TITLE PAGE

Protocol Title: An open-label, randomized three period cross-over relative bioavailability study to compare the pharmacokinetic parameters of a lower dose formulation of ambrisentan (GSK1325760) with marketed ambrisentan in healthy adult participants

Protocol Number: 205019

Compound Number: GSK1325760

Study Phase: Phase 1

Short Title: Relative bioavailability study of marketed and lower dose ambrisentan in healthy adult participants

Sponsor Name and Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
Brentford
Middlesex, TW8 9GS
UK

Medical Monitor Name and Contact Information can be found in the Study Reference Manual

Regulatory Agency Identifying Number(s):

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SPONSOR SIGNATORY:

PPD

June 19, 2019

Mary Ann Lukas, MD FACC
Senior Director, Physician Project Lead

Date

PPD

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title: An open-label, randomized three period cross-over relative bioavailability study to compare the pharmacokinetic parameters of a lower dose formulation of ambrisentan (GSK1325760) with marketed ambrisentan in healthy adult participants

Short Title: Relative bioavailability study of marketed and lower dose ambrisentan in healthy adult participants

Rationale:

Adult treatments are widely used off label for treatment of pulmonary arterial hypertension (PAH) in children; however, conventional drug delivery methods are not always suitable for younger patients who have difficulties with swallowing conventional tablet formulations. Therefore, the aim is to develop an ambrisentan (AMB) tablet for use in children <8 years, which may be dispersed in water or swallowed (dependent on the age or preference of the child), in order to improve patient compliance and acceptability, whilst maintaining safety and efficacy.

The primary objective of this study is to provide clinically relevant information on the pharmacokinetic (PK) and safety profile of a new lower dose formulation AMB tablet, which is intended for paediatric use. The study will compare the relative bioavailability of the lower dose tablet, dispersed in water and administered orally, with the reference marketed AMB tablet in healthy adults. This study is part of a Paediatric Investigational Plan (PIP) agreed with the European Medicines Agency's Paediatric Committee (PDCO).

Objectives and Endpoints:

Objective	Endpoint
Primary	
• To compare the relative bioavailability of AMB (1 mg x 5 tablets) as tablets dispersed in water or administered orally, with marketed AMB (5 mg x 1 tablet) administered orally, in healthy adult participants under fasted conditions.	• Plasma pharmacokinetic parameters of AMB, as data permit: C_{max}, t_{max}, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and $t_{½}$.

Objective	Endpoint
Secondary	
• To monitor the safety and tolerability of AMB (1 mg x 5 tablets) as tablets dispersed in water or administered orally, compared with marketed AMB (5 mg x 1 tablet) administered orally, in healthy adult participants.	• Adverse events (AE), vital signs, electrocardiogram (ECG), and clinical laboratory values

Overall Design:

This is a single centre, open-label, randomised, single dose, 3-period cross over study in healthy participants to compare the PK of a new lower dose formulation AMB tablet (dispersed in water and administered orally) with the reference marketed AMB tablet (administered orally).

Disclosure Statement: This is a cross-over relative bioavailability study with 3 arms with no masking.

Number of Participants:

Approximately 24 participants will be randomly assigned to study intervention, such that 20 evaluable participants complete the study.

Intervention Groups and Duration:

Each participant will have 3 treatment periods (single oral doses), and will be randomised to one of the following study interventions in each period:

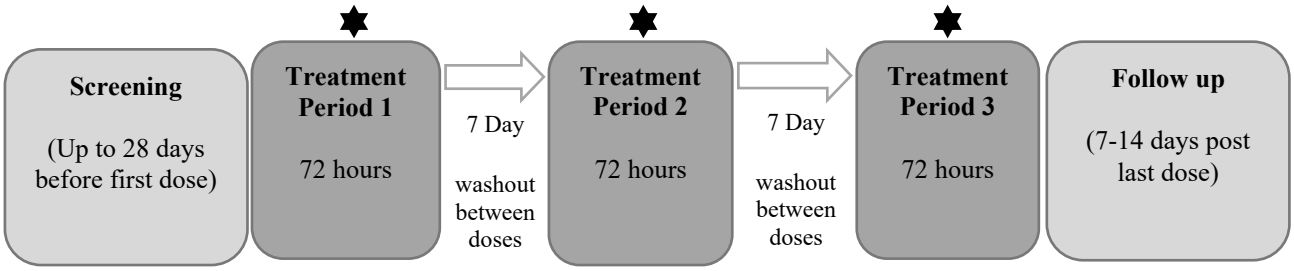
- **Test 1 (F1):** 5 mg (5 x 1 mg tablets) AMB tablet dispersed in water
- **Test 2 (F2):** 5 mg (5 x 1 mg tablets) AMB tablet administered orally
- **Reference (R):** 5 mg (1 x 5 mg tablet) AMB tablet administered orally

Each participant will:

- be screened (within 28 days of their first dose);
- have 3 treatment periods (3 overnight clinic stays, and 1 out-patient visit per treatment period) with a minimum of 7 days between doses in each treatment period; and
- have a follow-up visit (within 7 to 14 days after their last dose). The total study duration for each participant is expected to be approximately 9 weeks.

Data Monitoring Committee: No

1.2. Schema



Fasted from midnight prior to dose to 4 hours post dose

1.3. Schedule of Activities (SoA)

1.3.1. Screening and Follow-up Schedule of Activities

Procedure	**Screening[1] (up to 28 days before first dose)**	**Follow up/Early Withdrawal (7-14 days post last dose)**	**Notes**
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Medical history (includes substance usage)	X		Substances: Drugs, Alcohol, tobacco
Full physical exam, including height and weight	X		
Brief physical exam		X	
HIV, Hep B and Hep C screen	X		
Clinical chemistry, haematology and urinalysis	X	X	Only screening tests need to be fasted
FSH	X		Required only in women to confirm postmenopausal status
Alcohol breath test	X		
Urine drugs test	X		
Vital signs (blood pressure, heart rate and temperature)	X	X	Triplicate blood pressure and heart rate required at screening.
12-lead ECG	X	X	Triplicate ECG required at screening.
Concomitant Medication review	X	X	Con meds before dosing can be recorded in medical history
AE/SAE review	X	X	Refer to Section 8.3.1

