Division: Worldwide Development
Retention Category: GRS019
Information Type: Protocol Amendment

Title:	A randomized, open label study comparing safety and efficacy parameters for a high and a low dose of ambrisentan (adjusted for body weight) for the treatment of pulmonary arterial hypertension in paediatric patients aged 8 years up to 18 years.

Compound Number: GSK1325760

Effective Date: 02-FEB-2011

Protocol Amendment Number: 03

Description:

A 6-month (24-week), randomized, open label evaluation of the safety, tolerability, and efficacy of a high and low dose ambrisentan (adjusted for body weight) treatment group in subjects aged 8 years up to 18 years with pulmonary arterial hypertension (PAH). An additional objective is to determine the ambrisentan population pharmacokinetics in the paediatric population. The study will include a screening/baseline period and a treatment period. The treatment period will be 24 weeks or until the subject's clinical condition deteriorates to the point that alternative/additional treatment is necessary. Patients who participate in the study and in whom continued treatment with ambrisentan is desired will be eligible to enrol into a long term follow-up study. The primary comparison will be the safety and tolerability of the two ambrisentan dose groups (Low vs. High) in the paediatric PAH population The secondary comparison will be the change from baseline for the efficacy parameters between the two treatment groups.

Subject: Pulmonary Arterial Hypertension

Author: PPD

Revision Chronology:

RM2010/00053/00	2010-MAY-07	Original
RM2010/00053/01	2010-JUN-09	Amendment No.: 01 Clarify the inclusion criteria that existing drug treatment for PAH would continue unchanged throughout the study. Clarify that two forms of contraception is required only for female subjects of child bearing potential who are sexually active Expand the eligibility for the continuation study to all patients who participate in this study and in whom continued treatment with ambrisentan is desired. Specify that patients will be given a diary card to collect information about dosing and days missed from school. Remove references to "brain natriuretic peptide" and clarify that it is N-Terminal pro-B-type Natriuretic Peptide that is being assessed. Add more specific references for the Tanner development criteria. Change the wording of the questions regarding days missed from school to make it clear that the total number of days missed includes the days missed because of PAH and that the days missed because of PAH are due to symptoms of PAH and do not include clinic visits. Remove the requirement for an unblinded person to enter compliance data into InForm. Allow the investigator to be unblinded to treatment for an individual patient once that patient has completed the study.

RM2010/00053/02	2010-OCT-26	Amendment No.: 02 To clarify that it is hepatitis B surface antigen, and not hepatitis B surface antibody, that is being assessed as part of the exclusion criteria. Add the US IND number to the Sponsor Information Page and clarify that the medical monitor and Serious Adverse Events contact are the same person.
RM2010/00053/03	2011-FEB-02	Amendment No.: 03 Add oestrogen to the laboratory tests being performed on female subjects at all times that pubertal development assessments are performed. Remove testosterone from the laboratory tests being performed on female subjects at all times that the pubertal development assessments are performed. Change the storage requirements for the study medication to store below 30°C.

Sponsor Signatory:	**Signature:**	**Date:**
Rose G. Snipes, M.D. Vice President Metabolic Pathways and Cardiovascular Therapy Area Unit	PPD	Feb. 2. 2011

SPONSOR INFORMATION PAGE

Clinical Study Identifier: AMB112529

GlaxoSmithKline
Iron Bridge Road
Stockley Park West, Uxbridge, Middlesex, UB11 1BU, UK
Telephone: PPD

In some countries, the clinical trial sponsor may be the local GlaxoSmithKline affiliate company (or designee). Where applicable, the details of the Sponsor and contact person will be provided to the relevant regulatory authority as part of the clinical trial submission.

Sponsor Medical Monitor and Serious Adverse Events (SAE) Contact Information:

PPD FRCS
Clinical Development Physician
MDC Clinical Metabolic EU
GlaxoSminthKline
Stockley Park

Sponsor Serious Adverse Events (SAE) Contact Information:

Regulatory Agency Identifying Number(s): EudraCT 2010-019547-19

US IND 64,915

INVESTIGATOR AGREEMENT PAGE

For protocol AMB112529

I confirm agreement to conduct the study in compliance with the protocol, as amended by this protocol amendment.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name: _____

Investigator Signature	Date

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LIST OF ABBREVIATIONS

6MWD	6 minute walking distance test
ACE	Angiotensin-converting enzymes
AE	Adverse Event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	Body mass index
BSA	Body surface area
BUN	Blood urea nitrogen
CCBs	Calcium Channel Blockers
cGMP	Cyclic guanosine monophosphate
CHD	Congenital heart defects
COPD	Congestive obstructive pulmonary disease
CPK	Creatine phosphokinase
CTD	Connective tissue disease
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ERA	Endothelin receptor antagonist
ET	endothelin
EU	European Union
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GGT	gamma glutamyl transferase
GSK	GlaxoSmithKline
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
INR	International Normalized Ratio
IPAH	Idiopathic Pulmonary Arterial Hypertension
IRB	Institutional Review Board
ITT	Intent-to-Treat
IVRS	Central interactive voice recognition system
IVRS	Interactive Voice Response System
kg	kilogram
LDH	Lactate dehydrogenase
LEVDP	Left ventricular end diastolic pressure
LFT	Liver function test
LH	Luteinizing hormone
m	meter
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mPAP	mean pulmonary arterial pressure
MSDS	Material Safety Data Sheet

NT-Pro BNP	N-Terminal pro-B-type Natriuretic Peptide
PAH	Pulmonary Arterial Hypertension
PAH-CTD	pulmonary arterial hypertension associated with connective tissue disease
PCWP	Pulmonary capillary wedge pressure
PDCO	EMA Paediatric Committee
PDE-5	Phosphodiesterase type 5
PGx	Pharmacogenetic
PIP	Paediatric Investigational Plan
Pk	pharmacokinetic
PVR	Pulmonary vascular resistance
RA	Right atrial
RHC	Right heart catheterisation
RV	Right ventricle
RVF	Right ventricular failure
SAE	Serious Adverse Event
SEM	Standard error of the mean
SHBG	Sex hormone binding globulin
SPC	Summary of Product Characteristics
SPM	Study Procedures Manual
TAPSE	Tricuspid annular plane systolic excursion
TRAX	Tracleer excellence database
TRJ	Tricuspid regurgitant jet
ULN	Upper limit of normal
V/Q	Ventilation/perfusion
WHO	World Health Organization

Trademark Information

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PROTOCOL SUMMARY

Rationale

Pulmonary arterial hypertension (PAH) is a rare, progressive, highly debilitating disease characterized by vascular obstruction and the variable presence of vasoconstriction, leading to increased pulmonary vascular resistance and right-sided heart failure [Moledina, 2010; Newman, 2004]. If left untreated, PAH ultimately leads to right ventricular failure and death; adult subjects have a median survival of 2.8 years without treatment [Krum, 2000]. Epidemiological estimates vary but prevalence in Europe is thought to be of the order of 15 cases per million [Humbert, 2006]. Large scale epidemiology studies of PAH in children have not been conducted and there is no or limited outcome data in pediatric PAH patients. A register in France (1995-1996) estimates the prevalence in children is as low as 3.7 cases per million [Fraisse, 2010]. In a national, comprehensive country wide survey of the epidemiology of idiopathic PAH (IPAH) management and survival in the United Kingdom (UK) the incidence was 0.48 cases per million children per year and the prevalence was 2.1 cases per million children [Moledina, 2010].

Ambrisentan (VOLIBRIS™ tablets) is an endothelin receptor antagonist (ERA) marketed in the European Union (EU) and some other countries by GlaxoSmithKline (GSK) and in the United States as Letairis by Gilead Sciences Inc. Ambrisentan is indicated for the treatment of adult patients with PAH to improve exercise capacity, decrease the symptoms of PAH, and delay clinical worsening.

The primary purpose of this paediatric study is to provide clinically relevant information on the safety and pharmacokinetic profile of ambrisentan in children with the most common causes of PAH in this age group. The design of the study is also intended to provide information to guide dose selection and supportive efficacy data in this age group. Despite the fact that none of the currently available adult treatments are licensed for use in children <12 yrs, (with the exception of bosentan which was recently approved for use in paediatric population from 2 years of age) they are widely used off label. This study will provide useful prescribing information to the medical community for treating this orphan disease in children in this environment of rapidly changing medical practice.

This study is part of a Paediatric Investigational Plan (PIP; EMEA-000434-PIP01-08) agreed with the European Medicines Agency's Paediatric Committee (PDCO).

Objective(s)

The primary objective of this study is to evaluate the safety and tolerability of ambrisentan in the paediatric (aged 8 years up to 18 years) PAH population. Other objectives are to obtain supportive efficacy data (change from baseline in efficacy parameters) on the paediatric use of ambrisentan in PAH and to determine the ambrisentan population pharmacokinetics in the paediatric population. Because patient recruitment is limited by the low prevalence of the disease in children, powered clinical hypotheses testing cannot be performed.

Study Design

A 6-month (24-week), randomized, open label evaluation of the safety, tolerability, and efficacy of a high and low dose ambrisentan (adjusted for body weight) treatment group in 66 subjects (33 per treatment group) aged 8 years up to 18 years with PAH. The study will include a screening/baseline period and a treatment period. The treatment period will be 24 weeks or until the subject's clinical condition deteriorates to the point that alternative/additional treatment is necessary. Patients who participate in this study and in whom continued treatment with ambrisentan is desired will be eligible to enrol into a long term follow-up study. The primary comparison will be the safety and tolerability of the two ambrisentan dose groups (Low vs. High) in the paediatric PAH population The secondary comparison will be the change from baseline of the efficacy parameters between the two treatment groups. Because patient recruitment is limited by the low prevalence of the disease in children, powered clinical hypotheses testing cannot be performed. Sixty-six (66) subjects were chosen on the predicted recruitment rate based on historic data so that the study can be completed in a reasonable time frame (2 years) for it to be useful and informative to the medical community. A 10% drop out rate will leave 60 evaluable subjects.

Study Endpoints/Assessments

Primary

The safety and tolerability of ambrisentan in the paediatric PAH population (see Safety):

Safety

- Adverse Events.
- Serious Adverse Events.
- Clinical laboratory parameters.
- Physical examination (Including height, weight, body mass index / body surface area, oxygen saturation, jugular venous pressure, liver size, and presence of peripheral oedema and/or ascites.)
- Vital Signs.
- Pubertal development (change from baseline in endocrinology assessments at Weeks 12 and 24).

Secondary

Pharmcokinetics:

- Population pharmacokinetic assessment based on one plasma sample per subject at Weeks 4 (trough), 8 (0.5 to 4 hours post-dose), 12 (trough), 16 (0.5 to 4 hours post-dose), 20 (4 to 22 hours post-dose), and 24 (trough).
- Pharmacokinetic/pharmacodynamic modelling.

Efficacy

- The change from baseline in the 6 minute walking distance (6MWD) test evaluated after 24 weeks of therapy.
- Mean changes from baseline in the 6MWD test at Weeks 4, 8, 12, 16, and 20.
- The time to clinical worsening of PAH.
- The change from baseline in Subject Global Assessment to Week 24 using the SF-10 health survey for children.
- The change from baseline in WHO functional class to Week 24.
- Change from baseline in plasma N-Terminal pro-B-type Natriuretic Peptide (NT-Pro BNP) concentration at Week 24.

Exploratory

- The change from baseline in major prognostic factors based on echocardiogram: pericardial effusion, right atrial (RA) pressure, tricuspid annular plane systolic excursion (TAPSE), eccentricity index (systolic and diastolic), and right ventricular (RV) pressure by tricuspid regurgitant jet (TRJ) velocity to Week 24.

Other

- Change from baseline in cardiopulmonary hemodynamics at Week 24 (a sub-study in subjects enrolled at centres where the collection of hemodynamic data is considered part of the standard of care); see Appendix 1.

1. INTRODUCTION

1.1. Background

Pulmonary arterial hypertension (PAH) is a group of diseases characterised by a progressive increase of pulmonary vascular resistance (PVR) leading to right ventricular failure (RVF) and premature death. A recent meta-analysis estimates survival for untreated idiopathic PAH (IPAH) in adults to be only 79% at 1 year and 66% at 2 years [McLaughlin, 2006]. In the UK, survival in treated children with IPAH at 1, 3 and 5 years was 89%, 84%, and 75% respectively; whilst transplant free survival was 89%, 76%, and 57% respectively [Moledina, 2010].

Detailed, consensus, evidence-based guidelines for the treatment of PAH have been published [Badesch, 2004; Galiè, 2009]. Pharmacological approaches are divided into those that are supportive or background treatment (aimed at alleviating vasoconstriction, breathlessness, and thromboembolic complications) and those (such as endothelium receptor antagonist [ERAs]) that target the underlying pathophysiology.

