TITLE PAGE

Division: Worldwide Development

Information Type: Protocol Amendment

Title:	An escalating dose, randomized, placebo-controlled, incomplete-block, 2 period cross-over study to assess the dose response for topical efficacy via airway responsiveness to adenosine-5'-monophosphate (AMP) challenge and the dose response for systemic activity via 24 h plasma cortisol suppression and thereby the relative therapeutic index for fluticasone furoate (FF), fluticasone propionate (FP) and budesonide (BUD) in asthmatic subjects.

Compound Number: CCI18781 ((FP), fluticasone propionate), GW685698 ((FF), fluticasone furoate) and GR160288 **(**budesonide (BUD))

Development Phase IIa

Effective Date: 26-APR-2018

Protocol Amendment Number: 03

Author(s):

PPD

Revision Chronology

2016N281231_00	2016-SEP-29	Original
2016N281231_01	2017-JAN-16	Amendment No. 1 was to clarify the dose rationale, emergency unblinding procedure, and approval of substantial amendments procedure, as requested by the Medicines and Healthcare products Regulatory Agency (MHRA), as well as to clarify the blood sample time points and volumes and update the medical monitor contact details.
2016N281231_02	2017-OCT-03	Amendment No. 2 was to change the following: The protocol design, to allow subjects to complete either 1 or 2 treatment periods. To allow recruitment of subjects taking low-dose inhaled corticosteroids (ICS) with appropriate washout period. To allow inclusion of light smokers. Removal of the Run-in and treatment period 2 baseline visits for adenosine-5'-monophosphate (AMP) challenge. To allow the subjects to leave the unit after Day 7 evening procedures. To allow in-stream data review. Changes laid down in MEMOs to protocol amendment 1 were also incorporated into this protocol amendment.
2016N281231_03	2018-APR-26	Amendment No. 3 is being issued to update the serious adverse event (SAE) contact and processing information, the pregnancy reporting timelines, as well as to include administrative changes clarifying the screening peak expiratory flow rate (PEFR) procedure.

SPONSOR SIGNATORY:

PPD

PPD irtnPPD MD
Directc atory HUP

26 April 2018
Date

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	Site Address
SAE contact information (PAREXEL Global Pharmacovigilance Processing Group (GPPG)	PAREXEL Medical Services	Email: PPD PPD and PPD PPD and PPD Or Fax: PPD PPD	NA	NA
Primary Medical Monitor	PPD			GSK, Stockley Park West, Uxbridge, Middlesex, UB11 1BT, UK
Secondary Medical Monitor				GSK, 709 Swedeland Rd, King of Prussia, PA 19406, USA

Sponsor Legal Registered Address:

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

In some countries, the clinical study 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: 2016-003002-14.

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol 203162

- 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

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1. PROTOCOL SYNOPSIS FOR STUDY 203162

Rationale

This study aims to investigate whether higher potency confers an improvement in the topical efficacy to systemic activity ratio. It will compare the dose response for topical efficacy via airway responsiveness to adenosine-5'-monophosphate (AMP) challenge and the dose response for systemic activity via 24 hour plasma cortisol suppression and thereby the relative therapeutic index for fluticasone furoate (FF), fluticasone propionate (FP) and budesonide (BUD) in asthmatic subjects.

Metabolomics will also be included as an exploratory endpoint to study the systemic metabolic bioactivity profile of the three inhaled corticosteroids (ICS) with the aim of identifying whether qualitative or quantitative differences exists in on-target and off-target steroid receptor interactions. This will provide new information that may allow the systemic effects of the three ICS to be distinguished quantitatively and qualitatively with endpoints not previously identified.

Objectives/Endpoints

Objectives	Endpoints
Primary	
To characterize the dose response and relative potency for FF, FP and BUD in the adenosine monophosphate (AMP) challenge model at 12 hours after the last dose on Day 7	Provocative concentration (PC) of AMP causing a 20% fall in forced expiratory volume in 1 second (FEV_1) (AMP PC_{20}).
To characterize the dose response and relative potency for FF, FP and BUD on 24 hour plasma cortisol suppression on-treatment from pre-dose PM dose on Day 6 to pre-dose PM dose Day 7.	Suppression of 24 hour weighted mean plasma cortisol compared to placebo.
To assess the therapeutic index for FF, FP and BUD.	(ED_{20}) cortisol suppression (dose at which 20% cortisol suppression is reached) /ED_{80} for AMP PC_{20} (dose at which 80% of the maximum AMP PC_{20} is reached).
Secondary	
Safety and tolerability.	Safety and tolerability of all treatments as assessed by adverse events (AEs), peak expiratory flow rate (PEFR), vital signs, physical examinations, laboratory assessments, and spirometry.
Exploratory	
To estimate the dose of FF and BUD that gives the same AMP response as the FP doses administered in this study.	AMP PC_{20}.
To estimate the dose of FF and BUD that gives the same cortisol suppression as the FP doses administered in this study.	Suppression of 24 hour weighted mean plasma cortisol compared to placebo.

Objectives	Endpoints
To compare the systemic metabolic bioactivity profile of FF, FP and BUD with the aim of identifying qualitative, quantitative and dose related differences in on-target and off-target steroid receptor interactions using plasma metabolomics.	Metabolic profiling of plasma samples collected pre-dose and 12 hour after the last dose on Day 7 compared to placebo.

