TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	The effects of GSK2586881 on the responses to acute hypoxia and exercise

Compound Number: GSK2586881

Development Phase: I

Effective Date: 06-MAR-2018

Protocol Amendment Number: 03

Author (s): PPD

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2016N283626_00	2016-SEP-23	Original
2016N283626_01	2017-FEB-24	Amendment No. 1
Additional Inclusion Criteria (Section 5.2) and Subject Stopping Criteria (Section 5.4) added in response to comments from BfArM. A summary of changes is presented in Appendix 6.		
2016N283626_02	2017-MAR-07	Amendment No. 2
This is a substantial protocol amendment to: • Reduce the number of participants in the study from approximately 35 healthy volunteers to approximately 25 healthy volunteers. This is a non-substantial protocol amendment to: • Remove some of the exploratory biomarkers. • Add a spirometry assessment at screening. • Add a pre-dose pulse oximetry assessment. • Add continuous ECG telemetry. • Move the immunogenicity screening sample to Pre-dose in Treatment Period 1. • Remove height and weight assessments from the follow-up visit. • Clarify the sequence and priority of assessments. • Correct typographical errors. A summary of changes is presented in Appendix 6.		

2016N283626_03	2018-MAR-06	Amendment No. 3

This is a substantial protocol amendment to restart the trial following a temporary halt. The trial was halted following the planned interim analysis (when 10 subjects had completed both Treatment Periods) to allow the study team time to consider the data from the first part of the study (Part 1), and decide what changes would be made to the second part of the study (Part 2). The study team made the following changes to the study in Part 2:

- Increasing the altitude of the hypoxia chamber (Part 1: 4000 m, and Part 2: 5000 m).
- Changing the individual subject withdrawal criteria for oxygen saturation threshold during the hypoxia challenge (Part 1: below 65%; and Part 2: below 60% persistently for >15 seconds).
- Modification of the exercise challenge:
  - Changing from an upright ergometer (Part 1) to a semi-recumbent ergometer (Part 2).
  - Echo recordings taken *during* exercise (Part 2), rather than *after* exercise (Part 1).
  - Changing the workload of the exercise challenge, due to the change in chamber altitude (Part 1: 70% VO_2 max to Part 2: 50% VO_2 max).
- Modification of the echo recording procedure: calculation of pulmonary artery systolic pressure (PASP) conducted *after* image acquisition in Part 2 (rather than *during* the recording, Part 1); and the addition of a physician 'over-reader' in Part 2 to review all echo images/calculations and provide a quality statement (not used in Part 1).
- Addition of an exploratory echo endpoint in Part 2 allowing estimation of pulmonary vascular resistance.

A summary of changes is presented in Appendix 6.

SPONSOR SIGNATORY

PPD

6th March 2018.

Dr Tony Cahn

Project Physician Leader, Respiratory Therapy Area

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	Site Address
Primary Medical Monitor	PPD				Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
Secondary Medical Monitor					709 Swedeland Road UW2531 King of Prussia, PA 19406 USA
SAE contact information	[Medical monitor as above]				

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): 2016-002465-55

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol 204987.

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 204987

Rationale

The purpose of this study is to examine how GSK2586881, a recombinant human ACE2 peptide, modulates the acute hypoxic pulmonary vasoconstriction (HPV) response in healthy volunteers.

HPV is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO_2) levels in healthy and in pathophysiological settings, such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. The literature supports a role for the renin angiotensin system (RAS) in driving acute HPV and while there is a strong biological rationale for modulation of RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they blunt the normal HPV response and negatively impact on arterial oxygenation (PaO_2). Thus, from a safety (and efficacy) perspective it is important to understand the impact of modulation of the RAS system by GSK2586881 on the acute HPV response.

Protocol Changes Post Interim Analysis

An interim analysis was conducted when 10 subjects had completed both Treatment Periods. Adverse Event (AE) data indicated no safety concerns, and RAS peptide data indicated target engagement. However, review of pulmonary artery systolic pressure (PASP) data indicated that an HPV response could not consistently be detected in the first 10 subjects who completed the study. The study was placed on hold to allow the study team to consider the data from the first part of the study (Part 1), and decide what changes would be made to the second part of the study (Part 2). The study team made the following changes to the study in Part 2:

- Increasing the altitude of the hypoxia chamber (Part 1: 4000 m, and Part 2: 5000 m).
- Changing the individual subject withdrawal criteria for oxygen saturation threshold during the hypoxia challenge (Part 1: below 65%; and Part 2: below 60% persistently for >15 seconds).
- Modification of the exercise challenge:
  - Changing from an upright ergometer (Part 1) to a semi-recumbent ergometer (Part 2).
  - Echo recordings taken *during* exercise (Part 2), rather than *after* exercise (Part 1).
  - Changing the workload of the exercise challenge, due to the change in chamber altitude (Part 1: 70% VO2 max to Part 2: 50% VO2 max).
- Modification of the echo recording procedure: calculation of pulmonary artery systolic pressure conducted *after* image acquisition in Part 2 (rather than *during* the recording, Part 1); and the addition of a physician 'over-reader' in Part 2 to review all echo images/calculations and provide a quality statement (not used in Part 1).

- Addition of an exploratory echo endpoint in Part 2 allowing estimation of pulmonary vascular resistance (PVR).

Objective(s)/Endpoint(s)

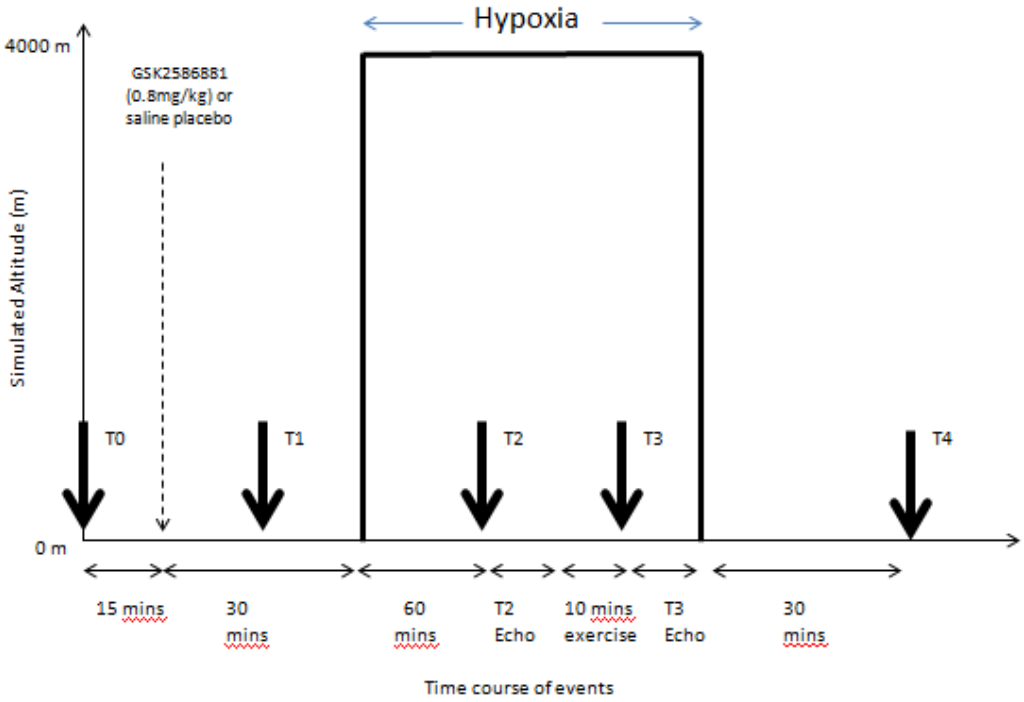
Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in Surfactant Protein-D and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers under hypoxic conditions (and during exercise under hypoxic conditions).	• Estimation of pulmonary vascular resistance (PVR) measured via Echocardiography

Overall Design

The study will be single-centre, randomised, placebo-controlled and double blind (sponsor open). Subjects will be randomised to receive a single IV dose of GSK2586881 or saline in a crossover design.

A schematic of the study is shown below. Echocardiograms (echo) approximate timings are indicated by bold arrows.

Part 1: Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2



The above schematic is applicable also to **Part 2** of the study, with the following modifications: simulated altitude will be 5000 m, and the T3 echo will be taken 2 minutes into the exercise challenge (rather than at the end of a 10-minute exercise challenge).

Treatment Groups, Randomisation Arms and Duration

The study is intended to follow a double blind (sponsor open) two-period cross-over design.

Treatment Group A: matching volume of placebo, administered as a single IV dose

Treatment Group B: GSK2586881 0.8 mg/kg, administered as a single IV dose.

Subjects will receive both treatments during the course of the study, and will be randomised to one of two sequences, each of which describes the order in which those treatments are received:

Sequence 1: AB: Placebo in the first period followed by GSK2586881 in the second.

Sequence 2: BA: GSK2586881 in the first period followed by Placebo in the second.

The total study duration for each subject is expected to be a maximum of 56 days.

Type and Number of Subjects

Approximately 25 subjects will be enrolled to ensure that a minimum of 20 subjects complete all dosing and critical assessments. Eleven subjects were enrolled into Part 1 of the study (before the first interim analysis), and up to 14 subjects will be enrolled into Part 2 of the study. The target of 20 evaluable subjects may be revised by the sample size re-estimation.

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

Analysis

This study is designed to estimate the effect of GSK2586881 relative to placebo on change from baseline in PASP following exercise under hypoxic conditions. No formal hypothesis will be tested. Point estimates will be calculated together with corresponding 95% confidence intervals (or credible intervals if utilising a Bayesian framework) for the difference between the mean of the test treatment and the mean of the reference treatment.

Analysis of PASP: Statistical modelling of changes from baseline (Ti-T0) will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). Subject will be included as a random effect. An unstructured covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements. Non-informative priors will be used for the model parameters. Posterior distributions will be obtained for the GSK2586881 Vs Placebo comparisons at each of the post dosing time-points (T1-T4). These distributions will be used to produce several posterior probability statements; the most important being the probability that the change from baseline in PASP is reduced by the GSK2586881 at time T3. Separate analyses will be performed for Part 1 and Part 2.

Oxygenation saturation will also be analysed in a similar way; although of interest is the posterior probability that the study drug causes (absolute) reductions in oxygenation saturation in excess of 5%.

An interim analysis was conducted when 10 subjects had completed Treatment Periods 1 and 2 (Part 1), and the following data were reviewed: PASP, oxygen saturation,

AE/SAE, and RAS peptide. The decision was taken to continue the study with modifications to the protocol (Part 2).

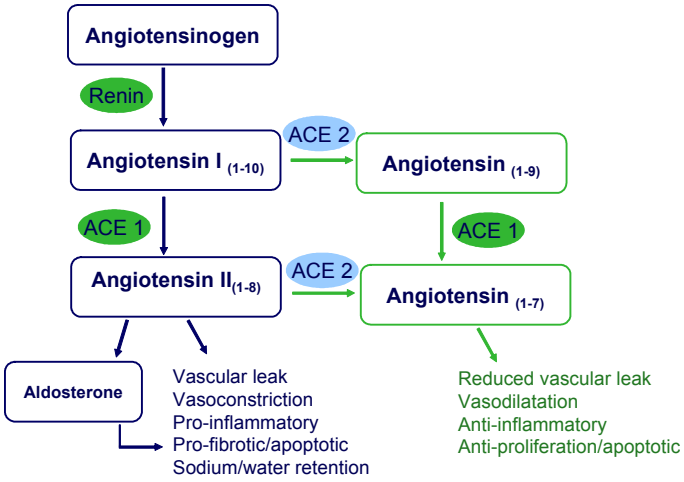
During Part 2 of the study, further interim analyses are planned, as follows:

- Review of PASP, oxygen saturation and AE data when approximately 3 subjects have completed Treatment Periods 1 and 2.
- Review of PASP, oxygen saturation and AE data when approximately 6 subjects have completed Treatment Periods 1 and 2. This second review may not be required and will be dependent on data observed during the first interim review. The timing of this review may be altered, as necessary, dependent on subject recruitment. Further interim reviews may take place, if appropriate.

2. INTRODUCTION

GSK2586881 is a purified intravenous formulation of soluble recombinant human angiotensin converting enzyme type 2 (rhACE2), which is expressed in Chinese Hamster Ovary cells. Angiotensin converting enzyme type 2 (ACE2) is a zinc carboxypeptidase involved in the Renin-Angiotensin System (RAS) that controls blood pressure, electrolytes, and intravascular fluid volume. A key function of ACE2 is believed to be the cleavage of Angiotensin II (Ang II) to Ang (1-7), which have opposing physiological roles. Elevated levels of Ang II are associated with vasoconstriction, inflammation, fibrosis, vascular leak, and sodium absorption. Ang (1-7) appears to be a counter-regulatory protein in the RAS; associated with vasodilation, anti-proliferation, anti-inflammation, and reduced vascular leak, as noted in Figure 1 below [Paul, 1992; Santos, 2005; Suzuki, 2003].

Figure 1 Renin Angiotensin System



Ang II binds to two distinct receptors called AT-1 and AT-2, with the AT-1 receptor mediating the vasoconstrictive, proliferative and pro-inflammatory actions of Ang II. The function of the AT-2 receptor has not been fully elucidated. Ang (1-7) initiates its effects by binding to the Mas-receptor, and also acts by inhibiting the activity of the carboxyterminal domain of angiotensin converting enzyme (ACE), which prevents ACE from fully acting on its substrates Angiotensin I and bradykinin.

ACE and Angiotensin II has been implicated in the pathogenesis of acute respiratory distress syndrome (ARDS), and pulmonary hypertension. It has been observed that circulating Ang II levels and lung ACE levels are increased in humans with ARDS and pulmonary hypertension. It has also been shown that the DD ACE polymorphism, which is associated with higher ACE activity, is associated with susceptibility to development of lung injury and worsened outcome (mortality) in patients with ARDS [Marshall, 2002] and to the development of pulmonary hypertension [Abraham, 2003].

It is expected that the reduction of Ang II and simultaneous formation of Ang (1-7) should have positive impacts in ARDS and on pulmonary haemodynamics in patients with pulmonary hypertension. This dual action can be achieved by ACE2, and thus, an

enhancement of the activity of this enzyme is seen as a promising approach for the treatment of diseases and conditions with an imbalance of the RAS system, insufficient natural ACE2 activity, and pathologically elevated Ang II levels or decreased Ang (1-7), such as is observed in ALI/ARDS [Tom, 2001; Idell S, 1987; Santos, 2003; Wenz, 2000] and pulmonary hypertension [Maron, 2014].

This study will demonstrate how GSK2586881 modulates the acute HPV response in healthy volunteers.

2.1. Study Rationale

Hypoxic pulmonary vasoconstriction (HPV) is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO2) levels in healthy and in pathophysiological settings, such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. However, in many ARDS patients with pulmonary microvascular injury and dystonia, the normal HPV reflex is compromised resulting in the mismatching of perfusion (Q) and ventilation (VA) and formation of areas of low perfusion to ventilated alveoli (high VA/Q; physiological deadspace), or perfusion of alveoli with minimal or no ventilation (low V/Q; physiological shunt).

The literature is conflicting as to the role of the RAS in modulating the HPV response; however, the majority of reports support a role for the RAS in driving acute HPV (Cargil, 1996; Kiely, 1996). While there is a strong biological rationale for modulation of the RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they impact on PaO2 and produce a blunted HPV response. Surprisingly, there are scant data regarding the effect of RAS modulation on HPV, V-Q matching or PaO2 in healthy human volunteers.

Inhibition of the acute HPV response would be expected to promote blood flow to areas of poorly-ventilated lung resulting in an increase in venous admixture and a reduction in PaO2 levels. In the context of acute lung injury, correction of any V/Q mismatch would be expected to be beneficial with respect to maintaining and improving PaO2 levels. Conversely, inhibition of HPV by pharmacological agents might be expected to worsen V/Q matching and compromise PaO2 levels. Thus, from a safety (and efficacy) perspective it is vital to understand the impact of modulation of the RAS system on the acute HPV response and subsequent PaO2.

Recombinant human ACE2 (rhACE2) delivered intravenously to pigs inhibits the HPV response to acute hypoxic challenge assessed by inhibition of mean pulmonary artery pressures and pulmonary vascular resistance. There was also a strong trend to increased shunt with administration of rhACE2; however PaO2 was not significantly affected. The authors concluded that the increased shunt may not have been sufficient to result in a reduction in PaO2 (Kleinsasser, 2012).

An elegant study by Wagner and colleagues demonstrated the importance of end arterial capillary diffusion limitations on PaO2 levels in healthy volunteers during hypoxia ± exercise (Torre-Bueno, 1985). Diffusion limitation made a significant contribution to PaO2 levels particularly under conditions where exercise and hypoxia were combined. In

addition, V/Q mismatching was suggested to increase with the combined stresses of exercise and hypoxia resulting in significantly more arterial oxygen desaturation than observed with either stressor by itself.

The main goals of this study are to examine whether GSK2586881 modulates the acute HPV response in healthy volunteers with a subsequent impact on O2 saturation. Should the application of GSK2586881 lead to accentuated arterial oxygen desaturation, further clinical studies to examine the therapeutic efficacy of GSK2586881 in acute lung injury should be approached with caution. Conversely, a reduction in HPV without augmented hypoxemia would provide supporting evidence that GSK2586881 could have a positive impact in patients with pulmonary hypertension.

2.1.1. Rationale for Changes to the Study following Interim Analysis

As planned in the original protocol, an interim analysis was conducted when 10 subjects had completed both Treatment Periods. Data from the first 11 subjects were reviewed, because Subject PP was withdrawn during Treatment Period 1 (withdrawn due to dizziness – investigator considered that this was unrelated to study treatment, and was most likely a vasovagal response to cannulation).

As planned, a core sub-set of the study team (as defined in Section 6.3) reviewed unblinded PASP, oxygen saturation, AE and RAS peptide data (RAS peptide data from only 5 subjects, as planned; see Section 9.3.2). The AE data indicated no safety concerns: in total, 5 AEs were reported, 1 was considered severe (as described above for Subject PPD and all were considered by the investigator to be unrelated to study treatment. The RAS peptide data indicated target engagement.

Review of PASP data indicated that an HPV response could not consistently be detected in the first 10 subjects who completed the study. An increase in PASP at T2 and T3 compared with baseline was not consistently observed. A decrease in oxygen saturation was observed at T2 (mean ~85%) and a further decrease at T3 (mean ~82%) compared with ~96% at baseline (similar in both Treatment Periods).

The study was placed on hold to allow the team to consider the data from the first part of the study (Part 1), and decide what changes would be made to the second part of the study (Part 2). The team concluded the following:

- In previous studies assessing HPV in healthy volunteers, increases in PASP were associated with more significant oxygen desaturation (Ricart, 2005; mean oxygen saturation in hypoxia was 79.4%, and simultaneous hypoxia and exercise was 63.5%). In Part 1 of the study, the altitude of the hypoxia chamber was 4000 m. In Part 2 of the study, the altitude will be increased to 5000 m in order to induce greater oxygen desaturation and associated increases in pulmonary pressures.
- It is anticipated that increasing the altitude of the chamber from 4000 to 5000 m will reduce oxygen saturation to as low as 65% in some subjects (when concurrently exercising). Therefore, the current individual subject withdrawal criteria of oxygen saturation falling *below 65% during the hypoxia challenge* (see Section 5.4) will be reduced to *below 60%* and a timeframe for the reduction added (persistently for >15 seconds). This is justified, because subjects will be continually monitored during the

challenge, and immediately removed from the chamber should oxygen saturation decline to the withdrawal limit. Oxygen saturation recovery on returning to normoxia is very rapid.

- The exercise challenge in Part 2 will be modified. In Part 1 of the study, PASP data at the T3 time-point were extremely variable. Review of the literature shows that any exercise-induced increase in PASP will decline to baseline extremely rapidly following cessation of exercise (Argiento, 2010). This likely explains the unreliability of data at T3. In Part 2, the exercise challenge will be conducted on a semi-recumbent ergometer, allowing acquisition of echo recordings during (rather than after) exercise. The workload of the exercise challenge will be reduced (from 70% VO_2max to 50% VO_2max), because of the increase in chamber altitude. Additionally, echo recordings will be taken within 2 minutes of starting exercise, rather than after exercise, based on reports that prolonged steady state exercise can conversely decrease pulmonary vascular pressures, due to a reduction in pulmonary blood volume as the systemic circulation adapts (Naeije, 2018).
- In Part 2 of the study, at all time-points, the protocol for echo recordings will be modified with the aim of improving data quality and reliability. The following changes will be made: calculation of PASP (outlined in Section 7.4) will be conducted *after* image acquisition (rather than *during* the recording); the sonographer will aim to capture at least 5 good quality images to estimate PASP; and a physician 'over-reader' process will be introduced. PASP will be calculated by a primary reader (the sonographer) and all echo images/calculations will be reviewed by a suitably qualified physician, who will provide a quality statement.
- In Part 2 of the study, an exploratory echo endpoint has been added, which will allow estimation of pulmonary vascular resistance (PVR).

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in Surfactant Protein (SP-D) and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers under hypoxic conditions (and during exercise under hypoxic conditions).	• Estimation of pulmonary vascular resistance (PVR) measured via echocardiography.

4. STUDY DESIGN

4.1. Overall Design

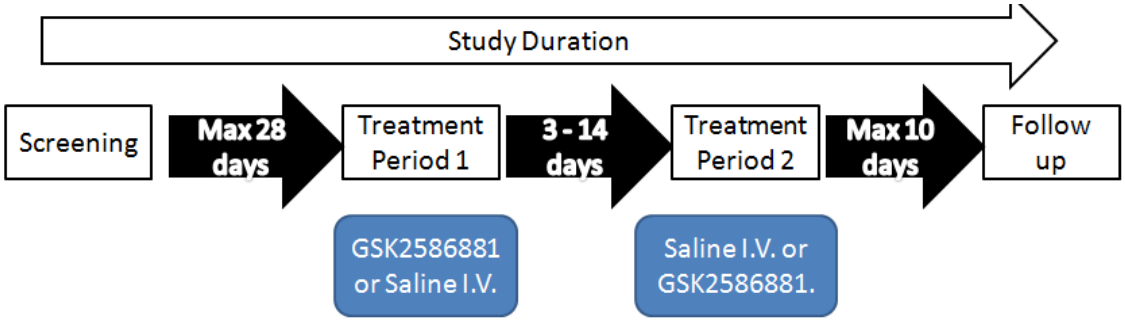
This is a single-centre, randomised, placebo-controlled and double blind (sponsor open), two-period crossover study in healthy subjects.

An interim analysis was conducted when 10 subjects had completed both Treatment Periods (Part 1). The study was placed on hold to allow the study team to consider the data from the first part of the study (Part 1). The study will now restart, with the modifications described in Section 2.1.1, and the second part of the study is designated Part 2.

The subjects will be required to attend the unit for a screening visit, Treatment Period 1, Treatment Period 2 and a follow up visit.

4.2. Treatment Arms and Duration

Figure 2 Subject participation flow



The subjects must participate in the procedures detailed in the Time and Events Table (Section 7.1) and the timings of the simulated altitude, exercise and echocardiograms is shown in Figure 3

Figure 3 Part 1: Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2

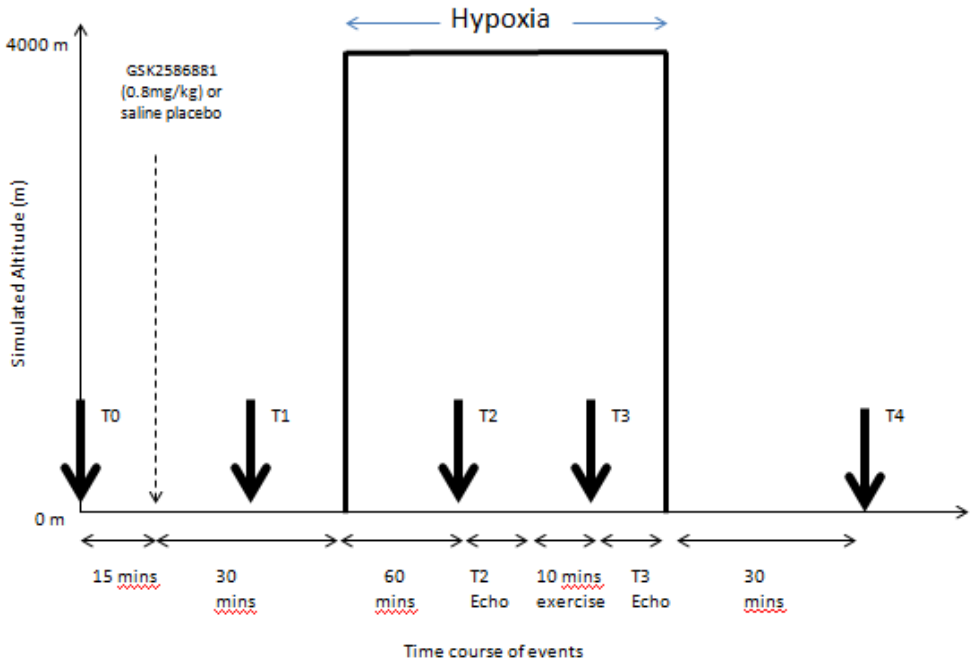


Figure 3 is applicable also to **Part 2** of the study, with the following modifications: simulated altitude will be 5000 m, and the T3 echo will be taken 2 minutes into the exercise challenge (rather than at the end of a 10-minute exercise challenge).

There will be a washout period of 3–14 days between treatments to ensure biomarkers return to pre-challenge baseline. Subjects then return to the site and repeat the same procedures as above, except that they will receive the treatment (GSK2586881 or Saline) that they did not receive in the first period.

4.2.1. Follow Up

The follow-up visit will occur up to 10 days after the end of the second treatment period. During the visit various safety tests will be conducted (see time and events table in Section 7.1).

The total study duration for each subject is expected to be a maximum of 56 days.

4.3. Type and Number of Subjects

Approximately 25 subjects will be randomised such that approximately 20 evaluable subjects complete the study. Eleven subjects were enrolled into Part 1 of the study (before the first interim analysis), and up to 14 subjects will be enrolled into Part 2 of the study. The target of 20 evaluable subjects may be revised following the sample size re-estimation, see Section 9.2.2.

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

4.4. Design Justification

The study design is based on a paper published by Ricart, 2005 and is considered to be feasible. The study will provide important information on whether GSK2586881 modulates the acute HPV response in healthy volunteers. In addition, the study will include assessments of PK and PD effects of GSK2586881. This will be achieved by assessing blood levels of GSK2586881 and RAS peptide responses throughout the duration of the hypoxia challenges.

The study will be placebo controlled (saline) so each subject can be used as their own control.

4.5. Dose Justification

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). In a concluded investigator-sponsored study in Pulmonary Arterial Hypertension (PAH) (Study 204696), 5 subjects have received a single dose of either 0.2 mg/kg or 0.4 mg/kg. There were no SAEs associated with the infusion of GSK2586881 or reported within the 2-week period of study observation. There were no clinically significant changes in laboratory or vital signs measurements for any subject.

A population PK model for GSK2586881 was derived from data obtained in healthy subjects and ARDS patients and showed that the systemic PK profile was adequately described by a two-compartment first order elimination model and that the PK profile was independent of population (healthy subjects or ARDS patients). Furthermore the PK/PD response (AngII) in healthy subjects and ARDS patients was consistent with a single direct Emax model after accounting for differences in baseline AngII concentrations between healthy subjects and ARDS patients.

Based on the population PK/PD model described above, single intravenous doses of 0.4–1.2 mg/kg GSK2586881 are predicted to reduce elevated levels of AngII (baseline AngII consistent with an ARDS population) to levels consistent with that observed in healthy subjects for the duration of the hypoxic challenge (approx 2.25 h). The dose of 0.8 mg/kg has been selected to ensure maximal reduction of AngII levels, whilst maintaining dosing volumes within acceptable limits, and will further aid the understanding of the PK/PD relationship for GSK2586881.

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with GSK2586881 can be found in the Investigator's Brochure (GlaxoSmithKline Document Number 2010N108777_04, 2017). The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2586881]		
Cardiovascular risk	During preclinical testing a brief period of non-sustained ventricular tachycardia was observed in one monkey receiving a dose of 20.8 mg/kg/day.	The dose used in this study is much lower and well below the No observed adverse effect level (NOAEL) for the 14 day repeat dose cynomolgus monkeys of 8 mg/kg/day.
Potential Reproductive/embryofetal risks	Preclinical studies have not been performed.	Women of childbearing potential will be excluded from the study.
Potential for Immunogenicity	There has been no induction of an immune response to rhACE2 in either of the clinical studies to date in healthy subjects or participants with ARDS.	Patients will have routine monitoring of any immunological response that may occur. If an immunological response is seen the patient will be asked to return for further monitoring and assessment(s).
Potential for rash	In study ACE114622, rash was reported more frequently in subjects receiving GSK2586881, although only one event was considered drug-related.	Patients will be monitored for rash in the clinical trials.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Effects of hypoxia to study subjects (light headiness, headaches, nausea)	The subjects will be exposed to hypoxic conditions for approximately 80 minutes with a simulated altitude of 5000 m.	The subjects will be continuously observed and monitored with telemetry and pulse oximetry. Stopping criteria are included in the protocol.
Effects of hypoxia to staff at the clinical unit (light headiness, headaches, nausea)	Clinical site staff performing the assessments within the hypoxia chamber will potentially be exposed to hypoxic conditions with a simulated altitude of 5000 m.	All clinical staff entering the hypoxia chamber will be provided with an ambulatory oxygen supply.

4.6.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the subjects taking part in the study as it is a healthy volunteer study. However, subjects will undergo a medical evaluation during screening including physical exams, ECGs and laboratory assessments which may provide important health information.

By taking part in this study, the subject will be contributing to the development of GSK2586881 for the treatment of pulmonary hypertension and ARDS.

4.6.3. Overall Benefit:Risk Conclusion

The design of the study is considered low risk to the subjects and justified based on the work carried out by other researchers, the safety information from the nonclinical studies and the two previous clinical trials carried out on GSK2586881.

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 the IB.

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 40 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, spirometry, laboratory tests and cardiac monitoring. 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 Medical Monitor if required) agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures. Note: Screened subjects with laboratory values outside of the normal range may be

TYPE OF SUBJECT
repeated once for inclusion into the study at the discretion of the Investigator. 3. Screening echocardiogram of good quality, without clinically significant abnormalities, and with mild-moderate tricuspid regurgitation sufficient for the reliable estimation of PASP, as determined by the echocardiography core laboratory or responsible cardiologist. *Screening PASP within the normal range according to site standards.* 4. Subjects have not resided at an altitude >1500 m for more than 7 days in the last 4 months. 5. Able to complete all study procedures. 6. Any contraindication (orthopaedic, cardiac etc.) to perform exercise on a bicycle ergometer.

WEIGHT
7. Body weight 50–100 kg (inclusive).

SEX
8. Male or female (non child bearing potential) Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication. a. Vasectomy with documentation of azoospermia. b. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant • Intrauterine device or intrauterine system • Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a] • Contraceptive vaginal ring [Hatcher, 2007a] • Percutaneous contraceptive patches [Hatcher, 2007a] This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH M3 (R2), 2009]. The investigator is responsible for ensuring that subjects understand how to properly use

SEX

these methods of contraception.
 	
Females:
 	
A female subject is eligible to participate if she is not pregnant (as confirmed by a negative urine human chorionic gonadotrophin (hCG) test), not lactating, and the following condition applies:
 	
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
• Postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels)]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 7.2, 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. ALT >1.5x Upper limit of normal (ULN). 2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%). 3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
4. Prior history of altitude sickness. 5. Recently been scuba diving (within 1 week before screening). 6. 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
7. Unable to refrain from prescription or non-prescription drugs, including agents active in the central nervous system, 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 and throughout the study, unless in the opinion of the Investigator and/or GSK Medical Monitor (if needed) the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
8. 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. 9. Urinary cotinine levels indicative of smoking or history or regular use of tobacco- or nicotine-containing products within 6 months prior to screening.

CONTRAINDICATIONS
10. 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
11. 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. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody (HBcAb) should also be excluded.
12. A positive pre-study drug/alcohol screen.
13. A positive test for HIV antibody.
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within 56 days.
15. 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: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
16. 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 randomised. 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 (Section 7.2.1.4).

Subjects that are not enrolled into the study within the allotted screening window may be re-screened once. If re-screening is performed, subjects are assigned a different unique subject Identification (ID) number for the re-screening, and all screening procedures must be repeated.

See the Study Reference Manual (SRM) for specific details.

5.4. Withdrawal/Stopping Criteria

Subjects may be withdrawn from the study for any of the following reasons:

- 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.
- If a subject's oxygen saturation falls below 60% (persistently for >15 seconds) at any point during the hypoxia challenge.

- If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

Once a subject has discontinued investigational product the subject may not re-enter the study. Dosing of the subjects with the investigational product may be stopped at any time, at the request of the subject or at the discretion of the Investigator (i.e. if clinically significant adverse events should occur). Withdrawal due to adverse events will be distinguished from withdrawal for other reasons.

If a subject decides to withdraw or is withdrawn by the Investigator, the reasons for withdrawal and the results of any relevant tests will be recorded in the Case Report Form (CRF) and the planned follow-up procedures will be performed, where possible.

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".

5.4.1. Study Stopping Criteria

An interim analysis was conducted when 10 subjects had completed both Treatment Periods (Part 1). The study team decided to continue the study (Part 2), with modifications to the protocol (see Section 2.1.1). In Part 2, an interim analysis will be conducted when approximately 3 subjects complete both Treatment Periods, and further interim analyses may be conducted (see Section 9.3.2). The study may be stopped if PASP results are highly variable, or if the PASP profile for the placebo group is not as expected (indicative of the model not working given the alterations to the study) or if review of the safety data suggest a change in benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.

Safety data that is collected on a continuous basis (oxygen saturation and telemetry) will be monitored live on site to determine whether a subject tolerates the hypoxia/exercise challenge; study procedures may be aborted on safety grounds if, in the Investigator's opinion, any of these measurements reach unsafe levels. The simulated altitude within the chamber may be reduced if too many participants fail to tolerate the challenge, and the study may be stopped if participants fail to tolerate the challenge at the minimum altitude. The full criteria for this procedure (which may lead to the study being stopped) are described in Section 7.3.

5.4.2. QTc Stopping Criteria

The *same* QT correction formula *must* be used for *each individual subject* to determine discontinuation from the study. For example, if a subject is eligible for the protocol based on QTcB, then QTcB must be used for discontinuation of this individual subject as well.

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

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

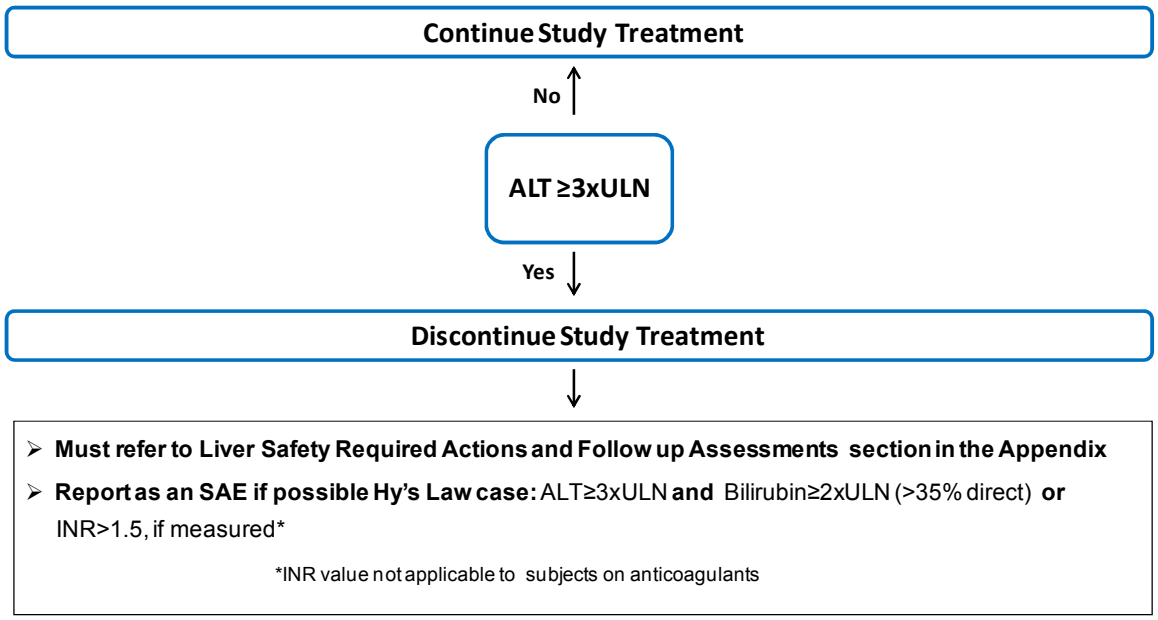
The QTc should be based on single or averaged QTc values of triplicate ECGs obtained over a brief (e.g. 5-10 minute) recording period.

5.4.3. 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 Food and Drug Administration (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.

5.4.3.1. Study Treatment Restart or Rechallenge

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

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 Treatment	
Product name: (Generic name and trade)	GSK2586881	Placebo
Formulation description:	rhACE2	Normal Saline (0.9%)
Dosage form:	IV	IV
Unit dose strength(s)/Dosage level(s):	0.8 mg/kg	Saline Placebo
Route of Administration	Intravenous	Intravenous
Dosing instructions:	Infuse over 3-5 minutes	Infuse over 3-5 minutes
Physical description:	Clear colourless liquid	Clear colourless liquid

At Screening a unique Subject Number (CRF number) will be assigned to any subject who has at least one Screening procedure performed, other than informed consent. The unique Subject Number will be used to identify individual subjects during the course of the study.

Subjects who meet screening eligibility criteria will be assigned to one of the sequences listed in Table 1 below, in accordance with the randomisation schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software. Once assigned, this number must not be reassigned to any other subject in the study.

Table 1 Description of sequences

Sequence	Period 1	Period 2
AB	Saline Placebo	GSK2586881
BA	GSK2586881	Saline Placebo

The subjects will be randomised using a central randomisation procedure created by GSK.

Further details on how and when a subject is allocated a randomisation number and the subject numbering convention is in the SRM.

6.2. Treatment after the End of the Study

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

6.2.1. Meals and Dietary Restrictions

- No dietary restrictions prior to the first treatment period.

6.2.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from caffeine for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.
- During each dosing session, subjects will abstain from alcohol for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.
- Use of tobacco products is not allowed as outlined in the exclusion criteria.

6.2.3. Activity

Subjects will abstain from strenuous exercise for 24 hours prior to screening and each treatment period Subjects may participate in light recreational activities between the planned study procedures (e.g., watch television, read).

6.3. Planned Dose Adjustments

No dose adjustments are allowed.

6.4. Blinding

This will be double blind (sponsor open) study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff), and the subject will be blinded to the treatment allocated to individual subjects. An unblinded qualified staff member will be required at site to prepare the study treatment for dosing. The unblinded staff member is not permitted to communicate the subject's treatment allocation to blinded site staff. Selected study team members working for the sponsor (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This will include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report.

The following will apply:

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment.
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the CRF

A subject whose treatment sequence assignment is unblinded for emergency reasons (as described above) will not be permitted to continue in the study (due to the emergency requiring unblinding rather than the unblinding itself). The event or condition that led to the unblinding will be recorded in the CRF as the primary reason for discontinuation.

A subject whose treatment sequence assignment is inadvertently unblinded (either to investigative staff or the subject themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the subject will be subject to review as to their inclusion in analyses as per the procedures set out in Section 9.3.1.

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

A description of the methods and materials required for GSK2586881 will be detailed in a Study Specific Technical Agreement/Memo (TTS) or Pharmacy Section of the SRM, which will be accompanied by a Quality Agreement.

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
- 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. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.
- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

6.7. Compliance with Study Treatment Administration

When the individual dose for a subject is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

Subjects will be dosed at the site, and 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.

GSK2586881 and the saline placebo will be intravenously administered to subjects at the site. Administration will be documented in the source documents and reported in the CRF.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK2586881 > 1.5 mg/kg within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose. The investigator will use clinical judgment to treat any overdose.

In the event of an overdose the investigator or treating physician should:

1. contact the Medical Monitor immediately
2. closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until GSK2586881can no longer be detected systemically (at least 3 days for GSK2586881).
3. obtain a plasma sample for pharmacokinetic (PK) analysis within 1 day from the date of the last dose of study treatment 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. Concomitant Medications and Non-Drug Therapies

6.9.1. 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.

6.9.2. 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. Ventilatory parameters
  2. Echocardiograms
  3. 12-lead ECG
  4. vital signs
  5. blood draws

  Note: The timing of the assessments should allow the echocardiogram to be performed as close as possible to the nominal time.
- The timing and number of planned study assessments, including: safety, echocardiogram, pharmacokinetic, pharmacodynamic/biomarker or other 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 Institutional Review Board (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

7.1.1. Screening and Follow up

Procedure	Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)	Follow up (7-10 days post last dose)	Notes
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam, including height and weight	X	X	Height and weight to be measured at screening only. Weight at screening will be used for dosing calculation.
Alcohol, Drugs of Abuse, Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: Drugs, Alcohol, tobacco
Past and current medical conditions [including cardiovascular medical history]	X		
Serum OR urine pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries)	X	X	Non-Fasting
Immunogenicity		X	
12-lead ECG	X	X	Triplicate ECG required at screening.
Vital signs	X	X	Triplicate vital signs required at screening.
Spirometry	X		
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		**Part 1:** Tolerance to 4000m for 10 mins followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max (to be used for the exercise challenge during the Treatment Periods). **Part 2:** Tolerance to 5000m for 10 mins followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 50% of VO2max (to be used for the exercise challenge during the Treatment Periods)
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.

Procedure	Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)	Follow up (7-10 days post last dose)	Notes
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

1. Screening assessments are allowed to be conducted on more than one day

7.1.2. Treatment Period 1 and 2

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Randomisation	X											Treatment Period 1 only. Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X[5]			X	
Immunogenicity	X											
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X[6 below]		X		
Subject enters chamber					X							Subject enters chamber approximately 30 min after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram, blood samples and vital signs have been taken.
Exercise challenge							X					**Part 1:** exercise at 70% VO2 max for ~5-10 min. Echo conducted immediately after. **Part 2:** exercise at 50% VO2 max for 2 min. Echo started at 2 min into the challenge, and the subject continues exercising.
Ventilatory parameters	X		X			X				X		Measurements to be taken 2 min before echocardiograms.

Procedure	**Treatment Period 1 and 2 (Washout 3-14 days)**											**Notes**
	Times relative to start of dosing				**Hypoxic Challenge ~80 min (Times relative to entry to Chamber)**							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Pulse Oximetry (O2 saturation)	←==========→											Will be continuously monitored for safety. A measurement should be recorded at the time of each echocardiogram and databased. **Part 2**: when the echo is recorded during the exercise challenge, pulse oximetry will be recorded immediately prior to initiation of the echo at 2 minutes into exercise.
Telemetry	←==========→											Will be continuously monitored for safety.
RAS Biomarkers	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
SP-D	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
PK sampling	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
AE/SAE review		←==========→										
Concomitant medication review	X											

1. Take at the end of the infusion
2. Taken immediately after echocardiogram
3. Immediately before entering the chamber
4. To be taken as soon as possible after leaving chamber
5. On this occasion ONLY, vital signs to be taken after the blood draw.
6. In Part 2, echo at this time-point taken 2 minutes into the exercise challenge (not immediately after exercise).

After written informed consent, screening assessments will be performed as outlined in the Time and Events Table (Section 7.1).

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

Cardiovascular medical history/risk factors (as detailed in the CRF) will be assessed at screening.

Cardiorespiratory fitness during bicycle exercise and hypoxia tolerance will be assessed.

Procedures conducted as part of the subject's routine clinical management (e.g. blood count) and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

7.2. 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 exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

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

The definitions of an AE or SAE can be found in Appendix 4.

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

7.2.1.1. 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 7.2.1.3), at the time-points 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 Appendix 4.

- 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 Appendix 4.

7.2.1.2. 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?"

7.2.1.3. 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 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 Appendix 4.

7.2.1.4. 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.

7.2.2. 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, at screening only.
- A brief physical examination will include, at a minimum assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). To be carried out at the start of each period.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

7.2.3. 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.
- Triplicate measurements will be taken at screening, and single measurements at other time-points. The first reading should be rejected. Second and third readings should be averaged to give the measurement to be recorded in the CRF.

7.2.4. Electrocardiogram (ECG)

- Triplicate 12-lead ECGs will be obtained at screening. At all other time-points, single 12-lead ECGs will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes rest.
- The same QT correction formula *must* be used for each individual subject for all QTc data being collected for data analysis. Safety ECGs and other non-protocol specified ECGs are an exception.

7.2.5. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled. Details for the preparation and shipment of samples will be provided by the laboratory. 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.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters
Haematology	Platelet Count; RBC Count; Hemoglobin; Hematocrit; *RBC Indices*: MCV; MCH; *WBC count with Differential:* Neutrophils; Lymphocytes; Monocytes; Eosinophils; Basophils
Clinical Chemistry [1]	BUN; Urea; Creatinine; Glucose; Potassium; Sodium; Calcium; AST (SGOT); ALT (SGPT); Alkaline phosphatise; Total and direct bilirubin; Total Protein; Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol, smoking and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, benzodiazepines and cotinine) performed at site • Urine hCG Pregnancy test (as needed) [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 5.4.2 and Appendix 2.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within 3 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.

7.3. Hypoxia Challenge

7.3.1. Part 1

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 4000 m ± 10% hypoxic conditions.

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O_2 saturation of <65%, then the simulated altitude will be adjusted in decrements of 500 m (i.e. from 4000 m to 3500 m) for all remaining subjects to a minimum of 3000 m.

7.3.2. Part 2

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 5000 m ± 10% hypoxic conditions.

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O_2 saturation of <60% (persistently for >15 seconds), then the simulated altitude will be adjusted in decrements of 500 m (i.e. from 5000 m to 4500 m) for all remaining subjects to a minimum of 4000 m.

Further detail about the hypoxia challenge is detailed in the SRM.

7.4. Exercise Challenge

7.4.1. Part 1

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on an upright cycle ergometer within the hypoxia chamber for 10 minutes.

Subjects must complete a minimum of 5 minutes exercise at 70% of maximumVO2 uptake (calculated from the incremental exhaustive exercise test at the screening visit).

7.4.2. Part 2

Subjects will perform an exercise challenge at 50% of maximum VO2 uptake on a semi-recumbent cycle ergometer within the hypoxia chamber (calculated from the incremental exhaustive exercise test at the screening visit). Two minutes into the exercise challenge, the echo recording will be initiated. The subject will exercise for a minimum of 5 minutes (or until the echo recording is completed, if the recording exceeds 5 minutes).

Further detail about the exercise challenge is detailed in the SRM.

7.5. Echocardiogram

Echocardiograms will be taken as detailed in the Time and Events table (Section 7.1).

Echocardiograms will be obtained with the subject resting supine or lying on their left side. In Part 2, the echo during the exercise challenge will be conducted with the subject on a semi-recumbent cycle ergometer tilted by 30-40 degrees. The subject will continue exercising during the echo recording.

PASP will be measured and recorded. PASP will be determined by measuring maximal tricuspid regurgitation velocity and applying the modified Bernoulli equation to convert this value into pressure values. Estimated right atrial pressure (RAP) must be added to this obtained value.