1. Screening assessments may be conducted at multiple visits, if required.

1.3.2. Treatment Periods 1, 2 and 3 (minimum of 7 days washout between doses)

Procedure	Day –1	Day 1 (hours)											Day 2		Day 3	Day 4	Notes
		Pre-dose	0	0.5	1	1.5	2	2.5	4	8	12	18	24	36	48	72	
Clinic Visits																	
Out-patient Visit																X	
Admission to clinic	X																
Discharge from clinic															X		If more convenient for participants, they can remain in the unit until 72 h, at the discretion of the investigator
Study Intervention																	
Randomisation		X															Can be done on Day –1 or Pre-dose Day 1. Only in Treatment Period 1
Study Intervention			X														Fasted from midnight until 4 h post-dose, except for water, allowed ad libitum, except for 1 h before and after dosing
Safety Assessments																	
Vital signs (blood pressure, heart rate and temperature)		X		X	X		X		X	X	X		X		X	X	Triplicate pre-dose. Single measurements at other timepoints, unless out of range, then triplicates should be performed
12-lead ECG		X			X		X		X		X		X			X	
Clinical chemistry, haematology and urinalysis	X														X		Fasting not required
Brief physical examination	X																
Alcohol breath test	X																
Urine drugs test	X																

Procedure	**Day –1**	**Day 1 (hours)**											**Day 2**		**Day 3**	**Day 4**	**Notes**
		Pre-dose	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**4**	**8**	**12**	**18**	**24**	**36**	**48**	**72**	
Concomitant Medication	◄==========►																Con meds before dosing can be recorded in medical history
SAE/AE review	◄==========►																Refer to Section 8.3.1
Other Assessments																	
PK blood samples		X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Palatability Questionnaire			X														Questionnaire only to be completed in the Treatment Period dosing AMB tablets dispersed in water. To be completed within 10 min of dosing

- When scheduled at the same time-points, 12-lead ECGs and vital signs should be completed before any blood draws.
- The timing of assessments should allow PK samples to be taken as close as possible to the nominal time-point.
- The timing and number of planned safety assessments and pharmacokinetic samples may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments as the result of emerging pharmacokinetic data from this study must be documented and approved by the relevant study team member and then archived in the sponsor and site study files but will not constitute a protocol amendment. The Competent Authority (CA) and ethics committee (EC) will be informed of any safety issues that constitute a substantial amendment and require alteration of the safety monitoring scheme or amendment of the informed consent form (ICF). The changes will be approved by the CA and the EC before implementation.

2. INTRODUCTION

Ambrisentan (AMB) (Volibris tablets) is an oral, once daily, propanoic acid-based, Endothelin Receptor Antagonist (ERA), which is selective for Endothelin Receptor Type A. AMB targets the phospholipase-C-dependent endothelin pathway, which is known to play an essential role in mammalian cardiovascular physiology. It is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of World Health Organization (WHO) Functional Class (FC) II to III, to improve exercise capacity, decrease the symptoms of PAH, and delay clinical worsening, including use in combination treatment. Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease [Volibris, 2018]. AMB is marketed in the European Union (EU), Japan and over thirty further countries by GlaxoSmithKline (GSK), and in the United States (US) by Gilead Sciences Inc, as Letairis.

2.1. Study Rationale

Adult treatments are widely used off label for treatment of PAH in children; however, conventional drug delivery methods are not always suitable for younger patients who have difficulties with swallowing conventional tablet formulations. Therefore, the aim is to develop an AMB tablet for use in children <8 years, which may be dispersed in water or swallowed (dependent on the age or preference of the child), in order to improve patient compliance and acceptability, whilst maintaining safety and efficacy.

The primary objective of this study is to provide clinically relevant information on the PK and safety profile of a new lower dose formulation AMB tablet, which is intended for paediatric use. The study will compare the relative bioavailability of the lower dose tablet, dispersed in water and administered orally, with the reference marketed AMB tablet in healthy adults. This study is part of a Paediatric Investigation Plan (PIP) agreed with the European Medicines Agency's Paediatric Committee (PDCO).

An exploratory objective of this study is to assess the palatability of the new lower dose formulation AMB tablet dispersed in water.

2.2. Background

PAH is a rare, progressive, highly debilitating and life-threatening disease characterized by pulmonary vascular functional and structural changes resulting in increased pulmonary vascular resistance (PVR) and eventual right-sided heart failure (RHF) and premature death [Moledina, 2010; Newman, 2004; Vorhies, 2014].

Epidemiological data estimates PAH incidence in Europe ranges from 1.1 to 7.6 per million; prevalence ranges from 6.6 to 26 per million [Hoeper, 2016]. 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 IPAH management and survival in the UK, the incidence was 0.48 cases per million children per year, and the prevalence was 2.1 cases per million children [Moledina, 2010].

Despite the emergence of new treatments, PAH still has a poor long-term prognosis (akin to many cancers). A recent meta-analysis estimates survival for untreated 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].

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 remodeling and pulmonary vasoconstriction. Although its role is not fully understood, endothelin is considered 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 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.

Treatments currently approved for the treatment of PAH target 3 biological pathways: endothelin, nitric oxide, and prostacyclin pathways. 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, or multiple inhalations) present substantial challenges to their successful use in a pediatric population.

The efficacy profile of AMB in adults is broadly comparable with other targeted oral treatments, but it has potentially important advantages. AMB is associated with a favourable liver safety profile and a low risk of drug-drug interactions. Data from the TRAX[1] database indicates that the risk of elevated aminotransferases with bosentan (another marketed ERA) is lower in children than adults [Beghetti, 2008]. Nevertheless, the liver safety profile of AMB in adults has been favorable to bosentan, 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 AMB, may provide a therapeutic option that simplifies treatment in this complex disease.

A detailed description of the pharmacology, efficacy, and safety of AMB is provided in the Volibris European Medicines Agency (EMA) Summary of Product Characteristics [SmPC, 2018].

[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 hospitalization [Segal, 2005].

2.3. Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of AMB (GSK1325760) may be found in the Volibris EMA Summary of Product Characteristics [SmPC, 2018].

2.3.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) AMB (GSK1325760)		
Identified and potential risks, from European Union Risk Management Plan (EURMP) for Volibris: • Teratogenicity • Decreased haemoglobin/haematocrit, anaemia, including anaemia requiring transfusion • Hepatotoxicity • Testicular tubular atrophy/Male infertility Consistent with the mechanism of action for AMB, there is a potential risk of hypotension in healthy participants.	• Refer to EURMP for Volibris v8.1 (EURMP, 2019) • Refer to Volibris EMA Summary of Product Characteristics (SmPC, 2018)	Exclusion criteria, ongoing safety assessments, study design and medical supervision. Specifically: • The use of single doses in this study. • Exclusion of women of child bearing potential. • Exclusion of potentially anaemic participants. • Haemoglobin reduction withdrawal criterion for individual participants. • Inclusion criteria to prevent enrolment of hypotensive participants. • Hypotension withdrawal criterion for individual participants. • Laboratory assessments per protocol. • Physical assessment per protocol. • Routine vital signs per protocol. • Participants remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48 hrs post dose.
Study Procedures		
Risk associated with blood draws	Fainting, mild pain, bruising, irritation or redness at phlebotomy site, may be associated with blood draws.	Experienced site staff will follow standard approaches for managing events related to blood draws.