Overall Design

This is a randomized, placebo-controlled, incomplete-block, 2-period cross-over, 7-day escalating repeat dose study in subjects with mild asthma.

Subjects will consent to participate in either:

- one treatment period; or
- two treatment periods, separated by a washout period of 25-42 days.

Each treatment period will comprise five consecutive 7-day dosing phases with escalating doses of one of three ICS products or placebo. Each 7-day period is referred to as an escalation phase. In each 7-day escalation phase, subjects will receive study treatment from the evening of Day 1 to the evening of Day 7.

Subjects who consent to completing 2 treatment periods will be assigned to one of the treatment sequences. Subjects who consent to completing 1 treatment period will be randomized to treatment. The randomization schedules will be generated by Clinical Statistics prior to the start of the study, using validated internal software.

Treatment Arms and Duration

There will be 5 different study treatments for subjects who consent to 2 treatment periods, these treatments will be administered in 12 different treatment sequences with 2 treatments each. Two subjects will be randomized to each of the 12 treatment sequences. Subjects who consent to 1 treatment period will be randomized to treatment, 6 subjects will be randomized to each active treatment and 3 subjects to each placebo treatment.

Study days for subjects who consent to 1 treatment period:

- Screening: Day -42 to Day -4.
- One treatment period of at least 35 days (i.e. 5 escalating doses administered for 7 days each).
- Follow-up period: 7 to 14 days after the last dose of treatment period 1.

Study days for subjects who consent to 2 treatment periods:

- Screening: Day -42 to Day -4.
- Treatment periods: two periods of at least 35 days (i.e. 5 escalating doses administered for 7 days each), separated by a washout period of between 25 to 42 days.
- Follow-up period: 7 to 14 days after the last dose of treatment period 2.

The study duration for subjects who consent to 1 treatment period will range from 46 to 91 days. The study duration for subjects who consent to 2 treatment periods will range from 106 to 168 days.

Table 1 Treatments regime proposed per dose escalation phase

Treatment	**1st escalation phase - 7 days**		**2nd escalation phase - 7 days**		**3rd escalation phase - 7 days**		**4th escalation phase – 7 days**		**5th escalation phase – 7 days**
A	FF ELLIPTA™ 25 µg 1 puff PM TDD = 25 µg	→	FF ELLIPTA™ 100 µg 1 puff PM TDD = 100 µg	→	FF ELLIPTA™ 200 µg 1 puff PM TDD = 200 µg	→	FF ELLIPTA™ 200 µg 2 puff PM TDD = 400 µg	→	FF ELLIPTA™ 200 µg 4 puff PM TDD = 800 µg
B	FP DISKUS™ 50 µg 1 puff PM TDD = 50 µg	→	FP DISKUS™ 100 µg 1 puff AM 1 puff PM TDD = 200 µg	→	FP DISKUS™ 250 µg 1 puff AM 1 puff PM TDD = 500 µg	→	FP DISKUS™ 500 µg 1 puff AM 1 puff PM TDD = 1000 µg	→	FP DISKUS™ 500 µg 2 puff AM 2 puff PM TDD = 2000 µg
C	BUD Turbuhaler 100 µg 1 puff PM TDD = 100 µg	→	BUD Turbuhaler 200 µg 1 puff AM 1 puff PM TDD = 400 µg	→	BUD Turbuhaler 400 µg 1 puff AM 1 puff PM TDD = 800 µg	→	BUD Turbuhaler 400 µg 2 puff AM 2 puff PM TDD = 1600 µg	→	BUD Turbuhaler 400 µg 4 puff AM 4 puff PM TDD = 3200 µg
D	ELLIPTA™ placebo 1 puff PM	→	ELLIPTA™ placebo 1 puff PM	→	ELLIPTA™ placebo 1 puff PM	→	ELLIPTA™ placebo 2 puff PM	→	ELLIPTA™ placebo 4 puff PM
E	DISKUS™ placebo 1 puff PM	→	DISKUS™ placebo 1 puff AM 1 puff PM	→	DISKUS™ placebo 1 puff AM 1 puff PM	→	DISKUS™ placebo 1 puff AM 1 puff PM	→	DISKUS™ placebo 2 puff AM 2 puff PM

BUD- Budesonide; FF-Fluticasone furoate; FP-Fluticasone propionate; TDD-Total daily dose

Type and Number of Subjects

Subjects will be recruited such that 48 subjects will complete the study. Twenty-four subjects will be enrolled to complete two treatment periods; and a further 24 subjects will be enrolled to complete one treatment period. This sample size is based on feasibility.

Analysis

The main purpose of this study is to characterize the dose response and relative potency following repeat inhaled doses of FF, FP and BUD on AMP PC_{20} at 12 hours after the last dose on Day 7 and also to characterize the dose response and relative potency for FF, FP and BUD on the 24 hour plasma cortisol suppression after 6 days' dosing.

To achieve these objectives a dose response maximum effect (E_{max}) model will be fitted to the AMP PC_{20} data and the cortisol suppression 0-24 hours weighted mean data. There will be no formal hypothesis tested, but point estimates and corresponding 95% confidence intervals will be constructed for each of the three parameters for the E_{max} model (response at dose 0 [E_0], E_{max}, and dose at which 50% of the maximum effect is reached [ED_{50}]).