In Part 2, the sonographer's priority will be to collect good quality images of the tricuspid regurgitation jet, allowing accurate calculation of PASP once image acquisition is completed. The sonographer will aim to collect at least 5 cardiac cycles. Once estimation of tricuspid regurgitation velocity is completed, the sonographer will measure the right ventricular outflow tract (RVOT) time velocity integral, which (along with the peak tricuspid regurgitant velocity) will allow an estimation of PVR (Rudski, 2010).

In Part 2, the sonographer conducting the echo will be considered the primary reader. All echo images/calculations will be reviewed by a suitably qualified physician (physician 'over-reader'). Any discrepancies will be discussed between the primary reader and physician over-reader, and an agreement reached. The physician over-reader will provide a quality statement for each echo. For the first 3 subjects in Part 2, the physician review will occur immediately following completion of each echo. If quality issues are identified, the sonographer and physician will consider if the echo should be repeated. If no echo quality issues are identified for the first 3 subjects, subsequent reviews will occur within a few hours.

In Part 2, when the echo recording is conducted during the exercise challenge, the sonographer will begin positioning the echo probe as soon as exercise is initiated. Image acquisition will occur at 2 minutes into the exercise challenge. The sonographer will aim to complete image acquisition within approximately 1–2 minutes.

Further details about the echo recording is detailed in the SRM.

Additional echocardiograms may be obtained for each subject as needed (in the judgement of the Investigator and GSK Medical Monitor, if required).

7.6. Ventilatory parameters

Ventilatory parameters will be measured as detailed in the Time and Events table (Section 7.1).

Ventilatory parameters will be recorded for 2 minutes before echocardiograms. The second minute of the recording will be averaged and used for data analysis.

Measurements may include change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2), total tidal volume, inspiratory tidal volume, expiratory tidal volume, total respiratory time, inspiratory time, expiratory time, duty cycle, mean respiratory flow and respiratory rate, as data permit.

7.7. Pulse Oximetry

Oxygen saturation will be monitored continuously via pulse oximetry as detailed in the SRM. Measurements will be taken at the same time as the echocardiograms and recorded in the eCRF.

7.8. Telemetry

Continuous ECG monitoring will occur via telemetry during both treatment periods.

7.9. Pharmacokinetics

7.9.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of GSK2586881 will be collected at the time points indicated in the Time and Events Table (Section 7.1). 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.

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

7.9.2. Sample Analysis

Plasma analysis will be performed under the control of PTS, In Vitro/In Vivo Translations (IVIVT) Department and Third Party Resourcing (TPR), GlaxoSmithKline. The details of the Bioanalytical Laboratory will be included in the SRM. Concentrations of GSK2586881 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site to be detailed in the SRM).

7.10. Biomarker(s)/Pharmacodynamic Markers

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

Details of biomarker/PD blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.10.1. Renin-angiotensin system biomarkers

RAS peptides including, but not limited to, AngII, Ang(1-7) and Ang(1-5) may be analysed as data permit. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site detailed in the SRM).

7.10.2. Surfactant Protein-D and other biomarkers

SP-D will be analysed. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site to be detailed in the SRM). Additional analytes may be analysed, as data permit.

7.11. Genetics

Information regarding genetic research is included in Appendix 3.

Genetic sampling is optional. Subjects can refuse genetic sampling, but will still be allowed to participate in the study.

7.11.1. Blood sample collection

A blood sample to investigate the association between the loss of function polymorphism rs1799752, representing the I/D polymorphism, in intron 16 of the Angiotensin Converting Enzyme (*ACE*) gene and Ang II (and possibly other RAS peptides) and hypoxic pulmonary vasoconstriction will be collected as specified in the Time and Events Table (Section 7.1).

Further information for blood sample collection, processing, storage and shipping procedures are provided in the SRM.

Information regarding genetic research is included in Appendix 3.

7.12. Immunogenicity

7.12.1. Sample collection

Blood samples for immunogenicity analysis will be collected at the time points indicated in Section 7.1, Time and Events Table. Additional visits to obtain immunogenicity samples may be required in the unlikely event that subjects develop a clinically relevant immunoglobulin response to the drug as described in the SRM.

Details of immunogenicity blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.12.2. Sample analysis

Immunogenicity analysis will be performed under the management of Clinical Immunology, Biopharm R&D, GlaxoSmithKline. All pre-dose and post-dose samples will be first tested for Anti-ACE2 binding antibodies by screening and confirmation assay steps. The post-dose samples tested positive for anti-ACE2 binding antibodies will be further characterized for anti-ACE2 neutralizing antibodies. Both positive incidences for anti-ACE2 binding and neutralizing antibodies will be reported.

8. DATA MANAGEMENT

- For this study subject data will be entered into GSK defined CRFs, transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g. removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials and

day/month of birth will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

The focus of this study is on the exploration of the effect of GSK2586881 on a range of echocardiography, pulse oximetry, biomarker, safety and PK endpoints, when subjecting healthy volunteers to an exercise challenge under hypoxic conditions. No formal statistical hypotheses are being tested. A Bayesian statistical analysis framework with non-informative priors for model parameters (unless otherwise specified) will be used to obtain posterior distributions for effects of interest for Part 1 and Part 2 of the study. These posterior distributions will be used to obtain a number of probability statements about the magnitude of treatment effects (e.g. Probability of **any** treatment related reduction in PASP, or Probability that the treatment related reduction in PASP ≥ 5 mmHg).

A rule of thumb for end of "study success" is if the probability of any treatment related reduction in PASP (T3-T0) exceeds 0.95 (success is also conditional on the probability of (absolute) treatment related reductions in oxygen saturation exceeding 5%, being small).

9.2. Sample Size Considerations

The sample size of 20 evaluable patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context (Note: precision in this context is defined as one half of the width of the 95% confidence interval). A sample size re-estimation was planned after 10 subjects, see Section 9.2.2.

9.2.1. Sample Size Assumptions

The precision of the primary treatment comparison (change from baseline PASP to hypoxic/exercise PASP) was estimated using simulation. PASP data points were simulated for each subject: normoxic at rest, and hypoxic after exercise, each for two study periods, giving a total of four PASP measurements for each subject. The four PASP measurements were drawn from a multivariate normal distribution to allow for different levels of within-subject correlation. One simulated study with a given sample size (N) was created by repeating this process of generating individual subject data N times, randomly allocating each subject to a treatment sequence in a 1:1 ratio, and calculating the half-width of the confidence interval of the treatment difference in change from baseline in PASP from that simulated dataset (using a linear mixed effects model including period baseline, subject baseline, treatment and period as fixed effects, and subject as a random effect). A full simulation run consisted of 1000 iterations of simulated studies, and a final estimate for precision was calculated as the mean precision across the 1000 iterations.

The following assumptions were made regarding the mean and Standard Deviation (SD) of PASP based on data from Ricart, 2005:

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) and 45 mmHg under hypoxia and exercise
- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise
- As the purpose of this simulation was to estimate precision by evaluating the SD of the treatment difference rather than the mean, there was no treatment effect assumed in the simulations
- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9).
- A range of sample sizes was considered: from 5 to 10 in increments of 1, and then up to 40 in increments of 5. Table 3 below shows a subset of these; the figure includes them all.

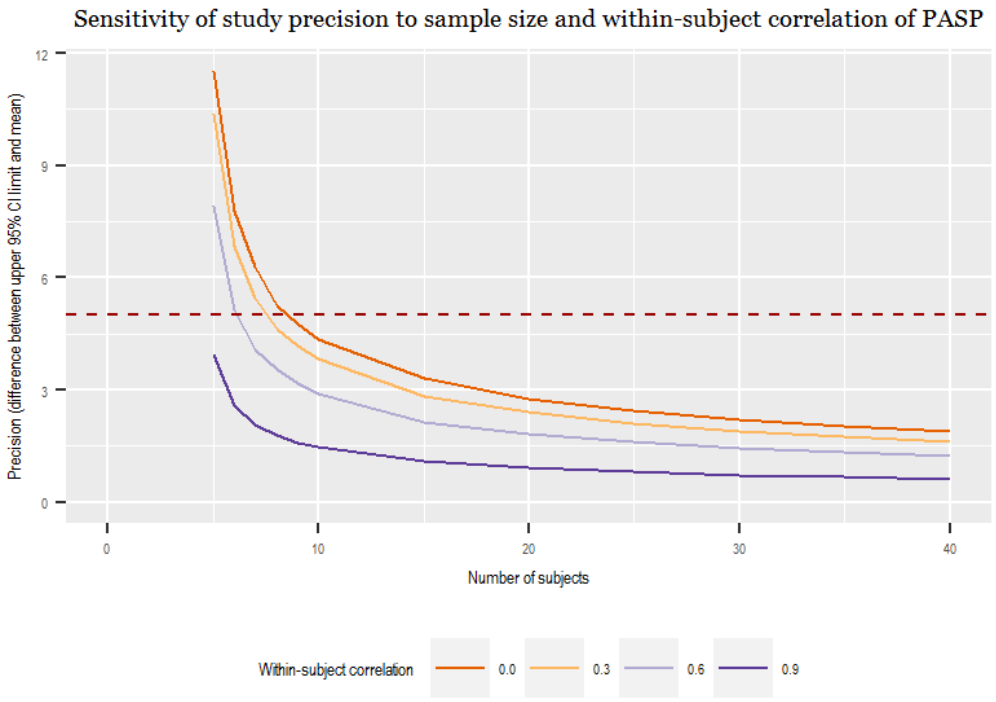
Based on these assumptions, the estimated precisions for these sample sizes and under these scenarios of within-subject correlation were as follows (smaller number for precision denotes more precise estimates):

Table 3 Estimated precision of treatment comparisons of change from baseline in PASP (mmHg) for a range of sample sizes and within-subject correlation scenarios

	Within-subject correlation			
Sample size (N)	**0**	**0.3**	**0.6**	**0.9**
10	4.35	3.83	2.92	1.48
20	2.76	2.40	1.83	0.91
30	2.22	1.91	1.46	0.72
40	1.91	1.63	1.23	0.62

Figure 4 illustrates precision estimates under the full range of sample sizes considered (from 5 to 40), and for all within-subject correlation scenarios. The red line at 5 mmHg is superimposed to indicate a rough guide to the magnitude of a clinically meaningful difference.

Figure 4 Sensitivity of study precision to sample size and within subject correlation of PASP



9.2.2. Sample Size Re-estimation

A sample size re-estimation was to be conducted at the interim analysis for Part 1 (see Section 9.3.2.1). The purpose of the sample size re-estimation was to determine whether the total number of subjects can be reduced below the anticipated 20, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP (i.e. T3-T0). The sample size re-estimation was to be considered advisory, and taken into account together with other considerations. These were to include:

- ensuring sufficient data is collected on secondary endpoints,
- non-conclusive safety data at the time of interim analysis (e.g. a weak signal of a reduction in oxygen saturation data that would require the full set of remaining subjects to be able to reach a conclusion),
- speed of recruitment.

An interim analysis was conducted when 10 subjects had completed Treatment Periods 1 and 2 (Part 1), and the following data were reviewed: PASP, oxygen saturation, AE/SAE, and RAS peptide. The decision was taken to continue the study with modifications to the protocol (Part 2), see Section 2.1.1.

9.3. Data Analysis Considerations

Data will be listed and summarised according to GSK integrated data standards library (IDSL) reporting standards where applicable. Complete details will be provided in the Reporting and Analysis Plan (RAP).

The key endpoints referred to in the text below comprise PASP measured by echocardiography, oxygen saturation measured by continuous pulse oximetry, RAS peptides and safety data.

9.3.1. Analysis Populations

All Subjects Screened Population: This population contains all subjects that complete at least one Visit 1 (Screening) procedure. This population will be used for the summary of subject disposition (including reasons for screening failures, run-in failures, and stabilization failures) and for the listing of AEs and SAEs for non-randomised subjects.

Modified Intent-to-Treat (mITT) Population: This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all safety and pharmacodynamic analyses.

Modified Intent-to-Treat (mITT1) Population Part 1: This population will include all subjects in the mITT population, who were randomised into Part 1 of the study (planned 4000 m altitude).

Modified Intent-to-Treat (mITT2) Population Part 2: This population will include all subjects in the mITT population, who were randomised into Part 2 of the study (planned 5000 m altitude)

Pharmacokinetic (PK) Population Part 1: This population will comprise all subjects in the mITT Population, randomised in Part 1 of the study, for whom a PK sample was obtained and analysed and on active treatment.

Pharmacokinetic (PK) Population Part 2: This population will comprise all subjects in the mITT Population, randomised in Part 2 of the study, for whom a PK sample was obtained and analysed and on active treatment.

In addition to the above populations, the effect of important protocol violations, including any subjects who failed the inclusion/exclusion criteria, may be assessed by means of sensitivity analyses. A blind review of all protocol violations will be performed prior to DBF in order to identify any important deviations and consequently identify any subjects who will be excluded from such sensitivity analyses.

9.3.2. Interim Analysis

9.3.2.1. Part 1

The details below are in reference to the completed interim analysis for Part 1 of the study. Following review of the data from Part 1, the study was modified before restarting Part 2. Section 9.3.2.2 details interim plans for Part 2 of the study.

An interim analysis is planned after 10 subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods
- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

The interim analysis will use the following study data:

- PASP at time T0 and T3 for both periods, for the first 10 subjects
- Oxygen saturation at T0 and T3 for both periods, for the first 10 subjects
- Adverse events for the first 10 subjects
- RAS peptide concentrations for the first 5 subjects (to allow time for the samples to be analysed by the external lab).

The interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

The aims of the interim analysis, and the endpoints to be used to address them, are as follows:

- Using the primary endpoint (treatment differences between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP [T3-T0]):
    - To decide whether to continue the study or stop based on futility, and
    - to conduct an advisory sample-size re-estimation with the view to potentially reduce the number of subjects participating in the study below 20.
- Using secondary endpoints:
    - to estimate treatment differences between GSK2586881 and Placebo in change from pre-dose oxygen saturation to hypoxic/exercised oxygen saturation [T3-T0], with a view to stopping the study if there is evidence that GSK2586881 causes a reduction in oxygen saturation in healthy

volunteers. As a non-binding guide high posterior probability of observing ≥5% absolute differences in **mean** oxygenation saturation values between placebo and active arms would be of concern; but clinical judgement would override any statistical methods if, for example, the majority of subjects displayed consistent patterns of reductions e.g. between 2-3%).

- to compare adverse events within the two treatment groups and explore any potential safety signals that may emerge

To assess the operating characteristics of the interim analysis decisions to be made based on the primary endpoint, a simulation was carried out to estimate the relative proportion of studies reaching conclusions to stop for futility, continue with no change to the sample size, and to continue with a reduced sample size, under various different scenarios of the true treatment effect. Broadly speaking, the aim of the simulation was to confirm that (in an overwhelming majority of cases) a decision to stop for futility would be reached in the case of no true treatment effect and a decision to continue (perhaps with reduced sample size) would be reached in the case of a true treatment effect.

Simulated datasets of PASP at baseline (i.e. normoxic and at rest) and under hypoxia and exercise for 10 subjects, each receiving both treatments in sequence, were generated using the following assumptions:

- 10 simulated subjects were randomised to one of the two available treatment sequences (see Section 6.2) using block randomisation with block size of 4 records

- PASP at baseline and under hypoxia and exercise for both treatment groups (a total of four PASP measurements per subject) were sampled from a multivariate normal distribution, using values for mean PASP, standard deviation of PASP and within-subject correlation of PASP as described below.

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) for both treatments, and 45 mmHg under hypoxia and exercise for Placebo (as in Section 9.2.1). For GSK2586881, mean PASP under hypoxia and exercise was (45 - Δ) mmHg, where Δ (the mean treatment difference) was varied according to four different scenarios:

    - no treatment difference (Δ = 0 mmHg)

    - weak treatment difference of a magnitude of half the clinically meaningful difference (Δ = 2.5 mmHg)

    - minimum clinically meaningful difference (Δ = 5 mmHg)

    - overwhelming treatment difference one-and-a-half times the clinically meaningful difference (Δ = 7.5 mmHg)

- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise, for both treatment groups (as in Section 9.2.1)

- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9) (as in Section 9.2.1). Within-subject correlation of PASP was assumed to be compound symmetric (i.e. the same correlation was used for each of the six possible pairs of the four PASP measurements within each subject).

- It was assumed that there would be no effect of period in the simulation, i.e. it was assumed that it would make no difference if the measurement was taken from the first or second chronological period in the treatment sequence.

Modelling took on a three-step process, with decisions after each. Firstly, the treatment difference and its associated standard error was then estimated for the dataset of 10 simulated subjects using a mixed effects linear regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. If the sign of treatment difference arising from the simulated data was in the wrong direction (i.e. the simulated outcome for GSK2586881 was worse than for Placebo), then the simulated study was stopped for futility.

Secondly, in the alternative case that the simulated treatment difference was in favour of GSK2586881 over Placebo, a posterior probability of success (where success was defined as a 97.5% posterior probability of the treatment difference being in favour of GSK2586881 over Placebo) was evaluated for 20 subjects. This was evaluated using a t-distribution, with the mean and standard deviation taken as the treatment difference and standard error (multiplied by a factor of the square root of 10 to estimate the standard deviation) from step one, respectively. If this posterior probability was less than 0.2, then the study was stopped for futility as being unlikely to show any benefit of GSK2586881 over Placebo.

Thirdly, provided that the simulated study hadn't been stopped for futility in either of the first two steps, posterior probabilities of success were calculated for N ranging from 10 to 19 subjects. If any of these posterior probabilities of success were greater than or equal to 0.9, then the study was continued with a reduced sample size equal to the lowest N at which that threshold was reached. Otherwise, the conclusion from the simulated interim analysis would be to continue the study with unchanged sample size.

This whole process (simulating data for 10 subjects, modelling the treatment difference, evaluating posterior probabilities of success and simulating the decision-making process) was replicated 10000 times for each scenario, with the decision ("futile", "no change to N" or "reduce N") recorded for each iteration. Each combination of the four treatment difference scenarios and the four correlation scenarios was evaluated for a total of 16 scenarios in all.

The estimated proportions of each decision being reached under each of the 16 scenarios are presented in Table 4.

Table 4 Operating characteristics of interim analysis at N=10 under four scenarios of treatment effect and four scenarios of within-subject correlation

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with 20 subjects	Continue with reduced sample size
Δ=0 mmHg (no treatment difference)				
	0.0	85%	15%	1%
	0.3	85%	15%	1%
	0.6	85%	15%	1%
	0.9	85%	14%	1%
Δ=2.5 mmHg (weak treatment effect, less than clinically meaningful difference)				
	0.0	44%	50%	6%
	0.3	36%	56%	9%
	0.6	21%	63%	16%
	0.9	1%	31%	68%
Δ=5 mmHg (good treatment effect, at threshold of clinical meaningful difference)				
	0.0	9%	60%	31%
	0.3	4%	54%	42%
	0.6	1%	31%	68%
	0.9	0%	0%	100%
Δ=7.5 mmHg (very strong treatment effect)				
	0.0	1%	32%	67%
	0.3	0%	18%	82%
	0.6	0%	3%	96%
	0.9	0%	0%	100%

These results suggest primarily that if there is no true treatment difference between GSK2586881 and Placebo, the study would be stopped for futility 85% of the time (irrespective of within-subject correlation of PASP). A drug with a weak treatment effect would most likely result in the study being continued without change or stopped for futility (except in the case where within-subject correlation is 0.9, which is presented here as an extreme case, unlikely to occur in practice). If the true treatment effect is at the clinically relevant threshold of 5 mmHg, then the study is unlikely to be stopped for futility and may indicate being reduced in sample size.

Secondarily, it should be noted that the higher the within-subject correlation, then the higher the probability of success. This is because the more correlation there is in measurements within the same individual, the less variability there will be in change from baseline. Results for correlations of 0.0 and 0.9 are presented as extreme cases unlikely to occur in practice, though the exact strength of within-subject correlation of PASP (particularly under different stressed conditions) is not known.

Full details of the interim analysis will be supplied in the RAP.

9.3.2.2. Part 2

Part 2 of the study will be based on modifications as detailed in Section 2.1.1. Two interim analysis reviews are planned when approximately 3 and 6 subjects, respectively have completed treatment periods 1 and 2.

The aims of the interim analyses are to assess the effectiveness of the Part 2 modifications on critical assessments (PASP, oxygen saturation and AEs) and the potential benefit/risk impact on subjects.

At each review, the study may be stopped for futility if PASP results are highly variable, or if the PASP profile for the placebo group is not as expected (indicative of the model not working given the alterations to the study) or if review of the safety data suggests a change in the benefit-risk profile. If clear criteria for stopping the study are not met, the study will continue to the next interim analysis review, or on to the planned maximum number of evaluable subjects.

If data suggest a further interim analysis review of data would be beneficial, then this will be documented.

Interim analysis reviews are planned as follows:

1. Review of PASP, oxygen saturation and AE data when approximately 3 subjects have completed Treatment Periods 1 and 2. Critical timepoints for PASP and oxygen saturation are T0 and T3 (a review of additional timepoints will also take place)

2. Review of PASP, oxygen saturation and AE data when approximately 6 subjects have completed Treatment Periods 1 and 2. Critical timepoints for PASP and oxygen saturation are T0 and T3 (a review of additional timepoints will also take place). Note that this second review may not be required and will be dependent on data observed during the first interim review. The timing of this review may be altered as necessary dependent on subject recruitment.

Each interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

9.3.3. Multiple Comparisons and Multiplicity

As this is an early-phase exploratory study, no adjustment for multiple comparisons will be made. Treatment comparisons will be presented as effect sizes with 95% confidence intervals.

9.4. Key Elements of Analysis Plan

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five key sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise (for Part 2, echo data will be measured during exercise)
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

PASP and Oxygen Saturation will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day. Biomarkers will be collected at times T0 thru T4, plus additional timepoints.

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline. Separate statistical analyses will be conducted for subjects in Part 1 and Part 2 of the study. In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). Subject will be fitted as a random effect and non-informative priors will be used for model parameters. An unstructured covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements.

Bayesian mixed effects regression models (as described above) will be fitted (using non-informative priors for the model parameters), and an estimate of the mean change from baseline in PASP for each treatment group and post-baseline timepoint, together with its 95% credible interval, will be obtained. Estimates for treatment differences will also be presented. Data may be log-transformed if necessary.

PASP data will be summarized separately by Part 1 and Part 2 by treatment and time-point in tabular and graphical format.

Details of the analysis of other endpoints will be described in the RAP.

9.4.1. Pharmacokinetic Analyses

Pharmacokinetic analysis will be performed by, or under the auspices, of Clinical Pharmacology Modelling and Simulation Department within GlaxoSmithKline. Plasma GSK2586881 concentration-time data will be analysed by non-compartmental methods with WinNonLin V6.3 or greater. 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 over the study period [nominally AUC(0-2.5h) post-dose], AUC over the hypoxia challenge (nominally AUC(0.5-2.0h post-dose), plasma clearance (CL), volume of distribution (V) and apparent terminal phase half-life (t1/2), if data permit. Other PK parameters may also be determined.

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively by Part 1 and Part 2. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals, R&D.

The relationship between the RAS biomarkers and other pharmacodynamic endpoints (echocardiography and pulse oximetry) and GSK2586881 concentrations and/or PK parameters may also be explored for Part 2, if appropriate.

If appropriate, a population PK analysis may also be conducted, in addition the plasma concentration-time data may be merged with historical data and analysed as part of a population PK meta-analysis.

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.
- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study; also current medical records must be available.

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 randomisation 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

Abraham WT, Raynolds MV, Badesch DB, Wynne KM, Groves BM, Roden RL,et al.. Angiotensin-converting enzyme DD genotype in patients with primary pulmonary hypertension: increased frequency and association with preserved haemodynamics. *J Renin Angiotensin Aldosterone Syst.* 2003 Mar;4(1):27-30.

Argiento P, Chesler N, Mulè M, D'Alto M, Bossone E, Unger P, Naeije R. Exercise stress echocardiography for the study of the pulmonary circulation. *Eur Respir J*. June 2010, 35 (6):1273 – 1278.

Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;49:818-822.

Cargil RI, Lipworth BJ. Lisinopril attenuates acute hypoxic pulmonary vasoconstriction in humans. *Chest*. 1996 Feb;109(2):424-9/

GlaxoSmithKline Document Number 2010N108777_04 Study ID GSK2586881. Investigator's Brochure for GSK2586881 (formerly known as APN01)– A Soluble Recombinant Human Angiotensin Converting Enzyme 2 (rhACE2). Report Date 28-NOV-2017.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(a): 24. Table 3-2.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(b): 28.

ICH Topic M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, 2009

Idell S, Kueppers, F, Lippmann, M, Rosen, H, Niederman, M, Fein, A. ACE elevated in ARDS BAL. Angiotensin converting enzyme in bronchoalveolar lavage in ARDS. *Chest.* 1987;91:52-56.

Kiely DG, Cargill RI, Lipworth BJ. Effects of hypercapnia on hemodynamic, inotropic, lusitropic, and electrophysiologic indices in humans. *Chest.* 1996; 109:1215–1221

Kiely DG1, Cargill RI, Wheeldon NM, Coutie WJ, Lipworth BJ. Haemodynamic and endocrine effects of type 1 angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale. *Cardiovasc Res*. 1997 Jan;33(1):201-8.

Kleinsasser A1, Pircher I, Treml B, Schwienbacher M, Schuster M, Janzek E, Loibner H, Penninger JM, Loeckinger A. Recombinant angiotensin-converting enzyme 2 suppresses pulmonary vasoconstriction in acute hypoxia. *Wilderness Environ Med*. 2012 Mar;23(1):24-30

Maron BA, Leopold JA. The role of the renin-angiotensin-aldosterone system in the pathobiology of pulmonary arterial hypertension. 2013 Grover Conference series. *Pulm Circ*. 2014 Jun;4(2):200-10. doi: 10.1086/675984

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, Chambers RJ, , GJ et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am. J. Resp. Crit. Care Med.* 2002;166:646-650.

Naeije R, Saggar R, Badesch D, Rajagopalan S, Gargani L, Rischard F, et al. Exercise induced pulmonary hypertension: Translating pathophysiological concepts into clinical practice. *Chest*. 2018; 3692(18)30161-2.

Paul M, Bachmann J, Ganten D. Angiotensin II-induced vasoconstriction and sodium resorption. The Tissue Renin-Angiotensin System in Cardiovascular Disease. *Trends Cardiovasc. Med.* 1992;2:94-99.

Ricart A, Maristany J, Fort N, Leal C, Pagés T, Viscor G. Effects of sildenafil on the human response to acute hypoxia and exercise. *High Alt Med Biol*. 2005 Spring;6(1):43-9.

Rubenfeld GD, Caldwell E, Peabody E. Incidence and Outcomes of Acute Lung Injury. *N Engl J Med.* 2005;353:1685-1693.

Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiograpy. *J am Soc Echocardiogr*. 2010; 23: 685 – 713

Santos RA, Ferreira AJ, Pinheiro SVB, Sampaio WO, Touyz R, Campangole-Santos MJ. Counter-regulatory effects of ANG (1-7) in ANG II. Angiotensin(1-7) and its receptor as potential targets for cardiovascular drugs *Expert Op. Investig. Drugs.* 2005;1019:1031.

Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, et al. Angiotensin (1-7) is an endogenous ligand for the G-protein coupled receptor Mas *Proc Natl Acad Sci USA.* 2003;100:8258-8263.

Suzuki Y, Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V., Egido, J. Inflammation and Angiotensin II. *Int J Biochem Cell Biol.* 2003;35:881-900.

The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med.* 2000;342:1301-1308.

The ARDS Network Authors for the ARDS network. Ketoconzaole for Early Treatment of Acute Lung Injury and Acute Respiratory Distress Syndrome. *JAMA.* 2000;283:1995-2002.

Tom B, de Vries R, Saxena PR, Danser AHJ. Bradykinin potentiation by angiotensin (1-7) and ACE inhibitors correlates with C- and N-terminal blockade. *Hypertension.* 2001;38:95-99.

Torre-Bueno JR, Wagner PD, Saltzman HA, Gale GE, Moon RE. Diffusion limitation in normal humans during exercise at sea level and simulated altitude. *J Appl Physiol* (1985). 1985 Mar;58(3):989-95

Wenz M, Hoffmann B, Bohlender J, Kaczmarczyk G. Angiotensin II formation and endothelin clearance in ARDS patients in supine and prone positions. *Intensive Care Med.* 2000;26:292-298.

Wheeler AP, Bernard GR, Thompson BT, Schoenfeld D, Wiedemann HP, deBoisblanc B,et al.. Pulmonary-Artery versus Central Venous Catheter to Guide Treatment of Acute Lung Injury. *N Engl J Med.* 2006;25:354(21):2213-24. Epub 2006 May 21.

Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B,et al. Comparison of Two Fluid-Management Strategies in Acute Lung Injury. *N Engl J Med.* 2006;354(24):2564-75. Epub 2006 May 21.

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

ACE2	Angiotensin converting enzyme type 2
AE	Adverse Event
ALI	Acute Lung Injury
ALT	Alanine aminotransferase (SGPT)
Ang II	Angiotensin II
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
BID	Bi-Daily
BUN	Blood urea nitrogen
CBP	Child Bearing Potential
CL	Clearance
CO2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CV	Cardiovascular
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HPV	Hypoxic pulmonary vasoconstriction
IB	Investigator's Brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identification
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board

ITT	Intent-to-Treat
IV	Intravenous
IVIVT	In Vitro/In Vivo Translations
Kg	Kilogram
LDH	Lactate dehydrogenase
LFTs	Liver function tests
m	Meters
MCH	Mean corpuscular hemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
MSDS	Material Safety Data Sheet
msec	Milliseconds
NOAEL	No observed adverse effect level
O2	Oxygen
PaO2	Partial Pressure of Oxygen in arterial blood
PAH	Pulmonary Arterial Hypertension
PASP	Pulmonary Artery Systolic Pressure
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
PVR	Pulmonary vascular resistance
Q	Perfusion
QC	Quality control
QTcB	QT duration corrected for heart rate by Bazett's formula
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RAS	Renin-Angiotensin System
RBC	Red blood cells
rhACE2	Recombinant human angiotensin converting enzyme type 2
RNA	Ribonucleic acid
RVOT	Right ventricular outflow tract
SAE	Serious adverse event(s)
SD	Standard deviation
SP-D	Surfactant Protein-D
SRM	Study Reference Manual
TPR	Third Party Resourcing
TTS	Technical Terms of Supply
t½	Terminal phase half-life
tmax	Time of occurrence of Cmax
V	Volume of Distribution
V_A	Ventilation
VO2	Oxygen Consumption

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
MedDRA
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 etiology (in alignment with the FDA premarketing clinical liver safety guidance).

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

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="2">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td>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="2">Required Actions and Follow up Assessments following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th>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>• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend.
• Blood sample for PK analysis, obtained 24 hours after last dose [4]
• 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,</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	other over the counter medications. • Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct 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. 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,et al.. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. Drug Metab Dispos 2009; 37:1779-1784.

12.3. Appendix 3: Genetic Research

Genetics – Background

Naturally occurring genetic variation may contribute to inter-individual variability in response to medicines, as well as an individual's risk of developing specific diseases. Genetic factors associated with disease characteristics may also be associated with response to therapy, and could help to explain some clinical study outcomes. For example, genetic variants associated with age-related macular degeneration (AMD) are reported to account for much of the risk for the condition [Gorin, 2012] with certain variants reported to influence treatment response [Chen, 2012]. Thus, knowledge of the genetic etiology of disease may better inform understanding of disease and the development of medicines. Additionally, genetic variability may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), or pharmacodynamics (relationship between concentration and pharmacologic effects or the time course of pharmacologic effects) of a specific medicine and/or clinical outcomes (efficacy and/or safety) observed in a clinical study.

Genetic Research Objectives and Analyses

The objectives of the genetic research are to investigate the relationship between genetic variants and:

- Response to medicine, including [any treatment regimens under investigation in this study] or any concomitant medicines;

Genetic data may be generated while the study is underway or following completion of the study. Genetic evaluations may include focused candidate gene approaches and/or examination of a large number of genetic variants throughout the genome (whole genome analyses). Genetic analyses will utilize data collected in the study and will be limited to understanding the objectives highlighted above. Analyses may be performed using data from multiple clinical studies to investigate these research objectives.

Appropriate descriptive and/or statistical analysis methods will be used. A detailed description of any planned analyses will be documented in a Reporting and Analysis Plan (RAP) prior to initiation of the analysis. Planned analyses and results of genetic investigations will be reported either as part of the clinical RAP and study report, or in a separate genetics RAP and report, as appropriate.

Study Population

Any subject who is enrolled in the study can participate in genetic research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the genetic research.

Study Assessments and Procedures

A key component of successful genetic research is the collection of samples during clinical studies. Collection of samples, even when no *a priori* hypothesis has been

identified, may enable future genetic analyses to be conducted to help understand variability in disease and medicine response.

- A 6 ml blood sample will be taken for Deoxyribonucleic acid (DNA) extraction. A blood sample is collected at the baseline visit, after the subject has been randomised and provided informed consent for genetic research. Instructions for collection and shipping of the genetic sample are described in the laboratory manual. The DNA from the blood sample may undergo quality control analyses to confirm the integrity of the sample. If there are concerns regarding the quality of the sample, then the sample may be destroyed. The blood sample is taken on a single occasion unless a duplicate sample is required due to an inability to utilize the original sample.

The genetic sample is labelled (or "coded") with the same study specific number used to label other samples and data in the study. This number can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number).

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study, or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will only use samples collected from the study for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

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

Informed Consent

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

Subject Withdrawal from Study

If a subject who has consented to participate in genetic research withdraws from the clinical study for any reason other than being lost to follow-up, the subject will be given a choice of one of the following options concerning the genetic sample, if already collected:

- Continue to participate in the genetic research in which case the genetic DNA sample is retained
- Discontinue participation in the genetic research and destroy the genetic DNA sample

If a subject withdraws consent for genetic research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records.

Genotype data may be generated during the study or after completion of the study and may be analyzed during the study or stored for future analysis.

- If a subject withdraws consent for genetic research and genotype data has not been analyzed, it will not be analyzed or used for future research.
- Genetic data that has been analyzed at the time of withdrawn consent will continue to be stored and used, as appropriate.

Screen and Baseline Failures

If a sample for genetic research has been collected and it is determined that the subject does not meet the entry criteria for participation in the study, then the investigator should instruct the subject that their genetic sample will be destroyed. No forms are required to complete this process as it will be completed as part of the consent and sample reconciliation process. In this instance a sample destruction form will not be available to include in the site files.

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

GSK may summarize the genetic research results in the clinical study report, or separately and may publish the results in scientific journals.

GSK may share genetic research data with other scientists to further scientific understanding in alignment with the informed consent. GSK does not inform the subject, family members, insurers, or employers of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from genetic studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined. Further, data generated in a research laboratory may not meet regulatory requirements for inclusion in clinical care.

Chen H, Yu KD, Xu GZ. Association between Variant Y402H in Age-Related Macular Degeneration (AMD) Susceptibility Gene CFH and Treatment Response of AMD: A Meta-Analysis. PloS ONE 2012; 7: e42464

Gorin MB. Genetic insights into age-related macular degeneration: Controversies addressing risk, causality, and therapeutics. Mol. Asp. Med. 2012; 33: 467-486.

12.4. Appendix 4: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.4.1. 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.

12.4.2. 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 and 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 Appendix 2 for the required liver chemistry follow-up instructions

12.4.3. 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

12.4.4. 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.

12.4.5. 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.

12.4.6. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool

- Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool
- If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor Site will enter the serious adverse event data into the electronic system as soon as it becomes available.
- The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.
- After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor telephone.
- Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

SAE reporting to GSK via paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail • 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.

12.5. Appendix 5: Modified List of Highly Effective Methods for Avoiding Pregnancy and Collection of Pregnancy Information

12.5.1. Modified List of Highly Effective Methods for Avoiding Pregnancy

The list does not apply to FRP with same sex partners or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

1. Contraceptive subdermal implant
2. Intrauterine device or intrauterine system
3. Combined estrogen and progestogen oral contraceptive [Hatcher RA, 2011]
4. Injectable progestogen [Hatcher RA, 2011]
5. Contraceptive vaginal ring [Hatcher RA, 2011]
6. Percutaneous contraceptive patches [Hatcher RA, 2011]
7. Male partner sterilization with documentation of azoospermia prior to the female subject's entry into the study, and this male is the sole partner for that subject [Hatcher RA, 2011]. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview provided by her or her partner.

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Contraceptive requirements for male subjects with female partners of reproductive potential (when applicable).

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until [at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives] after the last dose of study medication.

1. Vasectomy with documentation of azoospermia. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview.
2. Male condom plus partner use of one of the contraceptive options below that meets the Standard Operating Procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label:
    - Contraceptive subdermal implant
    - Intrauterine device or intrauterine system

- Combined estrogen and progestogen oral contraceptive [Hatcher RA, 2011]
- Injectable progestogen [Hatcher RA, 2011]
- Contraceptive vaginal ring [Hatcher RA, 2011]
- Percutaneous contraceptive patches [Hatcher RA, 2011]

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

12.5.2. Collection of Pregnancy Information

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study
- Information will be recorded on the appropriate form and submitted to GSK within 2 weeks of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on mother and infant, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Appendix 5. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating

- will discontinue study medication or be withdrawn from the study
- Investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. This applies only to subjects who are randomised to receive study medication.
- After obtaining the necessary signed informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy

- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

12.5.3. References

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, Policar MS, editors. Contraceptive Technology. 20th edition. Atlanta, Georgia: Ardent Media, Inc., 2011: 50. Table 3-2.

12.6. Appendix 6: Protocol Changes

12.6.1. Protocol Amendment 01 (24-Feb-2017) from Original Protocol (23-Sep-2016)

This is a global amendment.

12.6.1.1. Summary of Changes and Rationale for Amendment

Additional Inclusion Criteria (Section 5.2) and Subject Stopping Criteria (Section 5.4) added in response to comments from BfArM.

12.6.1.2. List of Specific Changes

Title Page, Author (s)

Rationale for change: Addition and removal of author(s).

PPD

Sponsor Signatory

Rationale for change: Change in sponsor signatory

~~Dr Richard Marshall~~ **Dr Aili Lazaar**

~~Vice President, Fibrosis & Lung Injury Discovery Performance Unit (DPU) Head, Respiratory Therapy Area Unit~~ **Director, Discovery Medicine**

Project Physician Leader, Respiratory Therapy Area

Section 5.2: Exclusion Criteria

Rationale for change: Addition of exclusion criteria number 4 and 5.

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
1. ALT >1.5x Upper limit of normal (ULN).
2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
4. Prior history of altitude sickness.
5. Recently been scuba diving (within 1 week before screening).

6. 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).

Section 5.4: Withdrawal/Stopping Criteria

Rationale for change: Addition of withdrawal criteria.

Subjects may be withdrawn from the study for any of the following reasons:

- 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.
- If a subjects oxygen saturation falls below 65% at any point during the hypoxia challenge.
- **If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.**
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

12.6.2. Protocol Amendment 02 (07-Mar-2017) from Protocol Amendment 01 (24-Feb-2017)

This is a global amendment.

12.6.2.1. Summary of Changes and Rationale for Amendment

This is a substantial protocol amendment to:

- Reduce the number of participants in the study from approximately 35 healthy volunteers to approximately 25 healthy volunteers.

This is a non-substantial protocol amendment to:

- Remove some of the exploratory biomarkers.
- Add a spirometry assessment at screening.
- Add a pre-dose pulse oximetry assessment.
- Add continuous ECG telemetry.
- Move the immunogenicity screening sample to Pre-dose in Treatment Period 1.
- Remove height and weight assessments from the follow-up visit.
- Clarify the sequence and priority of assessments.
- Correct typographical errors.

See Section 12.6.2.2 below for a rationale for each change.

12.6.2.2. List of Specific Changes

Protocol synopsis for study 204987

Protocol synopsis updated to reflect changes in the main body of the protocol.

Section 3: Objectives/Endpoints

Rationale for change: Removal of some of the exploratory biomarkers. SP-D will be analysed and reported, and additional biomarkers may be analysed, as data permit.

Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
~~• To evaluate the effect of a single IV dose of GSK2586881 on peptide hormone responses to hypoxia and exercise.~~	~~• Baseline levels and changes in response to hypoxia and exercise in kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin-13, pyr1-Apelin-13) and other systems (e.g. dynorphin A) as data permit~~.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in ~~other biomarkers which may include Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I,~~ Surfactant Protein D **(SP-D)** ~~, IL-6~~ and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

Section 4.3: Type and Number of Subjects

Rationale for change: The removal of some exploratory biomarkers, led to a re-assessment of the sample size; given that the larger prior sample size was to optimise data collection to include exploratory biomarkers. The sample size has been reduced from 35 to 25 enrolled subjects (30 to 20 completed subjects), because precision estimates show that a difference of between 3 and 5 mmHg in PASP (the primary endpoint) could be detected with an acceptable level of precision based on 20 completed subjects (see Section 9.2).

Approximately ~~35~~ **25** subjects healthy volunteers will be randomised such that approximately ~~30~~ **20** evaluable subjects complete the study (the target of ~~30~~ **20** may be revised following the sample size re-estimation, see Section 9.2.2).

Section 4.5: Dose Justification

Rationale for change: Additional subjects have been dosed with GSK2586881 since the protocol was finalised in September 2016.

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). **In a concluded (but not yet reported) investigator-sponsored study in Pulmonary Arterial Hypertension (PAH) (Study 204696), 5 subjects have received a single dose of either 0.2 mg/kg or 0.4 mg/kg**~~One subject with Pulmonary Arterial Hypertension (PAH) has received a single dose of 0.2 mg/kg~~.

Section 5.1: Inclusion Criteria

Rationale for change: Addition of a spirometry test at screening to ensure normal lung function before hypoxic challenge and exercise test.

TYPE OF SUBJECT
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, **spirometry**, laboratory tests and cardiac monitoring.

Section 5.1: Inclusion Criteria

Rationale for change: Correction of typographical errors.

SEX
8. Male or female (non child bearing potential) Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication. h. Vasectomy with documentation of azoospermia. i. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant • Intrauterine device or intrauterine system • Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a]

- Contraceptive vaginal ring [Hatcher, 2007a]
- Percutaneous contraceptive patches [Hatcher, 2007a]

This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH, M3 (R2) 2009].

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

~~This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH, M3 (R2) 2009].~~

~~The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.~~

Females:

A female subject is eligible to participate if she is not pregnant (as confirmed by a negative urine human chorionic gonadotrophin (hCG) test), not lactating, and the following condition applies:

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
- Postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels)]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

~~The investigator is responsible for ensuring that subjects understand how to properly use~~

~~these methods of contraception.~~

Section 5.4.1: Study Stopping Criteria

Rationale for change: The overall sample size has been reduced from 35 to 25 enrolled subjects (30 to 20 completed subjects), so the interim analysis will now be completed after 10 subjects have completed the study, rather than 15.

An interim analysis is planned after approximately ~~15~~ **10** subjects have completed treatment periods 1 and 2 and the study may be stopped if a reasonable change in PASP is not observed after dosing with GSK2586881 or if review of the safety data suggests a change in the benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.

Section 5.4.2: QTc Stopping Criteria

Rationale for change: QTc Stopping Criteria removed from Section 7.2.5, and inserted into Section 5.4 with the other subject stopping criteria.

The *same* QT correction formula *must* be used for *each individual subject* to determine discontinuation from the study. For example, if a subject is eligible for the protocol based on QTcB, then QTcB must be used for discontinuation of this individual subject as well.

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

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

The QTc should be based on single or averaged QTc values of triplicate ECGs obtained over a brief (e.g. 5-10 minute) recording period.

Section 6.1: Investigational Product and Other Study Treatment

Rationale for change: Order of treatment sequences altered to reflect GSK's standard practice to list placebo first.

Table 1 Description of sequences

Sequence	Period 1	Period 2
AB	~~GSK2586881~~**Saline Placebo**	~~Saline Placebo~~**GSK2586881**
BA	~~Saline Placebo~~**GSK2586881**	~~GSK2586881~~**Saline Placebo**

Section 7: Study Assessments and Procedures

Rationale for change: Addition of ventilatory parameters to the list of assessments, and clarification that echocardiograms should be taken as close as possible to the nominal time-point (rather than blood draws), because they are the primary endpoint.

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:

  1. **Ventilatory parameters**
  2. Echocardiograms
  3. 12-lead ECG
  4. vital signs
  5. blood draws

  Note: The timing of the assessments should allow the ~~blood draw to occur at the exact~~ **echocardiogram to be performed as close as possible to the** nominal time.

Section 7.1: Time and Events Table

Rationale for change:

- *Height and weight removed at follow-up (not required).*
- *Spirometry added at screening (see justification in Section 5.1).*
- *Immunogenicity moved from screening to Day 1, Treatment Period 1.*
- *Removal of exploratory ventilatory parameters after exercise, as this assessment would delay the echocardiogram, which is the primary endpoint.*
- *Addition of telemetry throughout the treatment periods.*
- *Correction of typographical errors.*

Section 7.1.1: Screening and Follow up

Procedure	**Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)**	**Follow up (7-10 days post last dose)**	**Notes**
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam, including height and weight	X	X	**Height and weight to be measured at screening only.** Weight at screening will be used for dosing calculation.
Alcohol, Drugs of Abuse, Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: Drugs, Alcohol, tobacco
Past and current medical conditions [including cardiovascular medical history]	X		
Serum OR urine pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries)	X	X	Non Fasting
Immunogenicity	~~X~~	X	
12-lead ECG	X	X	Triplicate ECG required at screening.
Vital signs	X	X	Triplicate vital signs required at screening.
Spirometry	**X**		
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		Tolerance to 4000m for 10 mins followed by ~~followed by~~ incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max ~~for the~~ **(to be used for the** exercise challenge **during the Treatment Periods).**
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

1. Screening assessments are allowed to be conducted on more than one day

Section 7.1.2: Treatment Period 1 and 2

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	~~30m rest~~ On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Randomisation	X											Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X[5]			X	
Immunogenicity	X											~~Treatment period 2 only~~
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X		X		Echocardiogram duration approx 5 min
Subject enters chamber					X							Subject enters chamber approximately 30 min after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram**, blood samples** and vital signs have been taken.
Exercise challenge							X					For approx 5-10 minutes
Ventilatory parameters	X		X			X	~~X[1]~~			X		Measurements to be taken ~~at the same time as the~~**2 min before** echocardiograms. ~~During the exercise challenge, an ECG integrated with the ventilatory assessment will be carried out.~~
Pulse Oximetry (O2 saturation)	←----------										→	Will be continuously monitored for safety. **A** measurement should be recorded at time of each echocardiogram ~~post-treatment~~ and databased.
Telemetry	←----------										→	**Will be continuously monitored for safety.**

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to <u>start of</u> dosing				**Hypoxic <u>Challenge ~80 min</u> (Times relative to entry to Chamber)**							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	~~30m rest~~ On exit from chamber	After 30 min rest	60 min after exit from Chamber	
RAS Biomarkers	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
~~Other~~ **~~Biomarkers~~SP-D**	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
PK sampling	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
AE/SAE review		←==========→										
Concomitant medication review	X											

1. ~~To be taken at the end of exercise~~ Take at the end of the infusion
2. Taken immediately after echocardiogram.
3. Immediately before entering the chamber
4. To be taken as soon as possible after leaving chamber
5. **<u>On this occasion ONLY, vital signs to be taken after the blood draw.</u>**

Section 7.2.1.3: Follow-up of AEs and SAEs

Rationale for change: Deletion of reference to AEs of special interest, as no AEs of special interest have been defined in the protocol.

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 Appendix 4.

Section 7.2.2: Physical Exams

Rationale for change: Height and Weight removed from follow-up visit, as not required.

