TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A Phase 1 study to demonstrate the relative bioavailability and bioequivalence of fixed dose combinations of ambrisentan and tadalafil in healthy subjects

Compound Number: GSK3380154, GSK1325760 and GF196960

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2015N232335_00	2015-DEC-16	Original
2015N232335_01	2016-APR-14	Amendment No. 1
Clarification of hypotension withdrawal criteria in Section 5.4.3, change to GSK medical monitor and minor administrative changes.		
2015N232335_02	2017-FEB-08	Amendment No. 2
• Inclusion of bioequivalence assessment in Part 3 to replace the optional food effect. • Inclusion of additional dosage strength in Part 3. • Change in Medical Monitor(s).		
2015N232335_03	2017-MAY-25	Amendment No. 3
Inclusion of revised potential risk of clinical significance for tadalafil (Adcirca) following an update to the Summary of Product Characteristics and Patient Information Leaflet Change in Primary Medical Monitor Changes to Data Analysis Considerations (section 9.3) and Pharmacokinetic Analyses 9.4.1 to satisfy EMA guidelines.		

SPONSOR SIGNATORY

PPD

25-MAY-2017

Sanjeev Khindri Date

Project Physician Ledd

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number 201964

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

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

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

Investigator Name:		
Investigator Address:		
Investigator Phone Number:		
Investigator Signature		Date

TABLE OF CONTENTS

PAGE

1. PROTOCOL SYNOPSIS FOR STUDY 201964

Rationale

This study is designed initially to compare the relative bioavailability of a number of fixed dose combinations (FDCs) of ambrisentan and tadalafil (Part 1 and 2) and consequently the bioequivalence of the FDC of different dose strength (Part 3A and 3B). Dependent on formulation work, the study will allow up to 9 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: C_{max}, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
To establish bioequivalence of the candidate FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fed and fasted conditions.	$AUC_{(0-t)}$,$AUC_{(0-inf)}$,C_{max}
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasted conditions.	$AUC_{(0-t)}$, $AUC_{(0-inf)}$,C_{max}
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 20 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 20 mg) taken concurrently in healthy human subjects under fasted conditions.	$AUC_{(0-t)}$, $AUC_{(0-inf)}$,C_{max}
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fed and fasted conditions.	Plasma PK parameters including; $AUC_{(0-t)}/AUC_{(0-inf)}$,t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fed and fasting conditions.	Safety and Tolerability of all treatments as assessed by vital signs, ECG, clinical laboratory safety data and review of adverse events

Overall Design

This is a single centre, Phase 1, single dose, randomised, open label crossover study in healthy volunteers with 3 study parts. Part 1 will include a 5 way cross-over. Part 2 and Part 3 (A&B) will each include a 4 way cross-over. All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in Parts 1 and 2. In Part 3 the washout will be at least 10 days. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part will include an evaluation of the bioavailability, safety and tolerability of 3 granulation forms of a single FDC (ambrisentan 10mg + tadalafil 40 mg) identified from Part 1. These data will be compared to that for the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

Part 3 A

Part 3A of the study is set to establish bioequivalence between the candidate FDC from Part 2. This study part will have 4 dose sessions and will assess the bioequivalence, in both the fed and fasted state, of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state. The fed arms of this part will have a standard high fat breakfast. (EMEA, 2010).

Part 3 B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg). This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference

ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

Treatment Arms and Duration

The proposed treatment arms for each study part are described here.

Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)**	**Part 3A (fed and fasted)**	**Part 3B (fasted)**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 1 (10mg/40mg)	ambrisentan and tadalafil FDC) (10mg/40mg), fed	Ambrisentan and tadalafil FDC (5mg/40mg), fasted
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 2 (10mg/40mg)	ambrisentan and tadalafil FDC (10mg/40mg) fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 3 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed	ambrisentan and tadalafil FDC (5mg/20mg), fasted
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

Type and Number of Subjects

Approximately 20 healthy adult subjects will be randomized to study Part 1 and an additional 20 healthy adult subjects in Part 2, such that at least 16 evaluable subjects complete each part of the study. Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subejcts will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study.

Analysis

No formal hypothesis will be tested for Part 1 and Part 2 of the study. An estimation approach will be used to estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

$$H(0): \mu(\text{test})/\mu(\text{reference}) < 0.80 \text{ or } \mu(\text{test})/\mu(\text{reference}) > 1.25,$$

i.e., treatments are not bioequivalent.

versus

$$H(1): 0.80 \leq \mu(\text{ test})/\mu(\text{reference}) \leq 1.25,$$

i.e, treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) and of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg and of ambrisentan 5 mg & tadalafil 20 mg respectively taken concurrently (reference) in

healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

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

2. INTRODUCTION

Ambrisentan (E.U. trade name: Volibris), is an orally active endothelin receptor antagonist (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, 2015; Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.

Tadalafil (E.U. trade name: Adcirca) is an orally active selective inhibitor of the enzyme PDE-5, the primary cGMP-hydrolyzing enzyme in smooth muscle. In the EU, Adcirca is indicated in adults for the treatment of PAH classified as WHO FC II and III, to improve exercise capacity (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015).

A recently completed study (Galiè, 2015a) has shown that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris, 2015, Section 5.1).

2.1. Study Rationale

This study is designed initially to compare the relative bioavailability of a number of fixed dose combinations (FDCs) of ambrisentan and tadalafil (Part 1 and 2) and consequently the bioequivalence of the FDC of different dose strengths (Part 3A and 3B). Dependent on formulation work, the study will allow up to 9 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently

The formulation(s) to be taken forward in Part 3A will be primarily chosen based on the test/reference ratio for both AUC and Cmax for both components. Ideally the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, tablet size, cost, ease of manufacture and stability would be considered.

Part 3 will include evaluation of bioequivalence for 3 dose strengths of the FDC; 10/40mg, 5/40mg and 5/20mg of ambrisentan and tadalafil.

2.2. Brief Background

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3 biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galiè, 2015b) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, 2015, Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease (Volibris, 2015). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015). Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5) inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112, 2008) in 26 healthy participants was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-

administered. There were no SAE's in the study. Three participants withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one participantbecause of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a listed event for ambrisentan. There were a total of 7 participants with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris, 2015, (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015).

The AMBITION clinical study (Galiè, 2015a and GSK document number 2014N193963_00), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galiè, 2015b) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, 2008).

3. OBJECTIVE(S) AND ENDPOINT(S)

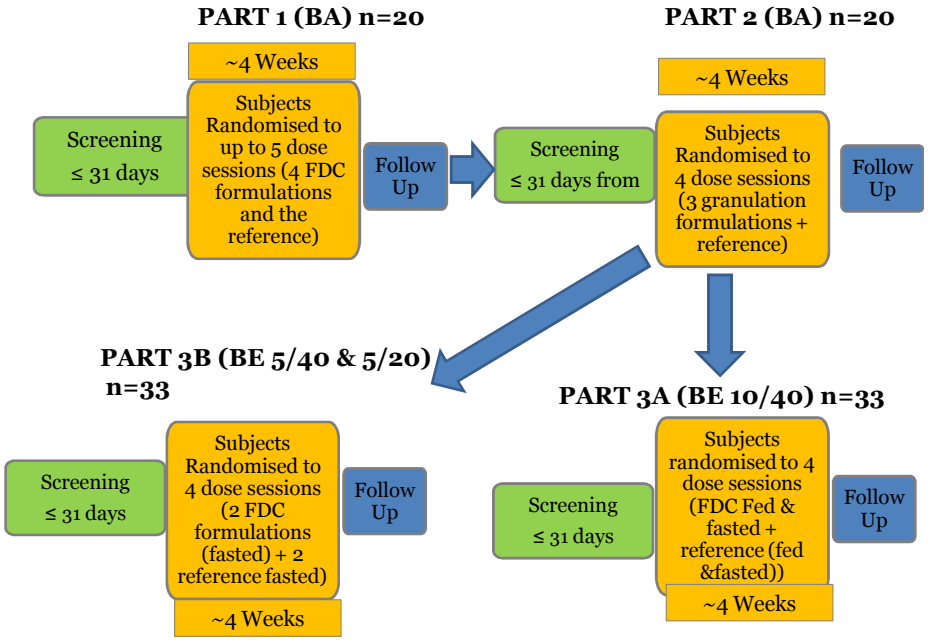
Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
To establish bioequivalence of the candidate FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fed and fasted conditions.	AUC(0-t), AUC(0-inf), Cmax
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasted conditions.	AUC(0-t), AUC (0-inf), Cmax
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 20 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 20 mg) taken concurrently in healthy human subjects under fasted conditions.	$AUC_{(0-t)}$, $AUC_{(0-inf)}$,C_{max}
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting and fed conditions.	Plasma PK parameters including; $AUC_{(0-t)}/AUC_{(0-inf)}$, t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fed and fasting conditions.	Safety and Tolerability of all treatments as assessed by vital signs, ECG, clinical laboratory safety data and review of adverse events

4. STUDY DESIGN

4.1. Overall Design

This is a single centre, Phase 1, single dose, randomised, open label, crossover study in healthy volunteers with 3 study parts; Part 1 will include a 5 way cross-over, and Part 2 and 3 (A&B) will each include a 4 way cross-over. See Figure 1 for study schematic.

Figure 1 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in study Part 1 and 2. In study Part 3 there will be a minimum of 10 days between each dose. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

Part 2

This study part will have 4 dose sessions and will evaluate the bioavailability, safety and tolerability of 3 different granulation sizes for a single FDC (ambrisentan 10 mg + tadalafil 40 mg) compared to the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

Part 3A

Part 3A of the study is set to establish bioequivalence between the candidate FDC from Part 2. This study part will have 4 dose sessions and will assess the bioequivalence, in both the fed and fasted state, of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state. The fed arms of this part will have a standard high fat breakfast (EMEA, 2010).

Part 3B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg) . This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference ambrisentan 5 mg + tadalafil 40mg monotherapies and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

4.2. Treatment and Duration

The treatments for each study part of the study are listed in Table 1. All treatments are single dose. Subjects will be randomised to the order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing analysis of pharmacokinetic data will be used to determine the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to evaluate 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2 and 3.

Following completion of Part 1 there will be a pause prior to Part 2, so that different granulation forms of a single FDC can be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for 3 granulation size forms of a single FDC formulation selected from Part 1. These will be based on a single successful formulation identified in Part 1 reformulated with the final active pharmaceutical ingredient (API). Success will be defined with the same criteria as those in Part 1.

Part 3 (A and B)

The BE part of the study will investigate the bioequivalence of the FDC ambrisentan 10 mg + tadalafil 40 mg in part 3A and the bioequivalence of the FDC ambrisentan 5 mg + tadalafil 40 mg and ambrisentan 5 mg + tadalafil 20 mg in Part 3B.

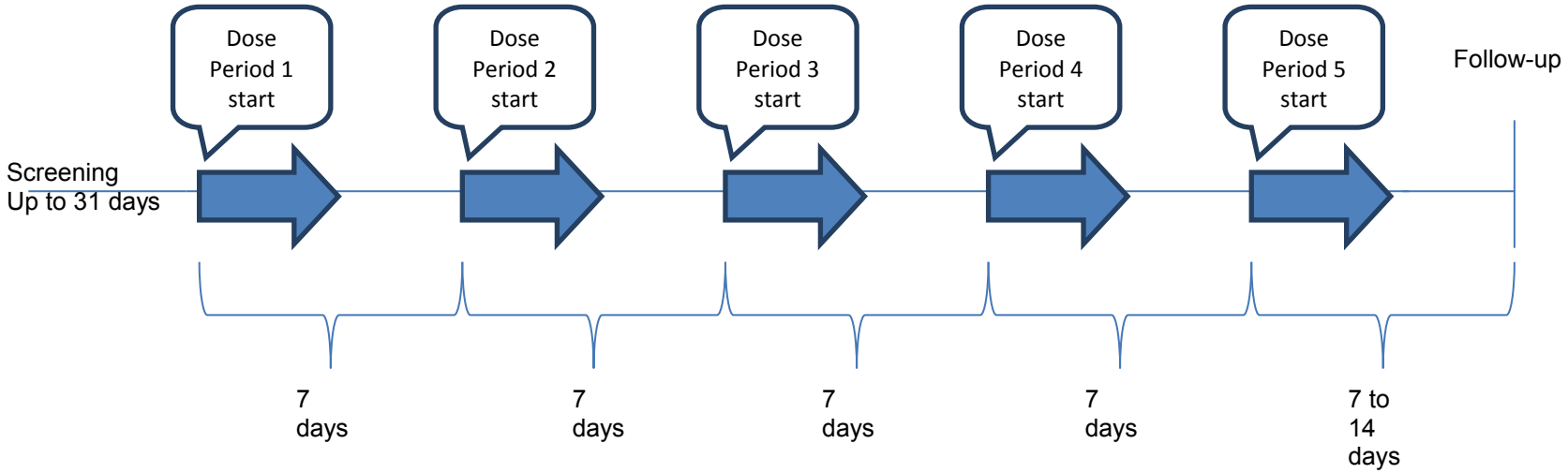
Table 1 Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)	Part 3A (fed and fasted)	Part 3B (fasted)
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 1 (10mg/40mg)	ambrisentan and tadalafil FDC (10mg/40mg), fed	Ambrisentan and tadalafil FDC (5mg/40mg), fasted
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDCgranulation 2 (10mg/40mg)	ambrisentan and tadalafil FDC (10mg/40mg) fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 3 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed	ambrisentan and tadalafil FDC (5mg/20mg), fasted
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

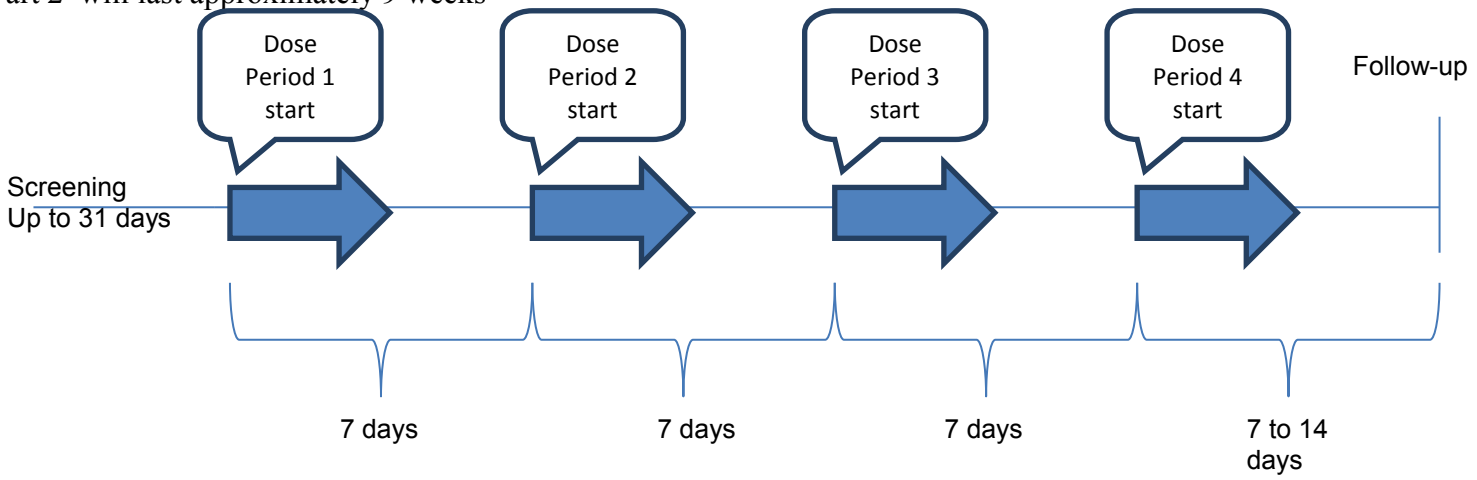
The duration for a subject on this study is described in Figure 2. This figure assumes all 5 dose sessions are used for Part 1. However, this is an approximation as minimum of 7 days is expected between doses in Parts 1 and 2 and a minimum of 10 days in Part 3.

Figure 2 Study Duration

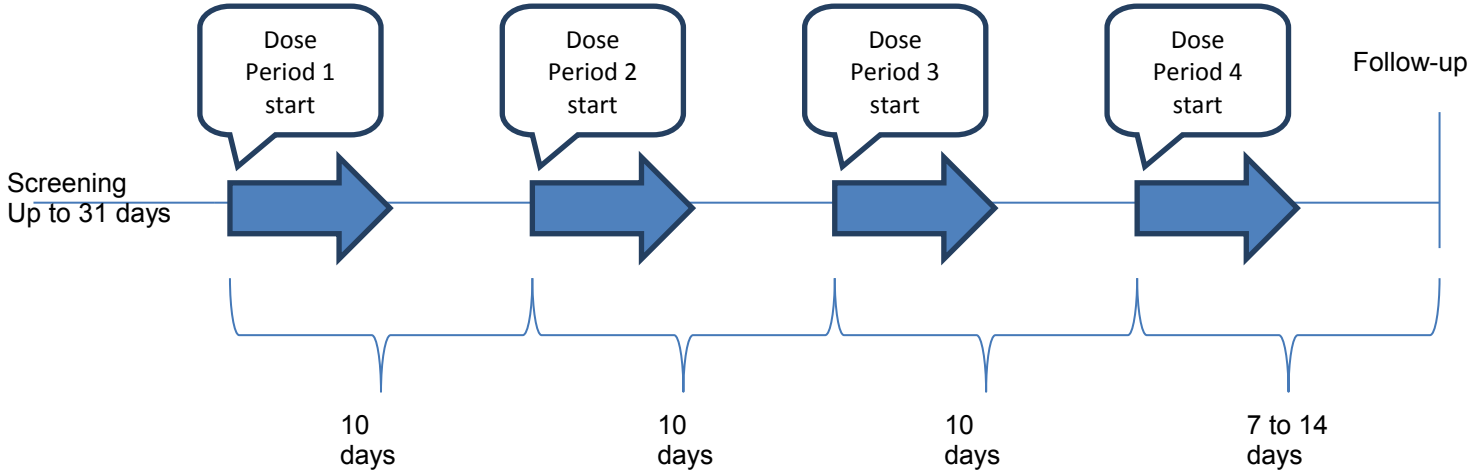
Part 1 can last up to 73 days.



Part 2 will last approximately 9 weeks



Part 3A and B will last approximately 11 weeks



A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

4.3. Type and Number of Subjects

Approximately 20 healthy adult subjects will be randomized to study Part 1 and an additional 20 healthy adult subjects in Part 2, such that at least 16 evaluable subjects complete each part of the study. Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subjects will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study.

The estimation of the number of subjects for each study part is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

4.4. Design Justification

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, nor is there any need to blind study treatment. The inclusion of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study contains three parts: Parts 1 and 2 will provide PK data for different candidate FDC formulations and will enable the assessment of bioequivalence of the FDC versus the combination of the individual reference products in Part 3. The interdependency of the 3 study parts is described in detail in Section 4.2.

4.5. Dose Justification

The oral dose for the new FDC formulations and the reference treatment proposed for the study of 40mg for tadalafil and 10mg for ambrisentan, are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to

be tolerated in healthy subjects. Within Part 3 of the study all dosage forms of the FDC that are proposed for marketing authorisation will be tested for bioequivalence versus the appropriate doses of reference products. These doses are required in clinical practice to allow safe up- and down-titration of the FDC in patients with PAH.

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates a minimumof 10 days, between doses, is also sufficient for clearance of the previous dose during the assessmet of bioequivalence.

4.6. Benefit: Risk Assessment

There is no direct benefit for healthy subjects participating in this study. The risks to the healthy subjects based upon previous experience indicate no expectation of SAE and mild to moderate AE. The benefit/risk remains satisfactory for this healthy subject study.

Summaries of findings from clinical studies conducted with both the Investigational Product taken as monotherapies and in combination can be found in:

- Ambrisentan IB (Volibris IB, 2015)
- Volibris (Ambrisentan) EMA SmPC (Volibris, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2017)

As described in Section 2.2, mild tachycardia was the most common (7/26, 27%) adverse event seen in healthy subjects following combination dosing with 10mg ambrisentan and 40mg tadalafil (Spence, 2009). There were three subjects (3/26, 11.5%) withdrawn due to AEs (e.g., anaemia [mild]; myalgia [severe], muscle fatigue, and dizziness; and headache [severe]). No SAEs or additional risks were observed with combination use in healthy subjects.

The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Risks of clinical significance, identified in subjects with PAH and the mitigation strategy identified for this study in healthy subjects are captured in Table 2.

Table 2 Risks of Clinical Significance and Mitigation Strategy

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product: GSK1325760 (ambrisentan-Volibris)		
• Teratogenicity • Anaemia (decreased haemoglobin, decreased haematocrit) • Hypersensitivity reactions (e.g. angioedema, rash, pruritus) • Headache (including sinus headache, migraine) • Dizziness • Cardiac failure • Palpitation • Hypotension • Flushing • Epistaxis • Dyspnoea • Upper respiratory congestion, sinusitis, nasopharyngitis, rhinitis • Abdominal pain • Constipation • Nausea, vomiting, diarrhoea • Hepatic transaminases increased • Peripheral oedema, fluid retention • Chest pain/discomfort • Asthenia and fatigue	Ambrisentan IB (Volibris IB, 2015) Volibris (Ambrisentan) EMA SmPC (Volibris, 2015):	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Exclusion of women of childbearing potential (teratogenicity) • Laboratory assessments per protocol • Physical assessment per protocol • Routine vital signs per protocol • Subjects remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48 hrs post dose. • Single doses used in the study
Investigational Product: GF196960 (tadalafil - Adcirca)		
• Hypersensitivity reactions • Headache • Syncope, • Migraine • Blurred vision • Sudden decrease or loss of vision (Non-Arteric Anterior Ischemic Optic Neuropathy (NAION)) • Palpitations	Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2017)	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Routine vital signs per protocol • Physical assessment per protocol • Subjects remain in the clinical unit for all doses, under medical supervision and until completion of

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
• Nasopharyngitis (including nasal congestion, sinus congestion and rhinitis) • Epistaxis • Nausea, • Dyspepsia (including abdominal pain/discomfort) • Vomiting, • Gastroesophageal reflux • Rash • Myalgia, • Back pain • Pain in extremity (including limb discomfort) • Increased uterine bleeding • Facial oedema, • Chest pain • Sudden decrease or loss of hearing		safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GSK3380154 (ambrisentan-tadalafil-FDC)		
The risks for the combination are the same as the 2 monotherapies; no extra risks have been identified for the combination.		As for GSK1325760 and GF196960 above

4.6.2. Benefit Assessment

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation and to demonstrate bioequivalence versus reference products for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

4.6.3. Overall Benefit: Risk Conclusion

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 2. In addition review of safety information from Part 1 of the study is consistent with this position. So, the benefit:risk is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in:

- Volibris (Ambrisentan) EMA SmPC (Volibris, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2017)

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

5.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

AGE
1. Between 18 and 60 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and cardiac monitoring (ECG and 24 hour Holter). A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical Monitor if required, judges and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.

WEIGHT
3. Body weight ≥ 50 kg (110 lbs) for men and ≥ 45 kg (99lbs) for women and body mass index (BMI) within the range 18– 30 kg/m^2 (inclusive)

SEX
4. Male or Female **Females must be the following**: Non-reproductive potential defined as:

• Pre-menopausal females with one of the following:
  • Documented tubal ligation
  • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion
  • Hysterectomy
  • Documented Bilateral Oophorectomy
• Documented Postmenopausal defined as 12 months of spontaneous amenorrhea

INFORMED CONSENT
5. Capable of giving signed informed consent as described in Section 10 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol.

5.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)

1. A blood pressure <100/55 mm Hg.
2. Haemoglobin below normal range:
  • Hb < 133 g/L for males
  • Hb< 114 g/L for females
3. ALT and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
4. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
5. 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.
  • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting

and Analysis Plan (RAP).

CONCOMITANT MEDICATIONS
6. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication, unless in the opinion of the Investigator and GSK Medical Monitor the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
7. History of regular alcohol consumption within 6 months of the study defined as: • An average weekly intake of >21 units for males or >14 units for females. 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. 8. Smoking more than 5 cigarettes per week and subjects must be able to abstain from smoking for a 24 hour period prior to dose and any time whilst in the clinical unit.

CONTRAINDICATIONS
9. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or Medical Monitor, contraindicates their participation.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
10. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at Screening or within 3 months prior to first dose of study treatment. . 11. A positive test for HIV antibody. 12. A positive pre-study drug/alcohol screen. 13. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within previous 3 months 14. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 3 months, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 15. Exposure to more than four new chemical entities within 12 months prior to the first

dosing day.

5.3. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.4.1.4).

5.4. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

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

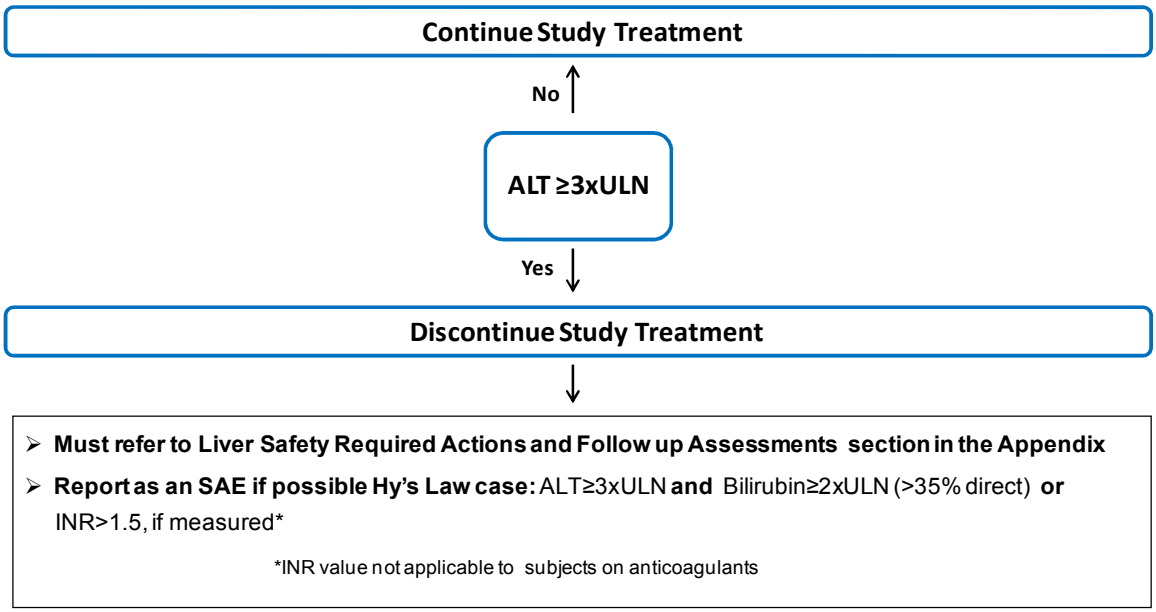
A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

5.4.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Study treatment will be discontinued **for a subject** if liver chemistry stopping criteria are met:

Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow-Up Assessments Section can be found in Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

5.4.1.1. Study Treatment Restart or Rechallenge

Study treatment restart or re-challenge after liver chemistry stopping criteria are met by any subject participating in this study is not allowed.

5.4.2. QTc Stopping Criteria

- The *same* QT correction formula *must* be used for *each individual subject* to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the subject has been enrolled.
  - For example, if a subject is eligible for the protocol based on QTcF, then QTcF must be used for discontinuation of this individual subject as well.
  - Once the QT correction formula has been chosen for a subject's eligibility, the *same formula* must continue to be used for that subject *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 averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A subject that meets either bulleted criterion below will be withdrawn from the study.

- QTc> 500 msec,

- Change from baseline: QTc >60 msec

5.4.3. Hypotension

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 mmHG and diastolic <50 mm HG confirmed by triplicate reading taken up to 5 minutes apart and is judged clinically significant and symptomatic by the investigator.

5.4.4. Other Dose Adjustment/Stopping Safety Criteria

For an individual study participant, stopping criteria include, but are not limited to:

Adverse events, or significant changes in any of the safety assessments, that put the safety of the individual at risk (e.g., ECG, vital signs, laboratory tests, etc), as judged by the Principal Investigator in consultation with the Medical Monitor if necessary.

5.5. Subject and Study Completion

A completed subject is one who has completed all dosing periods of the study including the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

The term 'study treatment' is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for Parts 1, 2 and 3 of the study are shown in Table 3, Table 4 and Table 5.

Table 3 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.45 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Address of reference manufacturer	NA	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

Table 4 Study Treatment for Part 2

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference	
Formulation description	FDC-G1 granulation 1 (10mg/40mg)	FDC-G2 granulation 2 (10mg/40mg)	FDC-G3 granulation 3 (10mg/40mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.45 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations), Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

Table 5 Study Treatment for Part 3

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Formulation description	FDC-G1 or 2 or3 (10mg/40mg)	FDC (5mg/40mg)	FDC (5mg/20mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each tablet contains 5 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura Red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

6.1.1. Retention Samples

GSK will send five times (5x) the drug product necessary for pivotal bioequivalency evaluation in Part 3 of the study for analytical testing for all tablet strengths used in the study. The retain samples are in addition to the supply of drug product sufficient to complete the study. Retention samples will be sent to the clinical CRO in the same bulk container. To ensure that reserve samples are representative of the same batches provided for the clinical study the clinical site will randomly select the supplies for the study from the supply received. The clinical site must retain enough reserve samples to permit FDA to perform five times all of the release tests required in the application.

6.2. Medical Devices

No GSK manufactured devices (or devices manufactured for GSK by a third party) are provided for use in this study.

6.3. Treatment Assignment

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

The treatment key for Parts 1, 2 and 3 are described in Table 6.

Table 6 Treatment Key for Part 1, Part 2 and Part 3

Treatment	Description
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 2	
FG1	ambrisentan and tadalafil FDC-G1 (10mg/40mg) granulation size 1
FG2	ambrisentan and tadalafil FDC-G2 (10mg/40mg) granulation size 2
FG3	ambrisentan and tadalafil FDC-G3 (10mg/40mg) granulation size 3
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 3A	
X1	ambrisentan and tadalafil FDC-G1 or 2 or 3 (10mg/40mg) fed
X2	ambrisentan and tadalafil FDC G1 or 2 or 3 (10mg/40mg) fasted

Treatment	**Description**
R1	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed
R2	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted
Part 3B	
Y1	ambrisentan and tadalafil FDC (5mg/40mg), fasted
Y2	ambrisentan and tadalafil FDC (5mg/20mg), fasted
R3	ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted
R4	ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 7. Additional treatment sequences may be created based using the Latin Squares for Williams Designs if needed.

Table 7 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1/ F2/ R/ F3/ F4 F2/ F3/ F1/ F4/ R F3/ F4/ F2/ R/ F1 F4/ R/ F3/ F1/ F2 R/ F1/ F4/ F2/ F3 F4/ F3/ R/ F2/ F1 R/ F4/ F1/ F3/ F2 F1/ R/ F2/ F4/ F3 F2/ F1/ F3/ R/ F4 F3/ F2/ F4/ F1/ R	1:1:1:1:1:1:1:1:1:1
Part 2	4	FG1/ FG2/ R/ FG3 FG2/ FG3/ FG1/ R FG3/ R/ FG2/ FG1 R/ FG1/ FG3/ FG2	1:1:1:1
Part 3A	4	X1/ R1/ R2/ X2 R1/ X2/ X1/ R2 X2/ R2/ R1/ X1 R2/ X1/ X2/ R1	1:1:1:1
Part 3B	4	Y1/ R3/ R4/ Y2 R3/ Y2/ Y1/ R4 Y2/ R4/ R3/ Y1 R4/ Y1/ Y2/ R3	1:1:1:1

6.4. Blinding

This will be an open-label study.

6.5. Packaging and Labeling

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

6.6. Preparation/Handling/Storage/Accountability

This will be detailed in a Study Specific Technical Agreement/Memo (TTS) which will be accompanied by a Quality Agreement.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.
- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the SRM.
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
- 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.

6.7. Compliance with Study Treatment Administration

When subjects are dosed at the site, they will receive study treatment 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 treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each subject's mouth to ensure that the study treatment was ingested.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK3380154 (ambrisentan/tadalafil-FDC), GSK1325760 (ambrisentan) and GF196960 (tadalafil) greater than the protocol defined dose and within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose.

Advice for the investigator is included in the product label for GSK1325760 (ambrisentan) and GF196960 (tadalafil).

In the event of an overdose the investigator should:

16. Contact the Medical Monitor immediately
17. Closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until compound number/name can no longer be detected systemically (at least 3 days for compound number/name)
18. Obtain a plasma sample for pharmacokinetic (PK) analysis if requested by the Medical Monitor (determined on a case-by-case basis)
19. 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 subject.

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because only healthy subjects are eligible for study participation.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until after the final dose.
- Dependent on the utility of the dose session subjects will be asked to fast or consume the FDA full fat breakfast prior to dosing (EMEA, 2010)
  - Fasting subjects will be required to fast from midnight before each full in-house dosing day (i.e. Day 1) with the exception of water, which will be allowed freely except for 1 hour either side of dosing. Subjects will be required to fast up to 4h hour post dose on Day 1 of each dose
  - For treatments given under fed conditions, a standard high-fat breakfast will be provided before dosing. See Section 12.4 for details of this meal. This meal should be eaten in its entirety within 30 minutes. The amount consumed will be recorded within the source documents and the CRF by the site staff. Subjects will be dosed within 5 minutes of completing the breakfast.
- Subjects should take each dose of investigational product with 240 ml (8 fl oz) of water.

6.10.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks and chocolate) for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- During each dosing session, subjects will abstain from alcohol for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- Smoking is not allowed for 24 hours prior to dosing and whilst subjects are in the clinic.

6.10.3. Activity

Subjects will abstain from strenuous exercise from Screening until Follow-Up. Subjects may participate in light recreational activities during studies (e.g., watch television, read).

6.11. Concomitant Medications and Non-Drug Therapies

6.11.1. Permitted Medications and Non-Drug Therapies

Paracetamol at doses of ≤ 2 grams/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.

6.11.2. Prohibited Medications and Non-Drug Therapies

Subjects 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) prior to the first dose of study medication until completion of the Follow-Up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 7.1

The following points must be noted:

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:
    1. 12-lead ECG
    2. Vital signs

3. Blood draws.

Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.

- The timing and number of planned study assessments, safety and pharmacokinetic assessments 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.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.

No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

7.1. Time and Events Table

Procedure	**Screen**	**Part 1, 2 and 3A and 3B. Each dose in each Part repeats this schedule.**																**FU**	**Notes**
Day	**≤-31**	**-1**	**1**											**2**		**3**	**4**	**≥7-14**	
Time (hrs)			**Pre-dose**	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**4**	**8**	**12**	**18**	**24**	**36**	**48**	**72**		
Outpatient visit	x																x	x	
Admission to unit		x																	
Informed consent	x																		
Inclusion and exclusion criteria	x	x																	
Demography	x																		
Full physical exam including height and weight	x																		
Brief Physical																		x	
Medical history (includes substance usage)	x																		
HIV, Hep B and Hep C screen]	x																		
Laboratory assessments (include liver chemistries)	x	x														x		x	Only Screening labs need to be taken in fasted state.
Serum hCG Pregnancy test	x																	x	Female subjects only
Urine hCG Pregnancy test		x																	Female subjects only
Breathalyser and Smokerlyzer	x	x																	
DOA testing	x	x																	
12-lead ECG	x		x			x		x		x		x		x			x	x	Triplicate at screen and baseline, single measure at other times, unless out of range then triplicates should be performed
Vital signs	x		x		x	x		x		x	x	x		x		x	x	x	
24hr Holter	x																		
Randomisation			x																Randomised prior to first dose only
Study Treatment				x															
AE/SAE review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	SAEs from Screen. AEs from first dose
Concomitant medication review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
PK Sample			x		x	x	x	x	x	x	x	x	x	x	x	x	x		
Discharge from Unit																x			For logistical reasons subjects may remain in-unit for the 72 hr assessments if they prefer.

7.2. Screening and Critical Baseline Assessments

All subjects must give written consent to participate in this trial. Consent for screening evaluations may be obtained using the ICF for the Hammersmith Medicines Research (HMR) healthy subject's panel, which has been approved by the HRA's Phase 1 Advert Review group. The study-specific information and consent form will be signed by the subject either before any screening evaluation or after the investigator confirms the eligibility of the subject for the study and before the subject is randomised to receive the first administration of IMP. Before giving consent, subjects must read the information sheet about the study. They must also read the consent form. They will then discuss the study with the investigator or his deputy and be given the opportunity to ask questions. The study-specific information sheet and the consent form must be approved by the REC.

7.2.1. Demographic/Medical History Assessments

Prior to enrolling in the study and having any study procedures completed, subjects must sign the informed consent.

During the Screening visit, each subject will undergo the assessments to determine eligibility for enrolment as detailed in Section 5.

The following demographic parameters will be captured: date of birth, gender, race and ethnicity.

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 5.

7.3. Pharmacokinetics

7.3.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of ambrisentan and tadalafil will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

For analysis of ambrisentan 2.7 mL of blood will be collected into sodium citrate tubes and for analysis of tadalafil 2.0 mL of blood will be collected into K2-EDTA tubes.

Processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

7.3.2. Sample Analysis

Plasma analysis will be performed under the control of Platform Technology and Science In vitro/In vivo Translation (PTS IVIVT) and Third Party Resource, GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of ambrisentan and tadalafil will be determined in plasma samples using

the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

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

7.4. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests such as vital signs, physical examinations and laboratory safety assessments may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

7.4.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The definitions of an AE or SAE can be found in Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.4.1.1. Time period and Frequency for collecting AE and SAE information

- Any SAEs assessed as related to study participation (e.g., 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 up to and including any follow up contact.
- AEs will be collected from the start of Study Treatment until the follow up contact (see Section 7.4.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.3.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Section 12.3.

7.4.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?

7.4.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Section 4.6.1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow up (as defined in Section 5.4). Further information on follow up procedures is given in Study Assessments and Procedures.

7.4.1.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs related to study treatment (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

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

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

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

7.4.2. Physical Exams

- A complete physical examination will include, at a minimum, assessment of the 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

7.4.3. Vital Signs

- Vital signs will be measured in semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure and pulse rate and respiratory rate.
- Three readings of blood pressure and pulse rate will be taken at Screening and baseline (pre-dose). All subsequent assessments will be single measures, unless the subjects blood pressure or pulse rate has changed from baseline by >15% and then 2 further readings should be taken and recorded:
- For triplicate readings:
    - All 3 readings will be recorded in the CRF
        - First reading should be rejected
        - Second and third readings should be averaged to give the measurement to be used and this will also be recorded in the CRF

7.4.4. Electrocardiogram (ECG)

- ECGs will be measured in semi-supine position after 5 minutes rest
- Triplicate 12-lead ECGs will be obtained at Screening and baseline (predose). All subsequent assessments will be a single measures, unless withdrawal criteria are meet and in which case 2 further readings should be taken to confirm if withdrawal is required. An ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- 24 hour continuous cardiac telemetry (Holter) will be performed at Screening. Full disclosures will be reviewed in detail and the review maintained as part of the subject's source documents

7.4.5. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 8, must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit dat. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

All study-required laboratory assessments will be performed by a local laboratory.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 8.

Table 8 Protocol Required Safety Laboratory Assessments

<table>
<tr><th>Laboratory Assessments</th><th>Parameters</th></tr>
<tr><td>Haematology</td><td>
<table>
<tr><td>Platelet Count</td><td><u>RBC Indices</u>:</td><td><u>WBC count with Differential</u>:</td></tr>
<tr><td>RBC Count</td><td>MCV</td><td>Neutrophils</td></tr>
<tr><td>Hemoglobin</td><td>MCH</td><td>Lymphocytes</td></tr>
<tr><td>Hematocrit</td><td>MCHC</td><td>Monocytes</td></tr>
<tr><td></td><td></td><td>Eosinophils</td></tr>
<tr><td></td><td></td><td>Basophils</td></tr>
</table>
</td></tr>
<tr><td>Clinical Chemistry [1]</td><td>
<table>
<tr><td>BUN</td><td>Potassium</td><td>AST (SGOT)</td><td>Total and direct bilirubin</td></tr>
<tr><td>Creatinine</td><td>Sodium</td><td>ALT (SGPT)</td><td>Total Protein</td></tr>
<tr><td>Glucose</td><td>Calcium</td><td>Alkaline phosphatise</td><td>Albumin</td></tr>
</table>
</td></tr>
<tr><td>Routine Urinalysis[3]</td><td>As a minimum, but not limited to the following tests and dependent on standard urinalysis dipstick used:
• Specific gravity
• pH, glucose, protein, blood and ketones by dipstick
• Microscopic examination (if blood or protein is abnormal)</td></tr>
<tr><td>Other Screening Tests</td><td>• HIV
• Hepatitis B (HBsAg)
• Hepatitis C (Hep C antibody)
• Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)
• Serum or urine hCG Pregnancy test[2]</td></tr>
<tr><td colspan="2">NOTES :

20. Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.4.1 and Appendix 2

21. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

22. Routine Urinalysis results will not be databased, unless a result is out of range and clinically significant then it would be captured as an AE</td></tr>
</table>

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within7 days after the last dose of study treatment should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

8. DATA MANAGEMENT

- Data will be double-entered into a clinical database management system (ClinPlus Version 3.3).
- Management of clinical data will be performed in accordance with applicable HMR standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- Original CRFs will be retained by GSK, while HMR will retain a copy. Subject initials will not be collected or transmitted to GSK according to GSK policy

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

No formal hypothesis will be tested for study Part 1 and Part 2. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently, (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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference). The objective of Part 2 is to assess whether differences in granulation size impact the pharmacokinetics of ambrisentan and tadalafil; the estimation approach for bioavailability is therefore applicable to Part 2.

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

$$H(0): \mu(\text{test})/\mu(\text{reference}) < 0.80 \text{ or } \mu(\text{test})/\mu(\text{reference}) > 1.25,$$

i.e., treatments are not bioequivalent.

versus

$H(1) : 0.80 \leq \mu(\text{ test})/\mu(\text{reference}) \leq 1.25,$

i.e., treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test (1) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg taken concurrently (reference) and (2) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 20 mg taken concurrently (reference), in healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

9.2. Sample Size Considerations

9.2.1. Sample Size Assumptions

For Part 1 and Part 2 of the study, the sample size assumptions are based on previously reported estimates of within subject CV for $AUC_{(0-\infty)}$ and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005). Table 9 summarizes the estimates of within subject CV for the primary endpoints $AUC_{(0-\infty)}$ and Cmax.

Table 9 Estimates of within subject CV for the primary end points $AUC_{(0-\infty)}$ and C_{max}

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
$AUC_{(0-\infty)}$	15%	13%

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 based on a sample size of 20 statistically evaluable subjects.

For Part 3A of the study, Table 10 summarized the observed estimates of between and within subject CV and observed ratio of geometric means for the primary endpoints $AUC_{(0-t)}$ and C_{max} from Part 1 of the study.

Table 10 Estimates of within subject CV for the primary end points AUC $_{(0-\infty)}$ and C_{max} for ambrisentan and tadalafil from part 1 of the study

CVw: within subject CV	ambrisentan	tadalafil
C_{max}	22.42%	14.53%
AUC $_{(0-\infty)}$	8.12%	10.32%

The largest of the within subject CV estimates 22.42% translates to a standard deviation (SD) of 0.221 on the natural log scale. Based on this SD, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. This calculation assumes:

- a true ratio of 1,
- the within-subject variability from the current study will not be larger than that used in the sample size calculations,
- data are log-normally distributed, and each t-test is made at the 5% level.

For Part 3B of the study, same assumptions are adopted as Part 3A. Thus, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. However, if the within subject CVs obtained after Part 2 are significantly higher than the ones in Part 1 (shown in Table 10), a sample size re-esimation may be conducted to adjust the sample size for Part 3A and 3B to ensure the study maintain enough power to demonstrate bioequivalence.

9.2.2. Sample Size Sensitivity

For Part 1 and Part 2, assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then Table 11 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 11 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%

For Part 3, based on the observed data from previous studies, the ratio of geometric mean for ambrisentan Cmax is unlikely to be 1. The effects on the power of declaring bioequivalence in the face of a shift in the expected ratio of the geometric means were examined.

If, under all other assumptions outlined above, the actual ratio of geometric means for ambrisentan Cmax is 0.97, then 26 evaluable subjects are needed to have 90% power to conclude bioequivalence.

With 26 evaluable subjects, the current study design has at least 90% power to conclude bioequivalance (with both Cmax and AUC for ambrisentan and tadalafil).

9.2.3. Sample Size Re-estimation or Adjustment

No sample size re-estimation will be performed. However, if the estimation of the within subject CV from Part 2 of the study is much larger than the within subject CV from Part 1 of the study, the sample size for Part3 may be re-evaluated.

9.3. Data Analysis Considerations

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed 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 analayis 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 Cmax $_{(tmax)}$, area under the plasma concentration-time curve [$AUC_{(0-t)}$ and $AUC_{(0-\infty)}$], and apparent terminal phase half-life ($t_{1/2}$).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in fixed effect ANOVA model and mixed effects model.

9.3.1. Analysis Populations

9.3.1.1. Safety Population

All subjects enrolled into the study who have received at least one dose of investigational product will be included in the Safety Population.

9.3.1.2. Pharmacokinetic Concentration Population

The PK Concentration Population will include all subjects for whom at least one PK sample was obtained and analyzed.

9.3.1.3. Pharmacokinetic Parameter Population

For each PK parameter, the PK Parameter Population will include all subjects who provide PK parameter data.

9.3.2. Interim Analysis

No formal interim analysis is planned. However, ongoing analyses of pharmacokinetic data will be performed in Part 1 and Part 2 of the study in order to direct development of the FDC for later parts of the study. Headline results based on statistical analysis using preliminary pharmacokinetic data with nominal time may be produced when 80% and 100% subjects complete Part 1 and Part 2 of the study to assist development of FDCs. Treatment and period information from the crossover design may be used in the analysis; sequence information may also be included depending on availability.

9.4. Key Elements of Analysis Plan

9.4.1. Pharmacokinetic Analyses

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

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

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.

The PK parameters, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, 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.

Actual visit day will be used for safety assessments. For the assessment of bioequivalence, both actual time of sampling, and nominal (planned) sampling time, will be used to estimate PK parameters at the end of the study. PK parameters estimation based on nominal sampling time will be used for generating headline results. According to EMEA guidilines on **GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE (**2010, Doc. Ref.:[CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **. 2010]), following log_e-transformation, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} of FDC formulations and reference will be separately analyzed using fixed effect ANOVA model with fixed effect terms for sequence, subject within sequence, period and treatment (formulation).

As a sensitivity analysis, mixed effect model with fixed effect terms for period, sequence and treatment (formulation), and subject as random effect term (i.e. ANOVA method using period, sequence and treatment as fixed effect, and subject as random effect) will be performed.

Point estimates and their associated 90% confidence intervals will be calculated for the differences: F1 - R, F2 - R, F3 -R, F4 - R for Part 1; FG1-R, FG2-R, FG3-R for Part 2; X1-R1, X2-R2 for Part 3A; and Y1-R3, Y2-R4 for Part 3B. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2:R, F3:R, F4:R; for Part 1; FG1:R, FG2:R, FG3:R for Part 2; X1:R1,X2:R2 for Part3A; and Y1:R3 and Y2:R4 for Part 3B.

9.4.2. Safety Analyses

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

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

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

Prior to initiation of a site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Obtaining signed informed consent
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.
- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

10.3. Quality Control (Study Monitoring)

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

GSK will monitor the study and site activity to verify that the:

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.
- If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.

- If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action.
- If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.
- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.
- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the

opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

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

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

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

11. REFERENCES

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
API	Active Pharmaceutical Ingredient
AUC	Area Under Curve
BE	Bioequivalence
CDISC	Clinical Data Interchange Standards Consortium
cGMP	Cyclic guanosine monophosphate
CI	Confidence Interval
C_{max}	Maximum concentration
CV	Coefficient of variance
CVw	Coefficient of variance within subject
EMA	European Medicines Agency
ERA	Endothelin Receptor Antagonists
ERS	European Respiratory Society
ESC	European Society of Cardiology
ET-1	Endothelin Receptor - 1
EU	European Union
FDA	Federal Drug Agency
FDC	Fixed Dose Combination
NAION	Non-arteritic anterior ischemic optic neuropathy
NO	Nitrous Oxide
PDE-5	Phosphodiesterase type 5
SAE	Serious Adverse Events
SRM	Study Reference Manual
$t_{1/2}$	Half Life
t_{max}	Time to maximum concentration
WHO	World Health Organisation

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Adcirca
WinNonlin

12.2. Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event aetiology (in alignment with the FDA premarketing clinical liver safety guidance).

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td colspan="2">ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >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 following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th colspan="2">Follow-Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment
• 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 subject until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline</td><td colspan="2">• Viral hepatitis serology[3]
• Blood sample for pharmacokinetic (PK) analysis, obtained within 96hrs of last dose
• 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
If ALT≥3xULN AND bilirubin ≥ 2xULN or</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
• A specialist or hepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	**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 HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. • 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 treatment 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, 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); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
4. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.

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12.3. Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.3.1. Definition of Adverse Events

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

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • 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 treatment administration even though it may have been present prior to the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae).

Events <u>NOT</u> meeting definition of an AE include:
• 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 subject'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 subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE. • Situations where an untoward medical occurrence did not occur (social and/or

convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

12.3.2. Definition of Serious Adverse Events

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, etc).

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

NOTE:

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

c. Requires hospitalization or prolongation of existing hospitalization

NOTE:

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

d. Results in disability/incapacity

NOTE:

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

e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. • Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse
g. Is associated with liver injury <u>and</u> impaired liver function defined as: • ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), **or** • ALT ≥ 3xULN and INR** > 1.5. * Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE. ** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.
• Refer to Section 12.2 for the required liver chemistry follow up instructions

12.3.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:
Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs: • Myocardial infarction/unstable angina • Congestive heart failure • Arrhythmias • Valvulopathy • Pulmonary hypertension • Cerebrovascular events/stroke and transient ischemic attack • Peripheral arterial thromboembolism • Deep venous thrombosis/pulmonary embolism • Revascularization

12.3.4. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF
- It is **not** acceptable for the investigator to send photocopies of the subject'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 instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.
- Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study.
- Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer.
- The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

12.3.5. Evaluating AEs and SAEs

Assessment of Intensity

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

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence 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 natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of 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 when 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 prior to the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow up information, amending the SAE data collection tool accordingly.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.
- The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.
- If a subject 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 the designated reporting time frames.

12.3.6. Reporting of SAEs to GSK

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 or the SAE coordinator • 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 • Initial notification via the 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 receipt can be found at this beginning of the protocol on the Sponsor/Medical Monitor Contact Information page.

12.4. Appendix 4 High Fat Meal Content

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the EMEA guidelines on the investigation of bioequivalence 2010. The meal should be a high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 kcal) meal. This test meal should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively.(EMEA, 2010)

12.5. Appendix 5: Protocol Changes

12.5.1. Protocol changes for Amendment 01 (14-Apr-2016) from Original Protocol (16-Dec-2015)

The purpose of this amendment is to clarify the hypotension withdrawal criteria in section 5.4.3, address changes to GSK medical monitor and minor administrative changes.

Summary of Changes

Section 1 PROTOCOL SYNOPSIS FOR STUDY 201964

AND

Section 3 OBJECTIVES AND ENDPOINTS

CHANGE FROM

Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; $t_{max,}$ $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assed by vital signs, ECG, clinical laboratory safety data and review of adverse events

CHANGE TO

Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; $t_{max,}$ $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as **assessed** by vital signs, ECG, clinical laboratory safety data and review of adverse events

Section 5.4.3 Hypotension

CHANGE FROM

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 or diastolic <50 confirmed by triplicate reading taken up to 5 minutes apart.

CHANGE TO

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 mm HG **and** diastolic <50 mm HG confirmed by triplicate reading taken up to 5 minutes apart **and is judged clinically significant and symptomatic by the investigator**.

Section 6.3 Treatment Assignment

ADDITION

Table 6 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Part 2 and Part 3 merged together	5 (Two FDCs selected from Part 1)	X1 X2 R Y1 Y2 X2 Y1 X1 Y2 R Y1 Y2 X2 R X1 Y2 R Y1 X1 X2 R X1 Y2 X2 Y1 Y2 Y1 R X2 X1 R Y2 X1 Y1 X2 X1 R X2 Y2 Y1 X2 X1 Y1 R Y2 Y1 X2 Y2 X1 R	1:1:1:1:1:1:1:1:1:1
	3 (One FDC selected from Part 1)	X1 X2 R R X2 X1 X2 R X1 F1 R X2 R X1 X2 X2 X1 R	3:3:3:3:3:3 + 2 random

12.5.2. Protocol changes for Amendment 2 (08-FEB-2017) from Amendment 1(14-Apr-2016)

Amendment 2 Summary of Changes

The purpose of this substantial amendment is to include bioequivalence assessment in Part 3 to replace the optional food effect and the addition of new dosage strengths. Furthermore GSK medical monitor contact have changed

List of Specific Changes

Medical Monitor/SAE Contact Information

Rationale for Change: GSK medical monitor contact have changed

CHANGE FROM

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Monitor				Site to scan and send to email
SAE contact information				Site to scan and send to email

CHANGE TO

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Medical Monitor				**Site to scan and send to email**

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
SAE contact information	PPD			**Site to scan and send to email**

Section 1 - Protocol Synopsis Overall design

CHANGE FROM**:**

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects.

All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast

CHANGE TO**:**

This is a single centre, Phase 1, single dose, randomised, open label crossover **study in healthy volunteers** with 3 study parts. **Part 1 will include a 5 way cross-over. Part 2 and Part 3 (A&B) will each include a 4 way cross-over. All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule**.

There will be a minimum washout of 7 days between each dose in **Parts 1 and 2. In Part 3 the washout will be at least 10 days.** A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part will **include an evaluation of the bioavailability, safety and tolerability of 3 granulation forms of a single FDC** (ambrisentan 10mg + tadalafil 40 mg) **identified from Part 1. These data will be compared to that for the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.**

Part 3 A

Part 3A of the study is set to establish bioequivalence between the candidate FDC from Part 2. This study part will have 4 dose sessions and will assess the bioequivalence, in both the fed and fasted state, of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state. The fed arms of this part will have a standard high fat breakfast. (EMEA, 2010).

Part 3 B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg). This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

Section 1 - Protocol Synopsis Treatment Arms and Duration

CHANGE FROM**:**

The proposed treatment arms for each study part are described here; however the treatments in Parts 2 and 3 may be changed dependent on the utility and results from the previous part.

Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)[1]**	**Part 3[2]**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less FDC treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. The minimum will be approximately 3 weeks and the maximum will be approximately 18 weeks.

CHANGE TO**:**

The proposed treatment arms for each study part are described here

Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)**	**Part 3A (fed and fasted)**	**Part 3B (fasted)**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 1 (10mg/40mg)	**ambrisentan and tadalafil FDC) (10mg/40mg), fed**	**Ambrisentan and tadalafil FDC (5mg/40mg), fasted**
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 2 (10mg/40mg)	**ambrisentan and tadalafil FDC (10mg/40mg) fasted**	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted**
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 3 (10mg/40mg)	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed**	**ambrisentan and tadalafil FDC (5mg/20mg), fasted**
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted**	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted**
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

Section 1 - Protocol Synopsis Type and Number of Subjects

CHANGE FROM:

A approximately of 20 healthy adult subjects will be randomized, to each study part, such that at least 16 evaluable subjects complete each part of the study

CHANGE TO:

Approximately 20 healthy adult subjects will be randomized to study Part 1 and an additional 20 healthy adult subjects in Part 2, such that at least 16 evaluable subjects complete each part of the study. Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subejcts will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study.

Section 1 - Protocol Synopsis Analysis

CHANGE FROM:

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and Part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in Part 2, if used for food effect and Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

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

CHANGE TO:

No formal hypothesis will be tested for Part 1 and Part 2 of the study. An estimation approach will be used to estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

H(0): μ(test)/μ(reference) < 0.80 or μ(test)/μ(reference) > 1.25,

i.e., treatments are not bioequivalent.

versus

H(1) : 0.80 ≤ μ(test)/μ(reference) ≤ 1.25,

i.e., treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) and of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg and of ambrisentan 5 mg & tadalafil 20 mg respectively taken concurrently (reference) in healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

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

Section 2 Introduction

CHANGE FROM

Ambrisentan (E.U. trade name: Volibris), an orally active endothelin receptor antagonists (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease

Tadalafil (E.U. trade name: Adcirca) is an orally active selective inhibitor of the enzyme PDE-5, the primary cGMP-hydrolyzing enzyme in smooth muscle. In the EU, Adcirca is indicated in adults for the treatment of PAH classified as WHO FC II and III, to improve exercise capacity (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015)A recently completed study (Galiè, 2015a) has show that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris EMA SmPC, 2015, Section 5.1).

This pilot study will investigate the relative bioavailability of new fixed dose combinations (FDC) of ambrisentan and tadalafil, compared to the two monotherapies taken concurrently in healthy subjects

CHANGE TO

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~~This pilot study will investigate the relative bioavailability of new fixed dose combinations (FDC) of ambrisentan and tadalafil, compared to the two monotherapies taken concurrently in healthy subjects~~

Section 2.1 Study Rationale

CHANGE FROM

This study is designed to select one, or more, FDCs of ambrisentan and tadalafil for further development and to provide pharmacokinetic data to enable a pivotal bioequivalence study (BE). Dependent on formulation work, the study will allow up to 8 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently. The study would also allow for up to 2 of the new formulations, that may be taken in to a BE study, to be tested for any effect on pharmacokinetics of the FDC in both fed and fasted state.

The formulation(s) to be taken forward into the BE study will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, between subject variability, tablet size, cost, ease of manufacture and stability would be considered.

CHANGE TO

This study is **designed initially to compare** the relative bioavailability of **a number of** fixed dose combinations **(FDCs)** of ambrisentan and tadalafil **(Part 1 and 2) and consequently** the bioequivalence **of the FDC of different dose strengths (Part 3A and 3B).** Dependent on formulation work, the study will allow up to 9 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently

The formulation(s) to be taken forward **in Part 3A** will be primarily chosen **based** on the test/reference ratio for both AUC and Cmax for both components. **Ideally** the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, tablet size, cost, ease of manufacture and stability would be considered.

Part 3 will include evaluation of bioequivalence for 3 dose strengths of the FDC; 10/40mg, 5/40mg and 5/20mg of ambrisentan and tadalafil.

Section 2.2 Brief Background

CHANGE FROM

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3 biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galiè, 2015b) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.(Volibris, EMA SmPC,2015). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015).Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5)

inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112, 2008) in 26 healthy subjects was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-administered. . There were no SAE's in the study. Three subjects withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one subject because of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a listed event for ambrisentan. There were a total of 7 subjects with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris, EMA SmPC, 2015;EMEA, 2010; Schuirmann, 1987;Adcirca, 2015).

The AMBITION clinical study (Galiè, 2015a and AMB112565 CSR 13Nov2014), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galiè, 2015b) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, CHMP/EWP/240/95) (2008).

CHANGE TO

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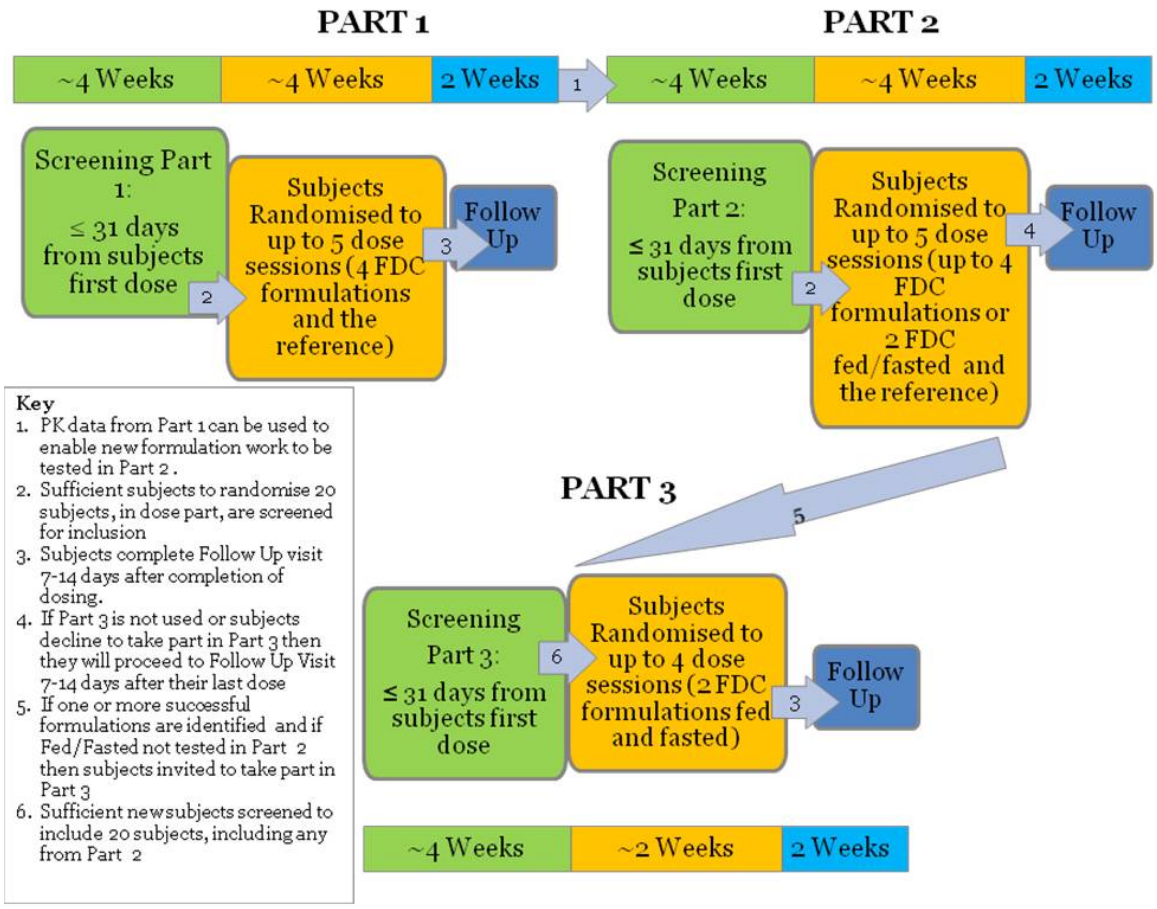
and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, 2008).

Section 4.1 Overall Design

CHANGE FROM

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects. See Figure 1 for study schematic.

Figure 2 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

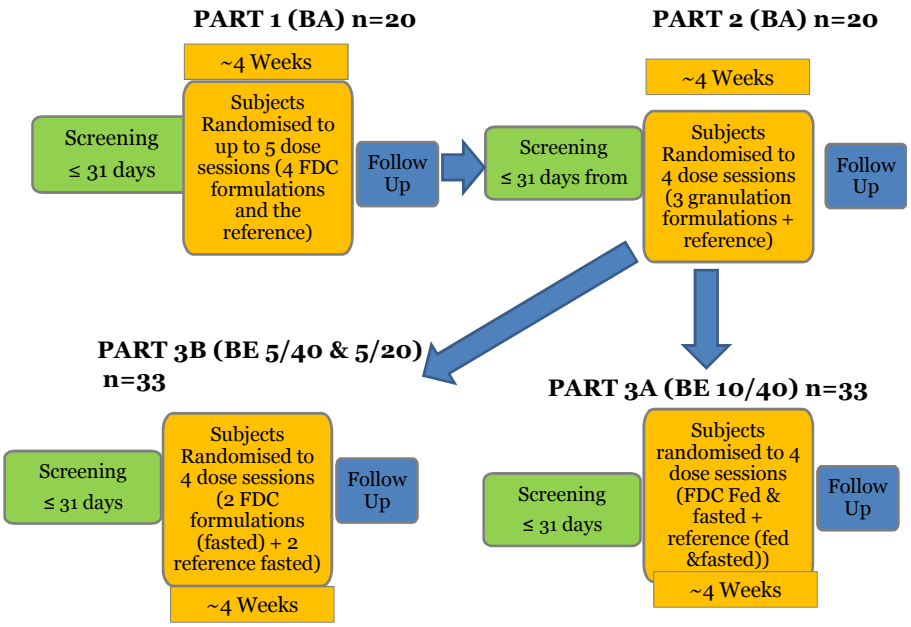
This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast.

Subjects who had taken part in the study part preceding Part 3, and which provided the successful formulations for a BE study could also be invited to take part in this study part. However, a subject's inclusion in more than one study part would be dependent on the subject not exceeding the maximum blood draw volume (500ml) for the study.

CHANGE TO

This is a single centre, Phase 1, single dose, randomised, open label, crossover study in **healthy volunteers with 3 study parts**; **Part 1 will include a 5 way cross-over, and Part 2 and 3 (A&B) will each include a 4 way cross-over.** See Figure 1 for study schematic.

Figure 1 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in **study Part 1 and 2. In study Part 3 there will be a minimum of 10 days between each dose**. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose

combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

Part 2

This study part will have **4** dose sessions **and** will **evaluate** the bioavailability, safety and tolerability of **3 different granulation sizes for** a **single FDC** (ambrisentan 10 mg + tadalafil 40 mg) **compared to** the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

Part 3A

Part **3A** of the study is **set to establish bioequivalence between** the **candidate FDC from Part 2**. This study part will have 4 dose sessions and **will assess the bioequivalence**, in both the fed and fasted state, **of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state**. The fed arms of this part will have a standard high fat breakfast **(EMEA, 2010)**.

Part 3B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg) . This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference ambrisentan 5 mg + tadalafil 40mg monotherapies and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

Section 4.2 Treatment and Duration

CHANGE FROM

The treatments for each study part of the study are listed in Table 1 All treatments are single dose. Subjects will be randomised to order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing analyse of pharmacokinetic data will be used to enable the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to look at up to 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2. If no successful formulations are identified from these criteria then the pharmacokinetic data would be used to enable further work to produce new FDC formulations for Part 2

Following completion of Part 1 there will be a pause prior to Part 2, so that up to a further 4 FDC formulations could be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for up to 4 FDC formulations. These could be either successful formulation identified in Part 1 reformulated with the final API and/or new formulations using the final API. Pharmacokinetic data from Part 2 of the study will be analysed following completion of each treatment arm by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to define any successful FDC formulation to be taken into Part 3 of this study and a pivotal BE study. If only two formulations are evaluated in Part 2 then the food effect may be added to Part 2 and Part 3 will not be required. Success will be defined with the same criteria as those in Part 1.

Part 3

Part 3 of the study will provide data for up to 2 successful formulations to be progressed to a pivotal BE study, in both the fed and fasted state.

Table 1 Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)[1]	Part 3[2]
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. Subjects may be included in Part 1, Part 2 or Part 3 of the study. However, if Part 2 of the study identifies any successful formulations to take into Part 3 then subjects from Part 2 may be invited to join Part 3.

The minimum and maximum duration for a subject on this study is described in Figure 2. This table assumes all 5 dose sessions are used for Part 1 and Part 2 and either 2 or 4 dose sessions are used in Part 3. However, this is an approximation as fewer sessions may be required in Part 2 and/or the washout between sessions of 7 days in each part may be longer, due to logistical reasons.

Table 2 Study Duration

Study Options		**Screen**	**Study Part 1or 2**	**Pause**	**Study part 3**	**Follow-Up Visit**	**Total Duration on Study**
		(1-31 days)	**(31 days)**	**(7-28 days)**	**(10-24 days)**	**(7-14 days)**	
Subjects included in only Part 1 or 2	Min	1	31			7	39
	Max	31	31			14	76
Subjects included in Part 2&3	Min	1	31	7	10	7	56
	Max	31	31	28	24	14	128
Subjects included in only Part 3	Min	1			10	7	18
	Max	31			24	14	69

A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

CHANGE TO

The treatments for each study part of the study are listed in Table 1. All treatments are single dose. Subjects will be randomised to **the** order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing **analysis** of pharmacokinetic data will be used to **determine** the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to **evaluate** 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each

FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2 **and 3**.

Following completion of Part 1 there will be a pause prior to Part 2, so that **different granulation forms of a single FDC can** be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for 3 **granulation size forms of a single** FDC **formulation selected from Part 1**. These **will** be **based on a single** successful formulation identified in Part 1 reformulated with the final **active pharmaceutical ingredient** (API). Success will be defined with the same criteria as those in Part 1.

Part 3 (A and B)

The BE part of the study will investigate the bioequivalence of the FDC ambrisentan 10 mg + tadalafil 40 mg in part 3A and the bioequivalence of the FDC ambrisentan 5 mg + tadalafil 40 mg and ambrisentan 5 mg + tadalafil 20 mg in Part 3B.

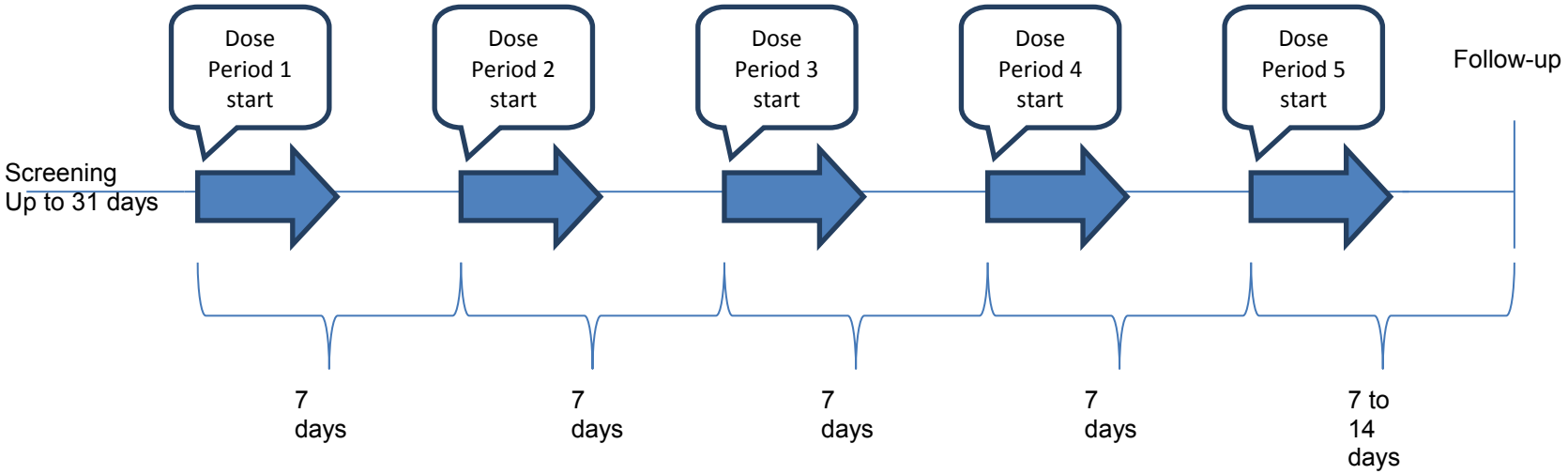
Table 1 Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)**	Part **3A (fed and fasted)**	**Part 3B (fasted)**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil **FDC granulation 1** (10mg/40mg)	ambrisentan and tadalafil **FDC** (10mg/40mg), fed	**Ambrisentan and tadalafil FDC (5mg/40mg), fasted**
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil **FDCgranulation 2** (10mg/40mg)	ambrisentan and tadalafil **FDC** (10mg/40mg) fasted	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted**
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil **FDC granulation 3** (10mg/40mg)	ambrisentan and tadalafil **monotherapies taken concurrently** (10mg/40mg), fed	**ambrisentan and tadalafil FDC (5mg/20mg), fasted**
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil **monotherapies taken concurrently** (10mg/40mg)	ambrisentan and tadalafil **monotherapies taken concurrently** (10mg/40mg), fasted	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted**
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

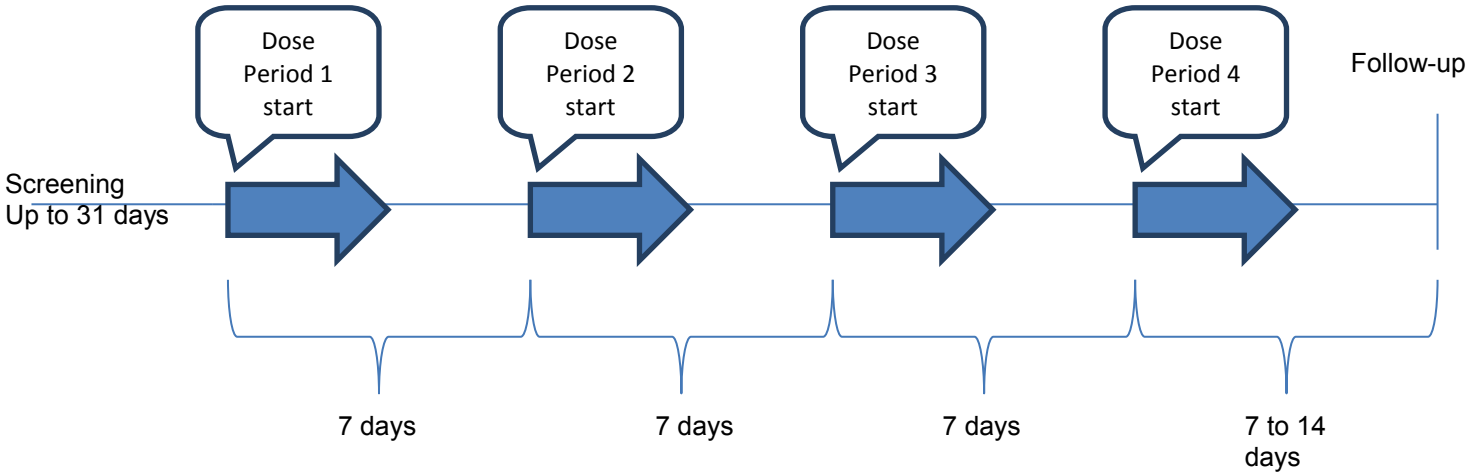
The duration for a subject on this study is described in Figure 2. This **figure** assumes all 5 dose sessions are used for Part 1. However, this is an approximation as **minimum** of 7 days **is expected between doses in Parts 1 and 2 and a minimum of 10 days in Part 3**.