2. INTRODUCTION

Inhaled corticosteroids are the mainstay of asthma treatment with currently eight inhaled corticosteroid (ICS) molecules approved for clinical use spanning a wide range of potencies and other pharmacological properties. However, the consensus amongst prescribers, regulators, treatment guidelines and patients is that the more recently approved molecules with greater selectivity, potency and improved targeting to the lung may not offer any therapeutic advantages.

One reason for this situation is the widely held assumption that the therapeutic index of corticosteroids cannot be improved by increasing their potency via enhanced glucocorticoid receptor binding affinity. This is probably valid for systemically administered corticosteroids since both the efficacy and safety are attributable to circulating drug concentrations and common receptor interactions [Mager, 2003]. However, the same rationale is generally applied to ICS and indeed embedded within asthma treatment guidelines. Consequently, ICS potency is not considered to affect the topical efficacy to systemic activity ratio [Kelly, 1998] and potency differences are thought to be overcome by giving larger doses of the less potent drug to achieve therapeutic equivalence [Kamada, 1996].

There are several reasons why this rationale may not be valid for ICS. First, they exert their anti-inflammatory activity at the site of action in the airways, which is not in equilibrium with the downstream systemic drug concentrations responsible for the unwanted systemic effects [Daley-Yates, 2010]. Secondly, it assumes that increasing ICS potency is not associated with changes in other features of the molecule [Hochhaus, 1997]. However, in reality, the molecular structural features that increase glucocorticoid receptor binding affinity and selectivity also result in physicochemical and

pharmacokinetic changes that together may enhance targeting to the airways and reduce systemic exposure [Daley-Yates, 2015].

Fluticasone furoate, ([FF]; GW685698) is a once daily ICS developed for the treatment of asthma in adults and adolescents aged 12 years and over. Fluticasone furoate is the most potent of the available ICS with a relative glucocorticoid receptor binding affinity ≈30 times that of dexamethasone. Fluticasone furoate is also a component of a combination product for both chronic obstructive pulmonary disease (COPD), and asthma with vilanterol (VI; GW642444), a long-acting beta2-agonist (LABA), and is also being developed as the ICS component in the following two combination treatments: a dual combination with umeclidinium bromide ([UMEC]; GSK573719) a long-acting muscarinic antagonist (LAMA) for the treatment of asthma-COPD overlap syndrome; and a triple combination with both UMEC and VI for the treatment of COPD [GSK Document Number GM2004/00283/12].

Fluticasone propionate (FP, CCI18781) is a twice daily ICS approved worldwide for the treatment of asthma in adults and children. It is also available as a combination product containing the long-acting bronchodilator salmeterol for the treatment of asthma and COPD. FP has a relative glucocorticoid receptor binding affinity ≈18 times that of dexamethasone [GSK Document Number RM2002/00280/01].

Budesonide is a twice daily ICS approved worldwide for the treatment of asthma in adults and children. It is also available as a combination product containing the long-acting bronchodilator formoterol for the treatment of asthma and COPD. Budesonide has a relative glucocorticoid receptor binding affinity ≈9 times that of dexamethasone.

Although it is widely recognized that glucocorticoids are the most effective therapy for asthma and other allergic and inflammatory diseases, the precise mechanism of action is not fully understood. The mode of action for glucocorticoids is through binding to the cytosolic glucocorticoid receptor eventually resulting in transactivation or trans repression of gene transcription. Glucocorticoids are therefore able to act as anti-inflammatory agents via multiple mechanisms to inhibit late phase allergic reactions including decreasing the density of mast cells along mucosal surfaces, decreasing chemotaxis and activation of eosinophils, decreasing cytokine production by lymphocytes, monocytes, mast cells and eosinophils, and inhibiting the metabolism of arachidonic acid [Daley-Yates, 2007].

The undesirable effects of inhaled corticosteroids comprise a broad range of class-related adverse events that include hoarseness/dysphonia, candidiasis of the mouth and throat, adrenal suppression, growth retardation in children and adolescents, decreased bone mineral density, cataract, glaucoma, hyperglycaemia, contusions and pneumonia (in patients with COPD). Some of the commonly reported and minor adverse effects, such as hoarseness/dysphonia, are related to local topical activity, whereas adverse effects related to systemic exposure, such as adrenal suppression, although more serious, are very rarely reported [Mortimer, 2006].

2.1. Study Rationale

Fluticasone furoate is the most recently approved ICS for the treatment of asthma and COPD. It is also the most potent and hence compared to the other ICS can be administered at much lower doses to achieve similar clinical efficacy [Busse, 2014]. There is also evidence from studies in healthy subjects and patients that FF doses in the therapeutic dose range are associated with less systemic effects than therapeutic doses of other ICS [Allen, 2013a; Allen, 2013b]. Although these findings support the hypothesis that higher potency can improve the therapeutic index, there is a need to confirm this in a single study that compares both efficacy and systemic safety endpoints for a range of ICS potencies in the target patient population.

This study aims to investigate whether higher potency confers an improvement in the topical efficacy to systemic activity ratio. It will compare the dose response for topical efficacy via airway responsiveness to adenosine-5'-monophosphate (AMP) challenge and the dose response for systemic activity via 24 hour plasma cortisol suppression and thereby the relative therapeutic index for fluticasone furoate (FF), fluticasone propionate (FP) and budesonide (BUD) in asthmatic subjects.

