endurance athletes [40].

The RAAS genotype also appears to play an important role in the response to treatment [45-47]. In a study by Kurland and colleagues of 115 males with mild-moderate hypertension, the treatment-induced reduction in LV mass was greater in those patients with one or more polymorphisms in the AGT and/or AT1R genes than in those without the pro-LVH genotype [47]. This difference was independent of the effect of treatment on the blood pressure. Given this background, we propose to study the influence of

polymorphisms of the following five RAAS genes on the response of the infant single ventricle to ACE inhibition therapy:

- ACE gene
- Angiotensinogen gene
- Angiotensin II receptor type 1 gene
- Aldosterone synthase gene
- Cardiac chymase A gene

B.1.4 Studies of ACE Inhibition

B.1.4.a Effect of ACE Inhibition on Ventricular Remodeling and Function

ACE inhibitors improve survival in adult patients with heart failure regardless of etiology and independent of the afterload-reducing effects. Treatment with ACE inhibitors decreases ventricular remodeling, even in the absence of clinical heart failure. In particular, ACE inhibition has been shown to:

- Slow left ventricular dilatation in patients with asymptomatic LV dysfunction [48]
- Reverse myocyte remodeling and prevent the development of ventricular hypertrophy and dysfunction in hypertensive animals independent of blood pressure-lowering effects [49]
- Prevent the development of heart failure in patients at high risk for cardiovascular complications who have no evidence of cardiac dysfunction [50]

A likely mechanism for these beneficial effects on remodeling is decreased angiotensin II production and increased kinin-dependent nitric oxide production. ACE inhibitors also decrease neurohormonal activation in association with clinical improvement in heart failure [51].

B.1.4.b Effect of ACE Inhibition on Growth in Infants with Heart Disease

Although ACE inhibitors are commonly administered to infants and children for treatment of heart failure [52], there are few reports regarding the use of ACE inhibitors in infants with complex congenital heart defects [11, 53]. The benefits of ACE inhibition in volume-loaded hearts were demonstrated in studies that reported both acute and long-term

reduction in LV volume and mass with ACE inhibitor use in children with chronic regurgitant lesions and other congenital heart defects [54, 55].

Besides the effects on ventricular remodeling and function, studies have demonstrated a rapid beneficial effect of ACE inhibition on growth in infants with severe congestive heart failure [9, 10], with a two-to-threefold increase in weight gain within a one-to-three month follow-up [8]. Improved weight gain was seen in association with an improvement in clinical symptoms of heart failure; however, small numbers of study subjects and relatively short follow-up limit the majority of reports. An informal survey of the seven Pediatric Heart Network centers showed that ACE inhibitors are administered to 40-60% of infants with single ventricle during the first year of life.

B.2 Rationale for this Trial

Impaired growth is common among infants with single ventricle. Although the etiology of growth failure in this group of patients is not well defined, cyanosis in the setting of abnormal ventricular loading conditions is likely to play an important role. In short term studies, ACE inhibition therapy improves growth in infants with ventricular volume overload. In addition, administration of ACE inhibitors reverses ventricular remodeling in adult patients with ventricular dysfunction and hypertrophy.

Despite the fact that administration of ACE inhibitors to infants with single ventricle physiology has not been studied systematically, empiric use is common. The results of this study will make an important contribution to the management of these infants by determining whether early initiation of ACE inhibition therapy improves clinical outcome, ventricular geometry, and function during the first 14 months of life. Possible deleterious effects of this therapy will also be assessed. The study will also establish a cohort of single ventricle patients who can be followed prospectively to assess the impact of early intervention on long-term outcome, and will provide important data to enable future evaluation of interventions in this complex group of patients.

B.3 Rationale for Selection of Outcome Measures

B.3.1 Measures of Somatic Growth

Growth failure is common in infants with congenital heart defects and is most severe in infants with both chronic ventricular volume overload and cyanosis, as is typically seen in single ventricle physiology [1, 14]. Therapy with ACE inhibitors may improve ventricular function and cardiac output, thereby improving growth in these patients. Weight-for-age is severely diminished in infants with single ventricle and is a readily measurable and easily interpretable parameter of growth. Weight- and head circumference-for-age and weight-for-height are also decreased in single ventricle patients. Growth parameters will be measured both before the Glenn shunt and at age 14±1 months to determine the period of maximal effect of ACE inhibition on growth.

B.3.2 Measures of Severity of Heart Failure

B.3.2.a Ross Heart Failure Class

ACE inhibitor therapy may improve ventricular function and thereby reduce signs and symptoms of circulatory insufficiency. The Ross Heart Failure Class for infants and children (See Appendix D) is comparable to the New York Heart Association (NYHA) heart failure class for adults. 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 [56, 57]. There are four classes that are defined by a composite assessment of respiratory effort, feeding difficulties and growth. Although growth failure is a component of the assessment, the inclusion of respiratory and feeding difficulties allows symptomatic heart failure to be evaluated in the study subjects.

B.3.2.b B-Type Natriuretic Peptide

BNP is a neurohormone primarily secreted by the ventricular myocardium in response to volume expansion or pressure overload. Early studies demonstrated a correlation of the plasma BNP level with ventricular dilatation in patients with dilated cardiomyopathy [58]. Normative data for BNP levels in infants are available [59]. There is accumulating evidence that BNP levels are a sensitive marker for abnormal systolic and diastolic

function in children with congenital heart disease [60, 61] and correlate with functional class and systemic ventricular function in older patients with congenital heart disease [33-36]. Therapy with ACE inhibitors has been shown to lower BNP [62-64]. BNP will be used to assess the effect of ACE inhibition on neurohormonal activation in the study subjects.

B.3.3 Neurodevelopmental Status

The Bayley Scales of Infant Development-Second Edition [65], the most recently standardized and most widely used instrument for assessing infant development (1 to 42 months of age), produces scores designated as the Psychomotor and Mental Development Index scores (PDI and MDI). The PDI tasks examine gross and fine motor function, whereas the MDI tasks examine cognitive functioning. The Bayley Scales have very good concurrent validity and reliability. Although the predictive validity of Bayley scores is low in a population of normally-developing children, their predictive validity is considerably higher in samples of at-risk infants, such as children with congenital heart disease [66]. In preliminary studies from Children's Hospital Boston, MDI and PDI scores at 12 months of age were significantly associated with intelligence quotient (IQ) score on the Wechsler Preschool and Primary Scale of Intelligence, Full-Scale, Fine and Gross Motor scores on the Peabody Developmental Motor Scales, and oromotor apraxia at four years of age. Although mild-to-moderate cognitive impairment may be missed by the BSID-II, the BSID-II is sensitive in detecting significant neurodevelopment delay, which has been demonstrated in preliminary studies of infants with single ventricle. Preliminary data from Children's Hospital Boston obtained in infants with hypoplastic left heart syndrome demonstrates the feasibility of using this instrument for assessing functional status in infants with single ventricle (see Section B.1.2)

The MacArthur Communicative Development Inventory/Words and Gestures (CDI) [67] is a parent-report instrument for assessing early language skills, designed for use in children 8 to 16 months of age. Use of the CDI will complement the Bayley Scales by providing a detailed assessment of several important aspects of early cognitive development (specifically symbolic) that are difficult to measure in a brief developmental assessment. The CDI/Words and Gestures should be sufficiently sensitive to identify any differences between treatment groups in terms of language development based on previous experience with the MacArthur instruments. In a study from Children's Hospital Boston, children assigned to a vital organ support technique

of deep hypothermic circulatory arrest achieved significantly lower scores than children assigned to low-flow cardiopulmonary bypass on several subscales of the Communicative Development Inventory/Word and Sentences, a similar instrument which is designed for 16 to 30 month old children [68].

The Functional Status II-Revised (FSII-R) is a parent report questionnaire that has been used to assess the health status of children with chronic disorders [69]. The instrument measures normal, daily, age-appropriate functions (how well the child eats, sleeps, plays, and temperament, etc.) and has been shown to correlate well with other markers of disease severity such as hospitalization rates, length of hospital stay, and other illness [70]. This questionnaire will provide important functional status information on the complete cohort in the event that administration of the Bayley Scales is not well tolerated by or not completed on all subjects.

The BSID-II, MacArthur CDI and the FSII-R will provide a profile of the motor and cognitive capabilities of the study subjects that will allow us to determine if ACE inhibition results in a change in functional and neurodevelopmental status.

B.3.4 Measures of Ventricular Geometry and Function

B.3.4.a Echocardiographic Indices of Cardiac Morphology and Function

Despite the limitations of quantitative two-dimensional echocardiography in single ventricle patients, it remains a universally available, standardized method for evaluation of cardiac morphology and function. Two-dimensional echocardiography will be utilized for assessment of ventricular function and to quantify the severity of atrioventricular (AV) valve insufficiency. Although measurements of Doppler indices of diastolic function have not been fully validated in single ventricle patients, the ease of obtaining these indices during the echocardiogram, and the absence of additional expense to the study, make it attractive to perform these measurements and to correlate them with the effect of ACE inhibition therapy.

B.3.4.b Hemodynamic Indices of Cardiac Function

The majority of infants undergo a diagnostic cardiac catheterization before the Glenn shunt; the ventricular end diastolic pressure and the pulmonary-to-systemic flow ratio will

be compared between treatment and placebo groups. These data will be obtained as part of routine catheterization with no additional time or expense required.

B.3.5 Relationship between Genotype and Response to ACE Inhibition

There is increasing evidence that the RAAS genotype modifies the response of the ventricle to load [44] and may also influence the response to therapy [47]. The frequency of RAAS polymorphisms will be assessed in the study population and their influence on the response to ACE inhibition will be evaluated by comparing the differences in ventricular mass between the enalapril and placebo groups in patients with and without the RAAS polymorphisms.

The list of potential candidate genes that may influence the progression of cardiac hypertrophy, remodeling and dysfunction is formidable. Candidate genes which have distinct associations with cardiac hypertrophy and are expressed with a relatively high frequency in the general population have been chosen as genes of interest to evaluate for specific mutations and to identify a "pro-hypertrophy" genotype in this study. These are:

- DD polymorphism in ACE gene (ACE)
- Met/Thr exchange in angiotensinogen gene (AGT)
- Polymorphic AC in angiotensin II receptor type 1 gene (AGTR1)
- C/T exchange at position -344 of the aldosterone synthase gene (CYP11B2)
- A/G exchange at position -1903 of the cardiac chymase A gene (CMA)

The frequency of homozygous alleles for a polymorphism in an individual RAAS gene is 20-25% in a control population [44]. However, when taken in combination, the likelihood of having at least one polymorphism in any of the RAAS genes is much higher, i.e. 60% [44]. Defining this as a "pro-hypertrophy" genotype will increase the likelihood of detecting the influence of genetic polymorphisms on the hypertrophic response to ACE inhibition. Genotyping at these loci could provide useful information that would permit tailoring of pharmacologic therapy in the future to those with the greatest likelihood of having a favorable outcome.