Diuretics and anticoagulants have been widely used in the management of PAH, yet response varies [Humbert, 2004]. Calcium channel blockers (CCBs) have shown improved survival in vasoreactive IPAH patients, yet the relatively low incidence of vasoreactivity make CCBs useful only in a minority of the population with PAH [Humbert, 2004; Rich, 1992].

The pathophysiologic mechanisms which underpin the disease spectrum of PAH involve pulmonary vascular dysfunction, which produces an imbalance in vasoactive substances as well as proliferation signals that lead to structural remodelling and pulmonary vasoconstriction. Although its role is not fully understood, endothelin (ET) is considered to be an important mediator of pathology in PAH. Almost all components of the endothelin system are upregulated in PAH [Giaid, 1993; Galiè, 2004]. There appears to be a significant correlation between serum endothelin 1 (ET-1) levels and disease severity, reflected by elevations in PVR, mean pulmonary artery pressure (mPAP) and right atrial pressure [Nootens, 1995]. Endothelin-1 (ET-1) receptor antagonists are a targeted pharmacological approach that limit both the vasoconstrictor and proliferative action of endothelin [Dingemanse, 2004], and in this way ameliorate the clinical manifestations of the disease.

Three signalling pathways involved in the pathogenesis of PAH have been targeted for therapeutic intervention [Humbert, 2004]: the cAMP-dependent prostacyclin (PGI_2) pathway, the cGMP-dependent nitric oxide (NO) pathway, and the phospholipase-C-dependent endothelin pathway. These pathways are targeted by the following classes of PAH medicines: prostanoids (FLOLAN™ [epoprostenol], Ventavis [iloprost] and Remodulin [terprostinil]); phosphodiesterase type-5 (PDE-5) inhibitors (Revatio [EU]/Viagra [sildenafil] and Adcirca [tadalafil])); and ERAs (VOLIBRIS [EU]/Letairis [US] (ambrisentan), Tracleer [bosentan] and Thelin [sitaxentan]).

There is evidence that prostanoid therapies are effective in children, but in common to their use in adults, the pharmacokinetic properties of these drugs and routes of administration (e.g., intravenous, subcutaneous injection, and multiple inhalations) present substantial challenges to their successful use in a pediatric population.

The clinical profile of ambrisentan (an ERA selective for the ETA receptor) in adults is that it has an efficacy profile broadly comparable with other targeted oral treatments, but that it has potentially important advantages. Ambrisentan has been associated with a favourable liver safety profile and a low risk of drug-drug interactions. It is noted that data from the TRAX[1] database indicates that the risk of elevated aminotransferases with bosentan is lower in children than adults [Beghetti, 2008]. Nevertheless the liver safety profile of ambrisentan in adults has been favourable and if replicated in children would provide a clinically useful option. Children, like adults with PAH, commonly require many concomitant medications. The low risk of drug-drug interactions and convenient once a day dosing associated with ambrisentan may provide a therapeutic option that simplifies treatment in this complex disease.

1.2. Rationale

Pulmonary arterial hypertension (PAH) is a rare, progressive, highly debilitating disease characterized by vascular obstruction and the variable presence of vasoconstriction, leading to increased pulmonary vascular resistance and right-sided heart failure [Moledina, 2010; Newman, 2004]. If left untreated, PAH ultimately leads to right ventricular failure and death; adult subjects have a median survival of 2.8 years without treatment [Krum, 2000]. Epidemiological estimates vary but prevalence in Europe is thought to be of the order of 15 cases per million [Humbert, 2006]. Large scale epidemiology studies of PAH in children have not been conducted and there is no or limited outcome data in paediatric PAH patients. A register in France (1995-1996) estimates the prevalence in children is as low as 3.7 cases per million [Fraisse, 2010]. In a national, comprehensive country wide survey of the epidemiology of idiopathic PAH (IPAH) management and survival in the United Kingdom (UK) the incidence was 0.48 cases per million children per year and the prevalence was 2.1 cases per million children [Moledina, 2010].

Ambrisentan (VOLIBRIS tablets) is an endothelin receptor antagonist (ERA) marketed in the European Union (EU) and some other countries by GlaxoSmithKline (GSK) and in the United States as Letairis by Gilead Sciences Inc. Ambrisentan is indicated for the treatment of adult patients with PAH to improve exercise capacity, decrease the symptoms of PAH, and delay clinical worsening.

The primary purpose of this paediatric study is to provide clinically relevant information on the safety and pharmacokinetic profile of ambrisentan in children with the most common causes of PAH in this age group. The design of the study is also intended to

[1] TRAX - the Tracleer Excellence database is a non-interventional, prospective, internet-based surveillance system initiated by the manufacturer in cooperation with Regulatory agencies to collect potential safety signals associated with bosentan use including adverse events, elevations of liver aminotransferase levels, other abnormal laboratory values, deaths, and hospitalisation [Segal, 2005].

provide information to guide dose selection and supportive efficacy data in this age group. Despite the fact that none of the currently available adult treatments are licensed for use in children <12 yrs, (with the exception of bosentan which was recently approved for use in paediatric population from 2 years of age) they are widely used off label. This study will provide useful prescribing information to the medical community for treating this orphan disease in children in this environment of rapidly changing medical practice.

This study is part of a Paediatric Investigational Plan (PIP; EMEA-000434-PIP01-08) agreed with the European Medicines Agency's Paediatric Committee (PDCO).

2. OBJECTIVE(S)

The primary objective of this study is to evaluate the safety and tolerability of ambrisentan in the paediatric (aged 8 years up to 18 years) PAH population. Other objectives are to obtain supportive efficacy data (change from baseline in efficacy parameters) on the paediatric use of ambrisentan in PAH and to determine the ambrisentan population pharmacokinetics in the paediatric population. Because patient recruitment is limited by the low prevalence of the disease in children, powered clinical hypotheses testing cannot be performed.

2.1. Primary

The primary objective is the safety and tolerability of ambrisentan in the paediatric PAH population (see Safety).

2.1.1. Safety

- Adverse Events.
- Serious Adverse Events.
- Clinical laboratory parameters.
- Physical examination (Including height, weight, body mass index / body surface area, oxygen saturation, jugular venous pressure, liver size, and presence of peripheral oedema and/or ascites.)
- Vital Signs.
- Pubertal development (change from baseline in endocrinology assessments at Weeks 12 and 24).

2.1.2. Secondary

2.1.2.1. Pharmcokinetics:

- Population pharmacokinetic assessment based on one plasma sample per subject at Weeks 4 (trough), 8 (0.5 to 4 hours post-dose), 12 (trough), 16 (0.5 to 4 hours post-dose), 20 (4 to 22 hours post-dose), and 24 (trough).

- Pharmacokinetic/pharmacodynamic modelling.

2.1.2.2. Efficacy

- The change from baseline in the 6 minute walking distance (6MWD) test evaluated after 24 weeks of therapy.
- Mean changes from baseline in the 6MWD test at Weeks 4, 8, 12, 16, and 20.
- The time to clinical worsening of PAH.
- The change from baseline in Subject Global Assessment to Week 24 using the SF-10 health survey for children.
- The change from baseline in WHO functional class to Week 24.
- Change from baseline in N-terminal pro-B-type natriuretic peptide (NT-Pro BNP) concentration at Week 24.

2.1.2.3. Exploratory

- The change from baseline in major prognostic factors based on echocardiograms: pericardial effusion, right atrial (RA) pressure, tricuspid annular plane systolic excursion (TAPSE), eccentricity index (systolic and diastolic), and right ventricular (RV) pressure by tricuspid regurgitant jet (TRJ) velocity to Week 24.

2.1.2.4. Other

- Change from baseline in cardiopulmonary hemodynamics at Week 24 (a sub-study in subjects enrolled at centres where the collection of hemodynamic data is considered part of the standard of care); see Appendix 1.

3. INVESTIGATIONAL PLAN

3.1. Study Design

A 6-month (24-week), randomized, open label evaluation of the safety, tolerability, and efficacy of a high and low dose ambrisentan (adjusted for body weight) in 66 subjects (33 per treatment group) aged 8 years up to 18 years with PAH. The study will include a screening/baseline period and a treatment period. The treatment period will be 24 weeks or until the subject's clinical condition deteriorates to the point that alternative/additional treatment is necessary. Subjects who participate in the study will be eligible to enrol into a long term follow-up study. The primary comparison will be the safety and tolerability of the two ambrisentan dose groups (Low vs. High) in the paediatric PAH population. The secondary comparison will be the change from baseline of the efficacy parameters between the two treatment groups. Because subject recruitment is limited by the low prevalence of the disease in children, powered clinical hypotheses testing cannot be performed. Sixty-six (66) subjects were chosen on the predicted recruitment rate based on historic data so that the study can be completed in a reasonable time frame (2 years)

for it to be useful and informative to the medical community. A 10% drop out rate will leave 60 evaluable subjects.

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying Study Procedures Manual (SPM). The SPM will provide the site personnel with administrative and detailed technical information on study conduct.

3.2. Discussion of Design

PAH is a serious and rapidly progressive disease in children. Despite the fact that none of the currently available adult treatments are licensed for use in children <12 yrs, (with the exception of bosentan which was recently approved for use in paediatric population from 2 years of age) they are widely used off label. In this context it is not considered appropriate to have a placebo control. This has been confirmed by discussion with expert clinicians in the treatment of PAH in children. The lack of robust data in children for the currently available treatments also means that there is little consistency regarding treatment selection. As a result there is no international consensus on what represents current standard of care. This makes a study comparing ambrisentan with 'standard of care' impractical across the number of sites required to conduct a study in children.

Recruitment into studies in children with PAH has been difficult and slow as evidenced by the 4 years the sildenafil study took to recruit [ClinicalTrials.gov identifier: NCT00159913]. A study with this duration of recruitment may be confounded by changes in routine management. This GSK study will be more tightly controlled in that it proposes to include patients with IPAH, PAH associated with connective tissue disease, and cases of sustained PAH following intracardiac repair. PAH associated with congenital heart disease, which is more common, will not be included because of the wide variation in causation and complexity. GSK has therefore based the size of the proposed study (in 8 up to 18 year-olds) on what is considered to be a feasible number of patients to recruit within a reasonable timeframe (18 months to 2 years). The estimate is based on the number of incident cases reported by a panel of clinical PAH experts at their centres. All patients will receive ambrisentan. On this basis the estimated sample size is 60 evaluable subjects. It is not possible to power the study for inferential analyses of between-group differences.

This is an open label study since there is no feasible method of blinding the study. All subjects will be randomised to one of two dose groups of ambrisentan. The risks and inconveniences associated with blinding the study as to dose outweigh any benefit of conducting the study in a blinded fashion. All patients will be receiving ambrisentan and, although the dose group assignment could be blinded the unsuitability of the resulting dosage form precludes blinding. The 2.5 mg, 5 mg, and 10 mg ambrisentan tablets differ in size and colour. Over-encapsulating the tablets so that all dose strengths appear the same would create a dosage form that would be more difficult for the younger children to swallow. Using all 2.5 mg tablets (with matching placebo) to disguise the dose would require every subject to take four tablets at each dose (which would be very difficult for the younger children) and could lead to an unacceptable risk of patients receiving the wrong dose (i.e., the wrong mix of active and placebo tablets).

However, to minimise bias, a person who is not involved in subject assessments will be designated at each investigation site to dispense the investigational product and to perform the subsequent compliance checks (i.e., pill counts). Subjects and their parents or legal guardian will be asked not to comment on the treatment (e.g., no. of tablets taken) with the individual making the assessments. Therefore, the individual making the subjects assessments would be unbiased.

4. SUBJECT SELECTION AND WITHDRAWAL CRITERIA

4.1. Number of Subjects

Approximately 80 subjects will enter the study to achieve 66 randomized subjects (33 per treatment group). It is anticipated that approximately 60 randomized subjects will complete the study and/or provide some evaluable data for the primary endpoint.

4.2. Inclusion Criteria

Subjects eligible for enrolment in the study must meet all of the following criteria:

1. Male or female at least 8 years of age and not yet 18 years of age at the time of randomization.
2. A current diagnosis of PAH (WHO Group 1) with WHO class II or III symptoms in one of the following categories:
    - Idiopathic
    - Heritable [familial]
    - Secondary to connective tissue disease (e.g., limited scleroderma, diffuse scleroderma, mixed connective tissue disease (CTD), systemic lupus erythematosus, or overlap syndrome).
    - Persistent PAH despite surgical repair (at least 6 months prior to the screening visit) of atrial septal defects, ventricular septal defects, atrio-ventricular septal defects, and persistent patent ductus.
3. Have met the following hemodynamic criteria for subjects with right heart catheterization (RHC) when performed as part of the diagnosis or routine care (see Appendix 1):
    - mean pulmonary arterial pressure (mPAP) of ≥25 mmHg
    - pulmonary vascular resistance (PVR) of ≥240 dyne·sec/cm5
    - left ventricular end diastolic pressure (LEVDP) or pulmonary capillary wedge pressure (PCWP) of ≤15 mmHg.