Metabolomics will also be included as an exploratory endpoint to study the systemic metabolic bioactivity profile of the three ICS with the aim of identifying whether qualitative or a quantitative difference exists in on-target and off-target steroid receptor interactions. This will provide new information that may allow the systemic effects of the three ICS to be distinguished quantitatively and qualitatively with endpoints not previously identified.

2.2. Brief Background

Asthma is a serious public health problem throughout the world, affecting about 300 million individuals of all ages. Asthma is a chronic inflammatory disorder of the airways, characterized by recurrent acute attacks of breathlessness and wheezing, in which many cells and cellular elements play a role [GINA, 2017]. When uncontrolled, asthma can place severe limitations on daily life, and is sometimes fatal. Although there is no cure, avoidance of asthma triggers and the use of appropriate long- and short-term medication offer patients options for control of their disease [WHO, 2015].

Inhaled corticosteroids are recommended as first-line treatment for patients with persistent asthma, and the addition of an inhaled LABA can be considered to improve lung function and symptoms in patients whose asthma is not well controlled on ICS alone.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To characterize the dose response and relative potency for FF, FP and BUD in the adenosine monophosphate (AMP) challenge model at	Provocative concentration (PC) of AMP causing a 20% fall in forced expiratory volume in 1 second (FEV_1) (AMP PC_{20}).

Objectives	Endpoints
12 hours after the last dose on Day 7	
To characterize the dose response and relative potency for FF, FP and BUD on 24hour plasma cortisol suppression on-treatment from pre-dose PM dose on Day 6 to pre-dose PM dose Day 7.	Suppression of 24 hour weighted mean plasma cortisol compared to placebo.
To assess the therapeutic index for FF, FP and BUD.	(ED_{20}) cortisol suppression (dose at which 20% cortisol suppression is reached) /ED_{80} for AMP PC_{20} (dose at which 80% of the maximum AMP PC_{20} is reached).
Secondary	
Safety and tolerability.	Safety and tolerability of all treatments as assessed by adverse events (AEs), peak expiratory flow rate (PEFR), vital signs, physical examinations, laboratory assessments, and spirometry.
Exploratory:	
To estimate the dose of FF and BUD that gives the same AMP response as the FP doses administered in this study.	AMP PC_{20}
To estimate the dose of FF and BUD that gives the same cortisol suppression as the FP doses administered in this study.	Suppression of 24 hour weighted mean plasma cortisol compared to placebo.
To compare the systemic metabolic bioactivity profile of FF, FP and BUD with the aim of identifying qualitative, quantitative and dose related differences in on-target and off-target steroid receptor interactions using plasma metabolomics.	Metabolic profiling of plasma samples collected pre-dose and 12 hour after the last dose on Day 7 compared to placebo.

4. STUDY DESIGN

4.1. Overall Design

This is a randomized, placebo-controlled, incomplete-block, 2-period cross-over, 7-day escalating repeat dose study in subjects with mild asthma.

Screening visit 1 will be conducted 4 – 42 days prior to the first treatment day. Subjects who are taking low-dose ICS will have a 4-week ICS washout during which their asthma symptoms must remain stable as defined in Section 5.1. Screening visit 2 will be conducted 4 – 14 days prior to the first treatment day and at least 4 weeks after screening visit 1. Subjects must have an AMP challenge PC_{20} of ≤80 mg/mL at screening visit 2 to be eligible. Procedures from screening visit 1 may be repeated at screening visit 2 as deemed necessary by the investigator/delegate.

Subjects who do not have to undergo ICS washout may only have 1 screening visit, and undergo screening visit 2 procedures at screening visit 1.

Subjects will consent to participate in either:

- one treatment period; or
- two treatment periods, separated by a washout period of 25-42 days.

Each treatment period will comprise five consecutive 7-day dosing phases with escalating doses of one of three ICS products or placebo. Each 7-day period is referred to as an escalation phase. In each 7-day escalation phase, subjects will receive study treatment from the evening of Day 1 to the evening of Day 7.

A follow-up visit will take place within 7-14 days after the last dose of study treatment. Time and events tables for screening and follow-up visits (Table 9) and Treatment Periods (Table 10) are provided in Section 7.1.

4.2. Treatment Arms and Duration

The treatments for each dose escalation phase are listed in Table 2 and regime to provide each dose in Table 3. Subjects who consent to completing 2 treatment periods will be randomized to order of treatments they are included in. Subjects who consent to completing 1 treatment period will be randomized to treatment.