Figure 2 Study Duration

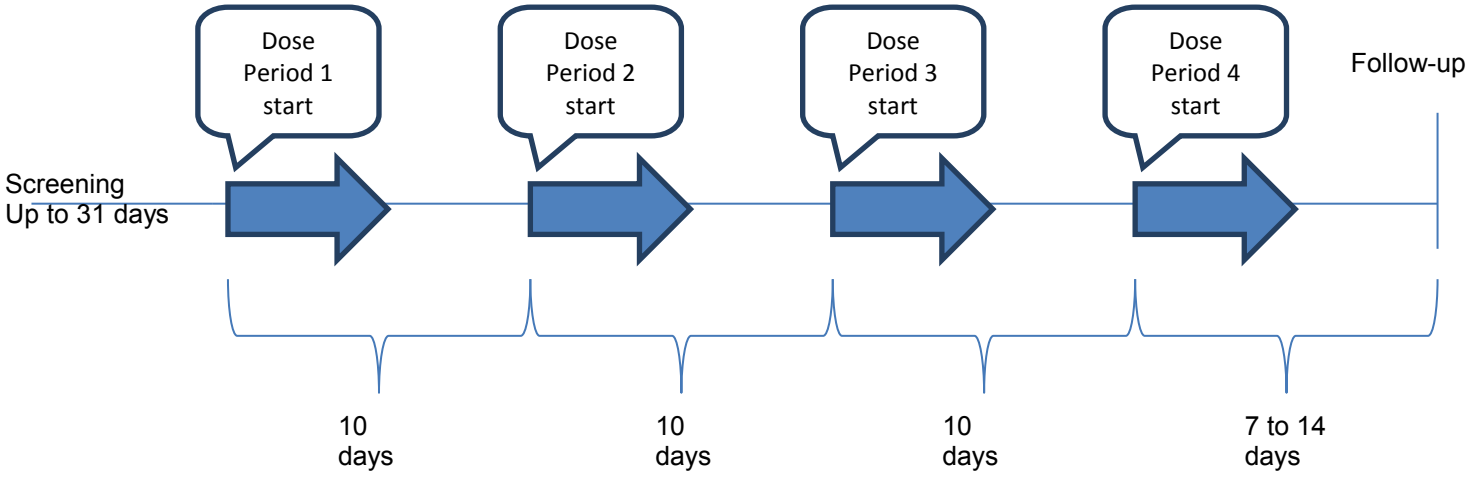
Part 1 can last up to 73 days.



Part 2 will last approximately 9 weeks



Part 3A and B will last approximately 11 weeks



A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

Section 4.3 Type and Number of Subjects

CHANGE FROM

A approximately of 20 healthy adult subjects will be randomized, to each study part, such that at least 16 evaluable subjects complete each part of the study.

The study is not powered. However, the number of subjects will be sufficient for the objectives of this study and this is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

CHANGE TO

Approximately 20 healthy adult subjects will be randomized to study **Part 1 and an additional 20 healthy adult subjects in Part 2**, such that at least 16 evaluable subjects complete each part of the study. **Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subjects will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study**.

The estimation of the number of subjects for **each** study **part** is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

Section 4.4 Design Justification

CHANGE FROM

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations to take into a pivotal BE study and to also provide pharmacokinetic data to enable the pivotal BE study to be powered sufficiently.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, or is there any need to blind study treatment. The Inclusion of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study is in three parts to enable PK data from Part 1 to enable formulation development for Part 2. Part 3 providing an opportunity to assess food effect in any FDC formulations suitable for development and inclusion in the pivotal bioequivalence study. The interdependency of the 3 study parts is described in detail in Section 4.2.

CHANGE TO

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, **nor** is there any need to blind study treatment. The **inclusion** of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study **contains** three parts: **Parts 1 and 2 will provide PK data for different candidate** FDC formulations and **will enable the assessment of bioequivalence of the FDC versus the combination of the individual reference products in Part 3.** The interdependency of the 3 study parts is described in detail in Section 4.2.

Section 4.5 Dose Justification

CHANGE FROM

The oral dose for the new FDC formulations and the reference treatment proposed for the study of, 40mg for tadalafil and 10mg for ambrisentan are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to be tolerated in healthy subjects.

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates a minimum washout of 7 days, between doses, is also sufficient for clearance of previous dose.

CHANGE TO

The oral dose for the new FDC formulations and the reference treatment proposed for the study of 40mg for tadalafil and 10mg for ambrisentan, are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to be tolerated in healthy subjects. **Within Part 3 of the study all dosage forms of the FDC that are proposed for marketing authorisation will be tested for bioequivalence versus the appropriate doses of reference products. These doses are required in clinical practice to allow safe up- and down-titration of the FDC in patients with PAH.**

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates **a minimumof 10** days, between doses, is also sufficient for clearance of **the** previous dose **during the assessmet of bioequivalence**.

Section 4.6.2 Benefit Assessment

CHANGE FROM

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

CHANGE TO

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation **and to demonstrate bioequivalence versus reference products** for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

Section 4.6.3 Overall Benefit: Risk Conclusion

CHANGE FROM

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 3. So, the risk: benefit is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

CHANGE TO

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 3. **In addition review of safety information from Part 1 of the study is consistent with this position.** So, the benefit:risk is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

Section 6.1 Investigational Product and Other Study Treatment

CHANGE FROM

The term ‘study treatment’ is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for Part 1 of the study are shown in Table 4, this will be amended once treatments for Part 2 and Part 3 are confirmed.

Table 4 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS**	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 233 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

CHANGE TO

The term ‘study treatment’ is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for **Parts** 1, 2 **and 3** of the study are shown in Table 4, **Table 5 and Table 6.**

Table 4 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately **90** mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.**45** mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, **245** mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Address of reference manufacturer	**NA**	**NA**	**NA**	**NA**	**Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom**	**Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain**

Table 5 Study Treatment for Part 2

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference	
Formulation description	FDC-G1 granulation 1 (10mg/40mg)	FDC-G2 granulation 2 (10mg/40mg)	FDC-G3 granulation 3 (10mg/40mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.45 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference	
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

Table 6 Study Treatment for Part 3

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Formulation description	FDC-G1 or 2 or3 (10mg/40mg)	FDC (5mg/40mg)	FDC (5mg/20mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each tablet contains 5 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura Red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

6.1.1 Retention Samples

GSK will send five times (5x) the drug product necessary for pivotal bioequivalency evaluation in Part 3 of the study for analytical testing for all tablet strengths used in the study. The retain samples are in addition to the supply of drug product sufficient to complete the study. Retention samples will be sent to the clinical CRO in the same bulk container. To ensure that reserve samples are representative of the same batches provided for the clinical study the clinical site will randomly select the supplies for the study from the supply received. The clinical site must retain enough reserve samples to permit FDA to perform five times all of the release tests required in the application.

Section 6.3 Treatment Assignment

CHANGE FROM

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

The treatments are denoted as F1 to F4 for Part 1, F5 to F8 for Part 2 and R for reference treatment for both parts; the selected formulations for Part 3 are denoted as X and Y. The treatment key for Parts 1, 2 and 3 are described in Table 6

Table 6 Treatment Key for Part 1, Part 2 and Part 3

Treatment	Description
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 2	
F5	ambrisentan and tadalafil FDC5 (10mg/40mg)
F6	ambrisentan and tadalafil FDC6 (10mg/40mg)
F7	ambrisentan and tadalafil FDC7 (10mg/40mg)
F8	ambrisentan and tadalafil FDC8 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 3	

Treatment	Description
X1	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
X2	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
Y1	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
Y2	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 7. Not all possible sequences are included here. Such as, if only one formulation taken into Part 2 or 3, or Part 2 is used for only one or two FDC formulations, fed and fasted. Additional treatment sequences will be created based on the Latin Squares for Williams Designs, as required

Table 7 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1 F2 R F3 F4 F2 F3 F1 F4 R F3 F4 F2 R F1 F4 R F3 F1 F2 R F1 F4 F2 F3 F4 F3 R F2 F1 R F4 F1 F3 F2 F1 R F2 F4 F3 F2 F1 F3 R F4 F3 F2 F4 F1 R	1:1:1:1:1:1:1:1:1:1
Part 2	5	F5 F6 R F7 F8 F6 F7 F5 F8 R F7 F8 F6 R F5 F8 R F7 F5 F6 R F5 F8 F6 F7 F8 F7 R F6 F5 R F8 F5 F7 F6 F5 R F6 F8 F7 F6 F5 F7 R F8 F7 F6 F8 F5 R	1:1:1:1:1:1:1:1:1:1
	4	F5 F6 R F7 F6 F7 F5 R F7 R F6 F5 R F5 F7 F6	1:1:1:1
	3	F5 F6 R R F6 F5 F6 R F5 F1 R F6 R F5 F6 F6 F5 R	3:3:3:3:3:3 + 2 random
	2	F5 R	1:1

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
		R F5	
Part 3	4	X1 X2 Y2 Y1 X2 Y1 X1 Y2 Y1 Y2 X2 X1 Y2 X1 Y1 X2	1:1:1:1
Part 2 and Part 3 merged together	5 (Two FDCs selected from Part 1)	X1 X2 R Y1 Y2 X2 Y1 X1 Y2 R Y1 Y2 X2 R X1 Y2 R Y1 X1 X2 R X1 Y2 X2 Y1 Y2 Y1 R X2 X1 R Y2 X1 Y1 X2 X1 R X2 Y2 Y1 X2 X1 Y1 R Y2 Y1 X2 Y2 X1 R	1:1:1:1:1:1:1:1:1:1
	3 (One FDC selected from Part 1)	X1 X2 R R X2 X1 X2 R X1 F1 R X2 R X1 X2 X2 X1 R	3:3:3:3:3:3 + 2 random

CHANGE TO

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software. ~~The treatments are denoted as F1 to F4 for Part 1, F5 to F8 for Part 2 and R for reference treatment for both parts; the selected formulations for Part 3 are denoted as X and Y~~

The treatment **key for Parts 1, 2 and 3 are described in Table 6**.

Table 6 Treatment Key for Part 1, Part 2 and Part 3

Treatment	**Description**
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)

Treatment	Description
Part 2	
FG1	**ambrisentan and tadalafil FDC-G1 (10mg/40mg) granulation size 1**
FG2	**ambrisentan and tadalafil FDC-G2 (10mg/40mg) granulation size 2**
FG3	**ambrisentan and tadalafil FDC-G3 (10mg/40mg) granulation size 3**
R	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)**
Part 3A	
X1	**ambrisentan and tadalafil FDC-G1 or 2 or 3 (10mg/40mg) fed**
X2	**ambrisentan and tadalafil FDC G1 or 2 or 3 (10mg/40mg) fasted**
R1	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed**
R2	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted**
Part 3B	
Y1	**ambrisentan and tadalafil FDC (5mg/40mg), fasted**
Y2	**ambrisentan and tadalafil FDC (5mg/20mg), fasted**
R3	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted**
R4	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted**

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 7. ~~Not all possible sequences are included here. Such as, if only one formulation taken into Part 2 or 3, or Part 2 is used for only one or two FDC formulations, fed and fasted~~ Additional treatment sequences may be created based using the Latin Squares for Williams Designs **if needed**.

Table 7 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1/ F2/ R/ F3/ F4 F2/ F3/ F1/ F4/ R F3/ F4/ F2/ R/ F1 F4/ R/ F3/ F1/ F2 R/ F1/ F4/ F2/ F3 F4/ F3/ R/ F2/ F1 R/ F4/ F1/ F3/ F2 F1/ R/ F2/ F4/ F3 F2/ F1/ F3/ R/ F4 F3/ F2/ F4/ F1/ R	1:1:1:1:1:1:1:1:1:1

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 2	4	FG1/ FG2/ R/ FG3 FG2/ FG3/ FG1/ R FG3/ R/ FG2/ FG1 R/ FG1/ FG3/ FG2	1:1:1:1
Part 3A	**4**	**X1/ R1/ R2/ X2** **R1/ X2/ X1/ R2** **X2/ R2/ R1/ X1** **R2/ X1/ X2/ R1**	**1:1:1:1**
Part 3B	**4**	**Y1/ R3/ R4/ Y2** **R3/ Y2/ Y1/ R4** **Y2/ R4/ R3/ Y1** **R4/ Y1/ Y2/ R3**	**1:1:1:1**

Section 9.1 Hypotheses

CHANGE FROM

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and if used, Study part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in to Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

CHANGE TO

No formal hypothesis will be tested **for study Part 1 and Part 2.** An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently**, (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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference). The objective of Part 2 is to assess whether differences in granulation size impact the pharmacokinetics of ambrisentan and tadalafil; the estimation approach for bioavailability is therefore applicable to Part 2.**

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric

mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

H(0): μ(test)/μ(reference) < 0.80 or μ(test)/μ(reference) > 1.25,

i.e., treatments are not bioequivalent.

versus

H(1) : 0.80 ≤ μ(test)/μ(reference) ≤ 1.25,

i.e. , treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test (1) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg taken concurrently (reference) and (2) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 20 mg taken concurrently (reference), in healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

Section 9.2 Sample Size Considerations

CHANGE FROM:

No formal sample size calculation has been performed. Sample sizes chosen for each study part in this study is considered sufficient for exploratory analysis based on PK variability data from previous studies

CHANGE TO

~~No formal sample size calculation has been performed. Sample sizes chosen for each study part in this study is considered sufficient for exploratory analysis based on PK variability data from previous studies~~

Section 9.2.1 Sample Size Assumptions

CHANGE FROM

The sample size assumptions are based on previously reported estimates of within subject CV for AUC(0-∞) and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005). Table 9 summarizes the estimates of within subject CV for the primary endpoints AUC (0-∞) and Cmax.

Table9 Estimates of within subject CV for the primary end points AUC (0-∞) and Cmax

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
AUC (0-∞)	15%	13%

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 based on a sample size of 20 statistically evaluable subjects.

CHANGE TO

For Part 1 and Part 2 of the study, the sample size assumptions are based on previously reported estimates of within subject CV for $AUC_{(0-\infty)}$ and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005**). Table 9** summarizes the estimates of within subject CV for the primary endpoints $AUC_{(0-\infty)}$ and Cmax.

Table 9 Estimates of within subject CV for the primary end points AUC (0-∞) and Cmax

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
$AUC_{(0-\infty)}$	15%	13%

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 based on a sample size of 20 statistically evaluable subjects.

For Part 3A of the study, Table 10 summarized the observed estimates of between and within subject CV and observed ratio of geometric means for the primary endpoints $AUC_{(0-t)}$ and C_{max} from Part 1 of the study.

Table 10 Estimates of within subject CV for the primary end points AUC $_{(0-\infty)}$ and C_{max} for ambrisentan and tadalafil from part 1 of the study

CVw: within subject CV	ambrisentan	tadalafil
C_{max}	22.42%	14.53%
AUC $_{(0-\infty)}$	8.12%	10.32%

The largest of the within subject CV estimates 22.42% translates to a standard deviation (SD) of 0.221 on the natural log scale. Based on this SD, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. This calculation assumes:

- **a true ratio of 1,**
- **the within-subject variability from the current study will not be larger than that used in the sample size calculations,**
- **data are log-normally distributed, and each t-test is made at the 5% level.**

For Part 3B of the study, same assumptions are adopted as Part 3A. Thus, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. However, if the within subject CVs obtained after Part 2 are significantly higher than the ones in Part 1 (shown in Table 10), a sample size re-esimation may be conducted to adjust the sample size for Part 3A and 3B to ensure the study maintain enough power to demonstrate bioequivalence.

Section 9.2.2 Sample Size Sensitivity

CHANGE FROM

Assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then Table 11 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 11 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%

CHANGE TO

For Part 1 and Part 2, assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then **Table 11** shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects**.**

Table 11 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%

For Part 3, based on the observed data from previous studies, the ratio of geometric mean for ambrisentan Cmax is unlikely to be 1. The effects on the power of declaring bioequivalence in the face of a shift in the expected ratio of the geometric means were examined.

If, under all other assumptions outlined above, the actual ratio of geometric means for ambrisentan Cmax is 0.97, then 26 evaluable subjects are needed to have 90% power to conclude bioequivalence.

With 26 evaluable subjects, the current study design has at least 90% power to conclude bioequivalance (with both Cmax and AUC for ambrisentan and tadalafil).

Section 9.2.3 Sample Size Re-estimation or Adjustment

CHANGE FROM

No sample size re-estimation will be performed.

CHANGE TO

No sample size re-estimation will be performed**. However, if the estimation of the within subject CV from Part 2 of the study is much larger than the within subject CV from Part 1 of the study, the sample size for Part3 may be re-evaluated.**

Section 9.3 Data Analysis Considerations

CHANGE FROM

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed by non-compartmental methods with WinNonlin [version 6.3 or above]. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data, the following pharmacokinetic parameters will be determined, as data permit: maximum observed plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curve [AUC(0-t) and AUC(0-∞)], and apparent terminal phase half-life (t1/2).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in a mixed effects model.

CHANGE TO

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed 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 analayis 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 Cmax $_{(tmax)}$, area under the plasma concentration-time curve [$AUC_{(0-t)}$ and $AUC_{(0-\infty)}$], and apparent terminal phase half-life ($t_{1/2}$).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using log_e-transformed AUC(0-∞) and Cmax in a mixed effects model

9.3.1 Analysis Populations

9.3.1.1. Safety Population

All subjects enrolled into the study who have received at least one dose of investigational product will be included in the Safety Population.

9.3.1.2. Pharmacokinetic Concentration Population

The PK Concentration Population will include all subjects for whom at least one PK sample was obtained and analyzed.

9.3.1.3. Pharmacokinetic Parameter Population

For each PK parameter, the PK Parameter Population will include all subjects who provide PK parameter data.

Section 9.3.2 Interim Analysis

CHANGE FROM

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed in order to direct development of fixed dose formulations for subsequent study parts and the planned BE study.

CHANGE TO

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed **in Part 1 and Part 2 of the study in order to direct development of the FDC for later parts of the study. Headline results based on statistical analysis using preliminary pharmacokinetic data with nominal time may be produced when 80% and 100% subjects complete Part 1 and Part 2 of the study to assist development of**

FDCs. Treatment and period information from the crossover design may be used in the analysis; sequence information may also be included depending on availability.

Section 9.4.1 Pharmacokinetic Analyses

CHANGE FROM

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

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

Following $\log_e$-transformation, AUC(0-∞), AUC(0-t) and Cmax of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period and treatment. Subject will be treated as a random effect in the model. Point estimates and their associated 90% confidence intervals will be constructed for the differences, F1 – R, F2 – R, F3 – R, F4 – R for Part 1 and similarly for Part 2 (as needed); and X1-X2, Y1-Y2 for Part 3. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2-R:F3-R, F4-R; and X1:X2, Y1:Y2.

Tmax of FDC formulations will be analyzed with the non-parametric Wilcoxon Matched Pairs Method to compute point estimate and associated 90% confidence intervals for the median difference, F1 R, F2 R, F3 R, F4 R and X1:X2, Y1:Y2.

CHANGE TO

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

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

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.

The PK parameters, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, 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.

Actual visit day will be used for safety assessments. For the assessment of bioequivalence, both actual time of sampling, and nominal (planned) sampling time, will be used to estimate PK parameters at the end of the study. PK parameters estimation based on nominal sampling time will be used for generating headline results. Following log_e-transformation, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period**, sequence** and treatment**, and subject** as random effect **term (i.e. ANOVA method using period, sequence and treatment as fixed effect, and subject as random effect)** Point estimates and their associated 90% confidence intervals will be **calculated** for the differences: F1 - R, F2 - R, F3 -R, F4 - R for Part 1**; FG1-R, FG2-R, FG3-R** for Part 2**; X1-R1, X2-R2 for Part 3A; and Y-R3 for Part 3B.** The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2:R, F3:R, F4:R**; for Part 1; FG1:R, FG2:R, FG3:R for Part 2; X1:R1,X2:R2 for Part3A; and Y:R3 for Part 3B.**

Section 11 REFERENCES

ADDITIONS

EMEA guidelines on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **. 2010

Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability, J Pharmacokin. Biopharm. 1987:15:657–680.

Section 12.4 Appendix 4 High Fat Meal Content

CHANGE FROM

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the US FDA in their 2002 draft guidance on food-effect bioavailability and bioequivalence studies. Approximately 50% of the caloric content of the meal is from fat and the meal is high in calories (approximately 1000 calories). Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

US Department of Health and Human Services. Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies. Guidance. Center for Drug Evaluation and Research (CDER). Food and Drug Administration; 2002.

CHANGE TO

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the **EMEA guidelines** on **the investigation of** bioequivalence **2010. The meal should be a high-fat** (**approximately** 50 **percent** of **total** caloric content of the meal) and high-**calorie** (approximately **800 to 1000 kcal) meal. This** test meal **should derive approximately 150, 250, and 500-600 kcal** from protein, carbohydrate, and fat, **respectively**(**EMEA, 2010).**

12.5.3. Protocol changes for Amendment 3 (25-MAY-2017) from Amendment 3 (8-Feb-2017)

Amendment 3 Summary of Changes

The SmPC and PIL for tadalafil (Adcirca) have recently been updated to reflect the risk of a sudden decrease or loss of vision or hearing. The risk of visual defects and Non-Arteritic Anterior Ischemic Optic Neuropathy (NAION) have been reported with the use of tadalafil (and other PDE5 inhibitors) for the management of erectile dysfunction though maybe relevant other patients exposed to tadalafil. Cases of sudden hearing loss have been reported with the use of tadalafil. Though other risk factors for hearing loss were present, this may be relevant to other patients exposed to tadalafil. Therefore, the protocol (and patient PIL) have been updated to reflect these changes. The GSK medical monitor will also change. Specific sections relating to Data Analyses considerations and PK Analysis have been updated to reflect EMA guidance.

List of Specific Changes

Section 4.6 Benefit: Risk Assessment

CHANGE FROM

Summaries of findings from clinical studies conducted with both the Investigational Product taken as monotherapies and in combination can be found in:

- Ambrisentan IB (Volibris IB, 2015)
- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015)

CHANGE TO

Summaries of findings from clinical studies conducted with both the Investigational Product taken as monotherapies and in combination can be found in:

- Ambrisentan IB (Volibris IB, 2015)
- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2017)

Section 4.6.1 Risk Assessment

Table 2 Risks of Clinical Significance and Mitigation Strategy

CHANGE FROM:

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product: GSK1325760 (ambrisentan-Volibris)		
• Teratogenicity • Anaemia (decreased haemoglobin, decreased haematocrit) • Hypersensitivity reactions (e.g. angioedema, rash, pruritus) • Headache (including sinus headache, migraine) • Dizziness • Cardiac failure • Palpitation • Hypotension • Flushing • Epistaxis • Dyspnoea • Upper respiratory congestion, sinusitis, nasopharyngitis, rhinitis • Abdominal pain • Constipation • Nausea, vomiting, diarrhoea • Hepatic transaminases increased • Peripheral oedema, fluid retention • Chest pain/discomfort • Asthenia and fatigue	Ambrisentan IB (Volibris IB, 2015) Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015):	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Exclusion of women of childbearing potential (teratogenicity) • Laboratory assessments per protocol • Physical assessment per protocol • Routine vital signs per protocol • Subjects remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GF196960 (tadalafil - Adcirca)		
• Hypersensitivity reactions • Headache • Syncope, • Migraine • Blurred vision • Palpitations • Nasopharyngitis (including nasal congestion, sinus congestion and rhinitis) • Epistaxis • Nausea, • Dyspepsia (including	Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015)	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Routine vital signs per protocol • Physical assessment per protocol • Subjects remain in the clinical unit for all doses, under medical supervision and until completion of safety assessments at

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
abdominal pain/discomfort) • Vomiting, • Gastroesophageal reflux • Rash • Myalgia, • Back pain • Pain in extremity (including limb discomfort) • Increased uterine bleeding • Facial oedema, • Chest pain		48hrs post dose. • Single doses used in the study
Investigational Product: GSK3380154 (ambrisentan-tadalafil-FDC)		
The risks for the combination are the same as the 2 monotherapies; no extra risks have been identified for the combination.		As for GSK1325760 and GF196960 above

CHANGE TO:

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product: GSK1325760 (ambrisentan-Volibris)		
• Teratogenicity • Anaemia (decreased haemoglobin, decreased haematocrit) • Hypersensitivity reactions (e.g. angioedema, rash, pruritus) • Headache (including sinus headache, migraine) • Dizziness • Cardiac failure • Palpitation • Hypotension • Flushing • Epistaxis • Dyspnoea • Upper respiratory congestion, sinusitis, nasopharyngitis, rhinitis • Abdominal pain • Constipation • Nausea, vomiting, diarrhoea • Hepatic transaminases increased • Peripheral oedema, fluid retention • Chest pain/discomfort • Asthenia and fatigue	Ambrisentan IB (Volibris IB, 2015) Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015):	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Exclusion of women of childbearing potential (teratogenicity) • Laboratory assessments per protocol • Physical assessment per protocol • Routine vital signs per protocol • Subjects remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GF196960 (tadalafil - Adcirca)		
• Hypersensitivity reactions • Headache • Syncope, • Migraine • Blurred vision • **Sudden decrease or loss of vision (Non-Arteritic Anterior Ischemic Optic Neuropathy (NAION))** • Palpitations • Nasopharyngitis (including nasal congestion, sinus	Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 201**7**)	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Routine vital signs per protocol • Physical assessment per protocol • Subjects remain in the clinical unit for all doses, under medical supervision and until completion of safety assessments at

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
congestion and rhinitis) • Epistaxis • Nausea, • Dyspepsia (including abdominal pain/discomfort) • Vomiting, • Gastroesophageal reflux • Rash • Myalgia, • Back pain • Pain in extremity (including limb discomfort) • Increased uterine bleeding • Facial oedema, • Chest pain • **Sudden decrease or loss of hearing**		48hrs post dose. • Single doses used in the study
Investigational Product: GSK3380154 (ambrisentan-tadalafil-FDC)		
The risks for the combination are the same as the 2 monotherapies; no extra risks have been identified for the combination.		As for GSK1325760 and GF196960 above

Section 5 Selection of Study Population and Withdrawal Criteria

Rationale for change: as above.

CHANGE FROM:

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in:

- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2015)

CHANGE TO:

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in:

- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010; Schuirmann, 1987;Adcirca, 2017)

Section 9.3 Data Analysis Consideration

Rationale for change: to reflect updated guidelines

CHANGE FROM :

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in a mixed effects model

CHANGE TO :

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax **in fixed effect ANOVA model and** mixed effects model

Section 9.4.1 Pharmacokinetic Analysis

Rationale for change: to reflect updated guidelines.

CHANGE FROM :

Actual visit day will be used for safety assessments. For the assessment of bioequivalence, both actual time of sampling, and nominal (planned) sampling time, will be used to estimate PK parameters at the end of the study. PK parameters estimation based on nominal sampling time will be used for generating headline results.Following $\log_e$-transformation, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period, sequence and treatment, and subject as random effect term (i.e. ANOVA method using period, sequence and treatment as fixed effect, and subject as random effect) Point estimates and their associated 90% confidence intervals will be calculated for the differences: F1 - R, F2 - R, F3 -R, F4 - R for Part 1; FG1-R, FG2-R, FG3-R for Part 2; X1-R1, X2-R2 for Part 3A; and Y-R3 for Part 3B. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2:R, F3:R, F4:R; for Part 1; FG1:R, FG2:R, FG3:R for Part 2; X1:R1,X2:R2 for Part3A; and Y:R3 for Part 3B.

CHANGE TO :

Actual visit day will be used for safety assessments. For the assessment of bioequivalence, both actual time of sampling, and nominal (planned) sampling time, will be used to estimate PK parameters at the end of the study. PK parameters estimation based on nominal sampling time will be used for generating headline results. **According to EMA guidilines on GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE (2010, Doc. Ref.: [**CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **. 2010**]), f**ollowing $\log_e$-transformation, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} of FDC formulations and reference will be separately analyzed using **fixed effect ANOVA model with fixed effect terms for sequence, subject within sequence, period and treatment (formulation).**

As a sensitivity analysis, mixed effect model with fixed effect terms for period, sequence and treatment **(formulation),** and subject as random effect term (i.e. ANOVA method using period, sequence and treatment as fixed effect, and subject as random effect) **will be performed.**

Point estimates and their associated 90% confidence intervals will be calculated for the differences: F1 - R, F2 - R, F3 -R, F4 - R for Part 1; FG1-R, FG2-R, FG3-R for Part 2; X1-R1, X2-R2 for Part 3A; and **Y1**-R3, **Y2-R4** for Part 3B. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2:R, F3:R, F4:R; for Part 1; FG1:R, FG2:R, FG3:R for Part 2; X1:R1,X2:R2 for Part3A; and **Y1**:R3 **and Y2:R4** for Part 3B.

TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A Phase 1 study to demonstrate the relative bioavailability and bioequivalence of fixed dose combinations of ambrisentan and tadalafil in healthy subjects

Compound Number: GSK3380154, GSK1325760 and GF196960

Development Phase: I

Effective Date: 08-FEB-2017

Protocol Amendment Number: 02

Author (s): PPD (Clinical Development Manager), PPD (Medical Advisor), PPD (Principal Statistician), PPD (Sr. Director Clin. Pharmacology) , PPD (Principle Data Manager), PPD (Medical Director SERM), PPD (SM Manager), PPD (Supply Chain Study Lead)

Revision Chronology:

GlaxoSmithKline Document Number	**Date**	**Version**
2015N232335_00	2015-DEC-16	Original
2015N232335_01	2016-APR-14	Amendment No. 1
Clarification of hypotension withdrawal criteria in Section 5.4.3, change to GSK medical monitor and minor administrative changes.		
2015N232335_02	2017-FEB-08	Amendment No. 2
• Inclusion of bioequivalence assessment in Part 3 to replace the optional food effect. • Inclusion of additional dosage strength in Part 3. • Change in Medical Monitor(s).		

SPONSOR SIGNATORY

PPD

8th Feb 2017

Andrew Zambanini
Physician Lead

Date

MEDICAL MONITOR/SPONSOR INFORMATION PAGE

Medical Monitor/SAE Contact Information:

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Medical Monitor				Site to scan and send to email
SAE contact information				Site to scan and send to email

Sponsor Legal Registered Address:

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UK

In some countries, the clinical trial sponsor may be the local GlaxoSmithKline Affiliate Company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number(s): EudraCT number 2015-004140-18

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number 201964

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

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

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

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

TABLE OF CONTENTS

PAGE

1. PROTOCOL SYNOPSIS FOR STUDY 201964

Rationale

This study is designed initially to compare the relative bioavailability of a number of fixed dose combinations (FDCs) of ambrisentan and tadalafil (Part 1 and 2) and consequently the bioequivalence of the FDC of different dose strength (Part 3A and 3B). Dependent on formulation work, the study will allow up to 9 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: C_{max}, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
To establish bioequivalence of the candidate FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fed and fasted conditions.	$AUC_{(0-t)}$,$AUC_{(0-inf)}$,C_{max}
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasted conditions.	$AUC_{(0-t)}$, $AUC_{(0-inf)}$,C_{max}
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 20 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 20 mg) taken concurrently in healthy human subjects under fasted conditions.	$AUC_{(0-t)}$, $AUC_{(0-inf)}$,C_{max}
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fed and fasted conditions.	Plasma PK parameters including; $AUC_{(0-t)}/AUC_{(0-inf)}$,t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fed and fasting conditions.	Safety and Tolerability of all treatments as assessed by vital signs, ECG, clinical laboratory safety data and review of adverse events

Overall Design

This is a single centre, Phase 1, single dose, randomised, open label crossover study in healthy volunteers with 3 study parts. Part 1 will include a 5 way cross-over. Part 2 and Part 3 (A&B) will each include a 4 way cross-over. All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in Parts 1 and 2. In Part 3 the washout will be at least 10 days. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part will include an evaluation of the bioavailability, safety and tolerability of 3 granulation forms of a single FDC (ambrisentan 10mg + tadalafil 40 mg) identified from Part 1. These data will be compared to that for the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

Part 3 A

Part 3A of the study is set to establish bioequivalence between the candidate FDC from Part 2. This study part will have 4 dose sessions and will assess the bioequivalence, in both the fed and fasted state, of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state. The fed arms of this part will have a standard high fat breakfast. (EMEA, 2010).

Part 3 B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg). This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference

ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

Treatment Arms and Duration

The proposed treatment arms for each study part are described here.

Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)**	**Part 3A (fed and fasted)**	**Part 3B (fasted)**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 1 (10mg/40mg)	ambrisentan and tadalafil FDC) (10mg/40mg), fed	Ambrisentan and tadalafil FDC (5mg/40mg), fasted
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 2 (10mg/40mg)	ambrisentan and tadalafil FDC (10mg/40mg) fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 3 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed	ambrisentan and tadalafil FDC (5mg/20mg), fasted
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

Type and Number of Subjects

Approximately 20 healthy adult subjects will be randomized to study Part 1 and an additional 20 healthy adult subjects in Part 2, such that at least 16 evaluable subjects complete each part of the study. Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subejcts will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study.

Analysis

No formal hypothesis will be tested for Part 1 and Part 2 of the study. An estimation approach will be used to estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

$$H(0): \mu(\text{test})/\mu(\text{reference}) < 0.80 \text{ or } \mu(\text{test})/\mu(\text{reference}) > 1.25,$$

i.e., treatments are not bioequivalent.

versus

$$H(1): 0.80 \leq \mu(\text{test})/\mu(\text{reference}) \leq 1.25,$$

i.e, treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) and of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg and of ambrisentan 5 mg & tadalafil 20 mg respectively taken concurrently (reference) in

healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

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

2. INTRODUCTION

Ambrisentan (E.U. trade name: Volibris), is an orally active endothelin receptor antagonist (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris EMA SmPc, 2015; Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.

Tadalafil (E.U. trade name: Adcirca) is an orally active selective inhibitor of the enzyme PDE-5, the primary cGMP-hydrolyzing enzyme in smooth muscle. In the EU, Adcirca is indicated in adults for the treatment of PAH classified as WHO FC II and III, to improve exercise capacity (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015).

A recently completed study (Galiè, 2015a) has shown that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris EMA SmPc, 2015, Section 5.1).

2.1. Study Rationale

This study is designed initially to compare the relative bioavailability of a number of fixed dose combinations (FDCs) of ambrisentan and tadalafil (Part 1 and 2) and consequently the bioequivalence of the FDC of different dose strengths (Part 3A and 3B). Dependent on formulation work, the study will allow up to 9 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently

The formulation(s) to be taken forward in Part 3A will be primarily chosen based on the test/reference ratio for both AUC and Cmax for both components. Ideally the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, tablet size, cost, ease of manufacture and stability would be considered.

Part 3 will include evaluation of bioequivalence for 3 dose strengths of the FDC; 10/40mg, 5/40mg and 5/20mg of ambrisentan and tadalafil.

2.2. Brief Background

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3 biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galiè, 2015b) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris EMA SmPC 2015, Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease (Volibris EMA SmPC, 2015). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015). Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5) inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112, 2008) in 26 healthy participants was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-

administered. There were no SAE's in the study. Three participants withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one participantbecause of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a listed event for ambrisentan. There were a total of 7 participants with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris EMA SmPC, 2015, (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015).

The AMBITION clinical study (Galiè, 2015a and GSK document number 2014N193963_00), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galiè, 2015b) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, 2008).

3. OBJECTIVE(S) AND ENDPOINT(S)

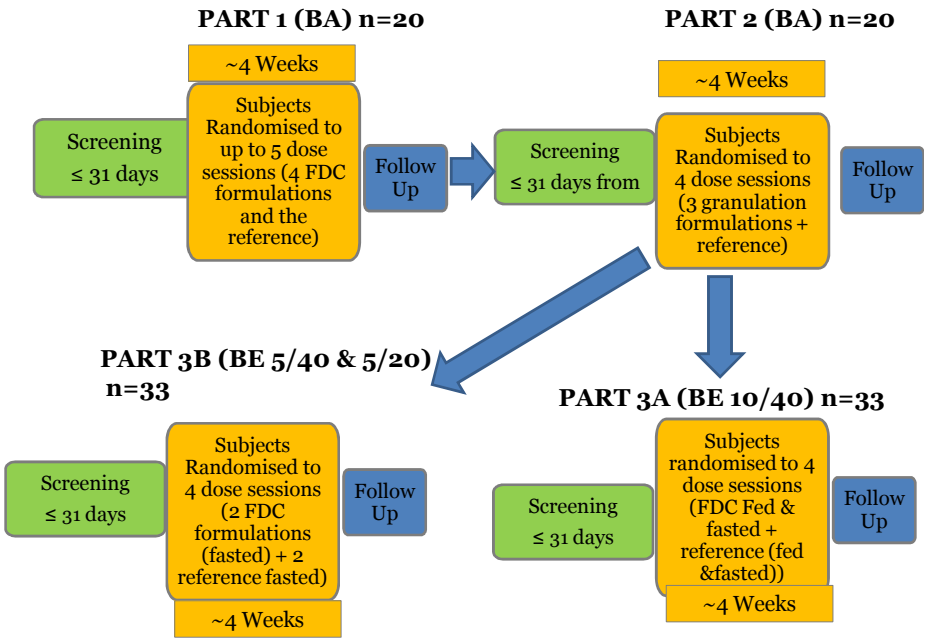
Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
To establish bioequivalence of the candidate FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fed and fasted conditions.	AUC(0-t), AUC(0-inf), Cmax
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasted conditions.	AUC(0-t), AUC (0-inf), Cmax
To establish bioequivalence of the FDC (ambrisentan 5 mg + tadalafil 20 mg) relative to reference monotherapies tested (ambrisentan 5 mg & tadalafil 20 mg) taken concurrently in healthy human subjects under fasted conditions.	$AUC_{(0-t)}$, $AUC_{(0-inf)}$,C_{max}
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting and fed conditions.	Plasma PK parameters including; $AUC_{(0-t)}/AUC_{(0-inf)}$, t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fed and fasting conditions.	Safety and Tolerability of all treatments as assessed by vital signs, ECG, clinical laboratory safety data and review of adverse events

4. STUDY DESIGN

4.1. Overall Design

This is a single centre, Phase 1, single dose, randomised, open label, crossover study in healthy volunteers with 3 study parts; Part 1 will include a 5 way cross-over, and Part 2 and 3 (A&B) will each include a 4 way cross-over. See Figure 1 for study schematic.