4. Subjects must either be treatment naïve, have discontinued treatment with another ERA (e.g., bosentan) at least 1 month previously because of elevated liver function tests (LFTs), or have been on a stable dose of drug therapy for PAH (e.g., sildenafil or prostacyclin) for at least one month prior to the Screening Visit. The baseline drug therapy for PAH, if any, should not change from the Screening Visit until the end of all Treatment Period assessments.
5. Subjects who discontinued ERA treatment due to elevated LFTs, must have LFTs of <3 x Upper Limit of Normal (ULN).
6. A female is eligible to participate in this study, as assessed by the investigator, if she is of:

a. Non-childbearing potential (i.e., physiologically incapable of becoming pregnant); or,

b. Child-bearing potential - has a negative pregnancy test and is not lactating at the Screening and Baseline/Randomisation Visits and, if sexually active, agrees to use 2 reliable methods of contraception from the Screening Visit until study completion and for at least 30 days following the last dose of study drug (reliable methods of contraception are listed in Appendix 2).

7. Subject or subject's legal guardian is able and willing to give written informed consent. As part of the consent, female subjects of childbearing potential will be informed of the risk of teratogenicity and will need to be counselled in a developmentally appropriate manner on the importance of pregnancy prevention; and male subjects will need to be informed of potential risk of testicular tubular atrophy and aspermia.

Complete information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the investigational product that may impact subject eligibility is provided in the Investigators Brochure and product label.

French subjects: In France, a subject will be eligible for inclusion in this study only if either affiliated to or a beneficiary of a social security category.

4.3. Exclusion Criteria

Subjects meeting any of the following criteria must not be enrolled in the study:

1. Subjects currently taking an ERA.
2. Subjects currently taking cyclosporine A.
3. Subjects whose body weight is less than 20 Kg.
4. Subjects who have not tolerated PAH therapy due to adverse effects which may be related to their mechanism of action (e.g., prostanoids, ERA, PDE-5 inhibitors) with the exception of liver abnormalities for those subjects who were receiving another ERA.
5. Female subjects who are pregnant or breastfeeding.

6. Subjects with diagnosis of active hepatitis (hepatitis B surface antigen and hepatitis C antibody), or clinically significant hepatic enzyme elevation (i.e., ALT, AST or AP >3xULN) at Screening.
7. Subjects with severe renal impairment (creatinine clearance <30 mL/min) at Screening.
8. Subject has clinically significant fluid retention in the opinion of the investigator.
9. Subject has clinically significant anaemia in the opinion of the investigator.
10. Subject has a known hypersensitivity to the study drug, the metabolites, or formulation excipients.
11. Subjects who have participated in another trial or have taken another investigational product during the previous 30 days.
12. Alcohol abuse, illicit drug use within 1 year.
13. Any concurrent condition or concurrent use of medication that would affect subject safety in the opinion of the investigator.

4.4. Withdrawal Criteria

Subjects who do not tolerate treatment or whose baseline drug therapy for PAH (i.e., PDE-5 or prostanoid) is changed will be withdrawn from the study. Treatment for subjects withdrawn from the study will be implemented at the Investigator's discretion and PAH guidelines.

Subjects whose clinical condition deteriorates to the point that additional drug treatment is indicated (including increasing the baseline doses of targeted PAH therapy) prior to Week 24 will be considered as having completed the study at that point.

The Independent Data Monitoring Committee (IDMC) may stop the study at any time if they consider that the potential risks outweigh the potential benefits (based on review of safety [adverse experiences] and exposure [plasma concentration] data after 20 subjects have completed Week 12 of the study and again after 40 subjects have completed Week 12).

A subject may also be discontinued prior to completion of the study for the following reasons:

- Liver chemistry values exceeding the threshold criteria (as outlined in Section 6.2.6).
- Adverse event which in the opinion of the investigator requires withdrawal.
- Pregnancy.
- Consent withdrawn.
- Lost to follow-up.
- Protocol violation.
- Termination of study by sponsor.

- Investigator's discretion (document reason in eCRF).

5. STUDY TREATMENTS

5.1. Investigational Product and Reference Therapy

The sponsor will provide commercially available ambrisentan 5 mg, and 10 mg tablets, as well as 2.5 mg tablets of equivalent quality. For centres in Japan, one or more 2.5 mg tablets will be used to achieve the appropriate dose. Subjects will be dosed orally (tablet must be swallowed whole) once daily for 24 weeks after randomization to either a high dose or a low dose group as shown below. Within each body weight group, subjects will start at the lower dose for that group and then, at Week 2 those randomized to the high dose group will receive the higher dose.

Body Weight	Low Dose	High Dose
≥ 50 kg	5 mg	10 mg
≥ 35 kg and < 50 kg	5 mg	7.5 mg
≥ 20 kg and <35 kg	2.5 mg	5 mg

The 7.5 mg dose will consist of one 2.5 mg tablet and one 5 mg tablet (three 2.5 mg tablets in Japan).

The contents of the label will be in accordance with all applicable regulatory requirements.

Under normal conditions of handling and administration, investigational product is not expected to pose significant safety risks to site staff. A Material Safety Data Sheet (MSDS) describing the occupational hazards and recommended handling precautions will be provided to site staff if required by local laws or will otherwise be available from GSK upon request.

Investigational product must be stored in a secure area under the appropriate physical conditions for the product. The Investigator, the hospital pharmacist, or other personnel allowed to store and dispense the investigational products will be responsible for ensuring that the investigational product used in the study will be securely maintained as specified by the Sponsor and in accordance with applicable regulatory requirements.

Study medication will be stored in secure (locked) areas at a temperature below30°C and dispensed according to the protocol under the supervision of the Investigator or his/her designee. Investigational product must be dispensed or administered only to subjects enrolled in the study and in accordance with the protocol. Subjects will be instructed to keep dispensed treatment in a secure place, out of reach of children, and at room temperature.

Subjects will be instructed to return unused medication to the Investigator. Any unused product will be returned by the Investigator to GSK or destroyed at the site based on local regulations.

5.2. Treatment Assignment

All subjects who meet the inclusion and exclusion criteria will be randomized to one of two dose groups (Low dose or High dose, based on body weight, see Section 5.1) of ambrisentan according to a computer-generated randomisation schedule. To ensure balance with respect to the number of patients assigned to each treatment group, the allocation schedule will be generated in blocks. Each subject will be assigned to a pack number according to the predefined randomisation list. A central Interactive Voice Response System (IVRS) will be used for treatment assignment.

Once a randomisation number has been assigned, the patient will be considered as definitely included and the number will not be re-assigned. The assigned study treatment should be initiated as soon as possible after randomisation.

Randomization will be stratified by the age groups 8 years up to 11 years and 12 years up to 18 years and by aetiology of PAH as follows:

- idiopathic;
- heritable [familial];
- secondary to connective tissue disease; and
- persistent despite surgical repair of atrial septal defects, ventricular septal defects, atrio-ventricular septal defects, and persistent patent ductus.

Subjects will be assigned to study treatment in accordance with the randomization schedule.

5.3. Blinding

This is an open label study. It is not feasible to blind the study in the classical sense; however, to minimise bias, a person who is not involved in subject assessments will be designated at each investigation site to dispense the investigational product and to perform the subsequent compliance checks (i.e., pill counts). Subjects and their parents or legal guardian will be asked not to comment on the treatment (e.g., no. of tablets taken) with the individual making the assessments. Therefore, the individual making the subjects assessments would be unbiased.

After each subject has completed the study, the randomised treatment will be unblinded to the investigators to allow for informed dose adjustments and follow-up treatment as may be necessary

5.4. Product Accountability

In accordance with local regulatory requirements, the investigator, designated site staff, or head of the medical institution (where applicable) must document the amount of GSK investigational product dispensed and/or administered to study subjects, the amount returned by study subjects, and the amount received from and returned to GSK, when applicable. Product accountability records must be maintained throughout the course of the study.

5.5. Treatment Compliance

Compliance will be assessed by the investigator or designee as specified in the Time and Events Table, (Table 1), and recorded in the eCRF. Compliance is calculated based on the number of tablets dispensed and the number of tablets returned, and the days between the date dispensed and the date returned. Compliance should be assessed for each child resistant blister pack to ensure an accurate assessment. The range of compliance at the appropriate visits will be recorded on the eCRF.

5.6. Concomitant Medications and Non-Drug Therapies

5.6.1. Permitted Medications and Non-Drug Therapies

Standard medical treatment(s) being taken by the subject upon study entry may be maintained throughout the study.

Subjects taking PAH specific medications (i.e., prostanoids [treprostinil, epoprostinol, iloprost], vasodilators [e.g., Angiotensin-converting enzymes [ACE] inhibitors, Calcium Channel blockers, nitric oxide] or Cyclic guanosine monophosphate (cGMP) inhibitors – specific phosphodiesterase type 5 [PDE-5; e.g., slidenafil, tadalafil]) have to have stable PAH therapy for 1 month before screening and the therapy has to be maintained throughout the study treatment period.

If a subjects targeted PAH background therapy (i.e., PDE-5 or prostanoid) is changed or discontinued due to tolerability issues, the subject should be withdrawn from the study.

Subjects may begin oral contraceptive therapy as per the prescribing information.

5.6.2. Prohibited Medications and Non-Drug Therapies

During the study, patients may not receive:

- Endothelin receptor antagonists other than ambrisentan (e.g., bosentan, sitaxentan).
- Cyclosporine A.

5.7. Treatment after the End of the Study

Patients who participate in the study and in whom continued treatment with ambrisentan is desired will be eligible to enrol into a long term follow-up study.

5.8. Treatment of Investigational Product Overdose

There is no experience in PAH patients of ambrisentan at daily doses greater than 10 mg. In healthy volunteers, single doses of 50 mg and 100 mg (5 to 10 times the maximum recommended dose) were associated with headache, flushing, dizziness, nausea, and nasal congestion.

Due to the mechanism of action, an overdose of ambrisentan could potentially result in hypotension. In case of pronounced hypotension, active cardiac support may be required. No specific antidote is available.

6. STUDY ASSESSMENTS AND PROCEDURES

The following measurements and evaluations will be conducted during the trial. Please refer to the Time and Events Table (Table 1) for additional details on the timing of the assessments. Every effort must be made to adhere to the protocol defined visit schedule.

The data collection tool for this study will be GSK defined eCRFs. In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

Table 1 Time and Events Table

Procedure	Screening/ Baseline[1]	Treatment						Early Withdrawal	End of Therapy	Follow-up[2]
	Max. 2 Wks	Wk 2 ±2 days	Wk 4 ±7 days	Wk 8 ±7 days	Wk 12 ±7 days	Wk 16 ±7 days	Wk 20 ±7 days		Week 24	
Written informed consent and assent	X									
Subject Demography	X									
Medical History	X									
Disease History	X									
Therapy History	X									
Inclusion/Exclusion Criteria	X									
Adverse Events	X	X	X	X	X	X	X	X	X	X
Serious Adverse Events	X	X	X	X	X	X	X	X	X	X
Clinical Chemistry[3]	X	X	X	X	X	X	X	X	X	X
Haematology[4]	X	X	X	X	X	X	X	X	X	X
Physical Examination	X				X			X	X	X
Vital Signs[5]	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X				X			X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X	X	X
6 Minute Walk Distance[6]	X		X	X	X	X	X	X	X	X
WHO Functional Class	X		X	X	X	X	X	X	X	X
Health Outcome Assessments[7]	X		X	X	X	X	X	X	X	X
Echocardiogram	X				X			X	X	X
Plasma NT-Pro BNP concentration	X				X			X	X	
Plasma Sample for Population Pharmacokinetic assessment[8]			X	X	X	X	X	X	X	
Hemodynamic assessment[9]	X							X	X	
PGx Sampling			X							
Pregnancy Test[10]	U		U	U	U	U	U	U	U	U
Pubertal Development Assessment[11]	X				X			X	X	
HBsAg and hepatitis C antibody[12]	X									
Dispense Investigational Product[13]	X	X	X	X	X	X	X			
Assess Investigational Product Compliance		X	X	X	X	X	X	X	X	

Table 1 Time and Events Table (Continued)