B.3.6 Incidence of Adverse Events with ACE Inhibition

ACE inhibitors are generally well-tolerated in infants and children with heart failure [52]. Although there are no large published studies of the use of ACE inhibitors in infants with single

ventricle, the fact that physicians in many centers use these drugs is suggestive of a favorable risk-to-benefit ratio. We selected enalapril as the ACE inhibitor in this study because it is FDA approved for use in children. In addition, enalapril only needs to be given twice each day. The pharmacokinetics and dose ranges of enalapril in children are well established [71-74].

There is a small risk of renal insufficiency with ACE inhibitor use [75]. Therefore, renal function will be closely monitored and infants with creatinine >1.0 mg/dL will not be enrolled in the study. Reduced blood pressure is a known effect of ACE inhibition. In infants with single ventricle, pulmonary blood flow is partly dependent on systemic blood pressure; thus a potential adverse effect of ACE inhibition may be decreased oxygenation. Blood pressure and oxygen saturation will be closely monitored in all subjects. Guidelines for adjustment or discontinuation of the study drug in the event of significant hypotension or systemic desaturation are included in section C.4.1.a.

Timing of the Glenn shunt and length of stay after the Glenn shunt are secondary outcome variables for safety assessment. The timing of the Glenn shunt is dictated by physician preference and by the degree of cyanosis and the severity of heart failure. If significant desaturation occurs in patients receiving ACE inhibition, the Glenn shunt surgery may be performed relatively early. We will determine if hospital stay following Glenn shunt is adversely affected by ACE inhibitor use. ACE inhibition may potentially improve the postoperative course and reduce length of stay after the Glenn shunt by improving systolic and diastolic ventricular function.

B.3.7 Rationale for Study Duration

The study duration of 14 months is based on the following rationale. Most centers perform the Glenn shunt between 4-8 months of age. A study duration of 14 months provides at least 6 months follow-up after the Glenn shunt to assess the effects of ACE inhibition independent of the acute effects of the surgery. A window of $\pm$1 month allows for flexibility in visit scheduling. To determine if ACE inhibition therapy improves growth before the Glenn shunt, outcome measures will also be analyzed immediately before this procedure. Although a fixed study duration may result in varying periods of follow-up after the Glenn shunt, this approach has the advantage of (i) standardizing the duration of ACE inhibition, which could be an important determinant of response to therapy, and (ii) keeping the duration of the study independent of the

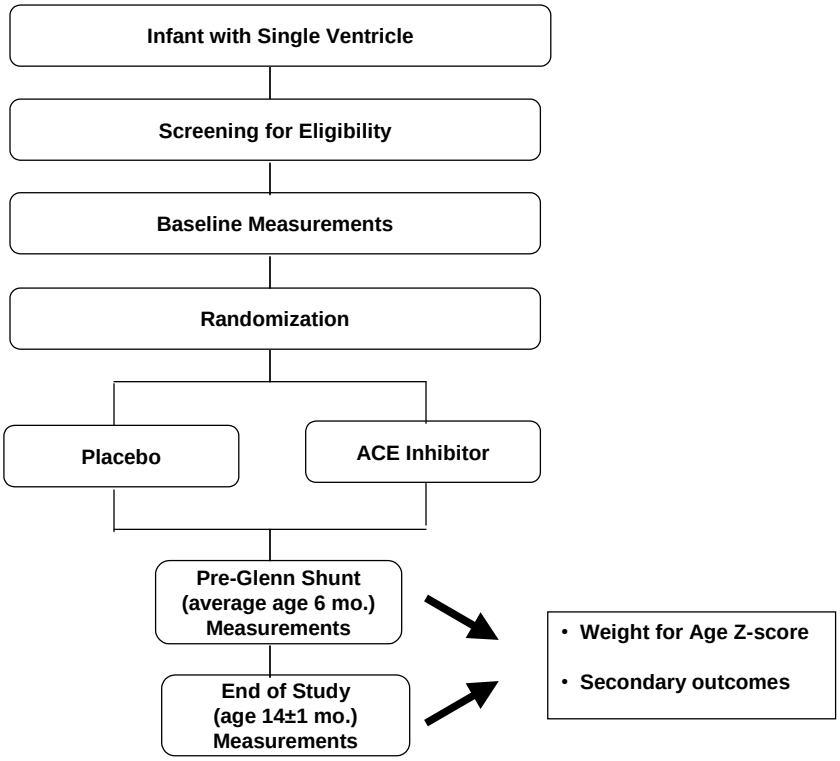
timing of the Glenn shunt, an event which may theoretically be influenced by ACE inhibition therapy.

C. STUDY DESIGN AND METHODS

C.1 Overview

A prospective, randomized, double-blind, placebo-controlled trial of ACE inhibition in infants with single ventricle.

Figure 1. Single Ventricle Trial Protocol



C.2 Participants

C.2.1 Inclusion Criteria

To be eligible for this trial the patient must meet all of the following inclusion criteria including:

- Age ≤ 45 days
- Age > 1 week if born at 35 weeks gestation
- Single ventricle physiology
- Stable systemic and pulmonary blood flow
- Planned Glenn shunt surgery (or variant known as hemi-Fontan)
- Informed consent of legal guardian

C.2.2 Exclusion Criteria

To be eligible for the trial, a patient must meet none of the following criteria:

- Birth weight ≤ 2.5 kg if gestational age is ≥ 38 weeks; birthweight < the 10th percentile for gestational age if gestational age is 35-37 weeks
- < 35 weeks gestation
- Anatomic diagnosis of pulmonary atresia with intact ventricular septum
- < 3 days after palliative cardiac surgical procedure, if performed
- Aortic oxygen saturation < 65%
- Current mechanical ventilatory support
- Current intravenous inotropic support
- Creatinine >1.0 mg/dL
- Absolute neutrophil count <1000 cells/mL
- Chromosomal or recognizable phenotypic syndrome of non-cardiac congenital abnormalities associated with growth failure (e.g. Trisomy 21, Noonan's syndrome, Turner's syndrome)
- Prior ACE inhibitor use for > 7 consecutive days

C.2.3 Patient Availability

Each Pediatric Heart Network investigator estimated the number of new infants with single ventricle physiology per year at their center. The projected Network total is 220 infants/year.

We project that 40% will be eligible and will also provide informed consent. With a target sample size of 230 patients, accrual is expected to be complete within 43 months due to the lower than planned eligibility and consent rate in the first year of the trial. However, enrollment will continue until the target of 230 is met.

C.2.4 Recruitment Protocol

The Principal Investigator at each clinical center, his or her designate, and the nurse coordinator will have responsibility for case-finding. Study centers will design a system by which infants with single ventricle are identified through review of inpatient records. A study investigator or the nurse coordinator will request informed consent from the parent(s) or legal guardian of all patients who meet study eligibility criteria. Data (demographic, eligibility criteria, and informed consent status) will be recorded on a screening form for all patients with single ventricle evaluated at the participating center, regardless of their inclusion in the study, for development of descriptive statistics and definition of the study population.

C.2.5 Human Subjects Considerations

The characteristics of the patient sample and sources of research material are specified in Sections C.2.1-C.2.4. Consent for the main study will be obtained from the parent(s) or legal guardian once eligibility has been established. Consent will be sought by site study investigators, study nurse coordinators, or assigned designates, and documented by the parent's or legal guardian's witnessed signature on an informed consent document (Appendix A). The consent process will require separate signatures indicating consent to use the subject's blood for a) RAAS genotyping and b) future genotyping studies. Informed consent for the genetic study components will typically be obtained from the parent(s) or legal guardian immediately prior to the Glenn shunt surgery, but may be obtained earlier depending on local procedures. If consent for either the RAAS genotyping and/or future genotyping studies is not obtained, the subject will participate in all study components except the RAAS and future genotyping, as consented to by the subject's parent/legal guardian (Appendix B).

C.2.5.a Potential Risks

The possible risks or discomforts of the study to the subjects include:

1. Treatment with enalapril may rarely induce clinically-important hypotension [11, 75]. The study drug will be initiated in-hospital at a low starting dose with frequent monitoring of blood pressure and oxygen saturation. The dose will not be up-titrated unless the blood pressure and oxygen saturation is stable. The decreased blood pressure observed after administration of enalapril has rarely resulted in discontinuation of the medication. Blood pressure and oxygen saturation will be monitored during the up-titration phase as described in Section C.4.1 and at every study visit.
2. Renal dysfunction has been reported in infants with cyanotic heart disease following ACE inhibition with an incidence of 0-13% [8, 75]. Serum creatinine will be measured during the up-titration phase and serially as described in Section C.5.1.
3. Other rare reported side effects of ACE inhibitors in infants have included hyperkalemia, angioedema and neutropenia. Study patients will be monitored for these effects as described in Section C.5.1.
4. All office visits will be part of the routine care of infants with single ventricle.
5. All echocardiograms performed before enrollment will be reviewed to evaluate ventricular function and atrioventricular valve regurgitation. If an echocardiographic study documenting ventricular function and atrioventricular valve regurgitation at the time of enrollment is not available, a limited study echocardiogram will be performed to evaluate ventricular function and atrioventricular valve regurgitation. No sedation will be required for this baseline echocardiographic study. The echocardiographic study performed at the pre-Glenn shunt time point is part of routine care. Whenever possible, performance of the echocardiogram at age 14 months will be planned to coincide with the timing of a clinically indicated follow-up echocardiogram. If an echocardiogram is not scheduled at age 14 months, a study echocardiogram will be performed. Moderate (conscious) sedation may be required to obtain the echocardiographic images necessary for analysis. If other sedated studies are being performed at the pre-Glenn shunt and age 14 months timepoints, every effort will be made to obtain the sedated echocardiogram at the same time. The use of sedation will be managed according to the practice at the individual center. All sedation protocols will adhere to practice guidelines for sedation and analgesia [76].

6. Safety laboratory examinations will be performed at 5 time points during the study as shown in Table 2. Two of these time points (4 day post-study drug initiation and before the Glenn shunt) are part of routine care. Safety laboratory examinations planned at 2 weeks post-study drug initiation, 7 days after restarting study drug post-Glenn shunt and age 14 months may not be routine at all Network centers. The amount of extra blood drawn at any venipuncture for laboratory tests that pertain only to this study will not exceed 4 mL. The total quantity of extra blood drawn over the 14 months of subject participation in the study will not exceed 8 mL, which includes 1 mL for safety laboratory examination at the 2 week post-study drug, 7 days after restarting study drug post-Glenn shunt, and age 14 month time points, one mL for each of two BNP measurements and three mL for genotyping.
7. The length of the subject's hospital stay will not be affected by any tests involved in this study.
8. All testing that is not part of routine care will be performed free-of-charge.
9. Investigators from the clinical center will have access to the medical record for up to 6 years following enrollment to review the results of follow-up clinical course, surgical intervention and other relevant studies.