- 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**, at screening only.** ~~This will be carried out at screening and follow up.~~

Section 7.2.3: Vital Signs

Rationale for change: Correction of typographical errors.

- **Triplicate measurements will be taken at screening, and single measurements at other time-points.** ~~Three readings of blood pressure and pulse rate will be taken.~~ The first reading should be rejected. Second and third readings should be averaged to give the measurement to be recorded in the CRF. ~~(Triplicate measurements will only be taken at screening and single measurements to be taken after that).~~

Section 7.2.4: Electrocardiogram (ECG)

Rationale for change: QTc Stopping Criteria removed from Section 7.2.5, and inserted into Section 5.4 with the other subject stopping criteria.

- Triplicate 12-lead ECGs will be obtained at screening. At all other time-points, a single 12-lead ECGs will be obtained ~~at each time point during the study~~ using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes rest.
- The same QT correction formula *must* be used for each individual subject **for all QTc data being collected for data analysis. Safety ECGs and other non-protocol specified ECGs are an exception.**

~~7.2.5 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 QTcB, then QTcB 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 single or 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.~~

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

Section 7.2.5: Clinical Safety Laboratory Assessments

Rationale for change: Correction of typographical errors. Addition of urea to clinical chemistry parameters, to allow the calculation of BUN.

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the 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 date.~~ Details for the preparation and shipment of samples will be provided by the laboratory ~~and are detailed in the laboratory manual.~~ 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.~~

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**			
Haematology	Platelet Count	*RBC Indices*:	*WBC count with Differential:*	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit		Monocytes	
			Eosinophils	
			Basophils	
Clinical Chemistry [1]	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Urea	Sodium	ALT (SGPT)	Total Protein
	Creatinine	Calcium	Alkaline phosphatise	Albumin
	Glucose			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol, smoking and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, benzodiazepines and cotinine) performed at site • Urine hCG Pregnancy test (as needed) [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 5.4.2 and Appendix 2.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

Section 7.3.1: Exercise Challenge

Rationale for change: Clarification of exercise test.

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on a cycle ergometer within the hypoxia chamber for 10 minutes ~~as detailed in the SRM.~~

Subjects must complete a minimum of 5 minutes exercise at 70% of maximumVO2 uptake **(calculated from the incremental exhaustive exercise test at the screening visit).**

Section 7.5: Ventilatory parameters

Rationale for change: Clarification of ventilatory parameter assessment.

Ventilatory parameters will be measured as detailed in the Time and Events table (Section 7.1).

Ventilatory parameters will be recorded for 2 minutes before echocardiograms. The second minute of the recording will be averaged and used for data analysis.

Measurements may include change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2**), total tidal volume, inspiratory tidal volume, expiratory tidal volume, total respiratory time, inspiratory time, expiratory time, duty cycle, mean respiratory flow and respiratory rate** ~~and other parameters~~ as data permit.

~~Further details can be found in the SRM~~

Section 7.7: Telemetry

Rationale for change: Addition of telemetry.

Continuous ECG monitoring will occur via telemetry during both treatment periods.

Section 7.9.1: Renin-angiotensin system biomarkers

Rationale for change: Clarification of archiving procedure.

RAS peptides including, but not limited to, AngII, Ang(1-7) and Ang(1-5) may be analysed as data permit**. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site detailed in the SRM).**

Section 7.9.2: **Surfactant Protein-D and** other biomarkers

Rationale for change: Removal of some exploratory biomarkers.

SP-D will be analysed. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site to be detailed in the SRM). Additional analytes may be analysed, as data permit. ~~Peptide hormones such as Kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) may be analysed as data permit.~~

~~Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined may also be analysed as data permit.~~

Section 9.2: Sample Size Considerations

Rationale for change: Refer to rationale stated for Section 4.3 and Section 5.4.1.

The sample size of ~~30~~ **20** patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context (Note: precision in this context is defined as one half of the width of the 95% confidence interval). A sample size re-estimation is planned after ~~15~~ **10** subjects.

Section 9.2.2: Sample Size Re-estimation

Rationale for change: Refer to rationale stated for Section 4.3 and Section 5.4.1.

A sample size re-estimation will be conducted at the interim analysis (see Section 9.3.2). The purpose of the sample size re-estimation is to determine whether the total number of subjects can be reduced below the anticipated ~~30~~**20**, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP (i.e. T3-T0). The sample size re-estimation is to be considered advisory, and taken into account together with other considerations.

Section 9.3.1: Analysis Populations

Rationale for change: Correction of typographical errors.

Intent-to-Treat (ITT) Population: This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, run-in failure, or stabilisation failure, but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all ~~efficacy and~~ safety **and pharmacodynamic** analyses.

Section 9.3.2: Interim Analysis

Rationale for change: Refer to rationale stated for Section 4.3 and Section 5.4.1.

An interim analysis is planned after ~~15~~ **10** subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods
- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

The interim analysis will use the following study data:

- PASP at time T0 and T3 for both periods, for the first ~~15~~ **10** subjects

- Oxygen saturation at T0 and T3 for both periods, for the first ~~15~~ **10** subjects
- Adverse events for the first ~~15~~ **10** subjects
- ~~Angiotensin biomarker~~**RAS peptide** concentrations for the first 5 subjects (to allow time for the samples to be analysed by the external lab).

The interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

The aims of the interim analysis, and the endpoints to be used to address them, are as follows:

- Using the primary endpoint (treatment differences between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP [T3-T0]):
    - To decide whether to continue the study or stop based on futility, and
    - to conduct an advisory sample-size re-estimation with the view to potentially reduce the number of subjects participating in the study below ~~30~~**20**.
- Using secondary endpoints:
    - to estimate treatment differences between GSK2586881 and Placebo in change from pre-dose oxygen saturation to hypoxic/exercised oxygen saturation [T3-T0], with a view to stopping the study if there is evidence that GSK2586881 causes a reduction in oxygen saturation in healthy volunteers. As a non-binding guide high posterior probability of observing ≥5% absolute differences in **mean** oxygenation saturation values between placebo and active arms would be of concern; but clinical judgement would override any statistical methods if, for example, the majority of subjects displayed consistent patterns of reductions e.g. between 2-3%).
    - to compare adverse events within the two treatment groups and explore any potential safety signals that may emerge

To assess the operating characteristics of the interim analysis decisions to be made based on the primary endpoint, a simulation was carried out to estimate the relative proportion of studies reaching conclusions to stop for futility, continue with no change to the sample size, and to continue with a reduced sample size, under various different scenarios of the true treatment effect. Broadly speaking, the aim of the simulation was to confirm that (in an overwhelming majority of cases) a decision to stop for futility would be reached in the case of no true treatment effect and a decision to continue (perhaps with reduced sample size) would be reached in the case of a true treatment effect.

Simulated datasets of PASP at baseline (i.e. normoxic and at rest) and under hypoxia and exercise for ~~15~~ **10** subjects, each receiving both treatments in sequence, were generated using the following assumptions:

- ~~15~~ **10** simulated subjects were randomised to one of the two available treatment sequences (see Section 6.2) using block randomisation with block size of 4 records

- PASP at baseline and under hypoxia and exercise for both treatment groups (a total of four PASP measurements per subject) were sampled from a multivariate normal distribution, using values for mean PASP, standard deviation of PASP and within-subject correlation of PASP as described below.

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) for both treatments, and 45 mmHg under hypoxia and exercise for Placebo (as in Section 9.2.1). For GSK2586881, mean PASP under hypoxia and exercise was (45 - Δ) mmHg, where Δ (the mean treatment difference) was varied according to four different scenarios:

    - no treatment difference (Δ = 0 mmHg)

    - weak treatment difference of a magnitude of half the clinically meaningful difference (Δ = 2.5 mmHg)

    - minimum clinically meaningful difference (Δ = 5 mmHg)

    - overwhelming treatment difference one-and-a-half times the clinically meaningful difference (Δ = 7.5 mmHg)

- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise, for both treatment groups (as in Section 9.2.1)

- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9) (as in Section 9.2.1). Within-subject correlation of PASP was assumed to be compound symmetric (i.e. the same correlation was used for each of the six possible pairs of the four PASP measurements within each subject).

- It was assumed that there would be no effect of period in the simulation, i.e. it was assumed that it would make no difference if the measurement was taken from the first or second chronological period in the treatment sequence.

Modelling took on a three-step process, with decisions after each. Firstly, the treatment difference and its associated standard error was then estimated for the dataset of ~~15~~ **10** simulated subjects using a mixed effects linear regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. If the sign of treatment difference arising from the simulated data was in the wrong direction (i.e. the simulated outcome for GSK2586881 was worse than for Placebo), then the simulated study was stopped for futility.

Secondly, in the alternative case that the simulated treatment difference was in favour of GSK2586881 over Placebo, a posterior probability of success (where success was defined as a 97.5% posterior probability of the treatment difference being in favour of GSK2586881 over Placebo) was evaluated for ~~30~~ **20** subjects. This was evaluated using a t-distribution, with the mean and standard deviation taken as the treatment difference and standard error (multiplied by a factor of the square root of ~~15~~ **10** to estimate the standard deviation) from step one, respectively. If this posterior probability was less than 0.2, then the study was stopped for futility as being unlikely to show any benefit of GSK2586881 over Placebo.

Thirdly, provided that the simulated study hadn't been stopped for futility in either of the first two steps, posterior probabilities of success were calculated for N ranging from ~~15~~ **10** to ~~29~~ **19** subjects. If any of these posterior probabilities of success were greater than or equal to 0.9, then the study was continued with a reduced sample size equal to the lowest N at which that threshold was reached. Otherwise, the conclusion from the simulated interim analysis would be to continue the study with unchanged sample size.

This whole process (simulating data for ~~15~~ **10** subjects, modelling the treatment difference, evaluating posterior probabilities of success and simulating the decision-making process) was replicated 1**0**000 times for each scenario, with the decision ("futile", "no change to N" or "reduce N") recorded for each iteration. Each combination of the four treatment difference scenarios and the four correlation scenarios was evaluated for a total of 16 scenarios in all.

The estimated proportions of each decision being reached under each of the 16 scenarios are presented in Table 4.

Table 4 Operating characteristics of interim analysis at N=10 under four scenarios of treatment effect and four scenarios of within-subject correlation

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with **20** ~~30~~ subjects	Continue with reduced sample size
Δ=0 mmHg (no treatment difference)				
	0.0	~~87~~ **85**%	~~13~~ **15**%	~~0~~ **1**%
	0.3	~~87~~ **85**%	~~13~~ **15**%	1%
	0.6	85%	15%	~~0~~ **1**%
	0.9	~~86~~ **85**%	14%	~~0~~ **1**%
Δ=2.5 mmHg (weak treatment effect, less than clinically meaningful difference)				
	0.0	**44**~~31~~%	**50** ~~60~~%	~~9~~ **6**%
	0.3	**36**~~24~~%	**56** ~~65~~%	~~11~~ **9**%
	0.6	**21**~~10~~%	**63** ~~66~~%	~~25~~ **16**%
	0.9	**1** ~~0~~%	**31**~~9~~%	~~91~~ **68**%
Δ=5 mmHg (good treatment effect, at threshold of clinical meaningful difference)				
	0.0	**9** ~~2~~%	**60** ~~46~~%	**31** ~~52~~%
	0.3	**4** ~~1~~%	**54** ~~35~~%	**42** ~~64~~%
	0.6	**1** ~~0~~%	**31** ~~11~~%	**68** ~~89~~%

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with **20** ~~30~~ subjects	Continue with reduced sample size
	0.9	0%	0%	100%
Δ=7.5 mmHg (very strong treatment effect)				
	0.0	**1** ~~0~~%	**32** ~~10~~%	**67** ~~90~~%
	0.3	0%	**18** ~~2~~%	**82** ~~98~~%
	0.6	0%	**3** ~~0~~%	**96** ~~100~~%
	0.9	0%	0%	100%

These results suggest primarily that if there is no true treatment difference between GSK2586881 and Placebo, the study would be stopped for futility **85**~~87~~% of the time (irrespective of within-subject correlation of PASP). A drug with a weak treatment effect would most likely result in the study being continued without change or stopped for futility (except in the case where within-subject correlation is 0.9, which is presented here as an extreme case, unlikely to occur in practice). If the true treatment effect is at the clinically relevant threshold of 5 mmHg, then the study is ~~highly~~ unlikely to be stopped for futility and may indicate being reduced in sample size.

Section 9.4: Key Elements of Analysis Plan

Rationale for change: Correction of typographical errors.

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five **key** sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

~~Pharmacodynamic endpoints~~ **PASP and Oxygen Saturation** will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day. **Biomarkers will be collected at times T0 thru T4, plus additional time-points.**

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline. In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). ~~as fixed effects and s~~Subject **will be fitted** as a random effect **and** non-informative priors **will be used** for model parameters. A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period.

Section 12.2: Appendix 2: Liver Safety Required Actions and Follow up Assessments

Rationale for change: Correction of typographical error.

Footnote 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.

Section 12.4.1: Definition of Adverse Events

Rationale for change: Removal of AE definitions related to efficacy, because this is not a patient study.

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

~~of an AE or SAE.~~

- ~~The signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.~~

12.6.3. Protocol Amendment 03 (06-Mar-2018) from Protocol Amendment 02 (07-Mar-2017)

This is a global amendment.

12.6.3.1. Summary of Changes and Rationale for Amendment

This is a substantial protocol amendment to restart the trial following a temporary halt. The trial was halted following the planned interim analysis (when 10 subjects had completed both Treatment Periods) to allow the study team time to consider the data from the first part of the study (Part 1), and decide what changes would be made to the second part of the study (Part 2). The study team made the following changes to the study in Part 2:

- Increasing the altitude of the hypoxia chamber (Part 1: 4000 m, and Part 2: 5000 m).
- Changing the individual subject withdrawal criteria for oxygen saturation threshold during the hypoxia challenge (Part 1: below 65%; and Part 2: below 60% persistently for >15 seconds).
- Modification of the exercise challenge:
  - Changing from an upright ergometer (Part 1) to a semi-recumbent ergometer (Part 2).
  - Echo recordings taken *during* exercise (Part 2), rather than *after* exercise (Part 1).
  - Changing the workload of the exercise challenge, due to the change in chamber altitude (Part 1: 70% VO_2 max to Part 2: 50% VO_2 max).
- Modification of the echo recording procedure: calculation of pulmonary artery systolic pressure (PASP) conducted *after* image acquisition in Part 2 (rather than *during* the recording, Part 1); and the addition of a physician 'over-reader' in Part 2 to review all echo images/calculations and provide a quality statement (not used in Part 1).
- Addition of an exploratory echo endpoint in Part 2 allowing estimation of pulmonary vascular resistance.

See Section 12.6.3.2 below for a rationale for each change.

12.6.3.2. List of Specific Changes

Title Page, Author (s)

Rationale for change: Addition and removal of author(s).

Sponsor Signatory

Rationale for change: Change in sponsor signatory.

~~Dr Aili Lazaar~~ **Dr Tony Cahn**

~~Director, Discovery Medicine~~, Project Physician Leader, Respiratory Therapy Area

Medical Monitor/SAE Contact Information:

Rationale for change: Switch in primary and secondary medical monitor roles.

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Fax Number	Site Address
Primary Medical Monitor	PPD				~~709 Swedeland Road UW2531 King of Prussia, PA 19406 USA~~ **Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK**
Secondary Medical Monitor					~~Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK~~ **709 Swedeland Road UW2531 King of Prussia, PA 19406 USA**
SAE contact information	[Medical monitor as above]				

Protocol synopsis for study 204987:

Protocol synopsis updated to reflect changes in the main body of the protocol.

Section 2.1.1: Rationale for changes to the study following interim analysis:

Rationale for change: Clarification of the rationale behind protocol changes following interim analysis.

As planned in the original protocol, an interim analysis was conducted when 10 subjects had completed both Treatment Periods. Data from the first 11 subjects were reviewed, because Subject PP was withdrawn during Treatment Period 1 (withdrawn due to dizziness – investigator considered that this was unrelated to study treatment, and was most likely a vasovagal response to cannulation).

As planned, a core sub-set of the study team (as defined in Section 6.3) reviewed unblinded PASP, oxygen saturation, AE and RAS peptide data (RAS peptide data from only 5 subjects, as planned; see Section 9.3.2). The AE data indicated no safety concerns: in total, 5 AEs were reported, 1 was considered severe (as described above for Subject PPD and all were considered by the investigator to be unrelated to study treatment. The RAS peptide data indicated target engagement.

Review of PASP data indicated that an HPV response could not consistently be detected in the first 10 subjects who completed the study. An increase in PASP at T2 and T3 compared with baseline was not consistently observed. A decrease in oxygen saturation was observed at T2 (mean ~85%) and a further decrease at T3 (mean ~82%) compared with ~96% at baseline (similar in both Treatment Periods).

The study was placed on hold to allow the team to consider the data from the first part of the study (Part 1), and decide what changes would be made to the second part of the study (Part 2). The team concluded the following:

- **In previous studies assessing HPV in healthy volunteers, increases in PASP were associated with more significant oxygen desaturation (Ricart, 2005; mean oxygen saturation in hypoxia was 79.4%, and simultaneous hypoxia and exercise was 63.5%). In Part 1 of the study, the altitude of the hypoxia chamber was 4000 m. In Part 2 of the study, the altitude will be increased to 5000 m in order to induce greater oxygen desaturation and associated increases in pulmonary pressures.**
- **It is anticipated that increasing the altitude of the chamber from 4000 to 5000 m will reduce oxygen saturation to as low as 65% in some subjects (when concurrently exercising). Therefore, the current individual subject withdrawal criteria of oxygen saturation falling below 65% during the hypoxia challenge (see Section 5.4) will be reduced to below 60% and persistently for >15 seconds added as a timeframe. This is justified, because subjects will be continually monitored during the challenge, and immediately removed from the chamber should oxygen saturation decline to the withdrawal limit. Oxygen saturation recovery on returning to normoxia is very rapid.**
- **The exercise challenge in Part 2 will be modified. In Part 1 of the study, PASP data at the T3 time-point were extremely variable. Review of the literature shows that any exercise-induced increase in PASP will decline to baseline extremely rapidly following cessation of exercise (Argiento, 2010). This likely**

explains the unreliability of data at T3. In Part 2, the exercise challenge will be conducted on a semi-recumbent ergometer, allowing acquisition of echo recordings during (rather than after) exercise. The workload of the exercise challenge will be reduced (from 70% VO_2max to 50% VO_2max), because of the increase in chamber altitude. Additionally, echo recordings will be taken within 2 minutes of starting exercise, rather than after exercise, based on reports that prolonged steady state exercise can conversely decrease pulmonary vascular pressures, due to a reduction in pulmonary blood volume as the systemic circulation adapts (Naeije, 2018).

- **In Part 2 of the study, at all time-points, the protocol for echo recordings will be modified with the aim of improving data quality and reliability. The following changes will be made: calculation of PASP (outlined in Section 7.4) will be conducted *after* image acquisition (rather than *during* the recording); the sonographer will aim to capture at least 5 good quality images to estimate PASP; and a physician 'over-reader' process will be introduced. PASP will be calculated by a primary reader (the sonographer) and all echo images/calculations will be reviewed by a suitably qualified physician, who will provide a quality statement.**
- **In Part 2 of the study, an exploratory echo endpoint has been added, which will allow the estimation of pulmonary vascular resistance (PVR).**

Section 3: Objectives and Endpoints:

Rationale for change: Addition of PVR as an exploratory endpoint.

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.

Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in Surfactant Protein (SP-D) and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.
• **To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers under hypoxic conditions (and during exercise under hypoxic conditions).**	• **Estimation of pulmonary vascular resistance (PVR) measured via echocardiography.**

Section 4.1: Overall Design

Rationale for change: Clarification of the change in study design following interim analysis

This is a single-centre, randomised, placebo-controlled and double blind (sponsor open), two-period crossover study in healthy subjects.

An interim analysis was conducted when 10 subjects had completed both Treatment Periods (Part 1). The study was placed on hold to allow the study team to consider the data from the first part of the study (Part 1). The study will now restart, with the modifications described in Section 2.1.1, and the second part of the study is designated Part 2.

The subjects will be required to attend the unit for a screening visit, Treatment Period 1, Treatment Period 2 and a follow up visit.

Section 4.2: Treatment arms and duration:

Rationale for change: Clarification to distinguish between Part 1 and Part 2 of the study.

Figure 3 **Part 1**: Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2

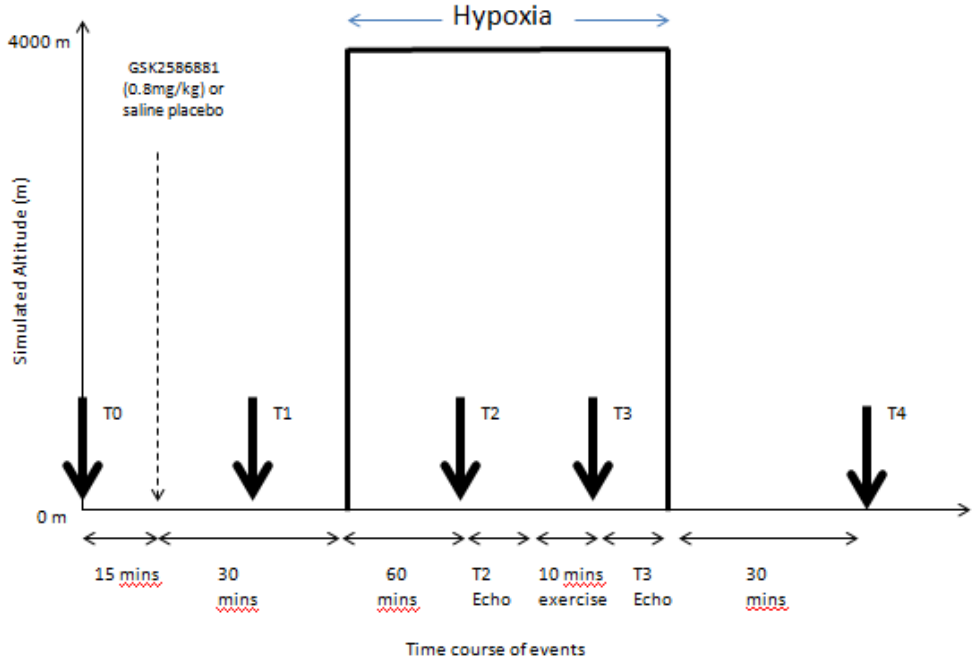


Figure 3 is applicable also to Part 2 of the study, with the following modifications: simulated altitude will be 5000 m, and the T3 echo will be taken 2 minutes into the exercise challenge (rather than at the end of a 10-minute exercise challenge).

There will be a washout period of 3–14 days between treatments to ensure biomarkers return to pre-challenge baseline. Subjects then return to the site and repeat the same procedures as above, except that they will receive the treatment (GSK2586881 or Saline) that they did not receive in the first period.

Section 4.3: Type and Number of Subjects:

Rationale for change: Clarification to distinguish between Part 1 and Part 2 of the study.

Approximately 25 subjects ~~healthy volunteers~~ will be randomised such that approximately 20 evaluable subjects complete the study. **Eleven subjects were enrolled into Part 1 of the study (before the first interim analysis), and up to 14 subjects will be enrolled into Part 2 of the study. The target of 20 evaluable subjects** ~~(the target of 20~~ may be revised following the sample size re-estimation, see Section 9.2.2.

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

Section 4.5: Dose Justification:

Rationale for change: Study 204696 has been completed and reported since the protocol was amended in March 2017.

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). In a concluded ~~(but not yet reported)~~ investigator-sponsored study in Pulmonary Arterial Hypertension (PAH) (Study 204696), 5 subjects have received a single dose of either 0.2 mg/kg or 0.4 mg/kg. **There were no SAEs associated with the infusion of GSK2586881 or reported within the 2-week period of study observation. There were no clinically significant changes in laboratory or vital signs measurements for any subject.**

Section 4.6.1: Risk Assessment:

Rationale for change: Inclusion of hypoxia risk and mitigation to clinical unit staff.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2586881]		
Cardiovascular risk	During preclinical testing a brief period of non-sustained ventricular tachycardia was observed in one monkey receiving a dose of 20.8 mg/kg/day.	The dose used in this study is much lower and well below the No observed adverse effect level (NOAEL) for the 14 day repeat dose cynomolgus monkeys of 8 mg/kg/day.
Potential Reproductive/embryofetal risks	Preclinical studies have not been performed.	Women of childbearing potential will be excluded from the study.
Potential for Immunogenicity	There has been no induction of an immune response to rhACE2 in either of the clinical studies to date in healthy subjects or participants with ARDS.	Patients will have routine monitoring of any immunological response that may occur. If an immunological response is seen the patient will be asked to return for further monitoring and assessment(s).
Potential for rash	In study ACE114622, rash was reported more frequently in subjects receiving GSK2586881, although only one event was considered drug-related.	Patients will be monitored for rash in the clinical trials.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Effects of hypoxia **to study subjects** (light headiness, headaches, nausea)	The subjects will be exposed to hypoxic conditions for approximately 80 minutes with a simulated altitude of ~~4000 m~~5000 m.	The subjects will be continuously observed and monitored with telemetry and pulse oximetry. Stopping criteria are included in the protocol.
Effects of hypoxia to staff at the clinical unit (light headiness, headaches, nausea)	**Clinical site staff performing the assessments within the hypoxia chamber will potentially be exposed to hypoxic conditions with a simulated altitude of 5000 m.**	**All clinical staff entering the hypoxia chamber will be provided with an ambulatory oxygen supply.**

Section 5.4: Withdrawal/Stopping Criteria

Rationale for change: Change in oxygen saturation withdrawal criteria.

Subjects may be withdrawn from the study for any of the following reasons:

- 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.
- If a subject's oxygen saturation falls below ~~65%~~**60% (persistently for >15 seconds)** at any point during the hypoxia challenge.
- If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

Section 5.4.1 Study Stopping Criteria

Rationale for change: Clarification to distinguish between Part 1 and Part 2 of the study.

~~An interim analysis is planned after approximately 10 subjects have completed treatment periods 1 and 2 and the study may be stopped if a reasonable change in PASP is not observed after dosing with GSK 2586881 or if review of the safety data suggests a change in the benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.~~**An interim analysis was conducted when 10 subjects had completed both Treatment Periods (Part 1). The study team decided to continue the study (Part 2), with modifications to the protocol (see Section 2.1.1). In Part 2, an interim analysis will be conducted when approximately 3 subjects complete both Treatment Periods, and further interim analyses may be conducted (see Section 9.3.2). The study may be stopped if PASP results are highly variable, or if the PASP profile for the placebo group is not as expected (indicative of the model not working given the alterations to the study) or if review of the safety data suggest a change in benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.**

Safety data that is collected on a continuous basis (oxygen saturation and telemetry) will be monitored live on site to determine whether a subject tolerates the hypoxia/exercise challenge; study procedures may be aborted on safety grounds if, in the Investigator's opinion, any of these measurements reach unsafe levels. The simulated altitude within the chamber may be reduced if too many participants fail to tolerate the challenge, and the study may be stopped if participants fail to tolerate the challenge at the minimum altitude. The full criteria for this procedure (which may lead to the study being stopped) are described in Section 7.3.

~~In addition, the study may be stopped at the interim analysis for a safety signal in oxygen saturation under hypoxia and exercise, combined with a lack of evidence of mechanistic effect on Angiotensin biomarkers (Ang II, Ang 1-7 and Ang 1-5). Full details on stopping criteria are described together with the full procedure for the interim analysis in Section 9.3.2.~~

Section 7.1: Time and Events Table:

Rationale for change:

- *Clarification to distinguish between Part 1 and Part 2 of the study.*
- *Change of echo and pulse oximetry recording from timepoint "immediately after exercise" to "2 minutes into exercise"*

Section 7.1.1: Screening and Follow up:

Procedure	Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)	Follow up (7-10 days post last dose)	Notes
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam, including height and weight	X	X	Height and weight to be measured at screening only. Weight at screening will be used for dosing calculation.
Alcohol, Drugs of Abuse, Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: Drugs, Alcohol, tobacco
Past and current medical conditions [including cardiovascular medical history]	X		
Serum OR urine pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries)	X	X	Non-Fasting
Immunogenicity		X	
12-lead ECG	X	X	Triplicate ECG required at screening.
Vital signs	X	X	Triplicate vital signs required at screening.
Spirometry	X		
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		**Part 1:** Tolerance to 4000m for 10 mins followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max (to be used for the exercise challenge during the Treatment Periods). **Part 2: Tolerance to 5000m for 10 mins followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 50% of VO2max (to be used for the exercise challenge during the Treatment Periods)**
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

3. Screening assessments are allowed to be conducted on more than one day

Section 7.1.2: Treatment Period 1 and 2:

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Randomisation	X											**Treatment Period 1 only.** Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X[5]			X	
Immunogenicity	X											
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X[6]		X		~~Echocardiogram duration approx 5 min~~
Subject enters chamber					X							Subject enters chamber approximately 30 min after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram, blood samples and vital signs have been taken.
Exercise challenge							X					~~For approx 5-10 min~~ **Part 1: exercise at 70% VO2 max for ~5-10 min. Echo conducted immediately after. Part 2: exercise at 50% VO2 max for 2 min. Echo started at 2 min into the challenge, and the subject continues exercising**.

Procedure	**Treatment Period 1 and 2 (Washout 3-14 days)**											**Notes**
	Times relative to start of dosing				**Hypoxic Challenge ~80 min (Times relative to entry to Chamber)**							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Ventilatory parameters	X		X			X				X		Measurements to be taken 2 min before echocardiograms.
Pulse Oximetry (O2 saturation)	←==========→											Will be continuously monitored for safety. A measurement should be recorded at time of each echocardiogram and databased. **Part 2: when the echo is recorded during the exercise challenge, pulse oximetry will be recorded immediately prior to initiation of the echo at 2 minutes into exercise.**
Telemetry	←==========→											Will be continuously monitored for safety.
RAS Biomarkers	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
SP-D	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
PK sampling	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
AE/SAE review		←==========→										
Concomitant medication review	X											

1. Take at the end of the infusion
2. Taken immediately after echocardiogram
3. Immediately before entering the chamber
4. To be taken as soon as possible after leaving chamber
5. On this occasion ONLY, vital signs to be taken after the blood draw.
6. **In Part 2, echo at this time-point taken 2 minutes into the exercise challenge (not immediately after exercise).**

Section 7.3: Hypoxia Challenge

Rationale for change: Clarification of the changes to the hypoxia challenge between Part 1 and Part 2 of the study

Section 7.3.1: **Part 1**

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 4000 m ± 10% hypoxic conditions.

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O_2 saturation of <65%, then the simulated altitude will be adjusted in decrements of 500 m (i.e. from 4000 m to 3500 m) for all remaining subjects to a minimum of 3000 m.

Section 7.3.2: **Part 2**

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 5000 m ± 10% hypoxic conditions.

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O_2 saturation of <60% (persistently for >15 seconds), then the simulated altitude will be adjusted in decrements of 500 m (i.e. from 5000 m to 4500 m) for all remaining subjects to a minimum of 4000 m.

Further detail about the hypoxia challenge is detailed in the SRM.

Section 7.4: Exercise Challenge

Rationale for change: Clarification of the changes to the exercise challenge between Part 1 and Part 2 of the study

Section 7.4.1: **Part 1**

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on a**n upright** cycle ergometer within the hypoxia chamber for 10 minutes.

Subjects must complete a minimum of 5 minutes exercise at 70% of maximumVO2 uptake (calculated from the incremental exhaustive exercise test at the screening visit).

Section 7.4.2: **Part 2**

Subjects will perform an exercise challenge at 50% of maximum VO2 uptake on a semi-recumbent cycle ergometer within the hypoxia chamber (calculated from the incremental exhaustive exercise test at the screening visit). Two minutes into the exercise challenge, the echo recording will be initiated. The subject will exercise for a minimum of 5 minutes (or until the echo recording is completed, if the recording exceeds 5 minutes).

Further detail about the exercise challenge is detailed in the SRM.

Section 7.5: Echocardiogram

Rationale for change:

- *Clarification of the changes to the echocardiogram between Part 1 and Part 2 of the study*
- *Addition of an order of priority for echo parameters*
- *Description of physician 'over-reader' procedure*

Echocardiograms will be taken as detailed in the Time and Events table (Section 7.1).

Echocardiograms will be obtained with the subject resting supine or lying on their left side. **In Part 2, the echo during the exercise challenge will be conducted with the subject on a semi-recumbent cycle ergometer tilted by 30-40 degrees. The subject will continue exercising during the echo recording.**

PASP will be measured and recorded. PASP will be determined by measuring maximal tricuspid regurgitation velocity and applying the modified Bernoulli equation to convert this value into pressure values. Estimated right atrial pressure (RAP) must be added to this obtained value.

In Part 2, the sonographer's priority will be to collect good quality images of the tricuspid regurgitation jet, allowing accurate calculation of PASP once image acquisition is completed. The sonographer will aim to collect at least 5 cardiac cycles. Once estimation of tricuspid regurgitation velocity is completed, the sonographer will measure the right ventricular outflow tract (RVOT) time velocity integral, which (along with the peak tricuspid regurgitant velocity) will allow an estimation of PVR (Rudski, 2010).

In Part 2, the sonographer conducting the echo will be considered the primary reader. All echo images/calculations will be reviewed by a suitably qualified physician (physician 'over-reader'). Any discrepancies will be discussed between the primary reader and physician over-reader, and an agreement reached. The physician over-reader will provide a quality statement for each echo. For the first 3 subjects in Part 2, the physician review will occur immediately following completion of each echo. If quality issues are identified, the sonographer and physician will consider if the echo should be repeated. If no echo quality issues are identified for the first 3 subjects, subsequent reviews will occur within a few hours.

In Part 2, when the echo recording is conducted during the exercise challenge, the sonographer will begin positioning the echo probe as soon as exercise is initiated. Image acquisition will occur at 2 minutes into the exercise challenge. The sonographer will aim to complete image acquisition within approximately 1–2 minutes.

Further details about the ~~PASP measurement~~**echo recording** is detailed in the SRM.

Additional echocardiograms may be obtained for each subject as needed (in the judgement of the Investigator and GSK Medical Monitor, if required).

Section 9.1: Hypotheses

Rationale for change: Clarification of statistical analysis for Part 1 and Part 2 of the study

The focus of this study is on the exploration of the effect of GSK2586881 on a range of echocardiography, pulse oximetry, biomarker, safety and PK endpoints, when subjecting healthy volunteers to an exercise challenge under hypoxic conditions. No formal statistical hypotheses are being tested. A Bayesian statistical analysis framework with non-informative priors for model parameters (unless otherwise specified) will be used to obtain posterior distributions for effects of interest **for Part 1 and Part 2 of the study**. These posterior distributions will be used to obtain a number of probability statements about the magnitude of treatment effects (e.g. Probability of **any** treatment related reduction in PASP, or Probability that the treatment related reduction in PASP ≥ 5 mmHg).

Section 9.2: Sample Size Considerations

Rationale for change: Correction of typographical errors

The sample size of 20 **evaluable** patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context (Note: precision in this context is defined as one half of the width of the 95% confidence interval). A sample size re-estimation **was**~~is~~ planned after 10 subjects**, see Section 9.2.2**.

Section 9.2.2: Sample Size Re-estimation

Rationale for change: Description of the completed interim analysis

A sample size re-estimation w**as to be** ~~ill be~~ conducted at the interim analysis **for Part 1** (see Section 9.3.2.1). The purpose of the sample size re-estimation **was**~~is~~ to determine whether the total number of subjects can be reduced below the anticipated 20, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP (i.e. T3-T0). The sample size re-estimation **was**~~is~~ to be considered advisory, and taken into account together with other considerations. These **were to**~~will~~ include:

- ensuring sufficient data is collected on secondary endpoints,
- non-conclusive safety data at the time of interim analysis (e.g. a weak signal of a reduction in oxygen saturation data that would require the full set of remaining subjects to be able to reach a conclusion),
- speed of recruitment.

An interim analysis was conducted when 10 subjects had completed Treatment Periods 1 and 2 (Part 1), and the following data were reviewed: PASP, oxygen

saturation, AE/SAE, and RAS peptide. The decision was taken to continue the study with modifications to the protocol (Part 2), see Section 2.1.1.

Section 9.3.1: Analysis Populations

Rationale for change: Separation of the analysis populations between Part 1 and Part 2 of the study.

All Subjects Screened Population: This population contains all subjects that complete at least one Visit 1 (Screening) procedure. This population will be used for the summary of subject disposition (including reasons for screening failures, run-in failures, and stabilization failures) and for the listing of AEs and SAEs for non-randomised subjects.

Modified *Intent-to-Treat (**m**ITT) Population:* This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, ~~run-in failure, or stabilisation failure,~~ but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all safety and pharmacodynamic analyses.

Modified Intent-to-Treat (mITT1) Population Part 1: **This population will include all subjects in the mITT population, who were randomised into Part 1 of the study (planned 4000 m altitude).**

Modified Intent-to-Treat (mITT2) Population Part 2: **This population will include all subjects in the mITT population, who were randomised into Part 2 of the study (planned 5000 m altitude)**

Pharmacokinetic (PK) Population Part 1: **This population will comprise all subjects in the mITT Population, randomised in Part 1 of the study, for whom a PK sample was obtained and analysed and on active treatment.**

Pharmacokinetic (PK) Population Part 2: **This population will comprise all subjects in the mITT Population, randomised in Part 2 of the study, for whom a PK sample was obtained and analysed and on active treatment.**

Section 9.3.2: Interim Analysis

Rationale for change: Clarification of the differences in interim analysis between Part 1 and Part 2 of the study

Section 9.3.2.1: **Part 1**

The details below are in reference to the completed interim analysis for Part 1 of the study. Following review of the data from Part 1, the study was modified before restarting Part 2. Section 9.3.2.2 details interim plans for Part 2 of the study.

An interim analysis is planned after 10 subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods
- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

Section 9.3.2.2: **Part 2**

Part 2 of the study will be based on modifications as detailed in Section 2.1.1. Two interim analysis reviews are planned when approximately 3 and 6 subjects, respectively have completed treatment periods 1 and 2.

The aims of the interim analyses are to assess the effectiveness of the Part 2 modifications on critical assessments (PASP, oxygen saturation and AEs) and the potential benefit/risk impact on subjects.

At each review, the study may be stopped for futility if PASP results are highly variable, or if the PASP profile for the placebo group is not as expected (indicative of the model not working given the alterations to the study) or if review of the safety data suggests a change in the benefit-risk profile. If clear criteria for stopping the study are not met, the study will continue to the next interim analysis review, or on to the planned maximum number of evaluable subjects.

If data suggest a further interim analysis review of data would be beneficial, then this will be documented.

Interim analysis reviews are planned as follows:

1. **Review of PASP, oxygen saturation and AE data when approximately 3 subjects have completed Treatment Periods 1 and 2. Critical timepoints for PASP and oxygen saturation are T0 and T3 (a review of additional timepoints will also take place)**

2. **Review of PASP, oxygen saturation and AE data when approximately 6 subjects have completed Treatment Periods 1 and 2. Critical timepoints for PASP and oxygen saturation are T0 and T3 (a review of additional timepoints will also take place). Note that this second review may not be required and will be dependent on data observed during the first interim review. The timing of this review may be altered as necessary dependent on subject recruitment.**

Each interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

Section 9.4: Key Elements of the Analysis Plan

Rationale for change: Clarification of the differences in statistical analysis between Part 1 and Part 2 of the study

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five key sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise **(for Part 2, echo data will be measured during exercise)**
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

PASP and Oxygen Saturation will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day. Biomarkers will be collected at times T0 thru T4, plus additional timepoints.

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline. **Separate statistical analyses will be conducted for subjects in Part 1 and Part 2 of the study.** In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). Subject will be fitted as a random effect and non-informative priors will be used for model parameters. **An unstructured** ~~compound symmetric~~ covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements ~~a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period.~~

~~PASP data will be summarized by treatment and time-point in tabular and graphical format. A~~ Bayesian mixed effects regression model**s** (as described above) will be fitted (using non-informative priors for the model parameters), and an estimate of the mean change from baseline in PASP for each treatment group and post-baseline timepoint,

together with its 95% credible interval, will be obtained. Estimates for treatment differences will also be presented. Data may be log-transformed if necessary.

PASP data will be summarized separately by Part 1 and Part 2 by treatment and time-point in tabular and graphical format.

Details of the analysis of other endpoints will be described in the RAP.

Section 9.4.1: Pharmacokinetic Analyses

Rationale for change: Clarification of PK analyses for Part 1 and Part 2 of the study.

Pharmacokinetic analysis will be performed by, or under the auspices, of Clinical Pharmacology Modelling and Simulation Department within GlaxoSmithKline. Plasma GSK2586881 concentration-time data will be analysed by non-compartmental methods with WinNonLin V6.3 or greater. 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 over the study period [nominally AUC(0-2.5h) post-dose], AUC over the hypoxia challenge (nominally AUC(0.5-2.0h post-dose), plasma clearance (CL), volume of distribution (V) and apparent terminal phase half-life (t1/2), if data permit. Other PK parameters may also be determined.

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively **by Part 1 and Part 2**. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals, R&D.

The relationship between the RAS biomarkers and other pharmacodynamic endpoints (echocardiography and pulse oximetry) and GSK2586881 concentrations and/or PK parameters may also be explored **for Part 2**, if appropriate.

Section 11: References

Rationale for change: Addition of references

Argiento P, Chesler N, Mulè M, D'Alto M, Bossone E, Unger P, Naeije R. Exercise stress echocardiography for the study of the pulmonary circulation. *Eur Respir J.* June 2010, 35 (6):1273 – 1278.

Naeije R, Saggar R, Badesch D, Rajagopalan S, Gargani L, Rischard F, et al. Exercise induced pulmonary hypertension: Translating pathophysiological concepts into clinical practice. *Chest.* 2018; 3692(18)30161-2.

Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a

report from the American Society of Echocardiograpy. *J am Soc Echocardiogr*. 2010; 23: 685 – 713

TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	The effects of GSK2586881 on the responses to acute hypoxia and exercise

Compound Number: GSK2586881

Development Phase: I

Effective Date: 07-MAR-2017

Protocol Amendment Number: 02

Author (s): PPD

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2016N283626_00	2016-SEP-23	Original
2016N283626_01	2017-FEB-24	Amendment No. 1
Additional Inclusion Criteria (Section 5.2) and Subject Stopping Criteria (Section 5.4) added in response to comments from BfArM. A summary of changes is presented in Appendix 6.		
2016N283626_02	2017-MAR-07	Amendment No. 2
This is a substantial protocol amendment to: • Reduce the number of participants in the study from approximately 35 healthy volunteers to approximately 25 healthy volunteers. This is a non-substantial protocol amendment to: • Remove some of the exploratory biomarkers. • Add a spirometry assessment at screening. • Add a pre-dose pulse oximetry assessment. • Add continuous ECG telemetry. • Move the immunogenicity screening sample to Pre-dose in Treatment Period 1. • Remove height and weight assessments from the follow-up visit. • Clarify the sequence and priority of assessments. • Correct typographical errors. A summary of changes is presented in Appendix 6.		

SPONSOR SIGNATORY

PPD

7-Mar-2017

PPD

Dr Aili Lazaar

Date

Director, Discovery Medicine
Project Physician Leader, Respiratory Therapy Area

PPD

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	Site Address
Primary Medical Monitor	PPD				709 Swedeland Road UW2531 King of Prussia, PA 19406 USA
Secondary Medical Monitor					Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
SAE contact information	[Medical monitor as above]				

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): 2016-002465-55

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol 204987

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 204987

Rationale

The purpose of this study is to examine how GSK2586881, a recombinant human ACE2 peptide, modulates the acute hypoxic pulmonary vasoconstriction (HPV) response in healthy volunteers.

HPV is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO_2) levels in healthy and in pathophysiological settings, such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. The literature supports a role for the renin angiotensin system (RAS) in driving acute HPV and while there is a strong biological rationale for modulation of RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they blunt the normal HPV response and negatively impact on arterial oxygenation (PaO_2). Thus, from a safety (and efficacy) perspective it is important to understand the impact of modulation of the RAS system by GSK2586881 on the acute HPV response.

Objective(s)/Endpoint(s)

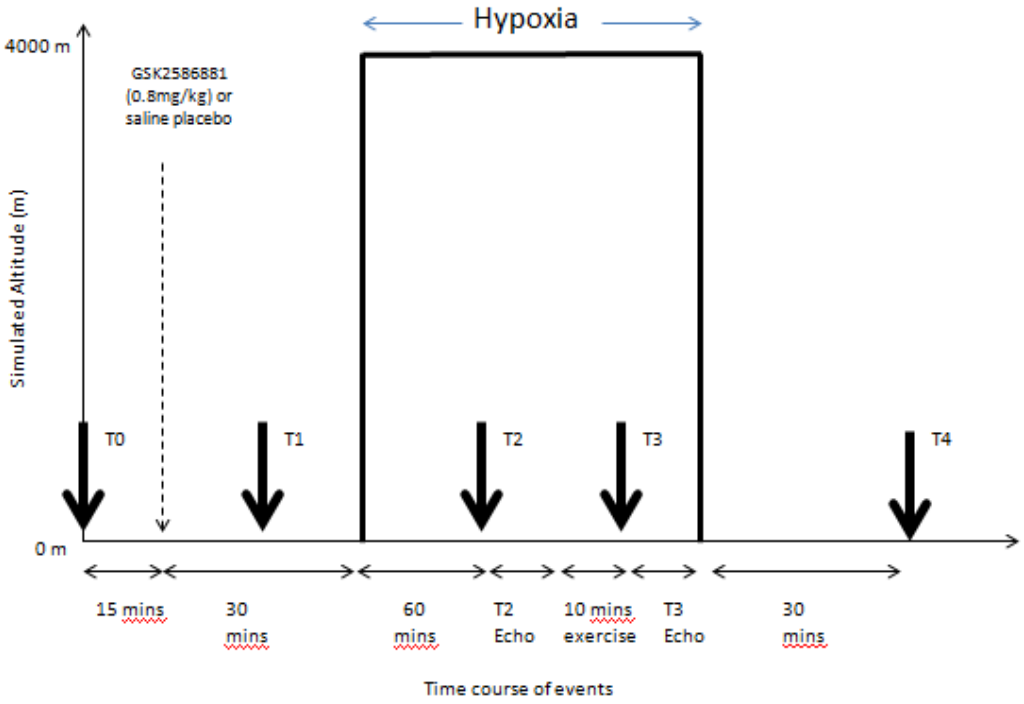
Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in Surfactant Protein-D and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

Overall Design

The study will be single-centre, randomised, placebo-controlled and double blind (sponsor open). Subjects will be randomised to receive a single IV dose of GSK2586881 or saline in a crossover design.

A schematic of the study is shown below. Echocardiograms approximate timings are indicated by bold arrows.

Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2.



Treatment Groups, Randomisation Arms and Duration

The study is intended to follow a double blind (sponsor open) two-period cross-over design.

Treatment Group A: matching volume of placebo, administered as a single IV dose

Treatment Group B: GSK2586881 0.8 mg/kg, administered as a single IV dose.

Subjects will receive both treatments during the course of the study, and will be randomised to one of two sequences, each of which describes the order in which those treatments are received:

Sequence 1: AB: Placebo in the first period followed by GSK2586881 in the second.

Sequence 2: BA: GSK2586881 in the first period followed by Placebo in the second.

The total study duration for each subject is expected to be a maximum of 56 days.

Type and Number of Subjects

Approximately 25 subjects will be enrolled to ensure that a minimum of 20 subjects complete all dosing and critical assessments (the target of 20 may be revised by the sample size re-estimation).