1. 2 weeks maximum between screening and baseline (randomization) visit; Note: screening and baseline can be the same day if local labs available to confirm eligibility. Central lab blood draw must also be done at this visit.
2. Only for subjects who are not participating in the long term follow-up study; within 4 to 6 weeks after last dose of investigational drug
3. Includes sodium, magnesium, potassium, calcium, glucose, chloride, bicarbonate (CO2,) phosphorus-inorganic, creatinine, total protein, albumin, ALT (SGPT), AST (SGOT), GGT, LDH, total bilirubin, creatine phosphokinase (CPK), blood urea nitrogen (BUN), uric acid, and alkaline phosphatase
4. Includes platelet count, RBC count, reticulocyte count, hematocrit, hemoglobin, RBC indices (mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], and mean corpuscular haemoglobin concentration [MCHC]), WBC count, automated WBC differential (neutrophils-total, lymphocytes, monocytes, eosinophils, basophils)
5. Includes height, weight, supine blood pressure, and heart rate
6. Subjects with a 20% decrease in 6MWD will need to return in 1 week to repeat the test, to confirm PAH deterioration.
7. SF 10 and a record of school days scheduled and missed. Two diary cards will be given to the subject at Baseline, and one diary card will be given to the subject at Weeks 4, 8, 12, 16, 20 and 24 on which to record the number of school days scheduled, the number missed for any reason, and the number missed because of symptoms of PAH. One baseline diary card will be completed and returned during the visit. The other baseline diary card should be returned at the Week 4 visit. All other diary cards will be returned at the next visit. Data from all diary cards will be transcribed into the eCRF.
8. One plasma sample per visit. A diary card will be given to the subject at Baseline to record date and time of study medication dosing 2 days prior to and 1 day prior to the Week 4 visit and at Weeks 4, 8, 12, and 20 to record date and time of study medication dosing 2 days prior to and 1 day prior to the next visit. At Week 16, a diary card will be given to the subject to record the date and time of study medication dosing 2 days prior to, 1 day prior to and on the day of the Week 20 visit. The diary card given out at Baseline should be returned at the Week 4 visit. All other diary cards will be returned at the next visit. The dates and times of study medication dosing will be transcribed into the eCRF. Subjects will have the option to receive a reminder SMS message on the days they should complete the diary card and to remind them of their next visit date.
9. Sub-study - Heart rate, mean blood pressure (systolic, diastolic), mean pulmonary artery (PA) pressure (systolic, diastolic), mean right atrium (RA) pressure, left ventricular end diastolic pressure (LEVDP) or pulmonary capillary wedge pressure (PCWP), cardiac output, cardiac index (calculated value), arterial oxygen saturation and mixed venous oxygen saturation. Record method used to calculate cardiac output measurement (if Fick's principle was used it must be stated if oxygen consumptions is measured or assumed).
10. Monthly pregnancy test for females of childbearing potential for the duration of the study: U = urine.
11. Pubertal development in male and female subjects will be assessed using Tanner criteria. In male subjects, testicular volume will be assessed using Prader's orchidometer, and blood samples will be obtained to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone binding globulin (SHBG) and inhibin B levels. In female subjects, blood samples will be obtained to measure FSH, LH, oestrogen, SHBG and inhibin B levels.
12. Hepatitis B surface antigen and hepatitis C antibody (if hepatitis C antibody positive, a hepatitis C RIBA immunoblot assay should be reflexively performed on the same sample to confirm the result).
13. Within each body weight group, subjects will start at the lower dose for that group and then, at Week 2 those randomized to the high dose group will receive the higher dose.

6.1. Critical Baseline Assessments

Review historical diagnostic tests, such as Right Heart Catheter (RHC); chest X-ray; echocardiogram; ventilation/perfusion scan, computed axial tomography (CT) or spiral CT, and pulmonary arteriogram (if applicable), to ensure that none of the exclusion criteria for PAH aetiology are met.

Historic hemodynamic data (e.g., mPAP, RAP, PVR, and cardiac index) based on the most recent RHC prior to starting this study will be collected.

Therapy history including PAH treatment and ERA discontinued due to elevated LFTs.

6.2. Safety

6.2.1. Physical examination

Physical examination at Baseline and Weeks 12 and 24 (including height, weight, body mass index [BMI] / body surface area [BSA], oxygen saturation, jugular venous pressure, liver size, and presence of peripheral oedema and/or ascites) and pubertal development (see Section 6.2.4).

Note: BMI and BSA to be calculated centrally using height and weight collected in the eCRF.

6.2.2. Electrocardiogram / Echocardiogram

A 12-lead ECG will be performed at Baseline and at Weeks 12 and 24 (or early withdrawal), and Follow-up. Any changes since Baseline felt to be significant in the medical and scientific judgement of the investigator are to be recorded as AEs or SAEs in the eCRF.

An echocardiogram will be performed at Baseline and at Weeks 12 and 24 (or early withdrawal), and Follow-up (if applicable). An echocardiogram (the results of which are considered exploratory in this study) will also be performed if there is PAH deterioration or if there is a change in PAH therapy.

6.2.3. Vital Signs, Body Weight and Height

Vital signs (including heart rate and supine blood pressure), height, and weight will be collected at each clinic visit.

All measures of blood pressure will be performed using standard sphygmomanometry. If possible, the same sphygmomanometer and arm should be used. Procedural details are provided in the study procedures manual.

6.2.4. Pubertal Development Assessment

Pubertal development in male and female subjects will be assessed at baseline, Week 12, and Week 24 or early termination using Tanner criteria [Marshall, 1969; Marshall, 1970; Cameron, 2004]. In male subjects, testicular volume will be assessed by Prader's orchidometer. These assessments will be performed by a paediatric endocrinologist or another individual with comparable experience employing the Tanner criteria and Prader's orchidometer. Preferably, the same assessor will evaluate an individual subject at each assessment time.

In addition, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (in male subjects only), oestrogen (in female subjects only), sex hormone binding globulin (SHBG), and inhibin B levels will be assessed at baseline, Week 12, and Week 24 or early withdrawal.

6.2.5. Clinical Laboratory Tests

Central labs (Quest Diagnostics) will be used for all laboratory assessments. However, results from local labs can be used if more readily available to confirm eligibility criteria at the Screening visit.

6.2.5.1. Safety Tests

The following tests are required at each clinic visits (including early withdrawal or end of therapy visits, and Follow-up visit [if applicable]).

- **Chemistry:** serum alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), alkaline phosphatase, gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), total bilirubin, creatinine, sodium, magnesium, potassium, chloride, bicarbonate (CO2), phosphorus-inorganic, calcium, blood urea nitrogen (BUN), uric acid, glucose, total protein, and albumin.
- **Haematology:** haemoglobin, hematocrit, red cell count, red cell indices (mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], and mean corpuscular haemoglobin concentration [MCHC]), white blood cell count (total and differential), reticulocyte count, and platelet count.
- **Pregnancy:** Urine pregnancy test at screening/baseline and end of treatment or early withdrawal visit and monthly urine pregnancy test for female subjects of child bearing potential.

6.2.5.2. Pharmacogenetics and NT-Pro BNP

- Blood samples for NT-Pro BNP concentration will be collected at screening/baseline, Week 12, early withdrawal or Week 24 (end of therapy) visits, and Follow-up visit (if applicable). Assays for plasma concentrations will be performed in the central laboratory.

- The first PK sample (at Week 4) will also be used for pharmacogenetics.

Collection, processing, labelling, and shipping of the NT-pro BNP and pharmacogenetic samples to central laboratory are detailed in the SPM.

6.2.6. Liver chemistry stopping and followup criteria

Phase II liver chemistry stopping and follow up criteria have been designed to assure subject safety and evaluate liver event aetiology.

Phase II liver chemistry stopping criteria 1-5 are defined below:

1. ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) (or ALT ≥ 3xULN and International Normalized Ratio [INR]>1.5, if INR measured).

NOTE: if serum bilirubin fractionation is not immediately available, study drug should be discontinued if ALT ≥ 3xULN **and** bilirubin ≥ 2xULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, **record presence of detectable urinary bilirubin on dipstick**, indicating direct bilirubin elevations and suggesting liver injury.

2. ALT ≥ 5xULN.
3. ALT ≥ 3xULN if associated with the appearance or worsening of symptoms of hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia.
4. ALT ≥ 3xULN persists for ≥4 weeks
5. ALT ≥ 3xULN and cannot be monitored weekly for 4 weeks.

When any of the liver chemistry stopping criteria 1-5 is met, do the following:

- **Immediately** withdraw investigational product
- Report the event to GSK **within 24 hours** of learning its occurrence
- Complete the liver event CRF and SAE data collection tool if the event also meets the criteria for an SAE. All events of ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct bilirubin) (or ALT ≥ 3xULN **and** INR>1.5, if INR measured; INR measurement is not required and the threshold value stated will not apply to patients receiving anticoagulants), termed 'Hy's Law', **must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis)**.

NOTE: if serum bilirubin fractionation is not immediately available, study drug should be discontinued if ALT ≥ 3xULN **and** bilirubin ≥ 2xULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, **record presence of detectable urinary bilirubin on dipstick,** indicating direct bilirubin elevations and suggesting liver injury.

- Complete the liver imaging and/or liver biopsy CRFs if these tests are performed

- Perform liver event follow up assessments, and monitor the subject until liver chemistries resolve, stabilize, or return to baseline values as described below.
- Withdraw the subject from the **study** (unless further safety follow up is required) after completion of the liver chemistry monitoring as described below.
- Do not re-challenge with investigational product.

In addition, for criterion 1:

- Make every reasonable attempt to have subjects return to clinic within **24 hours** for repeat liver chemistries, liver event follow up assessments (see below), and close monitoring
- A specialist or hepatology consultation is recommended.
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

For criteria 2, 3, 4, and 5:

- Make every reasonable attempt to have subjects return to clinic **within 24-72 hrs** for repeat liver chemistries and liver event follow up assessments (see below)
- Monitor subjects weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values; criterion 5 subjects should be monitored as frequently as possible.

Subjects with ALT ≥3xULN **but** <5xULN **and** bilirubin <2xULN, without hepatitis symptoms or rash, and who can be monitored weekly for 4 weeks

- Notify the GSK medical monitor within 24 hours of learning of the abnormality to discuss subject safety.
- Can continue investigational product [this can be deleted for single-dose studies]
- Must return weekly for repeat liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) until they resolve, stabilize or return to within baseline
- If at any time these subjects meet the liver chemistry stopping criteria, proceed as described above
- If, after 4 weeks of monitoring, ALT <3xULN and bilirubin <2xULN, monitor subjects twice monthly until liver chemistries normalize or return to within baseline values.

For criteria 1-5, make every attempt to carry out the **liver event follow up assessments** described below:

- Viral hepatitis serology including:
  - Hepatitis A IgM antibody;
  - Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM);

- Hepatitis C RNA;
- Cytomegalovirus IgM antibody;
- Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing);
- Hepatitis E IgM antibody (if subject resides outside the US or Canada, or has travelled outside US or Canada in past 3 months);

- Blood sample for pharmacokinetic (PK) analysis, obtained within 24 hours of last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of investigational product prior to blood sample draw on the eCRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SPM.
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Fractionate bilirubin, if total bilirubin≥2xULN.
- Obtain complete blood count with differential to assess eosinophilia.
- Record the appearance or worsening of clinical symptoms of hepatitis, or hypersensitivity, such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever rash or eosinophilia as relevant on the AE report form.
- Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins, on the concomitant medications report form.
- Record alcohol use on the liver event alcohol intake case report form

The following are required for subjects with ALT ≥3xULN and bilirubin ≥2xULN (>35% direct) but are optional for other abnormal liver chemistries:

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies.
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.

6.2.7. Adverse Events

The investigator or site staff will be responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

6.2.7.1. Definition of an AE

Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Note: An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.

Events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study
- Signs, symptoms, or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concomitant medication (overdose per se will not be reported as an AE/SAE).

“Lack of efficacy” or “failure of expected pharmacological action” per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE.

Events that **do not** meet the definition of an AE include:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject’s condition.

6.2.7.2. Definition of a SAE

A serious adverse event is any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

   NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires hospitalization or prolongation of existing hospitalization

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in disability/incapacity, or

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

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

g. All events of possible drug-induced liver injury with hyperbilirubinaemia defined as ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct) (or ALT ≥ 3xULN and INR>1.5, if INR measured) termed 'Hy's Law' events (INR measurement is not required and the threshold value stated will not apply to patients receiving anticoagulants).

NOTE: bilirubin fractionation is performed if testing is available. If testing is unavailable, record the presence of detectable urinary bilirubin on dipstick indicating direct bilirubin elevations and suggesting liver injury. If testing is unavailable and a subject meets the criterion of total bilirubin ≥ 2xULN, then the event is still reported as an SAE. If INR is obtained, include values on the SAE form. INR elevations >1.5 suggest severe liver injury.

6.2.8. Laboratory and Other Safety Assessment Abnormalities Reported as AEs and SAEs

Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator are to be recorded as AEs or SAEs.

However, any clinically significant safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition, are **not** to be reported as AEs or SAEs.