Figure 1 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in study Part 1 and 2. In study Part 3 there will be a minimum of 10 days between each dose. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

Part 2

This study part will have 4 dose sessions and will evaluate the bioavailability, safety and tolerability of 3 different granulation sizes for a single FDC (ambrisentan 10 mg + tadalafil 40 mg) compared to the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

Part 3A

Part 3A of the study is set to establish bioequivalence between the candidate FDC from Part 2. This study part will have 4 dose sessions and will assess the bioequivalence, in both the fed and fasted state, of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state. The fed arms of this part will have a standard high fat breakfast (EMEA, 2010).

Part 3B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg) . This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference ambrisentan 5 mg + tadalafil 40mg monotherapies and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

4.2. Treatment and Duration

The treatments for each study part of the study are listed in Table 1. All treatments are single dose. Subjects will be randomised to the order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing analysis of pharmacokinetic data will be used to determine the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to evaluate 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed

following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2 and 3.

Following completion of Part 1 there will be a pause prior to Part 2, so that different granulation forms of a single FDC can be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for 3 granulation size forms of a single FDC formulation selected from Part 1. These will be based on a single successful formulation identified in Part 1 reformulated with the final active pharmaceutical ingredient (API). Success will be defined with the same criteria as those in Part 1.

Part 3 (A and B)

The BE part of the study will investigate the bioequivalence of the FDC ambrisentan 10 mg + tadalafil 40 mg in part 3A and the bioequivalence of the FDC ambrisentan 5 mg + tadalafil 40 mg and ambrisentan 5 mg + tadalafil 20 mg in Part 3B.

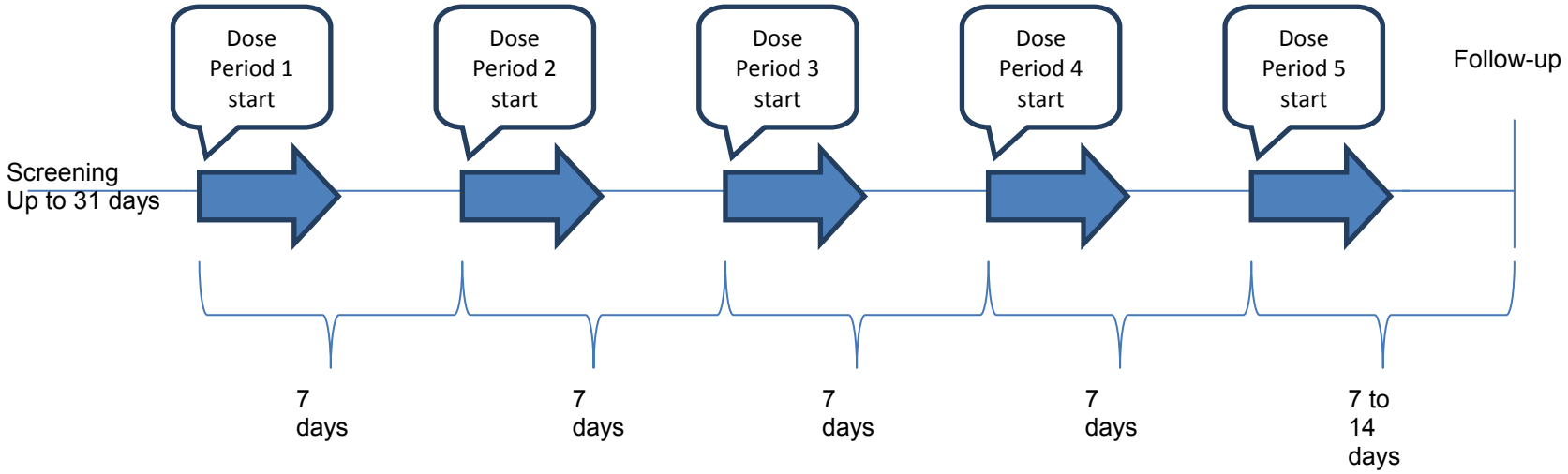
Table 1 Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)	Part 3A (fed and fasted)	Part 3B (fasted)
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 1 (10mg/40mg)	ambrisentan and tadalafil FDC (10mg/40mg), fed	Ambrisentan and tadalafil FDC (5mg/40mg), fasted
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDCgranulation 2 (10mg/40mg)	ambrisentan and tadalafil FDC (10mg/40mg) fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 3 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed	ambrisentan and tadalafil FDC (5mg/20mg), fasted
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted	ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

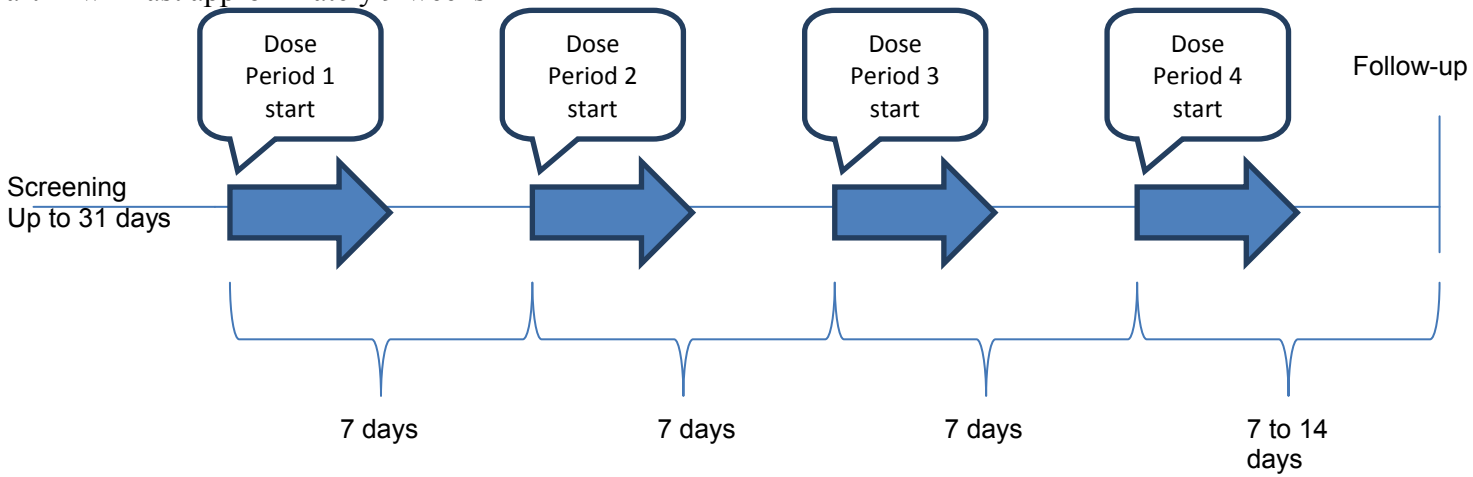
The duration for a subject on this study is described in Figure 2. This figure assumes all 5 dose sessions are used for Part 1. However, this is an approximation as minimum of 7 days is expected between doses in Parts 1 and 2 and a minimum of 10 days in Part 3.

Figure 2 Study Duration

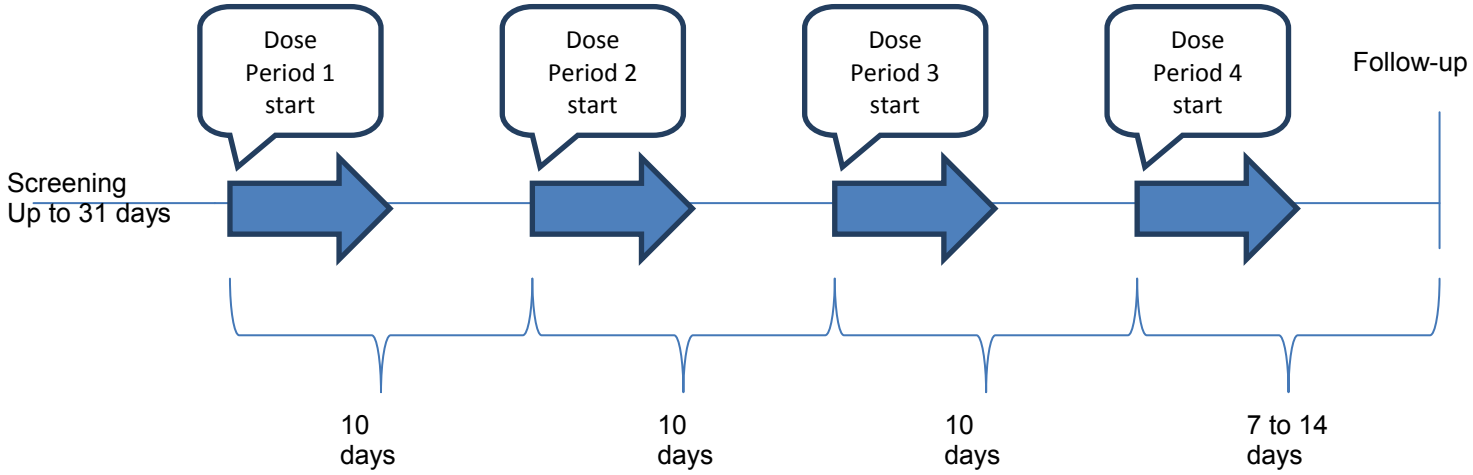
Part 1 can last up to 73 days.



Part 2 will last approximately 9 weeks



Part 3A and B will last approximately 11 weeks



A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

4.3. Type and Number of Subjects

Approximately 20 healthy adult subjects will be randomized to study Part 1 and an additional 20 healthy adult subjects in Part 2, such that at least 16 evaluable subjects complete each part of the study. Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subjects will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study.

The estimation of the number of subjects for each study part is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

4.4. Design Justification

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, nor is there any need to blind study treatment. The inclusion of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study contains three parts: Parts 1 and 2 will provide PK data for different candidate FDC formulations and will enable the assessment of bioequivalence of the FDC versus the combination of the individual reference products in Part 3. The interdependency of the 3 study parts is described in detail in Section 4.2.

4.5. Dose Justification

The oral dose for the new FDC formulations and the reference treatment proposed for the study of 40mg for tadalafil and 10mg for ambrisentan, are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to

be tolerated in healthy subjects. Within Part 3 of the study all dosage forms of the FDC that are proposed for marketing authorisation will be tested for bioequivalence versus the appropriate doses of reference products. These doses are required in clinical practice to allow safe up- and down-titration of the FDC in patients with PAH.

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates a minimumof 10 days, between doses, is also sufficient for clearance of the previous dose during the assessmet of bioequivalence.

4.6. Benefit: Risk Assessment

There is no direct benefit for healthy subjects participating in this study. The risks to the healthy subjects based upon previous experience indicate no expectation of SAE and mild to moderate AE. The benefit/risk remains satisfactory for this healthy subject study.

Summaries of findings from clinical studies conducted with both the Investigational Product taken as monotherapies and in combination can be found in:

- Ambrisentan IB (Volibris IB, 2015)
- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015)

As described in Section 2.2, mild tachycardia was the most common (7/26, 27%) adverse event seen in healthy subjects following combination dosing with 10mg ambrisentan and 40mg tadalafil (Spence, 2009). There were three subjects (3/26, 11.5%) withdrawn due to AEs (e.g., anaemia [mild]; myalgia [severe], muscle fatigue, and dizziness; and headache [severe]). No SAEs or additional risks were observed with combination use in healthy subjects.

The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Risks of clinical significance, identified in subjects with PAH and the mitigation strategy identified for this study in healthy subjects are captured in Table 2.

Table 2 Risks of Clinical Significance and Mitigation Strategy

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product: GSK1325760 (ambrisentan-Volibris)		
• Teratogenicity • Anaemia (decreased haemoglobin, decreased haematocrit) • Hypersensitivity reactions (e.g. angioedema, rash, pruritus) • Headache (including sinus headache, migraine) • Dizziness • Cardiac failure • Palpitation • Hypotension • Flushing • Epistaxis • Dyspnoea • Upper respiratory congestion, sinusitis, nasopharyngitis, rhinitis • Abdominal pain • Constipation • Nausea, vomiting, diarrhoea • Hepatic transaminases increased • Peripheral oedema, fluid retention • Chest pain/discomfort • Asthenia and fatigue	Ambrisentan IB (Volibris IB, 2015) Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015):	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Exclusion of women of childbearing potential (teratogenicity) • Laboratory assessments per protocol • Physical assessment per protocol • Routine vital signs per protocol • Subjects remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GF196960 (tadalafil - Adcirca)		
• Hypersensitivity reactions • Headache • Syncope, • Migraine • Blurred vision • Palpitations • Nasopharyngitis (including nasal congestion, sinus congestion and rhinitis) • Epistaxis	Adcirca (Tadalafil) EMA SmPC (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015)	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Routine vital signs per protocol • Physical assessment per protocol • Subjects remain in the clinical unit for all doses, under medical supervision and until completion of

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
• Nausea, • Dyspepsia (including abdominal pain/discomfort) • Vomiting, • Gastroesophageal reflux • Rash • Myalgia, • Back pain • Pain in extremity (including limb discomfort) • Increased uterine bleeding • Facial oedema, • Chest pain		safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GSK3380154 (ambrisentan-tadalafil-FDC)		
The risks for the combination are the same as the 2 monotherapies; no extra risks have been identified for the combination.		As for GSK1325760 and GF196960 above

4.6.2. Benefit Assessment

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation and to demonstrate bioequivalence versus reference products for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

4.6.3. Overall Benefit: Risk Conclusion

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 2. In addition review of safety information from Part 1 of the study is consistent with this position. So, the benefit:risk is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in:

- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)

- Adcirca (Tadalafil) EMA SmPC (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015)

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

5.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

AGE
1. Between 18 and 60 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and cardiac monitoring (ECG and 24 hour Holter). A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical Monitor if required, judges and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.

WEIGHT
3. Body weight ≥ 50 kg (110 lbs) for men and ≥ 45 kg (99lbs) for women and body mass index (BMI) within the range 18– 30 kg/m^2 (inclusive)

SEX

4. Male or Female
Females must be the following:
Non-reproductive potential defined as:
• Pre-menopausal females with one of the following:
  • Documented tubal ligation
  • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion
  • Hysterectomy
  • Documented Bilateral Oophorectomy

• Documented Postmenopausal defined as 12 months of spontaneous amenorrhea
INFORMED CONSENT
5. Capable of giving signed informed consent as described in Section 10 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol.

5.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)
1. A blood pressure <100/55 mm Hg. 2. Haemoglobin below normal range: • Hb < 133 g/L for males • Hb< 114 g/L for females 3. ALT and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%). 4. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) 5. 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. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP).

CONCOMITANT MEDICATIONS
6. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose

of study medication, unless in the opinion of the Investigator and GSK Medical Monitor the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
7. History of regular alcohol consumption within 6 months of the study defined as: • An average weekly intake of >21 units for males or >14 units for females. 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. 8. Smoking more than 5 cigarettes per week and subjects must be able to abstain from smoking for a 24 hour period prior to dose and any time whilst in the clinical unit.

CONTRAINDICATIONS
9. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or Medical Monitor, contraindicates their participation.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
10. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at Screening or within 3 months prior to first dose of study treatment. . 11. A positive test for HIV antibody. 12. A positive pre-study drug/alcohol screen. 13. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within previous 3 months 14. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 3 months, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 15. Exposure to more than four new chemical entities within 12 months prior to the first dosing day.

5.3. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal

set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.4.1.4).

5.4. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

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

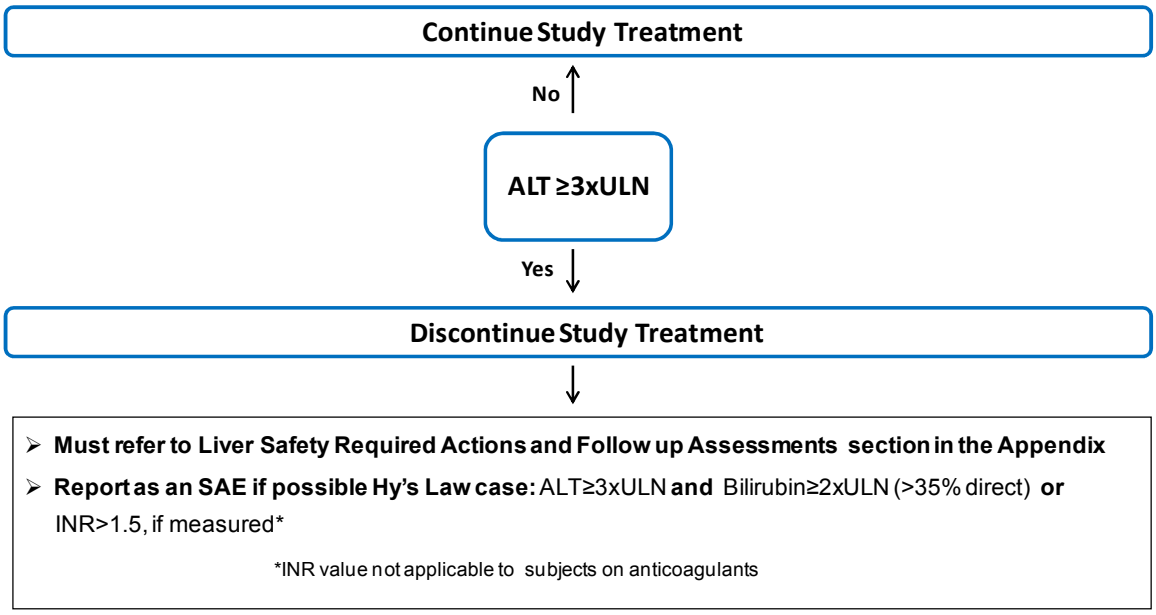
A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

5.4.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Study treatment will be discontinued **for a subject** if liver chemistry stopping criteria are met:

Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow-Up Assessments Section can be found in Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

5.4.1.1. Study Treatment Restart or Rechallenge

Study treatment restart or re-challenge after liver chemistry stopping criteria are met by any subject participating in this study is not allowed.

5.4.2. QTc Stopping Criteria

- The *same* QT correction formula *must* be used for *each individual subject* to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the subject has been enrolled.
  - For example, if a subject is eligible for the protocol based on QTcF, then QTcF must be used for discontinuation of this individual subject as well.
  - Once the QT correction formula has been chosen for a subject's eligibility, the *same formula* must continue to be used for that subject *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 averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A subject that meets either bulleted criterion below will be withdrawn from the study.

- QTc> 500 msec,

- Change from baseline: QTc >60 msec

5.4.3. Hypotension

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 mmHG and diastolic <50 mm HG confirmed by triplicate reading taken up to 5 minutes apart and is judged clinically significant and symptomatic by the investigator.

5.4.4. Other Dose Adjustment/Stopping Safety Criteria

For an individual study participant, stopping criteria include, but are not limited to:

Adverse events, or significant changes in any of the safety assessments, that put the safety of the individual at risk (e.g., ECG, vital signs, laboratory tests, etc), as judged by the Principal Investigator in consultation with the Medical Monitor if necessary.

5.5. Subject and Study Completion

A completed subject is one who has completed all dosing periods of the study including the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

The term 'study treatment' is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for Parts 1, 2 and 3 of the study are shown in Table 3, Table 4 and Table 5.

Table 3 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.45 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Address of reference manufacturer	NA	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

Table 4 Study Treatment for Part 2

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference	
Formulation description	FDC-G1 granulation 1 (10mg/40mg)	FDC-G2 granulation 2 (10mg/40mg)	FDC-G3 granulation 3 (10mg/40mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.45 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations), Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

Table 5 Study Treatment for Part 3

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Formulation description	FDC-G1 or 2 or3 (10mg/40mg)	FDC (5mg/40mg)	FDC (5mg/20mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each tablet contains 5 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura Red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

6.1.1. Retention Samples

GSK will send five times (5x) the drug product necessary for pivotal bioequivalency evaluation in Part 3 of the study for analytical testing for all tablet strengths used in the study. The retain samples are in addition to the supply of drug product sufficient to complete the study. Retention samples will be sent to the clinical CRO in the same bulk container. To ensure that reserve samples are representative of the same batches provided for the clinical study the clinical site will randomly select the supplies for the study from the supply received. The clinical site must retain enough reserve samples to permit FDA to perform five times all of the release tests required in the application.

6.2. Medical Devices

No GSK manufactured devices (or devices manufactured for GSK by a third party) are provided for use in this study.

6.3. Treatment Assignment

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

The treatment key for Parts 1, 2 and 3 are described in Table 6.

Table 6 Treatment Key for Part 1, Part 2 and Part 3

Treatment	Description
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 2	
FG1	ambrisentan and tadalafil FDC-G1 (10mg/40mg) granulation size 1
FG2	ambrisentan and tadalafil FDC-G2 (10mg/40mg) granulation size 2
FG3	ambrisentan and tadalafil FDC-G3 (10mg/40mg) granulation size 3
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 3A	
X1	ambrisentan and tadalafil FDC-G1 or 2 or 3 (10mg/40mg) fed
X2	ambrisentan and tadalafil FDC G1 or 2 or 3 (10mg/40mg) fasted

Treatment	**Description**
R1	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed
R2	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted
Part 3B	
Y1	ambrisentan and tadalafil FDC (5mg/40mg), fasted
Y2	ambrisentan and tadalafil FDC (5mg/20mg), fasted
R3	ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted
R4	ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 7. Additional treatment sequences may be created based using the Latin Squares for Williams Designs if needed.

Table 7 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1/ F2/ R/ F3/ F4 F2/ F3/ F1/ F4/ R F3/ F4/ F2/ R/ F1 F4/ R/ F3/ F1/ F2 R/ F1/ F4/ F2/ F3 F4/ F3/ R/ F2/ F1 R/ F4/ F1/ F3/ F2 F1/ R/ F2/ F4/ F3 F2/ F1/ F3/ R/ F4 F3/ F2/ F4/ F1/ R	1:1:1:1:1:1:1:1:1:1
Part 2	4	FG1/ FG2/ R/ FG3 FG2/ FG3/ FG1/ R FG3/ R/ FG2/ FG1 R/ FG1/ FG3/ FG2	1:1:1:1
Part 3A	4	X1/ R1/ R2/ X2 R1/ X2/ X1/ R2 X2/ R2/ R1/ X1 R2/ X1/ X2/ R1	1:1:1:1
Part 3B	4	Y1/ R3/ R4/ Y2 R3/ Y2/ Y1/ R4 Y2/ R4/ R3/ Y1 R4/ Y1/ Y2/ R3	1:1:1:1

6.4. Blinding

This will be an open-label study.

6.5. Packaging and Labeling

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

6.6. Preparation/Handling/Storage/Accountability

This will be detailed in a Study Specific Technical Agreement/Memo (TTS) which will be accompanied by a Quality Agreement.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.
- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the SRM.
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
- 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.

6.7. Compliance with Study Treatment Administration

When subjects are dosed at the site, they will receive study treatment 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 treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each subject's mouth to ensure that the study treatment was ingested.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK3380154 (ambrisentan/tadalafil-FDC), GSK1325760 (ambrisentan) and GF196960 (tadalafil) greater than the protocol defined dose and within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose.

Advice for the investigator is included in the product label for GSK1325760 (ambrisentan) and GF196960 (tadalafil).

In the event of an overdose the investigator should:

1. Contact the Medical Monitor immediately
2. Closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until compound number/name can no longer be detected systemically (at least 3 days for compound number/name)
3. Obtain a plasma sample for pharmacokinetic (PK) analysis 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 subject.

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because only healthy subjects are eligible for study participation.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until after the final dose.
- Dependent on the utility of the dose session subjects will be asked to fast or consume the FDA full fat breakfast prior to dosing (EMEA, 2010)
  - Fasting subjects will be required to fast from midnight before each full in-house dosing day (i.e. Day 1) with the exception of water, which will be allowed freely except for 1 hour either side of dosing. Subjects will be required to fast up to 4h hour post dose on Day 1 of each dose
  - For treatments given under fed conditions, a standard high-fat breakfast will be provided before dosing. See Section 12.4 for details of this meal. This meal should be eaten in its entirety within 30 minutes. The amount consumed will be recorded within the source documents and the CRF by the site staff. Subjects will be dosed within 5 minutes of completing the breakfast.
- Subjects should take each dose of investigational product with 240 ml (8 fl oz) of water.

6.10.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks and chocolate) for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- During each dosing session, subjects will abstain from alcohol for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- Smoking is not allowed for 24 hours prior to dosing and whilst subjects are in the clinic.

6.10.3. Activity

Subjects will abstain from strenuous exercise from Screening until Follow-Up. Subjects may participate in light recreational activities during studies (e.g., watch television, read).

6.11. Concomitant Medications and Non-Drug Therapies

6.11.1. Permitted Medications and Non-Drug Therapies

Paracetamol at doses of ≤ 2 grams/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.

6.11.2. Prohibited Medications and Non-Drug Therapies

Subjects 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) prior to the first dose of study medication until completion of the Follow-Up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 7.1

The following points must be noted:

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:
    1. 12-lead ECG
    2. Vital signs

3. Blood draws.

Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.

- The timing and number of planned study assessments, safety and pharmacokinetic assessments 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.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.

No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

7.1. Time and Events Table

Procedure	**Screen**	**Part 1, 2 and 3A and 3B. Each dose in each Part repeats this schedule.**																**FU**	**Notes**
Day	**≤-31**	**-1**	**1**											**2**		**3**	**4**	**≥7-14**	
Time (hrs)			**Pre-dose**	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**4**	**8**	**12**	**18**	**24**	**36**	**48**	**72**		
Outpatient visit	x																x	x	
Admission to unit		x																	
Informed consent	x																		
Inclusion and exclusion criteria	x	x																	
Demography	x																		
Full physical exam including height and weight	x																		
Brief Physical																		x	
Medical history (includes substance usage)	x																		
HIV, Hep B and Hep C screen]	x																		
Laboratory assessments (include liver chemistries)	x	x														x		x	Only Screening labs need to be taken in fasted state.
Serum hCG Pregnancy test	x																	x	Female subjects only
Urine hCG Pregnancy test		x																	Female subjects only
Breathalyser and Smokerlyzer	x	x																	
DOA testing	x	x																	
12-lead ECG	x		x			x		x		x		x		x			x	x	Triplicate at screen and baseline, single measure at other times, unless out of range then triplicates should be performed
Vital signs	x		x		x	x		x		x	x	x		x		x	x	x	
24hr Holter	x																		
Randomisation			x																Randomised prior to first dose only
Study Treatment				x															
AE/SAE review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	SAEs from Screen. AEs from first dose
Concomitant medication review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
PK Sample			x		x	x	x	x	x	x	x	x	x	x	x	x	x		
Discharge from Unit																x			For logistical reasons subjects may remain in-unit for the 72 hr assessments if they prefer.

7.2. Screening and Critical Baseline Assessments

All subjects must give written consent to participate in this trial. Consent for screening evaluations may be obtained using the ICF for the Hammersmith Medicines Research (HMR) healthy subject's panel, which has been approved by the HRA's Phase 1 Advert Review group. The study-specific information and consent form will be signed by the subject either before any screening evaluation or after the investigator confirms the eligibility of the subject for the study and before the subject is randomised to receive the first administration of IMP. Before giving consent, subjects must read the information sheet about the study. They must also read the consent form. They will then discuss the study with the investigator or his deputy and be given the opportunity to ask questions. The study-specific information sheet and the consent form must be approved by the REC.

7.2.1. Demographic/Medical History Assessments

Prior to enrolling in the study and having any study procedures completed, subjects must sign the informed consent.

During the Screening visit, each subject will undergo the assessments to determine eligibility for enrolment as detailed in Section 5.

The following demographic parameters will be captured: date of birth, gender, race and ethnicity.

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 5.

7.3. Pharmacokinetics

7.3.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of ambrisentan and tadalafil will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

For analysis of ambrisentan 2.7 mL of blood will be collected into sodium citrate tubes and for analysis of tadalafil 2.0 mL of blood will be collected into K2-EDTA tubes.

Processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

7.3.2. Sample Analysis

Plasma analysis will be performed under the control of Platform Technology and Science In vitro/In vivo Translation (PTS IVIVT) and Third Party Resource, GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of ambrisentan and tadalafil will be determined in plasma samples using

the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

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

7.4. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests such as vital signs, physical examinations and laboratory safety assessments may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

7.4.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The definitions of an AE or SAE can be found in Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.4.1.1. Time period and Frequency for collecting AE and SAE information

- Any SAEs assessed as related to study participation (e.g., 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 up to and including any follow up contact.
- AEs will be collected from the start of Study Treatment until the follow up contact (see Section 7.4.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.3.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Section 12.3.

7.4.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?

7.4.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Section 4.6.1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow up (as defined in Section 5.4). Further information on follow up procedures is given in Study Assessments and Procedures.

7.4.1.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs related to study treatment (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

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

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

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

7.4.2. Physical Exams

- A complete physical examination will include, at a minimum, assessment of the 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

7.4.3. Vital Signs

- Vital signs will be measured in semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure and pulse rate and respiratory rate.
- Three readings of blood pressure and pulse rate will be taken at Screening and baseline (pre-dose). All subsequent assessments will be single measures, unless the subjects blood pressure or pulse rate has changed from baseline by >15% and then 2 further readings should be taken and recorded:
- For triplicate readings:
    - All 3 readings will be recorded in the CRF
        - First reading should be rejected
        - Second and third readings should be averaged to give the measurement to be used and this will also be recorded in the CRF

7.4.4. Electrocardiogram (ECG)

- ECGs will be measured in semi-supine position after 5 minutes rest
- Triplicate 12-lead ECGs will be obtained at Screening and baseline (predose). All subsequent assessments will be a single measures, unless withdrawal criteria are meet and in which case 2 further readings should be taken to confirm if withdrawal is required . An ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- 24 hour continuous cardiac telemetry (Holter) will be performed at Screening. Full disclosures will be reviewed in detail and the review maintained as part of the subject's source documents

7.4.5. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 8, must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit dat. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

All study-required laboratory assessments will be performed by a local laboratory.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 8.

Table 8 Protocol Required Safety Laboratory Assessments

<table>
<tr><th>Laboratory Assessments</th><th>Parameters</th></tr>
<tr><td>Haematology</td><td>
<table>
<tr><td>Platelet Count</td><td><u>RBC Indices</u>:</td><td><u>WBC count with Differential</u>:</td></tr>
<tr><td>RBC Count</td><td>MCV</td><td>Neutrophils</td></tr>
<tr><td>Hemoglobin</td><td>MCH</td><td>Lymphocytes</td></tr>
<tr><td>Hematocrit</td><td>MCHC</td><td>Monocytes</td></tr>
<tr><td></td><td></td><td>Eosinophils</td></tr>
<tr><td></td><td></td><td>Basophils</td></tr>
</table>
</td></tr>
<tr><td>Clinical Chemistry [1]</td><td>
<table>
<tr><td>BUN</td><td>Potassium</td><td>AST (SGOT)</td><td>Total and direct bilirubin</td></tr>
<tr><td>Creatinine</td><td>Sodium</td><td>ALT (SGPT)</td><td>Total Protein</td></tr>
<tr><td>Glucose</td><td>Calcium</td><td>Alkaline phosphatise</td><td>Albumin</td></tr>
</table>
</td></tr>
<tr><td>Routine Urinalysis[3]</td><td>As a minimum, but not limited to the following tests and dependent on standard urinalysis dipstick used:
• Specific gravity
• pH, glucose, protein, blood and ketones by dipstick
• Microscopic examination (if blood or protein is abnormal)</td></tr>
<tr><td>Other Screening Tests</td><td>• HIV
• Hepatitis B (HBsAg)
• Hepatitis C (Hep C antibody)
• Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)
• Serum or urine hCG Pregnancy test[2]</td></tr>
<tr><td colspan="2">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 5.4.1 and Appendix 2
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.
3. Routine Urinalysis results will not be databased, unless a result is out of range and clinically significant then it would be captured as an AE</td></tr>
</table>

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within7 days after the last dose of study treatment should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

8. DATA MANAGEMENT

- Data will be double-entered into a clinical database management system (ClinPlus Version 3.3).
- Management of clinical data will be performed in accordance with applicable HMR standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- Original CRFs will be retained by GSK, while HMR will retain a copy. Subject initials will not be collected or transmitted to GSK according to GSK policy

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

No formal hypothesis will be tested for study Part 1 and Part 2. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently, (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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference). The objective of Part 2 is to assess whether differences in granulation size impact the pharmacokinetics of ambrisentan and tadalafil; the estimation approach for bioavailability is therefore applicable to Part 2.

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

$$H(0): \mu(\text{test})/\mu(\text{reference}) < 0.80 \text{ or } \mu(\text{test})/\mu(\text{reference}) > 1.25,$$

i.e., treatments are not bioequivalent.

versus

$H(1) : 0.80 \leq \mu(\text{ test})/\mu(\text{reference}) \leq 1.25,$

i.e., treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test (1) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg taken concurrently (reference) and (2) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 20 mg taken concurrently (reference), in healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

9.2. Sample Size Considerations

9.2.1. Sample Size Assumptions

For Part 1 and Part 2 of the study, the sample size assumptions are based on previously reported estimates of within subject CV for $AUC_{(0-\infty)}$ and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005). Table 9 summarizes the estimates of within subject CV for the primary endpoints $AUC_{(0-\infty)}$ and Cmax.

Table 9 Estimates of within subject CV for the primary end points $AUC_{(0-\infty)}$ and C_{max}

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
$AUC_{(0-\infty)}$	15%	13%

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 based on a sample size of 20 statistically evaluable subjects.

For Part 3A of the study, Table 10summarized the observed estimates of between and within subject CV and observed ratio of geometric means for the primary endpoints $AUC_{(0-t)}$ and C_{max} from Part 1 of the study.

Table 10 Estimates of within subject CV for the primary end points AUC $_{(0-\infty)}$ and C_{max} for ambrisentan and tadalafil from part 1 of the study

CVw: within subject CV	ambrisentan	tadalafil
C_{max}	22.42%	14.53%
AUC $_{(0-\infty)}$	8.12%	10.32%

The largest of the within subject CV estimates 22.42% translates to a standard deviation (SD) of 0.221 on the natural log scale. Based on this SD, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. This calculation assumes:

- a true ratio of 1,
- the within-subject variability from the current study will not be larger than that used in the sample size calculations,
- data are log-normally distributed, and each t-test is made at the 5% level.

For Part 3B of the study, same assumptions are adopted as Part 3A. Thus, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. However, if the within subject CVs obtained after Part 2 are significantly higher than the ones in Part 1 (shown in Table 10), a sample size re-esimation may be conducted to adjust the sample size for Part 3A and 3B to ensure the study maintain enough power to demonstrate bioequivalence.

9.2.2. Sample Size Sensitivity

For Part 1 and Part 2, assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then Table 11 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 11 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%

For Part 3, based on the observed data from previous studies, the ratio of geometric mean for ambrisentan Cmax is unlikely to be 1. The effects on the power of declaring bioequivalence in the face of a shift in the expected ratio of the geometric means were examined.

If, under all other assumptions outlined above, the actual ratio of geometric means for ambrisentan Cmax is 0.97, then 26 evaluable subjects are needed to have 90% power to conclude bioequivalence.

With 26 evaluable subjects, the current study design has at least 90% power to conclude bioequivalance (with both Cmax and AUC for ambrisentan and tadalafil).

9.2.3. Sample Size Re-estimation or Adjustment

No sample size re-estimation will be performed. However, if the estimation of the within subject CV from Part 2 of the study is much larger than the within subject CV from Part 1 of the study, the sample size for Part3 may be re-evaluated.

9.3. Data Analysis Considerations

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed 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 analayis 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 Cmax $_{(tmax)}$, area under the plasma concentration-time curve [$AUC_{(0-t)}$ and $AUC_{(0-\infty)}$], and apparent terminal phase half-life ($t_{1/2}$).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in a mixed effects model.

9.3.1. Analysis Populations

9.3.1.1. Safety Population

All subjects enrolled into the study who have received at least one dose of investigational product will be included in the Safety Population.