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

Analysis

This study is designed to estimate the effect of GSK2586881 relative to placebo on change from baseline in PASP following exercise under hypoxic conditions. No formal hypothesis will be tested. Point estimates will be calculated together with corresponding 95% confidence intervals (or credible intervals if utilising a Bayesian framework) for the difference between the mean of the test treatment and the mean of the reference treatment.

Analysis of PASP: Statistical modelling of changes from baseline (Ti-T0) will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). Subject will be included as a random effect. A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline time-points within each treatment period. Non-informative priors will be used for the model parameters. Posterior distributions will be obtained for the GSK2586881 Vs Placebo comparisons at each of the post dosing time-points (T1-T4). These distributions will be used to produce several posterior probability statements; the most important being the probability that the change from baseline in PASP is reduced by the GSK2586881 at time T3.

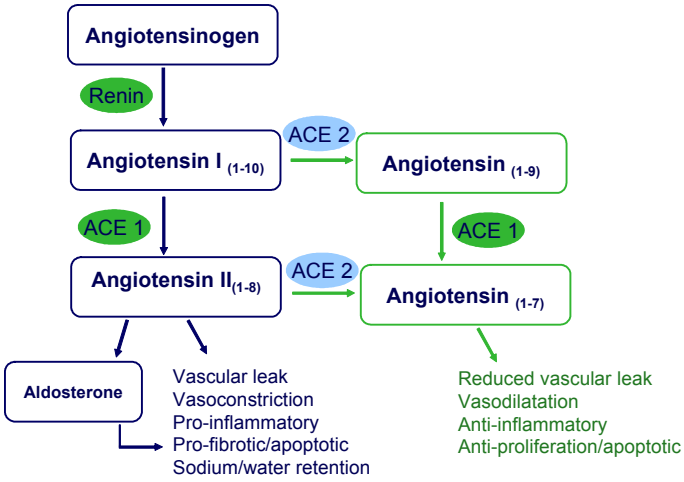
Oxygenation saturation will also be analysed in a similar way; although of interest is the posterior probability that the study drug causes (absolute) reductions in oxygenation saturation in excess of 5%.

An interim analysis is planned after 10 subjects have completed Treatment Period 1 and 2 assessments to i) evaluate safety and tolerability (SAE, AE and Oxygenation saturation), ii) determine whether the study should stop for futility based on expected PASP outcomes if the study were allowed to complete recruitment and iii) re-estimate the sample size based using the observed variance parameters.

2. INTRODUCTION

GSK2586881 is a purified intravenous formulation of soluble recombinant human angiotensin converting enzyme type 2 (rhACE2), which is expressed in Chinese Hamster Ovary cells. Angiotensin converting enzyme type 2 (ACE2) is a zinc carboxypeptidase involved in the Renin-Angiotensin System (RAS) that controls blood pressure, electrolytes, and intravascular fluid volume. A key function of ACE2 is believed to be the cleavage of Angiotensin II (Ang II) to Ang (1-7), which have opposing physiological roles. Elevated levels of Ang II are associated with vasoconstriction, inflammation, fibrosis, vascular leak, and sodium absorption. Ang (1-7) appears to be a counter-regulatory protein in the RAS; associated with vasodilation, anti-proliferation, anti-inflammation, and reduced vascular leak, as noted in Figure 1 below [Paul, 1992; Santos, 2005; Suzuki, 2003].

Figure 1 Renin Angiotensin System



Ang II binds to two distinct receptors called AT-1 and AT-2, with the AT-1 receptor mediating the vasoconstrictive, proliferative and pro-inflammatory actions of Ang II. The function of the AT-2 receptor has not been fully elucidated. Ang (1-7) initiates its effects by binding to the Mas-receptor, and also acts by inhibiting the activity of the carboxyterminal domain of angiotensin converting enzyme (ACE), which prevents ACE from fully acting on its substrates Angiotensin I and bradykinin.

ACE and Angiotensin II has been implicated in the pathogenesis of acute respiratory distress syndrome (ARDS), and pulmonary hypertension. It has been observed that circulating Ang II levels and lung ACE levels are increased in humans with ARDS and pulmonary hypertension. It has also been shown that the DD ACE polymorphism, which is associated with higher ACE activity, is associated with susceptibility to development of lung injury and worsened outcome (mortality) in patients with ARDS [Marshall, 2002] and to the development of pulmonary hypertension [Abraham, 2003].

It is expected that the reduction of Ang II and simultaneous formation of Ang (1-7) should have positive impacts in ARDS and on pulmonary haemodynamics in patients with pulmonary hypertension. This dual action can be achieved by ACE2, and thus, an

enhancement of the activity of this enzyme is seen as a promising approach for the treatment of diseases and conditions with an imbalance of the RAS system, insufficient natural ACE2 activity, and pathologically elevated Ang II levels or decreased Ang (1-7), such as is observed in ALI/ARDS [Tom, 2001; Idell S, 1987; Santos, 2003; Wenz, 2000] and pulmonary hypertension [Maron, 2014].

This study will demonstrate how GSK2586881 modulates the acute HPV response in healthy volunteers.

2.1. Study Rationale

Hypoxic pulmonary vasoconstriction (HPV) is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO2) levels in healthy and in pathophysiological settings, such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. However, in many ARDS patients with pulmonary microvascular injury and dystonia, the normal HPV reflex is compromised resulting in the mismatching of perfusion (Q) and ventilation (VA) and formation of areas of low perfusion to ventilated alveoli (high VA/Q; physiological deadspace), or perfusion of alveoli with minimal or no ventilation (low V/Q; physiological shunt).

The literature is conflicting as to the role of the RAS in modulating the HPV response; however, the majority of reports support a role for the RAS in driving acute HPV (Cargil, 1996; Kiely, 1996). While there is a strong biological rationale for modulation of the RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they impact on PaO2 and produce a blunted HPV response. Surprisingly, there are scant data regarding the effect of RAS modulation on HPV, V-Q matching or PaO2 in healthy human volunteers.

Inhibition of the acute HPV response would be expected to promote blood flow to areas of poorly-ventilated lung resulting in an increase in venous admixture and a reduction in PaO2 levels. In the context of acute lung injury, correction of any V/Q mismatch would be expected to be beneficial with respect to maintaining and improving PaO2 levels. Conversely, inhibition of HPV by pharmacological agents might be expected to worsen V/Q matching and compromise PaO2 levels. Thus, from a safety (and efficacy) perspective it is vital to understand the impact of modulation of the RAS system on the acute HPV response and subsequent PaO2.

Recombinant human ACE2 (rhACE2) delivered intravenously to pigs inhibits the HPV response to acute hypoxic challenge assessed by inhibition of mean pulmonary artery pressures and pulmonary vascular resistance. There was also a strong trend to increased shunt with administration of rhACE2; however PaO2 was not significantly affected. The authors concluded that the increased shunt may not have been sufficient to result in a reduction in PaO2 (Kleinsasser, 2012).

An elegant study by Wagner and colleagues demonstrated the importance of end arterial capillary diffusion limitations on PaO2 levels in healthy volunteers during hypoxia ± exercise (Torre-Bueno, 1985). Diffusion limitation made a significant contribution to PaO2 levels particularly under conditions where exercise and hypoxia were combined. In

addition, V/Q mismatching was suggested to increase with the combined stresses of exercise and hypoxia resulting in significantly more arterial oxygen desaturation than observed with either stressor by itself.

The main goals of this study are to examine whether GSK2586881 modulates the acute HPV response in healthy volunteers with a subsequent impact on O2 saturation. Should the application of GSK2586881 lead to accentuated arterial oxygen desaturation, further clinical studies to examine the therapeutic efficacy of GSK2586881 in acute lung injury should be approached with caution. Conversely, a reduction in HPV without augmented hypoxemia would provide supporting evidence that GSK2586881 could have a positive impact in patients with pulmonary hypertension.

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in Surfactant Protein (SP-D) and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

4. STUDY DESIGN

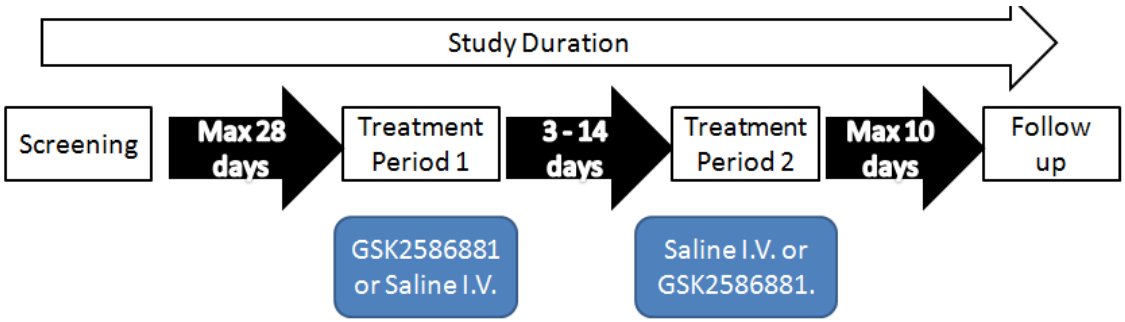
4.1. Overall Design

This is a single-centre, randomised, placebo-controlled and double blind (sponsor open), two-period crossover study in healthy subjects.

The subjects will be required to attend the unit for a screening visit, Treatment Period 1, Treatment Period 2 and a follow up visit.

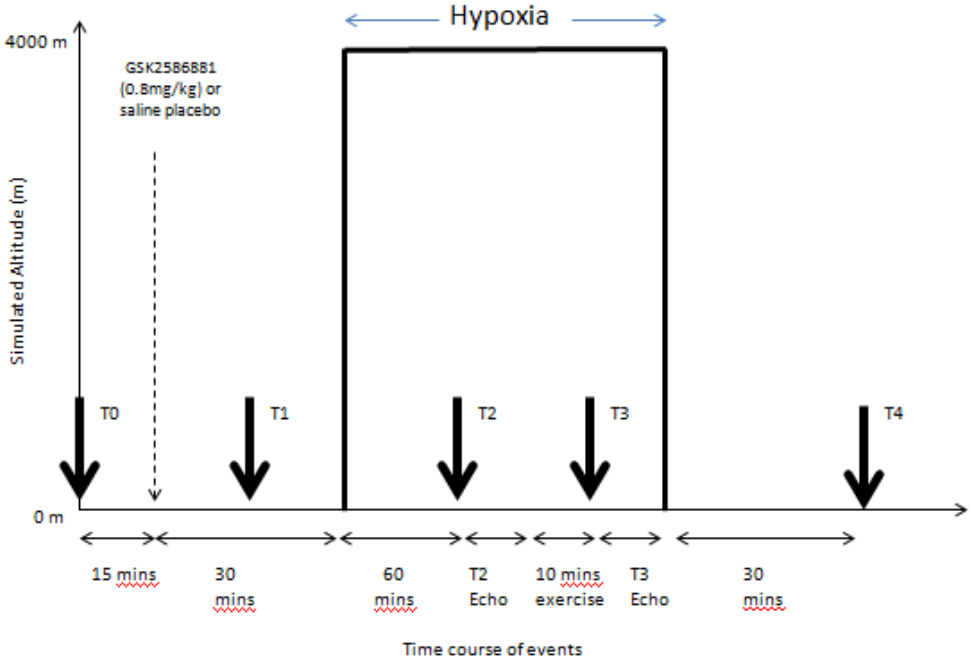
4.2. Treatment Arms and Duration

Figure 2 Subject participation flow



The subjects must participate in the procedures detailed in the Time and Events Table (Section 7.1) and the timings of the simulated altitude, exercise and echocardiograms is shown in Figure 3

Figure 3 Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2



There will be a washout period of 3–14 days between treatments to ensure biomarkers return to pre-challenge baseline. Subjects then return to the site and repeat the same procedures as above, except that they will receive the treatment (GSK2586881 or Saline) that they did not receive in the first period.

4.2.1. Follow Up

The follow-up visit will occur up to 10 days after the end of the second treatment period. During the visit various safety tests will be conducted (see time and events table in Section 7.1).

The total study duration for each subject is expected to be a maximum of 56 days.

4.3. Type and Number of Subjects

Approximately 25 subjects healthy volunteers will be randomised such that approximately 20 evaluable subjects complete the study (the target of 20 may be revised following the sample size re-estimation, see Section 9.2.2).

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

4.4. Design Justification

The study design is based on a paper published by Ricart, 2005 and is considered to be feasible. The study will provide important information on whether GSK2586881 modulates the acute HPV response in healthy volunteers. In addition, the study will include assessments of PK and PD effects of GSK2586881. This will be achieved by assessing blood levels of GSK2586881 and RAS peptide responses throughout the duration of the hypoxia challenges.

The study will be placebo controlled (saline) so each subject can be used as their own control.

4.5. Dose Justification

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). In a concluded (but not yet reported) investigator-sponsored study in Pulmonary Arterial Hypertension (PAH) (Study 204696), 5 subjects have received a single dose of either 0.2 mg/kg or 0.4 mg/kg.

A population PK model for GSK2586881 was derived from data obtained in healthy subjects and ARDS patients and showed that the systemic PK profile was adequately described by a two-compartment first order elimination model and that the PK profile was independent of population (healthy subjects or ARDS patients). Furthermore the PK/PD response (AngII) in healthy subjects and ARDS patients was consistent with a

single direct Emax model after accounting for differences in baseline AngII concentrations between healthy subjects and ARDS patients.

Based on the population PK/PD model described above, single intravenous doses of 0.4–1.2 mg/kg GSK2586881 are predicted to reduce elevated levels of AngII (baseline AngII consistent with an ARDS population) to levels consistent with that observed in healthy subjects for the duration of the hypoxic challenge (approx 2.25 h). The dose of 0.8 mg/kg has been selected to ensure maximal reduction of AngII levels, whilst maintaining dosing volumes within acceptable limits, and will further aid the understanding of the PK/PD relationship for GSK2586881.

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with GSK2586881 can be found in the Investigator's Brochure (GlaxoSmithKline Document Number 2010N108777_00, 2015). The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2586881]		
Cardiovascular risk	During preclinical testing a brief period of non-sustained ventricular tachycardia was observed in one monkey receiving a dose of 20.8 mg/kg/day.	The dose used in this study is much lower and well below the No observed adverse effect level (NOAEL) for the 14 day repeat dose cynomolgus monkeys of 8 mg/kg/day.
Potential Reproductive/embryofetal risks	Preclinical studies have not been performed.	Women of childbearing potential will be excluded from the study.
Potential for Immunogenicity	There has been no induction of an immune response to rhACE2 in either of the clinical studies to date in healthy subjects or participants with ARDS.	Patients will have routine monitoring of any immunological response that may occur. If an immunological response is seen the patient will be asked to return for further monitoring and assessment(s).
Potential for rash	In study ACE114622, rash was reported more frequently in subjects receiving GSK2586881, although only one event was considered drug-related.	Patients will be monitored for rash in the clinical trials.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Effects of hypoxia (light headiness, headaches, nausea)	The subjects will be exposed to hypoxic conditions for approximately 80 minutes with a simulated altitude of 4000 m.	The subjects will be continuously observed and monitored with telemetry and pulse oximetry. Stopping criteria are included in the protocol.

4.6.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the subjects taking part in the study as it is a healthy volunteer study. However, subjects will undergo a medical evaluation during screening including physical exams, ECGs and laboratory assessments which may provide important health information.

By taking part in this study, the subject will be contributing to the development of GSK2586881 for the treatment of pulmonary hypertension and ARDS.

4.6.3. Overall Benefit:Risk Conclusion

The design of the study is considered low risk to the subjects and justified based on the work carried out by other researchers, the safety information from the nonclinical studies and the two previous clinical trials carried out on GSK2586881.

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 the IB.

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 40 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, spirometry, laboratory tests and cardiac monitoring. 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 Medical Monitor if required) agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures. Note: Screened subjects with laboratory values outside of the normal range may be

repeated once for inclusion into the study at the discretion of the Investigator.

3. Screening echocardiogram of good quality, without clinically significant abnormalities, and with mild-moderate tricuspid regurgitation sufficient for the reliable estimation of PASP, as determined by the echocardiography core laboratory or responsible cardiologist.
   Screening PASP within the normal range according to site standards.
4. Subjects have not resided at an altitude >1500 m for more than 7 days in the last 4 months.
5. Able to complete all study procedures.
6. Any contraindication (orthopaedic, cardiac etc.) to perform exercise on a bicycle ergometer.

WEIGHT
7. Body weight 50–100 kg (inclusive).

SEX
8. Male or female (non child bearing potential)

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication.

a. Vasectomy with documentation of azoospermia.

b. Male condom plus partner use of one of the contraceptive options below:

- Contraceptive subdermal implant
- Intrauterine device or intrauterine system
- Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a]
- Contraceptive vaginal ring [Hatcher, 2007a]
- Percutaneous contraceptive patches [Hatcher, 2007a]

This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH M3 (R2), 2009].

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Females:

A female subject is eligible to participate if she is not pregnant (as confirmed by a negative urine human chorionic gonadotrophin (hCG) test), not lactating, and the following condition applies:

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
- Postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels)]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 7.2, 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. ALT >1.5x Upper limit of normal (ULN).
2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
4. Prior history of altitude sickness.
5. Recently been scuba diving (within 1 week before screening).

6. 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
7. Unable to refrain from prescription or non-prescription drugs, including agents active in the central nervous system, 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 and throughout the study, unless in the opinion of the Investigator and/or GSK Medical Monitor (if needed) the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
8. 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. 9. Urinary cotinine levels indicative of smoking or history or regular use of tobacco- or nicotine-containing products within 6 months prior to screening.

CONTRAINDICATIONS
10. 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
11. 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. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody

(HBcAb) should also be excluded.

12. A positive pre-study drug/alcohol screen.
13. A positive test for HIV antibody.
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within 56 days.
15. 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: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
16. 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 randomised. 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 (Section 7.2.1.4).

Subjects that are not enrolled into the study within the allotted screening window may be re-screened once. If re-screening is performed, subjects are assigned a different unique subject Identification (ID) number for the re-screening, and all screening procedures must be repeated.

See the Study Reference Manual (SRM) for specific details.

5.4. Withdrawal/Stopping Criteria

Subjects may be withdrawn from the study for any of the following reasons:

- 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.
- If a subjects oxygen saturation falls below 65% at any point during the hypoxia challenge.
- If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).

- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

Once a subject has discontinued investigational product the subject may not re-enter the study. Dosing of the subjects with the investigational product may be stopped at any time, at the request of the subject or at the discretion of the Investigator (i.e. if clinically significant adverse events should occur). Withdrawal due to adverse events will be distinguished from withdrawal for other reasons.

If a subject decides to withdraw or is withdrawn by the Investigator, the reasons for withdrawal and the results of any relevant tests will be recorded in the Case Report Form (CRF) and the planned follow-up procedures will be performed, where possible.

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".

5.4.1. Study Stopping Criteria

An interim analysis is planned after approximately 10 subjects have completed treatment periods 1 and 2 and the study may be stopped if a reasonable change in PASP is not observed after dosing with GSK 2586881 or if review of the safety data suggests a change in the benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.

Safety data that is collected on a continuous basis (oxygen saturation and telemetry) will be monitored live on site to determine whether a subject tolerates the hypoxia/exercise challenge; study procedures may be aborted on safety grounds if, in the Investigator's opinion, any of these measurements reach unsafe levels. The simulated altitude within the chamber may be reduced if too many participants fail to tolerate the challenge, and the study may be stopped if participants fail to tolerate the challenge at the minimum altitude. The full criteria for this procedure (which may lead to the study being stopped) are described in Section 7.3.

In addition, the study may be stopped at the interim analysis for a safety signal in oxygen saturation under hypoxia and exercise, combined with a lack of evidence of mechanistic effect on Angiotensin biomarkers (Ang II, Ang 1-7 and Ang 1-5). Full details on stopping criteria are described together with the full procedure for the interim analysis in Section 9.3.2.

5.4.2. QTc Stopping Criteria

The *same* QT correction formula *must* be used for *each individual subject* to determine discontinuation from the study. For example, if a subject is eligible for the protocol based on QTcB, then QTcB must be used for discontinuation of this individual subject as well.

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

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

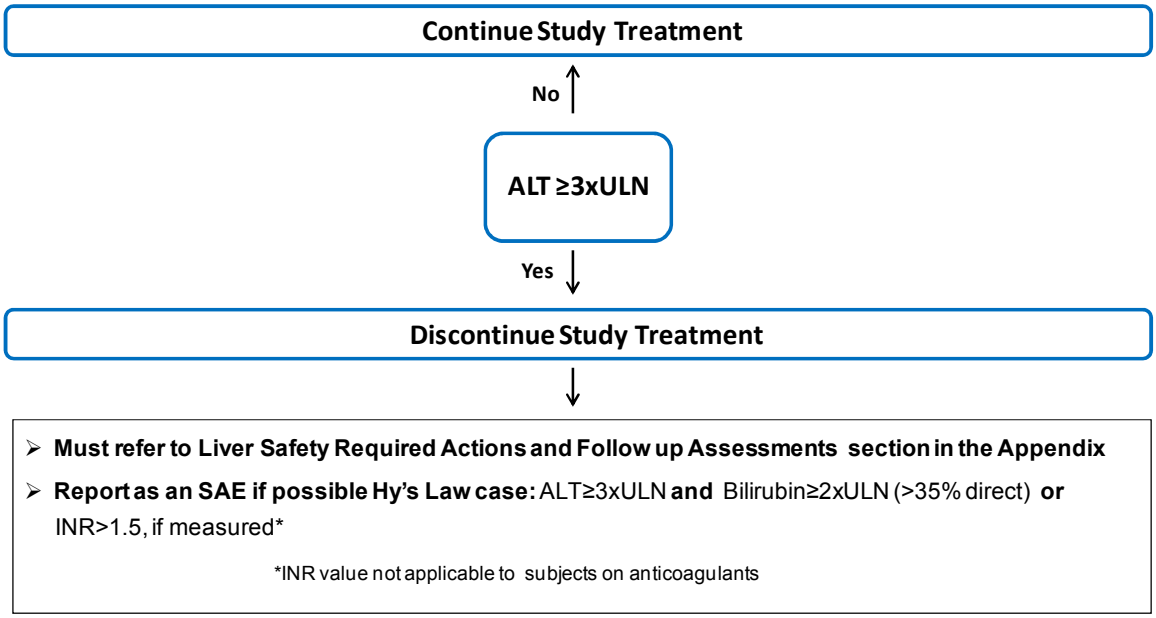
The QTc should be based on single or averaged QTc values of triplicate ECGs obtained over a brief (e.g. 5-10 minute) recording period.

5.4.3. 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 Food and Drug Administration (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.

5.4.3.1. Study Treatment Restart or Rechallenge

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

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 Treatment	
Product name: (Generic name and trade)	GSK2586881	Placebo
Formulation description:	rhACE2	Normal Saline (0.9%)
Dosage form:	IV	IV
Unit dose strength(s)/Dosage level(s):	0.8 mg/kg	Saline Placebo
Route of Administration	Intravenous	Intravenous
Dosing instructions:	Infuse over 3-5 minutes	Infuse over 3-5 minutes
Physical description:	Clear colourless liquid	Clear colourless liquid

At Screening a unique Subject Number (CRF number) will be assigned to any subject who has at least one Screening procedure performed, other than informed consent. The unique Subject Number will be used to identify individual subjects during the course of the study.

Subjects who meet screening eligibility criteria will be assigned to one of the sequences listed in Table 1 below, in accordance with the randomisation schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software. Once assigned, this number must not be reassigned to any other subject in the study.

Table 1 Description of sequences

Sequence	Period 1	Period 2
AB	Saline Placebo	GSK2586881
BA	GSK2586881	Saline Placebo

The subjects will be randomised using a central randomisation procedure created by GSK.

Further details on how and when a subject is allocated a randomisation number and the subject numbering convention is in the SRM.

6.2. Planned Dose Adjustments

No dose adjustments are allowed.

6.3. Blinding

This will be double blind (sponsor open) study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff), and the subject will be blinded to the treatment allocated to individual subjects. An unblinded qualified staff member will be required at site to prepare the study treatment for dosing. The unblinded staff member is not permitted to communicate the subject's treatment allocation to blinded site staff. Selected study team members working for the sponsor (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This will include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report.

The following will apply:

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment.
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the CRF

A subject whose treatment sequence assignment is unblinded for emergency reasons (as described above) will not be permitted to continue in the study (due to the emergency requiring unblinding rather than the unblinding itself). The event or condition that led to the unblinding will be recorded in the CRF as the primary reason for discontinuation.

A subject whose treatment sequence assignment is inadvertently unblinded (either to investigative staff or the subject themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the subject will be subject to review as to their inclusion in analyses as per the procedures set out in Section 9.3.1.

6.4. Packaging and Labeling

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

6.5. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for GSK2586881 will be detailed in a Study Specific Technical Agreement/Memo (TTS) or Pharmacy Section of the SRM, which will be accompanied by a Quality Agreement.

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
- 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. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.
- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

6.6. Compliance with Study Treatment Administration

When the individual dose for a subject is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

Subjects will be dosed at the site, and 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.

GSK2586881 and the saline placebo will be intravenously administered to subjects at the site. Administration will be documented in the source documents and reported in the CRF.

6.7. Treatment of Study Treatment Overdose

For this study, any dose of GSK2586881 > 1.5 mg/kg within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose. The investigator will use clinical judgment to treat any overdose.

In the event of an overdose the investigator or treating physician should:

1. contact the Medical Monitor immediately
2. closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until GSK2586881can no longer be detected systemically (at least 3 days for GSK2586881).
3. obtain a plasma sample for pharmacokinetic (PK) analysis within 1 day from the date of the last dose of study treatment 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.

6.8. Treatment after the End of the Study

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

6.8.1. Meals and Dietary Restrictions

- No dietary restrictions prior to the first treatment period.

6.8.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from caffeine for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.
- During each dosing session, subjects will abstain from alcohol for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.
- Use of tobacco products is not allowed as outlined in the exclusion criteria.

6.8.3. Activity

Subjects will abstain from strenuous exercise for 24 hours prior to screening and each treatment period Subjects may participate in light recreational activities between the planned study procedures (e.g., watch television, read).

6.9. Concomitant Medications and Non-Drug Therapies

6.9.1. 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.

6.9.2. 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. Ventilatory parameters
  2. Echocardiograms
  3. 12-lead ECG
  4. vital signs
  5. blood draws

  Note: The timing of the assessments should allow the echocardiogram to be performed as close as possible to the nominal time.
- The timing and number of planned study assessments, including: safety, echocardiogram, pharmacokinetic, pharmacodynamic/biomarker or other 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 Institutional Review Board (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

7.1.1. Screening and Follow up

Procedure	Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)	Follow up (7-10 days post last dose)	Notes
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam, including height and weight	X	X	Height and weight to be measured at screening only. Weight at screening will be used for dosing calculation.
Alcohol, Drugs of Abuse, Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: Drugs, Alcohol, tobacco
Past and current medical conditions [including cardiovascular medical history]	X		
Serum OR urine pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries)	X	X	Non Fasting
Immunogenicity		X	
12-lead ECG	X	X	Triplicate ECG required at screening.
Vital signs	X	X	Triplicate vital signs required at screening.
Spirometry	X		
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		Tolerance to 4000m for 10 mins followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max (to be used for the exercise challenge during the Treatment Periods).
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

1. Screening assessments are allowed to be conducted on more than one day

7.1.2. Treatment Period 1 and 2

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Randomisation	X											Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X[5]			X	
Immunogenicity	X											
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X		X		Echocardiogram duration approx 5 min
Subject enters chamber					X							Subject enters chamber approximately 30 min after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram, blood samples and vital signs have been taken.
Exercise challenge							X					For approx 5-10 min
Ventilatory parameters	X		X			X				X		Measurements to be taken 2 min before echocardiograms.
Pulse Oximetry (O2 saturation)	←==========										→	Will be continuously monitored for safety. A measurement should be recorded at time of each echocardiogram and databased.
Telemetry	←==========										→	Will be continuously monitored for safety.
RAS Biomarkers	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
SP-D	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	On exit from chamber	After 30 min rest	60 min after exit from Chamber	
PK sampling	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
AE/SAE review		←==========→										
Concomitant medication review	X											

1. Take at the end of the infusion
2. Taken immediately after echocardiogram
3. Immediately before entering the chamber
4. To be taken as soon as possible after leaving chamber
5. On this occasion ONLY, vital signs to be taken after the blood draw.

After written informed consent, screening assessments will be performed as outlined in the Time and Events Table (Section 7.1).

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

Cardiovascular medical history/risk factors (as detailed in the CRF) will be assessed at screening.

Cardiorespiratory fitness during bicycle exercise and hypoxia tolerance will be assessed.

Procedures conducted as part of the subject's routine clinical management (e.g. blood count) and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

7.2. 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 exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

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

The definitions of an AE or SAE can be found in Appendix 4.

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

7.2.1.1. 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 7.2.1.3), at the time-points 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 Appendix 4.

- 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 Appendix 4.

7.2.1.2. 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?"

7.2.1.3. 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 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 Appendix 4.

7.2.1.4. 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.

7.2.2. 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, at screening only.
- A brief physical examination will include, at a minimum assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). To be carried out at the start of each period.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

7.2.3. 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.
- Triplicate measurements will be taken at screening, and single measurements at other time-points. The first reading should be rejected. Second and third readings should be averaged to give the measurement to be recorded in the CRF.

7.2.4. Electrocardiogram (ECG)

- Triplicate 12-lead ECGs will be obtained at screening. At all other time-points, a single 12-lead ECGs will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes rest.
- The same QT correction formula *must* be used for each individual subject for all QTc data being collected for data analysis. Safety ECGs and other non-protocol specified ECGs are an exception.

7.2.5. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled. Details for the preparation and shipment of samples will be provided by the laboratory. 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.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**
Haematology	Platelet Count; RBC Count; Hemoglobin; Hematocrit *RBC Indices*: MCV; MCH *WBC count with Differential:* Neutrophils; Lymphocytes; Monocytes; Eosinophils; Basophils
Clinical Chemistry [1]	BUN; Urea; Creatinine; Glucose Potassium; Sodium; Calcium AST (SGOT); ALT (SGPT); Alkaline phosphatise Total and direct bilirubin; Total Protein; Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol, smoking and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, benzodiazepines and cotinine) performed at site • Urine hCG Pregnancy test (as needed) [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 5.4.2 and Appendix 2.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within 3 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.

7.3. Hypoxia Challenge

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 4000 m ± 10% hypoxic conditions.

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O_2 saturation of <65%, then the simulated altitude will be adjusted in decrements of 500 m (i.e. from 4000 m to 3500 m) for all remaining subjects to a minimum of 3000 m.

Further details about the hypoxia challenge is detailed in the SRM.

7.3.1. Exercise Challenge

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on a cycle ergometer within the hypoxia chamber for 10 minutes.

Subjects must complete a minimum of 5 minutes exercise at 70% of maximumVO2 uptake (calculated from the incremental exhaustive exercise test at the screening visit).

Further detail about the exercise challenge is detailed in the SRM.

7.4. Echocardiogram

Echocardiograms will be taken as detailed in the Time and Events table (Section 7.1).

Echocardiograms will be obtained with the subject resting supine or lying on their left side.

PASP will be measured and recorded.

PASP will be determined by measuring maximal tricuspid regurgitation velocity and applying the modified Bernoulli equation to convert this value into pressure values. Estimated right atrial pressure (RAP) must be added to this obtained value.

Further details about the PASP measurement is detailed in the SRM.

Additional echocardiograms may be obtained for each subject as needed (in the judgement of the Investigator and GSK Medical Monitor, if required).

7.5. Ventilatory parameters

Ventilatory parameters will be measured as detailed in the Time and Events table (Section 7.1).

Ventilatory parameters will be recorded for 2 minutes before echocardiograms. The second minute of the recording will be averaged and used for data analysis.

Measurements may include change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2), total tidal volume, inspiratory tidal volume, expiratory tidal volume, total respiratory time, inspiratory time, expiratory time, duty cycle, mean respiratory flow and respiratory rate, as data permit.

7.6. Pulse Oximetry

Oxygen saturation will be monitored continuously via pulse oximetry as detailed in the SRM. Measurements will be taken at the same time as the echocardiograms and recorded in the eCRF.

7.7. Telemetry

Continuous ECG monitoring will occur via telemetry during both treatment periods.

7.8. Pharmacokinetics

7.8.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of GSK2586881 will be collected at the time points indicated in the Time and Events Table (Section 7.1). 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.

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

7.8.2. Sample Analysis

Plasma analysis will be performed under the control of PTS, In Vitro/In Vivo Translations (IVIVT) Department and Third Party Resourcing (TPR), GlaxoSmithKline. The details of the Bioanalytical Laboratory will be included in the SRM. Concentrations of GSK2586881 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site to be detailed in the SRM).

7.9. Biomarker(s)/Pharmacodynamic Markers

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

Details of biomarker/PD blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.9.1. Renin-angiotensin system biomarkers

RAS peptides including, but not limited to, AngII, Ang(1-7) and Ang(1-5) may be analysed as data permit. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site detailed in the SRM).

7.9.2. Surfactant Protein-D and other biomarkers

SP-D will be analysed. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site to be detailed in the SRM). Additional analytes may be analysed, as data permit.

7.10. Genetics

Information regarding genetic research is included in Appendix 3.

Genetic sampling is optional. Subjects can refuse genetic sampling, but will still be allowed to participate in the study.

7.10.1. Blood sample collection

A blood sample to investigate the association between the loss of function polymorphism rs1799752, representing the I/D polymorphism, in intron 16 of the Angiotensin Converting Enzyme (*ACE*) gene and Ang II (and possibly other RAS peptides) and hypoxic pulmonary vasoconstriction will be collected as specified in the Time and Events Table (Section 7.1).

Further information for blood sample collection, processing, storage and shipping procedures are provided in the SRM.

Information regarding genetic research is included in Appendix 3.

7.11. Immunogenicity

7.11.1. Sample collection

Blood samples for immunogenicity analysis will be collected at the time points indicated in Section 7.1, Time and Events Table. Additional visits to obtain immunogenicity samples may be required in the unlikely event that subjects develop a clinically relevant immunoglobulin response to the drug as described in the SRM.

Details of immunogenicity blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.11.2. Sample analysis

Immunogenicity analysis will be performed under the management of Clinical Immunology, Biopharm R&D, GlaxoSmithKline. All pre-dose and post-dose samples will be first tested for Anti-ACE2 binding antibodies by screening and confirmation assay steps. The post-dose samples tested positive for anti-ACE2 binding antibodies will be further characterized for anti-ACE2 neutralizing antibodies. Both positive incidences for anti-ACE2 binding and neutralizing antibodies will be reported.

8. DATA MANAGEMENT

- For this study subject data will be entered into GSK defined CRFs, transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g. removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials and day/month of birth will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

The focus of this study is on the exploration of the effect of GSK2586881 on a range of echocardiography, pulse oximetry, biomarker, safety and PK endpoints, when subjecting healthy volunteers to an exercise challenge under hypoxic conditions. No formal statistical hypotheses are being tested. A Bayesian statistical analysis framework with non-informative priors for model parameters (unless otherwise specified) will be used to obtain posterior distributions for effects of interest. These posterior distributions will be used to obtain a number of probability statements about the magnitude of treatment effects (e.g. Probability of **any** treatment related reduction in PASP, or Probability that the treatment related reduction in PASP $\geq$ 5 mmHg).

A rule of thumb for end of "study success" is if the probability of any treatment related reduction in PASP (T3-T0) exceeds 0.95 (success is also conditional on the probability of (absolute) treatment related reductions in oxygen saturation exceeding 5%, being small).

9.2. Sample Size Considerations

The sample size of 20 patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context (Note: precision in this context is defined as one half of the width of the 95% confidence interval). A sample size re-estimation is planned after 10 subjects.

9.2.1. Sample Size Assumptions

The precision of the primary treatment comparison (change from baseline PASP to hypoxic/exercise PASP) was estimated using simulation. PASP data points were

simulated for each subject: normoxic at rest, and hypoxic after exercise, each for two study periods, giving a total of four PASP measurements for each subject. The four PASP measurements were drawn from a multivariate normal distribution to allow for different levels of within-subject correlation. One simulated study with a given sample size (N) was created by repeating this process of generating individual subject data N times, randomly allocating each subject to a treatment sequence in a 1:1 ratio, and calculating the half-width of the confidence interval of the treatment difference in change from baseline in PASP from that simulated dataset (using a linear mixed effects model including period baseline, subject baseline, treatment and period as fixed effects, and subject as a random effect). A full simulation run consisted of 1000 iterations of simulated studies, and a final estimate for precision was calculated as the mean precision across the 1000 iterations.

The following assumptions were made regarding the mean and Standard Deviation (SD) of PASP based on data from Antoni Ricart, 2005:

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) and 45 mmHg under hypoxia and exercise
- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise
- As the purpose of this simulation was to estimate precision by evaluating the SD of the treatment difference rather than the mean, there was no treatment effect assumed in the simulations
- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9).
- A range of sample sizes was considered: from 5 to 10 in increments of 1, and then up to 40 in increments of 5. The table below shows a subset of these; the figure includes them all.

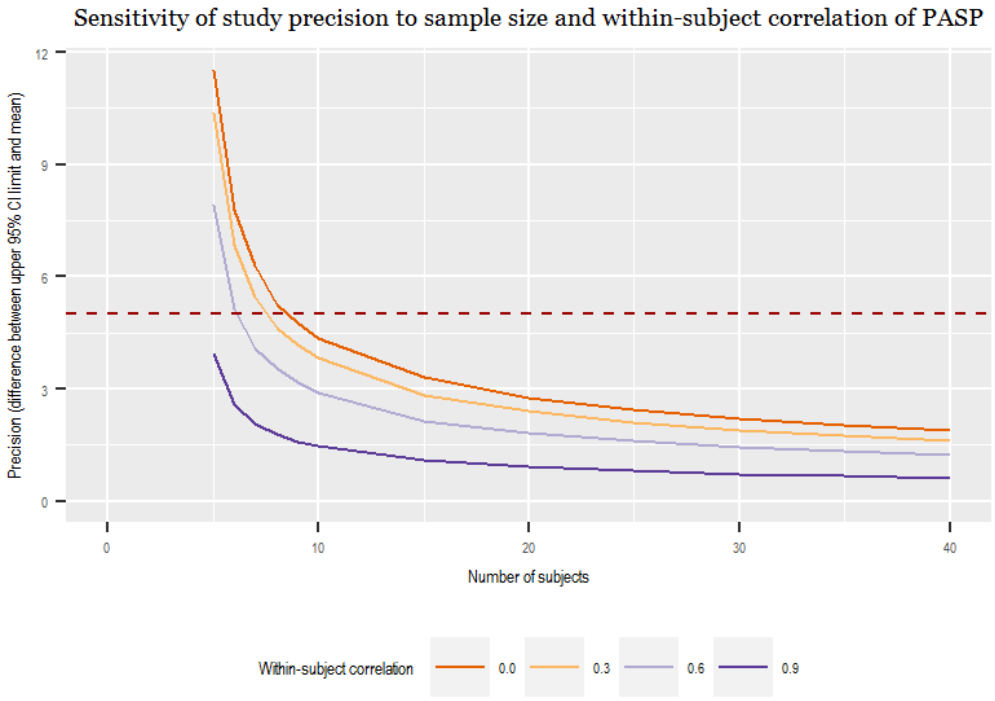
Based on these assumptions, the estimated precisions for these sample sizes and under these scenarios of within-subject correlation were as follows (smaller number for precision denotes more precise estimates):

Table 3 Estimated precision of treatment comparisons of change from baseline in PASP (mmHg) for a range of sample sizes and within-subject correlation scenarios

	Within-subject correlation			
Sample size (N)	**0**	**0.3**	**0.6**	**0.9**
10	4.35	3.83	2.92	1.48
20	2.76	2.40	1.83	0.91
30	2.22	1.91	1.46	0.72
40	1.91	1.63	1.23	0.62

Figure 4 illustrates precision estimates under the full range of sample sizes considered (from 5 to 40), and for all within-subject correlation scenarios. The red line at 5 mmHg is superimposed to indicate a rough guide to the magnitude of a clinically meaningful difference.

Figure 4 Sensitivity of study precision to sample size and within subject correlation of PASP



9.2.2. Sample Size Re-estimation

A sample size re-estimation will be conducted at the interim analysis (see Section 9.3.2). The purpose of the sample size re-estimation is to determine whether the total number of subjects can be reduced below the anticipated 20, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised

PASP (i.e. T3-T0). The sample size re-estimation is to be considered advisory, and taken into account together with other considerations. These will include:

- ensuring sufficient data is collected on secondary endpoints,
- non-conclusive safety data at the time of interim analysis (e.g. a weak signal of a reduction in oxygen saturation data that would require the full set of remaining subjects to be able to reach a conclusion),
- speed of recruitment.

9.3. Data Analysis Considerations

Data will be listed and summarised according to GSK integrated data standards library (IDSL) reporting standards where applicable. Complete details will be provided in the Reporting and Analysis Plan (RAP).

The key endpoints referred to in the text below comprise PASP measured by echocardiography, oxygen saturation measured by continuous pulse oximetry, RAS peptides and safety data.

9.3.1. Analysis Populations

All Subjects Screened Population: This population contains all subjects that complete at least one Visit 1 (Screening) procedure. This population will be used for the summary of subject disposition (including reasons for screening failures, run-in failures, and stabilization failures) and for the listing of AEs and SAEs for non-randomised subjects.

Intent-to-Treat (ITT) Population: This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, run-in failure, or stabilisation failure, but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all safety and pharmacodynamic analyses.

Pharmacokinetic (PK) population: This population will comprise all subjects in the ITT Population for whom a PK sample was obtained and analysed and on active treatment.

In addition to the above populations, the effect of important protocol violations, including any subjects who failed the inclusion/exclusion criteria, may be assessed by means of sensitivity analyses. A blind review of all protocol violations will be performed prior to DBF in order to identify any important deviations and consequently identify any subjects who will be excluded from such sensitivity analyses.

9.3.2. Interim Analysis

An interim analysis is planned after 10 subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods

- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

The interim analysis will use the following study data:

- PASP at time T0 and T3 for both periods, for the first 10 subjects
- Oxygen saturation at T0 and T3 for both periods, for the first 10 subjects
- Adverse events for the first 10 subjects
- RAS peptide concentrations for the first 5 subjects (to allow time for the samples to be analysed by the external lab).

The interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

The aims of the interim analysis, and the endpoints to be used to address them, are as follows:

- Using the primary endpoint (treatment differences between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP [T3-T0]):
    - To decide whether to continue the study or stop based on futility, and
    - to conduct an advisory sample-size re-estimation with the view to potentially reduce the number of subjects participating in the study below 20.
- Using secondary endpoints:
    - to estimate treatment differences between GSK2586881 and Placebo in change from pre-dose oxygen saturation to hypoxic/exercised oxygen saturation [T3-T0], with a view to stopping the study if there is evidence that GSK2586881 causes a reduction in oxygen saturation in healthy volunteers. As a non-binding guide high posterior probability of observing ≥5% absolute differences in **mean** oxygenation saturation values between placebo and active arms would be of concern; but clinical judgement would override any statistical methods if, for example, the majority of subjects displayed consistent patterns of reductions e.g. between 2-3%).
    - to compare adverse events within the two treatment groups and explore any potential safety signals that may emerge

To assess the operating characteristics of the interim analysis decisions to be made based on the primary endpoint, a simulation was carried out to estimate the relative proportion of studies reaching conclusions to stop for futility, continue with no change to the sample

size, and to continue with a reduced sample size, under various different scenarios of the true treatment effect. Broadly speaking, the aim of the simulation was to confirm that (in an overwhelming majority of cases) a decision to stop for futility would be reached in the case of no true treatment effect and a decision to continue (perhaps with reduced sample size) would be reached in the case of a true treatment effect.

Simulated datasets of PASP at baseline (i.e. normoxic and at rest) and under hypoxia and exercise for 10 subjects, each receiving both treatments in sequence, were generated using the following assumptions:

- 10 simulated subjects were randomised to one of the two available treatment sequences (see Section 6.2) using block randomisation with block size of 4 records

- PASP at baseline and under hypoxia and exercise for both treatment groups (a total of four PASP measurements per subject) were sampled from a multivariate normal distribution, using values for mean PASP, standard deviation of PASP and within-subject correlation of PASP as described below.

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) for both treatments, and 45 mmHg under hypoxia and exercise for Placebo (as in Section 9.2.1). For GSK2586881, mean PASP under hypoxia and exercise was (45 - Δ) mmHg, where Δ (the mean treatment difference) was varied according to four different scenarios:

    - no treatment difference (Δ = 0 mmHg)

    - weak treatment difference of a magnitude of half the clinically meaningful difference (Δ = 2.5 mmHg)

    - minimum clinically meaningful difference (Δ = 5 mmHg)

    - overwhelming treatment difference one-and-a-half times the clinically meaningful difference (Δ = 7.5 mmHg)

- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise, for both treatment groups (as in Section 9.2.1)

- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9) (as in Section 9.2.1). Within-subject correlation of PASP was assumed to be compound symmetric (i.e. the same correlation was used for each of the six possible pairs of the four PASP measurements within each subject).

- It was assumed that there would be no effect of period in the simulation, i.e. it was assumed that it would make no difference if the measurement was taken from the first or second chronological period in the treatment sequence.

Modelling took on a three-step process, with decisions after each. Firstly, the treatment difference and its associated standard error was then estimated for the dataset of 10

simulated subjects using a mixed effects linear regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. If the sign of treatment difference arising from the simulated data was in the wrong direction (i.e. the simulated outcome for GSK2586881 was worse than for Placebo), then the simulated study was stopped for futility.

Secondly, in the alternative case that the simulated treatment difference was in favour of GSK2586881 over Placebo, a posterior probability of success (where success was defined as a 97.5% posterior probability of the treatment difference being in favour of GSK2586881 over Placebo) was evaluated for 20 subjects. This was evaluated using a t-distribution, with the mean and standard deviation taken as the treatment difference and standard error (multiplied by a factor of the square root of 10 to estimate the standard deviation) from step one, respectively. If this posterior probability was less than 0.2, then the study was stopped for futility as being unlikely to show any benefit of GSK2586881 over Placebo.

Thirdly, provided that the simulated study hadn't been stopped for futility in either of the first two steps, posterior probabilities of success were calculated for N ranging from 10 to 19 subjects. If any of these posterior probabilities of success were greater than or equal to 0.9, then the study was continued with a reduced sample size equal to the lowest N at which that threshold was reached. Otherwise, the conclusion from the simulated interim analysis would be to continue the study with unchanged sample size.

This whole process (simulating data for 10 subjects, modelling the treatment difference, evaluating posterior probabilities of success and simulating the decision-making process) was replicated 10000 times for each scenario, with the decision ("futile", "no change to N" or "reduce N") recorded for each iteration. Each combination of the four treatment difference scenarios and the four correlation scenarios was evaluated for a total of 16 scenarios in all.

The estimated proportions of each decision being reached under each of the 16 scenarios are presented in Table 4.

