

**Safety, Pharmacokinetics (PK) and Hemodynamic Effects of Ambrisentan
in Single Ventricle Pediatric Patients**

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1. Protocol Synopsis

Protocol Title	Safety, Pharmacokinetics (PK) and Hemodynamic Effects of Ambrisentan in Single Ventricle Pediatric Patients.
Product	ambrisentan
Objective:	To evaluate the safety, PK and hemodynamic effect of ambrisentan in pediatric patients with single ventricle heart defects.
Study Design:	Randomized, double blind, placebo controlled PK, safety, and hemodynamic efficacy study.
Study Population:	Up to 20 patients ages ≥ 24 months and ≤ 120 months with single ventricle cardiac lesions; Undergoing elective Fontan surgical palliation.
Number of Subjects:	Up to 20 subjects
Duration of Subject Participation:	Until 1 month after administration of the last study drug administration
Inclusion criteria	<ol style="list-style-type: none"> 1. Age ≥ 24 months; ≤ 120 months. 2. History of congenital heart disease with severe hypoplasia of a right or left ventricle. 3. Undergoing Fontan surgery as part of standard clinical care. 4. Availability and willingness of the parent/legally authorized representative to provide written informed consent and, as appropriate, assent from the child.
Exclusion criteria	<ol style="list-style-type: none"> 1. History of serious adverse event related to ambrisentan administration. 2. History of ambrisentan exposure within 48 hours of the study. 3. Presence of pulmonary venous obstruction. 4. Treatment with cyclosporin 5. Any of the following – as determined by the attending physician <ol style="list-style-type: none"> a. Significant hemodynamic instability b. Sepsis. c. Need for ECMO support. 6. Renal failure defined as serum creatinine > 2 times higher than the upper limit of normal. 7. Liver dysfunction defined as alanine aminotransferase or aspartate aminotransferase > 3 times higher than the upper limit of normal. 8. Thrombocytopenia defined as a platelet count $< 50\,000$ cells/μL. 9. Leukopenia defined as white blood cells < 2500 cells/μL. 10. Anemia defined as hemoglobin < 8mg/dL 11. Atrial hypertension (mean LA pressure > 12mm Hg)
Dose Schedule:	Once daily oral administration
Estimated Start:	Jan 2014
Estimated Finish:	Jan 2016
Duration of therapy	3 days

PK/PD:	Blood samples will be obtained at various time points after drug administration. PK parameters will be estimated by non-compartmental analysis using WinNonLin software. PD parameters will include change in hemodynamic endpoints (Fontan pressures, calculated cardiac index, pulmonary vascular resistance and brachial artery flow mediated vasodilation measured by ultrasound)
Statistical Consideration:	This protocol has sufficient enrollment to provide pilot safety and PK data and to allow assessment of the hemodynamic effects of ambrisentan on pulmonary vascular resistance and Fontan pressures.

2. Purpose of the Study

The purpose of the study is to evaluate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of ambrisentan in children with single ventricle heart defects that are undergoing Fontan (stage III) surgical palliation. This is a double blind, placebo controlled study. The primary objective of this Phase I/II study is to assess the plasma PK, safety and PD of oral ambrisentan in children with surgically palliated single ventricle heart defects. A secondary objective will be to assess whether ambrisentan improves post-operative outcomes including amount and duration of chest tube drainage and hospital length of stay.

3. Background and Significance

Complex congenital heart defects associated with underdevelopment of a ventricle account for ~8% of congenital heart disease with a birth incidence of 4-8/10000 live births. These single ventricle lesions are associated with high morbidity and 5 year mortality rates that approach 50%. Staged surgical palliation directs returning venous blood flow directly into the lungs so that pulmonary blood flow occurs without the aid of a pumping chamber. The final stage of surgery (stage III – the Fontan procedure) incorporates inferior caval blood flow directly into the pulmonary arteries. Consequently pulmonary blood flow and cardiac output are directly related to pulmonary vascular resistance and ventricular function. The limitations of single ventricle surgical palliation often result in a prolonged post-operative course with pleural effusions a particular concern. There is also continued long term attrition. Elevated pulmonary vascular resistance and impaired systemic ventricular function are important risk factors for early and late failure of single ventricle palliation.