6.2.9. Pregnancy

Subjects who become pregnant during the study must discontinue study drug immediately. The Investigator should counsel the subject regarding the possible effects of prior study drug exposure on the foetus (See SmPC and USPI) and the need to inform the study site of the outcome of the pregnancy. Subjects should be instructed to notify the Investigator if they become pregnant at any time during the study, or if they become pregnant within 30 days of last study drug dose.

Any pregnancy that occurs during study participation must be reported using a clinical trial pregnancy form. To ensure subject safety, each pregnancy must be reported to GSK within 2 weeks of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to the investigational product, must be promptly reported to GSK.

6.2.10. Time Period and Frequency of Detecting AEs and SAEs

The investigator or site staff is responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

AEs will be collected from the start of investigational product and until 30 days after the last dose of investigational product.

SAEs will be collected over the same time period as stated above for AEs. However, any SAEs assessed **as related** to study participation (e.g., investigational product, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK concomitant medication, will be recorded from the time a subject consents to participate in the study up to and including any follow up contact. All SAEs will be reported to GSK within 24 hours, as indicated in Section 6.2.11.

6.2.11. Prompt Reporting of Serious Adverse Events and Other Events to GSK

SAEs, pregnancies, and liver function abnormalities meeting pre-defined criteria will be reported promptly by the investigator to GSK as described in the following table once the investigator determines that the event meets the protocol definition for that event.

	Initial Reports		Follow-up Information on a Previous Report	
Type of Event	**Time Frame**	**Documents**	**Time Frame**	**Documents**
All SAEs	24 hours	"SAE" data collection tool	24 hours	Updated "SAE" data collection tool
Pregnancy	2 Weeks	Pregnancy Notification Form	2 Weeks	Pregnancy Follow up Form
Liver chemistry abnormalities Phase II:				
ALT≥3xULN **and** Bilirubin≥2xULN (>35% direct) (or ALT≥3xULN **and** INR>1.5, if INR measured)***	24 hours*	SAE data collection tool. **Liver Event Case Report Form (CRF) and liver imaging and/or biopsy CRFs if applicable	24 hours	Updated SAE data collection tool. **Updated Liver Event CRF
ALT≥5xULN; ALT≥3xULN with hepatitis or rash **or** 3xULN ≥4 weeks	24 hours*	**Liver Event CRF	24 hours	**Updated Liver Event CRF
ALT≥3xULN and <5xULN and biliribin <2xULN	24 hours*	**Liver Event CRF does not need completing unless elevations persist for 4 weeks or subject cannot be monitored weekly for 4 weeks		

*GSK to be notified at onset of liver chemistry elevations to discuss subject safety.
** Liver event documents should be completed as soon as possible.
*** INR measurement is not required; if measured, the threshold value stated will not apply to patients receiving anticoagulants.

The method of detecting, recording, evaluating and follow-up of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in the SPM. Procedures for post-study AEs/SAEs are provided in the SPM.

Procedures for documenting, transmitting and follow-up of medical device incidents along with the regulatory reporting requirements for medical devices are provided in the SPM.

6.2.11.1. Regulatory reporting requirements for SAEs

Prompt notification of SAEs by the investigator to GSK is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

6.3. Pharmacokinetics

Blood samples for pharmacokinetic analysis of ambrisentan will be collected at the time points indicated in Table 2. The actual date and time of each blood sample collection will be recorded in the GSK-defined eCRF. The exact time of dosing on the day of the visit, 1 day prior to the visit and 2 days prior to the visit will be recorded in the eCRF by having the subject return a patient diary card on which this information is recorded.

Table 2 Pharmacokinetic Sampling

	Treatment					
Sample Collection Timepoint[1]	**Week 4**	**Week 8**	**Week 12**	**Week 16**	**Week 20**	**Week 24**
Pre-dose (Trough concentration)	X[2]		X[2]			X[2]
0.5-4 hours after dose (Absorption phase)		X		X		
4-22 hours after dose (Elimination phase)					X	

1. If the subject is withdrawn early, a blood sample for PK should be collected prior to discharge, if possible.
2. Subjects should be reminded to not take their study medication until after the PK sample is drawn.

Plasma analysis will be performed under the management of Worldwide Bioanalysis, DMPK, GSK. Plasma samples will be analyzed for ambrisentan only. The main circulating metabolite of ambrisentan, 4-hydroxymethyl ambrisentan, represents approximately 4% of parent AUC in plasma and the binding affinity of this metabolite is approximately 64-fold less than ambrisentan. Therefore, at concentrations observed in

the plasma, 4-hydroxymethyl ambrisentan is not expected to contribute to pharmacological activity of ambrisentan.

Details of PK blood sample collection (including volume to be collected), processing, storage, and shipping procedures are provided in the Study Procedures Manual (SPM).

6.4. Efficacy

6.4.1. Change from baseline in the 6 minute walking distance (6MWD) test.

The 6MWD will be assessed at Baseline, Weeks 4, 8, 12, 16, 20, and Week 24 or early withdrawal, and Follow –up (at Follow-up only for subjects who are not participating in the long term follow-up study). Subjects with a 20% decrease in 6MWD will need to return in 1 week to repeat the test, to confirm PAH deterioration.

6.4.2. Time to worsening of PAH

Time to clinical worsening of PAH is defined as the time from randomization to the first occurrence of:

- Death (all cause) or placed on active list for lung transplant;
- Hospitalisation due to PAH deterioration;
- Addition or increased dose of other targeted PAH therapeutic agents (prostanoids, PDE-5 inhibitors) and/or atrial septostomy;
- PAH related deterioration identified by:
    - increase in WHO functional class;
    - deterioration in exercise testing (i.e., 20% decrease in 6MWD on two consecutive tests - 1 week apart);
    - clinical signs or symptoms of right sided heart failure (i.e., new peripheral edema, increase in liver size, ascites, increase in jugular venous pressure, pericardial effusion, increased dyspnea).

6.4.3. Change from baseline (Week 0) in Subject Global Assessment to Week 24.

Global assessments will be performed at baseline and Week 24 or early withdrawal, and Follow–up (if appropriate).

6.4.4. Change from baseline in WHO functional class to Week 24.

WHO functional class will be determined at baseline and Weeks 4, 8, 12, 16, 20, and Week 24 or early withdrawal, and Follow –up (if appropriate).

6.4.5. Change from baseline plasma N-terminal pro-B type natriuretic peptide (NT-Pro BNP) at Week 24

Blood samples for determination of N-Terminal pro-B-type Natriuretic Peptide plasma concentrations will be determined at baseline, Week 12 and Week 24 or early withdrawal. A central lab (Quest Diagnostics) will analyze all samples.

6.4.6. Change from baseline in major prognostic factors to Week 24.

Echocardiograms will be performed at baseline and Weeks 12 and 24 or early withdrawal. Prognostic factors included pericardial effusion, RA pressure, tricuspid annular plane systolic excursion (TAPSE), eccentricity index (systolic and diastolic), and right ventricular (RV) pressure by tricuspid regurgitant jet (TRJ) velocity. These evaluations were considered exploratory in this study.

6.4.7. Change from baseline in cardiopulmonary hemodynamics at Week 24

Cardiopulmonary hemodynamic assessments will be performed at baseline and Week 24 or early withdrawal in a subgroup of subjects enrolled in centres where the collection of hemodynamic data is considered part of the standard of care (see Appendix 1).

6.5. Health Outcomes

Patient global assessments will be performed at Baseline, Weeks 4, 8, 12, 16, 20, and Week 24 or early withdrawal, and Follow-up (if appropriate). Health outcomes will be recorded using the parent-completed Short Form 10 (SF10) Health Survey for children.

The short-form 10 (SF-10) Health Survey for Children is a 10-item, 4-week recall, parent-completed health assessment that measures physical and psychosocial functioning for children ages five and over. Specific domains include Physical Functioning (2 items), Role/Social Emotional-Behavioral (1 item), Role/Social Physical (1 item), Bodily Pain (1 item), General Behavior (1 item), Mental Health (1 item), Self Esteem (2 items), and General Health Perceptions (1 item). This instrument provides a brief, reliable, and scientifically valid health status measurement that is easy to administer and interpret.

In addition to the SF10, specific questions will be asked regarding school days as follows:

1. Within the past month, how many days of school were scheduled? (at Baseline only)

   or

   Since your baseline visit, how many days of school were scheduled? (at Week 4 only)

   or

Since your last clinic visit, how many days of school were scheduled? (all other assessment times)

2. How many scheduled days of school were missed for any reason?
3. Of these, how many days were missed because of symptoms of pulmonary arterial hypertension (PAH)?

Subjects will record the days of school scheduled and missed on a patient diary card, which will be returned to the clinic at the scheduled visits.

6.6. Pharmacogenetics

Information regarding pharmacogenetic research is included in Appendix 3. The IEC/IRB and, where required, the applicable regulatory agency must approve the PGx assessments before these can be conducted at the site. The approval(s) must be in writing and will clearly specify approval of the PGx assessments (i.e., approval of X). In some cases, approval of the PGx assessments can occur after approval is obtained for the rest of the study. If so, then the written approval will clearly indicate approval of the PGx assessments is being deferred and the study, except for PGx assessments, can be initiated. When PGx assessments will not be approved, then the approval for the rest of the study will clearly indicate this and therefore, PGx assessments will not be conducted.

7. DATA MANAGEMENT

For this study, subject data will be entered into GSK defined electronic case report forms (eCRFs), transmitted electronically to GSK, and combined with data provided from other sources in a validated data system.

Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medications terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), and an internal validated medication dictionary, GSKDrug. eCRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. In all cases, subject initials will not be collected or transmitted to GSK according to GSK policy.

8. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

8.1. Study Objectives

The primary objective of this study is to evaluate the safety and tolerability of ambrisentan in the proposed paediatric PAH population. Secondary objectives are to obtain supportive efficacy data (change from baseline in efficacy parameters) on the paediatric use of ambrisentan in PAH. Other objectives are to determine the ambrisentan population pharmacokinetics in the paediatric population.

8.2. Study Design Considerations

8.2.1. Sample Size Assumptions

Due to the low prevalence of the disease in children, sample size was based on feasibility rather than formal power calculations. Recruitment of approximately 66 subjects (33 per treatment group with no prespecified number in each age group/aetiology stratum) is felt to be achievable within a reasonable period of time (~ 2 years) minimizing any impact of changing practice on the conduct and interpretation of the study.

8.2.2. Sample Size Sensitivity

Since the sample size is based on feasibility and no formal hypothesis testing is planned, no sample size sensitivity calculations were performed.

8.2.3. Sample Size Re-estimation

No sample size re-estimation is planned for this study.

8.3. Data Analysis Considerations

8.3.1. Analysis Populations

The Intention-to-Treat (ITT) Population will consist of all randomized subjects who received at least 1 dose of study drug. For the ITT population, subjects were considered as belonging to their randomized treatment group, regardless of the actual dose of ambrisentan received. The ITT population will be used in all efficacy summaries.

The Safety Population is defined as all randomized subjects who received at least 1 dose of study drug. Subjects were considered as belonging to the treatment group according to highest dose received. The safety population will be used in all safety summaries.

The Pharmacokinetic Population will consist of all randomized subjects from which a PK blood sample is obtained.

8.3.2. Analysis Data Sets

Analysis datasets will consist of all data collected in the study and evaluated according to the populations described in Section 8.3.1.

PK analysis data will contain all plasma ambrisentan concentrations with appropriate corresponding dosing records. Pharmacokinetic data from this study may be enriched with selected adult PK data to establish a structural PK model as described in Section 8.3.5.2.

8.3.3. Treatment Comparisons

8.3.3.1. Primary Comparisons of Interest

The primary comparison of interest will be between Low and High dose of Ambrisentan in terms of safety and tolerability during the treatment period as follows:

- Adverse Events.
- Serious Adverse Events.
- Clinical laboratory parameters.
- Physical examination (Including height, weight, body mass index / body surface area, oxygen saturation, jugular venous pressure, liver size, and presence of peripheral oedema and/or ascites.)
- Vital Signs.
- Pubertal development (change from baseline in endocrinology assessments at Weeks 12 and 24).

8.3.3.2. Secondary Comparisons of Interest

Secondary comparisons of interest will be the comparison of the two treatment groups in respect to the following:

Phamacokinetics:

- Population pharmacokinetic assessment based on one plasma sample per subject at Weeks 4, 8, 12, 16, 20, and 24.
- Pharmacokinetic/pharmacodynamic modelling.

Efficacy:

- The change from baseline in 6 minute walking distance (6MWD) test after 24 weeks
- Mean changes from baseline in the 6MWD test at each Weeks 4, 8, 12, 16, and 20
- Time to clinical worsening of PAH
- The change from baseline in Subject Global Assessment to Week 24.
- The change from baseline in WHO functional class to Week 24.
- Change from baseline in plasma N-Terminal pro-B-type Natriuretic Peptide concentration at Week 24.