2.3.2. Benefit Assessment

There is no clinical benefit for healthy participants taking part in this study. However, participants will undergo a medical evaluation during screening (including a physical examination, electrocardiogram (ECG), vital signs and laboratory assessments), which may provide important health information.

2.3.3. Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to participants participating in this study, the potential risks identified in association with AMB are justified by the anticipated benefits that may be afforded to paediatric patients with PAH.

3. OBJECTIVES AND ENDPOINTS

Objective	Endpoint
Primary	
• To compare the relative bioavailability of AMB (1 mg x 5 tablets) administered as tablets dispersed in water or administered orally, with marketed AMB (5 mg x 1 tablet) administered orally, in healthy adult participants under fasted conditions.	• Plasma pharmacokinetic parameters of AMB, as data permit: C_{max}, t_{max}, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and $t_{½}$.
Secondary	
• To monitor the safety and tolerability of AMB (1 mg x 5 tablets) administered as tablets dispersed in water or administered orally, compared with marketed AMB (5 mg x 1 tablet) administered orally, in healthy adult participants.	• Adverse events (AE), vital signs, electrocardiogram (ECG), and clinical laboratory values.
Exploratory	
• To investigate palatability of AMB (1 mg x 5 tablets) dispersed in water, in healthy adult participants.	• Palatability questionnaire scores

4. STUDY DESIGN

4.1. Overall Design

This is a single centre, open-label, randomised, single dose, 3-period cross over study in healthy participants to compare the PK of a new lower dose formulation AMB tablet

(dispersed in water and administered orally) with the reference marketed AMB tablet (administered orally). Each participant will have 3 treatment periods (single oral doses), and be randomised to one of the following study interventions in each period:

- **Test 1 (F1):** 5 mg (5 x 1 mg tablets) AMB tablet dispersed in water
- **Test 2 (F2):** 5 mg (5 x 1 mg tablets) AMB tablet administered orally
- **Reference (R):** 5 mg (1 x 5 mg tablet) AMB tablet administered orally

Each participant will:

- be screened (within 28 days of their first dose);
- have 3 treatment periods (3 overnight clinic stays, and 1 out-patient visit per treatment period), with a minimum of 7 days between doses in each treatment period; and
- have a follow-up visit (within 7 to 14 days after their last dose).

The total study duration for each participant is expected to be approximately 9 weeks.

4.2. Scientific Rationale for Study Design

This open-label, randomized, cross-over design is well-established for the evaluation of relative bioavailability of different oral dosage forms. Randomization of the treatment sequences is an attempt to prevent bias. No blinding or placebo control will be used, as these are not required for the primary study objective (PK assessment). The washout of 7 days between doses should eliminate the possibility of carryover of drug exposure from the previous dosing period, because AMB $t_{½}$ is 17 h.

4.3. Justification for Dose

The approved doses of AMB for the treatment of adult PAH patients are 5 mg and 10 mg, once daily [SmPC, 2018]. AMB is not approved for the treatment of paediatric PAH patients. An alternate tablet that may be dispersed in water or swallowed is therefore in development for paediatric patients <8 years old, who may have difficulty swallowing tablet formulations. The 5 mg AMB tablet has been studied in paediatric patients (8 to 18 years old) in GSK Study AMB112529 [Volibris, 2018].

The current study will compare the relative bioavailability of 5 x 1 mg tablets dispersed in water (administered as 'disperse in water and immediately take') and 5 x 1 mg tablets (administered orally), with that of 1 x 5 mg marketed tablet (reference) (administered orally).

Using the 5 mg AMB tablet as the reference dose allows assessment of the relative bioavailability comparison with the lower dose tablet but requires participants to take only 5 x 1 mg tablets – rather than 10 tablets (which would have been required if the 10 mg AMB tablet had been selected as the reference). This decreases the participant burden for the study. The reference 5 mg AMB tablet is quantitatively proportional to the 10 mg AMB tablet, and so, 2 x 5 mg tablets are equivalent to 1 x 10 mg tablet (Studies EE-001, EE-002 and AMB-103) [Volibris, 2018].

4.4. End of Study Definition

A participant is considered to have completed the study if he/she has completed all phases of the study including the follow-up visit.

The end of the study is defined as the date of the last visit of the last participant in the study.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age
1. Participant must be 18 to 65 years of age inclusive, at the time of signing the informed consent.

Type of Participant and Disease Characteristics
2. Participants who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests, vital signs and cardiac monitoring (refer to Section 5.4 for information about rescreening).
3. Average systolic blood pressure between 100-160 mmHg and diastolic between 55-90 mmHg (inclusive) over 3 readings at screening.

Weight
4. Body weight ≥ 50 kg for men and ≥ 45kg for women, and body mass index (BMI) within the range 18-30 kg/m^2 (inclusive).

Sex
5. Male or female **a. Male participants:** Male participants are eligible to participate if they agree to the following during the study and for at least 13 weeks afterwards corresponding to time needed to eliminate study intervention (5 terminal half-lives) plus an additional 90 days (a spermatogenesis cycle):

- Refrain from donating sperm

PLUS either:

- Be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

OR

- Must agree to use contraception/barrier, as follows:
  - Agree to use a male condom; and
  - Female partner to use an additional highly effective contraceptive method with a failure rate of <1% per year as described in Appendix 4.

b. Female participants:

A female participant is eligible to participate if she is not a woman of childbearing potential (WOCBP), as defined in Appendix 4.

Informed Consent
6. Capable of giving signed informed consent as described in Appendix 1 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions
1. History or presence of cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, haematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study intervention or interfering with the interpretation of data
2. History or presence of palpitations or tachyarrhythmias
3. Haemoglobin (Hb) below the normal range (Hb <133 g/L for male participants; and Hb <114 g/L for female participants)
4. Alanine transaminase (ALT) >1.5x upper limit of normal (ULN)
5. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)
6. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
7. QTc >450 msec

NOTES:

- The QTc is the QT interval corrected for heart rate according to Bazett's formula (QTcB), Fridericia's formula (QTcF), and/or another method, machine-read or manually over-read.
- The specific formula that will be used to determine eligibility and discontinuation for an individual subject should be determined prior to initiation of the study. In other words, several different formulae cannot be used to calculate the QTc for an individual subject and then the lowest QTc value used to include or discontinue the subject from the trial.

Prior / Concomitant Therapy
8. Past or intended use of over-the-counter or prescription medication (including vitamins and dietary or herbal supplements but excluding paracetamol ≤ 2 g/day) within 7 days (or 14 days if the drug is a potential enzyme inhibitor) or 5 half-lives (whichever is longer) prior to the first dose of study medication, unless approved by the Investigator in conjunction with GSK Medical Monitor.