Table 4 Operating characteristics of interim analysis at N=10 under four scenarios of treatment effect and four scenarios of within-subject correlation

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with 20 subjects	Continue with reduced sample size
Δ=0 mmHg (no treatment difference)				
	0.0	85%	15%	1%
	0.3	85%	15%	1%
	0.6	85%	15%	1%
	0.9	85%	14%	1%

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with 20 subjects	Continue with reduced sample size
Δ=2.5 mmHg (weak treatment effect, less than clinically meaningful difference)				
	0.0	44%	50%	6%
	0.3	36%	56%	9%
	0.6	21%	63%	16%
	0.9	1%	31%	68%
Δ=5 mmHg (good treatment effect, at threshold of clinical meaningful difference)				
	0.0	9%	60%	31%
	0.3	4%	54%	42%
	0.6	1%	31%	68%
	0.9	0%	0%	100%
Δ=7.5 mmHg (very strong treatment effect)				
	0.0	1%	32%	67%
	0.3	0%	18%	82%
	0.6	0%	3%	96%
	0.9	0%	0%	100%

These results suggest primarily that if there is no true treatment difference between GSK2586881 and Placebo, the study would be stopped for futility 85% of the time (irrespective of within-subject correlation of PASP). A drug with a weak treatment effect would most likely result in the study being continued without change or stopped for futility (except in the case where within-subject correlation is 0.9, which is presented here as an extreme case, unlikely to occur in practice). If the true treatment effect is at the clinically relevant threshold of 5 mmHg, then the study is unlikely to be stopped for futility and may indicate being reduced in sample size.

Secondarily, it should be noted that the higher the within-subject correlation, then the higher the probability of success. This is because the more correlation there is in measurements within the same individual, the less variability there will be in change from baseline. Results for correlations of 0.0 and 0.9 are presented as extreme cases unlikely to occur in practice, though the exact strength of within-subject correlation of PASP (particularly under different stressed conditions) is not known.

Full details of the interim analysis will be supplied in the RAP.

9.3.3. Multiple Comparisons and Multiplicity

As this is an early-phase exploratory study, no adjustment for multiple comparisons will be made. Treatment comparisons will be presented as effect sizes with 95% confidence intervals.

9.4. Key Elements of Analysis Plan

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five key sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

PASP and Oxygen Saturation will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day. Biomarkers will be collected at times T0 thru T4, plus additional timepoints.

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline. In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). Subject will be fitted as a random effect and non-informative priors will be used for model parameters. A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period.

PASP data will be summarized by treatment and time-point in tabular and graphical format. A Bayesian mixed effects regression model (as described above) will be fitted (using non-informative priors for the model parameters), and an estimate of the mean change from baseline in PASP for each treatment group and post-baseline timepoint, together with its 95% credible interval, will be obtained. Estimates for treatment differences will also be presented. Data may be log-transformed if necessary.

Details of the analysis of other endpoints will be described in the RAP.

9.4.1. Pharmacokinetic Analyses

Pharmacokinetic analysis will be performed by, or under the auspices, of Clinical Pharmacology Modelling and Simulation Department within GlaxoSmithKline. Plasma GSK2586881 concentration-time data will be analysed by non-compartmental methods with WinNonLin V6.3 or greater. 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 over the study period [nominally AUC(0-2.5h) post-dose], AUC over the hypoxia challenge (nominally AUC(0.5-2.0h post-dose), plasma clearance (CL), volume of distribution (V) and apparent terminal phase half-life (t1/2), if data permit. Other PK parameters may also be determined.

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.

The relationship between the RAS biomarkers and other pharmacodynamic endpoints (echocardiography and pulse oximetry) and GSK2586881 concentrations and/or PK parameters may also be explored, if appropriate.

If appropriate, a population PK analysis may also be conducted, in addition the plasma concentration-time data may be merged with historical data and analysed as part of a population PK meta-analysis.

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.
- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study; also current medical records must be available.

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 randomisation 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

Abraham WT, Raynolds MV, Badesch DB, Wynne KM, Groves BM, Roden RL,et al.. Angiotensin-converting enzyme DD genotype in patients with primary pulmonary hypertension: increased frequency and association with preserved haemodynamics. J Renin Angiotensin Aldosterone Syst. 2003 Mar;4(1):27-30.

Antoni Ricart, Jaume Maristany, Núria Fort, Conxita Leal, Teresa Pagés, and Ginés Viscor. High Altitude Medicine & Biology. March 2005, 6(1): 43-49. doi:10.1089/ham.2005.6.43

Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;49:818-822.

Cargil RI, Lipworth BJ. Lisinopril attenuates acute hypoxic pulmonary vasoconstriction in humans. Chest. 1996 Feb;109(2):424-9/

GlaxoSmithKline Document Number 2010N108777_00 Study ID GSK2586881. Investigator's Brochure for GSK2586881 (formerly known as APN01)– A Soluble Recombinant Human Angiotensin Converting Enzyme 2 (rhACE2). Report Date 16-FEB-2011.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(a): 24. Table 3-2.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(b): 28.

ICH Topic M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, 2009

Idell S, Kueppers, F, Lippmann, M, Rosen, H, Niederman, M, Fein, A. ACE elevated in ARDS BAL. *Angiotensin converting enzyme in bronchoalveolar lavage in ARDS. Chest.* 1987;91:52-56.

Kiely DG, Cargill RI, Lipworth BJ (1996) Effects of hypercapnia on hemodynamic, inotropic, lusitropic, and electrophysiologic indices in humans. Chest 109:1215–1221

Kiely DG1, Cargill RI, Wheeldon NM, Coutie WJ, Lipworth BJ. Haemodynamic and endocrine effects of type 1 angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale. Cardiovasc Res. 1997 Jan;33(1):201-8.

Kleinsasser A1, Pircher I, Treml B, Schwienbacher M, Schuster M, Janzek E, Loibner H, Penninger JM, Loeckinger A. Recombinant angiotensin-converting enzyme 2 suppresses pulmonary vasoconstriction in acute hypoxia. Wilderness Environ Med. 2012 Mar;23(1):24-30

Maron BA, Leopold JA. The role of the renin-angiotensin-aldosterone system in the pathobiology of pulmonary arterial hypertension (2013 Grover Conference series. Pulm Circ. 2014 Jun;4(2):200-10. doi: 10.1086/675984

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, Chambers RJ, , GJ et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am. J. Resp. Crit. Care Med.* 2002;166:646-650.

Paul M, Bachmann J, Ganten D. Angiotensin II-induced vasoconstriction and sodium resorption. *The Tissue Renin-Angiotensin System in Cardiovascular Disease. Trends Cardiovasc. Med.* 1992;2:94-99.

Ricart A1, Maristany J, Fort N, Leal C, Pagés T, Viscor G. Effects of sildenafil on the human response to acute hypoxia and exercise. High Alt Med Biol. 2005 Spring;6(1):43-9.

Rubenfeld GD, Caldwell E, Peabody E. Incidence and Outcomes of Acute Lung Injury. *N Engl J Med.* 2005;353:1685-1693.

Santos RAS, Ferreira AJ, Pinheiro SVB, Sampaio WO, Touyz R, Campangole-Santos MJ. Counter-regulatory effects of ANG (1-7) in ANG II. *Angiotensin(1-7) and its receptor as potential targets for cardiovascular drugs' Expert Op. Investig. Drugs.* 2005;1019:1031.

Santos RAS, Simoes e Silva, AC, Maric C, Silva DMR, Machado RP, de Buhr I, et al. ANG (1-7) as the agonist of mas. *'Angiotensin (1-7) is an endogenous ligand for the G-protein coupled receptor Mas' Proc. Natl. Acad. Sci. USA*. 2003;100:8258-8263.

Suzuki Y, Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V., Egido, J. Inflammatory and pro-fibrotic activity of ANG II. *) Inflammation and Angiotensin II. Int. J. Biochem. Cell Biol.* 2003;35:881-900.

The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med.* 2000;342:1301-1308.

The ARDS Network Authors for the ARDS network. Ketoconzaole for Early Treatment of Acute Lung Injury and Acute Respiratory Distress Syndrome. *JAMA.* 2000;283:1995-2002.

Tom B, deVries R, Saxena PR, Danser J. ANG (1-7) inhibiting ACE and modifying Bradykinin responses. *Bradykinin potentiation by angiotensin (1-7) and ACE inhibitors correlates with C- and N-terminal blockade' Hypertension.* 2001;38:95-99.

Torre-Bueno JR, Wagner PD, Saltzman HA, Gale GE, Moon RE. Diffusion limitation in normal humans during exercise at sea level and simulated altitude. J Appl Physiol (1985). 1985 Mar;58(3):989-95

Wenz M, Hoffmann B, Bohlender J, Kaczmarczyk G. ANG II elevated in ARDS. *Anfgiotensin II formation and endothelin clearance in ARDS patients in supine and prone positions. Intensive Care Med.* 2000;26:292-298.

Wheeler AP, Bernard GR, Thompson BT, Schoenfeld D, Wiedemann HP, deBoisblanc B,et al.. Pulmonary-Artery versus Central Venous Catheter to Guide Treatment of Acute Lung Injury. *N Engl J Med.* 2006;25:354(21):2213-24. Epub 2006 May 21.

Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B,et al. Comparison of Two Fluid-Management Strategies in Acute Lung Injury. *N Engl J Med.* 2006;354(24):2564-75. Epub 2006 May 21.

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

ACE2	Angiotensin converting enzyme type 2
AE	Adverse Event
ALI	Acute Lung Injury
ALT	Alanine aminotransferase (SGPT)
Ang II	Angiotensin II
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
BID	Bi-Daily
BUN	Blood urea nitrogen
CBP	Child Bearing Potential
CL	Clearance
CO2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CV	Cardiovascular
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HPV	Hypoxic pulmonary vasoconstriction
IB	Investigator's Brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identification
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board

ITT	Intent-to-Treat
IV	Intravenous
IVIVT	In Vitro/In Vivo Translations
Kg	Kilogram
LDH	Lactate dehydrogenase
LFTs	Liver function tests
m	Meters
MCH	Mean corpuscular hemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
MSDS	Material Safety Data Sheet
msec	Milliseconds
NOAEL	No observed adverse effect level
O2	Oxygen
PaO2	Partial Pressure of Oxygen in arterial blood
PAH	Pulmonary Arterial Hypertension
PASP	Pulmonary Artery Systolic Pressure
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
Q	Perfusion
QC	Quality control
QTcB	QT duration corrected for heart rate by Bazett's formula
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RAS	Renin-Angiotensin System
RBC	Red blood cells
rhACE2	Recombinant human angiotensin converting enzyme type 2
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SD	Standard deviation
SP-D	Surfactant Protein-D
SRM	Study Reference Manual
TPR	Third Party Resourcing
TTS	Technical Terms of Supply
t½	Terminal phase half-life
tmax	Time of occurrence of Cmax
V	Volume of Distribution
V_A	Ventilation
VO2	Oxygen Consumption

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
MedDRA
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 etiology (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="2">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td>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="2">Required Actions and Follow up Assessments following Liver Stopping Event</th></tr>
<tr><th>Actions</th><th>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
• A specialist or hepatology consultation is</td><td>• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend.
• Blood sample for PK analysis, obtained 24 hours after last dose [4]
• 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.</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
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	• Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct 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. 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,et al.. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. Drug Metab Dispos 2009; 37:1779-1784.

12.3. Appendix 3: Genetic Research

Genetics – Background

Naturally occurring genetic variation may contribute to inter-individual variability in response to medicines, as well as an individual's risk of developing specific diseases. Genetic factors associated with disease characteristics may also be associated with response to therapy, and could help to explain some clinical study outcomes. For example, genetic variants associated with age-related macular degeneration (AMD) are reported to account for much of the risk for the condition [Gorin, 2012] with certain variants reported to influence treatment response [Chen, 2012]. Thus, knowledge of the genetic etiology of disease may better inform understanding of disease and the development of medicines. Additionally, genetic variability may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), or pharmacodynamics (relationship between concentration and pharmacologic effects or the time course of pharmacologic effects) of a specific medicine and/or clinical outcomes (efficacy and/or safety) observed in a clinical study.

Genetic Research Objectives and Analyses

The objectives of the genetic research are to investigate the relationship between genetic variants and:

- Response to medicine, including [any treatment regimens under investigation in this study] or any concomitant medicines;

Genetic data may be generated while the study is underway or following completion of the study. Genetic evaluations may include focused candidate gene approaches and/or examination of a large number of genetic variants throughout the genome (whole genome analyses). Genetic analyses will utilize data collected in the study and will be limited to understanding the objectives highlighted above. Analyses may be performed using data from multiple clinical studies to investigate these research objectives.

Appropriate descriptive and/or statistical analysis methods will be used. A detailed description of any planned analyses will be documented in a Reporting and Analysis Plan (RAP) prior to initiation of the analysis. Planned analyses and results of genetic investigations will be reported either as part of the clinical RAP and study report, or in a separate genetics RAP and report, as appropriate.

Study Population

Any subject who is enrolled in the study can participate in genetic research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the genetic research.

Study Assessments and Procedures

A key component of successful genetic research is the collection of samples during clinical studies. Collection of samples, even when no *a priori* hypothesis has been

identified, may enable future genetic analyses to be conducted to help understand variability in disease and medicine response.

- A 6 ml blood sample will be taken for Deoxyribonucleic acid (DNA) extraction. A blood sample is collected at the baseline visit, after the subject has been randomised and provided informed consent for genetic research. Instructions for collection and shipping of the genetic sample are described in the laboratory manual. The DNA from the blood sample may undergo quality control analyses to confirm the integrity of the sample. If there are concerns regarding the quality of the sample, then the sample may be destroyed. The blood sample is taken on a single occasion unless a duplicate sample is required due to an inability to utilize the original sample.

The genetic sample is labelled (or "coded") with the same study specific number used to label other samples and data in the study. This number can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number).

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study, or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will only use samples collected from the study for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

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

Informed Consent

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

Subject Withdrawal from Study

If a subject who has consented to participate in genetic research withdraws from the clinical study for any reason other than being lost to follow-up, the subject will be given a choice of one of the following options concerning the genetic sample, if already collected:

- Continue to participate in the genetic research in which case the genetic DNA sample is retained
- Discontinue participation in the genetic research and destroy the genetic DNA sample

If a subject withdraws consent for genetic research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records.

Genotype data may be generated during the study or after completion of the study and may be analyzed during the study or stored for future analysis.

- If a subject withdraws consent for genetic research and genotype data has not been analyzed, it will not be analyzed or used for future research.
- Genetic data that has been analyzed at the time of withdrawn consent will continue to be stored and used, as appropriate.

Screen and Baseline Failures

If a sample for genetic research has been collected and it is determined that the subject does not meet the entry criteria for participation in the study, then the investigator should instruct the subject that their genetic sample will be destroyed. No forms are required to complete this process as it will be completed as part of the consent and sample reconciliation process. In this instance a sample destruction form will not be available to include in the site files.

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

GSK may summarize the genetic research results in the clinical study report, or separately and may publish the results in scientific journals.

GSK may share genetic research data with other scientists to further scientific understanding in alignment with the informed consent. GSK does not inform the subject, family members, insurers, or employers of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from genetic studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined. Further, data generated in a research laboratory may not meet regulatory requirements for inclusion in clinical care.

Chen H, Yu KD, Xu GZ. Association between Variant Y402H in Age-Related Macular Degeneration (AMD) Susceptibility Gene CFH and Treatment Response of AMD: A Meta-Analysis. PloS ONE 2012; 7: e42464

Gorin MB. Genetic insights into age-related macular degeneration: Controversies addressing risk, causality, and therapeutics. Mol. Asp. Med. 2012; 33: 467-486.

12.4. Appendix 4: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.4.1. 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.

12.4.2. 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 and 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 Appendix 2 for the required liver chemistry follow-up instructions

12.4.3. 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

12.4.4. 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.

12.4.5. 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.

12.4.6. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool

- Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool
- If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor Site will enter the serious adverse event data into the electronic system as soon as it becomes available.
- The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.
- After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor telephone.
- Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

SAE reporting to GSK via paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail • 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.

12.5. Appendix 5: Modified List of Highly Effective Methods for Avoiding Pregnancy and Collection of Pregnancy Information

12.5.1. Modified List of Highly Effective Methods for Avoiding Pregnancy

The list does not apply to FRP with same sex partners or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

1. Contraceptive subdermal implant
2. Intrauterine device or intrauterine system
3. Combined estrogen and progestogen oral contraceptive [Hatcher RA, 2011]
4. Injectable progestogen [Hatcher RA, 2011]
5. Contraceptive vaginal ring [Hatcher RA, 2011]
6. Percutaneous contraceptive patches [Hatcher RA, 2011]
7. Male partner sterilization with documentation of azoospermia prior to the female subject's entry into the study, and this male is the sole partner for that subject [Hatcher RA, 2011]. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview provided by her or her partner.

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Contraceptive requirements for male subjects with female partners of reproductive potential (when applicable).

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until [at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives] after the last dose of study medication.

1. Vasectomy with documentation of azoospermia. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview.
2. Male condom plus partner use of one of the contraceptive options below that meets the Standard Operating Procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label:
    - Contraceptive subdermal implant
    - Intrauterine device or intrauterine system

- Combined estrogen and progestogen oral contraceptive [Hatcher RA, 2011]
- Injectable progestogen [Hatcher RA, 2011]
- Contraceptive vaginal ring [Hatcher RA, 2011]
- Percutaneous contraceptive patches [Hatcher RA, 2011]

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

12.5.2. Collection of Pregnancy Information

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study
- Information will be recorded on the appropriate form and submitted to GSK within 2 weeks of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on mother and infant, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Appendix 5. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating

- will discontinue study medication or be withdrawn from the study
- Investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. This applies only to subjects who are randomised to receive study medication.
- After obtaining the necessary signed informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy

- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

12.5.3. References

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, Policar MS, editors. Contraceptive Technology. 20th edition. Atlanta, Georgia: Ardent Media, Inc., 2011: 50. Table 3-2.

12.6. Appendix 6: Protocol Changes

12.6.1. Protocol Amendment 01 (24-Feb-2017) from Original Protocol (23-Sep-2016)

This is a global amendment.

12.6.1.1. Summary of Changes and Rationale for Amendment

Additional Inclusion Criteria (Section 5.2) and Subject Stopping Criteria (Section 5.4) added in response to comments from BfArM.

12.6.1.2. List of Specific Changes

Title Page, Author (s)

Rationale for change: Addition and removal of author(s).

PPD

Sponsor Signatory

Rationale for change: Change in sponsor signatory

~~Dr Richard Marshall~~ **Dr Aili Lazaar**

~~Vice President, Fibrosis & Lung Injury Discovery Performance Unit (DPU) Head, Respiratory Therapy Area Unit~~ **Director, Discovery Medicine**
Project Physician Leader, Respiratory Therapy Area

Section 5.2: Exclusion Criteria

Rationale for change: Addition of exclusion criteria number 4 and 5.

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
1. ALT >1.5x Upper limit of normal (ULN).
2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
4. Prior history of altitude sickness.
5. Recently been scuba diving (within 1 week before screening).

6. 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).

Section 5.4: Withdrawal/Stopping Criteria

Rationale for change: Addition of withdrawal criteria.

Subjects may be withdrawn from the study for any of the following reasons:

- 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.
- If a subjects oxygen saturation falls below 65% at any point during the hypoxia challenge.
- **If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.**
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

12.6.2. Protocol Amendment 02 (07-Mar-2017) from Protocol Amendment 01 (24-Feb-2017)

This is a global amendment.

12.6.2.1. Summary of Changes and Rationale for Amendment

This is a substantial protocol amendment to:

- Reduce the number of participants in the study from approximately 35 healthy volunteers to approximately 25 healthy volunteers.

This is a non-substantial protocol amendment to:

- Remove some of the exploratory biomarkers.
- Add a spirometry assessment at screening.
- Add a pre-dose pulse oximetry assessment.
- Add continuous ECG telemetry.
- Move the immunogenicity screening sample to Pre-dose in Treatment Period 1.
- Remove height and weight assessments from the follow-up visit.
- Clarify the sequence and priority of assessments.
- Correct typographical errors.

See Section 12.6.2.2 below for a rationale for each change.

12.6.2.2. List of Specific Changes

Protocol synopsis for study 204987

Protocol synopsis updated to reflect changes in the main body of the protocol.

Section 3: Objectives/Endpoints

Rationale for change: Removal of some of the exploratory biomarkers. SP-D will be analysed and reported, and additional biomarkers may be analysed, as data permit.

Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• ~~To evaluate the effect of a single IV dose of GSK2586881 on peptide hormone responses to hypoxia and exercise.~~	• ~~Baseline levels and changes in response to hypoxia and exercise in kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) as data permit~~.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in ~~other biomarkers which may include Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I,~~ Surfactant Protein D **(SP-D)** ~~, IL-6~~ and/or additional analytes to be determined.
• To evaluate Pharmacogenetics.	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

Section 4.3: Type and Number of Subjects

Rationale for change: The removal of some exploratory biomarkers, led to a re-assessment of the sample size; given that the larger prior sample size was to optimise data collection to include exploratory biomarkers. The sample size has been reduced from 35 to 25 enrolled subjects (30 to 20 completed subjects), because precision estimates show that a difference of between 3 and 5 mmHg in PASP (the primary endpoint) could be detected with an acceptable level of precision based on 20 completed subjects (see Section 9.2).

Approximately ~~35~~ **25** subjects healthy volunteers will be randomised such that approximately ~~30~~ **20** evaluable subjects complete the study (the target of ~~30~~ **20** may be revised following the sample size re-estimation, see Section 9.2.2).

Section 4.5: Dose Justification

Rationale for change: Additional subjects have been dosed with GSK2586881 since the protocol was finalised in September 2016.

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). **In a concluded (but not yet reported) investigator-sponsored study in Pulmonary Arterial Hypertension (PAH) (Study 204696), 5 subjects have received a single dose of either 0.2 mg/kg or 0.4 mg/kg**~~One subject with Pulmonary Arterial Hypertension (PAH) has received a single dose of 0.2 mg/kg~~.

Section 5.1: Inclusion Criteria

Rationale for change: Addition of a spirometry test at screening to ensure normal lung function before hypoxic challenge and exercise test.

TYPE OF SUBJECT
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, **spirometry**, laboratory tests and cardiac monitoring.

Section 5.1: Inclusion Criteria

Rationale for change: Correction of typographical errors.

SEX
8. Male or female (non child bearing potential) Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication. h. Vasectomy with documentation of azoospermia. i. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant • Intrauterine device or intrauterine system • Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a]

- Contraceptive vaginal ring [Hatcher, 2007a]
- Percutaneous contraceptive patches [Hatcher, 2007a]

This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH, M3 (R2) 2009].

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

~~This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH, M3 (R2) 2009].~~

~~The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.~~

Females:

A female subject is eligible to participate if she is not pregnant (as confirmed by a negative urine human chorionic gonadotrophin (hCG) test), not lactating, and the following condition applies:

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
- Postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels)]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

~~The investigator is responsible for ensuring that subjects understand how to properly use~~

~~these methods of contraception.~~

Section 5.4.1: Study Stopping Criteria

Rationale for change: The overall sample size has been reduced from 35 to 25 enrolled subjects (30 to 20 completed subjects), so the interim analysis will now be completed after 10 subjects have completed the study, rather than 15.

An interim analysis is planned after approximately ~~15~~ **10** subjects have completed treatment periods 1 and 2 and the study may be stopped if a reasonable change in PASP is not observed after dosing with GSK2586881 or if review of the safety data suggests a change in the benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.

Section 5.4.2: QTc Stopping Criteria

Rationale for change: QTc Stopping Criteria removed from Section 7.2.5, and inserted into Section 5.4 with the other subject stopping criteria.

The *same* QT correction formula *must* be used for *each individual subject* to determine discontinuation from the study. For example, if a subject is eligible for the protocol based on QTcB, then QTcB must be used for discontinuation of this individual subject as well.

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

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

The QTc should be based on single or averaged QTc values of triplicate ECGs obtained over a brief (e.g. 5-10 minute) recording period.

Section 6.1: Investigational Product and Other Study Treatment

Rationale for change: Order of treatment sequences altered to reflect GSK's standard practice to list placebo first.

Table 1 Description of sequences

Sequence	Period 1	Period 2
AB	~~GSK2586881~~**Saline Placebo**	~~Saline Placebo~~**GSK2586881**
BA	~~Saline Placebo~~**GSK2586881**	~~GSK2586881~~**Saline Placebo**

Section 7: Study Assessments and Procedures

Rationale for change: Addition of ventilatory parameters to the list of assessments, and clarification that echocardiograms should be taken as close as possible to the nominal time-point (rather than blood draws), because they are the primary endpoint.

- If assessments are scheduled for the same nominal time, THEN the assessments should occur in the following order:

  1. **Ventilatory parameters**
  2. Echocardiograms
  3. 12-lead ECG
  4. vital signs
  5. blood draws

  Note: The timing of the assessments should allow the ~~blood draw to occur at the exact~~ **echocardiogram to be performed as close as possible to the** nominal time.

Section 7.1: Time and Events Table

Rationale for change:

- *Height and weight removed at follow-up (not required).*
- *Spirometry added at screening (see justification in Section 5.1).*
- *Immunogenicity moved from screening to Day 1, Treatment Period 1.*
- *Removal of exploratory ventilatory parameters after exercise, as this assessment would delay the echocardiogram, which is the primary endpoint.*
- *Addition of telemetry throughout the treatment periods.*
- *Correction of typographical errors.*

Section 7.1.1: Screening and Follow up

Procedure	**Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)**	**Follow up (7-10 days post last dose)**	**Notes**
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam, including height and weight	X	X	**Height and weight to be measured at screening only.** Weight at screening will be used for dosing calculation.
Alcohol, Drugs of Abuse, Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: Drugs, Alcohol, tobacco
Past and current medical conditions [including cardiovascular medical history]	X		
Serum OR urine pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries)	X	X	Non Fasting
Immunogenicity	~~X~~	X	
12-lead ECG	X	X	Triplicate ECG required at screening.
Vital signs	X	X	Triplicate vital signs required at screening.
Spirometry	**X**		
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		Tolerance to 4000m for 10 mins followed by ~~followed by~~ incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max ~~for the~~ **(to be used for the** exercise challenge **during the Treatment Periods).**
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

1. Screening assessments are allowed to be conducted on more than one day

Section 7.1.2: Treatment Period 1 and 2

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	~~30m rest~~ On exit from chamber	After 30 min rest	60 min after exit from Chamber	
Randomisation	X											Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X[5]			X	
Immunogenicity	X											~~Treatment period 2 only~~
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X		X		Echocardiogram duration approx 5 min
Subject enters chamber					X							Subject enters chamber approximately 30 min after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram**, blood samples** and vital signs have been taken.
Exercise challenge							X					For approx 5-10 minutes
Ventilatory parameters	X		X			X	~~X[1]~~			X		Measurements to be taken ~~at the same time as the~~**2 min before** echocardiograms. ~~During the exercise challenge, an ECG integrated with the ventilatory assessment will be carried out.~~
Pulse Oximetry (O2 saturation)	←----------										----------→	Will be continuously monitored for safety. **A** measurement should be recorded at time of each echocardiogram ~~post-treatment~~ and databased.
Telemetry	←----------										----------→	**Will be continuously monitored for safety.**

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to start of dosing				Hypoxic Challenge ~80 min (Times relative to entry to Chamber)							
	Pre-dose	0h	15 min	15-45 min	0-60 min	60 min	60-70 min	Immediately after exercise	~~30m rest~~ On exit from chamber	After 30 min rest	60 min after exit from Chamber	
RAS Biomarkers	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
~~Other~~ **~~Biomarkers~~SP-D**	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
PK sampling	X	X[1]	X[2]	X[3]		X[2]		X[2]	X[4]	X[2]		
AE/SAE review		←==========→										
Concomitant medication review	X											

1. ~~To be taken at the end of exercise~~ Take at the end of the infusion
2. Taken immediately after echocardiogram.
3. Immediately before entering the chamber
4. To be taken as soon as possible after leaving chamber
5. **On this occasion ONLY, vital signs to be taken after the blood draw.**

Section 7.2.1.3: Follow-up of AEs and SAEs

Rationale for change: Deletion of reference to AEs of special interest, as no AEs of special interest have been defined in the protocol.

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 Appendix 4.

Section 7.2.2: Physical Exams

Rationale for change: Height and Weight removed from follow-up visit, as not required.

- 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**, at screening only.** ~~This will be carried out at screening and follow up.~~

Section 7.2.3: Vital Signs

Rationale for change: Correction of typographical errors.

- **Triplicate measurements will be taken at screening, and single measurements at other time-points.** ~~Three readings of blood pressure and pulse rate will be taken.~~ The first reading should be rejected. Second and third readings should be averaged to give the measurement to be recorded in the CRF. ~~(Triplicate measurements will only be taken at screening and single measurements to be taken after that).~~

Section 7.2.4: Electrocardiogram (ECG)

Rationale for change: QTc Stopping Criteria removed from Section 7.2.5, and inserted into Section 5.4 with the other subject stopping criteria.

- Triplicate 12-lead ECGs will be obtained at screening. At all other time-points, a single 12-lead ECGs will be obtained ~~at each time point during the study~~ using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 5.4.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes rest.
- The same QT correction formula *must* be used for each individual subject **for all QTc data being collected for data analysis. Safety ECGs and other non-protocol specified ECGs are an exception.**

~~7.2.5 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 QTcB, then QTcB 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 single or 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.~~

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

Section 7.2.5: Clinical Safety Laboratory Assessments

Rationale for change: Correction of typographical errors. Addition of urea to clinical chemistry parameters, to allow the calculation of BUN.

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the 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 date.~~ Details for the preparation and shipment of samples will be provided by the laboratory ~~and are detailed in the laboratory manual.~~ 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.~~

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**			
Haematology	Platelet Count	*RBC Indices*:	*WBC count with Differential:*	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit		Monocytes	
			Eosinophils	
			Basophils	
Clinical Chemistry [1]	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Urea	Sodium	ALT (SGPT)	Total Protein
	Creatinine	Calcium	Alkaline phosphatise	Albumin
	Glucose			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol, smoking and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, benzodiazepines and cotinine) performed at site • Urine hCG Pregnancy test (as needed) [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 5.4.2 and Appendix 2.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

Section 7.3.1: Exercise Challenge

Rationale for change: Clarification of exercise test.

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on a cycle ergometer within the hypoxia chamber for 10 minutes ~~as detailed in the SRM.~~

Subjects must complete a minimum of 5 minutes exercise at 70% of maximumVO2 uptake **(calculated from the incremental exhaustive exercise test at the screening visit).**

Section 7.5: Ventilatory parameters

Rationale for change: Clarification of ventilatory parameter assessment.

Ventilatory parameters will be measured as detailed in the Time and Events table (Section 7.1).

Ventilatory parameters will be recorded for 2 minutes before echocardiograms. The second minute of the recording will be averaged and used for data analysis.

Measurements may include change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2**), total tidal volume, inspiratory tidal volume, expiratory tidal volume, total respiratory time, inspiratory time, expiratory time, duty cycle, mean respiratory flow and respiratory rate** ~~and other parameters~~ as data permit.

~~Further details can be found in the SRM~~

Section 7.7: Telemetry

Rationale for change: Addition of telemetry.

Continuous ECG monitoring will occur via telemetry during both treatment periods.

Section 7.9.1: Renin-angiotensin system biomarkers

Rationale for change: Clarification of archiving procedure.

RAS peptides including, but not limited to, AngII, Ang(1-7) and Ang(1-5) may be analysed as data permit**. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site detailed in the SRM).**

Section 7.9.2: **Surfactant Protein-D and** other biomarkers

Rationale for change: Removal of some exploratory biomarkers.

SP-D will be analysed. Raw data will be archived at the Bioanalytical Site (Bioanalytical Site to be detailed in the SRM). Additional analytes may be analysed, as data permit. ~~Peptide hormones such as Kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) may be analysed as data permit.~~

~~Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined may also be analysed as data permit.~~

Section 9.2: Sample Size Considerations

Rationale for change: Refer to rationale stated for Sections 4.3 and 5.4.1.

The sample size of ~~30~~ **20** patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context (Note: precision in this context is defined as one half of the width of the 95% confidence interval). A sample size re-estimation is planned after ~~15~~ **10** subjects.

Section 9.2.2: Sample Size Re-estimation

Rationale for change: Refer to rationale stated for Sections 4.3 and 5.4.1.

A sample size re-estimation will be conducted at the interim analysis (see Section 9.3.2). The purpose of the sample size re-estimation is to determine whether the total number of subjects can be reduced below the anticipated ~~30~~**20**, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP (i.e. T3-T0). The sample size re-estimation is to be considered advisory, and taken into account together with other considerations.

Section 9.3.1: Analysis Populations

Rationale for change: Correction of typographical errors.

Intent-to-Treat (ITT) Population: This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, run-in failure, or stabilisation failure, but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all ~~efficacy and~~ safety **and pharmacodynamic** analyses.

Section 9.3.2: Interim Analysis

Rationale for change: Refer to rationale stated for Sections 4.3 and 5.4.1.

An interim analysis is planned after ~~15~~ **10** subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods
- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

The interim analysis will use the following study data:

- PASP at time T0 and T3 for both periods, for the first ~~15~~ **10** subjects

- Oxygen saturation at T0 and T3 for both periods, for the first ~~15~~ **10** subjects
- Adverse events for the first ~~15~~ **10** subjects
- ~~Angiotensin biomarker~~**RAS peptide** concentrations for the first 5 subjects (to allow time for the samples to be analysed by the external lab).

The interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

The aims of the interim analysis, and the endpoints to be used to address them, are as follows:

- Using the primary endpoint (treatment differences between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP [T3-T0]):
    - To decide whether to continue the study or stop based on futility, and
    - to conduct an advisory sample-size re-estimation with the view to potentially reduce the number of subjects participating in the study below ~~30~~**20**.
- Using secondary endpoints:
    - to estimate treatment differences between GSK2586881 and Placebo in change from pre-dose oxygen saturation to hypoxic/exercised oxygen saturation [T3-T0], with a view to stopping the study if there is evidence that GSK2586881 causes a reduction in oxygen saturation in healthy volunteers. As a non-binding guide high posterior probability of observing ≥5% absolute differences in **mean** oxygenation saturation values between placebo and active arms would be of concern; but clinical judgement would override any statistical methods if, for example, the majority of subjects displayed consistent patterns of reductions e.g. between 2-3%).
    - to compare adverse events within the two treatment groups and explore any potential safety signals that may emerge

To assess the operating characteristics of the interim analysis decisions to be made based on the primary endpoint, a simulation was carried out to estimate the relative proportion of studies reaching conclusions to stop for futility, continue with no change to the sample size, and to continue with a reduced sample size, under various different scenarios of the true treatment effect. Broadly speaking, the aim of the simulation was to confirm that (in an overwhelming majority of cases) a decision to stop for futility would be reached in the case of no true treatment effect and a decision to continue (perhaps with reduced sample size) would be reached in the case of a true treatment effect.

Simulated datasets of PASP at baseline (i.e. normoxic and at rest) and under hypoxia and exercise for ~~15~~ **10** subjects, each receiving both treatments in sequence, were generated using the following assumptions:

- ~~15~~ **10** simulated subjects were randomised to one of the two available treatment sequences (see Section 6.2) using block randomisation with block size of 4 records

- PASP at baseline and under hypoxia and exercise for both treatment groups (a total of four PASP measurements per subject) were sampled from a multivariate normal distribution, using values for mean PASP, standard deviation of PASP and within-subject correlation of PASP as described below.

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) for both treatments, and 45 mmHg under hypoxia and exercise for Placebo (as in Section 9.2.1). For GSK2586881, mean PASP under hypoxia and exercise was (45 - Δ) mmHg, where Δ (the mean treatment difference) was varied according to four different scenarios:

    - no treatment difference (Δ = 0 mmHg)

    - weak treatment difference of a magnitude of half the clinically meaningful difference (Δ = 2.5 mmHg)

    - minimum clinically meaningful difference (Δ = 5 mmHg)

    - overwhelming treatment difference one-and-a-half times the clinically meaningful difference (Δ = 7.5 mmHg)

- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise, for both treatment groups (as in Section 9.2.1)

- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9) (as in Section 9.2.1). Within-subject correlation of PASP was assumed to be compound symmetric (i.e. the same correlation was used for each of the six possible pairs of the four PASP measurements within each subject).

- It was assumed that there would be no effect of period in the simulation, i.e. it was assumed that it would make no difference if the measurement was taken from the first or second chronological period in the treatment sequence.

Modelling took on a three-step process, with decisions after each. Firstly, the treatment difference and its associated standard error was then estimated for the dataset of ~~15~~ **10** simulated subjects using a mixed effects linear regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. If the sign of treatment difference arising from the simulated data was in the wrong direction (i.e. the simulated outcome for GSK2586881 was worse than for Placebo), then the simulated study was stopped for futility.

Secondly, in the alternative case that the simulated treatment difference was in favour of GSK2586881 over Placebo, a posterior probability of success (where success was defined as a 97.5% posterior probability of the treatment difference being in favour of GSK2586881 over Placebo) was evaluated for ~~30~~ **20** subjects. This was evaluated using a t-distribution, with the mean and standard deviation taken as the treatment difference and standard error (multiplied by a factor of the square root of ~~15~~ **10** to estimate the standard deviation) from step one, respectively. If this posterior probability was less than 0.2, then the study was stopped for futility as being unlikely to show any benefit of GSK2586881 over Placebo.

Thirdly, provided that the simulated study hadn't been stopped for futility in either of the first two steps, posterior probabilities of success were calculated for N ranging from ~~15~~ **10** to ~~29~~ **19** subjects. If any of these posterior probabilities of success were greater than or equal to 0.9, then the study was continued with a reduced sample size equal to the lowest N at which that threshold was reached. Otherwise, the conclusion from the simulated interim analysis would be to continue the study with unchanged sample size.

This whole process (simulating data for ~~15~~ **10** subjects, modelling the treatment difference, evaluating posterior probabilities of success and simulating the decision-making process) was replicated 1**0**000 times for each scenario, with the decision ("futile", "no change to N" or "reduce N") recorded for each iteration. Each combination of the four treatment difference scenarios and the four correlation scenarios was evaluated for a total of 16 scenarios in all.

The estimated proportions of each decision being reached under each of the 16 scenarios are presented in Table 4.

Table 4 Operating characteristics of interim analysis at N=10 under four scenarios of treatment effect and four scenarios of within-subject correlation

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with **20** ~~30~~ subjects	Continue with reduced sample size
Δ=0 mmHg (no treatment difference)				
	0.0	~~87~~ **85**%	~~13~~ **15**%	~~0~~ **1**%
	0.3	~~87~~ **85**%	~~13~~ **15**%	1%
	0.6	85%	15%	~~0~~ **1**%
	0.9	~~86~~ **85**%	14%	~~0~~ **1**%
Δ=2.5 mmHg (weak treatment effect, less than clinically meaningful difference)				
	0.0	**44**~~31~~%	**50** ~~60~~%	~~9~~ **6**%
	0.3	**36**~~24~~%	**56** ~~65~~%	~~11~~ **9**%
	0.6	**21**~~10~~%	**63** ~~66~~%	~~25~~ **16**%
	0.9	**1** ~~0~~%	**31**~~9~~%	~~91~~ **68**%
Δ=5 mmHg (good treatment effect, at threshold of clinical meaningful difference)				
	0.0	**9** ~~2~~%	**60** ~~46~~%	**31** ~~52~~%
	0.3	**4** ~~1~~%	**54** ~~35~~%	**42** ~~64~~%
	0.6	**1** ~~0~~%	**31** ~~11~~%	**68** ~~89~~%

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with **20** ~~30~~ subjects	Continue with reduced sample size
	0.9	0%	0%	100%
Δ=7.5 mmHg (very strong treatment effect)				
	0.0	**1** ~~0~~%	**32** ~~10~~%	**67** ~~90~~%
	0.3	0%	**18** ~~2~~%	**82** ~~98~~%
	0.6	0%	**3** ~~0~~%	**96** ~~100~~%
	0.9	0%	0%	100%

These results suggest primarily that if there is no true treatment difference between GSK2586881 and Placebo, the study would be stopped for futility **85**~~87~~% of the time (irrespective of within-subject correlation of PASP). A drug with a weak treatment effect would most likely result in the study being continued without change or stopped for futility (except in the case where within-subject correlation is 0.9, which is presented here as an extreme case, unlikely to occur in practice). If the true treatment effect is at the clinically relevant threshold of 5 mmHg, then the study is ~~highly~~ unlikely to be stopped for futility and may indicate being reduced in sample size.

Section 9.4: Key Elements of Analysis Plan

Rationale for change: Correction of typographical errors.

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five **key** sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

~~Pharmacodynamic endpoints~~ **PASP and Oxygen Saturation** will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day. **Biomarkers will be collected at times T0 thru T4, plus additional time-points.**

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline. In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines). ~~as fixed effects and s~~Subject **will be fitted** as a random effect **and** non-informative priors **will be used** for model parameters. A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period.

Section 12.2: Appendix 2: Liver Safety Required Actions and Follow up Assessments

Rationale for change: Correction of typographical error.

Footnote 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.

Section 12.4.1: Definition of Adverse Events

Rationale for change: Removal of AE definitions related to efficacy, because this is not a patient study.

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

~~of an AE or SAE.~~

- ~~The signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.~~

TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	The effects of GSK2586881 on the responses to acute hypoxia and exercise

Compound Number: GSK2586881

Development Phase: I

Effective Date: 24-FEB-2017

Protocol Amendment Number: 01

Author (s): PPD

Revision Chronology

GlaxoSmithKline Document Number	Date	Version
2016N283626_00	2016-SEP-23	Original
2016N283626_01	2017-FEB-24	Amendment No. 1
Additional Inclusion Criteria (Section 5.2) and Subject Stopping Criteria (Section 5.4) added in response to comments from BfArM. A summary of changes is presented in Appendix 6.		

SPONSOR SIGNATORY

PPD

24 Feb 2017

Dr Aili Lazaar — Date

Director, Discovery Medicine
Project Physician Leader, Respiratory Therapy Area

PPD

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	Site Address
Primary Medical Monitor	PPD				709 Swedeland Road UW2531 King of Prussia, PA 19406 USA
Secondary Medical Monitor					Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
SAE contact information	[Medical monitor as above]				

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): 2016-002465-55

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol 204987

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 204987

Rationale

The purpose of this study is to examine how GSK2586881, a recombinant human ACE2 peptide, modulates the acute hypoxic pulmonary vasoconstriction (HPV) response in healthy volunteers.

HPV is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO_2) levels in health and in pathophysiological settings such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. The literature supports a role for the renin angiotensin system (RAS) in driving acute HPV and while there is a strong biological rationale for modulation of RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they blunt the normal HPV response and negatively impact on arterial oxygenation (PaO_2). Thus, from a safety (and efficacy) perspective it is important to understand the impact of modulation of the RAS system by GSK2586881 on the acute HPV response.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.

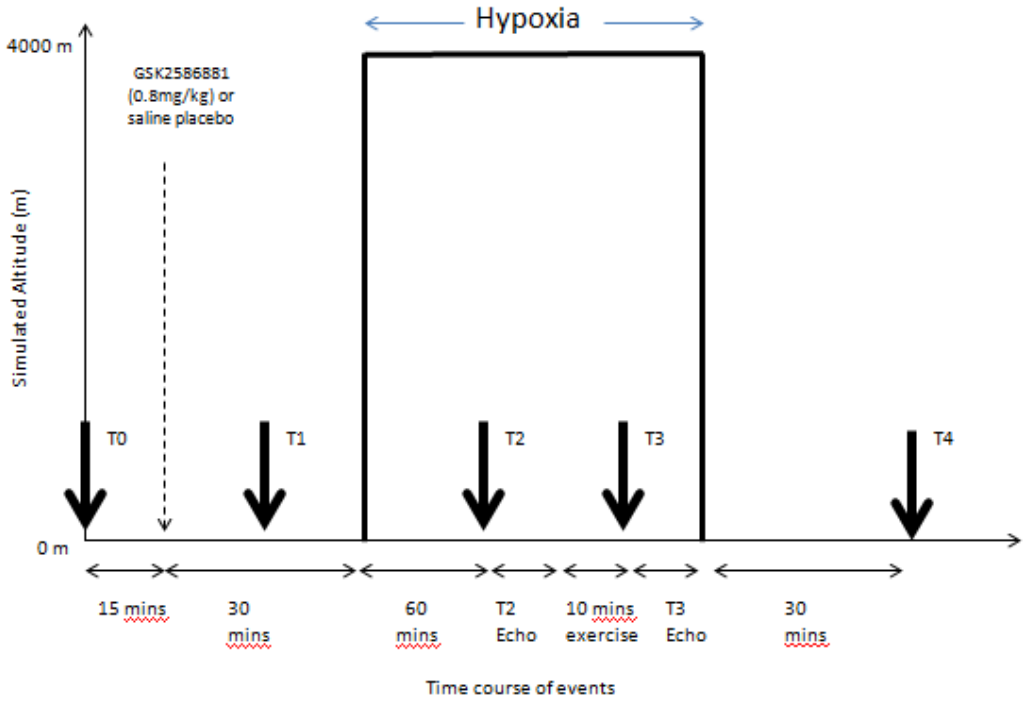
Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the effect of a single IV dose of GSK2586881 on peptide hormone responses to hypoxia and exercise.	• Baseline levels and changes in response to hypoxia and exercise in kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in other biomarkers which may include Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined.
• To evaluate Pharmacogenetics	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

Overall Design

The study will be single-centre , randomised, placebo-controlled and double blind (sponsor open). Subjects will be randomised to receive a single IV dose of GSK2586881 or saline in a crossover design.

A schematic of the study is shown below. Echocardiograms approximate timings are indicated by bold arrows.

APPROXIMATE TIMING OF SIMULATED ALTITUDE AND ECHOCARDIOGRAM MEASUREMENTS (BLACK ARROWS) DURING TREATMENT PERIODS 1 AND 2.



Treatment Groups, Randomisation Arms and Duration

The study is intended to follow a double blind (sponsor open) two-period cross-over design.

Treatment Group A: GSK2586881 0.8 mg/kg, administered as a single IV dose. .

Treatment Group B: matching volume of placebo administered as a single IV dose.

Subjects will receive both treatments during the course of the study, and will be randomised to one of two sequences, each of which describes the order in which those treatments are received:

Sequence 1: AB: GSK2586881 in the first period followed by placebo in the second.

Sequence 2: BA: Placebo in the first period followed by GSK2586881 in the second.

The total study duration for each subject is expected to be a maximum of 56 days.

Type and Number of Subjects

Approximately 35 subjects will be enrolled to ensure that a minimum of 30 subjects complete all dosing and critical assessments (the target of 30 may be revised by the sample size re-estimation).

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

Analysis

This study is designed to estimate the effect of GSK2586881 relative to placebo on change from baseline in PASP following exercise under hypoxic conditions. No formal hypothesis will be tested. Point estimates will be calculated together with corresponding 95% confidence intervals (or credible intervals if utilising a Bayesian framework) for the difference between the mean of the test treatment and the mean of the reference treatment.

Analysis of PASP: Statistical modelling of changes from baseline (Ti-T0) will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period. Non-informative priors will be used for the model parameters. Posterior distributions will be obtained for the GSK2586881 Vs Placebo comparisons at each of the post dosing timepoints (T1-T4). These distributions will be used to produce several posterior probability statements; the most important being the probability that the change from baseline in PASP is reduced by the GSK2586881at time T3.