8.3.3.3. Exploratory Comparisons of Interest

- The change from baseline in major prognostic factors based on echocardiograms: pericardial effusion, RA pressure, tricuspid annular plane systolic excursion (TAPSE), eccentricity index (systolic and diastolic), and right ventricular (RV) pressure by tricuspid regurgitant jet (TRJ) velocity to Week 24.

8.3.3.4. Other Comparisons of Interest

- Change from baseline in cardiopulmonary hemodynamics at Week 24 (a sub-study in subjects enrolled at centres where the collection of hemodynamic data is considered part of the standard of care), see Appendix 1.

8.3.4. Interim Analysis

There is no interim analysis planned for this study.

8.3.5. Key Elements of Analysis Plan

8.3.5.1. Safety Analyses

All subjects who received at least one dose of study medication will be assessed for clinical safety and tolerability and will be denoted as the Safety population.

Clinical interpretation will be based upon review of displays of adverse events, laboratory values, vital signs, body weight, pubertal development, and concomitant medications. Principal considerations in this evaluation of adverse events will be time to onset and investigator-reported relationship of either adverse events or laboratory abnormalities to study medication. . For each laboratory test, the number and percentage of subjects with values above the reference range will be displayed for each treatment group and overall. Appropriate monitoring of safety data will be conducted throughout the conduct of the study.

No formal statistical analysis is planned for safety parameters. All data will be presented descriptively or graphically, by treatment group (Low and High) and overall. Where data are available, it will be summarised by visit.

8.3.5.2. Pharmacokinetic Analyses

The objective of the pharmacokinetic analysis is to establish a population pharmacokinetic model adequate to describe the time-course and variability of plasma ambrisentan concentrations following repeat dosing in paediatric PAH patients. The specific objectives are:

1. To define a suitable structural PK model to characterize the PK of ambrisentan in paediatrics following oral administration.

2. To estimate the population pharmacokinetic parameters (e.g., absorption rate constant [ka], apparent volume of distribution [V/F] and apparent clearance [CL/F] of ambrisentan and their associated variability and precision.
3. To evaluate the influence of available covariates (e.g., subject demographics, physiological factors, patient status, ambrisentan doses and concomitant medications) on the population pharmacokinetic parameters and their associated variability.

The paediatric PK data may be enriched with selected adult PK data to establish a structural PK model. PK data will be analyzed using the nonlinear mixed effects modeling program (NONMEM). During each step in the model building process, improvements to the model will be assessed by evaluation of the agreement between the observed and predicted plasma concentrations, reductions in the range of weighted residuals, uniformity of the distribution of the weighted residuals versus the predicted concentrations about the line of identity, and increases in the precision of the parameter estimates, as well as reduction of the terms for inter-individual variability and random residual variability. Assessment of the log likelihood ratio test will also be conducted as a means of assessing improvement in the model.

Population PK analysis will be the responsibility of the Department of Clinical Pharmacology Modeling and Simulation (CPMS), Quantitative Sciences, GlaxoSmithKline.

8.3.5.3. Pharmacokinetic/Pharmacodynamic Analyses

The objective of the pharmacokinetic/pharmacodynamic (PK/PD) analysis is to evaluate the potential relationships between ambrisentan dose/exposure versus efficacy and/or safety measures in the paediatric patient population.

PK/PD data will be graphically assessed initially and maybe further analyzed with the use of the nonlinear mixed effects modeling program (NONMEM). Model selection will be conducted using the same criteria as described above for the PK analyses. Covariates examined during the development of the population PK model will also be evaluated to assess their effects on ambrisentan PK/PD.

Population PK/PD analysis will be the responsibility of the Department of Clinical Pharmacology Modeling and Simulation (CPMS), Quantitative Sciences, GlaxoSmithKline.

8.3.5.4. Efficacy Analyses

All subjects from the ITT population will be included in the analysis of efficacy data. Given the small sample size, all efficacy data will be summarized descriptively (including 95% CI) and graphically. Data will be summarised by treatment group (Low and High) and overall. In addition, summary analysis of the difference between the Low and High dose will be performed.

The summary analysis will include:

- mean change from baseline in the 6MWD after 24 weeks of therapy;
- mean changes from baseline in 6MWD for Weeks 4, 8, 12 16, and 20;
- mean time to clinical worsening of PAH;
- change from baseline in Subject Global Assessment to Week 24;
- change from baseline in WHO functional class to Week 24;
- The change from baseline in major prognostic factors: pericardial effusion, right atrial (RA) pressure, tricuspid annular plane systolic excursion (TAPSE), and eccentricity index (systolic and diastolic) to Week 24
- change from baseline in cardiopulmonary hemodynamics at Week 24; and
- change from baseline in plasma NT-Pro BNP concentration at Week 24.
- change form baseline in endocrinology assessments in male subjects at Weeks 12 and 24.

Time to clinical worsening will be presented graphically by Kaplan-Meier curves. Results will include the estimated hazard ratio, and confidence interval. The hazard ratios will express the rate of event occurrence for the high dose ambrisentan group relative to the low dose ambrisentan group.

Exploratory

- The change from baseline in major prognostic factors to Week 24 by echocardiogram: pericardial effusion, right atrial (RA) pressure, tricuspid annular plane systolic excursion (TAPSE), eccentricity index (systolic and diastolic), and right ventricular (RV) pressure by tricuspid regurgitant jet (TRJ) velocity.

Other

- Change from baseline in cardiopulmonary hemodynamics at Week 24 (a sub-study in subjects enrolled at centres where the collection of hemodynamic data is considered part of the standard of care); see Appendix 1.

8.3.5.5. Health Outcomes Analyses

Two sets of summary scores will be calculated based on responses to each item on the SF-10 Health Survey for Children (see Section 6.5):

- Physical Health Summary (PHS-10); and
- Psychosocial Summary (PSS-10).

The mean change from Baseline in SF10 scores will be calculated at Weeks 4, 8, 12, 16, 20, and 24.

8.3.5.6. Pharmacogenetic Analyses

8.3.5.7. Novel Biomarker(s) Analyses

The results of these biomarker investigations will be reported separately from the main clinical study report. All endpoints of interest from all comparisons will be descriptively and/or graphically summarised as appropriate to the data.

8.3.5.7.1. RNA Transcriptome Analysis

RNA transcriptome profile data will first be normalized to enable direct comparison of all data sets. Uninformative data (RNA species in all samples below detectable limits or levels unchanged across all samples under comparison) will be removed and multivariate statistical analyses will be performed on the remaining data to uncover intrinsic differences and similarities in the levels of RNAs between the different samples, and groups of samples. Statistical tools such as Principal Component Analysis, PLS-Discriminant Analysis, and ANOVA using either standard or customized software will be used for the profile analysis to assist identification of patterns/profiles which may associate with treatment outcome or PAH and medically related conditions.

8.3.5.7.2. RNA Expression Analysis of a Subset of RNA Species

RNA expression profile data are first normalised to enable direct comparison of all data sets. After data reduction to remove uninformative data (RNAs whose levels are below levels of detection for all samples, and those whose levels are unchanged across all samples) the remaining data will be subject to a series of multivariate statistical analyses to uncover intrinsic differences and similarities in the levels of RNAs between the different samples. This will include application of statistical tools such as Principal Component Analysis, and ANOVA using standard analysis software as well as software packages customised for such profile analysis to assist identification of patterns/profiles which may associate with treatment outcome and/or PAH.

9. STUDY CONDUCT CONSIDERATIONS

9.1. Posting of Information on Clinicaltrials.gov

Study information from this protocol will be posted on clinicaltrials.gov before enrolment of subjects begins.

9.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

Prior to initiation of a study site, GSK will obtain approval from the appropriate regulatory agency to conduct the study in accordance with applicable country-specific regulatory requirements, including those required under a US IND.

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with Good Clinical Practice (GCP), all applicable subject privacy requirements, and the ethical principles that are outlined in the Declaration of Helsinki 2008, including, but not limited to:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements.

GSK will provide full details of the above procedures, either verbally, in writing, or both.

Written informed consent must be obtained from each subject prior to participation in the study.

9.3. Quality Control (Study Monitoring)

In accordance with applicable regulations, GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK will monitor the study to ensure that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

9.4. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study. In the event of an audit or inspection, the investigator (and institution) must agree to grant the auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss any findings/relevant issues.

9.5. Study and Site Closure

Upon completion or termination of the study, the GSK monitor will conduct site closure activities with the investigator or site staff (as appropriate), in accordance with applicable regulations, GCP, and GSK Standard Operating Procedures.

GSK reserves the right to temporarily suspend or terminate the study at any time for reasons including (but not limited to) safety issues, ethical issues, or severe non-compliance. If GSK determines that such action is required, GSK will discuss the reasons for taking such action with the investigator or head of the medical institution (where applicable). When feasible, GSK will provide advance notice to the investigator or head of the medical institution of the impending action.

If a study is suspended or terminated for **safety reasons**, GSK will promptly inform all investigators, heads of the medical institutions (where applicable),and/or institutions conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension/termination along with the reasons for such action. Where required by applicable regulations, the investigator or head of the medical institution must inform the IRB/IEC promptly and provide the reason(s) for the suspension/termination.

9.6. Records Retention

Following closure of the study, the investigator or head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of the records may be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution must be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original. In addition, they must meet accessibility and retrieval standards, including regeneration of a hard copy, if required. The investigator must also ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for creating the reproductions.

GSK will inform the investigator of the time period for retaining the site records in order to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by local laws/regulations, GSK standard operating procedures, and/or institutional requirements.

The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to archival of records at an off-site facility or transfer of ownership of the records in the event that the investigator is no longer associated with the site.

9.7. Provision of Study Results to Investigators, Posting to the Clinical Trials Register and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.

The results summary will be posted to the Clinical Study Register no later than 12 months after the last subject's last visit (LSLV) or sooner if required by legal agreement, local law or regulation. In addition, a manuscript will be submitted to a peer-reviewed journal for publication within 18 months of LSLV. When manuscript publication in a peer-reviewed journal is not feasible, further study information will be posted to the GSK Clinical Study Register to supplement the results summary.

9.8. Independent Data Monitoring Committee (IDMC)

An IDMC will be utilized in this study to ensure external objective medical and/or statistical review of safety and/or efficacy issues in order to protect the ethical and safety interests of subjects and to protect the scientific validity of the study. The schedule of any planned interim analysis and the analysis plan for IDMC review is described in the charter, which is available upon request.

10. REFERENCES

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11. APPENDICES

11.1. Appendix 1: Cardiopulmonary Hemodynamic Sub-study

Hemodynamic assessments will be performed at baseline and Week 24 or early withdrawal in a subgroup of subjects enrolled in centres where the collection of hemodynamic data is considered part of the standard of care. A 'diagnostic catheter' must have been done within 3 months prior to randomization or re-catheterisation, and no change in PAH targeted medication since the most recent hemodynamic assessment.

Hemodynamic assessments will include:

- heart rate;
- mean blood pressure (systolic, diastolic);
- mean pulmonary arterial pressure (PA; systolic, diastolic);
- mean right atrial (RA) pressure;
- left ventricular end diastolic pressure (LEVDP); or
- pulmonary capillary wedge pressure (PCWP);
- cardiac output;
- cardiac index (calculated value);
- arterial and mixed venous oxygen saturation (Record method used to calculate cardiac output measurement, if Fick's principle was used it must be stated if oxygen consumptions is measured or assumed).

11.2. Appendix 2: List of Highly Effective Methods for Avoidance of Pregnancy in Women of Childbearing Potential

The following is the all inclusive list of the highly effective methods for avoiding pregnancy (i.e., have a failure rate of less than 1% per year).

- Abstinence [Hatcher, 2004]
- Combination Oral Contraceptive [Hatcher, 2004]
- Injectable progestogen [Hatcher, 2004]
- Implants of levonorgestrel [Hatcher, 2004]
- Estrogenic vaginal ring [Hatcher, 2004]
- Percutaneous contraceptive patches [Hatcher, 2004]
- Intrauterine device (IUD) or intrauterine system (IUS) that meets the SOP effectiveness criteria as stated in the product label [Hatcher, 2004]
- Male partner sterilization (vasectomy with documentation of azoospermia) prior to the **female subject's entry** into the study, and this male is the sole partner for that subject [Hatcher, 2004]. For this definition, “documented” refers to the outcome of the investigator's/designee’s medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject’s medical records.
- Double barrier method: condom and an occlusive cap (diaphragm or cervical/vault caps) with a vaginal spermicidal agent (foam/gel/film/cream/suppository) [Trussell, 2003]

  Nonoxynol-9 is the critical component in most spermicides, and is regarded as an acceptable spermicidal agent. Concern has been raised that nonoxynol-9 damages the epithelial lining of the vagina, and exposure may facilitate transmission of viruses, particularly human immunodeficiency virus (HIV). The World Health Organization (WHO) conducted a technical consultation in October 2001 and concluded that the increased risk for such transmission was low to minimal [Trussell, 2003].