9.3.1.2. Pharmacokinetic Concentration Population

The PK Concentration Population will include all subjects for whom at least one PK sample was obtained and analyzed.

9.3.1.3. Pharmacokinetic Parameter Population

For each PK parameter, the PK Parameter Population will include all subjects who provide PK parameter data.

9.3.2. Interim Analysis

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed in Part 1 and Part 2 of the study in order to direct development of the FDC for later parts of the study. Headline results based on statistical analysis using preliminary pharmacokinetic data with nominal time may be produced when 80% and 100% subjects complete Part 1 and Part 2 of the study to assist development of FDCs. Treatment and period information from the crossover design may be used in the analysis; sequence information may also be included depending on availability.

9.4. Key Elements of Analysis Plan

9.4.1. Pharmacokinetic Analyses

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

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

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.

The PK parameters, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, 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.

Actual visit day will be used for safety assessments. For the assessment of bioequivalence, both actual time of sampling, and nominal (planned) sampling time, will be used to estimate PK parameters at the end of the study. PK parameters estimation based on nominal sampling time will be used for generating headline results.Following log_e-transformation, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period, sequence and treatment, and subject as random effect term (i.e. ANOVA method using period, sequence and treatment as fixed effect, and subject as random effect) Point estimates and their associated 90% confidence intervals will be calculated for the differences: F1 - R, F2 - R, F3 -R, F4 - R for Part 1; FG1-R, FG2-R, FG3-R for Part 2; X1-R1, X2-R2 for Part 3A; and Y-R3 for Part 3B. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2:R, F3:R, F4:R; for Part 1; FG1:R, FG2:R, FG3:R for Part 2; X1:R1,X2:R2 for Part3A; and Y:R3 for Part 3B.

9.4.2. Safety Analyses

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

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

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

Prior to initiation of a site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Obtaining signed informed consent
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.
- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

10.3. Quality Control (Study Monitoring)

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

GSK will monitor the study and site activity to verify that the:

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.
- If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.

- If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action.
- If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.
- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.
- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the

opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

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

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

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

11. REFERENCES

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
API	Active Pharmaceutical Ingredient
AUC	Area Under Curve
BE	Bioequivalence
CDISC	Clinical Data Interchange Standards Consortium
cGMP	Cyclic guanosine monophosphate
CI	Confidence Interval
C_{max}	Maximum concentration
CV	Coefficient of variance
CVw	Coefficient of variance within subject
EMA	European Medicines Agency
ERA	Endothelin Receptor Antagonists
ERS	European Respiratory Society
ESC	European Society of Cardiology
ET-1	Endothelin Receptor - 1
EU	European Union
FDA	Federal Drug Agency
FDC	Fixed Dose Combination
NO	Nitrous Oxide
PDE-5	Phosphodiesterase type 5
SAE	Serious Adverse Events
SRM	Study Reference Manual
$t_{1/2}$	Half Life
t_{max}	Time to maximum concentration
WHO	World Health Organisation

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Adcirca
WinNonlin

12.2. Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event aetiology (in alignment with the FDA premarketing clinical liver safety guidance).

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td colspan="2">ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >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 following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th colspan="2">Follow-Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment
• 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 subject until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline</td><td colspan="2">• Viral hepatitis serology[3]
• Blood sample for pharmacokinetic (PK) analysis, obtained within 96hrs of last dose
• 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
If ALT≥3xULN AND bilirubin ≥ 2xULN or</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
• A specialist or hepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	**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 HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. • 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 treatment 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, 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); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
4. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.

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12.3. Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.3.1. Definition of Adverse Events

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

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • 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 treatment administration even though it may have been present prior to the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae).

Events <u>NOT</u> meeting definition of an AE include:
• 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 subject'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 subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE. • Situations where an untoward medical occurrence did not occur (social and/or

convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

12.3.2. Definition of Serious Adverse Events

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, etc).

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

NOTE:

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

c. Requires hospitalization or prolongation of existing hospitalization

NOTE:

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

d. Results in disability/incapacity

NOTE:

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

e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. • Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse
g. Is associated with liver injury <u>and</u> impaired liver function defined as: • ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), **or** • ALT ≥ 3xULN and INR** > 1.5. * Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE. ** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.
• Refer to Section 12.2 for the required liver chemistry follow up instructions

12.3.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:
Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs: • Myocardial infarction/unstable angina • Congestive heart failure • Arrhythmias • Valvulopathy • Pulmonary hypertension • Cerebrovascular events/stroke and transient ischemic attack • Peripheral arterial thromboembolism • Deep venous thrombosis/pulmonary embolism

- Revascularization

12.3.4. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF
- It is **not** acceptable for the investigator to send photocopies of the subject'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 instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.
- Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study.
- Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer.
- The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

12.3.5. Evaluating AEs and SAEs

Assessment of Intensity

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

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating

the intensity of an event; and both AEs and SAEs can be assessed as severe.

- An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence 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 natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of 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 when 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 prior to the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow up information, amending the SAE data collection tool accordingly.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.
- The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.
- If a subject 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 the designated reporting time frames.

12.3.6. Reporting of SAEs to GSK

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 or the SAE coordinator
- 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
- Initial notification via the 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 receipt can be found at this beginning of the protocol on the Sponsor/Medical Monitor Contact Information page.

12.4. Appendix 4 High Fat Meal Content

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the EMEA guidelines on the investigation of bioequivalence 2010. The meal should be a high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 kcal) meal. This test meal should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively.(EMEA, 2010)

12.5. Appendix 5: Protocol Changes

12.5.1. Protocol changes for Amendment 01 (14-Apr-2016) from Original Protocol (16-Dec-2015)

The purpose of this amendment is to clarify the hypotension withdrawal criteria in section 5.4.3, address changes to GSK medical monitor and minor administrative changes.

Summary of Changes

Section 1 PROTOCOL SYNOPSIS FOR STUDY 201964

AND

Section 3 OBJECTIVES AND ENDPOINTS

CHANGE FROM

Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assed by vital signs, ECG, clinical laboratory safety data and review of adverse events

CHANGE TO

Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as **assessed** by vital signs, ECG, clinical laboratory safety data and review of adverse events

Section 5.4.3 Hypotension

CHANGE FROM

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 or diastolic <50 confirmed by triplicate reading taken up to 5 minutes apart.

CHANGE TO

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 mm HG **and** diastolic <50 mm HG confirmed by triplicate reading taken up to 5 minutes apart **and is judged clinically significant and symptomatic by the investigator**.

Section 6.3 Treatment Assignment

ADDITION

Table 6 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Part 2 and Part 3 merged together	5 (Two FDCs selected from Part 1)	X1 X2 R Y1 Y2 X2 Y1 X1 Y2 R Y1 Y2 X2 R X1 Y2 R Y1 X1 X2 R X1 Y2 X2 Y1 Y2 Y1 R X2 X1 R Y2 X1 Y1 X2 X1 R X2 Y2 Y1 X2 X1 Y1 R Y2 Y1 X2 Y2 X1 R	1:1:1:1:1:1:1:1:1:1
	3 (One FDC selected from Part 1)	X1 X2 R R X2 X1 X2 R X1 F1 R X2 R X1 X2 X2 X1 R	3:3:3:3:3:3 + 2 random

12.5.2. Protocol changes for Amendment 2 () from Amendment 1(14-Apr-2016)

Amendment 2 Summary of Changes

The purpose of this substantial amendment is to include bioequivalence assessment in Part 3 to replace the optional food effect and the addition of new dosage strengths. Furthermore GSK medical monitor contact have changed

List of Specific Changes

Medical Monitor/SAE Contact Information

Rationale for Change: GSK medical monitor contact have changed

CHANGE FROM

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Monitor				Site to scan and send to email
SAE contact information				Site to scan and send to email

CHANGE TO

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Medical Monitor				**Site to scan and send to email**

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
SAE contact information	PPD			**Site to scan and send to email**

Section 1 - Protocol Synopsis Overall design

CHANGE FROM**:**

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects.

All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast

CHANGE TO**:**

This is a single centre, Phase 1, single dose, randomised, open label crossover **study in healthy volunteers** with 3 study parts. **Part 1 will include a 5 way cross-over. Part 2 and Part 3 (A&B) will each include a 4 way cross-over. All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule**.

There will be a minimum washout of 7 days between each dose in **Parts 1 and 2. In Part 3 the washout will be at least 10 days.** A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part will **include an evaluation of the bioavailability, safety and tolerability of 3 granulation forms of a single FDC** (ambrisentan 10mg + tadalafil 40 mg) **identified from Part 1. These data will be compared to that for the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.**

Part 3 A

Part 3A of the study is set to establish bioequivalence between the candidate FDC from Part 2. This study part will have 4 dose sessions and will assess the bioequivalence, in both the fed and fasted state, of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state. The fed arms of this part will have a standard high fat breakfast. (EMEA, 2010).

Part 3 B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg). This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

Section 1 - Protocol Synopsis Treatment Arms and Duration

CHANGE FROM**:**

The proposed treatment arms for each study part are described here; however the treatments in Parts 2 and 3 may be changed dependent on the utility and results from the previous part.

Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)[1]**	**Part 3[2]**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less FDC treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. The minimum will be approximately 3 weeks and the maximum will be approximately 18 weeks.

CHANGE TO**:**

The proposed treatment arms for each study part are described here

Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)**	**Part 3A (fed and fasted)**	**Part 3B (fasted)**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 1 (10mg/40mg)	**ambrisentan and tadalafil FDC) (10mg/40mg), fed**	**Ambrisentan and tadalafil FDC (5mg/40mg), fasted**
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 2 (10mg/40mg)	**ambrisentan and tadalafil FDC (10mg/40mg) fasted**	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted**
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC granulation 3 (10mg/40mg)	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed**	**ambrisentan and tadalafil FDC (5mg/20mg), fasted**
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted**	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted**
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

Section 1 - Protocol Synopsis Type and Number of Subjects

CHANGE FROM:

A approximately of 20 healthy adult subjects will be randomized, to each study part, such that at least 16 evaluable subjects complete each part of the study

CHANGE TO:

Approximately 20 healthy adult subjects will be randomized to study Part 1 and an additional 20 healthy adult subjects in Part 2, such that at least 16 evaluable subjects complete each part of the study. Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subejcts will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study.

Section 1 - Protocol Synopsis Analysis

CHANGE FROM:

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and Part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in Part 2, if used for food effect and Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

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

CHANGE TO:

No formal hypothesis will be tested for Part 1 and Part 2 of the study. An estimation approach will be used to estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

H(0): μ(test)/μ(reference) < 0.80 or μ(test)/μ(reference) > 1.25,

i.e., treatments are not bioequivalent.

versus

H(1) : 0.80 ≤ μ(test)/μ(reference) ≤ 1.25,

i.e., treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) and of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg and of ambrisentan 5 mg & tadalafil 20 mg respectively taken concurrently (reference) in healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

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

Section 2 Introduction

CHANGE FROM

Ambrisentan (E.U. trade name: Volibris), an orally active endothelin receptor antagonists (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease

Tadalafil (E.U. trade name: Adcirca) is an orally active selective inhibitor of the enzyme PDE-5, the primary cGMP-hydrolyzing enzyme in smooth muscle. In the EU, Adcirca is indicated in adults for the treatment of PAH classified as WHO FC II and III, to improve exercise capacity (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015)A recently completed study (Galiè, 2015a) has show that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris EMA SmPC, 2015, Section 5.1).

This pilot study will investigate the relative bioavailability of new fixed dose combinations (FDC) of ambrisentan and tadalafil, compared to the two monotherapies taken concurrently in healthy subjects

CHANGE TO

Ambrisentan (E.U. trade name: Volibris), is an orally active endothelin receptor antagonist (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.

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A recently completed study (Galiè, 2015a) has shown that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris EMA SmPC, 2015, Section 5.1).

~~This pilot study will investigate the relative bioavailability of new fixed dose combinations (FDC) of ambrisentan and tadalafil, compared to the two monotherapies taken concurrently in healthy subjects~~

Section 2.1 Study Rationale

CHANGE FROM

This study is designed to select one, or more, FDCs of ambrisentan and tadalafil for further development and to provide pharmacokinetic data to enable a pivotal bioequivalence study (BE). Dependent on formulation work, the study will allow up to 8 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently. The study would also allow for up to 2 of the new formulations, that may be taken in to a BE study, to be tested for any effect on pharmacokinetics of the FDC in both fed and fasted state.

The formulation(s) to be taken forward into the BE study will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, between subject variability, tablet size, cost, ease of manufacture and stability would be considered.

CHANGE TO

This study is **designed initially to compare** the relative bioavailability of **a number of** fixed dose combinations **(FDCs)** of ambrisentan and tadalafil **(Part 1 and 2) and consequently** the bioequivalence **of the FDC of different dose strengths (Part 3A and 3B).** Dependent on formulation work, the study will allow up to 9 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently

The formulation(s) to be taken forward **in Part 3A** will be primarily chosen **based** on the test/reference ratio for both AUC and Cmax for both components. **Ideally** the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, tablet size, cost, ease of manufacture and stability would be considered.

Part 3 will include evaluation of bioequivalence for 3 dose strengths of the FDC; 10/40mg, 5/40mg and 5/20mg of ambrisentan and tadalafil.

Section 2.1 Brief Background

CHANGE FROM

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3 biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galiè, 2015b) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.(Volibris, EMA SmPC,2015). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015).Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5)

inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112, 2008) in 26 healthy subjects was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-administered. . There were no SAE's in the study. Three subjects withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one subject because of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a listed event for ambrisentan. There were a total of 7 subjects with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris, EMA SmPC, 2015;EMEA, 2010;Schuirmann, 1987;Adcirca, 2015).

The AMBITION clinical study (Galiè, 2015a and AMB112565 CSR 13Nov2014), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galiè, 2015b) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, CHMP/EWP/240/95) (2008).

CHANGE TO

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3 biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galiè, 2015b) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease (Volibris, EMA SmPC, 2015). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (EMEA, 2010;Schuirmann, 1987;Adcirca, 2015). Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5) inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112, 2008) in 26 healthy **participants** was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-administered. There were no SAE's in the study. **Three participants** withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one **participant because** of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a listed event for ambrisentan. There were a total of 7 **participants** with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris, EMA SmPC, 2015;EMEA, 2010;Schuirmann, 1987;Adcirca, 2015)The AMBITION clinical study (Galiè, 2015a and GSK document number 2014N193963_00), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galiè, 2015b) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage

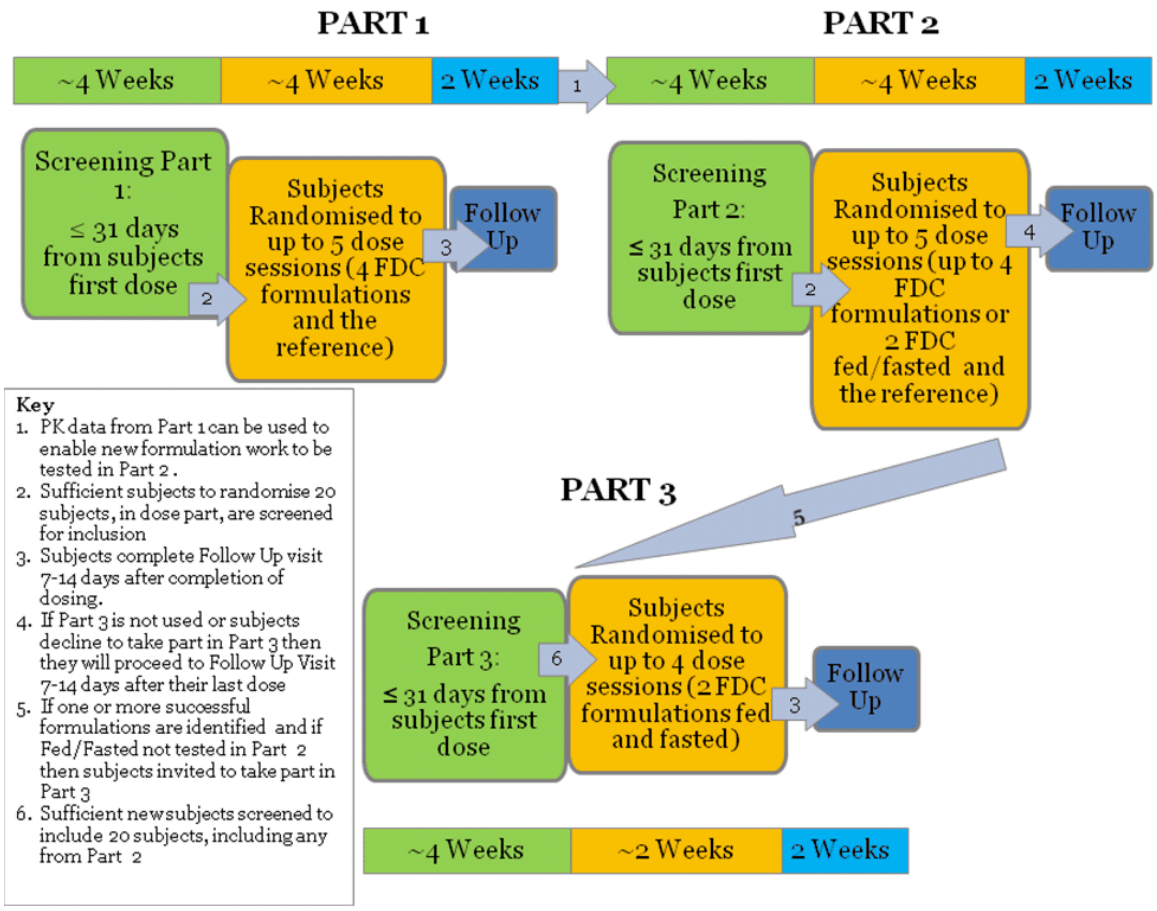
and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, 2008).

Section 4.1 Overall Design

CHANGE FROM

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects. See Figure 1 for study schematic.

Figure 2 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

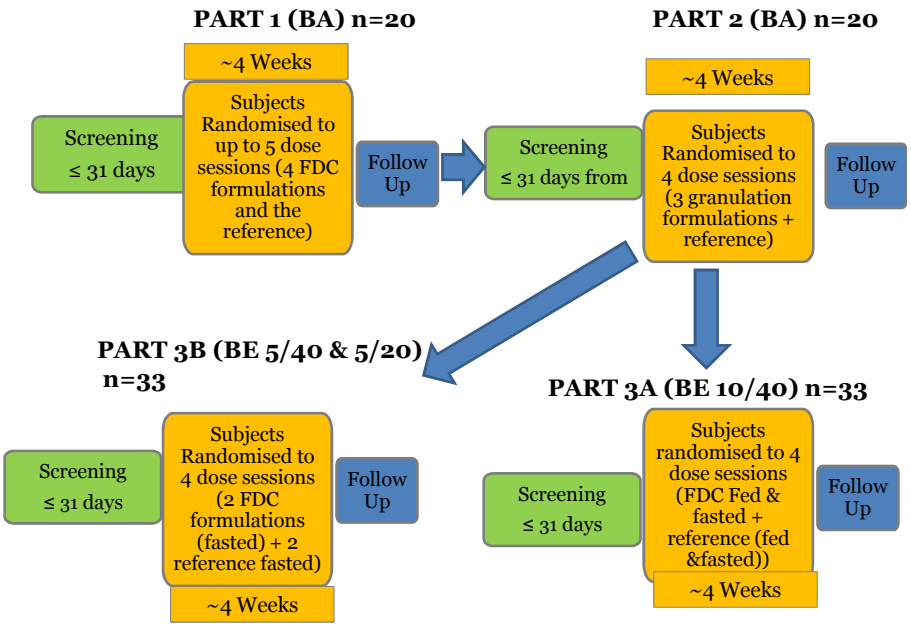
This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast.

Subjects who had taken part in the study part preceding Part 3, and which provided the successful formulations for a BE study could also be invited to take part in this study part. However, a subject's inclusion in more than one study part would be dependent on the subject not exceeding the maximum blood draw volume (500ml) for the study.

CHANGE TO

This is a single centre, Phase 1, single dose, randomised, open label, crossover study in **healthy volunteers with 3 study parts**; **Part 1 will include a 5 way cross-over, and Part 2 and 3 (A&B) will each include a 4 way cross-over.** See Figure 1 for study schematic.

Figure 1 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in **study Part 1 and 2. In study Part 3 there will be a minimum of 10 days between each dose**. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

Part 2

This study part will have **4** dose sessions **and** will **evaluate** the bioavailability, safety and tolerability of **3 different granulation sizes for** a **single FDC** (ambrisentan 10 mg + tadalafil 40 mg) **compared to** the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

Part 3A

Part **3A** of the study is **set to establish bioequivalence between** the **candidate FDC from Part 2**. This study part will have 4 dose sessions and **will assess the bioequivalence**, in both the fed and fasted state, **of the FDC against the reference ambrisentan 10 mg + tadalafil 40mg monotherapies in the fed and fasted state**. The fed arms of this part will have a standard high fat breakfast **(EMEA, 2010)**.

Part 3B

Part 3B of the study is set to establish bioequivalence between the Fixed Dose Combination of an additional two dose strengths (ambrisentan 5 mg + tadalafil 40mg and ambrisentan 5 mg + tadalafil 20mg) . This study part will have 4 dose sessions and will assess the bioequivalence of the two FDC dose strengths against the reference ambrisentan 5 mg + tadalafil 40mg monotherapies and ambrisentan 5 mg + tadalafil 20mg monotherapies in the fasted state.

Section 4.2 Treatment and Duration

CHANGE FROM

The treatments for each study part of the study are listed in Table 1 All treatments are single dose. Subjects will be randomised to order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing analyse of pharmacokinetic data will be used to enable the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to look at up to 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed

following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2. If no successful formulations are identified from these criteria then the pharmacokinetic data would be used to enable further work to produce new FDC formulations for Part 2

Following completion of Part 1 there will be a pause prior to Part 2, so that up to a further 4 FDC formulations could be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for up to 4 FDC formulations. These could be either successful formulation identified in Part 1 reformulated with the final API and/or new formulations using the final API. Pharmacokinetic data from Part 2 of the study will be analysed following completion of each treatment arm by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to define any successful FDC formulation to be taken into Part 3 of this study and a pivotal BE study. If only two formulations are evaluated in Part 2 then the food effect may be added to Part 2 and Part 3 will not be required. Success will be defined with the same criteria as those in Part 1.

Part 3

Part 3 of the study will provide data for up to 2 successful formulations to be progressed to a pivotal BE study, in both the fed and fasted state.

Table 1 Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)[1]	Part 3[2]
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. Subjects may be included in Part 1, Part 2 or Part 3 of the study. However, if Part 2 of the study identifies any successful formulations to take into Part 3 then subjects from Part 2 may be invited to join Part 3.

The minimum and maximum duration for a subject on this study is described in Figure 2. This table assumes all 5 dose sessions are used for Part 1 and Part 2 and either 2 or 4 dose sessions are used in Part 3. However, this is an approximation as fewer sessions may be required in Part 2 and/or the washout between sessions of 7 days in each part may be longer, due to logistical reasons.

Table 2 Study Duration

Study Options		**Screen**	**Study Part 1or 2**	**Pause**	**Study part 3**	**Follow-Up Visit**	**Total Duration on Study**
		(1-31 days)	**(31 days)**	**(7-28 days)**	**(10-24 days)**	**(7-14 days)**	
Subjects included in only Part 1 or 2	Min	1	31			7	39
	Max	31	31			14	76
Subjects included in Part 2&3	Min	1	31	7	10	7	56
	Max	31	31	28	24	14	128
Subjects included in only Part 3	Min	1			10	7	18
	Max	31			24	14	69

A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

CHANGE TO

The treatments for each study part of the study are listed in Table 1. All treatments are single dose. Subjects will be randomised to **the** order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing **analysis** of pharmacokinetic data will be used to **determine** the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to **evaluate** 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each

FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2 **and 3**.

Following completion of Part 1 there will be a pause prior to Part 2, so that **different granulation forms of a single FDC can** be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for 3 **granulation size forms of a single** FDC **formulation selected from Part 1**. These **will** be **based on a single** successful formulation identified in Part 1 reformulated with the final **active pharmaceutical ingredient** (API). Success will be defined with the same criteria as those in Part 1.

Part 3 (A and B)

The BE part of the study will investigate the bioequivalence of the FDC ambrisentan 10 mg + tadalafil 40 mg in part 3A and the bioequivalence of the FDC ambrisentan 5 mg + tadalafil 40 mg and ambrisentan 5 mg + tadalafil 20 mg in Part 3B.

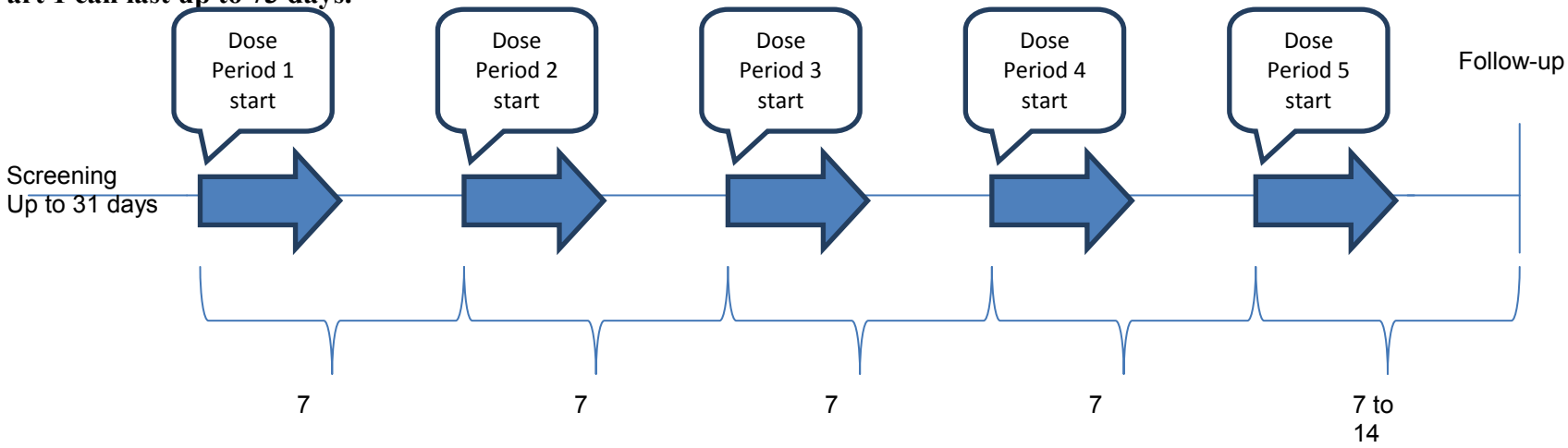
Table 1 Treatments proposed per study part

Part 1 (fasted)	**Part 2 (fasted)**	Part **3A (fed and fasted)**	**Part 3B (fasted)**
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil **FDC granulation 1** (10mg/40mg)	ambrisentan and tadalafil **FDC** (10mg/40mg), fed	**Ambrisentan and tadalafil FDC (5mg/40mg), fasted**
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil **FDCgranulation 2** (10mg/40mg)	ambrisentan and tadalafil **FDC** (10mg/40mg) fasted	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted**
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil **FDC granulation 3** (10mg/40mg)	ambrisentan and tadalafil **monotherapies taken concurrently** (10mg/40mg), fed	**ambrisentan and tadalafil FDC (5mg/20mg), fasted**
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil **monotherapies taken concurrently** (10mg/40mg)	ambrisentan and tadalafil **monotherapies taken concurrently** (10mg/40mg), fasted	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted**
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)			

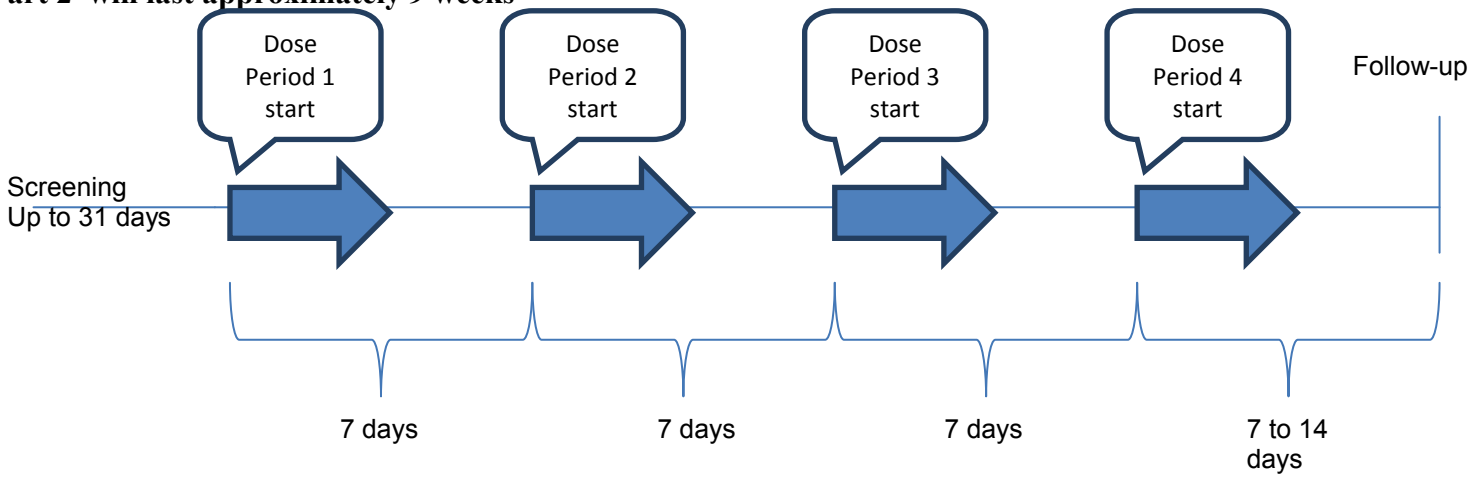
The duration for a subject on this study is described in Figure 2. This **figure** assumes all 5 dose sessions are used for Part 1. However, this is an approximation as **minimum** of 7 days **is expected between doses in Parts 1 and 2 and a minimum of 10 days in Part 3**.

Figure 2 Study Duration

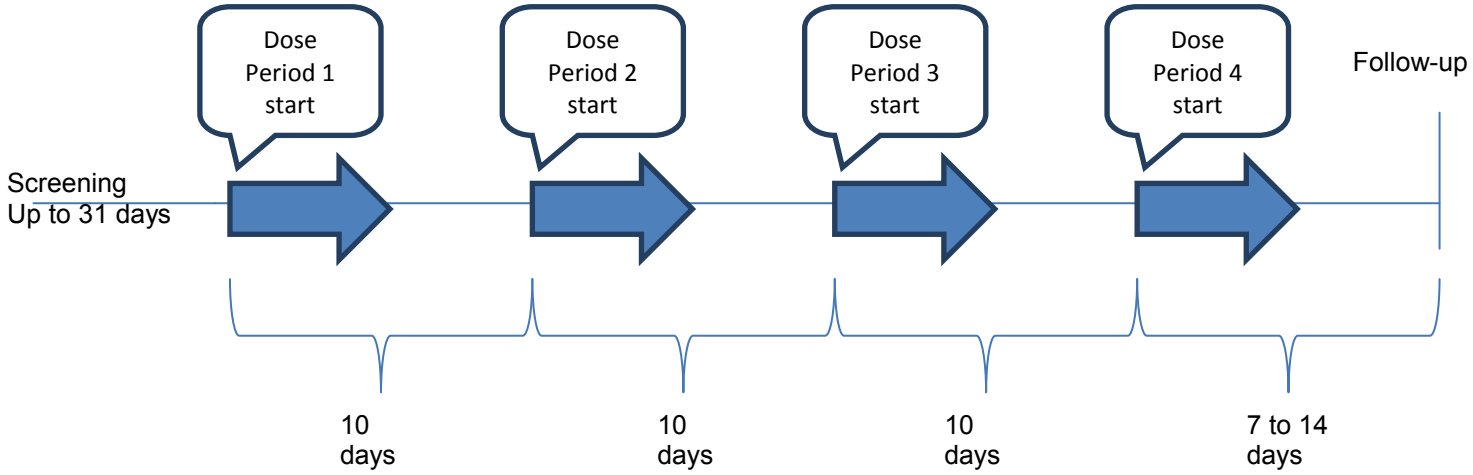
Part 1 can last up to 73 days.



Part 2 will last approximately 9 weeks



Part 3A and B will last approximately 11 weeks



A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

Section 4.3 Type and Number of Subjects

CHANGE FROM

A approximately of 20 healthy adult subjects will be randomized, to each study part, such that at least 16 evaluable subjects complete each part of the study.

The study is not powered. However, the number of subjects will be sufficient for the objectives of this study and this is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

CHANGE TO

Approximately 20 healthy adult subjects will be randomized to study **Part 1 and an additional 20 healthy adult subjects in Part 2**, such that at least 16 evaluable subjects complete each part of the study. **Approximately 33 healthy subjects will be randomized to study Part 3A, such that at least 26 evaluable subjects complete part 3A of the study. An additional 33 healthy subjects will be randomised to Part 3B, such that at least 26 evaluable subjects complete part 3B of the study**.

The estimation of the number of subjects for **each** study **part** is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

Section 4.4 Design Justification

CHANGE FROM

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations to take into a pivotal BE study and to also provide pharmacokinetic data to enable the pivotal BE study to be powered sufficiently.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, or is there any need to blind study treatment. The Inclusion of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study is in three parts to enable PK data from Part 1 to enable formulation development for Part 2. Part 3 providing an opportunity to assess food effect in any FDC formulations suitable for development and inclusion in the pivotal bioequivalence study. The interdependency of the 3 study parts is described in detail in Section 4.2.

CHANGE TO

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, **nor** is there any need to blind study treatment. The **inclusion** of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study **contains** three parts: **Parts 1 and 2 will provide PK data for different candidate** FDC formulations and **will enable the assessment of bioequivalence of the FDC versus the combination of the individual reference products in Part 3.** The interdependency of the 3 study parts is described in detail in Section 4.2.

Section 4.5 Dose Justification

CHANGE FROM

The oral dose for the new FDC formulations and the reference treatment proposed for the study of, 40mg for tadalafil and 10mg for ambrisentan are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to be tolerated in healthy subjects.

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates a minimum washout of 7 days, between doses, is also sufficient for clearance of previous dose.

CHANGE TO

The oral dose for the new FDC formulations and the reference treatment proposed for the study of 40mg for tadalafil and 10mg for ambrisentan, are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to be tolerated in healthy subjects. **Within Part 3 of the study all dosage forms of the FDC that are proposed for marketing authorisation will be tested for bioequivalence versus the appropriate doses of reference products. These doses are required in clinical practice to allow safe up- and down-titration of the FDC in patients with PAH.**

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates **a minimumof 10** days, between doses, is also sufficient for clearance of **the** previous dose **during the assessmet of bioequivalence**.

Section 4.6.2 Benefit Assessment

CHANGE FROM

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

CHANGE TO

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation **and to demonstrate bioequivalence versus reference products** for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

Section 4.6.3 Overall Benefit: Risk Conclusion

CHANGE FROM

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 3. So, the risk: benefit is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

CHANGE TO

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 3. **In addition review of safety information from Part 1 of the study is consistent with this position.** So, the benefit:risk is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

Section 6.1 Investigational Product and Other Study Treatment

CHANGE FROM

The term ‘study treatment’ is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for Part 1 of the study are shown in Table 4, this will be amended once treatments for Part 2 and Part 3 are confirmed.

Table 4 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS**	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 233 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

CHANGE TO

The term ‘study treatment’ is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for **Parts** 1, 2 **and 3** of the study are shown in Table 4, **Table 5 and Table 6.**

Table 4 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately **90** mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.**45** mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, **245** mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Address of reference manufacturer	**NA**	**NA**	**NA**	**NA**	**Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom**	**Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain**

Table 5 Study Treatment for Part 2

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference	
Formulation description	FDC-G1 granulation 1 (10mg/40mg)	FDC-G2 granulation 2 (10mg/40mg)	FDC-G3 granulation 3 (10mg/40mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.45 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference	
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

Table 6 Study Treatment for Part 3

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Formulation description	FDC-G1 or 2 or3 (10mg/40mg)	FDC (5mg/40mg)	FDC (5mg/20mg)	Each tablet contains 10 mg of ambrisentan, approximately 90 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each tablet contains 5 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura Red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 245 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil and 5 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 5 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral

Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC	FDC	FDC	Reference		
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed
Address of reference manufacturer	NA	NA	NA	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Glaxo Operations UK Ltd (trading as GlaxoWellcome Operations) Harmire Road Barnard Castle Co. Durham DL12 8DT United Kingdom	Lilly S.A Avda. de la Industria 30 28108 Alcobendas Madrid Spain

6.1.1 Retention Samples

GSK will send five times (5x) the drug product necessary for pivotal bioequivalency evaluation in Part 3 of the study for analytical testing for all tablet strengths used in the study. The retain samples are in addition to the supply of drug product sufficient to complete the study. Retention samples will be sent to the clinical CRO in the same bulk container. To ensure that reserve samples are representative of the same batches provided for the clinical study the clinical site will randomly select the supplies for the study from the supply received. The clinical site must retain enough reserve samples to permit FDA to perform five times all of the release tests required in the application.