Table 2 Doses proposed per dose escalation phase

Treatment	1st escalation phase – 7 days		2nd escalation phase – 7 days		3rd escalation phase – 7 days		4th escalation phase – 7 days		5th escalation phase – 7 days
A	FF 25 µg	→	FF 100 µg	→	FF 200 µg	→	FF 400 µg	→	FF 800 µg
B	FP 50 µg	→	FP 200 µg	→	FP 500 µg	→	FP 1000 µg	→	FP 2000 µg
C	BUD 100 µg	→	BUD 400 µg	→	BUD 800 µg	→	BUD 1600 µg	→	BUD 3200 µg
D	ELLIPTA™ Placebo	→	ELLIPTA™ Placebo	→	ELLIPTA™ Placebo	→	ELLIPTA™ Placebo	→	ELLIPTA™ Placebo
E	DISKUS™ Placebo	→	DISKUS™ Placebo	→	DISKUS™ Placebo	→	DISKUS™ Placebo	→	DISKUS™ Placebo

BUD- Budesonide; FF-Fluticasone furoate; FP-Fluticasone propionate

Table 3 Treatments regime proposed per dose escalation phase (total daily doses)

Treatment	1st escalation phase - 7 days		2nd escalation phase - 7 days		3rd escalation phase - 7 days		4th escalation phase – 7 days		5th escalation phase – 7 days
A	FF ELLIPTA™ 25 µg 1 puff PM TDD = 25 µg	→	FF ELLIPTA™ 100 µg 1 puff PM TDD = 100 µg	→	FF ELLIPTA™ 200 µg 1 puff PM TDD = 200 µg	→	FF ELLIPTA™ 200 µg 2 puff PM TDD = 400 µg	→	FF ELLIPTA™ 200 µg 4 puff PM TDD = 800 µg
B	FP DISKUS™ 50 µg 1 puff PM TDD = 50 µg	→	FP DISKUS™ 100 µg 1 puff AM 1 puff PM TDD = 200 µg	→	FP DISKUS™ 250 µg 1 puff AM 1 puff PM TDD = 500 µg	→	FP DISKUS™ 500 µg 1 puff AM 1 puff PM TDD = 1000 µg	→	FP DISKUS™ 500 µg 2 puff AM 2 puff PM TDD = 2000 µg
C	BUD Turbuhaler 100 µg 1 puff PM; TDD = 100 µg	→	BUD Turbuhaler 200 µg 1 puff AM 1 puff PM TDD = 400 µg	→	BUD Turbuhaler 400 µg 1 puff AM 1 puff PM TDD = 800 µg	→	BUD Turbuhaler 400 µg 2 puff AM 2 puff PM TDD = 1600 µg	→	BUD Turbuhaler 400 µg 4 puff AM 4 puff PM TDD = 3200 µg
D	ELLIPTA™ placebo 1 puff PM	→	ELLIPTA™ placebo 1 puff PM	→	ELLIPTA™ placebo 1 puff PM	→	ELLIPTA™ placebo 2 puff PM	→	ELLIPTA™ placebo 4 puff PM
E	DISKUS™ placebo 1 puff PM	→	DISKUS™ placebo 1 puff AM 1 puff PM	→	DISKUS™ placebo 1 puff AM 1 puff PM	→	DISKUS™ placebo 1 puff AM 1 puff PM	→	DISKUS™ placebo 2 puff AM 2 puff PM

BUD- Budesonide; FF-Fluticasone furoate; FP-Fluticasone propionate; TDD-Total daily dose

Twenty-four subjects completing 2 treatment periods will be randomized to one of the treatment sequences shown in Table 4.

Table 4 Treatments proposed per study period for subjects completing 2 treatment periods

Sequence	Period 1	Period 2	n
1	A	B	2
2	A	C	2
3	A	D	2
4	B	A	2
5	B	C	2
6	B	E	2
7	C	A	2
8	C	B	2
9	C	E	2
10	D	A	2
11	E	B	2
12	D	C	2
Total subjects			24
Subjects per treatment			12

Twenty-four subjects completing 1 treatment period will be randomized to one of the treatments shown in Table 5.

Table 5 Treatments proposed for subjects completing 1 treatment period

Treatment	n
A	6
B	6
C	6
D	3
E	3
Total subjects	24
Subjects per treatment	6

Therefore, a total of 48 subjects will complete the study, 18 subjects in total per study treatment (for each active treatment and for placebo combined).

Study durations for subjects completing either 2 or 1 treatment periods are shown in Table 6 and Table 7.

Table 6 Study Duration for subjects completing 2 treatment periods

Screening days	Period 1 days	Washout days	Period 2 days	Follow-Up days	Total days
4-42	35	25-42	35	7-14	106-168

Table 7 Study Duration for subjects completing 1 treatment period

Screening days	Period 1 days	Follow-Up days	Total days
4-42	35	7-14	46-91

4.3. Type and Number of Subjects

Subjects will be recruited such that 48 subjects will complete the study. Twenty-four subjects will be enrolled to complete 2 treatment periods and a further 24 subjects will be enrolled to complete 1 treatment period.

This sample size is based on feasibility.

If subjects are replaced they will be assigned to the same treatment or treatment sequence as the subject they are replacing. Refer to Section 6.2 for more details.

4.4. Design Justification

Three ICS are included in this study; FP which is the most commonly prescribed ICS with a glucocorticoid receptor (GR) binding potency in the mid to high range, BUD with a GR binding potency in the low to mid-range and FF which has the highest GR binding potency. The expected relative potency for efficacy in the AMP challenge model is 2:1 for FP:BUD. The corresponding relative potency for FF:FP is unknown but expected to be in the range 2-5 fold.

This study will assess the activity of FF, FP and BUD in the AMP bronchoprotection model to determine the dose response after 7 days of dosing, measured at 12 hours after the final dose when genomic actions were considered to be maximal. The study will also assess the corresponding dose responses on cortisol suppression, measured as 24 hour plasma cortisol from pre-dose evening of Day 6 to 24 hours later on Day 7.