C.2.5.b Potential Benefits

The possible benefits of participation in the proposed study to the subject are:

1. The subject's family, through the primary care provider and cardiologist, will receive extensive information regarding his/her cardiac status.
2. The evaluation of neurodevelopmental and functional status may provide valuable information to families and primary care providers that would not otherwise be available. This may lead to early intervention if evidence of developmental or functional abnormalities is detected.

C.2.5.c Risk/Benefit Ratio

The risk/benefit ratio of the study is favorable. Although an individual subject may not directly benefit from study participation, the results of this study will make an important contribution to the management of infants with single ventricle by determining whether early initiation of ACE inhibition therapy improves clinical outcome, including growth, ventricular geometry, and myocardial function during the first 14 months of life. This

study will also provide important information regarding the possible deleterious effects of this therapy in infants with complex cyanotic heart disease. In addition to the primary aim of this study, the information obtained concerning the interrelationships of measures of clinical status, laboratory evaluations, and medical and surgical therapies in infants with single ventricle will make an invaluable contribution to the overall management of these complex patients.

If parents decline to participate, their child's medical care will not be adversely affected in any way. If they agree to participate, they are free to withdraw their child from the study at any time.

Patients 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 (e.g., if the patient and family return to the study center for follow-up that otherwise would have been obtained closer to their home).

Patients will be enrolled without regard to gender, race, and ethnicity.

C.2.5.d Gender and Minority Recruitment

Based on current rosters of single ventricle patients at the Network sites, it is estimated that 40% of patients will be female and 15-30% of the patients will be of minority race/ethnicity, depending on geographic location of the clinical site.

C.2.5.e. Patient Confidentiality

Each subject will be assigned a study identification (I.D.) number so that study information will be confidential. The link between subject name and I.D. number will be stored only at the site where the subject received his/her clinical care.

The samples for RAAS genotyping will be sent to the Genetics Core laboratory by the study sites, labeled with the study I.D. and date of collection. The results of RAAS genotyping will be sent by the Core Laboratory to the Data Coordinating Center.

Samples for future genetic analysis will be stored at the Genetics Core Laboratory. The Data Coordinating Center will send a separate unique genetic identification number to

the Genetics Core Laboratory, which will be used to label the sample to be stored for future testing. The sample to be stored for future testing will not have the study identification number, subject's name or any other information that could identify the subject. The information at the Genetics Core Laboratory that links the unique genetic identifier to the study number will be destroyed immediately after the sample for future testing is relabeled, making it very difficult for the participating study site or the Genetics Core Laboratory to link future genotyping results with the study subject. Only the Data Coordinating Center will maintain the list linking the genetic identification number to the study identification number.

C.3 Trial Enrollment

C.3.1 Stratification

The study design will be a randomized, double-blind, placebo-controlled trial. The severity of growth impairment may be related to the underlying anatomy and the type of surgical procedure required in the neonatal period. Infants with hypoplastic left heart syndrome undergo a modified Norwood procedure (stage I surgical palliation) and may have the poorest clinical outcome over the first year of life. Therefore, we will randomize participating patients to treatment groups using randomly permuted blocks within strata defined by ventricular anatomy (hypoplastic left heart syndrome vs. no hypoplastic left heart syndrome). Dynamic balancing by center will be used to ensure that treatment arm totals are balanced within each center.

C.3.2 Blinding

All personnel at each Study Center, other than the research pharmacist, will be blinded to the patient's treatment assignment. Depending upon the policies and procedures at the individual clinical centers, treatment assignment will be obtained over the internet from the randomization computer at the Data Coordinating Center either by an investigator (or by his/her delegate) or by the responsible pharmacist. A numeric Treatment Allocation Code will be given, rather than the actual treatment assignment. Using a master list of assignments, the pharmacist will decipher the code with respect to whether the patient is assigned to the enalapril or placebo group. With this procedure, the randomization process can be completed by study staff who are to remain blinded to treatment assignment.

C.3.3 Randomization Procedure

Patients will be randomly assigned in a 1:1 ratio to the enalapril and placebo treatment groups. All eligibility criteria must be confirmed, and written informed consent obtained before randomization. Randomization will be accomplished over the Internet using the randomization computer at the Data Coordinating Center (available 24 hours a day, seven days a week). After supplying stratification information (see Section C.3.1) and verifying key eligibility criteria, the randomization computer will return a numeric code that the pharmacist will decipher into treatment group assignment (see Section C.3.2). Investigators at each institution, together with the research pharmacist where possible, must maintain a log containing the patient's study I.D. and name, date of enrollment, date of treatment and Treatment Allocation Code.

C.4 Treatment

C.4.1 Study Drug

C.4.1.a Administration

Up-titration Period

After the baseline clinical evaluation, all patients will be entered into the up-titration period, during which they will receive enalapril or placebo in addition to their usual medications. The goal of the up-titration period is to reach the highest dose that will then be continued throughout the maintenance phase. Based upon the patient's body weight, the pharmacist will determine the appropriate initial dose of enalapril (starting dose 0.1 mg/kg/day) or placebo. Enalapril will be given in a concentration of 1 mg/ml and placebo will be given as a similar volume of Ora-Plus and Ora-Sweet. A baseline blood pressure and oxygen saturation will be obtained immediately before the dose of study drug. Blood pressure and saturation will be measured every 30 minutes for the first four hours after the initial administration of study drug. If the subject's systolic blood pressure is sustained $\geq$ 70 mmHg, the monitoring period will be 2 hours during the up-titration phase. If the subject's systolic pressure is sustained < 70 mmHg after either the initial dose or up-titration dose, the monitoring period will remain at 4 hours. The initial dose of study drug may be administered in either the inpatient or outpatient setting; however, it must be given at the PHN clinical center. Subsequent up-titrations may take place outside the PHN clinical center provided that the patient can be monitored and data submitted according to the requirements outlined above.

The dose will be up-titrated as tolerated to a target dose of 0.4 mg/kg/day divided BID. The use of ACE inhibitors is standard practice in all Network centers; the exact up-titration schedule is therefore left to the discretion of the study cardiologist. We expect that the target dose will often be reached while the subject is in the hospital, but up-titration in the outpatient setting is acceptable. Every effort should be made to reach the target dose within two weeks of study drug initiation. Up-titration should be delayed and the dose of the study medication may be decreased if any of the following events occur:

- Sustained systolic blood pressure < 60 mmHg
- Sustained oxygen saturation < 65%
- Serum creatinine > 1.0 mg/dL
- Serum potassium > 5.5 mM/L obtained by venipuncture
- Absolute neutrophil count < 2000 cells/mL
- Other adverse events felt to be attributable to study drug by the treating cardiologist

Often, the doses of concomitant cardiovascular medications such as diuretics can be adjusted to optimize the opportunity for continuing or increasing the dose of the study drug. No patient shall be challenged more than three times at a given dose.

Maintenance Period

After completion of the up-titration period, the subject will enter the maintenance phase of the study. During this period, if the target dose of the medication was not achieved, the investigator may continue to increase the dose level, as tolerated. At each study visit the dose of study drug will be adjusted for weight gain to maintain the target dose. Study drug will routinely be withheld in the immediate post-Glenn surgical period. When the patient is stable post-operatively, study drug will be restarted at the discretion of the individual investigator while the subject is still in the hospital after the Glenn shunt. In the event of other hospitalizations or acute illnesses, study drug may be held and restarted at the discretion of the individual investigator. A baseline blood pressure and oxygen saturation will be obtained immediately before restarting study drug after the Glenn shunt procedure. Blood pressure and saturation will be measured every 30 minutes for two hours following study drug administration. If the subject's systolic blood

pressure is not sustained ≥ 70 mmHg, monitoring should continue for an additional two hours.

C.4.1.b Study Completion

A patient will be considered to have completed the study if he/she has completed the maintenance period of the study and undergone the end of maintenance period assessment at age 14±1 months.

C.4.1.c Indications for Permanent Discontinuation of Study Drug

- Anaphylactoid reaction
- Serum creatinine > 1.0 mg/dL after adjustment of study drug dose
- Open label use of an ACE inhibitor > 7 consecutive days
- Other adverse events that require discontinuation of study drug in the judgment of the study investigator

The reason and the circumstances for permanent discontinuation of study drug will be documented. If study drug is permanently discontinued the patient will continue to be followed until their planned completion date (age 14±1 months).

C.4.1.d Indications for Withdrawal From the Study

- Patient/guardian refusal to continue in the study
- Participation in the study felt to be not indicated by the attending cardiologist or study investigator
- Cardiac transplantation

The reason for withdrawal and the circumstances of withdrawal will be documented for all patients withdrawn from the study.

C.4.2 Study Drug Preparation and Dispensing

C.4.2.a Enalapril Drug Preparation (200 mL, 1 mg/mL)

1. Vasotec (Biovail Technologies, Chantilly, VA) will be used in the preparation.
2. Count out 40 5-mg Vasotec tablets and place in mortar. Ten-mg Vasotec tablets may not be used for this preparation.

3. Crush tablets to a fine powder.
4. Add a small volume of sweetened suspending agent (equal volumes of Ora-Plus and Ora-Sweet) and titrate to make a smooth paste.
5. Add increasing volumes of the vehicle to make the enalapril liquid pourable.
6. Transfer liquid to a graduated cylinder.
7. Add enough suspending agent to bring the final volume to 200 mL.
8. Label the bottle "Shake Well Before Using" and "Protect From Light."
9. Label with a 91-day expiration date (84).

C.4.2.b Drug Dispensing

The family will receive a medication bottle and a marked syringe to draw up the correct dose of the drug. The placebo group will receive an equal volume of sweetened suspending agent (equal volumes of Ora-Plus and Ora-Sweet). Study drug will be dispensed by the pharmacy on a 30-day basis for the first six months of the trial when patient weight may be changing rapidly and on a 60-day basis thereafter. Unused study drug will be returned to the pharmacy and measured to monitor compliance (see Section C.4.4).

C.4.3 Other Treatments

Subjects will be treated with other medications at the discretion of their cardiologist and primary care provider. All medications will be recorded on the study forms. The use of oral beta-adrenergic antagonists is discouraged during the trial period since beta-blockade therapy may independently alter cardiac function and remodeling.