Section 6.2 Treatment Assignment

CHANGE FROM

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

The treatments are denoted as F1 to F4 for Part 1, F5 to F8 for Part 2 and R for reference treatment for both parts; the selected formulations for Part 3 are denoted as X and Y. The treatment key for Parts 1, 2 and 3 are described in Table 6

Table 6 Treatment Key for Part 1, Part 2 and Part 3

Treatment	Description
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 2	
F5	ambrisentan and tadalafil FDC5 (10mg/40mg)
F6	ambrisentan and tadalafil FDC6 (10mg/40mg)
F7	ambrisentan and tadalafil FDC7 (10mg/40mg)
F8	ambrisentan and tadalafil FDC8 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 3	

Treatment	Description
X1	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
X2	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
Y1	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
Y2	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 7. Not all possible sequences are included here. Such as, if only one formulation taken into Part 2 or 3, or Part 2 is used for only one or two FDC formulations, fed and fasted. Additional treatment sequences will be created based on the Latin Squares for Williams Designs, as required

Table 7 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1 F2 R F3 F4 F2 F3 F1 F4 R F3 F4 F2 R F1 F4 R F3 F1 F2 R F1 F4 F2 F3 F4 F3 R F2 F1 R F4 F1 F3 F2 F1 R F2 F4 F3 F2 F1 F3 R F4 F3 F2 F4 F1 R	1:1:1:1:1:1:1:1:1:1
Part 2	5	F5 F6 R F7 F8 F6 F7 F5 F8 R F7 F8 F6 R F5 F8 R F7 F5 F6 R F5 F8 F6 F7 F8 F7 R F6 F5 R F8 F5 F7 F6 F5 R F6 F8 F7 F6 F5 F7 R F8 F7 F6 F8 F5 R	1:1:1:1:1:1:1:1:1:1
	4	F5 F6 R F7 F6 F7 F5 R F7 R F6 F5 R F5 F7 F6	1:1:1:1
	3	F5 F6 R R F6 F5 F6 R F5 F1 R F6 R F5 F6 F6 F5 R	3:3:3:3:3:3 + 2 random
	2	F5 R	1:1

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
		R F5	
Part 3	4	X1 X2 Y2 Y1 X2 Y1 X1 Y2 Y1 Y2 X2 X1 Y2 X1 Y1 X2	1:1:1:1
Part 2 and Part 3 merged together	5 (Two FDCs selected from Part 1)	X1 X2 R Y1 Y2 X2 Y1 X1 Y2 R Y1 Y2 X2 R X1 Y2 R Y1 X1 X2 R X1 Y2 X2 Y1 Y2 Y1 R X2 X1 R Y2 X1 Y1 X2 X1 R X2 Y2 Y1 X2 X1 Y1 R Y2 Y1 X2 Y2 X1 R	1:1:1:1:1:1:1:1:1:1
	3 (One FDC selected from Part 1)	X1 X2 R R X2 X1 X2 R X1 F1 R X2 R X1 X2 X2 X1 R	3:3:3:3:3:3 + 2 random

CHANGE TO

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software. ~~The treatments are denoted as F1 to F4 for Part 1, F5 to F8 for Part 2 and R for reference treatment for both parts; the selected formulations for Part 3 are denoted as X and Y~~

The treatment **key for Parts 1, 2 and 3 are described in Table 6**.

Table 6 Treatment Key for Part 1, Part 2 and Part 3

Treatment	**Description**
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)

Treatment	Description
Part 2	
FG1	**ambrisentan and tadalafil FDC-G1 (10mg/40mg) granulation size 1**
FG2	**ambrisentan and tadalafil FDC-G2 (10mg/40mg) granulation size 2**
FG3	**ambrisentan and tadalafil FDC-G3 (10mg/40mg) granulation size 3**
R	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)**
Part 3A	
X1	**ambrisentan and tadalafil FDC-G1 or 2 or 3 (10mg/40mg) fed**
X2	**ambrisentan and tadalafil FDC G1 or 2 or 3 (10mg/40mg) fasted**
R1	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fed**
R2	**ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg), fasted**
Part 3B	
Y1	**ambrisentan and tadalafil FDC (5mg/40mg), fasted**
Y2	**ambrisentan and tadalafil FDC (5mg/20mg), fasted**
R3	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/40mg), fasted**
R4	**ambrisentan and tadalafil monotherapies taken concurrently (5mg/20mg), fasted**

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 7. ~~Not all possible sequences are included here. Such as, if only one formulation taken into Part 2 or 3, or Part 2 is used for only one or two FDC formulations, fed and fasted~~ Additional treatment sequences may be created based using the Latin Squares for Williams Designs **if needed**.

Table 7 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1/ F2/ R/ F3/ F4 F2/ F3/ F1/ F4/ R F3/ F4/ F2/ R/ F1 F4/ R/ F3/ F1/ F2 R/ F1/ F4/ F2/ F3 F4/ F3/ R/ F2/ F1 R/ F4/ F1/ F3/ F2 F1/ R/ F2/ F4/ F3 F2/ F1/ F3/ R/ F4 F3/ F2/ F4/ F1/ R	1:1:1:1:1:1:1:1:1:1

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 2	4	FG1/ FG2/ R/ FG3 FG2/ FG3/ FG1/ R FG3/ R/ FG2/ FG1 R/ FG1/ FG3/ FG2	1:1:1:1
Part 3A	**4**	**X1/ R1/ R2/ X2** **R1/ X2/ X1/ R2** **X2/ R2/ R1/ X1** **R2/ X1/ X2/ R1**	**1:1:1:1**
Part 3B	**4**	**Y1/ R3/ R4/ Y2** **R3/ Y2/ Y1/ R4** **Y2/ R4/ R3/ Y1** **R4/ Y1/ Y2/ R3**	**1:1:1:1**

Section 9.1 Hypotheses

CHANGE FROM

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and if used, Study part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in to Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

CHANGE TO

No formal hypothesis will be tested **for study Part 1 and Part 2.** An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently**, (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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference). The objective of Part 2 is to assess whether differences in granulation size impact the pharmacokinetics of ambrisentan and tadalafil; the estimation approach for bioavailability is therefore applicable to Part 2.**

Part 3A of the study is designed to test the bioequivalence of a FDC of ambrisentan 10 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 10 mg & tadalafil 40 mg taken concurrently (reference) in healthy subjects in both the fed and fasted states. The null hypothesis is that the true ratio of the geometric

mean of the test treatment to the geometric mean of the reference treatment, μ(test)/μ(reference), for each primary PK endpoint, is either less than 0.80 or greater than 1.25. The alternate hypothesis is that the true ratio of the test treatment geometric mean to the reference treatment geometric mean is at least 0.80 and no greater than 1.25. Symbolically, this is expressed as follows:

H(0): μ(test)/μ(reference) < 0.80 or μ(test)/μ(reference) > 1.25,

i.e., treatments are not bioequivalent.

versus

H(1) : 0.80 ≤ μ(test)/μ(reference) ≤ 1.25,

i.e. , treatments are bioequivalent.

For each PK parameter designated as a primary endpoint, a two one-sided t-test (TOST) procedure (Schuirmann, 1987) with α=0.05 for each one-sided test will be used to test this set of hypotheses. This is equivalent to requiring that a 90% interval for the true ratio of test to reference geometric means falls entirely within the range of 0.80 to 1.25.

Part 3B of the study is designed similar to Part 3A and will test (1) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 40 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 40 mg taken concurrently (reference) and (2) the bioequivalence of a FDC of ambrisentan 5 mg + tadalafil 20 mg (test) relative to reference monotherapies of ambrisentan 5 mg & tadalafil 20 mg taken concurrently (reference), in healthy subjects in the fasted states. Therefore, the analysis approach for bioequivalence for Part 3A is applicable to Part 3B.

Section 9.2 Sample Size Considerations

CHANGE FROM:

No formal sample size calculation has been performed. Sample sizes chosen for each study part in this study is considered sufficient for exploratory analysis based on PK variability data from previous studies

CHANGE TO

~~No formal sample size calculation has been performed. Sample sizes chosen for each study part in this study is considered sufficient for exploratory analysis based on PK variability data from previous studies~~

Section 9.2.1 Sample Size Assumptions

CHANGE FROM

The sample size assumptions are based on previously reported estimates of within subject CV for AUC(0-∞) and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005). Table 9 summarizes the estimates of within subject CV for the primary endpoints AUC (0-∞) and Cmax.

Table9 Estimates of within subject CV for the primary end points AUC (0-∞) and Cmax

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
AUC (0-∞)	15%	13%

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 based on a sample size of 20 statistically evaluable subjects.

CHANGE TO

For Part 1 and Part 2 of the study, the sample size assumptions are based on previously reported estimates of within subject CV for $AUC_{(0-\infty)}$ and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005**). Table 9** summarizes the estimates of within subject CV for the primary endpoints $AUC_{(0-\infty)}$ and Cmax.

Table 9 Estimates of within subject CV for the primary end points AUC (0-∞) and Cmax

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
$AUC_{(0-\infty)}$	15%	13%

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 based on a sample size of 20 statistically evaluable subjects.

For Part 3A of the study, Table 10 summarized the observed estimates of between and within subject CV and observed ratio of geometric means for the primary endpoints $AUC_{(0-t)}$ and C_{max} from Part 1 of the study.

Table 10 Estimates of within subject CV for the primary end points AUC $_{(0-\infty)}$ and C_{max} for ambrisentan and tadalafil from part 1 of the study

CVw: within subject CV	ambrisentan	tadalafil
C_{max}	22.42%	14.53%
AUC $_{(0-\infty)}$	8.12%	10.32%

The largest of the within subject CV estimates 22.42% translates to a standard deviation (SD) of 0.221 on the natural log scale. Based on this SD, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. This calculation assumes:

- **a true ratio of 1,**
- **the within-subject variability from the current study will not be larger than that used in the sample size calculations,**
- **data are log-normally distributed, and each t-test is made at the 5% level.**

For Part 3B of the study, same assumptions are adopted as Part 3A. Thus, a sample size of 23 statistically evaluable subjects will have 90% power to demonstrate bioequivalence. However, if the within subject CVs obtained after Part 2 are significantly higher than the ones in Part 1 (shown in Table 10), a sample size re-esimation may be conducted to adjust the sample size for Part 3A and 3B to ensure the study maintain enough power to demonstrate bioequivalence.

Section 9.2.2 Sample Size Sensitivity

CHANGE FROM

Assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then Table 11 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 11 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%

CHANGE TO

For Part 1 and Part 2, assuming the within subject CV differs from 22% and the number of statistically evaluable subjects is lower than 20, then **Table 11** shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects**.**

Table 11 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%

For Part 3, based on the observed data from previous studies, the ratio of geometric mean for ambrisentan Cmax is unlikely to be 1. The effects on the power of declaring bioequivalence in the face of a shift in the expected ratio of the geometric means were examined.

If, under all other assumptions outlined above, the actual ratio of geometric means for ambrisentan Cmax is 0.97, then 26 evaluable subjects are needed to have 90% power to conclude bioequivalence.

With 26 evaluable subjects, the current study design has at least 90% power to conclude bioequivalance (with both Cmax and AUC for ambrisentan and tadalafil).

Section 9.2.3 Sample Size Re-estimation or Adjustment

CHANGE FROM

No sample size re-estimation will be performed.

CHANGE TO

No sample size re-estimation will be performed**. However, if the estimation of the within subject CV from Part 2 of the study is much larger than the within subject CV from Part 1 of the study, the sample size for Part3 may be re-evaluated.**

Section 9.3 Data Analysis Considerations

CHANGE FROM

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed by non-compartmental methods with WinNonlin [version 6.3 or above]. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data, the following pharmacokinetic parameters will be determined, as data permit: maximum observed plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curve [AUC(0-t) and AUC(0-∞)], and apparent terminal phase half-life (t1/2).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in a mixed effects model.

CHANGE TO

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed 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 analayis 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 Cmax $_{(tmax)}$, area under the plasma concentration-time curve [$AUC_{(0-t)}$ and $AUC_{(0-\infty)}$], and apparent terminal phase half-life ($t_{1/2}$).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using log_e-transformed AUC(0-∞) and Cmax in a mixed effects model

9.3.1 Analysis Populations

9.3.1.1. Safety Population

All subjects enrolled into the study who have received at least one dose of investigational product will be included in the Safety Population.

9.3.1.2. Pharmacokinetic Concentration Population

The PK Concentration Population will include all subjects for whom at least one PK sample was obtained and analyzed.

9.3.1.3. Pharmacokinetic Parameter Population

For each PK parameter, the PK Parameter Population will include all subjects who provide PK parameter data.

Section 9.3.2 Interim Analysis

CHANGE FROM

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed in order to direct development of fixed dose formulations for subsequent study parts and the planned BE study.

CHANGE TO

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed **in Part 1 and Part 2 of the study in order to direct development of the FDC for later parts of the study. Headline results based on statistical analysis using preliminary pharmacokinetic data with nominal time may be produced when 80% and 100% subjects complete Part 1 and Part 2 of the study to assist development of**

FDCs. Treatment and period information from the crossover design may be used in the analysis; sequence information may also be included depending on availability.

Section 9.4.1 Pharmacokinetic Analyses

CHANGE FROM

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

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

Following $\log_e$-transformation, AUC(0-∞), AUC(0-t) and Cmax of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period and treatment. Subject will be treated as a random effect in the model. Point estimates and their associated 90% confidence intervals will be constructed for the differences, F1 – R, F2 – R, F3 – R, F4 – R for Part 1 and similarly for Part 2 (as needed); and X1-X2, Y1-Y2 for Part 3. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2-R:F3-R, F4-R; and X1:X2, Y1:Y2.

Tmax of FDC formulations will be analyzed with the non-parametric Wilcoxon Matched Pairs Method to compute point estimate and associated 90% confidence intervals for the median difference, F1 R, F2 R, F3 R, F4 R and X1:X2, Y1:Y2.

CHANGE TO

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

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

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.

The PK parameters, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, 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.

Actual visit day will be used for safety assessments. For the assessment of bioequivalence, both actual time of sampling, and nominal (planned) sampling time, will be used to estimate PK parameters at the end of the study. PK parameters estimation based on nominal sampling time will be used for generating headline results. Following log_e-transformation, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max} of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period**, sequence** and treatment**, and subject** as random effect **term (i.e. ANOVA method using period, sequence and treatment as fixed effect, and subject as random effect)** Point estimates and their associated 90% confidence intervals will be **calculated** for the differences: F1 - R, F2 - R, F3 -R, F4 - R for Part 1**; FG1-R, FG2-R, FG3-R** for Part 2**; X1-R1, X2-R2 for Part 3A; and Y-R3 for Part 3B.** The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2:R, F3:R, F4:R**; for Part 1; FG1:R, FG2:R, FG3:R for Part 2; X1:R1,X2:R2 for Part3A; and Y:R3 for Part 3B.**

Section 11 REFERENCES

ADDITIONS

EMEA guidelines on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **. 2010

Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability, J Pharmacokin. Biopharm. 1987:15:657–680.

Section 12.4 Appendix 4 High Fat Meal Content

CHANGE FROM

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the US FDA in their 2002 draft guidance on food-effect bioavailability and bioequivalence studies. Approximately 50% of the caloric content of the meal is from fat and the meal is high in calories (approximately 1000 calories). Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

US Department of Health and Human Services. Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies. Guidance. Center for Drug Evaluation and Research (CDER). Food and Drug Administration; 2002.

CHANGE TO

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the **EMEA guidelines** on **the investigation of** bioequivalence **2010. The meal should be a high-fat** (**approximately** 50 **percent** of **total** caloric content of the meal) and high-**calorie** (approximately **800 to 1000 kcal) meal. This** test meal **should derive approximately 150, 250, and 500-600 kcal** from protein, carbohydrate, and fat, **respectively**(**EMEA, 2010).**

TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A Phase 1 study to demonstrate the relative bioavailability of fixed dose combinations of ambrisentan and tadalafil in healthy subjects

Compound Number: GSK3380154, GSK1325760 and GF196960

Development Phase: I

Effective Date: 14-APR-2016

Protocol Amendment Number: 01

Author (s): PPD

Revision Chronology

GlaxoSmithKline Document Number	Date	Version
2015N232335_00	2015-DEC-16	Original
2015N232335_01	2016-APR-14	Amendment No. 1
Clarification of hypotension withdrawal criteria in Section 5.4.3, change to GSK medical monitor and minor administrative changes.		

SPONSOR SIGNATORY

PPD

14/04/16

Hakim Bendjenana

Physician Lead

Date 14 April 2016

MEDICAL MONITOR/SPONSOR INFORMATION PAGE

Medical Monitor/SAE Contact Information:

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Monitor				Site to scan and send to email
SAE contact information				Site to scan and send to email

Sponsor Legal Registered Address:

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

In some countries, the clinical trial sponsor may be the local GlaxoSmithKline Affiliate Company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number(s): EudraCT number 2015-004140-18

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number 201964

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

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

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

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

TABLE OF CONTENTS

PAGE

1. PROTOCOL SYNOPSIS FOR STUDY 201964

Rationale

This study is designed to select one, or more fixed dose combinations (FDCs) of ambrisentan and tadalafil for further development and to provide pharmacokinetic data to enable a pivotal bioequivalence study (BE). Dependent on formulation work, the study will allow up to 8 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently. The study would also allow for up to 2 of the new formulations, that may be taken in to a BE study, to be tested for any effect on pharmacokinetics of the FDC in both fed and fasted state.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assessed by vital signs, ECG, clinical laboratory safety data and review of adverse events

Overall Design

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects.

All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast.

Treatment Arms and Duration

The proposed treatment arms for each study part are described here; however the treatments in Parts 2 and 3 may be changed dependent on the utility and results from the previous part.

Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)[1]	Part 3[2]
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less FDC treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. The minimum will be approximately 3 weeks and the maximum will be approximately 18 weeks.

Type and Number of Subjects

A approximately of 20 healthy adult subjects will be randomized, to each study part, such that at least 16 evaluable subjects complete each part of the study.

Analysis

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and Part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in Part 2, if used for food effect and Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

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

2. INTRODUCTION

Ambrisentan (E.U. trade name: Volibris), an orally active endothelin receptor antagonists (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease

Tadalafil (E.U. trade name: Adcirca) is an orally active selective inhibitor of the enzyme PDE-5, the primary cGMP-hydrolyzing enzyme in smooth muscle. In the EU, Adcirca is indicated in adults for the treatment of PAH classified as WHO FC II and III, to improve exercise capacity (Adcirca, EMA, SmPC, 2015).

A recently completed study (Galiè, 2015a) has show that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris, EMA SmPC, 2015, Section 5.1).

This pilot study will investigate the relative bioavailability of new fixed dose combinations (FDC) of ambrisentan and tadalafil, compared to the two monotherapies taken concurrently in healthy subjects

2.1. Study Rationale

This study is designed to select one, or more, FDCs of ambrisentan and tadalafil for further development and to provide pharmacokinetic data to enable a pivotal bioequivalence study (BE). Dependent on formulation work, the study will allow up to 8 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently. The study would also allow for up to 2 of the new formulations, that may be taken in to a BE study, to be tested for any effect on pharmacokinetics of the FDC in both fed and fasted state.

The formulation(s) to be taken forward into the BE study will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, between subject variability, tablet size, cost, ease of manufacture and stability would be considered.

2.2. Brief Background

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3

biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galiè, 2015b) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, 2015 Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.(Volibris, EMA SmPC,2015). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (Adcirca, EMA, SmPC, 2015). Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5) inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112, 2008) in 26 healthy subjects was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-administered. . There were no SAE's in the study. Three subjects withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one subject because of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a

listed event for ambrisentan. There were a total of 7 subjects with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris, EMA SmPC, 2015, Adcirca, EMA, SmPC, 2015).

The AMBITION clinical study (Galiè, 2015a and AMB112565 CSR 13Nov2014), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galiè, 2015b) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage and shipment requirements. This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, CHMP/EWP/240/95) (2008).

3. OBJECTIVE(S) AND ENDPOINT(S)

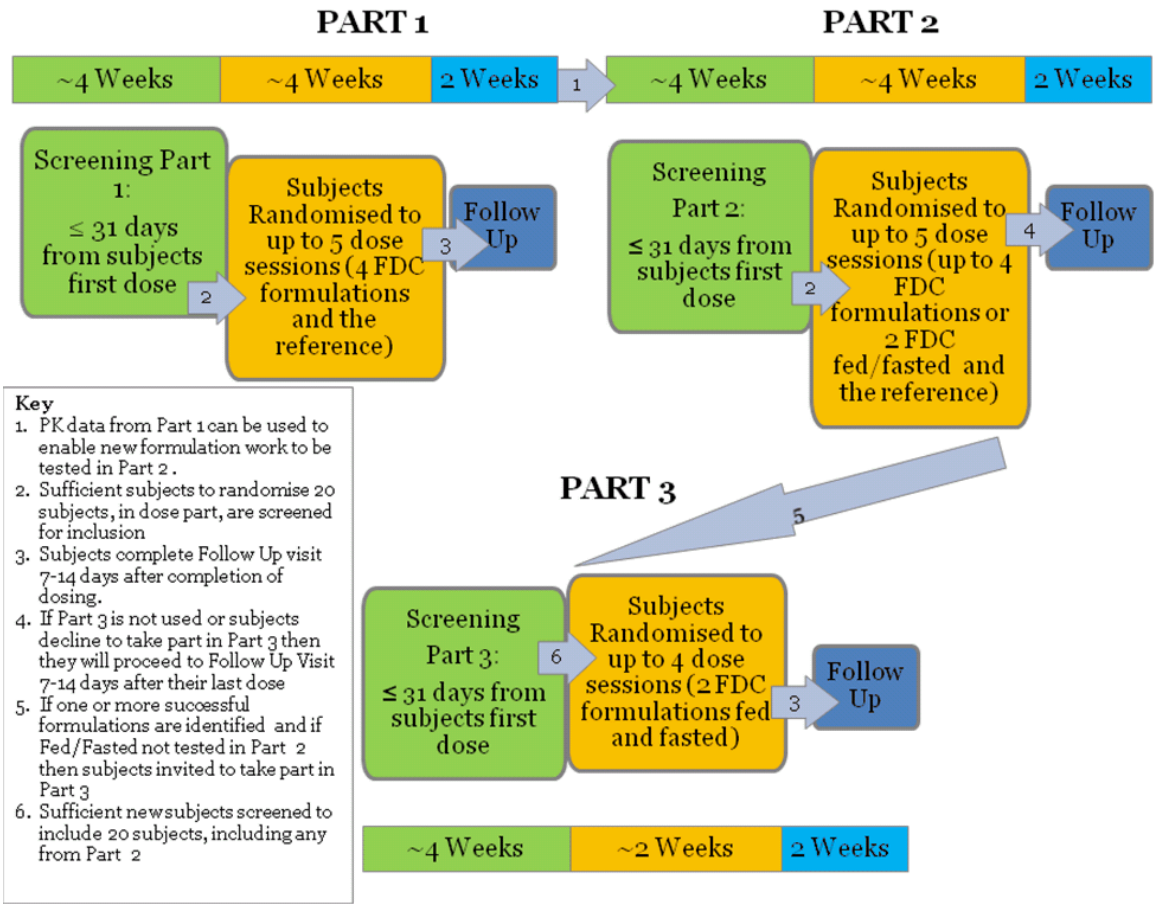
Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assessed by vital signs, ECG, clinical laboratory safety data and review of adverse events

4. STUDY DESIGN

4.1. Overall Design

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects. See Figure 1 for study schematic.

Figure 1 Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2

monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast.

Subjects who had taken part in the study part preceding Part 3, and which provided the successful formulations for a BE study could also be invited to take part in this study part. However, a subject's inclusion in more than one study part would be dependent on the subject not exceeding the maximum blood draw volume (500ml) for the study.

4.2. Treatment and Duration

The treatments for each study part of the study are listed in Table 1 All treatments are single dose. Subjects will be randomised to order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing analyse of pharmacokinetic data will be used to enable the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to look at up to 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2. If no successful formulations are identified from these criteria then the pharmacokinetic data would be used to enable further work to produce new FDC formulations for Part 2

Following completion of Part 1 there will be a pause prior to Part 2, so that up to a further 4 FDC formulations could be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for up to 4 FDC formulations. These could be either successful formulation identified in Part 1 reformulated with the final API and/or new formulations using the final API. Pharmacokinetic data from Part 2 of the study will be analysed following completion of each treatment arm by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to define any successful FDC formulation to be taken into Part 3 of this study and a pivotal BE study. If only two formulations are evaluated in Part 2 then the food effect may be added to Part 2 and Part 3 will not be required. Success will be defined with the same criteria as those in Part 1.

Part 3

Part 3 of the study will provide data for up to 2 successful formulations to be progressed to a pivotal BE study, in both the fed and fasted state.

Table 1 Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)[1]	Part 3[2]
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. Subjects may be included in Part 1, Part 2 or Part 3 of the study. However, if Part 2 of the study identifies any successful formulations to take into Part 3 then subjects from Part 2 may be invited to join Part 3.

The minimum and maximum duration for a subject on this study is described in Table 2. This table assumes all 5 dose sessions are used for Part 1 and Part 2 and either 2 or 4 dose sessions are used in Part 3. However, this is an approximation as fewer sessions may be required in Part 2 and/or the washout between sessions of 7 days in each part may be longer, due to logistical reasons.

Table 2 Study Duration

Study Options		Screen	Study Part 1or 2	Pause	Study part 3	Follow-Up Visit	Total Duration on Study
		(1-31 days)	(31 days)	(7-28 days)	(10-24 days)	(7-14 days)	
Subjects included in only Part 1 or 2	Min	1	31			7	39
	Max	31	31			14	76
Subjects included in Part 2&3	Min	1	31	7	10	7	56
	Max	31	31	28	24	14	128
Subjects included in only Part 3	Min	1			10	7	18
	Max	31			24	14	69

A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

4.3. Type and Number of Subjects

A approximately of 20 healthy adult subjects will be randomized, to each study part, such that at least 16 evaluable subjects complete each part of the study.

The study is not powered. However, the number of subjects will be sufficient for the objectives of this study and this is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

4.4. Design Justification

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations to take into a pivotal BE study and to also provide pharmacokinetic data to enable the pivotal BE study to be powered sufficiently.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, or is there any need to blind study treatment. The Inclusion of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study is in three parts to enable PK data from Part 1 to enable formulation development for Part 2. Part 3 providing an opportunity to assess food effect in any FDC formulations suitable for development and inclusion in the pivotal bioequivalence study. The interdependency of the 3 study parts is described in detail in Section 4.2.

4.5. Dose Justification

The oral dose for the new FDC formulations and the reference treatment proposed for the study of, 40mg for tadalafil and 10mg for ambrisentan are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to be tolerated in healthy subjects.

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates a minimum washout of 7 days, between doses, is also sufficient for clearance of previous dose.

4.6. Benefit: Risk Assessment

There is no direct benefit for healthy subjects participating in this study. The risks to the healthy subjects based upon previous experience indicate no expectation of SAE and mild to moderate AE. The benefit/risk remains satisfactory for this healthy subject study.

Summaries of findings from clinical studies conducted with both the Investigational Product taken as monotherapies and in combination can be found in:

- Ambrisentan IB (Volibris IB, 2015)
- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (Adcirca, EMA SmPC, 2015)

As described in Section 2.2, mild tachycardia was the most common (7/26, 27%) adverse event seen in healthy subjects following combination dosing with 10mg ambrisentan and 40mg tadalafil (Spence, 2009). There were three subjects (3/26, 11.5%) withdrawn due to AEs (e.g., anaemia [mild]; myalgia [severe], muscle fatigue, and dizziness; and headache [severe]). No SAEs or additional risks were observed with combination use in healthy subjects.

The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Risks of clinical significance, identified in subjects with PAH and the mitigation strategy identified for this study in healthy subjects are captured in Table 3.

Table 3 Risks of Clinical Significance and Mitigation Strategy

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product: GSK1325760 (ambrisentan-Volibris)		
• Teratogenicity • Anaemia (decreased haemoglobin, decreased haematocrit) • Hypersensitivity reactions (e.g. angioedema, rash, pruritus) • Headache (including sinus headache, migraine) • Dizziness • Cardiac failure • Palpitation • Hypotension • Flushing • Epistaxis • Dyspnoea • Upper respiratory congestion, sinusitis, nasopharyngitis, rhinitis • Abdominal pain • Constipation • Nausea, vomiting, diarrhoea • Hepatic transaminases increased • Peripheral oedema, fluid retention • Chest pain/discomfort • Asthenia and fatigue	Ambrisentan IB (Volibris IB, 2015) Volibris (Ambrisentan) EMA SmPC (Volibris, EMA SmPC, 2015):	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Exclusion of women of childbearing potential (teratogenicity) • Laboratory assessments per protocol • Physical assessment per protocol • Routine vital signs per protocol • Subjects remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GF196960 (tadalafil - Adcirca)		
• Hypersensitivity reactions • Headache • Syncope, • Migraine • Blurred vision • Palpitations • Nasopharyngitis (including nasal congestion, sinus congestion and rhinitis) • Epistaxis	Adcirca (Tadalafil) EMA SmPC (Adcirca, EMA SmPC, 2015))	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Routine vital signs per protocol • Physical assessment per protocol • Subjects remain in the clinical unit for all doses, under medical supervision and until completion of

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
• Nausea, • Dyspepsia (including abdominal pain/discomfort) • Vomiting, • Gastroesophageal reflux • Rash • Myalgia, • Back pain • Pain in extremity (including limb discomfort) • Increased uterine bleeding • Facial oedema, • Chest pain		safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GSK3380154 (ambrisentan-tadalafil-FDC)		
The risks for the combination are the same as the 2 monotherapies; no extra risks have been identified for the combination.		As for GSK1325760 and GF196960 above

4.6.2. Benefit Assessment

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

4.6.3. Overall Benefit: Risk Conclusion

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 3. So, the risk: benefit is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in:

- Volibris (Ambrisentan) EMA SmPC (Volibris, EMA SmPC, 2015)
- Adcirca (Tadalafil) EMA SmPC (Adcirca, EMA SmPC, 2015)

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

5.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

AGE
1. Between 18 and 60 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and cardiac monitoring (ECG and 24 hour Holter). A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical Monitor if required, judges and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.

WEIGHT
3. Body weight ≥ 50 kg (110 lbs) for men and ≥ 45 kg (99lbs) for women and body mass index (BMI) within the range 18 – 30 kg/m2 (inclusive)

SEX

4. Male or Female
Females must be the following:
Non-reproductive potential defined as:
• Pre-menopausal females with one of the following:
  • Documented tubal ligation
  • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion
  • Hysterectomy
  • Documented Bilateral Oophorectomy
• Documented Postmenopausal defined as 12 months of spontaneous amenorrhea

INFORMED CONSENT
5. Capable of giving signed informed consent as described in Section 10 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol.

5.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)
1. A blood pressure <100/55 mm Hg. 2. Haemoglobin below normal range: • Hb < 133 g/L for males • Hb< 114 g/L for females 3. ALT and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%). 4. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) 5. 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. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP).

CONCOMITANT MEDICATIONS
6. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose

of study medication, unless in the opinion of the Investigator and GSK Medical Monitor the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
7. History of regular alcohol consumption within 6 months of the study defined as: • An average weekly intake of >21 units for males or >14 units for females. 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. 8. Smoking more than 5 cigarettes per week and subjects must be able to abstain from smoking for a 24 hour period prior to dose and any time whilst in the clinical unit.

CONTRAINDICATIONS
9. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or Medical Monitor, contraindicates their participation.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
10. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at Screening or within 3 months prior to first dose of study treatment. . 11. A positive test for HIV antibody. 12. A positive pre-study drug/alcohol screen. 13. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within previous 3 months 14. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 3 months, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 15. Exposure to more than four new chemical entities within 12 months prior to the first dosing day.

5.3. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal

set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.4.1.4).

5.4. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

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

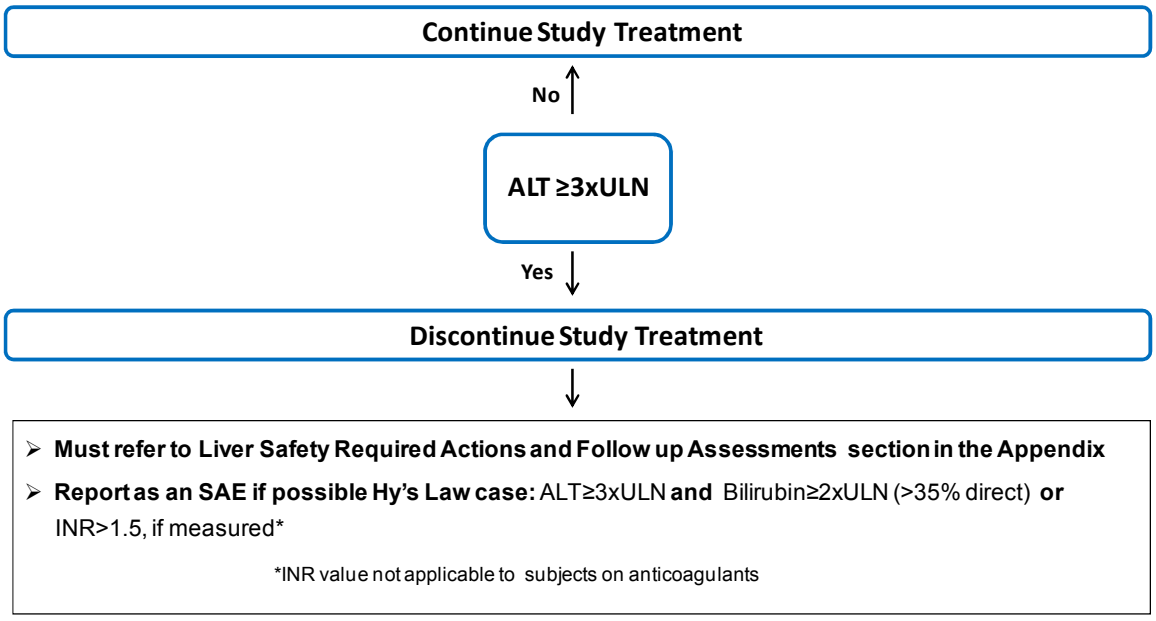
A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

5.4.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Study treatment will be discontinued **for a subject** if liver chemistry stopping criteria are met:

Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow-Up Assessments Section can be found in Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

5.4.1.1. Study Treatment Restart or Rechallenge

Study treatment restart or re-challenge after liver chemistry stopping criteria are met by any subject participating in this study is not allowed.

5.4.2. QTc Stopping Criteria

- The *same* QT correction formula *must* be used for *each individual subject* to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the subject has been enrolled.
  - For example, if a subject is eligible for the protocol based on QTcF, then QTcF must be used for discontinuation of this individual subject as well.
  - Once the QT correction formula has been chosen for a subject's eligibility, the *same formula* must continue to be used for that subject *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 averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A subject that meets either bulleted criterion below will be withdrawn from the study.

- QTc> 500 msec,

- Change from baseline: QTc >60 msec

5.4.3. Hypotension

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 mmHG and diastolic <50 mm HG confirmed by triplicate reading taken up to 5 minutes apart and is judged clinically significant and symptomatic by the investigator.

5.4.4. Other Dose Adjustment/Stopping Safety Criteria

For an individual study participant, stopping criteria include, but are not limited to:

Adverse events, or significant changes in any of the safety assessments, that put the safety of the individual at risk (e.g., ECG, vital signs, laboratory tests, etc), as judged by the Principal Investigator in consultation with the Medical Monitor if necessary.

5.5. Subject and Study Completion

A completed subject is one who has completed all phases of the study including the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

The term 'study treatment' is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for Part 1 of the study are shown in Table 4, this will be amended once treatments for Part 2 and Part 3 are confirmed.

Table 4 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS**	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 233 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

6.2. Medical Devices

No GSK manufactured devices (or devices manufactured for GSK by a third party) are provided for use in this study.

6.3. Treatment Assignment

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

The treatments are denoted as F1 to F4 for Part 1, F5 to F8 for Part 2 and R for reference treatment for both parts; the selected formulations for Part 3 are denoted as X and Y. The treatment key for Parts 1, 2 and 3 are described in Table 5

Table 5 Treatment Key for Part 1, Part 2 and Part 3

Treatment	Description
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 2	
F5	ambrisentan and tadalafil FDC5 (10mg/40mg)
F6	ambrisentan and tadalafil FDC6 (10mg/40mg)
F7	ambrisentan and tadalafil FDC7 (10mg/40mg)
F8	ambrisentan and tadalafil FDC8 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 3	
X1	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
X2	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
Y1	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
Y2	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 6. Not all possible sequences are included here. Such as, if only one formulation taken into Part 2 or 3, or Part 2 is used for

only one or two FDC formulations, fed and fasted. Additional treatment sequences will be created based on the Latin Squares for Williams Designs, as required.