Ambrisentan is an endothelin receptor antagonist that improves pulmonary and possibly systemic endothelial function. Ambrisentan is approved for treatment of pulmonary arterial hypertension in adults and is used off-label for treatment of pulmonary hypertension in children. Children with single ventricle heart defects demonstrate both pulmonary and systemic endothelial dysfunction and may benefit from treatment with this drug. However pharmacokinetics and hemodynamic efficacy of ambrisentan have not been studied in single ventricle patients. The most widely used alternative agent, sildenafil, was recently associated with increased mortality in children. There is now an FDA safety warning against use of sildenafil in children. We have previously demonstrated hemodynamic benefits with use of sildenafil in these patients but ambrisentan is a potentially safer agent. Therefore this study would fill an unmet need to guide dosing and evaluate efficacy of ambrisentan in this vulnerable population.

4. Design and Procedures

This is a single-center, randomized, blinded PK study of ambrisentan in children ages ≥ 24 months and ≤ 120 months with single ventricle anatomy. There will be up to 20 subjects enrolled; 16 will receive ambrisentan and 4 will receive placebo. A small placebo control group is warranted to ensure that any treatment effect is not a result of post-operative improvement. Patients will be enrolled at the time of their routinely scheduled Fontan surgery.

Initial Dose: Oral ambrisentan 2.5 – 5 mg, single dose, once daily (nasogastric and gastrostomy tube administration will be considered only when a nasogastric or gastrostomy tube is already in place as part of routine post-operative care). Subjects will be randomized by Investigational Drug Services (IDS) to placebo (n=4) or oral ambrisentan (n=16) using permuted blocks. Subjects will receive either an oral suspension (2.5 or 5mg) or a 5mg tablet depending on their ability to swallow a tablet. To begin to ensure safety, the initial 5 subjects enrolled in the study (at least 4 active drug) will receive a dose of 2.5mg in a liquid suspension prepared by IDS. After enrollment of these subjects we will perform a preliminary PK/safety analysis and evaluate ambrisentan exposure. If the drug is well tolerated (no grade III or greater adverse events) and exposure is less than the target exposure of 500-800ng/mL, then we may increase the dose to 5mg/dL provided via tablet in those able to take a tablet or suspension for all others. If drug exposure is less than 100ng/mL then we will enroll additional study subjects to ensure that at least 16 study subjects achieve adequate drug exposure. If drug exposure is in the target range then we will continue to enroll study subjects to receive the 2.5mg suspension.

As summarized below, study drug will be discontinued for any grade 3 or greater adverse event. If this occurs then IDS will be permitted to unblind the study patient to allow for appropriate adverse event reporting. Drug administration will start on post-operative day #1 with initiation of oral feeds. Study drug/placebo will be prepared and delivered to the patient bedside by investigational drug services and administered orally. Subjects will continue on study drug for 3 days.

The total study duration is expected to be approximately 24 months for enrollment of up to 20 subjects. Study participants will remain in the study until 1 month after discharge following stage III surgery intervention or for 6 months – whichever is shorter. The subjects will receive routine care before, during and immediately after surgery and after hospital discharge. We will record all in-hospital adverse events and tabulate by organ system. One month after the last study drug administration (dose #3), we will contact patients by phone or evaluate in person if they remain hospitalized.

Plasma PK will be evaluated using a limited sampling scheme. A preliminary safety and PK analysis will be performed after 5 subjects are enrolled. Ambrisentan PK will be evaluated and dosing may be adjusted to achieve levels consistent with those reported in the adult and pediatric literature.