C.4.4 Safety and Compliance Monitoring

C.4.4.a Safety Monitoring

For all subjects, blood pressure, heart rate and oxygen saturation will be measured before and at 30 minute intervals for the first two hours after administration of study drug during the up-titration phase. If the study investigator determines that the patient has developed an adverse reaction (e.g. significant hypotension, oxygen desaturation or elevated serum creatinine), further monitoring will be performed as clinically indicated. When restarting study drug after the Glenn procedure, vital signs and oxygen saturation

will be monitored for at least two hours. Patients will also be monitored for adverse events at each study visit and through phone contact monthly by the study coordinators.

C.4.4.b Compliance Monitoring

Study drug compliance will be assessed by comparing the expected to the measured residual volume of study drug returned to the pharmacy. Compliance will be assessed during the maintenance phase, when the pharmacy dispenses a 30- or 60-day supply of the study drug. Compliance will not be monitored during the uptitration phase or during hospitalizations, when the pharmacy will dispense primarily unit doses for patients. Patients will also be monitored for compliance at each study visit and through phone contact monthly by the study coordinators.

C.5 Measurements

C.5.1 Schedule of Measurement

Table 2. Schedule of Trial Measurements

	Baseline	Follow-Up					
Measurement	**Study entry**	**4 days on study drug**	**2 weeks on study drug**	**Before Glenn***	**7 days after restarting**	**Age 10 mo**	**Age 14 mo**
Clinical (BP, O_2 sat)	X	X	X	X	X	X	X
Ht, Wt, Head circ.	X	X	X	X	X	X	X
Safety labs	X	X	X	X	X		X
Heart failure class				X			X
BNP level				X			X
Neurodev./FSII-R							X
Echocardiogram	X			X			X
Cardiac cath**				X			
Gene Polymorphisms				X			
Adverse events		X	X	X	X	X	X

*Most centers perform the Glenn shunt at 4-8 months of age
**Not mandated by protocol; Performed at the discretion of the treating cardiologist

C.5.1.a Baseline Studies

At baseline, the resting blood pressure and oxygen saturation will be determined. The laboratory results obtained for routine post-operative care within 7 days prior to starting study drug will also be used to screen the patient for trial eligibility. Weight, recumbent

height and head circumference will be recorded. All echocardiograms performed prior to enrollment will be reviewed to evaluate ventricular function and atrioventricular valve regurgitation. The acceptable window for the baseline echocardiogram is 7 days prior to randomization to 2 days after randomization. If an echocardiographic study documenting ventricular function and atrioventricular valve regurgitation at the time of enrollment is not available, a limited study echocardiogram will be performed to evaluate ventricular function and atrioventricular valve regurgitation. The baseline echocardiogram will be submitted to the Echocardiogram Core Laboratory for analysis.

C.5.1.b Follow-Up Visits

Follow-up data will be obtained at 6 time points: 4 days on study drug, 2 weeks on study drug, before the Glenn shunt, 7 days after restarting study drug post-Glenn shunt, at age 10 months, and at age 14 months.

- At each visit, blood pressure, oxygen saturation, weight, recumbent height and head circumference will be determined.
- Safety laboratory examinations (complete blood count, electrolytes, and serum creatinine) will be performed at the following visits: 4 days on study drug, 2 weeks on study drug, before the Glenn shunt, 7 days after restarting study drug post-Glenn shunt, and age 14 months. The laboratory examinations at 4 days on study drug and before the Glenn shunt are obtained as part of routine care. The laboratory examinations at 2 weeks on study drug, 7 days after restarting study drug post-Glenn shunt, and age 14 months are not part of routine care.
- Ross Heart Failure Class will be determined before the Glenn shunt surgery and at age 14 months.
- BNP levels will be drawn before the Glenn shunt and the 14 month visit.
- The Bayley Scales of Infant Development-II, MacArthur Communicative Development Index and the Functional Status-II Revised questionnaire will be performed at 14 months of age.
- Echocardiographic images will be obtained for analysis at baseline, before the Glenn shunt surgery and at age 14 months. The echocardiogram before the Glenn shunt surgery will be obtained as part of routine care. In the majority of patients, the echocardiogram at baseline and age 14 months will

be part of routine care; however, if a routine echocardiogram is not performed, a study echocardiogram will be obtained.

- If a routine cardiac catheterization is performed before the Glenn shunt, data will be recorded.
- Blood samples will be obtained to test for RAAS gene polymorphisms during the study period. In most cases, the samples will be obtained at the time of the Glenn shunt catheterization or at the time of Glenn shunt surgery, if no catheterization is performed.
- Data regarding adverse events will be collected at each visit and at the time the adverse event occurs.

C.5.1.c Windows for Visits

Study medication will be started during hospitalization or in the outpatient setting at the PHN clinical center. Patients will be discharged when clinically stable. Measurements are scheduled for six subsequent time points. Subjects may or may not be hospitalized during the follow-up time points.

- The four-day on-study drug time point will occur with a window of ± 2 days.
- The two-week on-study drug time point will occur with a window of 10 days to 21 days.
- Cardiac catheterization will be performed at the discretion of the subject's cardiologist.
- The before the Glenn shunt time point will be obtained 2 weeks before the Glenn shunt surgery with a window of ±2 weeks.
- The seven day after restarting study drug time point will occur with a window of 3 days to 21 days.
- The age 10 month visit will occur with a window of ±1 month.
- The age 14 month visit will occur with a window of ±1 month.

C.5.2 Outcome Variables

Outcome variables have been chosen to reflect the impact of ACE inhibition therapy on the clinical status of the infant with single ventricle and to determine the effect of ACE inhibition on ventricular function and remodeling (Table 3).

Table 3. Outcome Variables

Primary Outcome

Weight-for-age Z-score

Secondary Outcomes

Growth

1) Height-for-age Z-score
2) Head circumference-for-age Z-score
3) Weight-for-height Z-score

Ross Heart Failure Score

Neurodevelopmental Status

1) Bayley Scales of Infant Development-II

Mental Development Index

Secondary Outcomes continued

Psychomotor Development Index

2) MacArthur CDI Index

Functional Status II-Revised Questionnaire

Echocardiogram

1) Measures of diastolic function
   - E:A ratio
   - Rate of deceleration of early inflow
   - Duration of pulmonary vein flow reversal during atrial systole
   - Atrioventricular valve annulus velocity in early diastole
   - Rate of ventricular flow propagation using M-mode color Doppler
2) Degree of AV valve regurgitation

Cardiac Catheterization

1) Ventricular end-diastolic pressure
2) Pulmonary to systemic flow ratio

BNP Level

C.5.2.a Measures of Somatic Growth

Weight, recumbent height and head circumference will be measured as recommended by the CDC and National Center for Health Statistics (see Appendix C). Study growth measurements will not be conducted during sick visits. Weights will be obtained on unclothed infants. Absolute weight values will be translated into the corresponding Z-scores for age and height as determined by the National Health and Nutrition

Examination Survey for comparison (see Appendix C) and the World Health Organization. Absolute height values will be transformed into Z-scores for age in a similar manner.

C.5.2.b Ross Heart Failure Class

A study investigator will determine the Ross Heart Failure Class from clinical history, physical examination and weight and height Z-scores (see Appendix D).

C.5.2.c B-Type Natriuretic Peptide Levels

A resting BNP level will be measured in plasma (see Appendix E). The sample for BNP will be drawn after the subject has been in a sitting/supine position in a quiet room for 15 minutes. A volume of 1 cc of whole blood will be collected and placed into a pre-chilled lavender-topped EDTA tube. The tube will be inverted to mix thoroughly and immediately placed on ice. The Serology Core Laboratory will provide centrifugation method and timing instructions to the clinical sites. The resulting plasma will be dispensed into vials for storage and frozen immediately at –20° to –80° C. Each vial will have a label identifying the patient identification number and sample date. Samples will be batched and shipped to the Serology Core Laboratory on dry ice.

C.5.2.d Neurodevelopmental and Functional Status

The Bayley Scales of Infant Development-II will be administered at age 14±1 months by trained personnel with the infant in a calm, quiet state. The MacArthur CDI and the Functional Status II-Revised are parent report questionnaires that will be completed during the age 14±1 month visit (see Appendix F). Both the MacArthur CDI and the Functional Status II-Revised will be self-administered. A Spanish version will be provided for parents who prefer Spanish. If the parent is unable to read either English or Spanish, the questionnaire will not be completed but the subject will remain in the study.

C.5.2.e Echocardiography

All echocardiograms performed prior to enrollment will be reviewed to evaluate ventricular function and atrioventricular valve regurgitation. If an echocardiographic study documenting ventricular function and atrioventricular valve regurgitation at the time of enrollment is not available, a limited study echocardiogram will be performed to evaluate ventricular function and atrioventricular valve regurgitation. Follow-up

echocardiograms are planned for the pre-Glenn shunt surgery time point and at age 14 months. The use of moderate (conscious) sedation will be managed according to the practice at the individual center. Each echocardiographic study will be recorded on a separate videotape or digital transfer medium, forwarded to the Data Coordinating Center (DCC) for blinding, and distributed to the Echocardiographic Core Laboratory for interpretation. If video medium is used, the original tape will be required and returned to the site immediately after a digital copy is made at the Core Laboratory. The follow-up echocardiograms will consist of a complete two-dimensional echocardiogram and Doppler evaluation. Systolic, diastolic, and mean blood pressure will be measured using an automated vital signs monitor (such as the Dynamap).

Ventricular diastolic function will be assessed using several indices. Assessment of ventricular diastolic filling will be performed using indices derived from pulsed Doppler interrogation of the atrioventricular valve(s) and of the pulmonary veins. The atrioventricular valve early deceleration time and E/A ratio will be calculated. The duration of pulmonary vein flow reversal during atrial systole will be measured from pulmonary vein Doppler. The rate of diastolic flow propagation will be obtained using mitral valve inflow color Doppler M-mode. Atrioventricular valve early diastolic annular velocity will be assessed using tissue Doppler. Atrioventricular valve regurgitation will be quantified using the PISA method (see Appendix G).

C.5.2.f Cardiac Catheterization

Cardiac catheterization will be performed before the Glenn shunt at the discretion of each subject's attending cardiologist as part of standard clinical care in the majority of study subjects. Data obtained from the catheterization include pulmonary to systemic flow ratio and ventricular end-diastolic pressure. If the patient does not have a catheterization, the patient will remain in the study and complete all other required study tests.

C.5.2.g Gene Polymorphisms

Patients will be screened for the presence of specific genetic polymorphisms in the RAAS genes (see Section B.3.5). In addition, with the consent of the participant's parent(s) or legal guardian, DNA will be isolated from the blood samples for genotyping and will be stored in the core laboratory. These samples may be used in the future for

the evaluation of new genetic polymorphisms related to ventricular hypertrophy or other cardiovascular diseases (see Appendix I).