The study is not double-blinded or double-dummy but a partially blinded study with the following design features mitigated for this aspect. The endpoints are objective rather than subjective. Subjects will receive one or two of the four possible treatments not knowing which are active or placebo. When receiving placebo they will be assigned either a DISKUS™ or an ELLIPTA™ device. There will be no BUD Turbuhaler placebo. Subjects assigned to placebo will follow the same dose escalation scheme taking the same number of inhalations in each phase as the corresponding active treatments. Additionally, this will provide a measure of stability of the AMP and cortisol response over the 5 week period where repeated AMP challenges are performed. For a completely balanced design there would be 14 treatment sequences for subjects completing 2 treatment periods; however, an assumption has been made that ELLIPTA™ and DISKUS™ placebo will have the same effect therefore two of the sequences including placebo (EC and CD) have been dropped to give an equal number of subjects on each treatment administered including placebo overall. Similarly, for subjects completing one treatment period, 3 subjects will be randomized to ELLIPTA™ and DISKUS™ placebo so that 6 subjects in total receive placebo overall, which is equal to the subjects on each active treatment.

In addition to the subjects, the investigators, nurses, technicians and other staff that interact with the subjects will also be blinded to the treatment designations. The subjects will be provided with one of three different dry powder inhalers for each period either active treatment or placebo. The subjects will not be informed which are the active or placebo treatments although no Turbuhaler placebo will be used in the study. The study sponsor will be unblinded to study treatment to allow selected sponsor study team members to perform unblinded in-stream analysis of available data. These findings will inform on the need for a sample size re-estimation or interim analysis (see Section 9.2.3).

Previous studies have indicated that conducting an AMP challenge may affect subsequent AMP challenges when multiple challenges are performed. Therefore by having 5 consecutive placebo challenges within a subject it will be possible to explore and if necessary control for this finding.

Metabolomics will be included as an exploratory endpoint in this study to assess the ability of metabolic profiling to provide additional insight into the direct effects of ICS on individual responses with a particular focus on dose related changes. The three ICS included in this study differ in their receptor binding potency, affinity and specificity for the various on-target and off-target steroid receptors. These interactions translate into the potential for systemic adverse effects of ICS. Of these the most is commonly measured side-effect is suppression the hypothalamic pituitary-adrenal axis, as assessed by monitoring changes in plasma cortisol. However, this provides no information about qualitative differences in the potential for adverse metabolic effects that may exist for different ICS. It is likely that perturbations of adrenal androgen secretion may be a better marker than cortisol for the risk of developing osteoporosis. Therefore, for example, it may be more appropriate to measure dehydroepiandrosterone sulphate (DHEA-S), an adrenal androgen precursor regulated by corticotrophin.

It also has the advantage of a long half-life (10-12 hours) and concentrations that do not fluctuate throughout the day like cortisol. It has also been shown that adrenal androgen secretion is more sensitive than cortisol production to the effects of glucocorticoid therapy [Kannisto, 2004]. The present study presents an opportunity to study the systemic metabolic bioactivity profile of the three ICS with the aim of identifying whether qualitative or quantitative differences exists in on-target and off-target steroid receptor interactions using metabolomics. Investigations will include the entire metabolome with particular focus on any pathways or metabolites detected and related to the steroid biome (e.g. DHEA-S). This will provide new information that may allow various ICS therapeutic indices to be distinguished quantitatively in class effects and by qualitative differences not previously identified.

4.5. Dose Justification

In order to fully characterize the dose response for the bronchoprotective effect of each ICS in the AMP challenge model and simultaneously for cortisol suppression it is necessary to study a wide dose range.

Previous studies have shown that the dose at which 50% of the maximum effect is reached (ED_{50}) for FP and BUD are likely to be less than the lowest clinical dose for

asthma [Phillips, 2004; GSK Document Number GM2005/00525/00, Study SIG103337]. Therefore, the lower doses to be used in the study will be of 25 µg of FF, 50 µg of FP and 100 µg of BUD administered once daily.

FF (100 and 200 µg per day), is a once daily ICS that is being developed for the treatment of asthma, and has been approved in the United States [Food and Drug Administration, 2014] and 5 other countries (Australia, Canada, Chile, Singapore, and Switzerland) for the treatment of asthma in adults and adolescents aged 12 years and over. FF (100 and 200 µg per day) is also approved worldwide (including the European Union - Relvar ELLIPTA™) as the ICS component of a combination product for COPD and asthma, with VI (GW642444). In the current study, it is proposed to dose escalate FF, to a maximum daily dose of 800 µg. FF has been administered to healthy volunteers at doses of 100 to 1600 µg once daily (OD) for 7 days (Study HZA113477, see Section 5.2.2.2 of the FF Investigator Brochure and Study FFA103096 [GSK Document Number GM2005/00082/00]) and as FF/VI at doses up to 800/100 µg OD for 7 days (Study HZA102936 [Kempsford, 2014]). These doses were well tolerated. FF was investigated in dose ranging studies in asthmatic subjects over the range 25 to 800 µg once daily for 8 weeks and was shown to be safe and well tolerated [Bateman, 2012, Bleecker, 2012, Busse, 2012].

FP is indicated in the UK and Germany for the treatment of asthma, at doses of up to 1000 µg twice per day, which also represents the maximum dose that subjects will be exposed to in this study.