Prior / Concurrent Clinical Study Experience
9. Participation in the study would result in loss of blood or blood products in excess of 500 mL within a 56-day period
10. Exposure to more than 4 new chemical entities within 12 months prior to the first dosing day
11. Current enrolment or past participation within 30 days before screening in any other clinical study involving an investigational study intervention or any other type of medical research

Diagnostics Assessments
12. Presence of Hepatitis B surface antigen (HBsAg) at screening or within 3 months prior to first dose of study intervention
13. Positive Hepatitis C antibody test result at screening or within 3 months prior to first dose of study intervention.
NOTE: Subjects with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA test is obtained
14. Positive Hepatitis C RNA test result at screening or within 3 months prior to first dose of study intervention.
NOTE: Test is optional and subjects with negative Hepatitis C antibody test are not required to also undergo Hepatitis C RNA testing
15. Positive human immunodeficiency virus (HIV) antibody test

16. Positive pre-study drug/alcohol screen
17. Regular use of known drugs of abuse

OTHER EXCLUSIONS

18. Regular alcohol consumption within 6 months prior to the study defined as:
    - An average weekly intake of >14 units. One unit is equivalent to 8 g of alcohol: a half-pint (~240 mL) of beer, 1 glass (125 mL) of wine or 1 (25 mL) measure of spirits.
19. Smoking > 5 cigarettes per week (or equivalent) and participants must be able to abstain from smoking for a 24-hour period prior to dose and any time whilst in the clinical unit.
20. Sensitivity to any of the study interventions, or components thereof, or drug or other allergy that, in the opinion of the investigator or medical monitor, contraindicates participation in the study

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice, pomelos, exotic citrus fruits, grapefruit hybrids, or fruit juices from 7 days before the start of study intervention until after the final dose.
- Participants will be required to fast from midnight before each dose until 4 hours afterwards, with the exception of water, which will be allowed ad libitum, except for 1 hour before and after dosing. At all other times whilst participants are in the unit they will receive standardised meals. The timing of the meals is at the unit's discretion.
- Participants will be required to fast for at least 6 hours before screening laboratory tests.

5.3.2. Caffeine, Alcohol, and Tobacco

- During each treatment period, participants will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) for 24 hours before each dose until after collection of the final PK sample.
- During each treatment period, participants will abstain from alcohol for 24 hours before each dose until after collection of the final PK sample.
- Participants who use tobacco products will be instructed that use of nicotine-containing products (including nicotine patches and other delivery devices such as vaporizers) will not be permitted while they are in the clinical unit. Use of tobacco

products will not be allowed from 24 hours before each dose and whilst participants are in the clinic.

5.3.3. Activity

- Participants will abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (e.g., walking, watching television, reading).

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized into the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Participants who fail eligibility may be rescreened once (restriction for rescreening not applicable for reserves). If rescreening is performed, participants must be assigned a different unique subject identification number for the rescreening, and all screening procedures must be repeated (unless agreed otherwise by the investigator and GSK medical monitor). See the study reference manual (SRM) for more details.

In the event of out-of-range results of safety tests, the tests may be repeated once within the screening window. If a retest result is again outside the reference range and considered clinically significant by the investigator and GSK medical monitor, the subject will be considered a screen failure.

6. STUDY INTERVENTION

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1. Study Intervention(s) Administered

ARM Name	**Test 1 (Dispersed)**	**Test 2 (Administered Orally)**	**Reference (Administered Orally)**
Intervention Name	Ambrisentan tablet dispersed in water (GSK1325760)	Ambrisentan tablet (GSK1325760)	Volibris* 5 mg film-coated tablet
Type	Drug	Drug	Drug

ARM Name	**Test 1 (Dispersed)**	**Test 2 (Administered Orally)**	**Reference (Administered Orally)**
Dose Formulation	Tablet	Tablet	Tablet
Unit Dose Strength(s)	1 mg	1 mg	5 mg
Dosage Level(s)	Single dose, 5 mg (5 x 1 mg)	Single dose, 5 mg (5 x 1 mg)	Single dose, 5 mg (1 x 5 mg)
Route of Administration	oral	oral	oral
Use	Experimental	Experimental	Experimental
IMP and NIMP	IMP	IMP	IMP
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor	Provided centrally by the Sponsor
Packaging and Labelling	Study Intervention will be provided in bottles. Each bottle will be labelled as required per country requirement.	Study Intervention will be provided in bottles. Each bottle will be labelled as required per country requirement.	Study Intervention will be provided in blister packs. Each blister pack will be labelled as required per country requirement.

* Volibris is the trademarked name for ambrisentan.

Detailed instructions for dosing will be provided in the SRM.

6.2. Preparation/Handling/Storage/Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.
2. Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.
3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

4. Further guidance and information for the final disposition of unused study intervention are provided in the Study Reference Manual.

Under normal conditions of handling and administration, study intervention is not expected to pose significant safety risks to site staff. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.

A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

6.3. Measures to Minimize Bias: Blinding and Randomization

6.3.1. Blinding

As this is an open label study, there will be no blinding.

6.3.2. Randomization

Pre-dose on Day 1 (or on Day –1, if needed), participants will be assigned a unique number (randomization number) in ascending numerical order. The randomization number encodes the participant's assignment to one of the treatment sequences, according to the randomization schedule generated prior to the study by the Statistics Department at GSK. In each Treatment Period, each participant will be dispensed study intervention, labelled with his/her unique randomization number.

6.3.3. Treatment Sequences

The treatment key is shown in Table 1.

Table 1 Treatment Key

Treatment	Description
F1	5 mg (5 x 1 mg tablets) AMB tablet dispersed in water
F2	5 mg (5 x 1 mg tablets) AMB tablet administered orally
R	5 mg (1 x 5 mg tablet) AMB tablet administered orally

The treatment sequence assignments, based on the Latin Squares balanced for carry-over effect, are shown in Table 2.

Table 2 Treatment Sequences

Number of Treatments	Sequence Assignments	Allocation ratio
6	F1 / F2 / R F2 / R / F1 R / F1 / F2 F1 / R / F2 F2 / F1 / R R / F2 / F1	1:1:1:1:1:1

6.4. Study Intervention Compliance

The individual doses for a participant will be prepared from a bulk supply; therefore, the preparation of the dose will be confirmed by a second member of the study clinic staff.

Participants will be dosed at the clinic and will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study clinic staff other than the person administering the study intervention. Study site personnel will examine each participant's mouth to ensure that the study intervention was ingested.

6.5. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrolment or receives during the study must be recorded along with:

- reason for use
- dates of administration including start and end dates
- dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Participants must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study intervention until completion of the follow-up visit, unless, in the opinion of the investigator and sponsor, the medication will not interfere with the study.

Paracetamol, at doses of ≤ 2grams/day, is permitted for use any time during the study. Other concomitant medication may be considered on a case-by-case basis by the investigator in consultation with the Medical Monitor if required.