C.6 Adverse Events

C.6.1 Definition

An adverse event is any untoward medical occurrence experienced by a subject. An event constitutes a disease, a set of related symptoms or signs, or a single symptom or sign.

C.6.2 Classification of Adverse Events

C.6.2.a Relationship

The relationship between study drug (enalapril or placebo) and any adverse event will be determined by the investigator using the following criteria:

Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject.

Possibly Related: The event follows a compatible temporal sequence from the time of drug administration, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject.

Probably Related: The event follows a reasonable temporal sequence from the time of drug administration, 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.6.2.b Severity

The severity of clinical adverse events and laboratory abnormalities will be assessed according to the following criteria:

1 = "Not Serious": Any event which:

(a) Results in minimal transient impairment of a body function or damage to a body structure; or
(b) Does not require any intervention other than monitoring.

2 = "Moderately Serious": Any event which:
(a) Results in moderate transient impairment of a body function or transient damage to a body structure; or
(b) Requires intervention, such as the administration of medication or a procedure, to prevent permanent impairment of a body function or damage to a body structure.

3 = "Serious": Any event which:
(a) Is fatal; or
(b) Is life-threatening (the patient was, in the view of the Principal Investigator, in immediate danger of death from the event as it occurred); or
(c) Is severely or permanently disabling; or
(d) Necessitates significant intervention, such as major surgery, to prevent permanent impairment of a body function or permanent damage to a body structure; or
(e) Requires or prolongs hospitalization (An elective hospitalization for a planned procedure will not be considered an adverse event and reporting is not required); or
(f) Involves a drug overdose; or
(g) The Principal Investigator considers to be a serious adverse event.

C.6.2.c Expectedness of the Event

All adverse events will be evaluated as to whether their occurrence was expected (as described in the protocol or consent forms), or whether it was not expected to occur.

1 = “Expected”: An event is considered expected if it is known to be associated with the underlying cardiovascular anomaly or with congenital heart surgery, and is mentioned in the protocol, informed consent, or other study documents. An event may be expected despite the study subject’s clinical state immediately prior to the event. For this protocol, expected events include:

(a) Sudden cardiac death
(b) Arrhythmias
(c) Tamponade
(d) Postoperative bleeding

(e) Postoperative open chest

(f) Postoperative chest re-exploration

(g) Postoperative electrolyte abnormalities

(h) Postoperative fever

(i) Postoperative renal dysfunction

(j) Postoperative liver dysfunction

(k) Shunt thrombosis

(l) Aortic coarctation with or without associated ventricular dysfunction

(m) Ventricular dysfunction not associated with coarctation

(n) Pericardial effusion

(o) Wound infection

(p) Reintubation after extubation following Norwood surgery

(q) Hypoxia

(r) Respiratory distress

(s) Pleural effusions

(t) Seizures

(u) Renal dysfunction associated with ventricular dysfunction

(v) Liver dysfunction associated with ventricular dysfunction

(w) Malrotation

(x) Failure to thrive

(y) Need for nasogastric tube feedings or gastrostomy

(z) Sepsis or bacteremia

(aa) General pediatric problems such as otitis media, reactive airways disease, gastroesophageal reflux, urinary tract infection, upper respiratory infection, croup, bronchiolitis including respiratory syncytial virus infection, pneumonia, gastroenteritis.

2 = “Unexpected”. An event is considered unexpected if there are no prior data linking this event with either the condition or intervention under study. Thus, a cardiac death would be expected regardless of timing or the prior condition of the infant, whereas an accidental death in a motor vehicle accident would be unexpected.

C.6.2.d Treatment or Action Taken

AEs and SAEs will result in:

(a) Intervention: Surgery or procedure

(b) Other Treatment: Medication initiation, change, or discontinuation

(c) None: No action is taken

C.6.2.e Outcome

The clinical outcome of the AE or SAE will be characterized as follows:

(a) Death

(b) Recovered: the patient returned to baseline status

(c) Symptoms continue

C.6.3 Data Collection Procedures for Adverse Events

Adverse Events will be recorded according to the date and time of first occurrence, severity, and their duration, as well as any treatment prescribed. Following initiation of study drug dosing, all new or continuing adverse events that were not present at enrollment will be recorded. Any medical condition present at the initial visit, which remains unchanged or improves, will not be recorded as an adverse event at subsequent visits. However, worsening of a medical condition that was present at the initial visit will be considered a new adverse event and reported. Abnormal laboratory values, if felt by the investigator to be clinically significant, will also be recorded on the Adverse Event Form and assessed in terms of severity and relationship to study drug. Laboratory values that are abnormal at study entry and that do not worsen will not be recorded on the Adverse Event Form.

C.6.4 Reporting Procedures

Reports of all serious adverse events will be submitted to the local Institutional Review Board (IRB) and the DCC within one working day of the event. The DCC will report the serious adverse event to the NHLBI as soon as possible and no later than seven calendar days after the event.

C.6.5 Post-study Adverse Events

All unresolved adverse events at the time of the patient's termination from the study will be followed by the investigators until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained or has stabilized. At the last scheduled contact, the investigator will instruct each parent to report any subsequent event(s) which the parent, or the subject's personal physician, believes might reasonably be related to administration of study

drug. Any death or other clinically serious adverse event that may be related to study drug and that occurs at any time after a subject has discontinued or terminated study participation will be reported as in C.6.3.

C.7 Statistical Methods

C.7.1 Sample Size and Power

C.7.1.a Primary Endpoint

The primary endpoint of the proposed trial is the weight-for-age Z-score determined at age 14±1 months. Existing data from the literature (4, 17) and Columbia University (Table 1) indicate that the standard deviation of weight-for-age Z-scores in this patient population is approximately 1.1. The Steering Committee has determined that a mean difference between treatment groups of half a standard deviation, that is, a mean Z-score difference of 0.5, is clinically meaningful. In terms of absolute weight, half a standard deviation at age 14 months is equivalent to a 0.52 kg mean difference between treatment groups. Based on Table 1, the mean weight-for-age Z-score of subjects in the placebo group at age 14 months is expected to be about the 10^{th} percentile for age. A mean group difference in Z-scores of 0.5 results in a mean Z-score equivalent to the 22.5^{th} weight percentile for age in the ACE inhibitor group. A total of 176 subjects (88 per arm) is required to detect this difference with 85% power using a two-sided .05 level test. Using a conservative inflation rate (20% for loss to follow-up and 3% for group sequential interim analysis), a total of 230 subjects will be enrolled into the trial (115 per arm).

C.7.1.b Secondary Endpoints

In this section we summarize statistical power for some of the main secondary endpoints with the projected sample size of 88 evaluable subjects per treatment group. Two-sided α=.05 tests are assumed throughout.

Growth before the Glenn shunt surgery. The secondary endpoint of weight-for-age Z-score measured immediately before the Glenn shunt surgery (average age 6 months) will have similar power (85%) given this sample size. The minimum clinically significant

difference between the ACE inhibitor and placebo groups in mean Z-score at this time point is assumed to be 0.5.

Height-for-Age Z-score. Infant height is more variable than infant weight. With 88 evaluable subjects per group, there will be 85% power to detect a mean Z-score difference of 0.68 between treatment groups assuming a standard deviation of 1.5. Power ranges from 75% to detect a mean Z-score difference of 0.60 to 95% power to detect a mean Z-score difference of 0.82.

Bayley Scales of Infant Development. Based on data collected at Children's Hospital Boston from single ventricle patients, the standard deviation of the Bayley Scale scores is 15, which is similar to that expected in a population of normal infants. The mean values for single ventricle patients are in the 75 to 85 range, depending on subscale (100 is the mean for normal healthy infants). With 88 evaluable subjects per group and desired power of 85%, the minimum mean detectable difference between groups is 6.8 points. Power ranges from 75% to detect a mean group difference of 6.0 points to 95% power to detect a mean group difference of 8.2 points.

B-Type Natriuretic Peptide. Based on Ationu et al. [77] and Puddy et al. [59], the standard deviation of BNP ranges from 4 pg/ml in normal children to 60 pg/ml in infants recovering from bypass surgery. There are no published data from single ventricle infants for this relatively new measure; thus a standard deviation of 30 pg/ml is conservatively assumed. With 88 evaluable patients per group and desired 85% power, the minimum mean detectable difference between groups is 14 pg/ml. If the standard deviation is 60 pg/ml, there will be 85% power to detect a 27 pg/ml difference.

C.7.1.c Interim Monitoring and Early Stopping

A Data and Safety Monitoring Board (DSMB) has been established by NHLBI to monitor this trial. The DSMB will meet one to two times a year. In addition to routine data reviews (see below) a formal early stopping procedure will be used to monitor the trial for large treatment differences. An O'Brien-Fleming stopping boundary, allowing for flexibility in the exact times of the interim analysis, will be used for this purpose [78, 79]. The O'Brien-Fleming plan is conservative in the sense that it is difficult to reach the boundary during the trial. Therefore, most of the Type I error is conserved for the final

analysis and the effect on statistical power is minimal. One formal interim analysis will be conducted when approximately half the patients have reached the 14±1 month visit. The Lan-DeMets methodology [79] will be used to adjust the boundary appropriately if the interim analysis does not fall at exactly the halfway point. The primary endpoint will be analyzed and the nominal p-value compared with the stopping boundary to judge the significance of the treatment effect while adjusting for multiple testing. At the interim analysis with half of the randomized patients having completed the 14 month visit, if the nominal p-value is less than 0.005, this would represent a statistically significant treatment effect with an overall experiment-wide false positive error rate of α=.05 (Table 4).

Table 4. Early Stopping Rule

Analysis	First Look (Half of total information)	Second Look (Final Analysis)
Nominal p-value to reject null:	0.005	0.048

Even if there is a statistically significant treatment difference at the interim analysis, the DSMB may decide that an ethical imperative to stop the trial is not present. Early stopping rules are only guidelines; the DSMB may take a more global view of the trial during data monitoring [80-82]. To provide this broader perspective, the DSMB reports will include summaries of accrual, patient characteristics, adverse events, compliance rates with therapy, frequency of protocol violations, data quality, primary and secondary endpoints, other information as requested by the DSMB, and any unanticipated special problems that arise during the conduct of the trial.

Judgment concerning the continuation or termination of the study may be based on not only the degree of statistical significance observed at the interim analysis, but also on the likelihood of achieving significance should enrollment continue to the target sample size. The Data Coordinating Center will supplement the group sequential analysis with a calculation of conditional power based on the method of stochastic curtailment [83]. This procedure evaluates the conditional probability that a particular statistical comparison will be significant (or not significant) at the end of the trial at the α=.05 level, given the hypothesized treatment difference and the observed data to date. Conditional power for the primary endpoint will be calculated and provided to the DSMB as part of the interim study report.