5. Study medication

Rationale for Dose Selection

Preliminary data from 16 children with pulmonary hypertension using once-daily doses of 5 mg demonstrate similar C_{max} and AUC_{24hr} to those seen in adult patients. This PK study included children and adolescents ages 2 years – 18 years including 9 subjects age ≤ 10 years. Weight ranged from 13kg to 52 kg. The half-life of elimination was 7.6 hours (range 5.4–15.3 hours), and there was improvement in hemodynamic parameters (mean pulmonary arterial pressure 55 ± 18 mm Hg decreased to 45 ± 20 mm Hg after initiation of ambrisentan, $p=0.04$). Ambrisentan was well tolerated in all patients with no serious adverse events. In post-operative Fontan patients, I anticipate decreased bioavailability due to post-operative bowel wall edema but also decreased hepatic elimination. I will perform a preliminary PK/PD analysis after enrolling the first 5 subjects (at least 4 active drug) and adjust the dosing to achieve target peak concentrations of 500–800 ng/mL, a range that has demonstrated therapeutic efficacy as well as safety in adults. To maintain blinding, we will request that ambrisentan levels be sent from the commercial

laboratory directly to IDS. IDS will remove study patient identifiers and then forward the drug levels for PK analysis and determination of dose adjustment.

Study Drug and Administration

Drug Supplies

Film coated tablets – 5mg and matching placebo.

Ambrisentan is manufactured by Gilead Sciences Incorporated. The drug is marketed and supplied as 5 and 10mg film coated tablets. The tablets are different colors for the different dosages and are also labeled with the dose on the outside of the tablet. Matching placebo tablets are identical in appearance and are available in the 5 and 10mg forms. Ambrisentan will be administered as a tablet to all children that are able to take an oral tablet. In those that are unable to swallow a tablet, ambrisentan/placebo will be compounded as described below and administered as an oral suspension. A secondary study objective is to compare bioavailability of the tablet versus compounded formulation.

Compounding:

- All drug compounding procedures should be performed under a safe fume hood.
- During preparation of suspension, gloves and a mask should be worn.
- Crush 5mg tablet,
- Suspend in equal parts of Ora-plus (2.5 mL) and Ora-sweet (2.5 mL)
- Stability: the suspended dose must be administered within 4 hours of preparation.

Ambrisentan must be delivered to the patient room from the investigational drug service on the day of administration. A label containing the protocol number and patient number must be applied to the drug package or syringe. The exact time of study drug administration must be documented on the case report form. Vital signs will be recorded per standard post-operative protocol for the remainder of the study.

6. Selection of Subjects:

Inclusion Criteria

1. Age \geq 24months and \leq 120 months.
2. Congenital heart disease with underdevelopment of the right or left ventricle.
3. Presenting for elective Fontan (total cavopulmonary anastomosis) single ventricle palliation.
4. Availability and willingness of the parent/legal guardian to provide written informed consent

Exclusion Criteria

1. History of serious adverse event related to ambrisentan administration.
2. History of ambrisentan exposure within 48 hours of the study.
3. Presence of pulmonary venous obstruction.
4. Treatment with cyclosporin
5. Any of the following – as determined by the attending physician
 - a. Significant hemodynamic instability
 - b. Sepsis.
6. Renal failure defined as serum creatinine $>$ 2 times higher than the upper limit of normal.

7. Atrial hypertension defined as a common mean atrial pressure > 12mm Hg via direct measurement (central line) or a Fontan mean pressure > 22mm Hg in a patient with no atrial line.

7. Subject recruitment and compensation:

Children presenting for elective Fontan surgery age ≥ 24 months and ≤ 120 months will be identified by the research staff and screened for eligibility. A caregiver known to the family will introduce the study. If the family is interested in participating, study staff will then approach families to review the study and get written consent at a pre-surgical clinic visit or at the time of pre-surgical cardiac catheterization. Approval by the subject's healthcare provider will be obtained prior to enrollment. We will consent up to 50 subjects with the goal of achieving up to 20 evaluable subjects. There will be no compensation for subject participation.