Table 6 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1 F2 R F3 F4 F2 F3 F1 F4 R F3 F4 F2 R F1 F4 R F3 F1 F2 R F1 F4 F2 F3 F4 F3 R F2 F1 R F4 F1 F3 F2 F1 R F2 F4 F3 F2 F1 F3 R F4 F3 F2 F4 F1 R	1:1:1:1:1:1:1:1:1:1
Part 2	5	F5 F6 R F7 F8 F6 F7 F5 F8 R F7 F8 F6 R F5 F8 R F7 F5 F6 R F5 F8 F6 F7 F8 F7 R F6 F5 R F8 F5 F7 F6 F5 R F6 F8 F7 F6 F5 F7 R F8 F7 F6 F8 F5 R	1:1:1:1:1:1:1:1:1:1
	4	F5 F6 R F7 F6 F7 F5 R F7 R F6 F5 R F5 F7 F6	1:1:1:1
	3	F5 F6 R R F6 F5 F6 R F5 F1 R F6 R F5 F6 F6 F5 R	3:3:3:3:3:3 + 2 random
	2	F5 R R F5	1:1
Part 3	4	X1 X2 Y2 Y1 X2 Y1 X1 Y2 Y1 Y2 X2 X1 Y2 X1 Y1 X2	1:1:1:1
Part 2 and Part 3 merged together	5 (Two FDCs selected from Part 1)	X1 X2 R Y1 Y2 X2 Y1 X1 Y2 R Y1 Y2 X2 R X1 Y2 R Y1 X1 X2 R X1 Y2 X2 Y1 Y2 Y1 R X2 X1	1:1:1:1:1:1:1:1:1:1

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
		R Y2 X1 Y1 X2 X1 R X2 Y2 Y1 X2 X1 Y1 R Y2 Y1 X2 Y2 X1 R	
	3 (One FDC selected from Part 1)	X1 X2 R R X2 X1 X2 R X1 F1 R X2 R X1 X2 X2 X1 R	3:3:3:3:3:3 + 2 random

6.4. Blinding

This will be an open-label study.

6.5. Packaging and Labeling

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

6.6. Preparation/Handling/Storage/Accountability

This will be detailed in a Study Specific Technical Agreement/Memo (TTS) which will be accompanied by a Quality Agreement.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.
- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the SRM.
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
- 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.

6.7. Compliance with Study Treatment Administration

When subjects are dosed at the site, they will receive study treatment 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 treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each subject's mouth to ensure that the study treatment was ingested.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK3380154 (ambrisentan/tadalafil-FDC), GSK1325760 (ambrisentan) and GF196960 (tadalafil) greater than the protocol defined dose and within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose.

Advice for the investigator is included in the product label for GSK1325760 (ambrisentan) and GF196960 (tadalafil).

In the event of an overdose the investigator should:

1. Contact the Medical Monitor immediately
2. Closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until compound number/name can no longer be detected systemically (at least 3 days for compound number/name)
3. Obtain a plasma sample for pharmacokinetic (PK) analysis 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 subject.

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because only healthy subjects are eligible for study participation.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until after the final dose.

- Dependent on the utility of the dose session subjects will be asked to fast or consume the FDA full fat breakfast prior to dosing:
  - Fasting subjects will be required to fast from midnight before each full in-house dosing day (i.e. Day 1) with the exception of water, which will be allowed freely except for 1 hour either side of dosing. Subjects will be required to fast up to 4h hour post dose on Day 1 of each dose
  - For treatments given under fed conditions, a standard high-fat breakfast will be provided before dosing. See Section 12.4 for details of this meal. This meal should be eaten in its entirety within 30 minutes. The amount consumed will be recorded within the source documents and the CRF by the site staff. Subjects will be dosed within 5 minutes of completing the breakfast.
- Subjects should take each dose of investigational product with 240 ml (8 fl oz) of water.

6.10.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks and chocolate) for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- During each dosing session, subjects will abstain from alcohol for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- Smoking is not allowed for 24 hours prior to dosing and whilst subjects are in the clinic.

6.10.3. Activity

Subjects will abstain from strenuous exercise from Screening until Follow-Up. Subjects may participate in light recreational activities during studies (e.g., watch television, read).

6.11. Concomitant Medications and Non-Drug Therapies

6.11.1. Permitted Medications and Non-Drug Therapies

Paracetamol at doses of ≤ 2 grams/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.

6.11.2. Prohibited Medications and Non-Drug Therapies

Subjects 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) prior to the first dose of study medication until completion of the Follow-Up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 7.1

The following points must be noted:

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:
    1. 12-lead ECG
    2. Vital signs
    3. Blood draws.

  Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.
- The timing and number of planned study assessments, safety and pharmacokinetic assessments 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.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.

No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

7.1. Time and Events Table

Procedure	**Screen**	**Part 1, 2 and 3. Each dose in each Part repeats this schedule.**																**FU**	**Notes**
Day	**≤-31**	**-1**	**1**											**2**		**3**	**4**	**≥7-14**	
Time (hrs)			**Pre-dose**	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**4**	**8**	**12**	**18**	**24**	**36**	**48**	**72**		
Outpatient visit	x																x	x	
Admission to unit		x																	
Informed consent	x																		
Inclusion and exclusion criteria	x	x																	
Demography	x																		
Full physical exam including height and weight	x																		
Brief Physical																		x	
Medical history (includes substance usage)	x																		
HIV, Hep B and Hep C screen]	x																		
Laboratory assessments (include liver chemistries)	x	x														x		x	Only Screening labs need to be taken in fasted state.
Serum hCG Pregnancy test	x																	x	Female subjects only
Urine hCG Pregnancy test		x																	Female subjects only
Breathalyser and Smokerlyzer	x	x																	
DOA testing	x	x																	
12-lead ECG	x		x			x		x		x		x		x			x	x	Triplicate at screen and baseline, single measure at other times, unless out of range then triplicates should be performed
Vital signs	x		x		x	x		x		x	x	x		x		x	x	x	
24hr Holter	x																		
Randomisation			x																Randomised prior to first dose only
Study Treatment				x															
AE/SAE review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	SAEs from Screen. AEs from first dose
Concomitant medication review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
PK Sample			x		x	x	x	x	x	x	x	x	x	x	x	x	x		
Discharge from Unit																x			For logistical reasons subjects may remain in-unit for the 72 hr assessments if they prefer.

7.2. Screening and Critical Baseline Assessments

All subjects must give written consent to participate in this trial. Consent for screening evaluations may be obtained using the ICF for the Hammersmith Medicines Research (HMR) healthy subject's panel, which has been approved by the HRA's Phase 1 Advert Review group. The study-specific information and consent form will be signed by the subject either before any screening evaluation or after the investigator confirms the eligibility of the subject for the study and before the subject is randomised to receive the first administration of IMP. Before giving consent, subjects must read the information sheet about the study. They must also read the consent form. They will then discuss the study with the investigator or his deputy and be given the opportunity to ask questions. The study-specific information sheet and the consent form must be approved by the REC.

7.2.1. Demographic/Medical History Assessments

Prior to enrolling in the study and having any study procedures completed, subjects must sign the informed consent.

During the Screening visit, each subject will undergo the assessments to determine eligibility for enrolment as detailed in Section 5.

The following demographic parameters will be captured: date of birth, gender, race and ethnicity.

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 5.

7.3. Pharmacokinetics

7.3.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of ambrisentan and tadalafil will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

For analysis of ambrisentan 2.7 mL of blood will be collected into sodium citrate tubes and for analysis of tadalafil 2.0 mL of blood will be collected into K2-EDTA tubes.

Processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

7.3.2. Sample Analysis

Plasma analysis will be performed under the control of Platform Technology and Science In vitro/In vivo Translation (PTS IVIVT) and Third Party Resource, GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of ambrisentan and tadalafil will be determined in plasma samples using

the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

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

7.4. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests such as vital signs, physical examinations and laboratory safety assessments may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

7.4.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The definitions of an AE or SAE can be found in Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.4.1.1. Time period and Frequency for collecting AE and SAE information

- Any SAEs assessed as related to study participation (e.g., 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 up to and including any follow up contact.
- AEs will be collected from the start of Study Treatment until the follow up contact (see Section 7.4.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.3.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Section 12.3.

7.4.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?

7.4.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Section 4.6.1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow up (as defined in Section 5.4). Further information on follow up procedures is given in Study Assessments and Procedures.

7.4.1.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs related to study treatment (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

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

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

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

7.4.2. Physical Exams

- A complete physical examination will include, at a minimum, assessment of the 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

7.4.3. Vital Signs

- Vital signs will be measured in semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure and pulse rate and respiratory rate.
- Three readings of blood pressure and pulse rate will be taken at Screening and baseline (pre-dose). All subsequent assessments will be single measures, unless the subjects blood pressure or pulse rate has changed from baseline by >15% and then 2 further readings should be taken and recorded:
- For triplicate readings:
    - All 3 readings will be recorded in the CRF
        - First reading should be rejected
        - Second and third readings should be averaged to give the measurement to be used and this will also be recorded in the CRF

7.4.4. Electrocardiogram (ECG)

- ECGs will be measured in semi-supine position after 5 minutes rest
- Triplicate 12-lead ECGs will be obtained at Screening and baseline (predose). All subsequent assessments will be a single measures, unless withdrawal criteria are meet and in which case 2 further readings should be taken to confirm if withdrawal is required . An ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- 24 hour continuous cardiac telemetry (Holter) will be performed at Screening. Full disclosures will be reviewed in detail and the review maintained as part of the subject's source documents

7.4.5. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 7, must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit dat. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

All study-required laboratory assessments will be performed by a local laboratory.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 7.

Table 7 Protocol Required Safety Laboratory Assessments

<table>
<tr><th>Laboratory Assessments</th><th>Parameters</th></tr>
<tr><td>Haematology</td><td>
<table>
<tr><td>Platelet Count</td><td><u>RBC Indices</u>:</td><td><u>WBC count with Differential:</u></td></tr>
<tr><td>RBC Count</td><td>MCV</td><td>Neutrophils</td></tr>
<tr><td>Hemoglobin</td><td>MCH</td><td>Lymphocytes</td></tr>
<tr><td>Hematocrit</td><td>MCHC</td><td>Monocytes</td></tr>
<tr><td></td><td></td><td>Eosinophils</td></tr>
<tr><td></td><td></td><td>Basophils</td></tr>
</table>
</td></tr>
<tr><td>Clinical Chemistry [1]</td><td>
<table>
<tr><td>BUN</td><td>Potassium</td><td>AST (SGOT)</td><td>Total and direct bilirubin</td></tr>
<tr><td>Creatinine</td><td>Sodium</td><td>ALT (SGPT)</td><td>Total Protein</td></tr>
<tr><td>Glucose</td><td>Calcium</td><td>Alkaline phosphatise</td><td>Albumin</td></tr>
</table>
</td></tr>
<tr><td>Routine Urinalysis[3]</td><td>As a minimum, but not limited to the following tests and dependent on standard urinalysis dipstick used:
• Specific gravity
• pH, glucose, protein, blood and ketones by dipstick
• Microscopic examination (if blood or protein is abnormal)</td></tr>
<tr><td>Other Screening Tests</td><td>• HIV
• Hepatitis B (HBsAg)
• Hepatitis C (Hep C antibody)
• Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)
• Serum or urine hCG Pregnancy test[2]</td></tr>
<tr><td colspan="2">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 5.4.1 and Appendix 2
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.
3. Routine Urinalysis results will not be databased, unless a result is out of range and clinically significant then it would be captured as an AE</td></tr>
</table>

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within7 days after the last dose of study treatment should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

8. DATA MANAGEMENT

- Data will be double-entered into a clinical database management system (ClinPlus Version 3.3).
- Management of clinical data will be performed in accordance with applicable HMR standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- Original CRFs will be retained by GSK, while HMR will retain a copy. Subject initials will not be collected or transmitted to GSK according to GSK policy

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and if used, Study part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in to Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

9.2. Sample Size Considerations

No formal sample size calculation has been performed. Sample sizes chosen for each study part in this study is considered sufficient for exploratory analysis based on PK variability data from previous studies

9.2.1. Sample Size Assumptions

The sample size assumptions are based on previously reported estimates of within subject CV for AUC(0-∞) and Cmax for ambrisentan (GS-US-300-0112, 2008) and tadalafil (Forgue, 2005). Table 8 summarizes the estimates of within subject CV for the primary endpoints AUC (0-∞) and Cmax.

Table 8 Estimates of within subject CV for the primary end points AUC (0-∞) and Cmax

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
AUC (0-∞)	15%	13%

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 based on a sample size of 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 9 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 9 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%

9.2.3. Sample Size Re-estimation or Adjustment

No sample size re-estimation will be performed.

9.3. Data Analysis Considerations

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed by non-compartmental methods with WinNonlin [version 6.3 or above]. Calculations will be based on the actual

sampling times recorded during the study. From the plasma concentration-time data, the following pharmacokinetic parameters will be determined, as data permit: maximum observed plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curve [AUC(0-t) and AUC(0-∞)], and apparent terminal phase half-life (t1/2).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in a mixed effects model.

9.3.1. Analysis Populations

9.3.1.1. Safety Population

All subjects enrolled into the study who have received at least one dose of investigational product will be included in the Safety Population.

9.3.1.2. Pharmacokinetic Concentration Population

The PK Concentration Population will include all subjects for whom at least one PK sample was obtained and analyzed.

9.3.1.3. Pharmacokinetic Parameter Population

For each PK parameter, the PK Parameter Population will include all subjects who provide PK parameter data.

9.3.2. Interim Analysis

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed in order to direct development of fixed dose formulations for subsequent study parts and the planned BE study.

9.4. Key Elements of Analysis Plan

9.4.1. Pharmacokinetic Analyses

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

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

Following $\log_e$-transformation, AUC(0-∞), AUC(0-t) and Cmax of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period and treatment. Subject will be treated as a random effect in the model. Point estimates and their associated 90% confidence intervals will be constructed for the differences, F1 – R, F2 – R, F3 – R, F4 – R for Part 1 and similarly for Part 2 (as needed); and X1-X2, Y1-Y2 for Part 3. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2-R:F3-R, F4-R; and X1:X2, Y1:Y2.

Tmax of FDC formulations will be analyzed with the non-parametric Wilcoxon Matched Pairs Method to compute point estimate and associated 90% confidence intervals for the median difference, F1 R, F2 R, F3 R, F4 R and X1:X2, Y1:Y2.

9.4.2. Safety Analyses

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

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

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

Prior to initiation of a site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Obtaining signed informed consent
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)

- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.
- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

10.3. Quality Control (Study Monitoring)

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

GSK will monitor the study and site activity to verify that the:

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.
- If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.
- If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action.
- If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.
- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

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

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

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

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

11. REFERENCES

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Volibris: EMA Summary of Product Characteristics, GSK, variation II/41, at time of publication, 2015.

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
API	Active Pharmaceutical Ingredient
AUC	Area Under Curve
BE	Bioequivalence
CDISC	Clinical Data Interchange Standards Consortium
cGMP	Cyclic guanosine monophosphate
C_{max}	Maximum concentration
CI	Confidence Interval
CV	Coefficient of variance
CVw	Coefficient of variance within subject
EMA	European Medicines Agency
ERA	Endothelin Receptor Antagonists
ERS	European Respiratory Society
ESC	European Society of Cardiology
ET-1	Endothelin Receptor - 1
EU	European Union
FDA	Federal Drug Agency
FDC	Fixed Dose Combination
NO	Nitrous Oxide
PDE-5	Phosphodiesterase type 5
SAE	Serious Adverse Events
SRM	Study Reference Manual
t_{max}	Time to maximum concentration
$t_{1/2}$	Half Life
WHO	World Health Organisation

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Adcirca
WinNonlin

12.2. Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event aetiology (in alignment with the FDA premarketing clinical liver safety guidance).

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td colspan="2">ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >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 following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th colspan="2">Follow-Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment
• 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 subject until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline</td><td colspan="2">• Viral hepatitis serology[3]
• Blood sample for pharmacokinetic (PK) analysis, obtained within 96hrs of last dose
• 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
If ALT≥3xULN AND bilirubin ≥ 2xULN or</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
• A specialist or hepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	**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 HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. • 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 treatment 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, 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); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
4. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.

References

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

12.3. Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.3.1. Definition of Adverse Events

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

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • 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 treatment administration even though it may have been present prior to the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae).

Events <u>NOT</u> meeting definition of an AE include:
• 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 subject'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 subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE. • Situations where an untoward medical occurrence did not occur (social and/or

convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

12.3.2. Definition of Serious Adverse Events

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, etc).

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

NOTE:

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

c. Requires hospitalization or prolongation of existing hospitalization

NOTE:

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

d. Results in disability/incapacity

NOTE:

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

e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. • Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse
g. Is associated with liver injury <u>and</u> impaired liver function defined as: • ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), **or** • ALT ≥ 3xULN and INR** > 1.5. * Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE. ** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.
• Refer to Section 12.2 for the required liver chemistry follow up instructions

12.3.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:
Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs: • Myocardial infarction/unstable angina • Congestive heart failure • Arrhythmias • Valvulopathy • Pulmonary hypertension • Cerebrovascular events/stroke and transient ischemic attack • Peripheral arterial thromboembolism • Deep venous thrombosis/pulmonary embolism

- Revascularization

12.3.4. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF
- It is **not** acceptable for the investigator to send photocopies of the subject'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 instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.
- Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study.
- Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer.
- The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

12.3.5. Evaluating AEs and SAEs

Assessment of Intensity

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

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating

the intensity of an event; and both AEs and SAEs can be assessed as severe.

- An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence 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 natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of 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 when 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 prior to the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow up information, amending the SAE data collection tool accordingly.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.
- The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.
- If a subject 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 the designated reporting time frames.

12.3.6. Reporting of SAEs to GSK

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 or the SAE coordinator
- 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
- Initial notification via the 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 receipt can be found at this beginning of the protocol on the Sponsor/Medical Monitor Contact Information page.

12.4. Appendix 4: High Fat Meal Content

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the US FDA in their 2002 draft guidance on food-effect bioavailability and bioequivalence studies. Approximately 50% of the caloric content of the meal is from fat and the meal is high in calories (approximately 1000 calories). Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

US Department of Health and Human Services. Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies. Guidance. Center for Drug Evaluation and Research (CDER). Food and Drug Administration; 2002.

12.5. Appendix 5: Protocol Changes

Amendment 01

The purpose of this amendment is to clarify the hypotension withdrawal criteria in section 5.4.3, address changes to GSK medical monitor and minor administrative changes.

Summary of Changes

Section 1 PROTOCOL SYNOPSIS FOR STUDY 201964

AND

Section 3 OBJECTIVES AND ENDPOINTS

CHANGE FROM

Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; $t_{max,}$ $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assed by vital signs, ECG, clinical laboratory safety data and review of adverse events

CHANGE TO

Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; $t_{max,}$ $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as **assessed** by vital signs, ECG, clinical laboratory safety data and review of adverse events

Section 5.4.3 Hypotension

CHANGE FROM

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 or diastolic <50 confirmed by triplicate reading taken up to 5 minutes apart.

CHANGE TO

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 mm HG **and** diastolic <50 mm HG confirmed by triplicate reading taken up to 5 minutes apart **and is judged clinically significant and symptomatic by the investigator**.

Section 6.3 Treatment Assignment

ADDITION

Table 6 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Part 2 and Part 3 merged together	5 (Two FDCs selected from Part 1)	X1 X2 R Y1 Y2 X2 Y1 X1 Y2 R Y1 Y2 X2 R X1 Y2 R Y1 X1 X2 R X1 Y2 X2 Y1 Y2 Y1 R X2 X1 R Y2 X1 Y1 X2 X1 R X2 Y2 Y1 X2 X1 Y1 R Y2 Y1 X2 Y2 X1 R	1:1:1:1:1:1:1:1:1:1
	3 (One FDC selected from Part 1)	X1 X2 R R X2 X1 X2 R X1 F1 R X2 R X1 X2 X2 X1 R	3:3:3:3:3:3 + 2 random

TITLE PAGE

Division: Worldwide Development

Information Type: Clinical Protocol

Title:	A Phase 1 study to demonstrate the relative bioavailability of fixed dose combinations of ambrisentan and tadalafil in healthy subjects

Compound Number: GSK3380154, GSK1325760 and GF196960

Development Phase I

Effective Date: 16-DEC-2015

Author(s): PPD

SPONSOR SIGNATORY:

PPD

Frank Gray
Physician Lead

16 DEC 2015
Date:

MEDICAL MONITOR/SPONSOR INFORMATION PAGE

Medical Monitor/SAE Contact Information:

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number
Primary Medical Monitor	PPD			Site to scan and send to email
Secondary Medical Monitor				Site to scan and send to email
SAE contact information				Site to scan and send to email

Sponsor Legal Registered Address:

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

In some countries, the clinical trial sponsor may be the local GlaxoSmithKline Affiliate Company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number(s): EudraCT number 2015-004140-18

INVESTIGATOR PROTOCOL AGREEMENT PAGE

- I confirm agreement to conduct the study in compliance with the protocol.
- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name: _____

Investigator Signature	Date

TABLE OF CONTENTS

PAGE

1. PROTOCOL SYNOPSIS FOR STUDY 201964

Rationale

This study is designed to select one, or more fixed dose combinations (FDCs) of ambrisentan and tadalafil for further development and to provide pharmacokinetic data to enable a pivotal bioequivalence study (BE). Dependent on formulation work, the study will allow up to 8 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently. The study would also allow for up to 2 of the new formulations, that may be taken in to a BE study, to be tested for any effect on pharmacokinetics of the FDC in both fed and fasted state.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assed by vital signs, ECG, clinical laboratory safety data and review of adverse events

Overall Design

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects.

All subjects will attend the unit for Screening within 31 days of their first dose. Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose. The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast.

Treatment Arms and Duration

The proposed treatment arms for each study part are described here; however the treatments in Parts 2 and 3 may be changed dependent on the utility and results from the previous part.

Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)[1]	Part 3[2]
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less FDC treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. The minimum will be approximately 3 weeks and the maximum will be approximately 18 weeks.

Type and Number of Subjects

A maximum of 20 healthy adult subjects will be randomized, to each study part, such that approximately 16 evaluable subjects complete each part of the study.

Analysis

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and Part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in Part 2, if used for food effect and Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

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

2. INTRODUCTION

Ambrisentan (E.U. trade name: Volibris), an orally active endothelin receptor antagonists (ERA) that is selective for ET_A. Once daily dosing at 5 or 10 mg, was first approved on 15 June 2007 in the US and on 21 April 2008 in the European Union (EU) and is currently approved in over 50 countries. In the EU, ambrisentan is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease

Tadalafil (E.U. trade name: Adcirca) is an orally active selective inhibitor of the enzyme PDE-5, the primary cGMP-hydrolyzing enzyme in smooth muscle. In the EU, Adcirca is indicated in adults for the treatment of PAH classified as WHO FC II and III, to improve exercise capacity (Adcirca, EMA, SmPC).

A recently completed study (Galiè N, 2015) has show that patients with PAH who started initial combination therapy with ambrisentan and tadalafil had a significantly lower risk of clinical-failure events compared to those that started with ambrisentan or tadalafil monotherapy. Ambrisentan has recently received EU approval (20 November 2015) for use in combination treatment with tadalafil (Volibris, EMA SmPC, Section 5.1).

This pilot study will investigate the relative bioavailability of new fixed dose combinations (FDC) of ambrisentan and tadalafil, compared to the two monotherapies taken concurrently in healthy subjects

2.1. Study Rationale

This study is designed to select one, or more, FDCs of ambrisentan and tadalafil for further development and to provide pharmacokinetic data to enable a pivotal bioequivalence study (BE). Dependent on formulation work, the study will allow up to 8 new FDCs to be compared with the reference of ambrisentan and tadalafil monotherapies taken concurrently. The study would also allow for up to 2 of the new formulations, that may be taken in to a BE study, to be tested for any effect on pharmacokinetics of the FDC in both fed and fasted state.

The formulation(s) to be taken forward into the BE study will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% confidence interval (CI) for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. If a number of candidate formulations successfully meet these criteria then other factors, including, between subject variability, tablet size, cost, ease of manufacture and stability would be considered.

2.2. Brief Background

Pulmonary Arterial Hypertension (PAH) is a progressive, life threatening disease that, despite the emergence of new treatments, still has a poor long term prognosis (akin to many cancers). Treatments currently approved for the treatment of PAH target 3

biological pathways, namely; endothelin (ET-1), nitric oxide (NO) and prostacyclin pathways. Due to the severity and progressive nature of the disease, combination therapy with agents targeting these different pathways has become increasingly utilised over the years. The evidence for sequential combination treatment has grown and it is now recommended in the latest treatment guidelines (Galie`, 2015) and the recent EU approval of Ambrisentan for combination treatment of Ambrisentan plus Tadalafil for PAH. In practice the combined use of medications targeting the different biological pathways is widespread as reflected in registry data and data from recently completed clinical trials such as SERAPHIN (Pulido, 2013) and PATENT (Ghofrani, 2013).

Ambrisentan (Volibris) is indicated for treatment of pulmonary arterial hypertension (PAH) in adult patients of WHO Functional Class (FC) II to III, including use in combination treatment (Volibris, EMA SmPC, Section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease.(Volibris, EMA SmPC). Ambrisentan is an oral, once daily, propanoic acid-based, ET_A-selective Endothelin receptor antagonist (ERA) which targets the phospholipase-C-dependent endothelin pathway and which is known to play an essential role in mammalian cardiovascular physiology.

Tadalafil (Adcirca) is indicated in adults for the treatment of PAH classified as WHO functional class II and III, to improve exercise capacity (Adcirca, EMA, SmPC). Tadalafil is an oral, once daily, phosphodiesterase type 5(PDE-5) inhibitor which targets the NO pathway. Through inhibition of PDE-5, tadalafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilatation of the vasculature.

An ambrisentan/tadalafil combination therapy is a rational treatment strategy for patients with PAH. Both components are orally administered once a day, have different mechanisms of action targeting different intracellular pathways, have no clinically relevant pharmacokinetic (PK) interactions and are well tolerated when co administered.

Nonclinical pharmacology data (Liang, 2012) demonstrates a synergistic effect of ambrisentan and tadalafil on vasodilatation, whilst a combination of tadalafil and other non selective ERAs (bosentan and macitentan) are additive.

A Phase 1 study (GS-US-300-0112) in 26 healthy subjects was performed to detect any significant PK interactions between tadalafil and ambrisentan when co-administered (Spence, 2009). From this study, it was concluded that there is no clinically significant PK interaction between ambrisentan (10 mg) and tadalafil (40 mg) when combined. Multiple doses of tadalafil had no clinically relevant effect on the PK of either ambrisentan or its metabolite, 4-hydroxymethyl ambrisentan. Similarly, the single-dose PK of tadalafil were unaffected by multiple doses of ambrisentan. Hence, no dose adjustments for ambrisentan or tadalafil should be necessary when these drugs are co-administered. . There were no SAE's in the study. Three subjects withdrew due to adverse events (AEs): one for anaemia (mild) in the last dosing session on combination, following ambrisentan alone and tadalafil alone; one subject because of myalgia (severe), muscle fatigue and dizziness on tadalafil alone and one because of headache (severe) on the first day of tadalafil and 3 days after ambrisentan. The anaemia was mild and is a

listed event for ambrisentan. There were a total of 7 subjects with mild tachycardia. There were 5 events on ambrisentan 10 mg, 4 days after tadalafil 40 mg. There were 3 events on the first day of tadalafil 40 mg given 3 days after ambrisentan 10 mg. There was one event on ambrisentan 10 mg and tadalafil 40 mg after 4 days of ambrisentan 10 mg. Taken together these data suggest that in healthy subjects a mild tachycardia may result from combination use, which is primarily transient.

Both the marketed products can be taken with or without food (Volibris, EMA SmPC, Adcirca, EMA, SmPC).

The AMBITION clinical study (Galiè N, 2015 and AMB112565 CSR 13Nov2014), which evaluated the time to first clinical failure event, a composite endpoint, shows a robust clinical benefit (50% hazard reduction) for PAH patients initiated on a combination of ambrisentan and tadalafil when compared to PAH patients initiated on either medication as monotherapy. The safety profile of the combination arm was consistent with the known safety data of the individual study drugs and no safety signals specific to combination treatment were identified.

ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (Galie`, 2015) have very recently been updated. The combination of ambrisentan – tadalafil is now recommended for efficacy of initial drug combination therapy for pulmonary arterial hypertension (group 1) according to World Health Organization functional class I-III.

The treatment of PAH is complex leading to significant patient burden. Patients require multiple medications, regular clinical review and repeated clinical assessment. The advances in the field, as described, has improved patient outcomes but at the same time added further complexity to the management of the disease. Therefore, GSK is proposing to develop a fixed dose combination formulation of ambrisentan and tadalafil for the treatment of PAH. This will reduce pill burden for patients, which may improve treatment compliance and offer a simplified treatment option for both patients and physicians. Further, there would be a reduced environmental impact from using a FDC, as opposed to separate monotherapies; these would include reduced packaging, storage and shipment requirements.This is in accordance with the EMEA guidelines on clinical development of fixed dose combination medicinal products (EMEA, CHMP/EWP/240/95).

3. OBJECTIVE(S) AND ENDPOINT(S)

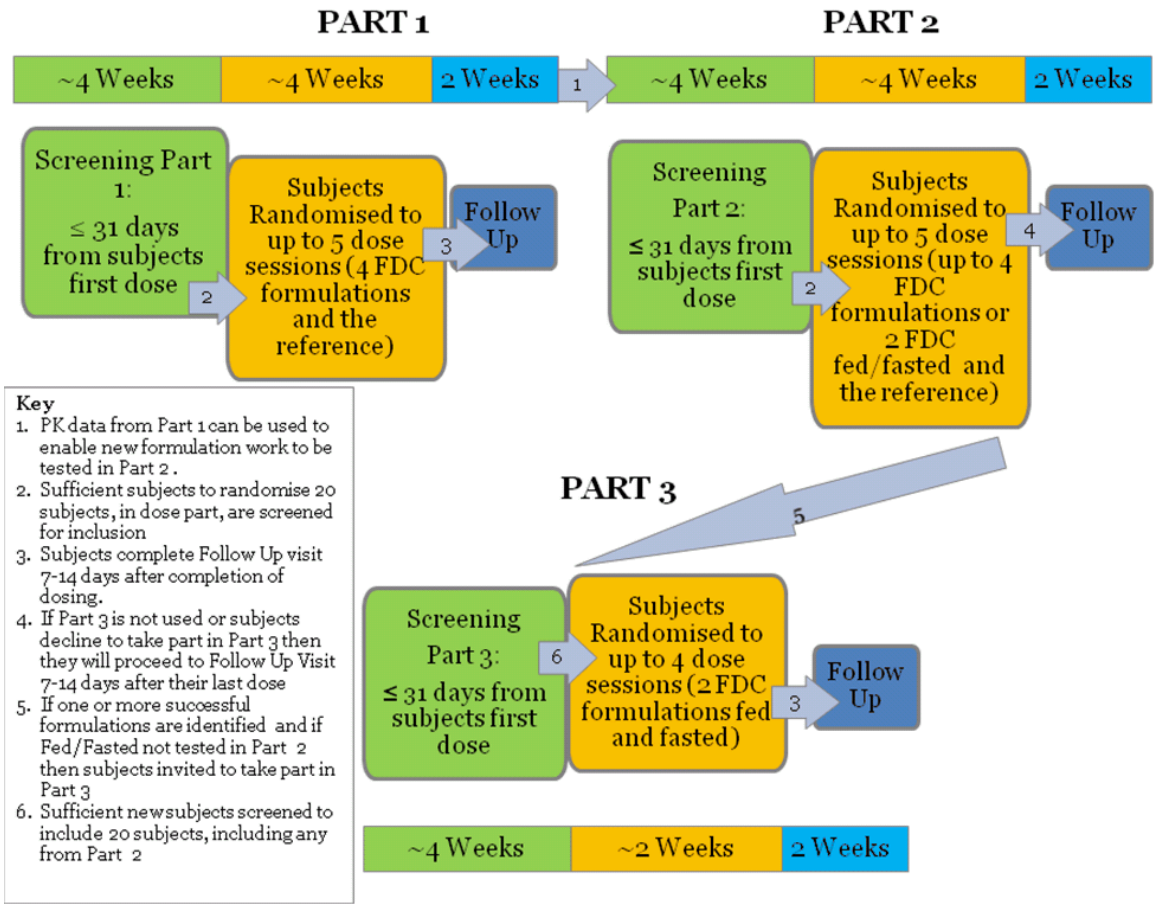
Objectives	Endpoints
Primary	
To characterise the relative bioavailability of ambrisentan and tadalafil following administration of candidate tablet formulations of the FDC (ambrisentan 10 mg + tadalafil 40 mg) relative to reference monotherapies tested (ambrisentan 10 mg & tadalafil 40 mg) taken concurrently in healthy human subjects under fasting conditions.	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, and $AUC_{(0-t)}$ of ambrisentan and tadalafil in FDC and reference treatments
Secondary	
To characterise the secondary PK parameters of the FDC and reference treatments in healthy human subjects under fasting conditions.	Plasma PK parameters including; t_{max}, $t_{1/2}$ of ambrisentan and tadalafil in FDC and reference treatments
To characterise the PK parameters of potential candidate FDC formulations in healthy human subjects under fed and fasted conditions	Plasma PK parameters: Cmax, $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, t_{max} and $t_{1/2}$ of ambrisentan and tadalafil in FDC
To monitor the safety and tolerability of the FDC candidate tablets compared to the reference in healthy human subjects under fasting conditions.	Safety and Tolerability of all treatments as assed by vital signs, ECG, clinical laboratory safety data and review of adverse events

4. STUDY DESIGN

4.1. Overall Design

This is a single centre, Phase 1, single dose, randomised, open label crossover study with 3 study parts; each study part of the study will be, up to, a 5 way cross over, in healthy subjects. See Figure 1 for study schematic.

Figure 1: Study Schematic



All subjects will attend the unit for Screening within 31 days of their first dose. The Clinical Unit has an approved panel screening protocol which may be utilised to identify eligible study candidates based on the defined study inclusion/ exclusion criteria. Further information on requirements for using the approved panel screen protocol is included in Section 7.2.

Eligible subjects will be randomised to order of treatment in that study parts in which they are included and the order in which these treatments are administered will be in accordance with the randomisation schedule.

There will be a minimum washout of 7 days between each dose in a study part. A Follow-Up Visit should be completed within 7-14 days of completion of a subject's last dose.

The utility of each of the 3 study parts is as follows:

Part 1

This study part, will have up to 5 dose sessions and will be used to characterise the relative bioavailability, safety and tolerability of up to, four formulations of the fixed dose combination (ambrisentan 10 mg +tadalafil 40 mg) and the reference of the 2

monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses will be taken in the fasted state.

If successful formulations are identified in this study part, then they may be reformulated and tested in Part 2. If no successful formulations are identified in this study part then Part 2 may be utilised to look at up to 4 new FDC formulations.

Part 2

This study part is flexible and will have up to 5 dose sessions. It will be used to characterise, but not limited to, the bioavailability, safety and tolerability of up to, a further, four formulations of the fixed dose combination (ambrisentan 10 mg + tadalafil 40 mg) and the reference of the 2 monotherapy components taken concurrently (ambrisentan 10 mg & tadalafil 40 mg). All doses are taken in the fasted state.

However, If only two formulations, or less, are evaluated in Part 2 then the FDC formulations may be tested both fed (FDA high fat breakfast) and fasted to assess food effect and Part 3 will not be required.

If successful formulations are identified in this study part, then up to 2 of these may be tested, for food effect, in Part 3 if not already assessed in this part.

Part 3

Part 3 of the study is optional and utility is dependent on the results of the previous study parts. This study part will only be required if formulation to be taken through to a pivotal BE study have been identified and the fed and fasted pharmacokinetics of any FDC formulations identified for progression have not been tested in Part 2.

This study part will have up to 4 dose sessions and be utilized to access the pharmacokinetics, safety and tolerability of up to 2 fixed dose combinations, in both the fed and fasted state and which have been identified for progression to the pivotal BE study. The fed arms of this part will have the standard FDA high fat breakfast.

Subjects who had taken part in the study part preceding Part 3, and which provided the successful formulations for a BE study could also be invited to take part in this study part. However, a subject's inclusion in more than one study part would be dependent on the subject not exceeding the maximum blood draw volume (500ml) for the study.

4.2. Treatment and Duration

The treatments for each study part of the study are listed in Table 1 All treatments are single dose. Subjects will be randomised to order of treatments in the parts of the study they are included in.

The study has 3 parts and ongoing analyse of pharmacokinetic data will be used to enable the formulations produced and tested in subsequent parts.

Part 1

Part 1 of the study will be utilised to look at up to 4 pilot FDC formulations and these are described in Section 6.1. Pharmacokinetic data from Part 1 of the study will be analysed following completion of the third, fourth and fifth treatment session by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to enable the formulation development work to produce the FDC formulations to be tested in Part 2 of the study.

Successful formulations will be primarily chosen on the test/reference ratio for both AUC and Cmax for both components ideally the 90% CI for the ratio would fall in the range of 0.80 and 1.25, with successful formulations having test/reference ratios close to unity and with low intra subject variability indicated by a narrow CI. Any formulations identified by these criteria would be reformulated, with the final intended API, for testing in Part 2. If no successful formulations are identified from these criteria then the pharmacokinetic data would be used to enable further work to produce new FDC formulations for Part 2

Following completion of Part 1 there will be a pause prior to Part 2, so that up to a further 4 FDC formulations could be produced and data included and approved in any required update to submissions to the oversight authorities.