C.7.1.d Safety Analyses

Analyses of clinical measures such as blood pressure, heart rate, oxygen saturation, and age at Glenn shunt surgery, as well as laboratory measures including complete blood count, electrolyte concentrations, and serum creatinine will be conducted periodically as the data accrue. The distributions of these measures and the incidence of adverse events in the two treatment groups will be compared. In addition, analyses of change will be conducted for serum creatinine in particular to determine whether renal function is stable for each subject.

C.7.2 Analysis Plan

C.7.2.a Primary Analysis of the Primary Endpoint

The primary analysis will be according to the intention-to-treat principle. All subjects will be analyzed according to their treatment group assignment regardless of actual treatment received. In addition, all measurements will be included in analysis regardless of whether the measurement was made within the recommended visit window. The primary endpoint is the weight-for-age Z-score from the age 14 month visit. A mixed model (repeated measures) regression will be conducted, using growth observations that were collected at the PHN center. The treatment difference in final visit (14-month) growth z-score will be estimated using a contrast of estimated 14-month means from the model. If necessary, a nonlinear fit for time will be employed. If there is a severe departure from normality with respect to these data, the Wilcoxon rank sum test comparing the distributions of growth at 14-month by treatment group will also be conducted to assess the robustness of the result obtained by regression, because the presence of negative Z-scores prevents simple transformation of the data. The data from Columbia University summarized in Table 1 suggest only minor departures from normality. The treatment groups will be compared descriptively with respect to demographics and baseline characteristics. Randomization should result in good balance across the two groups. If there are imbalances on covariates that are prognostic for study outcomes, then covariate-adjusted comparisons will be made. However, the primary analysis of the primary endpoint will not include an explicit adjustment for the age at which the Glenn shunt surgery is performed. The value of this variable is determined after randomization. Since the ACE inhibitor treatment may affect

the timing of the Glenn shunt surgery and the time of surgery may affect weight at 14 months of age, we can only test, at the end of the trial, whether ACE inhibitor treatment in conjunction with Glenn shunt surgery at a medically appropriate time has a significant effect on weight in comparison to treatment with placebo plus surgery.

C.7.2.b Secondary Analyses of the Primary Endpoint

The set of all weight measurements will be analyzed longitudinally using mixed model regression, utilizing the exact time of measurement for each subject, to determine the association over time between ACE inhibitor use and growth. The test of longitudinal treatment efficacy will be based on assessment of the treatment group by time interaction.

It is possible that in some study subjects, study drug will be permanently discontinued and weight at age 14 months will be obtained. Therefore, a secondary non-intent-to-treat analysis will be performed using a categorical measure of compliance. In this non-intent-to-treat analysis, subjects will be classified into two groups based on several factors, including their length of participation in the trial, reported compliance with the study drug, and knowledge regarding use of an open label ACE inhibitor. Patients will be excluded if compliance cannot be estimated or estimated total dose does not fit a secondary analysis definition of treated or untreated. If there is significant non-compliance with study drug assignment in the trial, then this analysis may provide a clearer understanding of the efficacy of ACE inhibitor therapy, with the caveat that there may be unmeasured differences between the two newly constructed treatment arms. Therefore bias in the treatment group comparison may be present due to subjects not being analyzed within the groups to which they were randomly assigned.

C.7.2.c Analyses of the Secondary Endpoints

Analytic methods for the secondary endpoints will be varied and depend on the form of the secondary endpoint. The Mantel-Haenszel test for linear trend will be used for comparison of the distributions of Ross Heart Failure Class and AV valve regurgitation grade in the two treatment groups. For continuous measures at a specific time point (e.g., before the Glenn shunt surgery and at age 14 months), such as the neurodevelopmental scales and BNP, Student's t-test will be used compare group

means, with a normalizing transformation as necessary. If such a transformation is not possible, then the Wilcoxon rank sum test will be employed. As described above, if any imbalances exist in baseline covariates, regression adjustment (analysis of covariance) will be used to assess treatment effect.

C.7.2.d Subgroup Analyses

To determine whether the effect of ACE inhibitor treatment differs across subgroups, separate treatment comparisons will be made within the following subgroups:

- Genotype: The presence or absence of RAAS gene polymorphisms
- Heterotaxy versus no heterotaxy
- Type of systemic single ventricle: Left ventricle versus non-left ventricle
- Type of Stage I surgical palliation: Norwood versus other palliations
- Type of surgery performed to control pulmonary blood flow in the Norwood procedure: Ventricular pulmonary conduit vs. aortopulmonary shunt
- The presence or absence of recurrent coarctation

Covariate by treatment group interaction tests will be performed to test whether the treatment effect is homogenous across subgroups. Statistical testing within subgroup will not be conducted unless the interaction test p-value is ≤ 0.10.

C.7.2.e Site and Cohort Differences

During the ongoing trial, analyses will be conducted on a periodic basis to assess site differences in protocol violation rates, enrollment rates, subject characteristics and adverse event rates. Differences identified may lead to a site visit to review subject data. The characteristics of patients who are screened for but do not participate in the trial will also be compared with enrolled subjects. This analysis will allow assessment of the generalizability of trial findings and whether the enrolled subject cohort is representative of the entire patient population.

C.7.2.f Missing Outcome Data

If a subject is lost to follow-up and weight at the final (14 month) visit is not obtained, data from this subject collected at visits prior to withdrawal or the missed visit will be utilized in regression modeling. Imputation of weight at 14 months, such as the last observation carried forward method, will not be conducted, because it is not known what

effect the Glenn shunt surgery may have on weight and weight at 10 months may not be a valid marker of weight at 14 months, depending on the timing of the Glenn shunt surgery. If study drug is withdrawn from the subject due to family or physician wishes, but the subject continues to participate in study tests and measurements, then the subject's data will be included in analyses.

C.8 Data Management

C.8.1 Information Flow

Data will be received from several sources, including the clinical sites and the Serology and Echocardiogram Core Laboratories. The flow of data among the units in this trial is illustrated in Figure 2. Clinical sites will enter data over the Internet using the Advanced Data

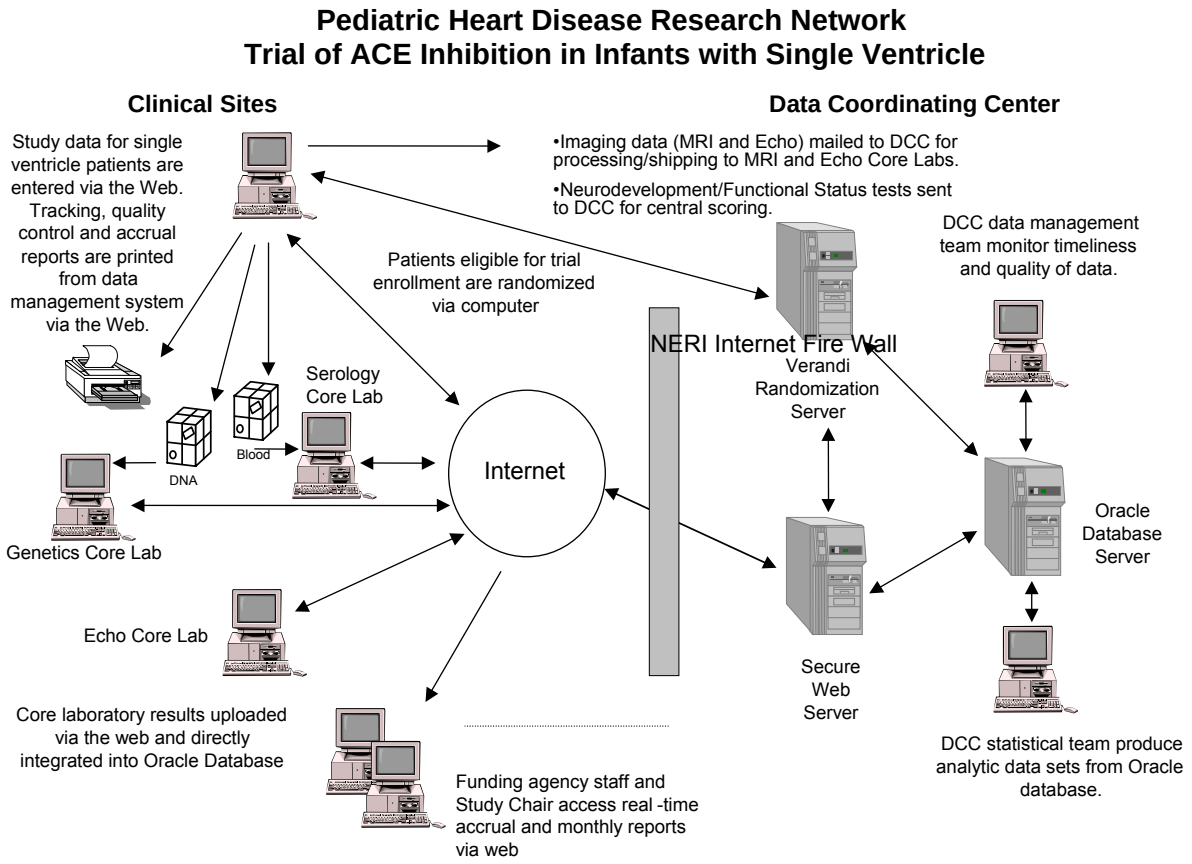


Figure 2. Data Management System and Information Flow

Entry and Protocol Tracking (ADEPT) software, a customized and secure Web application (see Section C.8.2). The DCC will also be able to perform central data entry and editing to accommodate sites or central labs that submit paper copies of data forms. Sites will send blood specimens directly to the Serology Core Laboratory for central processing, and results of tests performed by these laboratories will be electronically transmitted to the DCC and stored in the ADEPT Data Management System (DMS). Echocardiogram data files from clinical sites may be transmitted to the DCC via the Web using the PHN File Transfer Program (FTP) site at the New England Research Institutes (NERI) or submitted on other storage media, such as optical disk or CD-ROM. The DCC will forward the echocardiogram data to the Echocardiogram Core Laboratory either electronically or by FedEx. Results of studies performed by the Echocardiogram Core Laboratory will be directly uploaded to an Oracle database at the DCC or entered electronically using the ADEPT DMS.

C.8.2 Overview of Data Management System (DMS)

ADEPT uses a "browser-based" user interface together with an Oracle relational database engine which allows direct data entry from multiple study sites or at NERI, and then stores these data centrally at the DCC. Information entered into the data entry system will be by patient study identification number; names will not be linked with patient data in the database. Clinical sites will maintain records linking the patient name with the identification number assigned for the study in locked files. Sites will have full access to their own data and be able to view these data remotely, over the Internet.