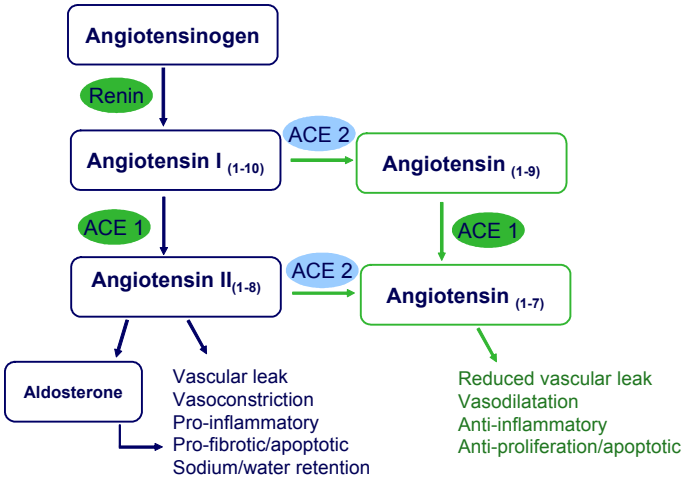
Oxygenation saturation will also be analysed in a similar way; although of interest is the posterior probability that the study drug causes (absolute) reductions in oxygenation saturation in excess of 5%.

An interim analysis is planned after 15 subjects have completed period 1 and 2 assessments to i) evaluate safety and tolerability (SAE, AE and Oxygenation saturation), ii) determine whether the study should stop for futility based on expected PASP outcomes if the study were allowed to complete recruitment and iii) Re-estimate the sample size based using the observed variance parameters.

2. INTRODUCTION

GSK2586881 is a purified intravenous formulation of soluble recombinant human angiotensin converting enzyme type 2 (rhACE2) which is expressed in Chinese Hamster Ovary cells. Angiotensin converting enzyme type 2 (ACE2) is a zinc carboxypeptidase involved in the Renin-Angiotensin System (RAS) that controls blood pressure, electrolytes, and intravascular fluid volume. A key function of ACE2 is believed to be the cleavage of Angiotensin II (Ang II) to Ang (1-7), which have opposing physiological roles. Elevated levels of Ang II are associated with vasoconstriction, inflammation, fibrosis, vascular leak, and sodium absorption. Ang (1-7) appears to be a counter-regulatory protein in the RAS; associated with vasodilation, anti-proliferation, anti-inflammation, and reduced vascular leak, as noted in Figure 1 below [Paul, 1992; Santos, 2005; Suzuki, 2003].

Figure 1 Renin Angiotensin System



Ang II binds to two distinct receptors called AT-1 and AT-2, with the AT-1 receptor mediating the vasoconstrictive, proliferative and pro-inflammatory actions of Ang II. The function of the AT-2 receptor has not been fully elucidated. Ang (1-7) initiates its effects by binding to the Mas-receptor, and also acts by inhibiting the activity of the carboxyterminal domain of angiotensin converting enzyme (ACE), which prevents ACE from fully acting on its substrates Angiotensin I and bradykinin.

ACE and Angiotensin II has been implicated in the pathogenesis of the acute respiratory distress syndrome (ARDS, and pulmonary hypertension. It has been observed that circulating Ang II levels and lung ACE levels are increased in humans with ARDS and pulmonary hypertension. It has also been shown that the DD ACE polymorphism, which is associated with higher ACE activity, is associated with susceptibility to development of lung injury and worsened outcome (mortality) in patients with ARDS [Marshall, 2002] and to the development of pulmonary hypertension [Abraham, 2003]

It is expected that the reduction of Ang II and simultaneous formation of Ang (1-7) should have positive impacts in ARDS and on pulmonary haemodynamics in patients with pulmonary hypertension. This dual action can be achieved by ACE2, and thus, an enhancement of the activity of this enzyme is seen as promising approach for the treatment of diseases and conditions with an imbalance of the RAS system, insufficient natural ACE2 activity, and pathologically elevated Ang II levels or decreased Ang (1-7) such as is observed in ALI/ARDS [Tom, 2001; Idell, 1987; Santos, 2003; Wenz, 2000] and pulmonary hypertension [Maron, 2014]

This study will demonstrate how GSK2586881 modulates the acute HPV response in healthy volunteers.

2.1. Study Rationale

Hypoxic pulmonary vasoconstriction (HPV) is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO_2) levels in health and in pathophysiological settings such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. However, in many ARDS patients with pulmonary microvascular injury and dystonia, the normal HPV reflex is compromised resulting in the mismatching of perfusion (Q) and ventilation (V_A) and formation of areas of low perfusion to ventilated alveoli (high V_A/Q; physiological deadspace), or perfusion of alveoli with minimal or no ventilation (low V/Q; physiological shunt).

The literature is conflicting as to the role of the RAS in modulating the HPV response; however, the majority of reports support a role for the RAS in driving acute HPV (Cargil, 1996; Kiely, 1996). While there is a strong biological rationale for modulation of the RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they impact on PaO_2 and produce a blunted HPV response. Surprisingly, there are scant data regarding the effect of RAS modulation on HPV, V-Q matching or PaO_2 in healthy human volunteers.

Inhibition of the acute HPV response would be expected to promote blood flow to areas of poorly-ventilated lung resulting in an increase in venous admixture and a reduction in PaO_2 levels. In the context of acute lung injury, correction of any V/Q mismatch would be expected to be beneficial with respect to maintaining and improving PaO_2 levels. Conversely, inhibition of HPV by pharmacological agents might be expected to worsen V/Q matching and compromise PaO_2 levels. Thus, from a safety (and efficacy) perspective it is vital to understand the impact of modulation of the RAS system on the acute HPV response and subsequent PaO_2.

Recombinant human ACE2 (rhACE2) delivered intravenously to pigs inhibits the HPV response to acute hypoxic challenge assessed by inhibition of mean pulmonary artery pressures and pulmonary vascular resistance. There was also a strong trend to increased shunt with administration of rhACE2; however PaO2 was not significantly affected. The authors concluded that the increased shunt may not have been sufficient to result in a reduction in PaO_2 (Kleinsasser, 2012).

An elegant study by Wagner and colleagues demonstrated the importance of end arterial capillary diffusion limitations on PaO_2 levels in healthy volunteers during hypoxia ± exercise (Torre-Bueno, 1985). Diffusion limitation made a significant contribution to PaO_2 levels particularly under conditions where exercise and hypoxia were combined. In addition, V/Q mismatching was suggested to increase with the combined stresses of exercise and hypoxia resulting in significantly more arterial oxygen desaturation than observed with either stressor by itself.

The main goals of this study are to examine whether GSK2586881 modulates the acute HPV response in healthy volunteers with a subsequent impact on O_2 saturation. Should the application of GSK2586881 lead to accentuated arterial oxygen desaturation, further clinical studies to examine the therapeutic efficacy of GSK2586881 in acute lung injury should be approached with caution. Conversely, a reduction in HPV without augmented hypoxemia would provide supporting evidence that GSK2586881 could have a positive impact in patients with pulmonary hypertension.

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.

Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the effect of a single IV dose of GSK2586881 on peptide hormone responses to hypoxia and exercise.	• Baseline levels and changes in response to hypoxia and exercise in kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in other biomarkers which may include Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined.
• To evaluate Pharmacogenetics	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

4. STUDY DESIGN

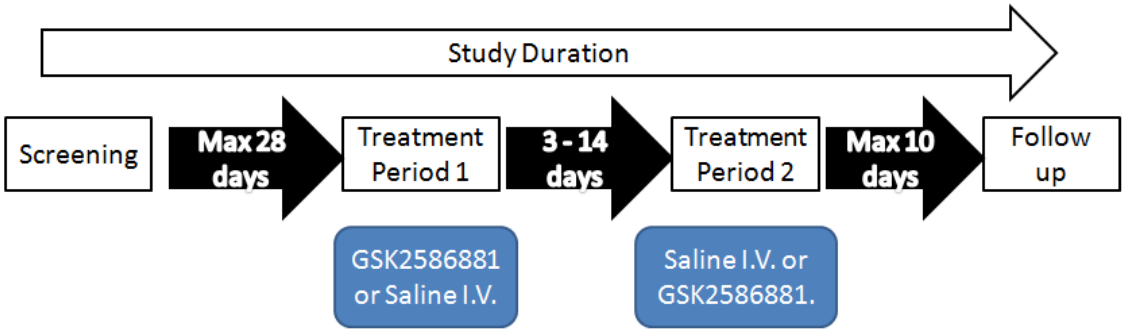
4.1. Overall Design

This is a single-centre, randomised, placebo-controlled and double blind (sponsor open), two-period crossover study in healthy subjects.

The subjects will be required to attend the unit for a screening visit, treatment period 1, treatment period 2 and a follow up visit.

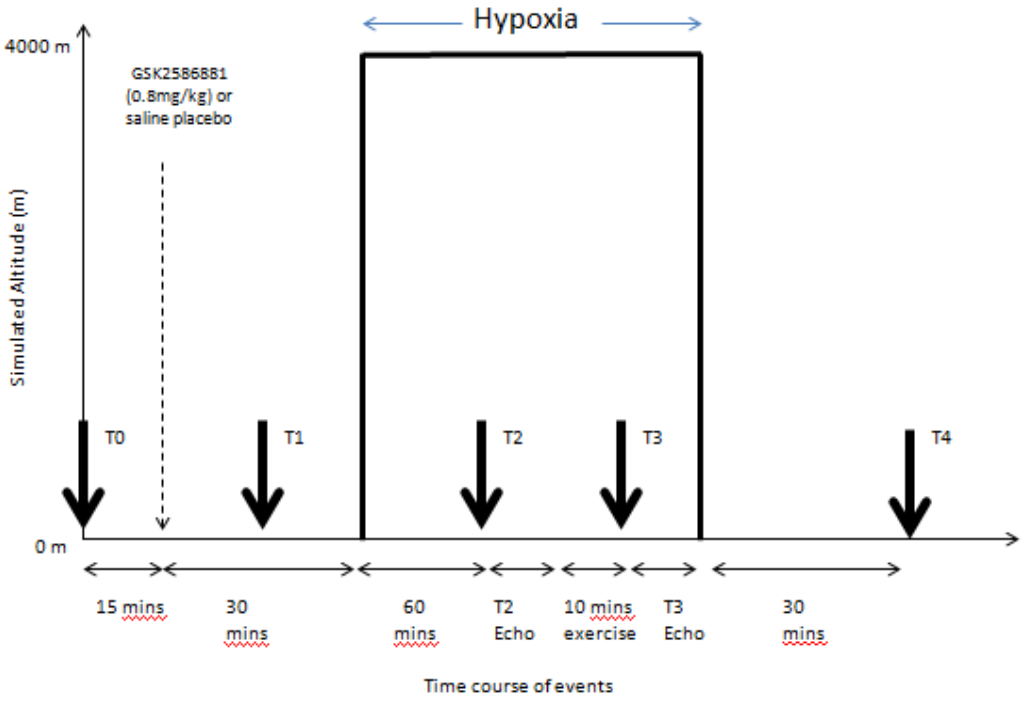
4.2. Treatment Arms and Duration

Figure 2 Subject participation flow



The subjects must participate in the procedures detailed in the Time and Events Table (Section 7.1) and the timings of the simulated altitude, exercise and echocardiograms is shown in Figure 3.

Figure 3 Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2



There will be a washout period of 3-14 days between treatments to ensure biomarkers return to pre-challenge baseline. Subjects then return to the site and repeat the same procedures as above, except that they will receive the treatment (GSK2586881 or Saline) that they did not receive in the first period.

Follow Up

This will occur up to 10 days after the end of the second treatment period. During the visit various safety tests will be conducted (see time and events table in Section 7.1).

The total study duration for each subject is expected to be a maximum of 56 days.

4.3. Type and Number of Subjects

Approximately 35 subjects healthy volunteers will be randomised such that approximately 30 evaluable subjects complete the study (the target of 30 may be revised following the sample size re-estimation) see Section 9.2.2).

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

4.4. Design Justification

The study design is based on a paper published by Ricart, 2005 and is considered to be feasible. The study will provide important information on whether GSK2586881 modulates the acute HPV response in healthy volunteers. In addition, the study will include assessments of PK and PD effects of GSK2586881. This will be achieved by assessing blood levels of GSK2586881 and RAS peptide responses throughout the duration of the hypoxia challenges.

The study will be placebo controlled (saline) so each subject can be used as their own control.

4.5. Dose Justification

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). One subject with Pulmonary Arterial Hypertension (PAH) has received a single dose of 0.2 mg/kg.

A population PK model for GSK2586881 was derived from data obtained in healthy subjects and ARDS patients and showed that the systemic PK profile was adequately described by a two-compartment first order elimination model and that the PK profile was independent of population (health subjects or ARDS patients). Furthermore the PKPD response (AngII) in healthy subjects and ARDS patients was consistent with a single direct Emax model after accounting for differences in baseline AngII concentrations between healthy subjects and ARDS patients.

Based on the population PK/PD model described above, single intravenous doses of 0.4 - 1.2 mg/kg GSK2586881 are predicted to reduce elevated levels of AngII (baseline AngII consistent with an ARDS population) to levels consistent with that observed in healthy subjects for the duration of the hypoxic challenge (approx 2.25h). The dose of 0.8 mg/kg has been selected to ensure maximal reduction of AngII levels, whilst maintaining dosing volumes within acceptable limits, and will further aid the understanding of the PK/PD relationship for GSK2586881.

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with GSK2586881 can be found in the Investigator's Brochure (GlaxoSmithKline Document Number 2010N108777_00, 2015). The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2586881]		
Cardiovascular risk	During preclinical testing a brief period of non-sustained ventricular tachycardia was observed in one monkey receiving a dose of 20.8 mg/kg/day.	The dose used in this study is much lower.and well below the No observed adverse effect level (NOAEL) for the 14 day repeat dose cynomolgus monkeys of 8 mg/kg/day.
Potential Reproductive/embryofetal risks	Preclinical studies have not been performed	Women of childbearing potential will be excluded from the study.
Potential for Immunogenicity	There has been no induction of an immune response to rhACE2 in either of the clinical studies to date in healthy subjects or participants with ARDS.	Patients will have routine monitoring of any immunological response that may occur. If an immunological response is seen the patient will be asked to return for further monitoring and assessment(s).
Potential for rash	In study ACE114622, rash was reported more frequently in subjects receiving GSK2586881, although only one event was considered drug-related.	Patients will be monitored for rash in the clinical trials.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Effects of hypoxia (light headiness, headaches, nausea)	The subjects will be exposed to hypoxic conditions for approximately 90 minutes with a simulated altitude of 4000m	The subjects will be continuously observed and monitored with telemetry and pulse oximetry. Stopping criteria are included in the protocol.

4.6.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the subjects taking part in the study as it is a healthy volunteer study. However, subjects will undergo a medical evaluation during screening including physical exams, ECGs and laboratory assessments which may provide important health information.

By taking part in this study the subject will be contributing to the development of GSK2586881 for the treatment of pulmonary hypertension and ARDS.

4.6.3. Overall Benefit:Risk Conclusion

The design of the study is considered low risk to the subjects and justified based on the work carried out by other researchers, the safety information from the nonclinical studies and the two previous clinical trials carried out on GSK2586881.

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 the IB.

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 40 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring. 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 Medical Monitor if required] agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures. Note: Screened subjects with laboratory values outside of the normal range may be

repeated once for inclusion into the study at the discretion of the Investigator.

3. Screening echocardiogram of good quality, without clinically significant abnormalities, and with mild-moderate tricuspid regurgitation sufficient for the reliable estimation of PASP, as determined by the echocardiography core laboratory or responsible cardiologist.

Screening PASP within the normal range according to site standards.

4. Subjects have not resided at an altitude >1500m for more than 7 days in the last 4 month

5. Able to complete all study procedures.

6. Any contraindication (orthopaedic, cardiac etc.) to perform exercise on a bicycle ergometer.

WEIGHT
7. Body weight 50-100 kg (inclusive).

SEX
8. Male or female (non CBP)

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication.

a. Vasectomy with documentation of azoospermia.

b. Male condom plus partner use of one of the contraceptive options below:

- Contraceptive subdermal implant
- Intrauterine device or intrauterine system
- Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a]
- Contraceptive vaginal ring [Hatcher, 2007a]
- Percutaneous contraceptive patches [Hatcher, 2007a]

This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g., male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH, M3 (R2) 2009]."

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

This is an all inclusive list of those methods that meet the GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and, correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by ICH [ICH, M3 (R2) 2009].

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Females:

A female subject is eligible to participate if she is not pregnant (as confirmed by a negative urine human chorionic gonadotrophin (hCG) test), not lactating, and the following condition applies:

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
- Postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels)]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 7.2 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. ALT >1.5x Upper limit of normal (ULN). 2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%). 3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones). 4. Prior history of altitude sickness. 5. Recently been scuba diving (within 1 week before screening). 6. 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
7. Unable to refrain from prescription or non-prescription drugs, including agents active in the central nervous system, 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 and throughout the study, unless in the opinion of the Investigator and/or GSK Medical Monitor (if needed) the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
8. 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 9. Urinary cotinine levels indicative of smoking or history or regular use of tobacco- or nicotine-containing products within 6 months prior to screening

CONTRAINDICATIONS
10. 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
11. 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. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody (HBcAb) should also be excluded. 12. A positive pre-study drug/alcohol screen. 13. A positive test for HIV antibody. 14. Where participation in the study would result in donation of blood or blood products in excess of 500 ml within 56 days. 15. 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: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 16. 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 randomised. 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 (Section 7.2.1.4).

Subjects that are not enrolled into the study within the allotted screening window may be re-screened once. If re-screening is performed, subjects are assigned a different unique subject Identification (ID) number for the re-screening, and all screening procedures must be repeated.

See the SRM for specific details.

5.4. Withdrawal/Stopping Criteria

Subjects may be withdrawn from the study for any of the following reasons: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.

- If a subjects oxygen saturation falls below 65% at any point during the hypoxia challenge.
- If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

Once a subject has discontinued investigational product the subject may not re-enter the study. Dosing of the subjects with the investigational product may be stopped at any time, at the request of the subject or at the discretion of the Investigator (i.e. if clinically significant adverse events should occur). Withdrawal due to adverse events will be distinguished from withdrawal for other reasons.

If a subject decides to withdraw or is withdrawn by the Investigator, the reasons for withdrawal and the results of any relevant tests will be recorded in the Case Report Form (CRF) and the planned follow-up procedures will be performed, where possible.

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".

5.4.1. Study Stopping Criteria

An interim analysis is planned after approximately 15 subjects have completed treatment periods 1 and 2 and the study may be stopped if a reasonable change in PASP is not observed after dosing with GSK 2586881 or if review of the safety data suggests a change in the benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.

Safety data that is collected on a continuous basis (oxygen saturation and telemetry) will be monitored live on site to determine whether a subject tolerates the hypoxia/exercise challenge; study procedures may be aborted on safety grounds if, in the Investigator's opinion, any of these measurements reach unsafe levels. The simulated altitude within the chamber may be reduced if too many participants fail to tolerate the challenge, and the study may be stopped if participants fail to tolerate the challenge at the minimum altitude. The full criteria for this procedure (which may lead to the study being stopped) are described in Section 7.3.

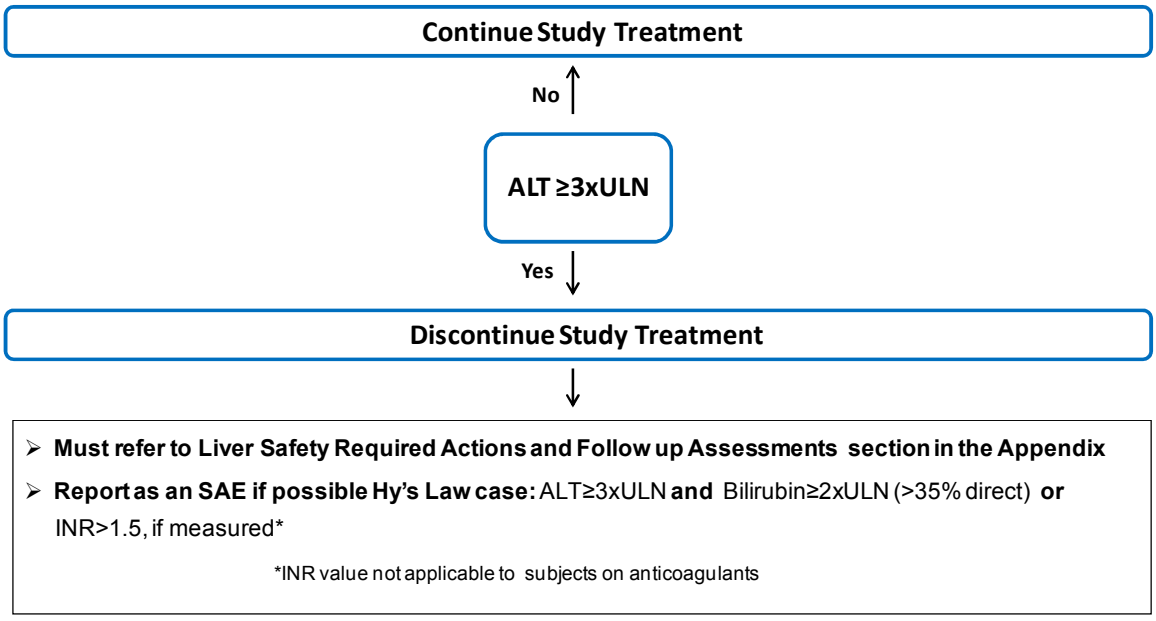
In addition, the study may be stopped at the interim analysis for a safety signal in oxygen saturation under hypoxia and exercise, combined with a lack of evidence of mechanistic effect on Angiotensin biomarkers (Ang II, Ang 1-7 and Ang 1-5). Full details on stopping criteria are described together with the full procedure for the interim analysis in Section 9.3.2.

5.4.2. 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 Food and Drug Administration (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.

5.4.2.1. Study Treatment Restart or Rechallenge

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

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 subjects 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 Treatment	
Product name: (Generic name and trade)	GSK2586881	Placebo
[Formulation description:]	rhACE2	Normal Saline (0.9%)
Dosage form:	IV	IV
Unit dose strength(s)/Dosage level(s):	0.8 mg/kg	Saline Placebo
Route of Administration E.g. oral, for IV infusion, for IV injection, intravitreal use etc	Intravenous	Intravenous
Dosing instructions:	Infuse over 3-5 minutes	Infuse over 3-5 minutes
[Physical description:]	Clear colourless liquid	Clear colourless liquid

At Screening a unique Subject Number (CRF number) will be assigned to any subject who has at least one Screening procedure performed, other than informed consent. The unique Subject Number will be used to identify individual subjects during the course of the study.

Subjects who meet screening eligibility criteria will be assigned to one of the sequences listed in Table 1 below, in accordance with the randomisation schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software. Once assigned, this number must not be reassigned to any other subject in the study.

Table 1 Description of sequences

Sequence	**Period 1**	**Period 2**
AB	GSK2586881	Saline Placebo
BA	Saline Placebo	GSK2586881

The subjects will be randomised using a central randomisation procedure created by GSK.

Further details on how and when a subject is allocated a randomisation number and the subject numbering convention is in the SRM

6.2. Planned Dose Adjustments

No dose adjustments are allowed.

6.3. Blinding

This will be double blind (sponsor open) study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff), and the subject will be blinded to the treatment allocated to individual subjects. An unblinded qualified staff member will be required at site to prepare the study treatment for dosing. The unblindedstaff member is not permitted to communicate the subject's treatment allocation to blinded site staff. Selected study team members working for the sponsor (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This will include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report.

The following will apply:

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment.
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the CRF

A subject whose treatment sequence assignment is unblinded for emergency reasons (as described above) will not be permitted to continue in the study (due to the emergency requiring unblinding rather than the unblinding itself). The event or condition that led to the unblinding will be recorded in the CRF as the primary reason for discontinuation.

A subject whose treatment sequence assignment is inadvertently unblinded (either to investigative staff or the subject themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the subject will be subject to review as to their inclusion in analyses as per the procedures set out in Section 9.3.1.

6.4. Packaging and Labeling

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

6.5. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for GSK2586881 will be detailed in a Study Specific Technical Agreement/Memo (TTS) or Pharmacy Manual which will be accompanied by a Quality Agreement.

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
- 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 Study Reference Manual (SRM).
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.
- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

6.6. Compliance with Study Treatment Administration

When the individual dose for a subject is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

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.

GSK2586881 and the saline placebo will be intravenously administered to subjects at the site. Administration will be documented in the source documents and reported in the CRF.

6.7. Treatment of Study Treatment Overdose

For this study, any dose of GSK2586881 > 1.5 mg/kg within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose. The investigator will use clinical judgment to treat any overdose.

In the event of an overdose the investigator or treating physician should:

1. contact the Medical Monitor immediately
2. closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until GSK2586881can no longer be detected systemically (at least 3 days for GSK2586881).
3. obtain a plasma sample for pharmacokinetic (PK) analysis within 1 day from the date of the last dose of study treatment 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.

6.8. Treatment after the End of the Study

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

6.8.1. Meals and Dietary Restrictions

- No dietary restrictions prior to first treatment.

6.8.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from caffeine for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.

- During each dosing session, subjects will abstain from alcohol for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.
- Use of tobacco products is not allowed as outlined in the exclusion criteria.

6.8.3. Activity

Subjects will abstain from strenuous exercise for 24 hours prior to screening and each treatment period Subjects may participate in light recreational activities between the planned study procedures (e.g., watch television, read).

6.9. Concomitant Medications and Non-Drug Therapies

6.9.1. 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.

6.9.2. 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. Echocardiograms
    2. 12-lead ECG
    3. vital signs
    4. 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, including: safety, echocardiogram, pharmacokinetic, pharmacodynamic/biomarker or other 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 Institutional Review Board (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

7.1.1. Screening and Follow up

Procedure	**Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)**	**Follow up (7- 10 days post lastdose)**	**Notes**
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam including height and weight	X	X	Weight at screening will be used for dosing calculation.
Alcohol, DoA , Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: [Drugs, Alcohol, tobacco]
Past and current medical conditions [including cardiovascular medical history]	X		
[Serum OR urine] pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries	X	X	Non Fasting
Immunogenicity	X	X	
12-lead ECG)	X	X	Triplicate ECG required at screening.
Vital signs)	X	X	Triplicate vital signs required at screening.
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		Tolerance to 4000m for 10 mins followed by followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max for the exercise challenge d
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

1. Screening assessments are allowed to be conducted on more than one day

7.1.2. Treatment Period 1 and 2

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to dosing				Hypoxia (Times relative to entry to Chamber)							
	Predose	0h	15 m	15-45m	0-60m	60m	60-70m	Immediately after exercise	30m rest on exit from chamber	After 30 mins rest	60 minutes after exit from Chamber	
Randomisation	X											Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X			X	
Immunogenicity	X											Treatment period 2 only
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X		X		Echo duration approx 5 mins
Subject enters chamber					X							Subject enters chamber approximately 30 minutes after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram and vital signs have been taken.
Exercise challenge							X					For approx 5-10 minutes
Ventilatory parameters	X		X			X	X[1]			X		Measurements to be taken at the same time as the Echocardiograms. During the exercise challenge, an ECG integrated with the ventilatory assessment will be carried out.
Pulse Oximetry (O2 saturation)		←----------									→	Will be continuously monitored for safety. Measurement should be recorded at time of each echocardiogram post-treatment and databased.
RAS Biomarkers	X	X[2]	X[3]	X[4]		X[3]		X[3]	X[5]	X[3]		
Other Biomarkers	X	X[2]	X[3]	X[4]		X[3]		X[3]	X[5]	X[3]		
PK sampling	X	X[2]	X[3]	X[4]		X[3]		X[3]	X[5]	X[3]		

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to dosing				Hypoxia (Times relative to entry to Chamber)							
	Predose	0h	15 m	15-45m	0-60m	60m	60-70m	Immediately after exercise	30m rest on exit from chamber	After 30 mins rest	60 minutes after exit from Chamber	
AE/SAE review		←==========→										
Concomitant medication review	X											

1. To be taken at the end of exercise
2. Take at the end of the infusion
3. Taken immediately after echocardiogram
4. Immediately before entering the chamber
5. To be taken as soon as possible after leaving chamber

After written informed consent, screening assessments will be performed as outlined in the Time and Events Table (Section 7.1).

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

Cardiovascular medical history/risk factors (as detailed in the CRF) will be assessed at screening.

Cardiorespiratory fitness during bicycle exercise and hypoxia tolerance will be assessed.

Procedures conducted as part of the subject's routine clinical management (e.g. blood count) and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

7.2. 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 exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

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

The definitions of an AE or SAE can be found in Appendix 4.

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

7.2.1.1. 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 7.2.1.3), 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 Appendix 4.

- 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 Appendix 4

7.2.1.2. 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?"

7.2.1.3. 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 Appendix 4.

7.2.1.4. 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.

7.2.2. 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. This will be carried out at screening and follow up.
- A brief physical examination will include, at a minimum assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). To be carried out at the start of each period.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

7.2.3. 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. Three readings of blood pressure and pulse rate will be taken.
- First reading should be rejected.
- Second and third readings should be averaged to give the measurement to be recorded in the CRF. (Triplicate measurements will only be taken at screening and single measurements to be taken after that).

7.2.4. Electrocardiogram (ECG)

- Triplicate 12-lead ECGs will be obtained at screening. At all other time-points a single 12-lead ECGs will be obtained at each time point during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 7.2.5 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes rest.

7.2.5. 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 QTcB, then QTcB 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 single or 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.

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

7.2.6. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the 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 date. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the laboratory manual. 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.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**			
Haematology	Platelet Count	*RBC Indices*:	*WBC count with Differential:*	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit		Monocytes	
			Eosinophils	
			Basophils	
Clinical Chemistry [1]	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium	Alkaline phosphatise	Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol, smoking and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, benzodiazepines and cotinine) performed at site • Urine hCG Pregnancy test (as needed) [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 5.4.2 and Appendix 2.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within 3 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.

7.3. Hypoxia Challenge

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 4000m ± 10%. hypoxic conditions

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O2 saturation of <65%, then the simulated altitude will be adjusted in decrements of 500m (i.e. from 4000m to 3500m) for all remaining subjects to a minimum of 3000m.

Further details about the hypoxia challenge is detailed in the SRM.

7.3.1. Exercise Challenge

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on a cycle ergometer within the hypoxia chamber for 10 minutes as detailed in the SRM.

Subjects must complete a minimum of 5 minutes exercises at 70% of maximum$_{max}$ $_{max}$VO2 uptake.

Further details about the exercise challenge is detailed in the SRM.

7.4. Echocardiogram

Echocardiograms will be taken as detailed in the Time and Events table (Section 7.1).

Echocardiograms will be obtained with the subject resting supine or lying on their left side.

PASP will be measured and recorded.

PASP will be determined by measuring maximal tricuspid regurgitation velocity and applying the modified Bernoulli equation to convert this value into pressure values. Estimated right atrial pressure (RAP) must be added to this obtained value.

Further details about the PASP measurement is detailed in the SRM.

Additional echocardiograms may be obtained for each subject as needed (in the judgement of the Investigator and GSK Medical Monitor if required.

Further details can be found in the SRM.

7.5. Ventilatory parameters

Ventilatory parameters will be measured as detailed in the Time and Events table (Section 7.1).

Measurements may include change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit

Further details can be found in the SRM.

7.6. Pulse Oximetry

Oxygen saturation will be monitored continuously via pulse oximetry as detailed in the SRM. Measurements to be taken at the same time as the echocardiograms and recorded in the eCRF.

7.7. Pharmacokinetics

7.7.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of GSK2586881 will be collected at the time points indicated in the Time and Events Table (Section 7.1). 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.

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

7.7.2. Sample Analysis

Plasma analysis will be performed under the control of PTS, In Vitro/In Vivo Translations (IVIVT) Department and Third Party Resourcing (TPR), GlaxoSmithKline. The details of the Bioanalytical Laboratory will be included in the Study Reference Manual (SRM). Concentrations of GSK2586881 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will archived at the Bioanalytical Site (to be detailed in the SRM).

7.8. Biomarker(s)/Pharmacodynamic Markers

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

Details of biomarker/PD blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.8.1. Renin-angiotensin system biomarkers

RAS peptides including but not limited to AngII, Ang(1-7) and Ang(1-5) may be analysed as data permit.

7.8.2. Other Biomarkers

Peptide hormones such as Kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) may be analysed as data permit.

Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined may also be analysed as data permit.

7.9. Genetics

Information regarding genetic research is included in Appendix 3.

Genetic sampling is optional. Subjects can refuse PGx sampling but will still be allowed to participate in the study.

7.9.1. Blood sample collection

Blood samples to investigate the association between the loss of function polymorphism rs1799752, representing the I/D polymorphism, in intron 16 of the Angiotensin Converting Enzyme (*ACE*) gene and Ang II (and possibly other RAS peptides) and hypoxic pulmonary vasoconstriction will be collected as specified in the Time and Events Table (Section 7.1).

Further information for blood sample collection, processing, storage and shipping procedures are provided in the SRM.

Information regarding genetic research is included in Appendix 3.

7.10. Immunogenicity

7.10.1. Sample collection

Blood samples for immunogenicity analysis will be collected at the time points indicated in Section 7.1, Time and Events Table. Additional visits to obtain immunogenicity samples may be required in the unlikely event that subjects develop a clinically relevant immunoglobulin response to the drug as described in the SRM.

Details of immunogenicity blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.10.2. Sample analysis

Immunogenicity analysis will be performed under the management of Clinical Immunology, Biopharm R&D, GlaxoSmithKline. All pre-dose and post-dose samples will be first tested for Anti-ACE2 binding antibodies by screening and confirmation assay steps. The post-dose samples tested positive for anti-ACE2 binding antibodies will be further characterized for anti-ACE2 neutralizing antibodies. Both positive incidences for anti-ACE2 binding and neutralizing antibodies will be reported.

8. DATA MANAGEMENT

- For this study subject data will be entered into GSK defined CRFs, transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials and day/month of birth will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

The focus of this study is on the exploration the effect of GSK2586881 on a range of echocardiography, pulse oximetry, biomarker, safety and PK endpoints, when subjecting healthy volunteers to an exercise challenge under hypoxic conditions. No formal statistical hypotheses are being tested. A Bayesian statistical analysis framework with non-informative priors for model parameters (unless otherwise specified) will be used to obtain posterior distributions for effects of interest. These posterior distributions will be used to obtain a number of probability statements about the magnitude of treatment effects (e.g. Probability of **any** treatment related reduction in PASP, or Probability that the treatment related reduction in PASP $\geq$ 5 mmHg).

A rule of thumb for end of "study success" is if the probability of any treatment related reduction in PASP (T3-T0) exceeds 0.95 (success is also conditional on the probability of (absolute) treatment related reductions in oxygen saturation exceeding 5% being small).

9.2. Sample Size Considerations

The sample size of 30 patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context. (Note: precision in this context is defined as one half of the width of the 95% confidence interval.) A sample size re-estimation is planned after 15 subjects.

9.2.1. Sample Size Assumptions

The precision of the primary treatment comparison (change from baseline PASP to hypoxic/exercise PASP) was estimated using simulation. PASP data points were simulated for each subject: normoxic at rest, and hypoxic after exercise, each for two study periods, giving a total of four PASP measurements for each subject. The four PASP measurements were drawn from a multivariate normal distribution to allow for different levels of within-subject correlation. One simulated study with a given sample size (N) was created by repeating this process of generating individual subject data N times, randomly allocating each subject to a treatment sequence in a 1:1 ratio, and calculating the half-width of the confidence interval of the treatment difference in change from baseline in PASP from that simulated dataset (using a linear mixed effects model including period baseline, subject baseline, treatment and period as fixed effects, and subject as a random effect). A full simulation run consisted of 1000 iterations of simulated studies, and a final estimate for precision was calculated as the mean precision across the 1000 iterations.

The following assumptions were made regarding the mean and Standard Deviation (SD) of PASP based on data from Antoni, 2005

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) and 45 mmHg under hypoxia and exercise
- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise
- As the purpose of this simulation was to estimate precision by evaluating the SD of the treatment difference rather than the mean, there was no treatment effect assumed in the simulations
- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9).
- A range of sample sizes was considered: from 5 to 10 in increments of 1, and then up to 40 in increments of 5. The table below shows a subset of these; the figure includes them all.

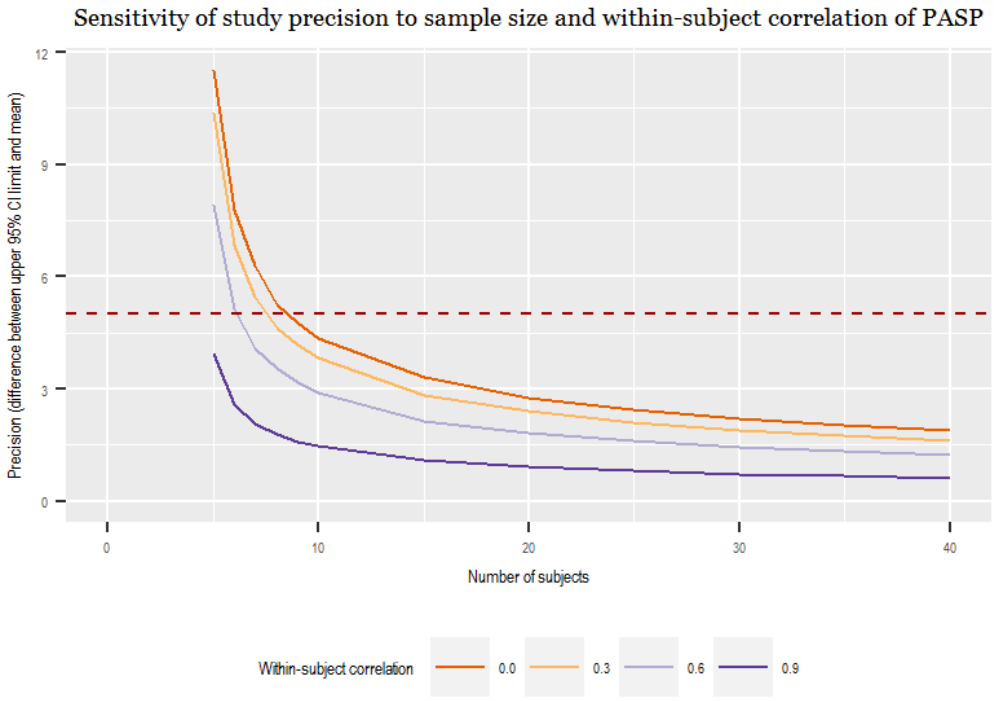
Based on these assumptions, the estimated precisions for these sample sizes and under these scenarios of within-subject correlation were as follows (smaller number for precision denotes more precise estimates):

Table 3 Estimated precision of treatment comparisons of change from baseline in PASP (mmHg) for a range of sample sizes and within-subject correlation scenarios

	Within-subject correlation			
Sample size (N)	**0**	**0.3**	**0.6**	**0.9**
10	4.35	3.83	2.92	1.48
20	2.76	2.40	1.83	0.91
30	2.22	1.91	1.46	0.72
40	1.91	1.63	1.23	0.62

Figure 4 illustrates precision estimates under the full range of sample sizes considered (from 5 to 40), and for all within-subject correlation scenarios. The red line at 5 mmHg is superimposed to indicate a rough guide to the magnitude of a clinically meaningful difference.

Figure 4 Sensitivity of study precision to sample size and within subject correlation of PASP



9.2.2. Sample Size Re-estimation

A sample size re-estimation will be conducted at the interim analysis (see Section 9.3.2). The purpose of the sample size re-estimation is to determine whether the total number of subjects can be reduced below the anticipated 30, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised

PASP (i.e. T3-T0). The sample size re-estimation is to be considered advisory, and taken into account together with other considerations. These will include:

- ensuring sufficient data is collected on secondary endpoints,
- non-conclusive safety data at the time of interim analysis (e.g. a weak signal of a reduction in oxygen saturation data that would require the full set of remaining subjects to be able to reach a conclusion),
- speed of recruitment.

9.3. Data Analysis Considerations

Data will be listed and summarised according to GSK integrated data standards library (IDSL) reporting standards where applicable. Complete details will be provided in the Reporting and Analysis Plan (RAP).

The key endpoints referred to in the text below comprise PASP measured by echocardiography, oxygen saturation measured by continuous pulse oximetry, RAS peptides and safety data.

9.3.1. Analysis Populations

All Subjects Screened Population: This population contains all subjects that complete at least one Visit 1 (Screening) procedure. This population will be used for the summary of subject disposition (including reasons for screening failures, run-in failures, and stabilization failures) and for the listing of AEs and SAEs for non-randomised subjects.

Intent-to-Treat (ITT) Population: This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, run-in failure, or stabilisation failure, but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all efficacy and safety analyses.

Pharmacokinetic (PK) population: This population will comprise all subject in the ITT Population for whom a PK sample was obtained and analysed and on active treatment.

In addition to the above populations, the effect of important protocol violations, including any subjects who failed the inclusion/exclusion criteria, may be assessed by means of sensitivity analyses. A blind review of all protocol violations will be performed prior to DBF in order to identify any important deviations and consequently identify any subjects who will be excluded from such sensitivity analyses.

9.3.2. Interim Analysis

An interim analysis is planned after 15 subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods
- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

The interim analysis will use the following study data:

- PASP at time T0 and T3 for both periods, for the first 15 subjects
- Oxygen saturation at T0 and T3 for both periods, for the first 15 subjects
- Adverse events for the first 15 subjects
- Angiotensin biomarker concentrations for the first 5 subjects (to allow time for the samples to be analysed by the external lab).

The interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

The aims of the interim analysis, and the endpoints to be used to address them, are as follows:

- Using the primary endpoint (treatment differences between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP [T3-T0]):
    - To decide whether to continue the study or stop based on futility, and
    - to conduct an advisory sample-size re-estimation with the view to potentially reduce the number of subjects participating in the study below 30.
- Using secondary endpoints:
    - to estimate treatment differences between GSK2586881 and Placebo in change from pre-dose oxygen saturation to hypoxic/exercised oxygen saturation [T3-T0], with a view to stopping the study if there is evidence that GSK2586881 causes a reduction in oxygen saturation in healthy volunteers. As a non-binding guide high posterior probability of observing ≥5% absolute differences in **mean** oxygenation saturation values between placebo and active arms would be of concern; but clinical judgement would override any statistical methods if, for example, the majority of subjects displayed consistent patterns of reductions e.g. between 2-3%).

- to compare adverse events within the two treatment groups and explore any potential safety signals that may emerge

To assess the operating characteristics of the interim analysis decisions to be made based on the primary endpoint, a simulation was carried out to estimate the relative proportion of studies reaching conclusions to stop for futility, continue with no change to the sample size, and to continue with a reduced sample size, under various different scenarios of the true treatment effect. Broadly speaking, the aim of the simulation was to confirm that (in an overwhelming majority of cases) a decision to stop for futility would be reached in the case of no true treatment effect and a decision to continue (perhaps with reduced sample size) would be reached in the case of a true treatment effect.

Simulated datasets of PASP at baseline (i.e. normoxic and at rest) and under hypoxia and exercise for 15 subjects, each receiving both treatments in sequence, were generated using the following assumptions:

- 15 simulated subjects were randomised to one of the two available treatment sequences (see Section 6.2) using block randomisation with block size of 4 records

- PASP at baseline and under hypoxia and exercise for both treatment groups (a total of four PASP measurements per subject) were sampled from a multivariate normal distribution, using values for mean PASP, standard deviation of PASP and within-subject correlation of PASP as described below.

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) for both treatments, and 45 mmHg under hypoxia and exercise for Placebo (as in Section 9.2.1). For GSK2586881, mean PASP under hypoxia and exercise was (45 - Δ) mmHg, where Δ (the mean treatment difference) was varied according to four different scenarios:

    - no treatment difference (Δ = 0 mmHg)

    - weak treatment difference of a magnitude of half the clinically meaningful difference (Δ = 2.5 mmHg)

    - minimum clinically meaningful difference (Δ = 5 mmHg)

    - overwhelming treatment difference one-and-a-half times the clinically meaningful difference (Δ = 7.5 mmHg)

- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise, for both treatment groups (as in Section 9.2.1)

- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9) (as in Section 9.2.1). Within-subject correlation of PASP was assumed to be compound symmetric (i.e. the same correlation was used for each of the six possible pairs of the four PASP measurements within each subject).

- It was assumed that there would be no effect of period in the simulation, i.e. it was assumed that it would make no difference if the measurement was taken from the first or second chronological period in the treatment sequence.

Modelling took on a three-step process, with decisions after each. Firstly, the treatment difference and its associated standard error was then estimated for the dataset of 15 simulated subjects using a mixed effects linear regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. If the sign of treatment difference arising from the simulated data was in the wrong direction (i.e. the simulated outcome for GSK2586881 was worse than for Placebo), then the simulated study was stopped for futility.

Secondly, in the alternative case that the simulated treatment difference was in favour of GSK2586881 over Placebo, a posterior probability of success (where success was defined as a 97.5% posterior probability of the treatment difference being in favour of GSK2586881 over Placebo) was evaluated for 30 subjects. This was evaluated using a t-distribution, with the mean and standard deviation taken as the treatment difference and standard error (multiplied by a factor of the square root of 15 to estimate the standard deviation) from step one respectively, and . If this posterior probability was less than 0.2, then the study was stopped for futility as being unlikely to show any benefit of GSK2586881 over Placebo.

Thirdly, provided that the simulated study hadn't been stopped for futility in either of the first two steps, posterior probabilities of success were calculated for N ranging from 15 to 29 subjects. If any of these posterior probabilities of success were greater than or equal to 0.9, then the study was continued with a reduced sample size equal to the lowest N at which that threshold was reached. Otherwise, the conclusion from the simulated interim analysis would be to continue the study with unchanged sample size.

This whole process (simulating data for 15 subjects, modelling the treatment difference, evaluating posterior probabilities of success and simulating the decision-making process) was replicated 1000 times for each scenario, with the decision ("futile", "no change to N" or "reduce N") recorded for each iteration. Each combination of the four treatment difference scenarios and the four correlation scenarios was evaluated for a total of 16 scenarios in all.

The estimated proportions of each decision being reached under each of the 16 scenarios are presented in Table 4.

Table 4 Operating characteristics of interim analysis under four scenarios of treatment effect and four scenarios of within-subject correlation

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with 30 subjects	Continue with reduced sample size
Δ=0 mmHg (no treatment difference)				
	0.0	87%	13%	0%
	0.3	87%	13%	1%
	0.6	85%	15%	0%
	0.9	86%	14%	0%
Δ=2.5 mmHg (weak treatment effect, less than clinically meaningful difference)				
	0.0	31%	60%	9%
	0.3	24%	65%	11%
	0.6	10%	66%	25%
	0.9	0%	9%	91%
Δ=5 mmHg (good treatment effect, at threshold of clinical meaningful difference)				
	0.0	2%	46%	52%
	0.3	1%	35%	64%
	0.6	0%	11%	89%
	0.9	0%	0%	100%
Δ=7.5 mmHg (very strong treatment effect)				
	0.0	0%	10%	90%
	0.3	0%	2%	98%
	0.6	0%	0%	100%
	0.9	0%	0%	100%

These results suggest primarily that if there is no true treatment difference between GSK2586881 and Placebo, the study would be stopped for futility 85-87% of the time (irrespective of within-subject correlation of PASP). A drug with a weak treatment effect would most likely result in the study being continued without change or stopped for futility (except in the case where within-subject correlation is 0.9, which is presented here as an extreme case, unlikely to occur in practice). If the true treatment effect is at the clinically relevant threshold of 5 mmHg, then the study is highly unlikely to be stopped for futility and may indicate being reduced in sample size.

Secondarily, it should be noted that the higher the within-subject correlation, then the higher the probability of success. This is because the more correlation there is in measurements within the same individual, the less variability there will be in change from baseline. Results for correlations of 0.0 and 0.9 are presented as extreme cases unlikely to occur in practice, though the exact strength of within-subject correlation of PASP (particularly under different stressed conditions) is not known.