6.6. Dose Modification

Not applicable

6.7. Intervention after the End of the Study

As this is a healthy participant study, no intervention will be given to participants following the end of study. Participants will receive a follow up visit 7–14 days after their last dose.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Intervention

In rare instances, it may be necessary for a participant to permanently discontinue study intervention. If study intervention is permanently discontinued, the participant will not remain in the study. The participant will remain at the clinic until the investigator or delegate discharges the participant. An early withdrawal visit should be conducted (see SOA).

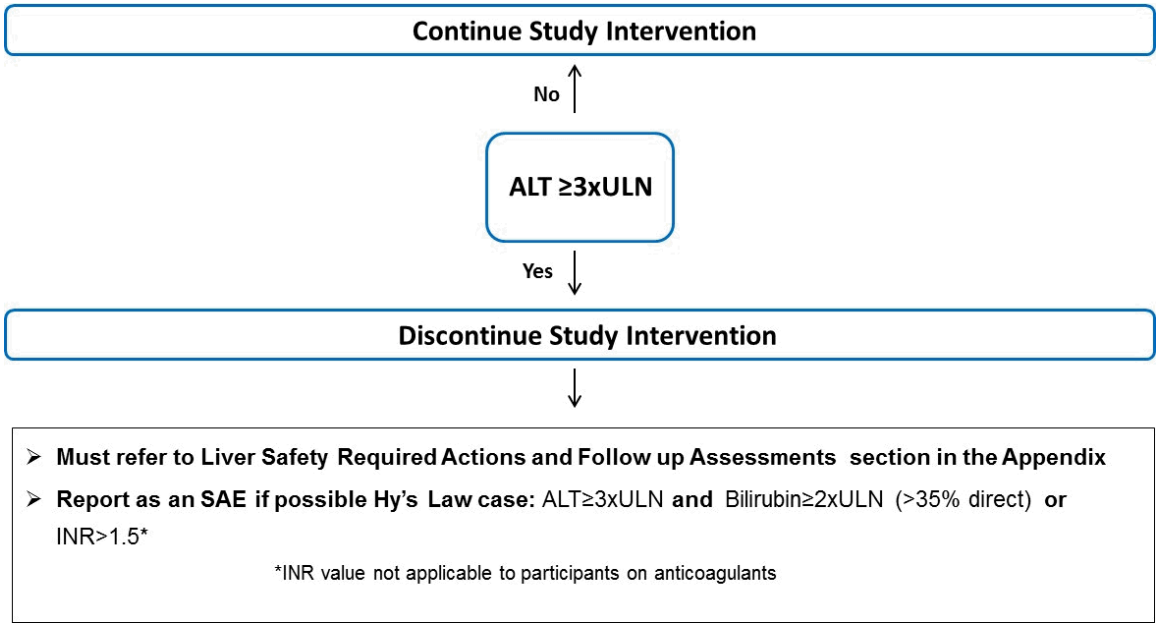
7.1.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology.

Discontinuation of study intervention for abnormal liver tests is required when:

- a participant meets one of the conditions outlined in the algorithm; or
- when in the presence of abnormal liver chemistries not meeting protocol-specified stopping rules, the investigator believes study intervention discontinuation is in the best interest of the participant.

Phase 1 Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Abbreviations: ALT = alanine transaminase; INR = international normalized ratio; SAE = serious adverse event; ULN = upper limit of normal.

Refer to Appendix 5 for required Liver Safety Actions and Follow up Assessments

7.1.1.1. Study Intervention Restart or Rechallenge after liver stopping criteria met

Study intervention restart or rechallenge after liver chemistry stopping criteria are met by any participant in this study is not allowed.

7.1.2. QTc Stopping Criteria

A participant that meets either bulleted criterion based on the average of triplicate ECG readings will be withdrawn from study intervention.

- QTc, QTcB, QTcF > 500 msec,
- Change from baseline: QTc >60 msec

- The *same* QT correction formula *must* be used for *each individual participant* to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the participant has been enrolled.
  - For example, if a participant is eligible for the protocol based on QTcB, then QTcB must be used for discontinuation of this individual participant as well.
  - Once the QT correction formula has been chosen for a participant's eligibility, the *same formula* must continue to be used for that participant *for all QTc data being collected for data analysis*. Safety ECGs and other non-protocol specified ECGs are an exception.

- The QTc should be based on the average of triplicate ECG readings obtained over a brief (e.g., 5-10 minute) recording period.

7.1.3. Hypotension Stopping Criteria

A participant that meets the criterion below will be withdrawn from study intervention:

- systolic <90 mmHg and diastolic <50 mmHg confirmed by triplicate readings (taken up to 5 minutes apart) and is judged clinically significant / symptomatic by the investigator.

7.1.4. Haemoglobin Stopping Criteria

A participant that meets the criterion below will be withdrawn from the study intervention:

- Decrease in haemoglobin from baseline of ≥ 10.4 g/L over the course of the study.

7.1.5. Temporary Discontinuation

Withdrawal from study intervention will require the participant to withdraw from the study.

7.2. Participant Discontinuation/Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons. This is expected to be uncommon.
- At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted, as shown in the SoA. See SoA for data to be collected at the time of study discontinuation and follow-up.
- The participant will be permanently discontinued both from the study intervention and from the study at that time.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

7.3. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

Information regarding discontinuation of the study as a whole is given in Appendix 1.

8. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA.
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1. Efficacy Assessments

Not applicable

8.2. Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

8.2.1. Physical Examinations

- A full physical examination will include, at a minimum, assessments of the Skin, Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height and weight will also be measured and recorded.
- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure, and pulse.
- At screening and pre-dose, three readings of blood pressure and pulse will be taken. At screening, the average of the three blood pressure readings will be used to confirm eligibility. At all other time-points, single measurements will be taken, unless values go out of range, then triplicates should be performed.

8.2.3. Electrocardiograms

- An ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals will be used. Refer to Section 7 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECGs will be recorded in a semi-supine position after 5 minutes rest.
- Triplicate 12-lead ECGs will be obtained at screening and pre-dose. At all other time-points, single measurements will be taken, unless values go out of range, then triplicates should be performed. Triplicate ECGs should be obtained over a brief (e.g 5 to 10 minute) recording period.

8.2.4. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 7–14 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal by the investigator or medical monitor.
- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the aetiology should be identified and the sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the local laboratory manual and the SoA.

8.3. Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3.

The investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or the study, or that caused the participant to discontinue the study (see Section 7).

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of intervention until the follow-up visit at the time points specified in the SoA (Section 1.3). However, any SAEs assessed as related to study participation (e.g., study intervention, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study.
- All AEs will be collected from the start of intervention until the follow-up visit at the time points specified in the SoA (Section 1.3).
- Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded as Medical History.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs after the conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.2. Method of Detecting AEs and SAEs

- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.
- Care will be taken not to introduce bias when detecting AE and/or SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 3.