The ADEPT data entry system will include real-time field level validations and context sensitive help. Electronic data entry forms will be formatted using HTML to resemble closely the paper-based study instruments. These forms will be enhanced with client side JavaScript code to ensure rapid data entry, proper validations of all data fields, and proper skip patterns within study data forms. Data will be 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 of the Internet infrastructure, the ADEPT system has a dial-in backup system to allow for dial-up access to the DMS.

NERI's proprietary VERANDI randomization system, which is capable of supporting blinded medication trials and supports a number of different allocation methodologies, will be used for randomizing patients. Site personnel will randomize patients over the Internet.

Key capabilities of the ADEPT system are described below.

C.8.2.a Data Entry and Editing

The data entry system will include a number of standard features designed to ensure consistently high quality data. Each question on a study form will be associated with a validation, and validations will be executed in real-time during data entry. If the response to a particular question falls outside the range of allowable values specified in the validation for that question, the user will be alerted so that the error can be corrected immediately. Validations will include both inter- and intra-instrument data checks. In addition to alerting the user to invalid entry of items, edit reports will be automatically generated at the completion of data entry for a form. These edit reports will provide the

information necessary to correct any data entry errors or resolve questions regarding out-of-range or questionable values. Edit reports will list the participant identification number, instrument name, and a detailed description of why each specific data item was flagged. These edit reports can be printed out and reviewed by a supervisor, or returned to the data collector for resolution.

The ADEPT system will track expected, partially completed, and missing data entry forms by instrument and data collector. Data entry quality will be monitored through a sample-based, double data entry quality control system. This quality control system utilizes a self-adjusting algorithm to enforce higher double data entry rates on data entry staff that have higher error rates. This system also allows for a minimum double data entry rate to be specified for each individual study instrument. This minimum rate of double data entry is adhered to regardless of a data entry staff's error rate.

C.8.2.b Reporting

The ADEPT system will produce visit schedules to assist Clinical Site staff in scheduling of appointments, and visit control sheets that will list all of the forms and procedures for a scheduled visit. In addition, the system will produce a variety of reports in both graphical and tabular format, as applicable, for the Study Chair, Program Officer, clinical site and Core laboratory management staff. These will include:

- Study Instruments pending entry
- Study Instruments pending edit resolution
- Missing data rates
- Time between collection and entry of data
- Time to physically key each study instrument
- Audit logs for all edits to study data
- Patients with overdue visits
- Reimbursement information for sites and Core Laboratories
- Other customized reports will be developed within the ADEPT system as needed.

C.8.2.c Data Security and Integrity

The Web-based components of the data management system utilize several levels of security to ensure privacy and integrity of the study data as noted below:

- Web access to ADEPT requires use of assigned user names and passwords;
- Passwords are changed every 90 days;
- Web-based data entry uses secure socket layer (SSL) data encryption;
- Access to any study-specific system features are controlled by Oracle database rights and privileges;
- Oracle archive files are backed up daily;
- There is a full Oracle back up weekly;
- Back-up files are stored off site in safety deposit box;
- Duplicate NT servers are available to replace the Oracle or Web Server;
- Primary Identification is via study I.D.;
- Access to electronic linkage limited by Oracle Database Administrator;
- Access to hard copies of linkage kept in locked cabinets by Clinical Center Coordinators;
- NERI firewall limits which internet protocols are allowed to access the Web server;
- No direct access is allowed to the Oracle server from the Internet;
- NERI's firewall monitors for unusual (hacker) activity and automatically notifies NERI IS staff.

All study data will be stored on NERI's Microsoft Windows NT-based, Oracle server. Access to data on this server (from both inside and outside the data center) is controlled by Oracle's extensive security features. The Oracle archiving and back-up system ensures minimal data loss, even in the most catastrophic system failure.

C.9 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 (MOO) in collaboration with study investigators and Core Laboratory directors. In addition, an ADEPT Manual will be developed

for clinical site and Core Laboratory personnel who will be using the ADEPT data management system. The two manuals will serve as both training and reference manuals and will be accessible on NERI's PHN website.

C.9.1 Clinical Center Coordinator Training

The DCC (working closely with the Program Officer), Study Chair, and the Infant Single Ventricle Protocol Subcommittee, will provide central training of clinical center staff in the areas of protocol implementation, data collection and management, measurement of growth, specimen collection and handling, collection and handling of imaging data, medical records abstraction, interview techniques, questionnaire administration, neurodevelopmental assessments, and quality control expectations. Training manuals 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.9.2 Certification of Personnel

Echocardiography personnel at each center will undergo sessions on standardization of technique as required by the Core Laboratory, and all echocardiographic will be read in a Core Laboratory. Poor quality echocardiograms may necessitate site visits.

C.9.3 Data Monitoring/Site Visits

Each clinical site will be visited once by representatives from the DCC and the NHLBI during the study period. The primary roles of the site visit team will be to evaluate general protocol compliance and adherence to Institutional Review Board 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.

The DCC will conduct site visits to each Core Laboratory during the first year to review in-house quality assurance (QA) and quality control (QC) procedures and data transfer to the DCC. Review of central laboratory-related reports will be conducted at least monthly to identify overall or site-specific problems in data or specimen acquisition and reporting of results.

D. STUDY LIMITATIONS

Study limitations include the following:

- The study may be underpowered for subgroup analysis and some secondary endpoints.
- Because the latest endpoint is at age 14 months, the present study will not show the effect of ACE inhibition on later outcomes (such as after the modified Fontan procedure).

REFERENCES

1. Leitch CA. Growth, nutrition and energy expenditure in pediatric heart failure. Prog in Ped Cardiology. 2000; 11: 195-202.
2. Cameron JW, Rosenthal A, Olson AD. Malnutrition in hospitalized children with congenital heart disease. Arch Pediatr Adolesc Med. 1995; 149: 1098-1102.
3. Cohen DM. Surgical management of congenital heart disease in the 1990's. Am J Dis Child. 1992; 146: 1447-1452.
4. Day RW, Denton DM, Jackson WD. Growth of children with a functionally single ventricle following palliation at moderately increased altitude. Cardiol Young. 2000; 10: 193-200.
5. Cheung YF, Penny DJ, Redington AN. Serial Assessment of Left Ventricular Diastolic Function after Fontan Procedure. Heart. 2000; 83: 420-424.
6. Gentles TA, Gauvreau K, Mayer JE, et al. Functional outcome after the Fontan operation: factors influencing late outcome. J Thoracic Cardiovasc Surg. 1997; 114: 392-403.
7. Seliem M, Muster AJ, Paul MH, et al. Relation between preoperative left ventricular muscle mass and outcome of the Fontan procedure in patients with tricuspid atresia. J Am Coll Card. 1989; 14: 750-755.
8. Frenneaux M, Stewart RAH, Newman CMH, et al. Enalapril for severe heart failure in infancy. Arch Dis Child. 1989; 64: 219-223.
9. Shaw NJ, Wilson N, Dickinson DF. Captopril in heart failure secondary to a left to right shunt. Arch Dis Child. 1988; 63: 360-363.
10. Scammell AM, Arnold R, Wilkinson JL. Captopril in treatment of infant heart failure: a preliminary report. Int J Cardiol. 1987; 16: 295-301.
11. Montigny M, Davignon A, Fouron JC, et al. Captopril in infants for congestive heart failure secondary to a large ventricular left-to-right shunt. Am J Cardiol. 1989; 63: 631-633.
12. Steinberger E, Ferencz C, Loffredo C. Infants with single ventricle: a population-based epidemiological study. Teratology. 2002; 65: 106-115.
13. Gentles TA, Mayer JE, Gauvreau K, et al. Fontan operation in five hundred consecutive patients: factors influencing early and late outcome. J Thoracic Cardiovasc Surg. 1997; 114: 376-391.
14. Leitch CA, Karn CA, Peppard RJ, et al. Increased energy expenditure in infants with cyanotic congenital heart disease. J Pediatr. 1998; 133: 755-760.

15. Farrell AG SM, Leitch CA. Energy expenditure in infants with hypoplastic left heart syndrome. Pediatr Cardiol. 2002; 23 (Abstr 2): 663.
16. Rudd N, Mussatto K, Steltzer M, et al. Nutritional management and growth velocity of infants with hypoplastic left heart syndrome. Ped Card. 2002; 23 (Abstr 20): 671.
17. Cohen MI, Bush DM, Perry RJ, et al. Somatic growth failure after the Fontan operation. Cardiol Young. 2002; 10: 447-457.
18. Kern JH, Hinton VJ, Nereo NE, et al. Early developmental outcome after the Norwood procedure for hypoplastic left heart syndrome. Pediatrics. 1998; 102: 1148-1152.
19. Rogers B, Msall M, Buck G, et al. Neurodevelopmental outcome of infants with hypoplastic left heart syndrome. J Pediatr. 1995; 126: 496-498.
20. Mahle W. Neurologic and cognitive outcomes in children with congenital heart disease. Curr Opin Pediatr. 2001; 13: 482-486.
21. Miceli PJ, Goeke-Morey MC, Whitman TL, et al. Brief Report: Birth status, medical complications, and social environment: individual differences in development of preterm, very low birth weight infants. J. Pediatr. Psychol. 2000; 25: 353-358.
22. Leonard CH, Piecuch RE, Cooper BA. Use of the Bayley infant neurodevelopmental screener with low birth weight infants. J. Pediatr. Psychol. 2001; 26: 33-40.
23. Rappaport LA, Wypij D, Bellinger DC, et al. Relation of seizures after cardiac surgery in early infancy to neurodevelopmental outcome. Circulation. 1998; 97: 773-779.
24. Singer L, Siegel A, Lewis B, et al. Preschool language outcomes of children with history of bronchopulmonary dysplasia and very low birth weight. J Dev Behav Pediatr. 2001; 22: 19-26.
25. Mahle W, Clancy R, Moss E, et al. Neurodevelopmental outcome and lifestyle assessment in school-aged and adolescent children with hypoplastic left heart syndrome. Pediatrics. 2000; 105: 1082-1089.
26. Forbes TJ, Gajarski R, Johnson GL, et al. Influence of age on the effect of bidirectional cavopulmonary anastomosis on left ventricular volume, mass and ejection fraction. J Am Coll Card. 1996; 28: 1301-1307.
27. Fogel MA, Weinberg PW, Chin AJ, et al. Late ventricular geometry and performance changes of functional single ventricle throughout staged Fontan reconstruction assessed by magnetic resonance imaging. J Am Coll Card. 1996; 28: 212-221.
28. Allgood NL, Alejos N, Drinkwater DC, et al. Effectiveness of the bidirectional Glenn shunt procedure for volume unloading in the single ventricle. Am J Cardiol. 1994; 74: 834-836.