References

Hatcher RA, Trussell J, Stewart F, Nelson AL, Cates W, Guest F, Kowal DD, editors. Contraceptive Technology. New York: Ardent Media, 2004: 226. Table 9-2, “% of Women Experiencing an Unintended Pregnancy During the First Year of Typical Use and the First Year of Perfect Use of Contraception and the Percentage Continuing Use at the End of the First Year. United States”, column entitled, “% of Women Experiencing an Unintended Pregnancy Within the First Year of Use. Perfect Use”.

Trussell J. Personal communication WHO/CONRAD Technical consultation on nonoxynol-9. WHO, Geneva, 9-10 October 2001. Summary Report. World Health Organization, 2003

11.3. Appendix 3: Pharmacogenetic Research

Pharmacogenetics – Background

Pharmacogenetics (PGx) is the study of variability in drug response due to hereditary factors in different populations. There is increasing evidence that an individual's genetic composition (i.e., genotype) may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), pharmacodynamics (relationship between concentrations and pharmacologic effects or the time course of pharmacologic effects) and/or clinical outcome (in terms of efficacy and/or safety and tolerability). Some reported examples of PGx analysis include:

Drug	Disease	Gene	Outcome
Abacavir	HIV [Hetherington, 2002; Mallal, 2002]	HLA –B*5701	Individuals with HLA-B*5701 variant may be at increased risk for experiencing hypersensitivity to abacavir. Clinical assays are available for HLA-B*5701 but none has been validated. HLA-B*5701 screening would supplement but never replace abacavir clinical risk management strategies aimed at minimising rare but serious outcomes associated with abacavir hypersensitivity.
Warfarin	Cardiovascular [Neergard, 2006; Wilke, 2005]	CYP2C9	Serious adverse events (SAEs) experienced by some patients on warfarin may be explained by variations in the CYP2C9 gene that influences the degree of anticoagulation achieved.
Irinotecan	Cancer [FDA News Release, 2005]	UGT1A1	Variations in the UGT1A1 gene can influence a patient's ability to break down irinotecan, which can lead to increased blood levels of the drug and a higher risk of side effects. A dose of irinotecan that is safe for one patient with a particular UGT1A1 gene variation, might be too high for another patient without this variation, raising the risk of certain side-effects. A genetic blood test (Invader UGT1A1 molecular assay) is available that can detect variations in the gene.

A key component to successful PGx research is the collection of samples during the conduct of clinical studies.

Collection of whole blood samples, even when no *a priori* hypothesis has been identified, may enable PGx analysis to be conducted if at any time it appears that there is a potential unexpected or unexplained variation in handling or response to ambrisentan.

Pharmacogenetic Research Objectives

The objective of the PGx research (if there is a potential unexpected or unexplained variation) is to investigate a possible genetic relationship to handling or response to ambrisentan. If at any time it appears there is potential variability in response in this clinical study or in a series of clinical studies with ambrisentan that may be attributable to genetic variations of subjects, the following objectives may be investigated:

- Relationship between genetic variants and the pharmacokinetics and/or pharmacodynamics of investigational product;
- Relationship between genetic variants and safety and/or tolerability of investigational product; and
- Relationship between genetic variants and efficacy of investigational product.

Study Population

Any subject who has given informed consent to participate in the clinical study, has met all the entry criteria for the clinical study, and receives investigational product may take part in the PGx research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the PGx research.

Subject participation in the PGx research is voluntary and refusal to participate will not indicate withdrawal from the clinical study. Refusal to participate will involve no penalty or loss of benefits to which the subject would otherwise be entitled.

Study Assessments and Procedures

Blood taken for pharmacokinetics will also be used for the PGx sample. It is recommended that the first PK sample (Week 4) is used to prepare the PGx sample, but any of the PK samples can be used. Full details of sample preparation are provided in the SPM.

The PGx sample is labelled (or "coded") with a study specific number that can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number). The blood sample is taken on a single occasion unless a duplicate sample is required due to inability to utilize the original sample.

The DNA extracted from the blood sample may be subjected to sample quality control analysis. This analysis will involve the genotyping of several genetic markers to confirm the integrity of individual samples. If inconsistencies are noted in the analysis, then those samples may be destroyed.

The need to conduct PGx analysis may be identified after a study (or a set of studies) of ambrisentan has been completed and the study data reviewed.

In some cases, the samples may not be studied. e.g., no questions are raised about how people respond to ambrisentan.

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will use samples collected from the study for the purpose stated in this protocol and in the informed consent form.

Subjects can request their sample to be destroyed at any time.

Subject Withdrawal from Study

If a subject who has consented to participate in PGx research and has a sample taken for PGx research withdraws from the clinical study for any reason other than lost to follow-up, the subject will be given the following options:

- The sample is retained for PGx research
- Any PGx sample is destroyed.

If a subject withdraws consent from the PGx research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records. If the sample has already been processed, it will be destroyed after all steps are complete. GSK will ensure that any data related to the sample will not be analysed. The sample will be destroyed after processing is complete.

Pharmacogenetics Analyses

Generally GSK will utilize three approaches to explore genetic variation in drug response.

1. Specific sections of DNA may be selected from areas of the genome (e.g., candidate genes) known to encode the drug target, drug metabolizing enzymes, areas associated with mechanisms underlying adverse events, and those linked to study disease and, thus, linked to drug response.

The candidate genes that may be investigated in this study include the following: the GSK Absorption, Distribution, Metabolism and Excretion genes. These play a central role in drug pharmacokinetics and pharmacodynamics. In addition, continuing research may identify other enzymes, transporters, proteins or receptors that may be involved in response to ambrisentan. The genes that may code for these proteins may also be studied.

2. By evaluating large numbers of polymorphic markers (e.g., single nucleotide polymorphisms or SNPs) throughout the genome, sets of markers may be identified that correspond to differential drug response.

The results of PGx investigations will be reported either as part of the main clinical study report or as a separate report. All endpoints of interest from all comparisons will be descriptively and/or graphically summarised as appropriate to the data. In all cases, appropriate statistical methods will be used to analyse the genetic markers in the context of other clinical data. Statistical methods may include, but are not limited to Hardy-Weinberg Equilibrium testing, Comparison of Demographic and Baseline Characteristics by Genotype, Evaluation of Genotypic Effects, Evaluation of Treatment by Genotype and Gene-Gene Interaction, Linkage Disequilibrium, Multiple Comparison and Multiplicity and/or Power and Sample Size Considerations. Detailed description of the analyses to be conducted will be documented in the Pharmacogenetics Reporting and Analysis Plan.

3. Genome-wide scans involving a large number of polymorphic markers (e.g., single nucleotide polymorphisms) located throughout the genome. This approach is often employed when potential genetic effects are not well understood.

Informed Consent

Subjects who do not wish to participate in the PGx research may still participate in the clinical study. PGx informed consent must be obtained prior to any blood being taken for PGx research.

Provision of Study Results and Confidentiality of Subject's PGx Data

GSK may summarize the cumulative PGx research results in the clinical study report.

In general, GSK does not inform the investigator, subject, or anyone else (e.g., family members, study investigators, primary care physicians, insurers, or employers) of the PGx research results that are not known to be relevant to the subject's medical care at the time of the study, because the information generated from PGx studies is preliminary in nature, and the significance and scientific validity of the results are undetermined at such an early stage of research, under any circumstances unless required by law.

References

Hetherington S, Hughes AR, Mosteller M, Shortino D, Baker KL, Spreen W, Lai E, Davies K, Handley A, Dow DJ, Fling ME, Stocum M, Bowman C, Thurmond LM, Roses AD. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. Lancet. 2002; 359:1121-2.

Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, Sayer D, Castley A, Mamotte C, Maxwell D, James I. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet. 2002; 359:727-32.

Neergard. Reducing the risk of blood thinners. Associated press, September 2006.

U.S. Food and Drug Administration, FDA Clears Genetic Test That Advances Personalized Medicine Test Helps Determine Safety of Drug Therapy 22 August 2005, http://www.fda.gov/bbs/topics/NEWS/2005/NEW01220.html.

Wilke RA, Musana AK, Weber WW. Cytochrome P450 gene-based drug prescribing, and factors impacting translation into routine clinical practice. Personalized Med 2005; 2: 213-224.

11.4. Appendix 4: Country Specific Requirements

11.5. Appendix 5: Protocol Amendment 01

Statement of Intent

The intents of this amendment are to clarify procedural issues to insure a better global understanding of intent of the protocol and to open the long term extension study to those patients who have reached an endpoint in this study prior to week 24 but in whom continued treatment with ambrisentan is desirable.

Amended Sections of the Protocol

Deletions are noted in *Italics*

Insertions are noted in **bold face**.

Section 2.1.2.2 Efficacy

Item 6

Was:

- Change from baseline in plasma brain natriuretic peptide (NT-Pro BNP) concentration at Week 24.

Is:

- Change from baseline in plasma **N-terminal pro-B-type** natriuretic peptide (NT-Pro BNP) concentration at Week 24.

Section 3.1 – Study Design

Was:

Subjects who *complete 24 weeks of treatment* will be eligible to enrol into a long term follow-up study.

Is:

Subjects who **participate in this study and in whom continued treatment with ambrisentan is desired** will be eligible to enrol into a long term follow-up study.

Section 3.2 – Discussion of Design

Last paragraph

Was:

However, to minimise bias, a person who is not involved in subject assessments will be designated at each investigation site to dispense the investigational product, to perform the subsequent compliance checks (i.e., pill counts), and to complete the InForm data entry. Subjects and their parents or legal guardian will be asked not to comment on the treatment (e.g., no. of tablets taken) with the individual making the assessments. Therefore, the individual making the subjects assessments would be unbiased.

Is:

However, to minimise bias, a person who is not involved in subject assessments will be designated at each investigation site to dispense the investigational product and to perform the subsequent compliance checks (i.e., pill counts). Subjects and their parents or legal guardian will be asked not to comment on the treatment (e.g., no. of tablets taken) with the individual making the assessments. Therefore, the individual making the subjects assessments would be unbiased.

Section 4.2 – Inclusion Criteria

Item 4

Was:

Subjects must either be treatment naïve, have discontinued treatment with another ERA (e.g., bosentan) at least 1 month previously because of elevated liver function tests (LFTs), or have been on a stable dose of *background treatment* for PAH (e.g., sildenafil or prostacyclin) for at least one month prior to the Screening Visit. *Background treatment* for PAH, if any, should not change from the Screening Visit until the end of all Treatment Period assessments.

Is:

Subjects must either be treatment naïve, have discontinued treatment with another ERA (e.g., bosentan) at least 1 month previously because of elevated liver function tests (LFTs), or have been on a stable dose of **drug therapy** for PAH (e.g., sildenafil or prostacyclin) for at least one month prior to the Screening Visit. **The baseline drug therapy** for PAH, if any, should not change from the Screening Visit until the end of all Treatment Period assessments.

Rationale:

The intent of the protocol was to ensure that subjects who were still symptomatic despite other PAH treatment (excluding another ERA) could enter the study, as long as the previous treatment was maintained throughout the study. Some people interpreted "background treatment" to include only supportive therapy such as supplemental oxygen; therefore, the protocol is amended to make it clear that it is the baseline drug therapy targeted to PAH that is allowed and is not to be changed during the course of the study.

Item 6 b

Was:

Child-bearing potential - has a negative pregnancy test and is not lactating at the Screening and Baseline/Randomisation Visits and agrees to use 2 reliable methods of contraception from the Screening Visit until study completion and for at least 30 days following the last dose of study drug (reliable methods of contraception are listed in Appendix 2.

Is:

Child-bearing potential - has a negative pregnancy test and is not lactating at the Screening and Baseline/Randomisation Visits and**, if sexually active,** agrees to use 2 reliable methods of contraception from the Screening Visit until study completion and for at least 30 days following the last dose of study drug (reliable methods of contraception are listed in Appendix 2**)**.

Rationale:

Abstinence is one of the acceptable methods of contraception listed in Appendix 2, and it makes no sense to require another form of contraception in subjects who are not sexually active.

Section 4.4 – Withdrawal Criteria

Was:

Subjects who do not tolerate treatment or whose *background targeted PAH therapy* (i.e., PDE-5 or prostanoid) is changed will be withdrawn from the study. Treatment for subjects withdrawn from the study will be implemented at the Investigator's discretion and PAH guidelines.

Subjects whose clinical condition deteriorates to the point that additional treatment is indicated prior to Week 24 will be considered as having completed the study at that point.

Is:

Subjects who do not tolerate treatment or whose **baseline drug therapy for PAH** (i.e., PDE-5 or prostanoid) is changed will be withdrawn from the study. Treatment for subjects withdrawn from the study will be implemented at the Investigator's discretion and PAH guidelines.