Part 2

Part 2 of the study will be providing data for up to 4 FDC formulations. These could be either successful formulation identified in Part 1 reformulated with the final API and/or new formulations using the final API. Pharmacokinetic data from Part 2 of the study will be analysed following completion of each treatment arm by subjects. This ongoing review of the pharmacokinetic data will enable rapid ongoing assessment of each FDC formulation and will be used to define any successful FDC formulation to be taken into Part 3 of this study and a pivotal BE study. If only two formulations are evaluated in Part 2 then the food effect may be added to Part 2 and Part 3 will not be required. Success will be defined with the same criteria as those in Part 1.

Part 3

Part 3 of the study will provide data for up to 2 successful formulations to be progressed to a pivotal BE study, in both the fed and fasted state.

Table 1 Treatments proposed per study part

Part 1 (fasted)	Part 2 (fasted)[1]	Part 3[2]
ambrisentan and tadalafil FDC1 (10mg/40mg)	ambrisentan and tadalafil FDC5 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
ambrisentan and tadalafil FDC2 (10mg/40mg)	ambrisentan and tadalafil FDC6 (10mg/40mg)	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
ambrisentan and tadalafil FDC3 (10mg/40mg)	ambrisentan and tadalafil FDC7 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
ambrisentan and tadalafil FDC4 (10mg/40mg)	ambrisentan and tadalafil FDC8 (10mg/40mg)	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted
ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)	

1. If 2 or less treatments are tested in Part 2, then these could be tested in both fed and fasted state
2. If food effect is tested in Part 2 then part 3 may not be required

Due to the flexibility of the protocol there is a range for a subject's possible duration on study. Subjects may be included in Part 1, Part 2 or Part 3 of the study. However, if Part 2 of the study identifies any successful formulations to take into Part 3 then subjects from Part 2 may be invited to join Part 3.

The minimum and maximum duration for a subject on this study is described in Table 2. This table assumes all 5 dose sessions are used for Part 1 and Part 2 and either 2 or 4 dose sessions are used in Part 3. However, this is an approximation as fewer sessions may be required in Part 2 and/or the washout between sessions of 7 days in each part may be longer, due to logistical reasons.

Table 2 Study Duration

Study Options		Screen	Study Part 1or 2	Pause	Study part 3	Follow-Up Visit	Total Duration on Study
		(1-31 days)	(31 days)	(7-28 days)	(10-24 days)	(7-14 days)	
Subjects included in only Part 1 or 2	Min	1	31			7	39
	Max	31	31			14	76
Subjects included in Part 2&3	Min	1	31	7	10	7	56
	Max	31	31	28	24	14	128
Subjects included in only Part 3	Min	1			10	7	18
	Max	31			24	14	69

A completed subject is one who has completed all study parts they have been randomised to and the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

4.3. Type and Number of Subjects

A maximum of 20 healthy adult subjects will be randomized, to each study part, such that approximately 16 evaluable subjects complete each part of the study.

The study is not powered. However, the number of subjects will be sufficient for the objectives of this study and this is detailed further in Section 9.2 Sample Size Considerations.

If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

4.4. Design Justification

The rationale for why ambrisentan and tadalafil should be co-formulated in a new FDC, for the treatment of PAH, have been explained in Section 2.2. This study provides the first data to enable this and has been designed to identify suitable candidate FDC formulations to take into a pivotal BE study and to also provide pharmacokinetic data to enable the pivotal BE study to be powered sufficiently.

The single dose, cross over design, used in each study part, is a standard design and sufficient to enable the objectives of the study.

The primary endpoints of the study are pharmacokinetic and as such placebo is not warranted, or is there any need to blind study treatment. The Inclusion of the two monotherapies taken concurrently provides the reference for the primary pharmacokinetic objective and will also provide a comparison for the secondary safety endpoints, when compared to any FDC formulations tested.

The study is in three parts to enable PK data from Part 1 to enable formulation development for Part 2. Part 3 providing an opportunity to assess food effect in any FDC formulations suitable for development and inclusion in the pivotal bioequivalence study. The interdependency of the 3 study parts is described in detail in Section 4.2.

4.5. Dose Justification

The oral dose for the new FDC formulations and the reference treatment proposed for the study of, 40mg for tadalafil and 10mg for ambrisentan are the approved maximum doses for the drugs and the strength at which these drugs are marketed, for the treatment of PAH. These doses will be tested in this protocol and will provide sufficient exposure for the pharmacokinetic study endpoints for both components and have previously shown to be tolerated in healthy subjects.

All treatments are single dose, which is a sufficient duration for assessment of relative bioavailability and effect of food on the pharmacokinetics of selected FDC formulations. The terminal half life of each component indicates a minimum washout of 7 days, between doses, is also sufficient for clearance of previous dose.

4.6. Benefit: Risk Assessment

There is no direct benefit for healthy subjects participating in this study. The risks to the healthy subjects based upon previous experience indicate no expectation of SAE and mild to moderate AE. The benefit/risk remains satisfactory for this healthy subject study.

Summaries of findings from clinical studies conducted with both the Investigational Product taken as monotherapies and in combination can be found in:

- Ambrisentan IB (Volibris IB, 2015)
- Volibris (Ambrisentan) EMA SmPC (Volibris EMA SmPC)
- Adcirca (Tadalafil) EMA SmPC (Adcirca, EMA SmPC)

As described in Section 2.2, mild tachycardia was the most common (7/26, 27%) adverse event seen in healthy subjects following combination dosing with 10mg ambrisentan and 40mg tadalafil (Spence, 2009). There were three subjects (3/26, 11.5%) withdrawn due to AEs (e.g., anaemia [mild]; myalgia [severe], muscle fatigue, and dizziness; and headache [severe]). No SAEs or additional risks were observed with combination use in healthy subjects.

The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Risks of clinical significance, identified in subjects with PAH and the mitigation strategy identified for this study in healthy subjects are captured in Table 3.

Table 3 Risks of Clinical Significance and Mitigation Strategy

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product: GSK1325760 (ambrisentan-Volibris)		
• Teratogenicity • Anaemia (decreased haemoglobin, decreased haematocrit) • Hypersensitivity reactions (e.g. angioedema, rash, pruritus) • Headache (including sinus headache, migraine) • Dizziness • Cardiac failure • Palpitation • Hypotension • Flushing • Epistaxis • Dyspnoea • Upper respiratory congestion, sinusitis, nasopharyngitis, rhinitis • Abdominal pain • Constipation • Nausea, vomiting, diarrhoea • Hepatic transaminases increased • Peripheral oedema, fluid retention • Chest pain/discomfort • Asthenia and fatigue	Ambrisentan IB (Volibris IB, 2015) Volibris (Ambrisentan) EMA SmPC (Volibris, EMA SmPC):	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Exclusion of women of childbearing potential (teratogenicity) • Laboratory assessments per protocol • Physical assessment per protocol • Routine vital signs per protocol • Subjects remain in the clinical unit, under medical supervision, for all doses and until completion of safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GF196960 (tadalafil - Adcirca)		
• Hypersensitivity reactions • Headache • Syncope, • Migraine • Blurred vision • Palpitations • Nasopharyngitis (including nasal congestion, sinus congestion and rhinitis) • Epistaxis	Adcirca (Tadalafil) EMA SmPC (Adcirca, EMA SmPC))	Exclusion criteria, ongoing safety assessments, study design and medical supervision. • Routine vital signs per protocol • Physical assessment per protocol • Subjects remain in the clinical unit for all doses, under medical supervision and until completion of

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
• Nausea, • Dyspepsia (including abdominal pain/discomfort) • Vomiting, • Gastroesophageal reflux • Rash • Myalgia, • Back pain • Pain in extremity (including limb discomfort) • Increased uterine bleeding • Facial oedema, • Chest pain		safety assessments at 48hrs post dose. • Single doses used in the study
Investigational Product: GSK3380154 (ambrisentan-tadalafil-FDC)		
The risks for the combination are the same as the 2 monotherapies; no extra risks have been identified for the combination.		As for GSK1325760 and GF196960 above

4.6.2. Benefit Assessment

There is no direct benefit for healthy subjects taking part in this study.

However, the intended benefit of this study is to inform the correct formulation for a fixed dose combination of ambrisentan plus tadalafil to use as first line therapy in PAH patients.

4.6.3. Overall Benefit: Risk Conclusion

Though there is no direct benefit for healthy subjects, based on observations from previous healthy subject studies, the adverse event burden is mild, consistent with observations in patients and all risks have been mitigated as described in Table 3. So, the risk: benefit is appropriate for this study and to enable development of this investigational product. The overall benefit: risk therefore remains positive.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in:

- Volibris (Ambrisentan) EMA SmPC (Volibris, EMA SmPC)
- Adcirca (Tadalafil) EMA SmPC (Adcirca, EMA SmPC)

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

5.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

AGE
1. Between 18 and 60 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests, vital signs and cardiac monitoring (ECG and 24 hour Holter). A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical Monitor if required, judges and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.

WEIGHT
3. Body weight ≥ 50 kg (110 lbs) for men and ≥ 45 kg (99lbs) for women and body mass index (BMI) within the range 18 – 30 kg/m2 (inclusive)

SEX

4. Male or Female
Females must be the following:
Non-reproductive potential defined as:
• Pre-menopausal females with one of the following:
  • Documented tubal ligation
  • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion
  • Hysterectomy
  • Documented Bilateral Oophorectomy
• Documented Postmenopausal defined as 12 months of spontaneous amenorrhea

INFORMED CONSENT
5. Capable of giving signed informed consent as described in Section 10 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol.

5.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)
1. A blood pressure <100/55 mm Hg. 2. Haemoglobin below normal range: • Hb < 133 g/L for males • Hb< 114 g/L for females 3. ALT and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%). 4. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) 5. 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. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP).

CONCOMITANT MEDICATIONS
6. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication, unless in the opinion of the Investigator and GSK Medical Monitor the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
7. History of regular alcohol consumption within 6 months of the study defined as: • An average weekly intake of >21 units for males or >14 units for females. 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. 8. Smoking more than 5 cigarettes per week and subjects must be able to abstain from smoking for a 24 hour period prior to dose and any time whilst in the clinical unit.

CONTRAINDICATIONS
9. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or Medical Monitor, contraindicates their participation.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
10. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at Screening or within 3 months prior to first dose of study treatment. . 11. A positive test for HIV antibody. 12. A positive pre-study drug/alcohol screen. 13. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within previous 3 months 14. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 3 months, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 15. Exposure to more than four new chemical entities within 12 months prior to the first dosing day.

5.3. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.4.1.4).

5.4. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

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

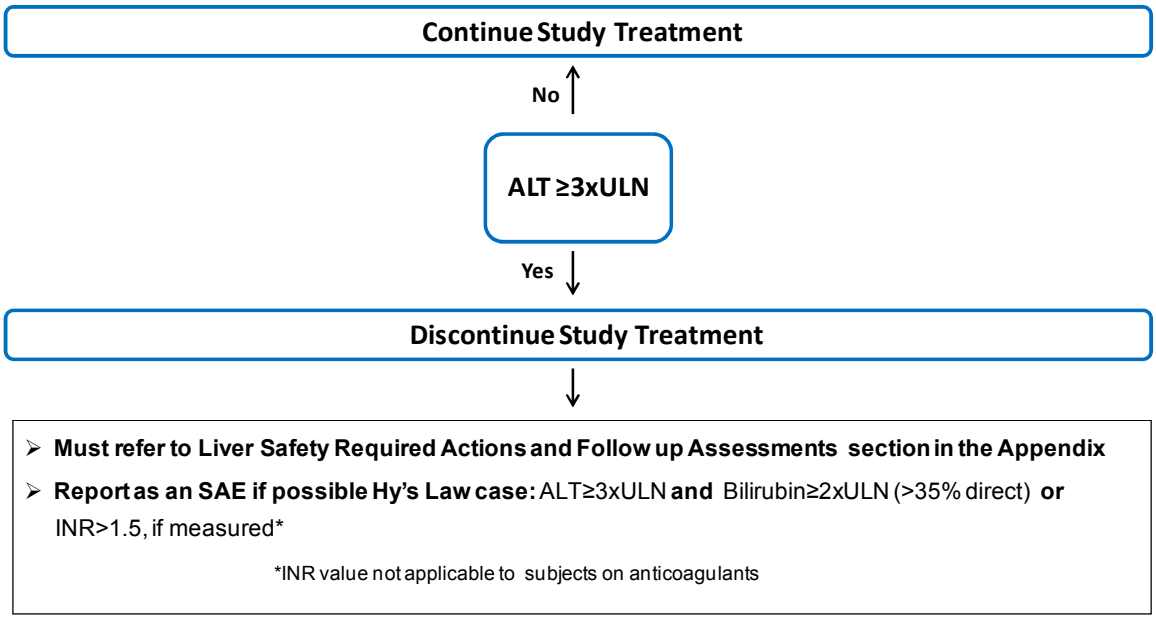
A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

5.4.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Study treatment will be discontinued **for a subject** if liver chemistry stopping criteria are met:

Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow-Up Assessments Section can be found in Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

5.4.1.1. Study Treatment Restart or Rechallenge

Study treatment restart or re-challenge after liver chemistry stopping criteria are met by any subject participating in this study is not allowed.

5.4.2. QTc Stopping Criteria

- The *same* QT correction formula *must* be used for *each individual subject* to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the subject has been enrolled.
  - For example, if a subject is eligible for the protocol based on QTcF, then QTcF must be used for discontinuation of this individual subject as well.
  - Once the QT correction formula has been chosen for a subject's eligibility, the *same formula* must continue to be used for that subject *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 averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A subject that meets either bulleted criterion below will be withdrawn from the study.

- QTc> 500 msec,

- Change from baseline: QTc >60 msec

5.4.3. Hypotension

A subject that meets bulleted criterion below will be withdrawn from the study.

- For hypotension: if systolic <90 or diastolic <50 confirmed by triplicate reading taken upto 5 minutes apart.

5.4.4. Other Dose Adjustment/Stopping Safety Criteria

For an individual study participant, stopping criteria include, but are not limited to:

Adverse events, or significant changes in any of the safety assessments, that put the safety of the individual at risk (e.g., ECG, vital signs, laboratory tests, etc), as judged by the Principal Investigator in consultation with the Medical Monitor if necessary.

5.5. Subject and Study Completion

A completed subject is one who has completed all phases of the study including the Follow-Up visit.

The end of the study is defined as the last subject's last visit.

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

The term 'study treatment' is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments. Study treatments for Part 1 of the study are shown in Table 4, this will be amended once treatments for Part 2 and Part 3 are confirmed.

Table 4 Study Treatments for Part 1

	Study Treatment					
Product name:	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK3380154 (ambrisentan-tadalafil-FDC)	GSK1325760 (ambrisentan-Volibris)	GF196960 (tadalafil - Adcirca)
Treatment	FDC1	FDC2	FDC3	FDC4	Reference	
Formulation description	GSK3380154, TAB-A, Tablet Weight 840mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 840mg/4mg SLS**	GSK3380154, TAB-A, Tablet Weight 560mg/2mg SLS*	GSK3380154, TAB-A, Tablet Weight 560mg/4mg SLS	Each tablet contains 10 mg of ambrisentan, approximately 95 mg of lactose (as monohydrate), approximately 0.25 mg of lecithin (soya) (E322) and approximately 0.11 mg of Allura red AC Aluminium Lake	Each film-coated tablet contains 20 mg tadalafil, 233 mg lactose (as monohydrate).
Dosage form:	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet	Film-coated tablet
Unit dose strength(s)/ Dosage level(s):	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 40 mg tadalafil and 10 mg of ambrisentan.	Each tablet contains 10 mg of ambrisentan.	Each tablet contains 20 mg tadalafil.
Route of Administration	Oral	Oral	Oral	Oral	Oral	Oral
Dosing instructions	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed	Take as directed

6.2. Medical Devices

No GSK manufactured devices (or devices manufactured for GSK by a third party) are provided for use in this study.

6.3. Treatment Assignment

Subjects will be assigned to treatment sequence, for the study part/s that they are included in and in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

The treatments are denoted as F1 to F4 for Part 1, F5 to F8 for Part 2 and R for reference treatment for both parts; the selected formulations for Part 3 are denoted as X and Y. The treatment key for Parts 1, 2 and 3 are described in Table 5

Table 5 Treatment Key for Part 1, Part 2 and Part 3

Treatment	Description
Part 1	
F1	ambrisentan and tadalafil FDC1 (10mg/40mg)
F2	ambrisentan and tadalafil FDC2 (10mg/40mg)
F3	ambrisentan and tadalafil FDC3 (10mg/40mg)
F4	ambrisentan and tadalafil FDC4 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 2	
F5	ambrisentan and tadalafil FDC5 (10mg/40mg)
F6	ambrisentan and tadalafil FDC6 (10mg/40mg)
F7	ambrisentan and tadalafil FDC7 (10mg/40mg)
F8	ambrisentan and tadalafil FDC8 (10mg/40mg)
R	ambrisentan and tadalafil monotherapies taken concurrently (10mg/40mg)
Part 3	
X1	ambrisentan and tadalafil FDCX1 (10mg/40mg), fed
X2	ambrisentan and tadalafil FDCX1 (10mg/40mg) fasted
Y1	ambrisentan and tadalafil FDCX2 (10mg/40mg), fed
Y2	ambrisentan and tadalafil FDCX2 (10mg/40mg), fasted

The treatment sequence assignments for each part of the study, based on the Latin Squares for Williams Designs are shown in Table 6. Not all possible sequences are included here. Such as, if only one formulation taken into Part 2 or 3, or Part 2 is used for

only one or two FDC formulations, fed and fasted. Additional treatment sequences will be created based on the Latin Squares for Williams Designs, as required.

Table 6 Treatment Sequence assignments for Part 1, Part 2 and Part 3

Study Parts	Number of Treatments	Sequence assignments	Allocation ratio
Part 1	5	F1 F2 R F3 F4 F2 F3 F1 F4 R F3 F4 F2 R F1, F4 R F3 F1 F2 R F1 F4 F2 F3, F4 F3 R F2 F1 R F4 F1 F3 F2, F1 R F2 F4 F3, F2 F1 F3 R F4, F3 F2 F4 F1 R	1:1:1:1:1:1:1:1:1:1
Part 2	5	F5 F6 R F7 F8 F6 F7 F5 F8 R F7 F8 F6 R F5 F8 R F7 F5 F6 R F5 F8 F6 F7 F8 F7 R F6 F5 R F8 F5 F7 F6 F5 R F6 F8 F7 F6 F5 F7 R F8 F7 F6 F8 F5 R	1:1:1:1:1:1:1:1:1:1
	4	F5 F6 R F7 F6 F7 F5 R F7 R F6 F5 R F5 F7 F6	1:1:1:1
	3	F5 F6 R R F6 F5 F6 R F5 F1 R F6 R F5 F6 F6 F5 R	3:3:3:3:3:3 + 2 random
	2	F5 R R F5	1:1
Part 3	4	X1 X2 Y2 Y1 X2 Y1 X1 Y2 Y1 Y2 X2 X1 Y2 X1 Y1 X2	1:1:1:1

6.4. Blinding

This will be an open-label study.

6.5. Packaging and Labeling

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

6.6. Preparation/Handling/Storage/Accountability

This will be detailed in a Study Specific Technical Agreement/Memo (TTS) which will be accompanied by a Quality Agreement.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.
- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the SRM.
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
- 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.

6.7. Compliance with Study Treatment Administration

When subjects are dosed at the site, they will receive study treatment 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 treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each subject's mouth to ensure that the study treatment was ingested.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK3380154 (ambrisentan/tadalafil-FDC), GSK1325760 (ambrisentan) and GF196960 (tadalafil) greater than the protocol defined dose and within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose.

Advice for the investigator is included in the product label for GSK1325760 (ambrisentan) and GF196960 (tadalafil).

In the event of an overdose the investigator should:

1. Contact the Medical Monitor immediately
2. Closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until compound number/name can no longer be detected systemically (at least 3 days for compound number/name)
3. Obtain a plasma sample for pharmacokinetic (PK) analysis 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 subject.

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because only healthy subjects are eligible for study participation.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until after the final dose.
- Dependent on the utility of the dose session subjects will be asked to fast or consume the FDA full fat breakfast prior to dosing:
  - Fasting subjects will be required to fast from midnight before each full in-house dosing day (i.e. Day 1) with the exception of water, which will be allowed freely except for 1 hour either side of dosing. Subjects will be required to fast up to 4h hour post dose on Day 1 of each dose
  - For treatments given under fed conditions, a standard high-fat breakfast will be provided before dosing. See Section 12.4 for details of this meal. This meal should be eaten in its entirety within 30 minutes. The amount consumed will be recorded within the source documents and the CRF by the site staff. Subjects will be dosed within 5 minutes of completing the breakfast.
- Subjects should take each dose of investigational product with 240 ml (8 fl oz) of water.

6.10.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks and chocolate) for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- During each dosing session, subjects will abstain from alcohol for 24 hours prior to the start of dosing until collection of the final pharmacokinetic sample during each session.
- Smoking is not allowed for 24 hours prior to dosing and whilst subjects are in the clinic.

6.10.3. Activity

Subjects will abstain from strenuous exercise from Screening until Follow-Up. Subjects may participate in light recreational activities during studies (e.g., watch television, read).

6.11. Concomitant Medications and Non-Drug Therapies

6.11.1. Permitted Medications and Non-Drug Therapies

Paracetamol at doses of ≤ 2 grams/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.

6.11.2. Prohibited Medications and Non-Drug Therapies

Subjects 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) prior to the first dose of study medication until completion of the Follow-Up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 7.1

The following points must be noted:

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:
    1. 12-lead ECG
    2. Vital signs

3. Blood draws.

Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.

- The timing and number of planned study assessments, safety and pharmacokinetic assessments 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.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.

No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

7.1. Time and Events Table

Procedure	**Screen**	**Part 1, 2 and 3. Each dose in each Part repeats this schedule.**																**FU**	**Notes**
Day	**≤-31**	**-1**	**1**											**2**		**3**	**4**	**≥7-14**	
Time (hrs)			**Pre-dose**	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**4**	**8**	**12**	**18**	**24**	**36**	**48**	**72**		
Outpatient visit	x																x	x	
Admission to unit		x																	
Informed consent	x																		
Inclusion and exclusion criteria	x	x																	
Demography	x																		
Full physical exam including height and weight	x																		
Brief Physical																		x	
Medical history (includes substance usage)	x																		
HIV, Hep B and Hep C screen]	x																		
Laboratory assessments (include liver chemistries)	x	x														x		x	Only Screening labs need to be taken in fasted state.
Serum hCG Pregnancy test	x																	x	Female subjects only
Urine hCG Pregnancy test		x																	Female subjects only
Breathalyser and Smokerlyzer	x	x																	
DOA testing	x	x																	
12-lead ECG	x		x			x		x		x		x		x			x	x	Triplicate at screen and baseline, single measure at other times, unless out of range then triplicates should be performed
Vital signs	x		x		x	x		x		x	x	x		x		x	x	x	
24hr Holter	x																		
Randomisation			x																Randomised prior to first dose only
Study Treatment				x															
AE/SAE review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	SAEs from Screen. AEs from first dose
Concomitant medication review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
PK Sample			x		x	x	x	x	x	x	x	x	x	x	x	x	x		
Discharge from Unit																x			For logistical reasons subjects may remain in-unit for the 72 hr assessments if they prefer.

7.2. Screening and Critical Baseline Assessments

All subjects must give written consent to participate in this trial. Consent for screening evaluations may be obtained using the ICF for the Hammersmith Medicines Research (HMR) healthy subject's panel, which has been approved by the HRA's Phase 1 Advert Review group. The study-specific information and consent form will be signed by the subject either before any screening evaluation or after the investigator confirms the eligibility of the subject for the study and before the subject is randomised to receive the first administration of IMP. Before giving consent, subjects must read the information sheet about the study. They must also read the consent form. They will then discuss the study with the investigator or his deputy and be given the opportunity to ask questions. The study-specific information sheet and the consent form must be approved by the REC.

7.2.1. Demographic/Medical History Assessments

Prior to enrolling in the study and having any study procedures completed, subjects must sign the informed consent.

During the Screening visit, each subject will undergo the assessments to determine eligibility for enrolment as detailed in Section 5.

The following demographic parameters will be captured: date of birth, gender, race and ethnicity.

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 5.

7.3. Pharmacokinetics

7.3.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of ambrisentan and tadalafil will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

For analysis of ambrisentan 2.7 mL of blood will be collected into sodium citrate tubes and for analysis of tadalafil 2.0 mL of blood will be collected into K2-EDTA tubes.

Processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

7.3.2. Sample Analysis

Plasma analysis will be performed under the control of PTS-DMPK/Scinovo, GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of ambrisentan and tadalafil will be determined in plasma

samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Once the plasma has been analyzed for ambrisentan and tadalafil any remaining plasma may be analyzed for other compound-related metabolites and the results reported under a separate PTS-DMPK/Scinovo, GlaxoSmithKline protocol.

7.4. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests such as vital signs, physical examinations and laboratory safety assessments may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

7.4.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The definitions of an AE or SAE can be found in Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.4.1.1. Time period and Frequency for collecting AE and SAE information

- Any SAEs assessed as related to study participation (e.g., 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 up to and including any follow up contact.
- AEs will be collected from the start of Study Treatment until the follow up contact (see Section 7.4.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.3.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Section 12.3.

7.4.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?

7.4.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Section 4.6.1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow up (as defined in Section 5.4). Further information on follow up procedures is given in Study Assessments and Procedures.

7.4.1.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs related to study treatment (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

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

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

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

7.4.2. Physical Exams

- A complete physical examination will include, at a minimum, assessment of the 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

7.4.3. Vital Signs

- Vital signs will be measured in semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure and pulse rate and respiratory rate.
- Three readings of blood pressure and pulse rate will be taken at Screening and baseline (pre-dose). All subsequent assessments will be single measures, unless the subjects blood pressure or pulse rate has changed from baseline by >15% and then 2 further readings should be taken and recorded:
- For triplicate readings:
    - All 3 readings will be recorded in the CRF
        - First reading should be rejected
        - Second and third readings should be averaged to give the measurement to be used and this will also be recorded in the CRF

7.4.4. Electrocardiogram (ECG)

- ECGs will be measured in semi-supine position after 5 minutes rest
- Triplicate 12-lead ECGs will be obtained at Screening and baseline (predose). All subsequent assessments will be a single measures, unless withdrawal criteria are meet and in which case 2 further readings should be taken to confirm if withdrawal is required . An ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- 24 hour continuous cardiac telemetry (Holter) will be performed at Screening. Full disclosures will be reviewed in detail and the review maintained as part of the subject's source documents

7.4.5. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 7, must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit dat. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

All study-required laboratory assessments will be performed by a local laboratory.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 7.

Table 7 Protocol Required Safety Laboratory Assessments

<table>
<tr><th>Laboratory Assessments</th><th>Parameters</th></tr>
<tr><td>Haematology</td><td>
<table>
<tr><td>Platelet Count</td><td><u>RBC Indices</u>:</td><td><u>WBC count with Differential:</u></td></tr>
<tr><td>RBC Count</td><td>MCV</td><td>Neutrophils</td></tr>
<tr><td>Hemoglobin</td><td>MCH</td><td>Lymphocytes</td></tr>
<tr><td>Hematocrit</td><td>MCHC</td><td>Monocytes</td></tr>
<tr><td></td><td></td><td>Eosinophils</td></tr>
<tr><td></td><td></td><td>Basophils</td></tr>
</table>
</td></tr>
<tr><td>Clinical Chemistry [1]</td><td>
<table>
<tr><td>BUN</td><td>Potassium</td><td>AST (SGOT)</td><td>Total and direct bilirubin</td></tr>
<tr><td>Creatinine</td><td>Sodium</td><td>ALT (SGPT)</td><td>Total Protein</td></tr>
<tr><td>Glucose</td><td>Calcium</td><td>Alkaline phosphatise</td><td>Albumin</td></tr>
</table>
</td></tr>
<tr><td>Routine Urinalysis[3]</td><td>As a minimum, but not limited to the following tests and dependent on standard urinalysis dipstick used:
• Specific gravity
• pH, glucose, protein, blood and ketones by dipstick
• Microscopic examination (if blood or protein is abnormal)</td></tr>
<tr><td>Other Screening Tests</td><td>• HIV
• Hepatitis B (HBsAg)
• Hepatitis C (Hep C antibody)
• Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)
• Serum or urine hCG Pregnancy test[2]</td></tr>
<tr><td colspan="2">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 5.4.1 and Appendix 2
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.
3. Routine Urinalysis results will not be databased, unless a result is out of range and clinically significant then it would be captured as an AE</td></tr>
</table>

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within7 days after the last dose of study treatment should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

8. DATA MANAGEMENT

- Data will be double-entered into a clinical database management system (ClinPlus Version 3.3).
- Management of clinical data will be performed in accordance with applicable HMR standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- Original CRFs will be retained by GSK, while HMR will retain a copy. Subject initials will not be collected or transmitted to GSK according to GSK policy

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

No formal hypothesis will be tested. An estimation approach will be used to (i) estimate the bioavailability of the FDC formulations relative to the monotherapies taken concurrently in Part 1 and if used, Study part 2, (ii) estimate the bioavailability of the formulation(s) of the FDC formulations, taken in to Part 3, in the fed state relative to the fasting state.

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 treatment (FDC formulations) to the geometric mean of the reference treatment (co-administration of ambrisentan and tadalafil), μ(test)/ μ(reference).

9.2. Sample Size Considerations

No formal sample size calculation has been performed. Sample sizes chosen for each study part in this study is considered sufficient for exploratory analysis based on PK variability data from previous studies

9.2.1. Sample Size Assumptions

The sample size assumptions are based on previously reported estimates of within subject CV for AUC(0-∞) and Cmax for ambrisentan (GS-US-300-0112) and tadalafil (Forgue, 2005). Table 8 summarizes the estimates of within subject CV for the primary endpoints AUC (0-∞) and Cmax.

Table 8 Estimates of within subject CV for the primary end points AUC (0-∞) and Cmax

CVw: within subject CV	ambrisentan	tadalafil
Cmax	22%	16%
AUC (0-∞)	15%	13%

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 based on a sample size of 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 9 shows the precision (the half width of the 90% confidence interval) for a range of evaluable subjects.

Table 9 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%

9.2.3. Sample Size Re-estimation or Adjustment

No sample size re-estimation will be performed.

9.3. Data Analysis Considerations

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling and Simulation Department, CPDM, GlaxoSmithKline. Plasma concentration-time data for ambrisentan and tadalafil will be analyzed by non-compartmental methods with WinNonlin [version 6.3 or above]. Calculations will be based on the actual

sampling times recorded during the study. From the plasma concentration-time data, the following pharmacokinetic parameters will be determined, as data permit: maximum observed plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curve [AUC(0-t) and AUC(0-∞)], and apparent terminal phase half-life (t1/2).

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals R&D.

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

Systemic exposure of ambrisentan and tadalafil will be evaluated when administered as the FDC compared to administration as the monotherapies concurrently, using $\log_e$-transformed AUC(0-∞) and Cmax in a mixed effects model.

9.3.1. Analysis Populations

9.3.1.1. Safety Population

All subjects enrolled into the study who have received at least one dose of investigational product will be included in the Safety Population.

9.3.1.2. Pharmacokinetic Concentration Population

The PK Concentration Population will include all subjects for whom at least one PK sample was obtained and analyzed.

9.3.1.3. Pharmacokinetic Parameter Population

For each PK parameter, the PK Parameter Population will include all subjects who provide PK parameter data.

9.3.2. Interim Analysis

No formal interim is planned. However, ongoing analyses of pharmacokinetic data will be performed in order to direct development of fixed dose formulations for subsequent study parts and the planned BE study.

9.4. Key Elements of Analysis Plan

9.4.1. Pharmacokinetic Analyses

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

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

Following $\log_e$-transformation, AUC(0-∞), AUC(0-t) and Cmax of FDC formulations and reference will be separately analyzed using a mixed effects model with fixed effect terms for period and treatment. Subject will be treated as a random effect in the model. Point estimates and their associated 90% confidence intervals will be constructed for the differences, F1 – R, F2 – R, F3 – R, F4 – R for Part 1 and similarly for Part 2 (as needed); and X1-X2, Y1-Y2 for Part 3. The point estimates and their associated 90% confidence intervals will then be back-transformed to provide point estimates and 90% confidence intervals for the ratios, F1:R, F2-R:F3-R, F4-R; and X1:X2, Y1:Y2.

Tmax of FDC formulations will be analyzed with the non-parametric Wilcoxon Matched Pairs Method to compute point estimate and associated 90% confidence intervals for the median difference, F1 R, F2 R, F3 R, F4 R and X1:X2, Y1:Y2.

9.4.2. Safety Analyses

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

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

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

Prior to initiation of a site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Obtaining signed informed consent
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)

- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.
- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

10.3. Quality Control (Study Monitoring)

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

GSK will monitor the study and site activity to verify that the:

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.
- If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.
- If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action.
- If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.
- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

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

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

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

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

11. REFERENCES

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EMEA guidelines on clinical development of fixed dose combination medicinal products. CHMP/EWP/240/95 Rev.1

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Spence R, Mandagere A, Harrison B, et al. J Pharm Sci. 2009 Dec;98(12):4962-74. No clinically relevant pharmacokinetic and safety interactions of ambrisentan in combination with tadalafil in healthy volunteers.

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
API	Active Pharmaceutical Ingredient
AUC	Area Under Curve
BE	Bioequivalence
CDISC	Clinical Data Interchange Standards Consortium
cGMP	Cyclic guanosine monophosphate
C_{max}	Maximum concentration
CI	Confidence Interval
CV	Coefficient of variance
CVw	Coefficient of variance within subject
EMA	European Medicines Agency
ERA	Endothelin Receptor Antagonists
ERS	European Respiratory Society
ESC	European Society of Cardiology
ET-1	Endothelin Receptor - 1
EU	European Union
FDA	Federal Drug Agency
FDC	Fixed Dose Combination
NO	Nitrous Oxide
PDE-5	Phosphodiesterase type 5
SAE	Serious Adverse Events
SRM	Study Reference Manual
t_{max}	Time to maximum concentration
$t_{1/2}$	Half Life
WHO	World Health Organisation

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Adcirca
WinNonlin

12.2. Appendix 2: Liver Safety Required Actions and Follow-Up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event aetiology (in alignment with the FDA premarketing clinical liver safety guidance).

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td colspan="2">ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >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 following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th colspan="2">Follow-Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment
• 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 subject until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline</td><td colspan="2">• Viral hepatitis serology3
• Blood sample for pharmacokinetic (PK) analysis, obtained within 96hrs of last dose
• 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
If ALT≥3xULN AND bilirubin ≥ 2xULN or</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
• A specialist or hepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	**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 HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. • 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 treatment 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, 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); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
4. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.

References

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

12.3. Appendix 3: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.3.1. Definition of Adverse Events

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

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • 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 treatment administration even though it may have been present prior to the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae).

Events <u>NOT</u> meeting definition of an AE include:
• 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 subject'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 subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is an AE. • Situations where an untoward medical occurrence did not occur (social and/or

convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

12.3.2. Definition of Serious Adverse Events

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, etc).

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

NOTE:

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

c. Requires hospitalization or prolongation of existing hospitalization

NOTE:

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

d. Results in disability/incapacity

NOTE:

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

e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. • Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse
g. Is associated with liver injury <u>and</u> impaired liver function defined as: • ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), **or** • ALT ≥ 3xULN and INR** > 1.5. * Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE. ** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.
• Refer to Section 12.2 for the required liver chemistry follow up instructions

12.3.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:
Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs: • Myocardial infarction/unstable angina • Congestive heart failure • Arrhythmias • Valvulopathy • Pulmonary hypertension • Cerebrovascular events/stroke and transient ischemic attack • Peripheral arterial thromboembolism • Deep venous thrombosis/pulmonary embolism

- Revascularization

12.3.4. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF
- It is **not** acceptable for the investigator to send photocopies of the subject'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 instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.
- Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study.
- Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer.
- The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

12.3.5. Evaluating AEs and SAEs

Assessment of Intensity

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

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating

the intensity of an event; and both AEs and SAEs can be assessed as severe.

- An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence 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 natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of 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 when 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 prior to the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow up information, amending the SAE data collection tool accordingly.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.
- The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.
- If a subject 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 the designated reporting time frames.

12.3.6. Reporting of SAEs to GSK

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 or the SAE coordinator
- 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
- Initial notification via the 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 receipt can be found at this beginning of the protocol on the Sponsor/Medical Monitor Contact Information page.

12.4. Appendix 4: High Fat Meal Content

- 2 eggs fried in butter,
- 2 strips of bacon,
- 2 slices of toast with butter,
- 120 g hash brown potatoes, and
- 240 mls of whole milk.

The standard high-fat meal will be the meal suggested by the US FDA in their 2002 draft guidance on food-effect bioavailability and bioequivalence studies. Approximately 50% of the caloric content of the meal is from fat and the meal is high in calories (approximately 1000 calories). Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

US Department of Health and Human Services. Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies. Guidance. Center for Drug Evaluation and Research (CDER). Food and Drug Administration; 2002.