8.3.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information e.g., summary or listing of SAE) from the sponsor will review and then file it in the Investigator Site File and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5. Pregnancy

- Details of all pregnancies in female partners of male participants will be collected after the start of study intervention and until the follow-up visit.
- If a pregnancy is reported, the investigator should inform GSK within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4.
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAE.

8.4. Treatment of Overdose

For this study, any dose of AMB (GSK1325760) greater than the protocol defined dose will be considered an overdose.

Due to the mechanism of action, an overdose of AMB could potentially result in hypotension. In case of pronounced hypotension, active cardiovascular support may be required. There are no specific antidotes available for AMB.

In the event of an overdose, the investigator/treating physician should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until AMB (GSK1325760) can no longer be detected systemically (at least 3 days).
3. Obtain a plasma sample for PK analysis within 3days from the date of the last dose of study intervention if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

8.5. Pharmacokinetics

8.5.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of AMB will be collected at the time points indicated in the SOA (Section 1.3). The actual date and time of each blood sample collection will be recorded.

For analysis of AMB, 2.7 mL of blood will be collected into sodium citrate tubes. Processing, storage and shipping procedures are provided in the SRM.

8.5.2. Sample Analysis

Plasma analysis will be performed under the control of In vitro/In vivo Translation (IVIVT) and Third Party Resource, GlaxoSmithKline. Concentrations of AMB will be determined in plasma samples using the currently approved bioanalytical method. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Once the plasma has been analysed for AMB, any remaining plasma may be analysed for other compound-related metabolites and the results reported under a separate IVIVT, GlaxoSmithKline protocol.

8.6. Palatability Questionnaire

Participants will complete a palatability questionnaire after dosing of AMB tablets dispersed in water. Participants will complete 3 short questions about the smell, taste and feel of the study intervention.

Participants will be shown the questionnaire before dosing, so they know to consider the smell, taste and feel of the study intervention during dosing. Participants will complete the questionnaire within 10 min of dosing. Refer to Appendix 7 for the palatability questionnaire.

8.7. Pharmacodynamics

Pharmacodynamic parameters will not be evaluated in this study.

8.8. Genetics

Genetics will not be evaluated in this study.

8.9. Biomarkers

Biomarkers are not evaluated in this study.

8.10. Health Economics or MEDICAL Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

No formal hypothesis will be tested. An estimation approach will be used to i) estimate the bioavailability of the test formulation relative to the reference formulation, and ii) for each primary pharmacokinetic endpoint, point estimates and corresponding 90% confidence intervals will be constructed for the ratio of the geometric mean of the test formulation to the geometric mean of the reference formulation.

Success for this study is defined quantitatively as the observed AUC and C_{max} geometric mean ratio of the test formulation to reference formulation falling within 0.6 to 2.5. Both AUC and C_{max} will be used, but AUC (average concentration) is most relevant, because AMB is administered chronically.

The lower and upper limits of success are defined accordingly:

- *Lower limit of success (based on efficacy)*: PKPD analysis of adult AMB data (reference formulation) did not show a correlation between exposure and efficacy. However, the lowest approved dose (in Japan), which has shown efficacy is 2.5 mg.

Therefore, we have assumed our lower limit to be the 2.5 mg PK exposure. In relation to the therapeutic dose of 5 mg (to be tested in this study), and assuming linearity (observed in the adult studies), a 0.5-fold change in PK exposure (AUC or C_{max}) for the test formulation relative to the reference formulation, is the lower limit for success.

- *Upper limit of success (based on safety)*: In the first time in human study (EE-001), doses up to 15 mg were generally well tolerated (higher doses were associated with an increased frequency of AEs, although there were no SAEs). As the dose to be tested in this study is 5 mg, and 15 mg is considered a tolerated dose, a 3-fold increase in PK exposure (AUC or C_{max}) for the test formulation relative to the reference formulation, is the upper limit for success.

Therefore, the PK exposure (AUC and C_{max}) will be considered both safe and efficacious if the observed geometric mean ratio of test formulation to reference formulation falls within 0.5 to 3.

9.2. Sample Size Determination

Approximately 24 participants will be randomly assigned to study intervention such that 20 evaluable participants complete the study.

An evaluable participant is one who receives at least one active dose of AMB for whom a pharmacokinetic sample was obtained and provides data on pharmacokinetic parameters.

9.2.1. Sample Size Assumptions

The sample size assumptions are based on previously reported estimates of within subject CV for AUC $_{(0-\infty)}$ and C_{max} for AMB (study GS-US-300-0112, 2008) [Volibris, 2018]. Table 3 summarizes the estimates of within subject CV for the primary endpoints AUC (0-∞) and C_{max}.

Table 3 Estimates of within subject CV for the primary endpoints AUC (0-∞) and C_{max}

CVw: within subject CV	AMB
C_{max}	22%
AUC $_{(0-\infty)}$	15%

The largest of the within subject CV estimates is about 22%, which translate to a standard deviation (SD) of 0.217 on the natural log scale. Based on this SD, the half width of the 90% confidence interval will be 12.5% of the point estimate, approximately 24 subjects will be enrolled to provide at least 20 statistically evaluable subjects.

9.2.2. Sample Size Sensitivity

Assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then Table 4 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 4 Sample Size Sensitivity

Evaluable Subjects	CVw	Precision
20	22%	12.5%
20	34%	19.6%
18	22%	13.2%
18	32%	19.6%
16	22%	14.2%
16	30%	19.6%

Assuming a within subject CV for AUC of 15% (see Section 9.2.1), and with a sample size of 20, if the observed mean AUC ratio of test formulation to reference is 0.6, the probability of the true mean ratio being < 0.5 is < 0.5%. Similarly, if the observed mean AUC ratio of test formulation to reference is 2.5-fold, the probability of the true mean ratio being > 3 is < 0.5%. **So, there is at least 99% probability that the observed AUC will not exceed the boundary of (0.5, 3).**

Assuming a within subject CV for C_{max} of 22% (see Section 9.2.1), and with a sample size of 20, the probability of observing a mean C_{max} ratio of test formulation to reference below 0.5 is < 2% when the true PK ratio is 0.6. Similarly, if the observed C_{max} ratio of test formulation to reference is 2.5-fold, the probability of the true mean ratio being > 3-fold is < 2%. **So, there is at least 98% probability that the observed C_{max} will not exceed the boundary of (0.5, 3).**

9.2.3. Sample Size Re-estimation or Adjustment

No sample size re-estimation is planned to be performed.

9.3. Populations for Analyses

Population	Description
Safety	All randomized participants who take at least 1 dose of study intervention. Participants will be analysed according to the intervention they received.
Pharmacokinetic Concentration	The PK Concentration Population will include all participants for whom at least one PK sample was obtained and analysed.
Pharmacokinetic Parameter	For each PK parameter, the PK Parameter Population will include all participants who provide PK parameter data.