BUD is indicated in the UK and Germany for the treatment of asthma, at doses up to 1600 µg in divided doses. In the current study, it is proposed to dose escalate BUD, to a maximum daily dose of 3200 µg. BUD has been administered to asthmatic patients at doses of up to 3200 µg for up to 6 weeks [Kaiser, 1999]. These doses were well tolerated.

For the AMP challenge quadrupling dose increments are expected to show a well-defined dose response [Phillips, 2004] for this reason the following total daily doses of 25 µg, 100 µg, 400 µg for FF; 50 µg, 200 µg, 1000 µg for FP and 100 µg, 400 µg, 1600 µg for BUD have been chosen. However, three doses are insufficient to define the dose response for both AMP challenge and cortisol suppression and include all the doses used therapeutically since there is little overlap in the doses needed [Daley-Yates, 2015; Möllmann, 2001; GSK Document Number GM2005/00082/00]. Therefore, FF 200 µg/day, FP 500 µg/day, and 2000 µg/day and BUD 800 µg/day are included to ensure the dose responses are adequately defined. Likewise, an 800 µg/day FF dose and 3200 µg/day BUD dose will also be included to fully define the cortisol suppression dose response [GSK Document Number GM2005/00082/00] and ensure the dose escalation increments are matched between the three ICS under investigation.

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and nonclinical studies conducted with fluticasone propionate, fluticasone furoate and budesonide can be found in the relevant Investigator's Brochure [GSK Document Number RM2002/00280/01 and GSK

Document Number GM2004/00283/12] and product label, respectively. 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
Corticosteroids: Fluticasone furoate, fluticasone propionate and budesonide		
Exposure to corticosteroid induced side effects including, oropharyngeal pain, candidiasis, hoarseness and potential systemic effect on the hypothalamic-pituitary-adrenal (HPA) axis including adrenal suppression. Other adverse effects include eye disorders, respiratory infections including pneumonia and hypersensitivity.	Inhaled corticosteroids have a number of potential side effects, both locally and systemically. Oral candidiasis is observed as a common event associated with the use of ICS. The incidence and severity of oral candidiasis is similar for various ICS. An increased incidence of pneumonia with higher doses of ICS cannot be ruled out; however, the absolute risk of pneumonia appears to be very small and consistent for various ICS. Hypersensitivity adverse reactions may include anaphylaxis, angioedema, urticaria and rash.	Administered doses in this study will not exceed the previous clinical experience for tolerated doses of FF, FP and budesonide. Significant corticosteroid mediated adverse effects are more likely with chronic dosing of high doses; subjects will receive at least of 35 days' continuous administration including sub-therapeutic doses. The highest doses in the doses escalation are only administered for 7 days. Subjects will be informed about the risk of candidiasis, adrenal suppression, eye disorders, pneumonia and hypersensitivity reactions in the informed consent. If any symptoms appear, investigators should implement an appropriate treatment while observing the subject's asthma symptoms. Subjects will be advised to seek medical treatment if any signs of eye disorder occur. Subjects with concurrent respiratory disease are excluded from the study. Subjects will be advised to seek medical

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		treatment if any signs of hypersensitivity occur. Subjects with milk protein allergy or known hypersensitivity to ICS will be excluded from participating in the study.
AMP Challenge		
Exposure to inhaled AMP challenge agent	Subject may experience bronchospasm	Adenosine monophosphate challenges will be conducted in specialist clinical units, with trained technicians. The normal bronchoconstrictor response to AMP disappears within 30 to 45 minutes after PC_{20} has been achieved. Subjects will remain in the unit until their FEV_1 has returned to at least 90% of pre-diluent baseline. Inhaled salbutamol will be allowed to facilitate recovery as needed.
Withdrawal of Inhaled Corticosteroids		
Subjects receiving low-dose ICS as defined in Appendix 5 may take part after a 4 week ICS washout prior to AMP challenge. There is a risk associated with withdrawing inhaled corticosteroids in these subjects who are currently taking them	Subject may experience worsening of asthma symptoms	Subjects washing out from ICS will be monitored via weekly calls from site, and maintenance of diary to capture PEFR and SABA use. Subjects are instructed to contact the PI/site for assessment if they have a deterioration of their asthma (refer to Section 7.3.8.4. Diary Assessments) or if they have an exacerbation. Any subject whose asthma is defined as not

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		stable will not be eligible to participate in the study.

4.6.2. Benefit Assessment

The study procedures e.g. blood samples, are normally associated with low risk of short-term tolerability e.g. pain and bruising from blood sampling.

All subjects will be closely monitored during the treatment and during the washout period for deterioration of lung function and if necessary withdrawal criteria applied as specified in the protocol (Section 5.4).

This is an exploratory study in otherwise healthy asthmatic subjects, to define dose equivalence relationships for new and established inhaled corticosteroids. The subjects will derive no direct benefit from study participation.

The benefit/risk profile supports the proposed short-term dose escalations of low mid and high doses of currently marketed inhaled corticosteroids study in asthmatic subjects

4.6.3. Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to subjects participating in the study, the potential risks identified in association with FF, FP and BUD are justified by the anticipated benefits that may be afforded to subjects with asthma.