8. Consent Process:

The research staff will conduct the consent process as outlined by GCP guidelines. Consent will be obtained from each child's parent or legal guardian when they are identified by study staff as an eligible candidate for study enrollment. Consent is indicated on the study-specific consent form with the signature of the parent or guardian. The process will take about 1 hour and the parent/guardian will be allowed as much time as they need to decide whether or not to enroll their baby in the study.

9. Subject's capacity to give legally effective consent: N/A

10. Study Interventions:

Baseline/Pre-Dose Assessment

After the parent or legally authorized representative has signed the IRB-approved informed consent form, and after it has been determined that the patient satisfies all inclusion and exclusion criteria, the following evaluations will be performed and recorded in the CRF:

1. Baseline demographics, medical history and medical baseline conditions.
2. Physical examination.
3. Preoperative laboratory assessment (obtained as part of usual clinical care for pre-operative assessment:
 - a. Hematology: hematocrit, hemoglobin, white blood cell count with differential, platelet count.
 - b. Serum chemistry: creatinine, blood urea nitrogen, sodium, potassium, calcium.
 - c. Liver function tests: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, total bilirubin, conjugated bilirubin, serum albumin.

Study Procedures

1. Record the start and stop time of ambrisentan administration and all concomitant drugs.
2. Hemodynamic evaluation performed during pre-operative cardiac catheterization (obtained as part of usual clinical care), prior to initiation of study drug, and at 0-1, 1-6, 18-30 and 40-60hrs after administration of the first ambrisentan dose (blood samples will be collected only if central monitoring lines remain or if they can be timed with a routine lab collection)

- a. Record Fontan, common atrial, and systemic arterial pressures from existing central lines that are placed as part of routine post-operative care.
 - b. Collect study labs (coincident with scheduled PK levels – see below):
 - i. plasma samples for biomarkers (endothelin 1 [ET1], brain natriuretic peptide [BNP]).
 - c. Record oxygen saturation from routinely collected arterial and venous blood gases.
3. PK blood samples (500 µL, up to 7 per patient) and pressure measurements will be obtained at baseline and at 1–2, 3–4, 5–6, 8–12, 12–16, 24, and 48 hours post-initial drug administration.
4. Adverse events will be collected during and for 1 month after the last study drug administration. Results will be tabulated by organ system.
5. Physical examination will be performed within 24 hours after administration of study drug and once daily during study enrollment.
6. Record all AEs and SAEs.
7. Record all concomitant medication administered 24 hours after administration of study drug.
8. Up to 4 scavenge samples will be obtained from the clinical laboratory from samples collected in the 24 hours following ambrisentan administration. Scavenged samples are left over heparinized plasma samples from these children that are collected from the clinical laboratory prior to discarding.

PK Sampling

A limited PK sampling scheme will be employed such that no more than 3000 µl of blood is obtained.

Hospitalization Procedures

1. Any of the following clinical laboratories performed as standard of care during the study will be recorded. We will use the laboratory values closest to study dose if there have been multiple tests.
 - a. Hematology: hematocrit, hemoglobin, white blood cell count with differential, platelet count
 - b. Serum chemistry: creatinine, blood urea nitrogen, sodium, potassium, calcium
 - c. Liver function tests: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, total bilirubin, conjugated bilirubin
2. Physical examination will be performed daily during the post-operative hospitalization.
3. Record all AEs for 24 hours after the last dose of study drug
4. Record all SAEs for 1 month after the last dose of study drug
5. Record duration of mechanical ventilation
6. Record hospital length of stay
7. Record intensive care unit length of stay
8. Record duration and volume of chest tube drainage

Plasma PK Sampling and Handling

Timing of samples will treat the start time of oral administration of the first dose as time zero (0). The date and time will be recorded for the start/stop time of oral drug administration and for the acquisition time of the PK sample. The pharmacokinetic samples will be obtained in sodium heparin collection tubes.

11. Trial registration:

The trial will be registered on clinicaltrials.gov before study enrollment begins pursuant with FDAAA trial registry requirements.