29. Gewelling M, Lundstrom U, Deanfield J, et al. Impact of Fontan operation on left ventricular size and contractility in tricuspid atresia. Circulation. 1990; 81: 118-127.
30. Apfel HD, Solowiejczyk DE, Printz BF, et al. Feasibility of a two-dimensional echocardiographic method for the clinical assessment of right ventricular volume and function in children. J Am Coll Card. 1996; 5: 637-645.
31. Kim S,Iwao H. Molecular and cellular mechanisms of angiotensin II-medicated cardiovascular and renal diseases. Pharmacol Rev. 2000; 52: 11-34.
32. Ondrej L, Redfied M, Jovanovic S, et al. Mechanical unloading versus neurohormonal stimulation on myocardial structure and endocrine function in vivo. Circulation. 2000; 102: 338-343.
33. Ationu A, Singer D, Smith A, et al. Studies of cardiopulmonary bypass in children: implications for the regulation of brain natriuretic peptide. Cardiovasc Res. 1993; 27: 1538-1541.
34. Hjortdal VE, Stenbog EV, Ravn HB, et al. Neurohormonal activation later after cavopulmonary connection. Heart. 2000; 83: 439-443.
35. Tulevski II, Groenink M, van der Wall EE, et al. Increased brain and atrial natriuretic peptides in patients with chronic right ventricular pressure overload: correlation between plasma neurohormones and right ventricular dysfunction. Heart. 2001; 86: 27-30.
36. Bolger AP, Sharma R, Li W, et al. Neurohormonal activation and the chronic heart failure syndrome in adults with congenital heart disease. Circulation. 2002; 106: 92-99.
37. Mainwaring RD, Lamberti JJ, Moore JW, et al. Comparison of the hormonal response after bidirectional Glenn and Fontan procedures. Ann Thorac Surg. 1994; 57: 59-63.
38. Sen I,Rajasekaran AK. Angiotensin II-binding protein in adult and neonatal rat heart. J Mol Cell Cardiol. 1991; 23: 563-572.
39. Suzuki J, Matubara H, Urakami M, et al. Rat angiotensin II (Type 1A) receptor and mRNA regulation and subtype expression in myocardial growth and hypertrophy. Circ Res. 1993; 73: 439-447.
40. Diet F, Graf C, Mahnke N, et al. ACE and angiotensinogen gene genotypes and left ventricular mass in athletes. Eur J Clin Invest. 2001; 31: 836-842.
41. Patel R, Lim D, Reddy D, et al. Variants of trophic factors and expression of cardiac hypertrophy in patients with hypertrophic cardiomyopathy. J Mol Cell Cardiol. 2000; 32: 2369-2377.

42. Semsarian C, Healey MJ, Fatkin D, et al. A polymorphic modifier gene alters the hypertrophic response in a murine model of familial hypertrophic cardiomyopathy. Journal of Molecular and Cellular Cardiology. 2001; 33: 2055-2060.
43. Tiret L, Mallet C, Poirier O, et al. Lack of association between polymorphisms of eight candidate genes and idiopathic dilated cardiomyopathy: The CARDIGENE study. Journal of the American College of Cardiology. 2000; 35: 29-35.
44. Ortlepp JR, Vosberg HP, Reith S, et al. Genetic polymorphisms in the renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: a study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. Heart. 2002; 87: 270-275.
45. Kurland L, Melhus H, Karlsson J, et al. Angiotensin converting enzyme gene polymorphism predicts blood pressure response to angiotensin II receptor type 1 antagonist treatment in hypertensive patients. J Hypertens. 2001; 19: 1783-1787.
46. Hallberg P, Karlsson J, Kurland L, et al. The CYP2C9 genotype predicts the blood pressure response to irbesartan: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs Atenolol (SILVHIA) trial. J Hypertens. 2002; 20: 2089-2093.
47. Kurland L, Melhus H, Karlsson J, et al. Polymorphisms in the angiotensinogen and angiotensin II type 1 receptor gene are related to change in left ventricular mass during antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. J Hypertens. 2002; 20: 657-663.
48. Konstam MA, Kronenberg MW, Rousseau MF, et al. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dilatation in patients with asymptomatic systolic dysfunction. Circulation. 1993; 88: 2277-2283.
49. Tamura T, Said S, Harris J, et al. Reverse remodeling of cardiac myocyte hypertrophy in hypertension and failure by targeting of the renin-angiotensin system. Circulation. 2000; 102: 253-259.
50. Yusuf S, Sleight P, Pogue J, et al. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcome Prevention Evaluation Study Investigators. N Eng J Med. 2000; 342: 145-153.
51. Buchhorn R, Ross RD, Bartmus D, et al. Activity of the renin-angiotensin-aldosterone and sympathetic nervous system and their relation to hemodynamic and clinical

abnormalities in infants with left-to-right shunts. International Journal of Cardiology. 2001; 78: 225-230.

52. Grenier MA, Fioravanti J, Truesdell SC, et al. Angiotensin-converting enzyme inhibitor therapy for ventricular dysfunction in infants, children and adolescents: a review. Progress in Pediatric Cardiology. 2000; 12: 91-111.
53. Seguchi M, Nakazawa M, Momma K. Effect of enalapril on infants and children with congestive heart failure. Cardiol Young. 1992; 14-19.
54. Mori Y, Nakazawa M, Tomimatsu H, et al. Long term effect of angiotensin-converting enzyme inhibitor in volume overload heart during growth: a controlled pilot study. J Am Coll Card. 2000; 36: 270-275.
55. Calabro R, Piscane C, Pacileo G, et al. Hemodynamic effects of a single oral dose of enalapril among children with asymptomatic chronic mitral regurgitation. Am J Cardiol. 1999; 138: 955-961.
56. Ross RD, Daniels SR, Schwartz DC, et al. Plasma norepinephrine levels in infants and children with congestive heart failure. Am J Cardiol. 1987; 59: 911-914.
57. Wu J, Chang H, Huang T, et al. Reduction in lymphocyte beta-adrenergic receptor density in infants and children with heart failure secondary to congenital heart disease. Am J Cardiol. 1996; 77: 170-174.
58. Yoshimura M, Yasue H, Okumura K, et al. Different secretion patterns of atrial and natriuretic peptide and brain natriuretic peptide in patients with congestive heart failure. Circulation. 1993; 87: 464-469.
59. Puddy V, Amirmansour C, Williams A, et al. Plasma brain natriuretic peptide as a predictor of haemodynamically significant patent ductus arteriosus in preterm infants. Clin Sci (Lond). 2002; 103: 75-77.
60. Cowley CG BJ, Shaddy RE. Plasma B-type natriuretic peptide (BNP) levels in children with left ventricular outflow tract obstruction. Ped Card. 2002; 23 (Abstr 18): 671.
61. Koulouri S AR, Wong PC, Lewis AB. Utility of B-type natriuretic peptide in differentiating congestive heart failure from lung disease in pediatric patients with respiratory distress. Ped Card. 2002; 23 (Abstr 26): 673.
62. Brunner-La Rocca H, Weilenmann D, Kiowski W, et al. Within-patient comparison of effects of different dosages of enalapril on functional capacity and neurohormone levels in patients with chronic heart failure. Am Heart J. 1999; 138: 654-662.

63. Lang C, Motwani J, Rahman A, et al. Effect of angiotensin-converting enzyme inhibition on plasma brain natriuretic peptide levels in patients with heart failure. Clin Sci (Lond). 1992; 83: 143-147.
64. Inoko M, Fujita M, Nakae I, et al. Effect of angiotensin-converting enzyme inhibition on sympathetic tone in patients with mild to moderate heart failure. Jpn Circ J. 2001; 65: 395-398.
65. Bayley N, *Bayley Scales of Infant Development, Second Edition*. 1993, San Antonio, TX: Psychol. Corp.
66. Kopp C,McCall R, *Predicting later mental performance for normal, at risk and handicapped infants*. Life-span development and behavior, ed. P. Baltes and O. Brim. 1982, New York: Academic Press. 33.
67. Fenson L, Dale P, Reznick S, et al., *MacArthur Communicative Development Inventories: User's Guide and Technical Manual*. 1993, San Diego, CA: Singular Publishing Group.
68. Bellinger DC, Rappaport LA, Wypij D, et al. Patterns of developmental dysfunction after surgery during infancy to correct transposition of the great arteries. J Dev Behav Ped. 1997; 18: 75-83.
69. Bernstein D, Starnes VA, Baum D. Pediatric heart transplantation. Adv Pediatr. 1990; 37: 413-439.
70. Missmer S, Spiegelman D, Gorbach S, et al. Predictors of change in the functional status of children with human immunodeficiency virus infection. Pediatrics. 2000; 106: http://www.pediatrics.org/cgi/content/full/106/102/e124.
71. Wells T, Rippley R, Hogg R, et al. The pharmacokinetics of enalapril in children and infants with hypertension. J Clin Pharmacol. 2001; 41: 1064-1074.
72. Irvin J,Viau J. Safety profiles of the angiotensin converting enzyme inhibitors captopril and enalapril. Am J Med. 1986; 81: 46-50.
73. Nakamura H, Ishii M, Sugimura T, et al. The kinetic profiles of enalapril and enalaprilat and their possible developmental changes in pediatric patients with congestive heart failure. Clin Pharmacol Ther. 1994; 56: 160-168.
74. Rippley R, Connor J, Boyle J, et al. Pharmacokinetic assessment of an oral enalapril suspension for use in children. Biopharm Drug Dispos. 2000; 21: 339-344.
75. Leversha A, Wilson N, Clarkson P, et al. Efficacy and dosage of enalapril in congenital and acquired heart disease. Arch Dis Child. 1994; 70: 35-39.

76. Gross J, Bailey P, Connis R, et al. Practice guidelines for sedation and analgesia by non-anesthesiologist. Anesthesiology. 2002; 96: 1004-10017.
77. Ationu A,Carter N. Brain and atrial natriuretic peptide plasma concentrations in normal healthy children. Br J Biomed Sci. 1993; 50: 92-95.
78. O'Brien P,Fleming T. A multiple testing procedure for clinical trials. Biometr. 1979; 35: 549-556.
79. Lan K,DeMets D. Discrete sequential boundaries for clinical trials. Biometrika. 1983; 70: 659-663.
80. DeMets D. Stopping guidelines vs. stopping rules: A practitioner's point of view. Commun Stat-Theory Methods. 1984; 13: 2395-2417.
81. Fleming T, Harrington D, O'Brien P. Designs for group sequential tests. Control Clin Trials. 1984; 5: 348-361.
82. Pocock S. When to stop a clinical trial. BMJ. 1992; 305: 235-240.
83. Lan K,Wittes J. The B-value: a tool for monitoring data. Biometrics. 1988; 44: 579-585.