9.4. Statistical Analyses

9.4.1. Pharmacokinetic Analyses

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modelling and Simulation Department, CPKMS, GlaxoSmithKline. Statistical analyses

of the pharmacokinetic parameter data will be the responsibility of Clinical Statistics, GlaxoSmithKline.

Plasma concentration time data for AMB will be analysed by non-compartmental methods with WinNonlin [version 6.3 or above]. Calculations will be based on the actual sampling times recorded during the study although supplementary analysis will be available based on the nominal times. From the plasma concentration-time data, the following pharmacokinetic parameters will be determined, as data permit: maximum observed plasma concentration (C_{max}), time to C_{max} (tmax), area under the plasma concentration-time curve [AUC(0-t) and AUC (0-∞)], and apparent terminal phase half-life ($t_{1/2}$).

The PK parameters, AUC (0-∞), AUC(0-t), C_{max}, and $t_{½}$ will be transformed using natural logarithms. Missing PK parameters will not be imputed. All data from withdrawn subjects will be listed. No adjustment for multiple tests or comparisons is planned.

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively by treatment. Descriptive summary table, graphics, and treatment will also be provided.

Data will be listed and summarized according to GlaxoSmithKline reporting standards, where applicable. Listings will be sorted by subject, period, noting treatment. Summaries will be presented by treatment. Unless stated otherwise, descriptive summaries will include n, mean, standard deviation (SD), coefficient of variation (CV), median, minimum, and maximum for continuous variables, n and percent for categorical variables and geometric mean, 95% confidence interval (CI), and the between-subject CV (CVb) based on geometric mean for the log-transformed PK parameters.

Version 9.3 (or higher) of the SAS system will be used for statistical analysis of the data as well as to generate tables, figures, and listings.

Any deviation(s) from the original analyses planned in the protocol will be reported in the Reporting and Analysis Plan (RAP) and/or in the Clinical Pharmacology Study Report.

All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

9.5. Interim Analyses

No interim analyses are planned.

9.6. Safety Analyses

Safety data will be presented in tabular and/or graphical format and summarized descriptively according to GSK's IDSL standards.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
  - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study centre.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant.
- Participants who are rescreened are required to sign a new ICF.

10.1.4. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5. Dissemination of Clinical Study Data

- 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.

- The procedures and timing for public disclosure of the protocol and results summary and for development of a manuscript for publication for this study will be in accordance with GSK Policy.
- GSK intends to make anonymized patient-level data from this trial available to external researchers for scientific analyses or to conduct further research that can help advance medical science or improve patient care. This helps ensure the data provided by trial participants are used to maximum effect in the creation of knowledge and understanding

10.1.6. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.7. Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in Source Document Agreement.

10.1.8. Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development

10.1.9. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 5 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 5 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Haemoglobin Haematocrit	RBC Indices: MCV MCH %Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	BUN	Potassium	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	Total Protein
	Glucose (fasting at screening)	Calcium	Alkaline phosphatase	
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening (or Pre-dose) Tests	• Follicle-stimulating hormone (as needed in women of non-childbearing potential only) • Urine drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)			

NOTES :

1. Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1 and Appendix 5. All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.

Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • 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 study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events <u>NOT</u> Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.

- Situations in which 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.

10.3.2. Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
○ Results in death
○ Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant 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.
Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant 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 outpatient setting. Complications that occur during hospitalization are AE. 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.
Results in persistent disability/incapacity • 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, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
Is a congenital anomaly/birth defect
Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE 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 participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include 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.

10.3.3. Recording and Follow-Up of AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality
• The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. • A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated. • The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment. • For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to** GSK**.** • The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. • The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed CRF. • The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

10.3.4. Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool
• The primary mechanism for reporting SAE to GSK will be the electronic data collection tool. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE data into the electronic system as soon as it becomes available. • The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor by telephone. • Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in SRM.

10.4. Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

10.4.1. Definitions:

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

   For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., mullerian agenesis, androgen insensitivity), investigator discretion should be applied to determining study entry.

   Note: Documentation can come from the site personnel's: review of the participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
     - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement in the postmenopausal range is required.
   - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-oestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

10.4.2. Contraception Guidance:

CONTRACEPTIVES [a] REQUIRED DURING THE STUDY FOR FEMALE PARTNERS OF MALE PARTICIPANTS:
• **Highly Effective Methods [b] That Have Low User Dependency** *Failure rate of <1% per year when used consistently and correctly.*
• Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c]
• Intrauterine device (IUD)
• Intrauterine hormone-releasing system (IUS)[c]
• Bilateral tubal occlusion
• **Highly Effective Methods [b] That Are User Dependent** *Failure rate of <1% per year when used consistently and correctly.*

• Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation
  • oral
  • intravaginal
  • transdermal
  • injectable

• Progestogen-only hormone contraception associated with inhibition of ovulation
  • oral
  • injectable

a. Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies. b. Failure rate of <1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly. Note: Periodic abstinence (calendar, sympto-thermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Male condom and female condom should not be used together (due to risk of failure with friction)

10.4.3. Collection of Pregnancy Information:

Male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 24 hours of learning of the partner's pregnancy.
- The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.

- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of foetal status (presence or absence of anomalies) or indication for procedure.

10.5. Appendix 5: Liver Safety: Required Actions and Follow-up Assessments

Phase 1 Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event etiology

Phase 1 liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria</th></tr>
<tr><td>ALT-absolute</td><td colspan="2">ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or <u>international normalized ratio (INR)</u> >1.5, Report as an SAE.
See additional Actions and Follow Up Assessments listed below</td></tr>
<tr><th colspan="3">Required Actions and Follow up Assessments</th></tr>
<tr><th colspan="2">Actions</th><th>Follow Up Assessments</th></tr>
<tr><td colspan="2">• Report the event to GSK within 24 hours
• Complete the liver event CRF, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2]
• Perform liver event follow up assessments
• Monitor the participant until liver chemistries resolve, stabilise, or return to within baseline (Day –1) (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, aspartate transaminase [AST], alkaline phosphatase, bilirubin and INR) and perform liver event follow up assessments within 24 hours
• Monitor participant twice weekly until liver chemistries resolve, stabilise or return to within baseline (Day –1)
• A specialist or hepatology consultation is recommended
If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:</td><td>• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend
• Obtain blood sample for pharmacokinetic (PK) analysis, obtained within 96 hrs of last dose of last dose4
• 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 liver injury, or hypersensitivity, on the AE report form
• Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications.
• Record alcohol use on the liver event alcohol intake case report form</td></tr>
</table>

Liver Chemistry Stopping Criteria	
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin and INR) and perform liver event follow up assessments within **24-72 hours** • Monitor participant weekly until liver chemistries resolve, stabilize or return to within baseline (Day –1)	**If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Serum acetaminophen adduct high performance liquid chromatography (HPLC) assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week) [James, 2009]. **Not required if participant has been in the clinical unit, and agreed by the GSK Medical Monitor.** • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study intervention for that subject if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); the INR threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A immunoglobulin (gM) antibody; HBsAg and HBcAb; Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing) and Hepatitis E IgM antibody
4. Record the date/time of the PK blood sample draw and the date/time of the last dose of study intervention prior to PK blood sample draw on the CRF. If 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 SRM.