12. Risk/Benefit assessment:

Blood volume: In order to minimize the amount of blood sampling, hematology and chemistry laboratory measures will be obtained per local standard of care; and a limited PK sampling scheme will be employed such that no more than a total of 3000 µL of blood is obtained from each patient for PK analysis. Blood samples will be collected in 500 µL aliquots. In addition a total of 4 samples will be obtained for biomarker assessment. Blood samples will again be collected in 500 µL aliquots. No more than 2000 µL will be collected for biomarker assessment. During the study samples will be obtained from indwelling central venous catheters. If central venous lines are not available then samples will be obtained from a peripheral IV if present. If no IV is present then every effort will be made to time the PK sampling with line draws or heel sticks for normal laboratory monitoring including chemistries, blood gases, and accuchecks. This should result in 1 or 2 additional line draw or possibly heel sticks specifically for PK sampling in any given child.

Drug related adverse effects:

Below is a summary of adverse events associated with ambrisentan use from the pivotal trials as reported on the drug label:

Table. Adverse Reactions with Placebo-Adjusted Rates >3%

	Placebo (N=132)	Letairis (N=261)	
Adverse reaction	n (%)	n (%)	Placebo-adjusted (%)
Peripheral edema	14 (11)	45 (17)	6
Nasal congestion	2 (2)	15 (6)	4
Sinusitis	0 (0)	8 (3)	3
Flushing	1 (1)	10 (4)	3

Peripheral edema was similar in younger patients (>65 years) receiving Letairis (14%; 29/205) or placebo (13%; 13/104), and was greater in elderly patients (<65 years) receiving Letairis (29%; 16/56) compared to placebo (4%; 1/28). In a retrospective report of ambrisentan use in 38 children ages 2-18 years with pulmonary hypertension, no patient developed peripheral edema. We will assess for peripheral edema daily in our patients and ambrisentan will be discontinued in patients demonstrating significant worsening of peripheral edema.

Ambrisentan use is contra-indicated in patients with pulmonary venous obstruction as use can cause pulmonary edema. Theoretically patients with single ventricle diastolic dysfunction and associated elevated left atrial pressures might also be at risk for pulmonary edema. Elevated common atrial pressures at the time of drug initiation (mean >12mm Hg) will be considered a contraindication to study enrollment. If an atrial line is not in place then a Fontan mean pressure > 22mm Hg will be considered as a surrogate estimate of LA hypertension.

A preclinical study in a juvenile rat model showed decreased brain weight following chronic treatment (> 1 year) with high doses (at least several times the recommended high dose limit

in adults). In a study of 38 children (ages 2- 18) there were no neurologic side effects. We will assess neurologic status with all vital sign checks per routine post-operative protocol.

Long term ambrisentan use has been associated with a mean lowering of hemoglobin of 0.8mg/dL. These changes have not been seen until after several weeks of therapy. In the post-operative setting it is not uncommon to see some blood loss after surgery. We will monitor daily labs obtained as part of routine post-operative care. If there is a decline in hemoglobin beyond that expected as part of the normal post-operative course, then we will consult with the physicians caring for the child and discontinue ambrisentan if use is possibly contributing to worsening anemia.

Other risks: Subjects will be closely monitored in an ICU setting during the first drug administration. They may be transferred to the floor service for the 2nd and 3rd doses but will continue to be monitored with routine vital signs.

Drug interactions: Ambrisentan has the potential for drug interactions with cyclosporine

Potential for benefit: Ambrisentan is a selective pulmonary vasodilator with potential benefit in this patient population. Use in the post-operative setting may improve post-operative course with the potential to decrease duration of chest tube drainage as well as ICU or hospital length of stay. Whatever the findings may conclude, this study will provide additional information about ambrisentan to physicians and may provide preliminary data for a multi-center pK study in this population. Therefore, future children may benefit from the increased knowledge of physicians who treat them. Participation in this research study is optional; if parents decide not to allow their child to participate in the study their child's routine standard of care will not be altered.

12. Costs to the subject and compensation:

The subject will incur no charges or fees for participating in the study. Gilead will supply the study drug. The remaining study costs will be covered by the Gilead Sciences Research Scholars Program in Cardiovascular Disease, including cost of pK and biomarker analysis.