Full details of the interim analysis will be supplied in the RAP.

9.3.3. Multiple Comparisons and Multiplicity

As this is an early-phase exploratory study, no adjustment for multiple comparisons will be made. Treatment comparisons will be presented as effect sizes with 95% confidence intervals.

9.4. Key Elements of Analysis Plan

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

Pharmacodynamic endpoints will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day.

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline.In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect (non-informative priors for model parameters). A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period.

PASP data will be summarized by treatment and time-point in tabular and graphical format. A Bayesian mixed effects regression model (as described above) will be fitted (using non-informative priors for the model parameters), and an estimate of the mean change from baseline in PASP for each treatment group and post-baseline timepoint,

together with its 95% credible interval, will be obtained. Estimates for treatment differences will also be presented. Data may be log-transformed if necessary.

Details of the analysis of other endpoints will be described in the RAP.

9.4.1. Pharmacokinetic Analyses

Pharmacokinetic analysis will be performed by, or under the auspices, of Clinical Pharmacology Modelling and Simulation Depertment with GlasxoSmithKline. Plasma GSK2586881 concnetration-time data will be analysed by non-compartmental methods with WinNonLin V6.3 or greater. 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 over the study period [nominally AUC(0-2.5h) post-dose], AUC over the hypoxia challenge (nominally AUC(0.5-2.0h post-dose), plasma clearance (CL), volume of distribution (V) and apparent terminal phase half-life (t1/2), if data permit. Other PK parameters may also be determined.

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.

The relationship between the RAS biomarkers and other pharmacodynamic endpoints (echocardiography and pulse oximetry) and GSK2586881 concentrations and/or PK parameters may also be explored, if appropriate.

If appropriate, a population PK analysis may also be conducted, in addition the plasma concentration-time data may be merged with historical data and analysed as part of a population PK meta-analysis.

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.
- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study; also current medical records must be available.

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 randomisation 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

Abraham WT, Raynolds MV, Badesch DB, Wynne KM, Groves BM, Roden RL,et al.. Angiotensin-converting enzyme DD genotype in patients with primary pulmonary hypertension: increased frequency and association with preserved haemodynamics. J Renin Angiotensin Aldosterone Syst. 2003 Mar;4(1):27-30.

Antoni Ricart, Jaume Maristany, Núria Fort, Conxita Leal, Teresa Pagés, and Ginés Viscor. High Altitude Medicine & Biology. March 2005, 6(1): 43-49. doi:10.1089/ham.2005.6.43

Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;49:818-822.

Cargil RI, Lipworth BJ. Lisinopril attenuates acute hypoxic pulmonary vasoconstriction in humans. Chest. 1996 Feb;109(2):424-9/

GlaxoSmithKline Document Number 2010N108777_00 Study ID GSK2586881. Investigator's Brochure for GSK2586881 (formerly known as APN01)– A Soluble Recombinant Human Angiotensin Converting Enzyme 2 (rhACE2). Report Date 16-FEB-2011.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(a): 24. Table 3-2.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(b): 28.

ICH Topic M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, 2009

Idell S, Kueppers, F, Lippmann, M, Rosen, H, Niederman, M, Fein, A. ACE elevated in ARDS BAL. *Angiotensin converting enzyme in bronchoalveolar lavage in ARDS. Chest.* 1987;91:52-56.

Kiely DG, Cargill RI, Lipworth BJ (1996) Effects of hypercapnia on hemodynamic, inotropic, lusitropic, and electrophysiologic indices in humans. Chest 109:1215–1221

Kiely DG1, Cargill RI, Wheeldon NM, Coutie WJ, Lipworth BJ. Haemodynamic and endocrine effects of type 1 angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale. Cardiovasc Res. 1997 Jan;33(1):201-8.

Kleinsasser A1, Pircher I, Treml B, Schwienbacher M, Schuster M, Janzek E, Loibner H, Penninger JM, Loeckinger A. Recombinant angiotensin-converting enzyme 2 suppresses pulmonary vasoconstriction in acute hypoxia. Wilderness Environ Med. 2012 Mar;23(1):24-30

Maron BA, Leopold JA. The role of the renin-angiotensin-aldosterone system in the pathobiology of pulmonary arterial hypertension (2013 Grover Conference series. Pulm Circ. 2014 Jun;4(2):200-10. doi: 10.1086/675984

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, Chambers RJ, , GJ et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am. J. Resp. Crit. Care Med.* 2002;166:646-650.

Paul M, Bachmann J, Ganten D. Angiotensin II-induced vasoconstriction and sodium resorption. *The Tissue Renin-Angiotensin System in Cardiovascular Disease. Trends Cardiovasc. Med.* 1992;2:94-99.

Ricart A1, Maristany J, Fort N, Leal C, Pagés T, Viscor G. Effects of sildenafil on the human response to acute hypoxia and exercise. High Alt Med Biol. 2005 Spring;6(1):43-9.

Rubenfeld GD, Caldwell E, Peabody E. Incidence and Outcomes of Acute Lung Injury. *N Engl J Med.* 2005;353:1685-1693.

Santos RAS, Ferreira AJ, Pinheiro SVB, Sampaio WO, Touyz R, Campangole-Santos MJ. Counter-regulatory effects of ANG (1-7) in ANG II. *Angiotensin(1-7) and its receptor as potential targets for cardiovascular drugs' Expert Op. Investig. Drugs.* 2005;1019:1031.

Santos RAS, Simoes e Silva, AC, Maric C, Silva DMR, Machado RP, de Buhr I, et al. ANG (1-7) as the agonist of mas. *'Angiotensin (1-7) is an endogenous ligand for the G-protein coupled receptor Mas' Proc. Natl. Acad. Sci. USA*. 2003;100:8258-8263.

Suzuki Y, Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V., Egido, J. Inflammatory and pro-fibrotic activity of ANG II. *) Inflammation and Angiotensin II. Int. J. Biochem. Cell Biol.* 2003;35:881-900.

The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med.* 2000;342:1301-1308.

The ARDS Network Authors for the ARDS network. Ketoconzaole for Early Treatment of Acute Lung Injury and Acute Respiratory Distress Syndrome. *JAMA.* 2000;283:1995-2002.

Tom B, deVries R, Saxena PR, Danser J. ANG (1-7) inhibiting ACE and modifying Bradykinin responses. *Bradykinin potentiation by angiotensin (1-7) and ACE inhibitors correlates with C- and N-terminal blockade' Hypertension.* 2001;38:95-99.

Torre-Bueno JR, Wagner PD, Saltzman HA, Gale GE, Moon RE. Diffusion limitation in normal humans during exercise at sea level and simulated altitude. J Appl Physiol (1985). 1985 Mar;58(3):989-95

Wenz M, Hoffmann B, Bohlender J, Kaczmarczyk G. ANG II elevated in ARDS. *Anfgiotensin II formation and endothelin clearance in ARDS patients in supine and prone positions. Intensive Care Med.* 2000;26:292-298.

Wheeler AP, Bernard GR, Thompson BT, Schoenfeld D, Wiedemann HP, deBoisblanc B,et al.. Pulmonary-Artery versus Central Venous Catheter to Guide Treatment of Acute Lung Injury. *N Engl J Med.* 2006;25:354(21):2213-24. Epub 2006 May 21.

Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B,et al. Comparison of Two Fluid-Management Strategies in Acute Lung Injury. *N Engl J Med.* 2006;354(24):2564-75. Epub 2006 May 21.

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

ACE2	Angiotensin converting enzyme type 2
AE	Adverse Event
ALI	Acute Lung Injury
ALT	Alanine aminotransferase (SGPT)
Ang II	Angiotensin II
ANP	Atrial Naturetic peptide
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
BID	Bi-Daily
BUN	Blood urea nitrogen
CBP	Child Bearing Potential
CL	Clearance
CO2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CV	Cardiovascular
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HPV	Hypoxic pulmonary vasoconstriction
IB	Investigator's Brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identification
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IND	Investigational New Drug
IP	Investigational Product

IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
IVIVT	In Vitro/In Vivo Translations
Kg	Kilogram
LDH	Lactate dehydrogenase
LFTs	Liver function tests
m	Meters
MCH	Mean corpuscular hemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
MSDS	Material Safety Data Sheet
msec	Milliseconds
NOAEL	No observed adverse effect level
NT-proBNP	Brain Naturetic Peptide
O2	Oxygen
PaO2	Partial Pressure of Oxygen in arterial blood
PAH	Pulmonary Arterial Hypertension
PASP	Pulmonary Artery Systolic Pressure
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
Q	Perfusion
QC	Quality control
QTcB	QT duration corrected for heart rate by Bazett's formula
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RAS	Renin-Angiotensin System
RBC	Red blood cells
rhACE2	Recombinant human angiotensin converting enzyme type 2
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SD	Standard deviation
SRM	Study Reference Manual
TPR	Third Party Resourcing
TTS	Technical Terms of Supply
t½	Terminal phase half-life
tmax	Time of occurrence of Cmax
V	Volume of Distribution
V_A	Ventilation
VO2	Oxygen Consumption

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
MedDRA
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 etiology (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
• A specialist or hepatology consultation is</td><td colspan="2">• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend.
• Blood sample for pharmacokinetic (PK) analysis, obtained 24 hours after 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.</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
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	• Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. **NOTE: not required in China**. • 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,et al.. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. Drug Metab Dispos 2009; 37:1779-1784.

12.3. Appendix 3: Genetic Research

Genetics – Background

Naturally occurring genetic variation may contribute to inter-individual variability in response to medicines, as well as an individual's risk of developing specific diseases. Genetic factors associated with disease characteristics may also be associated with response to therapy, and could help to explain some clinical study outcomes. For example, genetic variants associated with age-related macular degeneration (AMD) are reported to account for much of the risk for the condition [Gorin, 2012] with certain variants reported to influence treatment response [Chen, 2012]. Thus, knowledge of the genetic etiology of disease may better inform understanding of disease and the development of medicines. Additionally, genetic variability may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), or pharmacodynamics (relationship between concentration and pharmacologic effects or the time course of pharmacologic effects) of a specific medicine and/or clinical outcomes (efficacy and/or safety) observed in a clinical study.

Genetic Research Objectives and Analyses

The objectives of the genetic research are to investigate the relationship between genetic variants and:

- Response to medicine, including [any treatment regimens under investigation in this study] or any concomitant medicines;

Genetic data may be generated while the study is underway or following completion of the study. Genetic evaluations may include focused candidate gene approaches and/or examination of a large number of genetic variants throughout the genome (whole genome analyses). Genetic analyses will utilize data collected in the study and will be limited to understanding the objectives highlighted above. Analyses may be performed using data from multiple clinical studies to investigate these research objectives.

Appropriate descriptive and/or statistical analysis methods will be used. A detailed description of any planned analyses will be documented in a Reporting and Analysis Plan (RAP) prior to initiation of the analysis. Planned analyses and results of genetic investigations will be reported either as part of the clinical RAP and study report, or in a separate genetics RAP and report, as appropriate.

Study Population

Any subject who is enrolled in the study can participate in genetic research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the genetic research.

Study Assessments and Procedures

A key component of successful genetic research is the collection of samples during clinical studies. Collection of samples, even when no *a priori* hypothesis has been

identified, may enable future genetic analyses to be conducted to help understand variability in disease and medicine response.

- A 6 ml blood sample will be taken for Deoxyribonucleic acid (DNA) extraction. A blood sample is collected at the baseline visit, after the subject has been randomised and provided informed consent for genetic research. Instructions for collection and shipping of the genetic sample are described in the laboratory manual. The DNA from the blood sample may undergo quality control analyses to confirm the integrity of the sample. If there are concerns regarding the quality of the sample, then the sample may be destroyed. The blood sample is taken on a single occasion unless a duplicate sample is required due to an inability to utilize the original sample.

The genetic sample is labelled (or "coded") with the same study specific number used to label other samples and data in the study. This number can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number).

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study, or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will only use samples collected from the study for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

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

Informed Consent

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

Subject Withdrawal from Study

If a subject who has consented to participate in genetic research withdraws from the clinical study for any reason other than being lost to follow-up, the subject will be given a choice of one of the following options concerning the genetic sample, if already collected:

- Continue to participate in the genetic research in which case the genetic DNA sample is retained
- Discontinue participation in the genetic research and destroy the genetic DNA sample

If a subject withdraws consent for genetic research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records.

Genotype data may be generated during the study or after completion of the study and may be analyzed during the study or stored for future analysis.

- If a subject withdraws consent for genetic research and genotype data has not been analyzed, it will not be analyzed or used for future research.
- Genetic data that has been analyzed at the time of withdrawn consent will continue to be stored and used, as appropriate.

Screen and Baseline Failures

If a sample for genetic research has been collected and it is determined that the subject does not meet the entry criteria for participation in the study, then the investigator should instruct the subject that their genetic sample will be destroyed. No forms are required to complete this process as it will be completed as part of the consent and sample reconciliation process. In this instance a sample destruction form will not be available to include in the site files.

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

GSK may summarize the genetic research results in the clinical study report, or separately and may publish the results in scientific journals.

GSK may share genetic research data with other scientists to further scientific understanding in alignment with the informed consent. GSK does not inform the subject, family members, insurers, or employers of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from genetic studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined. Further, data generated in a research laboratory may not meet regulatory requirements for inclusion in clinical care.

Chen H, Yu KD, Xu GZ. Association between Variant Y402H in Age-Related Macular Degeneration (AMD) Susceptibility Gene CFH and Treatment Response of AMD: A Meta-Analysis. PloS ONE 2012; 7: e42464

Gorin MB. Genetic insights into age-related macular degeneration: Controversies addressing risk, causality, and therapeutics. Mol. Asp. Med. 2012; 33: 467-486.

12.4. Appendix 4: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.4.1. 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). • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. • The signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.

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.

12.4.2. 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 and 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 Appendix 2 for the required liver chemistry follow-up instructions

12.4.3. 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

12.4.4. 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.

12.4.5. 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.

12.4.6. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool

- Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool
- If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor Site will enter the serious adverse event data into the electronic system as soon as it becomes available.
- The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.
- After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor telephone.
- Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

SAE reporting to GSK via paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail • 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.

12.5. Appendix 5: Modified List of Highly Effective Methods for Avoiding Pregnancy and Collection of Pregnancy Information

12.5.1. Modified List of Highly Effective Methods for Avoiding Pregnancy

The list does not apply to FRP with same sex partners or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

1. Contraceptive subdermal implant
2. Intrauterine device or intrauterine system
3. Combined estrogen and progestogen oral contraceptive [Hatcher, 2011]
4. Injectable progestogen [Hatcher, 2011]
5. Contraceptive vaginal ring [Hatcher, 2011]
6. Percutaneous contraceptive patches [Hatcher, 2011]
7. Male partner sterilization with documentation of azoospermia prior to the female subject's entry into the study, and this male is the sole partner for that subject [Hatcher, 2011]. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview provided by her or her partner.

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Contraceptive requirements for male subjects with female partners of reproductive potential (when applicable).

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until [at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives] after the last dose of study medication.

1. Vasectomy with documentation of azoospermia. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview.
2. Male condom plus partner use of one of the contraceptive options below that meets the Standard Operating Procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label:
    - Contraceptive subdermal implant
    - Intrauterine device or intrauterine system

- Combined estrogen and progestogen oral contraceptive [Hatcher, 2011]
- Injectable progestogen [Hatcher, 2011]
- Contraceptive vaginal ring [Hatcher, 2011]
- Percutaneous contraceptive patches [Hatcher, 2011]

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

12.5.2. Collection of Pregnancy Information

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study
- Information will be recorded on the appropriate form and submitted to GSK within 2 weeks of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on mother and infant, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Appendix 5. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating

- will discontinue study medication or be withdrawn from the study
- Investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. This applies only to subjects who are randomised to receive study medication.
- After obtaining the necessary signed informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy

- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

12.5.3. References

Hatcher R A, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, Policar MS, editors. Contraceptive Technology. 20th edition. Atlanta, Georgia: Ardent Media, Inc., 2011: 50. Table 3-2.

12.6. Appendix 6: Protocol Changes

12.6.1. Protocol Amendment 01 (24-Feb-2017) from Original Protocol (23-Sep-2016)

This is a global amendment.

12.6.1.1. Summary of Changes and Rationale for Amendment

Additional Inclusion Criteria (Section 5.2) and Subject Stopping Criteria (Section 5.4) added in response to comments from BfArM.

12.6.1.2. List of Specific Changes

Title Page, Author (s)

Rationale for change: Addition and removal of author(s).

PPD

Sponsor Signatory

Rationale for change: Change in sponsor signatory

~~Dr Richard Marshall~~ **Dr Aili Lazaar**

~~Vice President, Fibrosis & Lung Injury Discovery Performance Unit (DPU) Head, Respiratory Therapy Area Unit~~ **Director, Discovery Medicine**
Project Physician Leader, Respiratory Therapy Area

Section 5.2: Exclusion Criteria

Rationale for change: Addition of exclusion criteria number 4 and 5.

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
1. ALT >1.5x Upper limit of normal (ULN).
2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
4. Prior history of altitude sickness.
5. Recently been scuba diving (within 1 week before screening).

6. 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).

Section 5.4: Withdrawal/Stopping Criteria

Rationale for change: Addition of withdrawal criteria.

Subjects may be withdrawn from the study for any of the following reasons:

- 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.
- If a subjects oxygen saturation falls below 65% at any point during the hypoxia challenge.
- **If the subject experiences worsening shortness of breath or other symptoms, which in the opinion of the investigator are consistent with altitude sickness.**
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

TITLE PAGE

Division: Worldwide Development

Information Type: Clinical Protocol

Title:	The effects of GSK2586881 on the responses to acute hypoxia and exercise

Compound Number: GSK2586881

Development Phase I

Effective Date: 23-SEP-2016

Author(s): PPD

SPONSOR SIGNATORY:

PPD

28th Sep 2016

Dr Richard Marshall

Date

Vice President, Fibrosis & Lung Injury Discovery
Performance Unit (DPU) Head

Respiratory Therapy Area Unit

PPD

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	Site Address
Primary Medical Monitor	PPD				709 Swedeland Road UW2531 King of Prussia, PA 19406 USA
Secondary Medical Monitor					Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
SAE contact information	[Medical monitor as above]				

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): 2016-002465-55

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 204987

Rationale

The purpose of this study is to examine how GSK2586881, a recombinant human ACE2 peptide, modulates the acute hypoxic pulmonary vasoconstriction (HPV) response in healthy volunteers.

HPV is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO_2) levels in health and in pathophysiological settings such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. The literature supports a role for the renin angiotensin system (RAS) in driving acute HPV and while there is a strong biological rationale for modulation of RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they blunt the normal HPV response and negatively impact on arterial oxygenation (PaO_2). Thus, from a safety (and efficacy) perspective it is important to understand the impact of modulation of the RAS system by GSK2586881 on the acute HPV response.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.

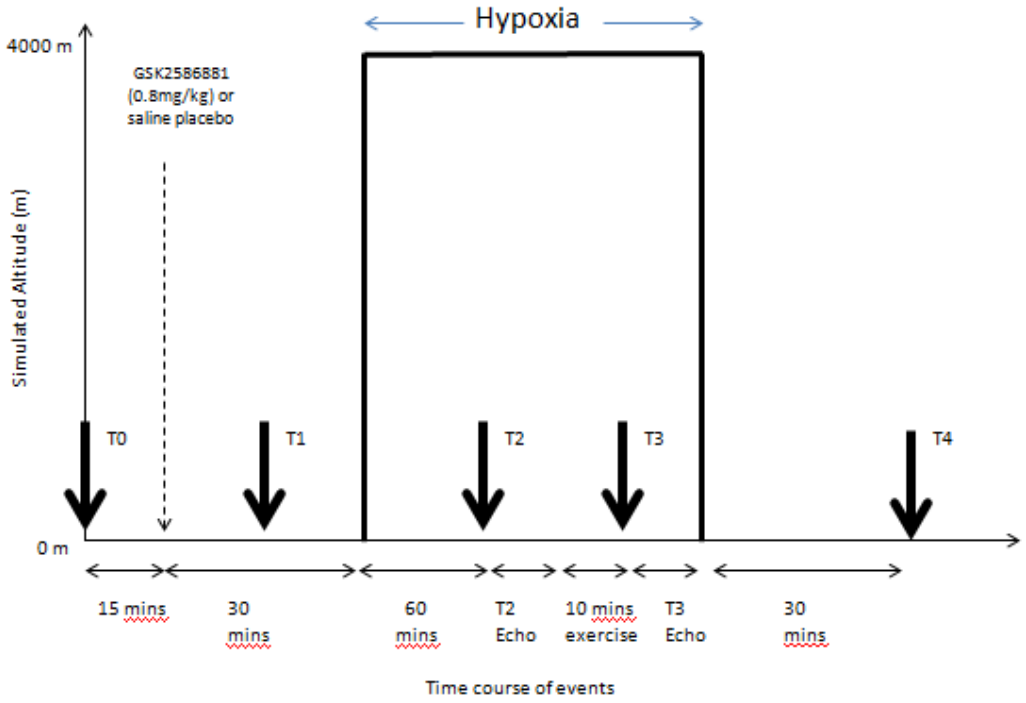
Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the effect of a single IV dose of GSK2586881 on peptide hormone responses to hypoxia and exercise.	• Baseline levels and changes in response to hypoxia and exercise in kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in other biomarkers which may include Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined.
• To evaluate Pharmacogenetics	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

Overall Design

The study will be single-centre , randomised, placebo-controlled and double blind (sponsor open). Subjects will be randomised to receive a single IV dose of GSK2586881 or saline in a crossover design.

A schematic of the study is shown below. Echocardiograms approximate timings are indicated by bold arrows.

APPROXIMATE TIMING OF SIMULATED ALTITUDE AND ECHOCARDIOGRAM MEASUREMENTS (BLACK ARROWS) DURING TREATMENT PERIODS 1 AND 2.



Treatment Groups, Randomisation Arms and Duration

The study is intended to follow a double blind (sponsor open) two-period cross-over design.

Treatment Group A: GSK2586881 0.8 mg/kg, administered as a single IV dose. .

Treatment Group B: matching volume of placebo administered as a single IV dose.

Subjects will receive both treatments during the course of the study, and will be randomised to one of two sequences, each of which describes the order in which those treatments are received:

Sequence 1: AB: GSK2586881 in the first period followed by placebo in the second.

Sequence 2: BA: Placebo in the first period followed by GSK2586881 in the second.

The total study duration for each subject is expected to be a maximum of 56 days.

Type and Number of Subjects

Approximately 35 subjects will be enrolled to ensure that a minimum of 30 subjects complete all dosing and critical assessments (the target of 30 may be revised by the sample size re-estimation).

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

Analysis

This study is designed to estimate the effect of GSK2586881 relative to placebo on change from baseline in PASP following exercise under hypoxic conditions. No formal hypothesis will be tested. Point estimates will be calculated together with corresponding 95% confidence intervals (or credible intervals if utilising a Bayesian framework) for the difference between the mean of the test treatment and the mean of the reference treatment.

Analysis of PASP: Statistical modelling of changes from baseline (Ti-T0) will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period. Non-informative priors will be used for the model parameters. Posterior distributions will be obtained for the GSK2586881 Vs Placebo comparisons at each of the post dosing timepoints (T1-T4). These distributions will be used to produce several posterior probability statements; the most important being the probability that the change from baseline in PASP is reduced by the GSK2586881at time T3.

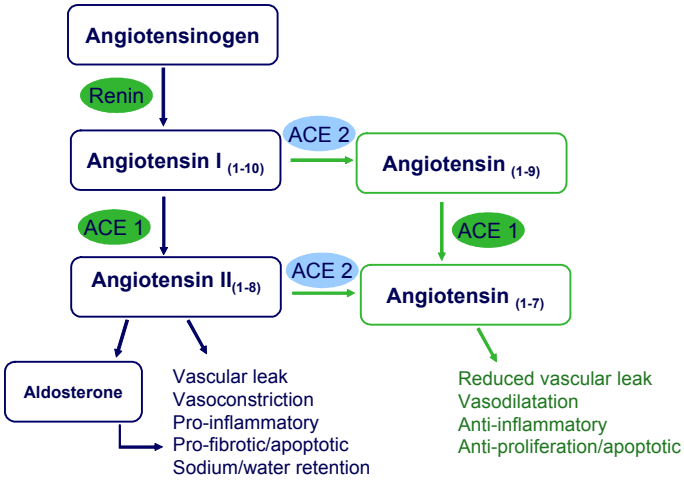
Oxygenation saturation will also be analysed in a similar way; although of interest is the posterior probability that the study drug causes (absolute) reductions in oxygenation saturation in excess of 5%.

An interim analysis is planned after 15 subjects have completed period 1 and 2 assessments to i) evaluate safety and tolerability (SAE, AE and Oxygenation saturation), ii) determine whether the study should stop for futility based on expected PASP outcomes if the study were allowed to complete recruitment and iii) Re-estimate the sample size based using the observed variance parameters.

2. INTRODUCTION

GSK2586881 is a purified intravenous formulation of soluble recombinant human angiotensin converting enzyme type 2 (rhACE2) which is expressed in Chinese Hamster Ovary cells. Angiotensin converting enzyme type 2 (ACE2) is a zinc carboxypeptidase involved in the Renin-Angiotensin System (RAS) that controls blood pressure, electrolytes, and intravascular fluid volume. A key function of ACE2 is believed to be the cleavage of Angiotensin II (Ang II) to Ang (1-7), which have opposing physiological roles. Elevated levels of Ang II are associated with vasoconstriction, inflammation, fibrosis, vascular leak, and sodium absorption. Ang (1-7) appears to be a counter-regulatory protein in the RAS; associated with vasodilation, anti-proliferation, anti-inflammation, and reduced vascular leak, as noted in Figure 1 below [Paul, 1992; Santos, 2005; Suzuki, 2003].

Figure 1 Renin Angiotensin System



Ang II binds to two distinct receptors called AT-1 and AT-2, with the AT-1 receptor mediating the vasoconstrictive, proliferative and pro-inflammatory actions of Ang II. The function of the AT-2 receptor has not been fully elucidated. Ang (1-7) initiates its effects by binding to the Mas-receptor, and also acts by inhibiting the activity of the carboxyterminal domain of angiotensin converting enzyme (ACE), which prevents ACE from fully acting on its substrates Angiotensin I and bradykinin.

ACE and Angiotensin II has been implicated in the pathogenesis of the acute respiratory distress syndrome (ARDS, and pulmonary hypertension. It has been observed that circulating Ang II levels and lung ACE levels are increased in humans with ARDS and pulmonary hypertension. It has also been shown that the DD ACE polymorphism, which is associated with higher ACE activity, is associated with susceptibility to development of lung injury and worsened outcome (mortality) in patients with ARDS [Marshall, 2002] and to the development of pulmonary hypertension [Abraham, 2003]

It is expected that the reduction of Ang II and simultaneous formation of Ang (1-7) should have positive impacts in ARDS and on pulmonary haemodynamics in patients with pulmonary hypertension. This dual action can be achieved by ACE2, and thus, an enhancement of the activity of this enzyme is seen as promising approach for the treatment of diseases and conditions with an imbalance of the RAS system, insufficient natural ACE2 activity, and pathologically elevated Ang II levels or decreased Ang (1-7) such as is observed in ALI/ARDS [Tom, 2001; Idell S, 1987; Santos, 2003; Wenz, 2000] and pulmonary hypertension [Maron, 2014]

This study will demonstrate how GSK2586881 modulates the acute HPV response in healthy volunteers.

2.1. Study Rationale

Hypoxic pulmonary vasoconstriction (HPV) is an acute physiological response that plays a fundamental role in maintaining optimal arterial oxygen (PaO_2) levels in health and in pathophysiological settings such as acute respiratory distress syndrome (ARDS) by facilitating re-distribution of pulmonary blood flow to areas of ventilated lung. However, in many ARDS patients with pulmonary microvascular injury and dystonia, the normal HPV reflex is compromised resulting in the mismatching of perfusion (Q) and ventilation (V_A) and formation of areas of low perfusion to ventilated alveoli (high V_A/Q; physiological deadspace), or perfusion of alveoli with minimal or no ventilation (low V/Q; physiological shunt).

The literature is conflicting as to the role of the RAS in modulating the HPV response; however, the majority of reports support a role for the RAS in driving acute HPV (Cargil, 1996; Kiely, 1996). While there is a strong biological rationale for modulation of the RAS in the treatment of ARDS patients, therapeutic strategies aimed at RAS may have unintended, negative consequences for ARDS patients if they impact on PaO_2 and produce a blunted HPV response. Surprisingly, there are scant data regarding the effect of RAS modulation on HPV, V-Q matching or PaO_2 in healthy human volunteers.

Inhibition of the acute HPV response would be expected to promote blood flow to areas of poorly-ventilated lung resulting in an increase in venous admixture and a reduction in PaO_2 levels. In the context of acute lung injury, correction of any V/Q mismatch would be expected to be beneficial with respect to maintaining and improving PaO_2 levels. Conversely, inhibition of HPV by pharmacological agents might be expected to worsen V/Q matching and compromise PaO_2 levels. Thus, from a safety (and efficacy) perspective it is vital to understand the impact of modulation of the RAS system on the acute HPV response and subsequent PaO_2.

Recombinant human ACE2 (rhACE2) delivered intravenously to pigs inhibits the HPV response to acute hypoxic challenge assessed by inhibition of mean pulmonary artery pressures and pulmonary vascular resistance. There was also a strong trend to increased shunt with administration of rhACE2; however PaO2 was not significantly affected. The authors concluded that the increased shunt may not have been sufficient to result in a reduction in PaO_2 (Kleinsasser., 2012).

An elegant study by Wagner and colleagues demonstrated the importance of end arterial capillary diffusion limitations on PaO_2 levels in healthy volunteers during hypoxia ± exercise (Torre-Bueno, 1985). Diffusion limitation made a significant contribution to PaO_2 levels particularly under conditions where exercise and hypoxia were combined. In addition, V/Q mismatching was suggested to increase with the combined stresses of exercise and hypoxia resulting in significantly more arterial oxygen desaturation than observed with either stressor by itself.

The main goals of this study are to examine whether GSK2586881 modulates the acute HPV response in healthy volunteers with a subsequent impact on O_2 saturation. Should the application of GSK2586881 lead to accentuated arterial oxygen desaturation, further clinical studies to examine the therapeutic efficacy of GSK2586881 in acute lung injury should be approached with caution. Conversely, a reduction in HPV without augmented hypoxemia would provide supporting evidence that GSK2586881 could have a positive impact in patients with pulmonary hypertension.

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To evaluate the effect of a single IV dose of GSK2586881 on the HPV response in healthy volunteers during exercise under hypoxic conditions.	• Change from baseline of Pulmonary Artery Systolic Pressure (PASP) measured via Echocardiography.
Secondary	
• To evaluate the effect of a single IV dose GSK2586881 on RAS peptide responses.	• Effect of GSK2586881 on baseline levels and changes in response to hypoxia and exercise of RAS peptides (e.g. Ang II, Ang(1-7), Ang(1-5)).
• To evaluate the safety of a single IV dose of GSK2586881 in healthy volunteers.	• Vital signs (heart rate, systolic and diastolic blood pressure), oxygen saturation, 12-lead ECGs, AEs, immunogenicity and clinical laboratory assessments and continuous pulse oximetry.
• To evaluate the pharmacokinetics of a single IV dose of GSK2586881.	• Plasma concentrations of GSK2586881 and derived pharmacokinetic parameters.

Objectives	Endpoints
Exploratory	
• To evaluate the effect of a single IV dose of GSK2586881 on ventilatory parameters in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit.
• To evaluate the effect of a single IV dose of GSK2586881 on peptide hormone responses to hypoxia and exercise.	• Baseline levels and changes in response to hypoxia and exercise in kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) as data permit.
• To evaluate the pharmacodynamic activity of a single IV dose of GSK2586881 in healthy volunteers during exercise in hypoxic conditions.	• Change from baseline in other biomarkers which may include Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined.
• To evaluate Pharmacogenetics	• Evaluate I/D polymorphisms in the Angiotensin Converting Enzyme (ACE) gene and analyse the impact on Ang II (and possibly other RAS peptides), hypoxic pulmonary vasoconstriction and responses to GSK2586881 administration.

4. STUDY DESIGN

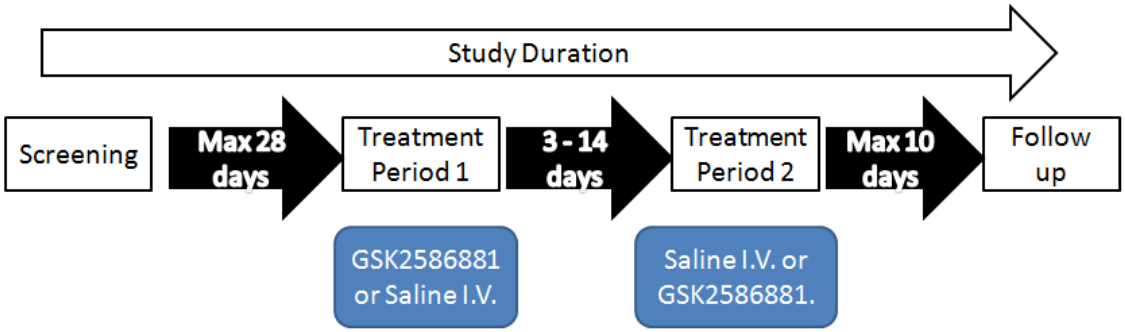
4.1. Overall Design

This is a single-centre, randomised, placebo-controlled and double blind (sponsor open), two-period crossover study in healthy subjects.

The subjects will be required to attend the unit for a screening visit, treatment period 1, treatment period 2 and a follow up visit.

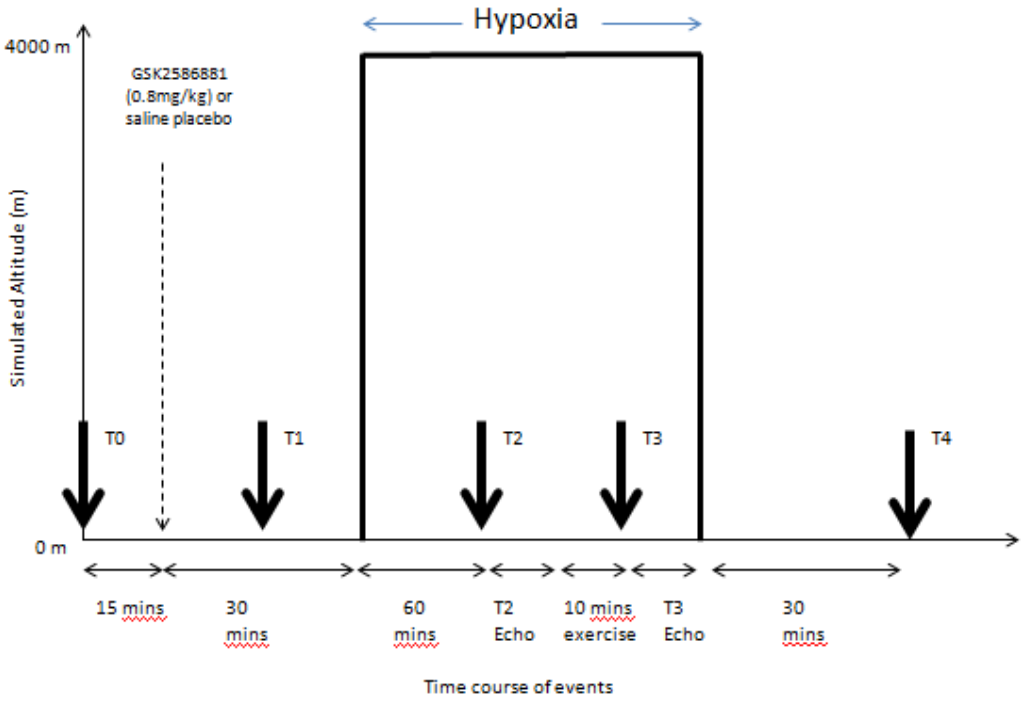
4.2. Treatment Arms and Duration

Figure 2 Subject participation flow



The subjects must participate in the procedures detailed in the Time and Events Table (Section 7.1) and the timings of the simulated altitude, exercise and echocardiograms is shown in Figure 3 .

Figure 3 Approximate timing of simulated altitude and echocardiogram measurements (black arrows) during Treatment Periods 1 and 2



There will be a washout period of 3-14 days between treatments to ensure biomarkers return to pre-challenge baseline. Subjects then return to the site and repeat the same procedures as above, except that they will receive the treatment (GSK2586881 or Saline) that they did not receive in the first period.

Follow Up

This will occur up to 10 days after the end of the second treatment period. During the visit various safety tests will be conducted (see time and events table in Section 7.1).

The total study duration for each subject is expected to be a maximum of 56 days.

4.3. Type and Number of Subjects

Approximately 35 subjects healthy volunteers will be randomised such that approximately 30 evaluable subjects complete the study (the target of 30 may be revised following the sample size re-estimation) see Section 9.2.2).

If subjects prematurely discontinue the study, additional replacement subjects will not be recruited in order to preserve randomisation integrity.

4.4. Design Justification

The study design is based on a paper published by Ricart, 2005 and is considered to be feasible. The study will provide important information on whether GSK2586881 modulates the acute HPV response in healthy volunteers. In addition, the study will include assessments of PK and PD effects of GSK2586881. This will be achieved by assessing blood levels of GSK2586881 and RAS peptide responses throughout the duration of the hypoxia challenges.

The study will be placebo controlled (saline) so each subject can be used as their own control.

4.5. Dose Justification

GSK2586881 has been studied in 21 healthy subjects as single, intravenous doses over 30 minutes from 0.1 mg/kg to 1.2 mg/kg, and in repeated daily doses for up to six days of 0.4 mg/kg once a day. In addition, ARDS patients have received four doses of GSK2586881: 0.1 mg/kg, 0.2 mg.kg, 0.4 mg/kg and 0.8 mg/kg over a 24 hour period (n=5) or 0.4 mg/kg BID twice daily for 3 days (n=19). One subject with Pulmonary Arterial Hypertension (PAH) has received a single dose of 0.2 mg/kg.

A population PK model for GSK2586881 was derived from data obtained in healthy subjects and ARDS patients and showed that the systemic PK profile was adequately described by a two-compartment first order elimination model and that the PK profile was independent of population (health subjects or ARDS patients). Furthermore the PKPD response (AngII) in healthy subjects and ARDS patients was consistent with a single direct Emax model after accounting for differences in baseline AngII concentrations between healthy subjects and ARDS patients.

Based on the population PK/PD model described above, single intravenous doses of 0.4 - 1.2 mg/kg GSK2586881 are predicted to reduce elevated levels of AngII (baseline AngII consistent with an ARDS population) to levels consistent with that observed in healthy subjects for the duration of the hypoxic challenge (approx 2.25h). The dose of 0.8 mg/kg has been selected to ensure maximal reduction of AngII levels, whilst maintaining dosing volumes within acceptable limits, and will further aid the understanding of the PK/PD relationship for GSK2586881.

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with GSK2586881 can be found in the Investigator's Brochure (GlaxoSmithKline Document Number 2010N108777_00 , 2015). The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2586881]		
Cardiovascular risk	During preclinical testing a brief period of non-sustained ventricular tachycardia was observed in one monkey receiving a dose of 20.8 mg/kg/day.	The dose used in this study is much lower.and well below the No observed adverse effect level (NOAEL) for the 14 day repeat dose cynomolgus monkeys of 8 mg/kg/day.
Potential Reproductive/embryofetal risks	Preclinical studies have not been performed	Women of childbearing potential will be excluded from the study.
Potential for Immunogenicity	There has been no induction of an immune response to rhACE2 in either of the clinical studies to date in healthy subjects or participants with ARDS.	Patients will have routine monitoring of any immunological response that may occur. If an immunological response is seen the patient will be asked to return for further monitoring and assessment(s).
Potential for rash	In study ACE114622, rash was reported more frequently in subjects receiving GSK2586881, although only one event was considered drug-related.	Patients will be monitored for rash in the clinical trials.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Effects of hypoxia (light headiness, headaches, nausea)	The subjects will be exposed to hypoxic conditions for approximately 90 minutes with a simulated altitude of 4000m	The subjects will be continuously observed and monitored with telemetry and pulse oximetry. Stopping criteria are included in the protocol.

4.6.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the subjects taking part in the study as it is a healthy volunteer study. However, subjects will undergo a medical evaluation during screening including physical exams, ECGs and laboratory assessments which may provide important health information.

By taking part in this study the subject will be contributing to the development of GSK2586881 for the treatment of pulmonary hypertension and ARDS.

4.6.3. Overall Benefit:Risk Conclusion

The design of the study is considered low risk to the subjects and justified based on the work carried out by other researchers, the safety information from the nonclinical studies and the two previous clinical trials carried out on GSK2586881.

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 the IB.

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 40 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT
2. Healthy as determined by the investigator or medically qualified designee based on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring. 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 Medical Monitor if required] agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures. Note: Screened subjects with laboratory values outside of the normal range may be

repeated once for inclusion into the study at the discretion of the Investigator.

3. Screening echocardiogram of good quality, without clinically significant abnormalities, and with mild-moderate tricuspid regurgitation sufficient for the reliable estimation of PASP, as determined by the echocardiography core laboratory or responsible cardiologist.

Screening PASP within the normal range according to site standards.

4. Subjects have not resided at an altitude >1500m for more than 7 days in the last 4 month

5. Able to complete all study procedures.

6. Any contraindication (orthopaedic, cardiac etc.) to perform exercise on a bicycle ergometer.

WEIGHT
7. Body weight 50-100 kg (inclusive).

SEX
8. Male or female (non CBP)

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication.

a. Vasectomy with documentation of azoospermia.

b. Male condom plus partner use of one of the contraceptive options below:

- Contraceptive subdermal implant
- Intrauterine device or intrauterine system
- Oral Contraceptive, either combined or progestogen alone [Hatcher, 2007a] Injectable progestogen [Hatcher, 2007a]
- Contraceptive vaginal ring [Hatcher, 2007a]
- Percutaneous contraceptive patches [Hatcher, 2007a]

This is an all-inclusive list of those methods that meet the following GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For non-product methods (e.g., male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the International Conference on Harmonization (ICH) [ICH, M3 (R2) 2009]."

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

This is an all inclusive list of those methods that meet the GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and, correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by ICH [ICH, M3 (R2) 2009].

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Females:

A female subject is eligible to participate if she is not pregnant (as confirmed by a negative urine human chorionic gonadotrophin (hCG) test), not lactating, and the following condition applies:

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
- Postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) and estradiol levels consistent with menopause (refer to laboratory reference ranges for confirmatory levels)]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 7.2 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. ALT >1.5x Upper limit of normal (ULN). 2. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%). 3. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones). 4. 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
5. Unable to refrain from prescription or non-prescription drugs, including agents active in the central nervous system, 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 and throughout the study, unless in the opinion of the Investigator and/or GSK Medical Monitor (if needed) the medication will not interfere with the study procedures or compromise subject safety.

RELEVANT HABITS
6. 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 7. Urinary cotinine levels indicative of smoking or history or regular use of tobacco- or nicotine-containing products within 6 months prior to screening

CONTRAINDICATIONS
8. 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
9. 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. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody (HBcAb) should also be excluded. 10. A positive pre-study drug/alcohol screen. 11. A positive test for HIV antibody. 12. Where participation in the study would result in donation of blood or blood products in excess of 500 ml within 56 days. 13. 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: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 14. 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 randomised. 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 (Section 7.2.1.4).

Subjects that are not enrolled into the study within the allotted screening window may be re-screened once. If re-screening is performed, subjects are assigned a different unique subject Identification (ID) number for the re-screening, and all screening procedures must be repeated.

See the SRM for specific details.

5.4. Withdrawal/Stopping Criteria

- Subjects may be withdrawn from the study for any of the following reasons: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.
- If a subjects oxygen saturation falls below 65% at any point during the hypoxia challenge.
- The Sponsor may request a subject withdraw for reasons such as significant protocol deviations (after discussion with the investigator).
- Study is terminated by the Sponsor.

A subject will be considered to have prematurely withdrawn from the investigational product if they do not complete all doses in the treatment phase having received the first dose of investigational product, for any reason.

Once a subject has discontinued investigational product the subject may not re-enter the study. Dosing of the subjects with the investigational product may be stopped at any time, at the request of the subject or at the discretion of the Investigator (i.e. if clinically significant adverse events should occur). Withdrawal due to adverse events will be distinguished from withdrawal for other reasons.

If a subject decides to withdraw or is withdrawn by the Investigator, the reasons for withdrawal and the results of any relevant tests will be recorded in the Case Report Form (CRF) and the planned follow-up procedures will be performed, where possible.

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".

5.4.1. Study Stopping Criteria

An interim analysis is planned after approximately 15 subjects have completed treatment periods 1 and 2 and the study may be stopped if a reasonable change in PASP is not observed after dosing with GSK 2586881 or if review of the safety data suggests a change in the benefit-risk profile. The decision to stop the study will be made by the core members of the GSK study team.

Safety data that is collected on a continuous basis (oxygen saturation and telemetry) will be monitored live on site to determine whether a subject tolerates the hypoxia/exercise challenge; study procedures may be aborted on safety grounds if, in the Investigator's opinion, any of these measurements reach unsafe levels. The simulated altitude within the chamber may be reduced if too many participants fail to tolerate the challenge, and the study may be stopped if participants fail to tolerate the challenge at the minimum altitude. The full criteria for this procedure (which may lead to the study being stopped) are described in Section 7.3.

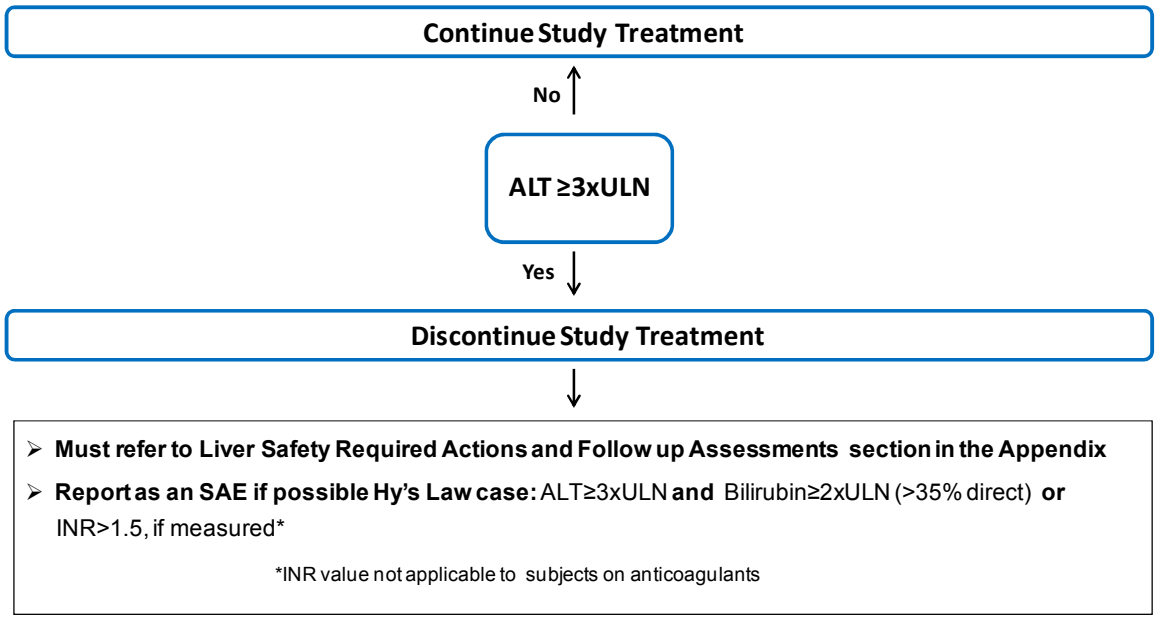
In addition, the study may be stopped at the interim analysis for a safety signal in oxygen saturation under hypoxia and exercise, combined with a lack of evidence of mechanistic effect on Angiotensin biomarkers (Ang II, Ang 1-7 and Ang 1-5). Full details on stopping criteria are described together with the full procedure for the interim analysis in Section 9.3.2.