10.6. Appendix 6: Abbreviations and Trademarks

AE	Adverse event
ALT	Alanine aminotransferase
AMB	Ambrisentan
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
CA	Competent Authority
CFR	Code of Federal Regulation
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
Cmax	Maximum observed plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case report form
CSR	Clinical Study Report
CV	Coefficient of Variation
CVb	Between subject coefficient of variation
EC	Ethics committee
ECG	Electrocardiogram
EMA	European Medicines Agency
ERA	Endothelin receptor antagonist
ET-1	Endothelin-1
EU	European Union
EURMP	European Union Risk Management Plan
FC	Functional Class
FSH	Follicle stimulating hormone
g/L	Gram per Litre
GCP	Good clinical practice
GSK	GlaxoSmithKline
h	Hour
Hb	Haemoglobin
HBsAg	Hepatitis B surface antigen
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HRT	Hormonal replacement therapy
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council on Harmonisation
IRB	Institutional Review Boards
IEC	Independent ethics committee
Ig	Immunoglobulin
INR	International normalised ratio
IP	Investigational product
iPAH	Idiopathic pulmonary arterial hypertension
IUD	Intrauterine device

IUS	Intrauterine hormone-releasing system
IVIVT	In vitro/In vivo Translation
Kg	Kilogram
LSLV	Last subject last visit
LV	Left ventricular
m	Metre
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
mg	Milligram
min	Minute
mL	Millilitre
mmHg	millimetre of mercury
MSDS	Material Safety Data Sheet
PAH	Pulmonary arterial hypertension
PAP	Pulmonary artery pressure
PDCO	Paediatric Committee
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic
PKPD	Pharmacokinetic Pharmacodynamic analysis
PVR	Pulmonary vascular resistance
QTc	Corrected QT interval
QTcB	QT interval corrected for heart rate according to Bazett's formula
QTcF	QT interval corrected for heart rate according to Fridericia's formula
RAP	Reporting and analysis plan
RHF	Right-sided heart failure
RNA	Ribonucleic acid
SA	Safety analysis
SAE	Serious adverse event
SD	Standard deviation
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SUSAR	Suspected unexpected serious adverse reactions
SRM	Study reference manual
Tmax	Time to Cmax
t1/2	Apparent terminal phase half-life
ULN	Upper limit of normal
US	United States
WBC	Whir Blood Cells
WHO	World Health Organisation
WOCBP	Woman of childbearing potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
None

Trademarks not owned by the GlaxoSmithKline group of companies
Letairis
VOLIBRIS is a trade mark of Gilead Sciences, Inc.

10.7. Appendix 7: Palatability Questionnaire

PALATABILITY QUESTIONNAIRE

**Please read each question carefully and answer by checking the box that best applies to you.
Check only ONE box for each question. Answer ALL questions. Thank you.**

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.

UK:ENG (United Kingdom/English) Non-Standard [Palatability Questionnaire]

11. REFERENCES

Beghetti M, Hoeper MM, Kiely DG, Carlesen J, Schwierin B, Segal ES, et al. Safety experience with bosentan in 146 children 2-11 years with pulmonary arterial hypertension: results from the European post-marketing surveillance program. *Pediar Res*. 2008;64(2):200-4.

Clinical study report EE-001: A randomized, double-blind, placebo-controlled single ascending dose trial to investigate the tolerability, pharmacodynamics, pharmacokinetics and possible food effect of the endothelin receptor antagonist BSF 208075 in healthy male volunteers. 14 February 2001.

Clinical study report EE-002: A randomized, double-blind, placebo-controlled, multiple ascending dose trial to investigate the tolerability, safety, pharmacokinetics and – dynamics of oral BSF 208075 in healthy male subjects. 6 February 2001.

Dingemanse J, van Giersbergen P. Clinical pharmacology of bosentan, a dual endothelin receptor antagonist. *Clin Pharmacokinet.* 2004;43(15):1089-1115.

European Union Risk Management Plan (EURMP) for VOLIBRIS (ambrisentan). Version 8.1. 2019.

Fraisse A, Xavier J, Schliech JM, di Flippo S, Maragnes P, Beghetti M, et al. Characteristics and prospective 2-year follow-up of children with pulmonary arterial hypertension in France. *Arch Cardiovasc Dis* (2010), doi:10.1016/j.acvd.2009.12.001; in press

Galiè N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res*. 2004;61:227–37.

Giaid A, Yanagisawa M, Langleben D Michel R, Levy R, Shennib H. Expression of endothelin-1 in the lungs of subjects with pulmonary hypertension. *N Engl J Med* 1993;328:1732–9.

Hoeper MM, Huscher D, Pittrow D., Incidence and prevalence of pulmonary arterial hypertension in Germany. *International Journal of Cardiology*. 2016; 203:612-13

James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA,et al. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. *Drug Metab Dispos* 2009; 37:1779-1784.

McLaughlin VV. Survival in subjects with pulmonary arterial hypertension treated with first-line bosentan. *Eur J Clin Invest*. 2006;36(Suppl. 3):10–15.

Moledina S, Hislop A, Foster H, Schulze-Neick I, Haworth S. Childhood idiopathic pulmonary hypertension – A national cohort study. *Heart* 2010, in press.

Newman JH, Fanburg BL, Archer SL, Badesch DB, Barst RJ, Garcia JGN, et al., Pulmonary Arterial Hypertension: Future Directions: Report of a National Heart, Lung

and Blood Institute/Office of Rare Diseases Workshop. *Circulation* 2004; 109(24): 2947-52.

Nootens M, Kaufmann E, Rector T, Fisher C, Judd D, Francis G. Neurohormonal activation in subjects with right ventricular failure from pulmonary hypertension: relation to hemodynamic variables and endothelin levels. *J Am Coll Cardiol.* 1995;26:1581–5.

Segal E, Valette C, Oster L, Bouley L, Edfjall C, et. al. Risk management strategies in the postmarketing period – Safety experience with the US and EU bosentan surveillance programmes. *Drug Safety*.2005;28(11);971-980

Volibris Investigators Brochure. Seventeenth Edition. March 2018.

Volibris Summary of Product Characteristics (SmPC). Electronic Medicines Compendium. Nov 2018.

Vorhies EE and Ivy DD. Drug Treatment of Pulmonary Hypertension in Children. *Paediatric Drugs.* 2014;16(1):43-65