Subjects whose clinical condition deteriorates to the point that additional **drug** treatment is indicated **(including increasing the baseline doses of targeted PAH therapy)** prior to Week 24 will be considered as having completed the study at that point.

Rationale:

To clarify that increasing the dose from baseline of targeted PAH therapy meets the criteria of "additional drug treatment" and to be consistent with the amended wording in Section 4.2 item 4.

Section 5.3 Blinding

Was:

This is an open label study. It is not feasible to blind the study in the classical sense; however, to minimise bias, a person who is not involved in subject assessments will be designated at each investigation site to dispense the investigational product, to perform the subsequent compliance checks (i.e., pill counts)*, and to complete the InForm data entry*. Subjects and their parents or legal guardian will be asked not to comment on the treatment (e.g., no. of tablets taken) with the individual making the assessments. Therefore, the individual making the subjects assessments would be unbiased.

Is:

This is an open label study. It is not feasible to blind the study in the classical sense; however, to minimise bias, a person who is not involved in subject assessments will be designated at each investigation site to dispense the investigational product **and** to perform the subsequent compliance checks (i.e., pill counts). Subjects and their parents or legal guardian will be asked not to comment on the treatment (e.g., no. of tablets taken) with the individual making the assessments. Therefore, the individual making the subjects assessments would be unbiased.

After each subject has completed the study, the randomised treatment will be unblinded to the investigators to allow for informed dose adjustments and follow-up treatment as may be necessary

Rationale

Since the compliance data that are entered into InForm are only categorical estimates of the percent compliance, there is no reason for the unblinded person to physically enter those data into the system. It is important that the investigator know which dose the patient was receiving to manage the patient's care properly once the patient has completed the study.

Section 5.7 – Treatment after the End of the Study

Was

Patients who *complete 24 weeks of treatment* will be eligible to enrol into a long term follow-up study.

Is

Patients who **participate in the study and in whom continued treatment with ambrisentan is desired** will be eligible to enrol into a long term follow-up study.

Rationale

Subjects could complete the study prior to week 24 (e.g., if additional PAH treatment or if a dose adjustment to their baseline PAH drug therapy is necessary). In some of these patients, it might be desirable to continue treatment with ambrisentan and add or change the dose of other targeted PAH treatment.

Section 6.2.2 – Electrocardiogram / Echocardiogram

Was:

A 12-lead ECG will be performed at Baseline and at Weeks 12 and 24 (or early withdrawal), and Follow-up. Any changes since the Screening Visit felt to be significant in the medical and scientific judgement of the investigator are to be recorded as AEs or SAEs in the eCRF.

Is:

A 12-lead ECG will be performed at Baseline and at Weeks 12 and 24 (or early withdrawal), and Follow-up. Any changes since **Baseline** felt to be significant in the medical and scientific judgement of the investigator are to be recorded as AEs or SAEs in the eCRF.

Section 6.2.4 – Pubertal Development Assessment

Was:

Pubertal development in male and female subjects will be assessed at baseline, Week 12, and Week 24 or early termination *by a paediatric endocrinologist* using Tanner criteria [Cameron, 2004]. In male subjects, testicular volume will be assessed by Prader's orchidometer.

Is:

Pubertal development in male and female subjects will be assessed at baseline, Week 12, and Week 24 or early termination using Tanner criteria [**Marshall, 1969; Marshall, 1970;** Cameron, 2004]. In male subjects, testicular volume will be assessed by Prader's orchidometer. **These assessments will be performed by a paediatric endocrinologist or another individual with comparable experience employing the Tanner criteria and Prader's orchidometer. Preferably, the same assessor will evaluate an individual subject at each assessment time.**

Rationale:

"Paediatric endocrinologist" is not a recognized speciality in all territories. The intent is that the person performing these assessments be someone who is competent and experienced with using the Tanner criteria and Prader's orchidometer. In some centres, the individual who has that experience might not have the title "paediatric endocrinologist".

Section 6.3 – Pharmacokinetics

Was

Blood samples for pharmacokinetic analysis of ambrisentan will be collected at the time points indicated in Table 2. The actual date and time of each blood sample collection will be recorded in the GSK-defined eCRF. The exact time of dosing on the day of the visit, 1 day prior to the visit and 2 days prior to the visit will be recorded in the eCRF by having the subject return a *medication* card on which this information is recorded.

Is

Blood samples for pharmacokinetic analysis of ambrisentan will be collected at the time points indicated in Table 2. The actual date and time of each blood sample collection will be recorded in the GSK-defined eCRF. The exact time of dosing on the day of the visit, 1 day prior to the visit and 2 days prior to the visit will be recorded in the eCRF by having the subject return a **patient diary** card on which this information is recorded.

Section 6.4.5

Heading

Was:

Change from baseline plasma *brain* natriuretic peptide (NT-Pro BNP) at Week 24

Is:

Change from baseline plasma **N-terminal pro-B type** natriuretic peptide (NT-Pro BNP) at Week 24

Section 6.5 – Health Outcomes

Was:

In addition to the SF10, specific question will be asked regarding school days as follows:

1. Within the past month, how many days of school were scheduled?
2. How many days of school were missed for any reason?
3. How many days *of school* were missed because of pulmonary arterial hypertension (PAH)?

Is:

In addition to the SF10, specific question will be asked regarding school days as follows:

1. Within the past month, how many days of school were scheduled? **(at Baseline only)**

   or

   Since your baseline visit, how many days of school were scheduled? (at Week 4 only)

   or

   Since your last clinic visit, how many days of school were scheduled? (all other assessment times)
2. How many **scheduled** days of school were missed for any reason?
3. **Of these,** how many days were missed because of **symptoms of** pulmonary arterial hypertension (PAH)?

Subjects will record the days of school scheduled and missed on a patient diary card, which will be returned to the clinic at the scheduled visits.

Rationale:

To clarify that the number of days missed in question 2 is inclusive of the days missed in question 3 and to differentiate between days missed because of clinic visits and days missed because of symptoms of the disease.

Section 8.3.3.2 Secondary Comparisons of Interest

Efficacy bullet item 6

Was:

- Change from baseline in plasma brain natriuretic peptide (BNP or N-Terminal pro-B-type Natriuretic Peptide) concentration at Week 24.

Is:

- Change from baseline in plasma **NT-Pro BNP** concentration at Week 24.

Section 8.3.5.4 Efficacy Analyses

Item 8

Was:

- change from baseline in plasma BNP concentration at Week 24

Is:

- change from baseline in plasma NT-Pro BNP concentration at Week 24

Table 1 – Time and Events Table

Change "*Patient Global Assessment (SF10)*" to "**Health Outcomes Assessments**"

Add a foot note for "Health Outcomes Assessments" as follows: "**SF 10 and a record of school days scheduled and missed. Two diary cards will be given to the subject at Baseline, and one diary card will be given to the subject at Weeks 4, 8, 12, 16, 20 and 24 on which to record the number of school days scheduled, the number missed for any reason, and the number missed because of symptoms of PAH. One baseline diary card will be completed and returned during the visit. The other baseline diary card should be returned at the Week 4 visit. All other diary cards will be returned at the next visit. Data from all diary cards will be transcribed into the eCRF.**"

To footnote for "Plasma Sample for Population Pharmacokinetic Assessment" add "**A diary card will be given to the subject at Baseline to record date and time of study medication dosing 2 days prior to and 1 day prior to the Week 4 visit and at Weeks 4, 8, 12, and 20 to record date and time of study medication dosing 2 days prior to and 1 day prior to the next visit. At Week 16, a diary card will be given to the**

subject to record the date and time of study medication dosing 2 days prior to, 1 day prior to and on the day of the Week 20 visit. The diary card given out at Baseline should be returned at the Week 4 visit. All other diary cards will be returned at the next visit. The dates and times of study medication dosing will be transcribed into the eCRF. Subjects will have the option to receive a reminder SMS message on the days they should complete the diary card and to remind them of their next visit date."

Footnote for "Pubertal Development Assessment"

Was:

Pubertal development in male and female subjects will be assessed *by a paediatric endocrinologist* using Tanner criteria. In male subjects, testicular volume will be assessed using Prader's orchidometer. In addition, blood samples will be obtained to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone binding globulin (SBGH) and inhibin B levels.

Is:

Pubertal development in male and female subjects will be using Tanner criteria. In male subjects, testicular volume will be assessed using Prader's orchidometer. In addition, blood samples will be obtained to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone binding globulin (SBGH) and inhibin B levels.

Rationale

To be consistent with the text of the protocol and to clarify the use of patient diary cards.

11.6. Appendix 6: Protocol Amendment 02

Statement of Intent

The intents of this amendment are to clarify procedural issues to insure a better global understanding of intent of the protocol and to correct that it is hepatitis B surface antigen that is being assessed as part of the exclusion criteria.

Amended Sections of the Protocol

Deletions are noted in *Italics*

Insertions are noted in **bold face**.

Sponsor Information Page

Was

Sponsor Medical Monitor Contact Information:

PPD FRCS
Clinical Development Physician
MDC Clinical Metabolic EU
GlaxoSmithKline
Stockley Park

Sponsor Serious Adverse Events (SAE) Contact Information:

Regulatory Agency Identifying Number(s): EudraCT 2010-019547-19

Is

Sponsor Medical Monitor **and Serious Adverse Events (SAE)** Contact Information:

PPD FRCS
Clinical Development Physician
MDC Clinical Metabolic EU
GlaxoSmithKline
Stockley Park

Regulatory Agency Identifying Number(s): EudraCT 2010-019547-19

US IND 64,915

Rationale

To confirm that for the sites in the US, the study is being conducted under a US IND and that the Medical Monitor and SAE contact are the same person.

Table 1 – Time and Events Table

Footnote 12

Was

12. Hepatitis B surface *antibody* and hepatitis C antibody (if hepatitis C antibody positive, a hepatitis C RIBA immunoblot assay should be reflexively performed on the same sample to confirm the result).

Is

12. Hepatitis B surface **antigen** and hepatitis C antibody (if hepatitis C antibody positive, a hepatitis C RIBA immunoblot assay should be reflexively performed on the same sample to confirm the result).

Rationale

Antibody was a mistake. The intent was to assess the hepatitis B surface antigen, and the line item in the table to which the footnote applied correctly listed "HBsAg"

Section 4.3 Exclusion Criteria

Item 6

Was

6. Subjects with diagnosis of active hepatitis (hepatitis B surface *antibody* and hepatitis C antibody), or clinically significant hepatic enzyme elevation (i.e., ALT, AST or AP >3xULN) at Screening.

Is

6. Subjects with diagnosis of active hepatitis (hepatitis B surface **antigen** and hepatitis C antibody), or clinically significant hepatic enzyme elevation (i.e., ALT, AST or AP >3xULN) at Screening.

Rationale

Antibody was a mistake. The intent was to assess the hepatitis B surface antigen, and the line item in the time and events table correctly listed "HBsAg".

11.7. Appendix 7: Protocol Amendment 03

Statement of Intent

The intents of this amendment are to add oestrogen and remove testosterone from laboratory assessments being conducted on female subjects and to align the storage conditions requirements in the protocol with those that are printed on the study medication package.

Amended Sections of the Protocol

Deletions are noted in *Italics*

Insertions are noted in **bold face**.

In Section 5.1 Investigational Product and Reference Therapy

Was:

Study medication will be stored in secure (locked) areas at a temperature *between 15° and* 30°C and dispensed according to the protocol under the supervision of the Investigator or his/her designee.

Is

Study medication will be stored in secure (locked) areas at a temperature **below** 30°C and dispensed according to the protocol under the supervision of the Investigator or his/her designee.

Table 1 – Time and Events Table

Footnote 11

Was

11. Pubertal development in male and female subjects will be assessed using Tanner criteria. In male subjects, testicular volume will be assessed using Prader's orchidometer. *In addition,* blood samples will be obtained to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone binding globulin (*SBGH*) and inhibin B levels.

Is

11. Pubertal development in male and female subjects will be assessed using Tanner criteria. In male subjects, testicular volume will be assessed using Prader's orchidometer, **and** blood samples will be obtained to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone binding globulin (**SHBG**) and

inhibin B levels. **In female subjects, blood samples will be obtained to measure FSH, LH, oestrogen, SHBG and inhibin B levels.**

In Section 6.2.4 Pubertal Development Assessment

Was:

In addition, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sex hormone binding globulin (SHBG), and inhibin B levels will be assessed at baseline, Week 12, and Week 24 or early withdrawal.

Is:

In addition, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone **(in male subjects only), oestrogen (in female subjects only)**, sex hormone binding globulin (SHBG), and inhibin B levels will be assessed at baseline, Week 12, and Week 24 or early withdrawal.