5.4.2. 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 Food and Drug Administration (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.

5.4.2.1. Study Treatment Restart or Rechallenge

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

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 subjects 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 Treatment	
Product name: (Generic name and trade)	GSK2586881	Placebo
[Formulation description:]	rhACE2	Normal Saline (0.9%)
Dosage form:	IV	IV
Unit dose strength(s)/Dosage level(s):	0.8 mg/kg	Saline Placebo
Route of Administration E.g. oral, for IV infusion, for IV injection, intravitreal use etc	Intravenous	Intravenous
Dosing instructions:	Infuse over 3-5 minutes	Infuse over 3-5 minutes
[Physical description:]	Clear colourless liquid	Clear colourless liquid

At Screening a unique Subject Number (CRF number) will be assigned to any subject who has at least one Screening procedure performed, other than informed consent. The unique Subject Number will be used to identify individual subjects during the course of the study.

Subjects who meet screening eligibility criteria will be assigned to one of the sequences listed in Table 1 below, in accordance with the randomisation schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software. Once assigned, this number must not be reassigned to any other subject in the study.

Table 1 Description of sequences

Sequence	**Period 1**	**Period 2**
AB	GSK2586881	Saline Placebo
BA	Saline Placebo	GSK2586881

The subjects will be randomised using a central randomisation procedure created by GSK.

Further details on how and when a subject is allocated a randomisation number and the subject numbering convention is in the SRM

6.2. Planned Dose Adjustments

No dose adjustments are allowed.

6.3. Blinding

This will be double blind (sponsor open) study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff), and the subject will be blinded to the treatment allocated to individual subjects. An unblinded qualified staff member will be required at site to prepare the study treatment for dosing. The unblindedstaff member is not permitted to communicate the subject's treatment allocation to blinded site staff. Selected study team members working for the sponsor (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This will include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report.

The following will apply:

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment.
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the CRF

A subject whose treatment sequence assignment is unblinded for emergency reasons (as described above) will not be permitted to continue in the study (due to the emergency requiring unblinding rather than the unblinding itself). The event or condition that led to the unblinding will be recorded in the CRF as the primary reason for discontinuation.

A subject whose treatment sequence assignment is inadvertently unblinded (either to investigative staff or the subject themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the subject will be subject to review as to their inclusion in analyses as per the procedures set out in Section 9.3.1.

6.4. Packaging and Labeling

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

6.5. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for GSK2586881 will be detailed in a Study Specific Technical Agreement/Memo (TTS) or Pharmacy Manual which will be accompanied by a Quality Agreement.

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
- 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 Study Reference Manual (SRM).
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.
- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

6.6. Compliance with Study Treatment Administration

When the individual dose for a subject is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

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.

GSK2586881 and the saline placebo will be intravenously administered to subjects at the site. Administration will be documented in the source documents and reported in the CRF.

6.7. Treatment of Study Treatment Overdose

For this study, any dose of GSK2586881 > 1.5 mg/kg within a 24 hour time period will be considered an overdose.

GSK does not recommend specific treatment for an overdose. The investigator will use clinical judgment to treat any overdose.

In the event of an overdose the investigator or treating physician should:

1. contact the Medical Monitor immediately
2. closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until GSK2586881can no longer be detected systemically (at least 3 days for GSK2586881).
3. obtain a plasma sample for pharmacokinetic (PK) analysis within 1 day from the date of the last dose of study treatment 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.

6.8. Treatment after the End of the Study

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

6.8.1. Meals and Dietary Restrictions

- No dietary restrictions prior to first treatment.

6.8.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from caffeine for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.

- During each dosing session, subjects will abstain from alcohol for 12 hours prior to the start of dosing until collection of the final pharmacokinetic and or pharmacodynamic sample during each session.
- Use of tobacco products is not allowed as outlined in the exclusion criteria.

6.8.3. Activity

Subjects will abstain from strenuous exercise for 24 hours prior to screening and each treatment period Subjects may participate in light recreational activities between the planned study procedures (e.g., watch television, read).

6.9. Concomitant Medications and Non-Drug Therapies

6.9.1. 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.

6.9.2. 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. Echocardiograms
    2. 12-lead ECG
    3. vital signs
    4. 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, including: safety, echocardiogram, pharmacokinetic, pharmacodynamic/biomarker or other 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 Institutional Review Board (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

7.1.1. Screening and Follow up

Procedure	Screening[1] (up to 28 days prior to Treatment Period 1, Day 1)	Follow up (7- 10 days post lastdose)	Notes
Informed consent	X		
Inclusion and exclusion criteria	X		
Demography	X		
Full physical exam including height and weight	X	X	Weight at screening will be used for dosing calculation.
Alcohol, DoA , Smoking test	X		
Medical history (includes substance usage [and family history of premature CV disease])	X		Substances: [Drugs, Alcohol, tobacco]
Past and current medical conditions [including cardiovascular medical history]	X		
[Serum OR urine] pregnancy test (WCBP)	X		
HIV, Hep B and Hep C screen	X		
Laboratory assessments (include liver chemistries	X	X	Non Fasting
Immunogenicity	X	X	
12-lead ECG)	X	X	Triplicate ECG required at screening.
Vital signs)	X	X	Triplicate vital signs required at screening.
Echocardiogram	X		
Concomitant Medication review	X	X	
Hypoxia chamber plus exercise	X		Tolerance to 4000m for 10 mins followed by followed by incremental exercise testing to determine maximum oxygen uptake (VO2max) and calculate 70% of VO2max for the exercise challenge d
Pharmacogenetic sample (PGx)	X		Can be taken any time after consent has been signed. Only required once and is optional.
AE/SAE review	X	X	As per timings detailed in Section 7.2.1.1

1. Screening assessments are allowed to be conducted on more than one day

7.1.2. Treatment Period 1 and 2

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to dosing				Hypoxia (Times relative to entry to Chamber)							
	Predose	0h	15 m	15-45m	0-60m	60m	60-70m	Immediately after exercise	30m rest on exit from chamber	After 30 mins rest	60 minutes after exit from Chamber	
Randomisation	X											Randomisation can occur up to the day before the first treatment period
Brief physical exam	X											
Vital signs	X			X				X			X	
Immunogenicity	X											Treatment period 2 only
12-lead ECG	X			X							X	
Echocardiogram	X		X			X		X		X		Echo duration approx 5 mins
Subject enters chamber					X							Subject enters chamber approximately 30 minutes after study treatment
Study Treatment (Dosing)		X										
Subject leaves chamber								X				Subject leaves chamber after the fourth echocardiogram and vital signs have been taken.
Exercise challenge							X					For approx 5-10 minutes
Ventilatory parameters	X		X			X	X[1]			X		Measurements to be taken at the same time as the Echocardiograms. During the exercise challenge, an ECG integrated with the ventilatory assessment will be carried out.
Pulse Oximetry (O2 saturation)		←----------									→	Will be continuously monitored for safety. Measurement should be recorded at time of each echocardiogram post-treatment and databased.
RAS Biomarkers	X	X[2]	X[3]	X[4]		X[3]		X[3]	X[5]	X[3]		
Other Biomarkers	X	X[2]	X[3]	X[4]		X[3]		X[3]	X[5]	X[3]		
PK sampling	X	X[2]	X[3]	X[4]		X[3]		X[3]	X[5]	X[3]		

Procedure	Treatment Period 1 and 2 (Washout 3-14 days)											Notes
	Times relative to dosing				Hypoxia (Times relative to entry to Chamber)							
	Predose	0h	15 m	15-45m	0-60m	60m	60-70m	Immediately after exercise	30m rest on exit from chamber	After 30 mins rest	60 minutes after exit from Chamber	
AE/SAE review		←==========→										
Concomitant medication review	X											

1. To be taken at the end of exercise
2. Take at the end of the infusion
3. Taken immediately after echocardiogram
4. Immediately before entering the chamber
5. To be taken as soon as possible after leaving chamber

After written informed consent, screening assessments will be performed as outlined in the Time and Events Table (Section 7.1).

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

Cardiovascular medical history/risk factors (as detailed in the CRF) will be assessed at screening.

Cardiorespiratory fitness during bicycle exercise and hypoxia tolerance will be assessed.

Procedures conducted as part of the subject's routine clinical management (e.g. blood count) and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

7.2. 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 exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

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

The definitions of an AE or SAE can be found in Appendix 4.

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

7.2.1.1. 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 7.2.1.3), 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 Appendix 4.

- 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 Appendix 4

7.2.1.2. 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?"

7.2.1.3. 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 Appendix 4.

7.2.1.4. 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.

7.2.2. 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. This will be carried out at screening and follow up.
- A brief physical examination will include, at a minimum assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). To be carried out at the start of each period.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

7.2.3. 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. Three readings of blood pressure and pulse rate will be taken.
- First reading should be rejected.
- Second and third readings should be averaged to give the measurement to be recorded in the CRF. (Triplicate measurements will only be taken at screening and single measurements to be taken after that).

7.2.4. Electrocardiogram (ECG)

- Triplicate 12-lead ECGs will be obtained at screening. At all other time-points a single 12-lead ECGs will be obtained at each time point during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 7.2.5 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes rest.

7.2.5. 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 QTcB, then QTcB 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 single or 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.

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

7.2.6. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 2, must be conducted in accordance with the 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 date. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the laboratory manual. 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.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 2.

Table 2 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters
Haematology	Platelet Count; RBC Count; Hemoglobin; Hematocrit; *RBC Indices*: MCV, MCH; *WBC count with Differential:* Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils
Clinical Chemistry [1]	BUN, Potassium, Creatinine, Sodium, Glucose, Calcium, AST (SGOT), ALT (SGPT), Alkaline phosphatise, Total and direct bilirubin, Total Protein, Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal)
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Alcohol, smoking and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, benzodiazepines and cotinine) performed at site • Urine hCG Pregnancy test (as needed) [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 5.4.2 and Appendix 2.
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within 3 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.

7.3. Hypoxia Challenge

Each subject will be inside the hypoxia chamber for approximately 80 minutes per period, during which time they will be under the full 4000m ± 10%. hypoxic conditions

If three or more subjects do not tolerate the hypoxic challenge, either due to symptoms or O2 saturation of <65%, then the simulated altitude will be adjusted in decrements of 500m (i.e. from 4000m to 3500m) for all remaining subjects to a minimum of 3000m.

Further details about the hypoxia challenge is detailed in the SRM.

7.3.1. Exercise Challenge

Subjects will perform an exercise challenge at 70% of maximum VO2 uptake on a cycle ergometer within the hypoxia chamber for 10 minutes as detailed in the SRM.

Subjects must complete a minimum of 5 minutes exercises at 70% of maximum$_{max}$ $_{max}$VO2 uptake.

Further details about the exercise challenge is detailed in the SRM.

7.4. Echocardiogram

Echocardiograms will be taken as detailed in the Time and Events table (Section 7.1).

Echocardiograms will be obtained with the subject resting supine or lying on their left side.

PASP will be measured and recorded.

PASP will be determined by measuring maximal tricuspid regurgitation velocity and applying the modified Bernoulli equation to convert this value into pressure values. Estimated right atrial pressure (RAP) must be added to this obtained value.

Further details about the PASP measurement is detailed in the SRM.

Additional echocardiograms may be obtained for each subject as needed (in the judgement of the Investigator and GSK Medical Monitor if required.

Further details can be found in the SRM.

7.5. Ventilatory parameters

Ventilatory parameters will be measured as detailed in the Time and Events table (Section 7.1).

Measurements may include change from baseline of oxygen consumption (VO2), carbon dioxide production (CO2) and other parameters as data permit

Further details can be found in the SRM.

7.6. Pulse Oximetry

Oxygen saturation will be monitored continuously via pulse oximetry as detailed in the SRM. Measurements to be taken at the same time as the echocardiograms and recorded in the eCRF.

7.7. Pharmacokinetics

7.7.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of GSK2586881 will be collected at the time points indicated in the Time and Events Table (Section 7.1). 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.

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

7.7.2. Sample Analysis

Plasma analysis will be performed under the control of PTS, In Vitro/In Vivo Translations (IVIVT) Department and Third Party Resourcing (TPR), GlaxoSmithKline. The details of the Bioanalytical Laboratory will be included in the Study Reference Manual (SRM). Concentrations of GSK2586881 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will archived at the Bioanalytical Site (to be detailed in the SRM).

7.8. Biomarker(s)/Pharmacodynamic Markers

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

Details of biomarker/PD blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.8.1. Renin-angiotensin system biomarkers

RAS peptides including but not limited to AngII, Ang(1-7) and Ang(1-5) may be analysed as data permit.

7.8.2. Other Biomarkers

Peptide hormones such as Kinins (e.g. des-Arg-bradykinin), apelins (e.g. Apelin 13, pyr1-Apelin, 13) and other systems (e.g. dynorphin A) may be analysed as data permit.

Atrial Naturetic peptide (ANP), Brain Naturetic Peptide (NT-proBNP), Troponin I, Surfactant Protein D, IL-6 and/or additional analytes to be determined may also be analysed as data permit.

7.9. Genetics

Information regarding genetic research is included in Appendix 3.

Genetic sampling is optional. Subjects can refuse PGx sampling but will still be allowed to participate in the study.

7.9.1. Blood sample collection

Blood samples to investigate the association between the loss of function polymorphism rs1799752, representing the I/D polymorphism, in intron 16 of the Angiotensin Converting Enzyme (*ACE*) gene and Ang II (and possibly other RAS peptides) and hypoxic pulmonary vasoconstriction will be collected as specified in the Time and Events Table (Section 7.1).

Further information for blood sample collection, processing, storage and shipping procedures are provided in the SRM.

Information regarding genetic research is included in Appendix 3.

7.10. Immunogenicity

7.10.1. Sample collection

Blood samples for immunogenicity analysis will be collected at the time points indicated in Section 7.1, Time and Events Table. Additional visits to obtain immunogenicity samples may be required in the unlikely event that subjects develop a clinically relevant immunoglobulin response to the drug as described in the SRM.

Details of immunogenicity blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

7.10.2. Sample analysis

Immunogenicity analysis will be performed under the management of Clinical Immunology, Biopharm R&D, GlaxoSmithKline. All pre-dose and post-dose samples will be first tested for Anti-ACE2 binding antibodies by screening and confirmation assay steps. The post-dose samples tested positive for anti-ACE2 binding antibodies will be further characterized for anti-ACE2 neutralizing antibodies. Both positive incidences for anti-ACE2 binding and neutralizing antibodies will be reported.

8. DATA MANAGEMENT

- For this study subject data will be entered into GSK defined CRFs, transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.
- CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials and day/month of birth will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

The focus of this study is on the exploration the effect of GSK2586881 on a range of echocardiography, pulse oximetry, biomarker, safety and PK endpoints, when subjecting healthy volunteers to an exercise challenge under hypoxic conditions. No formal statistical hypotheses are being tested. A Bayesian statistical analysis framework with non-informative priors for model parameters (unless otherwise specified) will be used to obtain posterior distributions for effects of interest. These posterior distributions will be used to obtain a number of probability statements about the magnitude of treatment effects (e.g. Probability of **any** treatment related reduction in PASP, or Probability that the treatment related reduction in PASP $\geq$ 5 mmHg).

A rule of thumb for end of "study success" is if the probability of any treatment related reduction in PASP (T3-T0) exceeds 0.95 (success is also conditional on the probability of (absolute) treatment related reductions in oxygen saturation exceeding 5% being small).

9.2. Sample Size Considerations

The sample size of 30 patients is based on logistical considerations and is not based on formal sample size calculations. However, estimates of precision for the treatment difference in the primary endpoint are presented for context. (Note: precision in this context is defined as one half of the width of the 95% confidence interval.) A sample size re-estimation is planned after 15 subjects.

9.2.1. Sample Size Assumptions

The precision of the primary treatment comparison (change from baseline PASP to hypoxic/exercise PASP) was estimated using simulation. PASP data points were simulated for each subject: normoxic at rest, and hypoxic after exercise, each for two study periods, giving a total of four PASP measurements for each subject. The four PASP measurements were drawn from a multivariate normal distribution to allow for different levels of within-subject correlation. One simulated study with a given sample size (N) was created by repeating this process of generating individual subject data N times, randomly allocating each subject to a treatment sequence in a 1:1 ratio, and calculating the half-width of the confidence interval of the treatment difference in change from baseline in PASP from that simulated dataset (using a linear mixed effects model including period baseline, subject baseline, treatment and period as fixed effects, and subject as a random effect). A full simulation run consisted of 1000 iterations of simulated studies, and a final estimate for precision was calculated as the mean precision across the 1000 iterations.

The following assumptions were made regarding the mean and Standard Deviation (SD) of PASP based on data from Antoni Ricart, 2005

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) and 45 mmHg under hypoxia and exercise
- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise
- As the purpose of this simulation was to estimate precision by evaluating the SD of the treatment difference rather than the mean, there was no treatment effect assumed in the simulations
- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9).
- A range of sample sizes was considered: from 5 to 10 in increments of 1, and then up to 40 in increments of 5. The table below shows a subset of these; the figure includes them all.

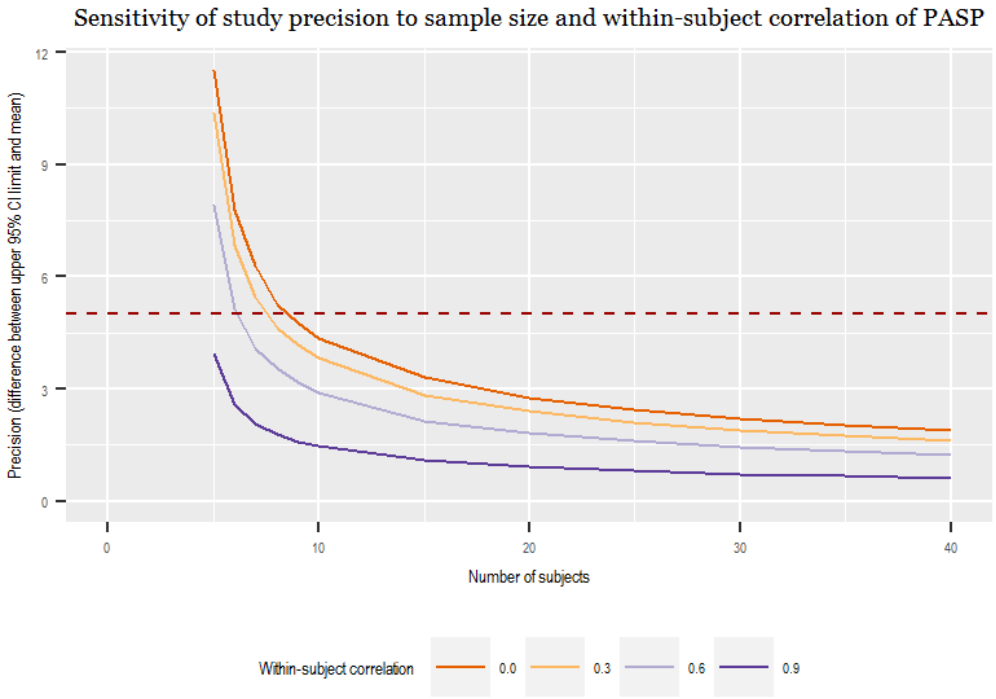
Based on these assumptions, the estimated precisions for these sample sizes and under these scenarios of within-subject correlation were as follows (smaller number for precision denotes more precise estimates):

Table 3 Estimated precision of treatment comparisons of change from baseline in PASP (mmHg) for a range of sample sizes and within-subject correlation scenarios

	Within-subject correlation			
Sample size (N)	**0**	**0.3**	**0.6**	**0.9**
10	4.35	3.83	2.92	1.48
20	2.76	2.40	1.83	0.91
30	2.22	1.91	1.46	0.72
40	1.91	1.63	1.23	0.62

Figure 4 illustrates precision estimates under the full range of sample sizes considered (from 5 to 40), and for all within-subject correlation scenarios. The red line at 5 mmHg is superimposed to indicate a rough guide to the magnitude of a clinically meaningful difference.

Figure 4 Sensitivity of study precision to sample size and within subject correlation of PASP



9.2.2. Sample Size Re-estimation

A sample size re-estimation will be conducted at the interim analysis (see Section 9.3.2). The purpose of the sample size re-estimation is to determine whether the total number of subjects can be reduced below the anticipated 30, based on treatment comparison between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised

PASP (i.e. T3-T0). The sample size re-estimation is to be considered advisory, and taken into account together with other considerations. These will include:

- ensuring sufficient data is collected on secondary endpoints,
- non-conclusive safety data at the time of interim analysis (e.g. a weak signal of a reduction in oxygen saturation data that would require the full set of remaining subjects to be able to reach a conclusion),
- speed of recruitment.

9.3. Data Analysis Considerations

Data will be listed and summarised according to GSK integrated data standards library (IDSL) reporting standards where applicable. Complete details will be provided in the Reporting and Analysis Plan (RAP).

The key endpoints referred to in the text below comprise PASP measured by echocardiography, oxygen saturation measured by continuous pulse oximetry, RAS peptides and safety data.

9.3.1. Analysis Populations

All Subjects Screened Population: This population contains all subjects that complete at least one Visit 1 (Screening) procedure. This population will be used for the summary of subject disposition (including reasons for screening failures, run-in failures, and stabilization failures) and for the listing of AEs and SAEs for non-randomised subjects.

Intent-to-Treat (ITT) Population: This population will comprise all randomised subjects, excluding those who were randomised in error. A subject who is recorded as a screen failure, run-in failure, or stabilisation failure, but is randomised and does not receive a dose of study treatment, is considered to be randomised in error. Any other subject who receives a randomisation number will be considered to have been randomised. This will constitute the primary population for all efficacy and safety analyses.

Pharmacokinetic (PK) population: This population will comprise all subject in the ITT Population for whom a PK sample was obtained and analysed and on active treatment.

In addition to the above populations, the effect of important protocol violations, including any subjects who failed the inclusion/exclusion criteria, may be assessed by means of sensitivity analyses. A blind review of all protocol violations will be performed prior to DBF in order to identify any important deviations and consequently identify any subjects who will be excluded from such sensitivity analyses.

9.3.2. Interim Analysis

An interim analysis is planned after 15 subjects have completed the following critical assessments:

- PASP at T0 and T3 for both study periods
- Oxygen saturation at T0 and T3 for both study periods
- Adverse events

The interim analysis will use the following study data:

- PASP at time T0 and T3 for both periods, for the first 15 subjects
- Oxygen saturation at T0 and T3 for both periods, for the first 15 subjects
- Adverse events for the first 15 subjects
- Angiotensin biomarker concentrations for the first 5 subjects (to allow time for the samples to be analysed by the external lab).

The interim analysis will use a data snapshot that has been cleaned to the best extent allowed by the timeframes (i.e. the interim data will not be fully cleaned or fully QC'd data and there will not be a locked database).

The aims of the interim analysis, and the endpoints to be used to address them, are as follows:

- Using the primary endpoint (treatment differences between GSK2586881 and Placebo in change from pre-dose PASP to hypoxic/exercised PASP [T3-T0]):
    - To decide whether to continue the study or stop based on futility, and
    - to conduct an advisory sample-size re-estimation with the view to potentially reduce the number of subjects participating in the study below 30.
- Using secondary endpoints:
    - to estimate treatment differences between GSK2586881 and Placebo in change from pre-dose oxygen saturation to hypoxic/exercised oxygen saturation [T3-T0], with a view to stopping the study if there is evidence that GSK2586881 causes a reduction in oxygen saturation in healthy volunteers. As a non-binding guide high posterior probability of observing ≥5% absolute differences in **mean** oxygenation saturation values between placebo and active arms would be of concern; but clinical judgement

would override any statistical methods if, for example, the majority of subjects displayed consistent patterns of reductions e.g. between 2-3%).

- to compare adverse events within the two treatment groups and explore any potential safety signals that may emerge

To assess the operating characteristics of the interim analysis decisions to be made based on the primary endpoint, a simulation was carried out to estimate the relative proportion of studies reaching conclusions to stop for futility, continue with no change to the sample size, and to continue with a reduced sample size, under various different scenarios of the true treatment effect. Broadly speaking, the aim of the simulation was to confirm that (in an overwhelming majority of cases) a decision to stop for futility would be reached in the case of no true treatment effect and a decision to continue (perhaps with reduced sample size) would be reached in the case of a true treatment effect.

Simulated datasets of PASP at baseline (i.e. normoxic and at rest) and under hypoxia and exercise for 15 subjects, each receiving both treatments in sequence, were generated using the following assumptions:

- 15 simulated subjects were randomised to one of the two available treatment sequences (see Section 6.2) using block randomisation with block size of 4 records

- PASP at baseline and under hypoxia and exercise for both treatment groups (a total of four PASP measurements per subject) were sampled from a multivariate normal distribution, using values for mean PASP, standard deviation of PASP and within-subject correlation of PASP as described below.

- Mean PASP was 30 mmHg at baseline (i.e. normoxic and at rest) for both treatments, and 45 mmHg under hypoxia and exercise for Placebo (as in Section 9.2.1). For GSK2586881, mean PASP under hypoxia and exercise was (45 - Δ) mmHg, where Δ (the mean treatment difference) was varied according to four different scenarios:

    - no treatment difference (Δ = 0 mmHg)

    - weak treatment difference of a magnitude of half the clinically meaningful difference (Δ = 2.5 mmHg)

    - minimum clinically meaningful difference (Δ = 5 mmHg)

    - overwhelming treatment difference one-and-a-half times the clinically meaningful difference (Δ = 7.5 mmHg)

- SD of PASP was 2.81 mmHg at baseline and 4.30 mmHg under hypoxia and exercise, for both treatment groups (as in Section 9.2.1)

- The within-subject correlation of PASP was unknown, therefore four different scenarios of within-subject correlation were evaluated: zero correlation, low correlation (0.3), high correlation (0.6) and very high correlation (0.9) (as in Section 9.2.1). Within-subject correlation of PASP was assumed to be compound symmetric

(i.e. the same correlation was used for each of the six possible pairs of the four PASP measurements within each subject).

- It was assumed that there would be no effect of period in the simulation, i.e. it was assumed that it would make no difference if the measurement was taken from the first or second chronological period in the treatment sequence.

Modelling took on a three-step process, with decisions after each. Firstly, the treatment difference and its associated standard error was then estimated for the dataset of 15 simulated subjects using a mixed effects linear regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect. If the sign of treatment difference arising from the simulated data was in the wrong direction (i.e. the simulated outcome for GSK2586881 was worse than for Placebo), then the simulated study was stopped for futility.

Secondly, in the alternative case that the simulated treatment difference was in favour of GSK2586881 over Placebo, a posterior probability of success (where success was defined as a 97.5% posterior probability of the treatment difference being in favour of GSK2586881 over Placebo) was evaluated for 30 subjects. This was evaluated using a t-distribution, with the mean and standard deviation taken as the treatment difference and standard error (multiplied by a factor of the square root of 15 to estimate the standard deviation) from step one respectively, and . If this posterior probability was less than 0.2, then the study was stopped for futility as being unlikely to show any benefit of GSK2586881 over Placebo.

Thirdly, provided that the simulated study hadn't been stopped for futility in either of the first two steps, posterior probabilities of success were calculated for N ranging from 15 to 29 subjects. If any of these posterior probabilities of success were greater than or equal to 0.9, then the study was continued with a reduced sample size equal to the lowest N at which that threshold was reached. Otherwise, the conclusion from the simulated interim analysis would be to continue the study with unchanged sample size.

This whole process (simulating data for 15 subjects, modelling the treatment difference, evaluating posterior probabilities of success and simulating the decision-making process) was replicated 1000 times for each scenario, with the decision ("futile", "no change to N" or "reduce N") recorded for each iteration. Each combination of the four treatment difference scenarios and the four correlation scenarios was evaluated for a total of 16 scenarios in all.

The estimated proportions of each decision being reached under each of the 16 scenarios are presented in Table 4.

Table 4 Operating characteristics of interim analysis under four scenarios of treatment effect and four scenarios of within-subject correlation

		Frequency of decisions (%)		
Treatment effect Scenario	Within-subject correlation of PASP	Futile	Continue with 30 subjects	Continue with reduced sample size
Δ=0 mmHg (no treatment difference)				
	0.0	87%	13%	0%
	0.3	87%	13%	1%
	0.6	85%	15%	0%
	0.9	86%	14%	0%
Δ=2.5 mmHg (weak treatment effect, less than clinically meaningful difference)				
	0.0	31%	60%	9%
	0.3	24%	65%	11%
	0.6	10%	66%	25%
	0.9	0%	9%	91%
Δ=5 mmHg (good treatment effect, at threshold of clinical meaningful difference)				
	0.0	2%	46%	52%
	0.3	1%	35%	64%
	0.6	0%	11%	89%
	0.9	0%	0%	100%
Δ=7.5 mmHg (very strong treatment effect)				
	0.0	0%	10%	90%
	0.3	0%	2%	98%
	0.6	0%	0%	100%
	0.9	0%	0%	100%

These results suggest primarily that if there is no true treatment difference between GSK2586881 and Placebo, the study would be stopped for futility 85-87% of the time (irrespective of within-subject correlation of PASP). A drug with a weak treatment effect would most likely result in the study being continued without change or stopped for futility (except in the case where within-subject correlation is 0.9, which is presented here as an extreme case, unlikely to occur in practice). If the true treatment effect is at the clinically relevant threshold of 5 mmHg, then the study is highly unlikely to be stopped for futility and may indicate being reduced in sample size.

Secondarily, it should be noted that the higher the within-subject correlation, then the higher the probability of success. This is because the more correlation there is in measurements within the same individual, the less variability there will be in change from baseline. Results for correlations of 0.0 and 0.9 are presented as extreme cases unlikely to occur in practice, though the exact strength of within-subject correlation of PASP (particularly under different stressed conditions) is not known.

Full details of the interim analysis will be supplied in the RAP.

9.3.3. Multiple Comparisons and Multiplicity

As this is an early-phase exploratory study, no adjustment for multiple comparisons will be made. Treatment comparisons will be presented as effect sizes with 95% confidence intervals.

9.4. Key Elements of Analysis Plan

Each subject will undergo two treatment periods in this crossover study, each one of which being subdivided into five sub-epochs (labelled T0-T4) as follows:

- T0: Pre-dose (period baseline)
- T1: Post-dose, normoxic, at rest, pre-challenge
- T2: Hypoxic, at rest
- T3: Hypoxic, immediately after exercise
- T4: Post-challenge, normoxic, at rest

This follows the principle illustrated in the diagram of echocardiograph measurements (Figure 2).

Pharmacodynamic endpoints will be assigned to T0 thru T4 timepoints based on when they happened relative to the sequence of events that the subject undertakes during the course of the study day.

Pharmacokinetic endpoints will be timed based on elapsed time since dose administration.

In addition to T0 being defined as the period baseline, an overall subject baseline will be defined as the mean of the two T0 measurements (one from each period).

Change from baseline at time points T1 - T4 will be calculated as the difference between the measurement at the time point in question and the period baseline.In general, statistical modelling of changes from baseline will be done using a Bayesian mixed effects regression model, including covariates for period, treatment, period baseline and subject baseline (the mean of the two period baselines) as fixed effects and subject as a random effect (non-informative priors for model parameters). A compound symmetric covariance structure within each subject-period-treatment cell will be assumed to account for repeated measurements a) inherent in the two-period crossover design and b) due to the four post-baseline timepoints within each treatment period.

PASP data will be summarized by treatment and time-point in tabular and graphical format. A Bayesian mixed effects regression model (as described above) will be fitted (using non-informative priors for the model parameters), and an estimate of the mean change from baseline in PASP for each treatment group and post-baseline timepoint,

together with its 95% credible interval, will be obtained. Estimates for treatment differences will also be presented. Data may be log-transformed if necessary.

Details of the analysis of other endpoints will be described in the RAP.

9.4.1. Pharmacokinetic Analyses

Pharmacokinetic analysis will be performed by, or under the auspices, of Clinical Pharmacology Modelling and Simulation Depertment with GlasxoSmithKline. Plasma GSK2586881 concnetration-time data will be analysed by non-compartmental methods with WinNonLin V6.3 or greater. 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 over the study period [nominally AUC(0-2.5h) post-dose], AUC over the hypoxia challenge (nominally AUC(0.5-2.0h post-dose), plasma clearance (CL), volume of distribution (V) and apparent terminal phase half-life (t1/2), if data permit. Other PK parameters may also be determined.

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.

The relationship between the RAS biomarkers and other pharmacodynamic endpoints (echocardiography and pulse oximetry) and GSK2586881 concentrations and/or PK parameters may also be explored, if appropriate.

If appropriate, a population PK analysis may also be conducted, in addition the plasma concentration-time data may be merged with historical data and analysed as part of a population PK meta-analysis.

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.
- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study; also current medical records must be available.

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 randomisation 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

Abraham WT, Raynolds MV, Badesch DB, Wynne KM, Groves BM, Roden RL,et al.. Angiotensin-converting enzyme DD genotype in patients with primary pulmonary hypertension: increased frequency and association with preserved haemodynamics. J Renin Angiotensin Aldosterone Syst. 2003 Mar;4(1):27-30.

Antoni Ricart, Jaume Maristany, Núria Fort, Conxita Leal, Teresa Pagés, and Ginés Viscor. High Altitude Medicine & Biology. March 2005, 6(1): 43-49. doi:10.1089/ham.2005.6.43

Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;49:818-822.

Cargil RI, Lipworth BJ. Lisinopril attenuates acute hypoxic pulmonary vasoconstriction in humans. Chest. 1996 Feb;109(2):424-9/

GlaxoSmithKline Document Number 2010N108777_00 Study ID GSK2586881. Investigator's Brochure for GSK2586881 (formerly known as APN01)– A Soluble Recombinant Human Angiotensin Converting Enzyme 2 (rhACE2). Report Date 16-FEB-2011.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(a): 24. Table 3-2.

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, editors. Contraceptive Technology. 19th edition. New York: Ardent Media, 2007(b): 28.

ICH Topic M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, 2009

Idell S, Kueppers, F, Lippmann, M, Rosen, H, Niederman, M, Fein, A. ACE elevated in ARDS BAL. *Angiotensin converting enzyme in bronchoalveolar lavage in ARDS. Chest.* 1987;91:52-56.

Kiely DG, Cargill RI, Lipworth BJ (1996) Effects of hypercapnia on hemodynamic, inotropic, lusitropic, and electrophysiologic indices in humans. Chest 109:1215–1221

Kiely DG1, Cargill RI, Wheeldon NM, Coutie WJ, Lipworth BJ. Haemodynamic and endocrine effects of type 1 angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale. Cardiovasc Res. 1997 Jan;33(1):201-8.

Kleinsasser A1, Pircher I, Treml B, Schwienbacher M, Schuster M, Janzek E, Loibner H, Penninger JM, Loeckinger A. Recombinant angiotensin-converting enzyme 2 suppresses pulmonary vasoconstriction in acute hypoxia. Wilderness Environ Med. 2012 Mar;23(1):24-30

Maron BA, Leopold JA. The role of the renin-angiotensin-aldosterone system in the pathobiology of pulmonary arterial hypertension (2013 Grover Conference series. Pulm Circ. 2014 Jun;4(2):200-10. doi: 10.1086/675984

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, Chambers RJ, , GJ et al. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am. J. Resp. Crit. Care Med.* 2002;166:646-650.

Paul M, Bachmann J, Ganten D. Angiotensin II-induced vasoconstriction and sodium resorption. *The Tissue Renin-Angiotensin System in Cardiovascular Disease. Trends Cardiovasc. Med.* 1992;2:94-99.

Ricart A1, Maristany J, Fort N, Leal C, Pagés T, Viscor G. Effects of sildenafil on the human response to acute hypoxia and exercise. High Alt Med Biol. 2005 Spring;6(1):43-9.

Rubenfeld GD, Caldwell E, Peabody E. Incidence and Outcomes of Acute Lung Injury. *N Engl J Med.* 2005;353:1685-1693.

Santos RAS, Ferreira AJ, Pinheiro SVB, Sampaio WO, Touyz R, Campangole-Santos MJ. Counter-regulatory effects of ANG (1-7) in ANG II. *Angiotensin(1-7) and its receptor as potential targets for cardiovascular drugs' Expert Op. Investig. Drugs.* 2005;1019:1031.

Santos RAS, Simoes e Silva, AC, Maric C, Silva DMR, Machado RP, de Buhr I, et al. ANG (1-7) as the agonist of mas. *'Angiotensin (1-7) is an endogenous ligand for the G-protein coupled receptor Mas' Proc. Natl. Acad. Sci. USA*. 2003;100:8258-8263.

Suzuki Y, Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V., Egido, J. Inflammatory and pro-fibrotic activity of ANG II. *) Inflammation and Angiotensin II. Int. J. Biochem. Cell Biol.* 2003;35:881-900.

The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med.* 2000;342:1301-1308.

The ARDS Network Authors for the ARDS network. Ketoconzaole for Early Treatment of Acute Lung Injury and Acute Respiratory Distress Syndrome. *JAMA.* 2000;283:1995-2002.

Tom B, deVries R, Saxena PR, Danser J. ANG (1-7) inhibiting ACE and modifying Bradykinin responses. *Bradykinin potentiation by angiotensin (1-7) and ACE inhibitors correlates with C- and N-terminal blockade' Hypertension.* 2001;38:95-99.

Torre-Bueno JR, Wagner PD, Saltzman HA, Gale GE, Moon RE. Diffusion limitation in normal humans during exercise at sea level and simulated altitude. J Appl Physiol (1985). 1985 Mar;58(3):989-95

Wenz M, Hoffmann B, Bohlender J, Kaczmarczyk G. ANG II elevated in ARDS. *Anfgiotensin II formation and endothelin clearance in ARDS patients in supine and prone positions. Intensive Care Med.* 2000;26:292-298.

Wheeler AP, Bernard GR, Thompson BT, Schoenfeld D, Wiedemann HP, deBoisblanc B,et al.. Pulmonary-Artery versus Central Venous Catheter to Guide Treatment of Acute Lung Injury. *N Engl J Med.* 2006;25:354(21):2213-24. Epub 2006 May 21.

Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B,et al. Comparison of Two Fluid-Management Strategies in Acute Lung Injury. *N Engl J Med.* 2006;354(24):2564-75. Epub 2006 May 21.

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

ACE2	Angiotensin converting enzyme type 2
AE	Adverse Event
ALI	Acute Lung Injury
ALT	Alanine aminotransferase (SGPT)
Ang II	Angiotensin II
ANP	Atrial Naturetic peptide
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
BID	Bi-Daily
BUN	Blood urea nitrogen
CBP	Child Bearing Potential
CL	Clearance
CO2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CV	Cardiovascular
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HPV	Hypoxic pulmonary vasoconstriction
IB	Investigator's Brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identification
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IND	Investigational New Drug
IP	Investigational Product

IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
IVIVT	In Vitro/In Vivo Translations
Kg	Kilogram
LDH	Lactate dehydrogenase
LFTs	Liver function tests
m	Meters
MCH	Mean corpuscular hemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mL	Milliliter
MSDS	Material Safety Data Sheet
msec	Milliseconds
NOAEL	No observed adverse effect level
NT-proBNP	Brain Naturetic Peptide
O2	Oxygen
PaO2	Partial Pressure of Oxygen in arterial blood
PAH	Pulmonary Arterial Hypertension
PASP	Pulmonary Artery Systolic Pressure
PD	Pharmacodynamic
PGx	Pharmacogenetics
PK	Pharmacokinetic
Q	Perfusion
QC	Quality control
QTcB	QT duration corrected for heart rate by Bazett's formula
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RAS	Renin-Angiotensin System
RBC	Red blood cells
rhACE2	Recombinant human angiotensin converting enzyme type 2
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SD	Standard deviation
SRM	Study Reference Manual
TPR	Third Party Resourcing
TTS	Technical Terms of Supply
t½	Terminal phase half-life
tmax	Time of occurrence of Cmax
V	Volume of Distribution
V_A	Ventilation
VO2	Oxygen Consumption

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
MedDRA
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 etiology (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 colspan="2">Actions</th><th>Follow Up Assessments</th></tr>
<tr><td colspan="2">• 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
• A specialist or hepatology consultation is</td><td>• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend.
• Blood sample for pharmacokinetic (PK) analysis, obtained 24 hours after 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.</td></tr>
</table>

Liver Chemistry Stopping Criteria – Liver Stopping Event	
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	• Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]. **NOTE: not required in China**. • 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,et al.. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. Drug Metab Dispos 2009; 37:1779-1784.

12.3. Appendix 3: Genetic Research

Genetics – Background

Naturally occurring genetic variation may contribute to inter-individual variability in response to medicines, as well as an individual's risk of developing specific diseases. Genetic factors associated with disease characteristics may also be associated with response to therapy, and could help to explain some clinical study outcomes. For example, genetic variants associated with age-related macular degeneration (AMD) are reported to account for much of the risk for the condition [Gorin, 2012] with certain variants reported to influence treatment response [Chen, 2012]. Thus, knowledge of the genetic etiology of disease may better inform understanding of disease and the development of medicines. Additionally, genetic variability may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), or pharmacodynamics (relationship between concentration and pharmacologic effects or the time course of pharmacologic effects) of a specific medicine and/or clinical outcomes (efficacy and/or safety) observed in a clinical study.

Genetic Research Objectives and Analyses

The objectives of the genetic research are to investigate the relationship between genetic variants and:

- Response to medicine, including [any treatment regimens under investigation in this study] or any concomitant medicines;

Genetic data may be generated while the study is underway or following completion of the study. Genetic evaluations may include focused candidate gene approaches and/or examination of a large number of genetic variants throughout the genome (whole genome analyses). Genetic analyses will utilize data collected in the study and will be limited to understanding the objectives highlighted above. Analyses may be performed using data from multiple clinical studies to investigate these research objectives.

Appropriate descriptive and/or statistical analysis methods will be used. A detailed description of any planned analyses will be documented in a Reporting and Analysis Plan (RAP) prior to initiation of the analysis. Planned analyses and results of genetic investigations will be reported either as part of the clinical RAP and study report, or in a separate genetics RAP and report, as appropriate.

Study Population

Any subject who is enrolled in the study can participate in genetic research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the genetic research.

Study Assessments and Procedures

A key component of successful genetic research is the collection of samples during clinical studies. Collection of samples, even when no *a priori* hypothesis has been

identified, may enable future genetic analyses to be conducted to help understand variability in disease and medicine response.

- A 6 ml blood sample will be taken for Deoxyribonucleic acid (DNA) extraction. A blood sample is collected at the baseline visit, after the subject has been randomised and provided informed consent for genetic research. Instructions for collection and shipping of the genetic sample are described in the laboratory manual. The DNA from the blood sample may undergo quality control analyses to confirm the integrity of the sample. If there are concerns regarding the quality of the sample, then the sample may be destroyed. The blood sample is taken on a single occasion unless a duplicate sample is required due to an inability to utilize the original sample.

The genetic sample is labelled (or "coded") with the same study specific number used to label other samples and data in the study. This number can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number).

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study, or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will only use samples collected from the study for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

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

Informed Consent

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

Subject Withdrawal from Study

If a subject who has consented to participate in genetic research withdraws from the clinical study for any reason other than being lost to follow-up, the subject will be given a choice of one of the following options concerning the genetic sample, if already collected:

- Continue to participate in the genetic research in which case the genetic DNA sample is retained
- Discontinue participation in the genetic research and destroy the genetic DNA sample

If a subject withdraws consent for genetic research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records.

Genotype data may be generated during the study or after completion of the study and may be analyzed during the study or stored for future analysis.

- If a subject withdraws consent for genetic research and genotype data has not been analyzed, it will not be analyzed or used for future research.
- Genetic data that has been analyzed at the time of withdrawn consent will continue to be stored and used, as appropriate.

Screen and Baseline Failures

If a sample for genetic research has been collected and it is determined that the subject does not meet the entry criteria for participation in the study, then the investigator should instruct the subject that their genetic sample will be destroyed. No forms are required to complete this process as it will be completed as part of the consent and sample reconciliation process. In this instance a sample destruction form will not be available to include in the site files.

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

GSK may summarize the genetic research results in the clinical study report, or separately and may publish the results in scientific journals.

GSK may share genetic research data with other scientists to further scientific understanding in alignment with the informed consent. GSK does not inform the subject, family members, insurers, or employers of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from genetic studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined. Further, data generated in a research laboratory may not meet regulatory requirements for inclusion in clinical care.

Chen H, Yu KD, Xu GZ. Association between Variant Y402H in Age-Related Macular Degeneration (AMD) Susceptibility Gene CFH and Treatment Response of AMD: A Meta-Analysis. PloS ONE 2012; 7: e42464

Gorin MB. Genetic insights into age-related macular degeneration: Controversies addressing risk, causality, and therapeutics. Mol. Asp. Med. 2012; 33: 467-486.

12.4. Appendix 4: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.4.1. 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). • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. • The signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.

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.

12.4.2. 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 and 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 Appendix 2 for the required liver chemistry follow-up instructions

12.4.3. 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

12.4.4. 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.

12.4.5. 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.

12.4.6. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool

- Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool
- If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor Site will enter the serious adverse event data into the electronic system as soon as it becomes available.
- The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.
- After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor telephone.
- Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

SAE reporting to GSK via paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail • 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.

12.5. Appendix 5: Modified List of Highly Effective Methods for Avoiding Pregnancy and Collection of Pregnancy Information

12.5.1. Modified List of Highly Effective Methods for Avoiding Pregnancy

The list does not apply to FRP with same sex partners or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

1. Contraceptive subdermal implant
2. Intrauterine device or intrauterine system
3. Combined estrogen and progestogen oral contraceptive [Hatcher RA, 2011]
4. Injectable progestogen [Hatcher RA, 2011]
5. Contraceptive vaginal ring [Hatcher RA, 2011]
6. Percutaneous contraceptive patches [Hatcher RA, 2011]
7. Male partner sterilization with documentation of azoospermia prior to the female subject's entry into the study, and this male is the sole partner for that subject [Hatcher RA, 2011]. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview provided by her or her partner.

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

Contraceptive requirements for male subjects with female partners of reproductive potential (when applicable).

Male subjects with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until [at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives] after the last dose of study medication.

1. Vasectomy with documentation of azoospermia. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview.
2. Male condom plus partner use of one of the contraceptive options below that meets the Standard Operating Procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label:
    - Contraceptive subdermal implant
    - Intrauterine device or intrauterine system

- Combined estrogen and progestogen oral contraceptive [Hatcher RA, 2011]
- Injectable progestogen [Hatcher RA, 2011]
- Contraceptive vaginal ring [Hatcher RA, 2011]
- Percutaneous contraceptive patches [Hatcher RA, 2011]

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

12.5.2. Collection of Pregnancy Information

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study
- Information will be recorded on the appropriate form and submitted to GSK within 2 weeks of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on mother and infant, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Appendix 5. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating

- will discontinue study medication or be withdrawn from the study
- Investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. This applies only to subjects who are randomised to receive study medication.
- After obtaining the necessary signed informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy

- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

12.5.3. References

Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, Policar MS, editors. Contraceptive Technology. 20th edition. Atlanta, Georgia: Ardent Media, Inc., 2011: 50. Table 3-2.