13. Data Analysis & Statistical Considerations:

There will be up to 50 subjects enrolled and receiving drug in this study. Additional subjects will be enrolled to ensure that at least 20 subjects have at least 4 evaluable PK samples and that at least 16 subjects have ambrisentan peak exposure > 100ng/dL. Subjects who have only 1 evaluable sample will be included in the analysis. Descriptive statistics such as number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum will be presented for continuous variables (such as age, weight, etc). Other descriptive statistics such as counts, proportions, and/or percentages will be presented to summarize discrete variables (such as race, sex, etc.). Pharmacokinetic parameters will be calculated using non-compartmental analysis using WinNonlin software (Pharsight Corporation, Mountain View, CA). As previously mentioned, there will be an interim PK analysis that may alter the dosage.

14. Data & Safety monitoring:

Laboratory data, such as hematology and serum chemistry data will be tabulated by dosage group. Summary statistics for changes from baseline will be presented. Hemodynamic measurements will be described using univariable descriptive statistics (mean median, etc.); observed values and changes from baseline will be summarized.

Safety

Adverse events will be collected during study participation.

Study termination

Adverse events will be defined based on the Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (1).

In accordance with the CTCAE guidelines, adverse events will be evaluated by organ system and broadly defined as follows:

- | | |
|----------|--|
| Grade 1: | Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. |
| Grade 2: | Moderate; minimal, local or non-invasive intervention indicated; limiting age appropriate instrumental activity of daily living. |
| Grade 3: | Severe or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL. |
| Grade 4: | Life threatening consequences; urgent intervention indicated. |
| Grade 5: | Death related to AE. |

Study drug must be discontinued and the patient will be withdrawn from the study if one of the following occurs:

- Patient develops a grade 3 or greater adverse event thought to be related to study drug.
- The study team decides it is in the patient's best interest to discontinue study drug

The study will be stopped if one of the following occurs:

- Any 3 participants experience a grade 3 adverse event.
- Any single participant experiences a grade 4 or grade 5 adverse event.

Serious Adverse Events

Serious adverse events (SAE) will be defined as Grade 3 or greater adverse events. Investigators will submit safety reports of all SAE as required by the Institutional Review Board (IRB) and Pfizer Inc.

Dose Interruptions for Serious Adverse Events

If the patient experiences an AE felt to be related to study drug, the drug will be held at the discretion of the investigator. For an AE felt to be at least possibly related to study drug, study drug can be held until the AE resolves. Resume drug at the original dose. If the same AE recurs, study drug should be held. For an AE not felt to be due to study drug, no interruption is necessary.

All patients who receive study medication will be followed for safety. Peer investigators including Dr. Michael Cohen-Wolkowicz and Dr. Jennifer Li will conduct an internal review of study safety following the completion 6 subjects. The safety review will include all SAEs, all AEs thought to be possibly or probably related to the study drug, and all patients who discontinued participation in the study early. Enrollment will be allowed to continue during the safety evaluation.

15. Data storage & confidentiality:

Data generated by this study will be available for inspection by any regulatory authorities, by the sponsor, and by the Duke IRB, as appropriate, although no routine monitoring is planned. Medical information obtained from subjects during the course of this study is confidential and disclosure to third parties other than those noted above is prohibited. Data will be collected from the medical record and identified with a code number. The link between this subject's identity and the code number will be kept locked in a research office at Duke and in a password protected computer. All study data will be securely stored. Data about subjects and their samples will be maintained on case report forms under lock and key in the Pediatric cardiology offices. De-identified pK samples (using study code number) will be sent to an external laboratory for analysis. No PHI will leave Duke. If data resulting from this protocol is presented at scientific meetings or published in scientific journals, the subjects will not be identified. Study records will be retained until subjects reach 21 years of age.

1. U.S. National Institutes of Health: Common Terminology Criteria for Adverse Events v4.03: Published June 14, 2010. http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40_conversion.