All three study treatments administered in this study are marketed for the treatment of asthma, and therefore, they have already clearly demonstrated a favourable benefit: risk ratio.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational products or other study treatment that may impact subject eligibility is provided in the relevant Investigator Brochure (IB) [GSK Document Number RM2002/00280/01 and GSK Document Number GM2004/00283/12].

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. **Age of subject**: 18 to 65 years of age inclusive, at the time of signing the informed consent.

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. **Asthma**: Documented history of bronchial asthma, first diagnosed at least 6 months prior to the screening visit.
3. **Severity of disease:** a) Pre-bronchodilator $FEV_1 \geq 65\%$ of predicted at screening. b) The pre-dose baseline FEV_1 should not have changed significantly in the opinion of the investigator from the screening baseline value and should be $\geq 65\%$ predicted for the subject to continue. c) Documented sensitivity to AMP with a provocative concentration of AMP resulting in a fall of $\geq 20\%$ FEV_1 with a PC_{20} AMP ≤ 80 mg/mL at the screening visit.

4. **Current Therapy:**
 	
• **Short-Acting Beta2-Agonists (SABA):** prescribed SABA for at least 12 weeks prior to screening, if needed.
 	
• Subjects receiving low-dose ICS as defined in Appendix 5 may take part after a 4-week ICS washout prior to AMP challenge. The subject's asthma symptoms must remain stable during this 4-week period as assessed at screening visit 2. Stable asthma is defined as:
 	
  • No more than 2 consecutive days where ≥ 12 inhalations/day of salbutamol were used.
 	
  • No severe asthma exacerbations requiring use of systemic corticosteroids (tablets, suspension, or injection) or an in-patient hospitalization or emergency department visit due to asthma that required systemic corticosteroids.
 	
  • No clinical asthma worsening which in the opinion of the investigator requires additional asthma treatment other than study medication or salbutamol.
 	
• Subjects taking LABA, LAMA, leukotriene receptor antagonist (LTRA) therapy within 3 months prior to the start of the study are not eligible.
 	
• Subjects taking biological therapies within 6 months prior to start of the study are not eligible.

WEIGHT
5. **Bodyweight and Body Mass Index (BMI):** Bodyweight ≥ 50 kg and BMI within the range 18.0-35.0 kg/m^2 (inclusive)

SEX
6. **Gender:** Male and female. **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 at least one of the following conditions applies prior to randomization: a. 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 postmenopausal status prior to study enrolment. b. Reproductive potential and agrees to follow one of the options listed in the Modified List of Highly Effective Methods for Avoiding Pregnancy in Females of Reproductive Potential from 30 days prior to the first dose of study medication and until at least five terminal half-lives after the last dose of study medication (Appendix 4). The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.
OTHER
7. **Liver criteria:** Aspartate aminotransferase (AST) and Alanine transaminase (ALT) < 2x upper limit of normal (ULN); alkaline phosphatase and bilirubin ≤ 1.5x ULN (isolated bilirubin > 1.5x ULN is acceptable if bilirubin is fractionated and direct bilirubin < 35%). 8. Light smokers, who smoke ≤ 20 cigarettes per week or equivalent unit dose of other tobacco products or e-cigarettes, are eligible for the study. Smokers who intend to stop, reduce or increase their smoking habit during the study period are not eligible.
INFORMED CONSENT
9. Having provided written informed consent, which includes compliance with the requirements and restrictions listed in the consent form.

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 CORRECTED QT (QTc) INTERVAL)
1. **A history of life-threatening asthma.** NOTE: Life-threatening asthma is defined as an asthma episode that required intubation and/or was associated with hypercapnia, respiratory arrest or hypoxic seizures within the last 5 years.
2. **Other significant pulmonary diseases to include (but not limited to):** severe pneumonia within 6 months of the screening visit, pneumothorax, atelectasis, pulmonary fibrotic disease, bronchopulmonary dysplasia, chronic bronchitis, emphysema, chronic obstructive pulmonary disease, tuberculosis or other respiratory abnormalities other than asthma.
3. **Respiratory Infection:** Culture-documented or suspected bacterial or viral infection of the upper or lower respiratory tract, sinus or middle ear within 4 weeks of screening that: • In the opinion of the investigator, is expected to affect the subject's asthma status or the subject's ability to participate in the study.
4. **Oropharyngeal examination**: A subject will not be eligible if he/she has clinical visual evidence of oral candidiasis at screening.
5. **Asthma Exacerbation:** Any asthma exacerbation requiring oral corticosteroids within 12 weeks of screening or that resulted in overnight hospitalization requiring additional treatment for asthma within 6 months prior to randomization.
CONCOMITANT MEDICATIONS
6. Use of prohibited medications: Any other medications including, anti-depressant drugs, and anti-asthma drugs (other than short-acting inhaled β-agonists supplied as rescue medication, oral contraceptives not listed in Appendix 4, non-steroidal anti-inflammatory drugs, stable doses of antihistamines, and paracetamol) for 1 week prior to screening and throughout the course of the study. Antihistamines should be withheld 4 days prior to AMP challenge. • Subjects must also be able to abstain from short-acting inhaled β-agonists, for 8 hours prior to spirometry. 7. Subjects undergoing de-sensitization therapy.
RELEVANT HABITS
8. **Tobacco Use:** Current smokers who smoke > 20 cigarettes per week or the equivalent unit dose of other tobacco products or e-cigarettes, or smokers with a smoking history of ≥ 10 pack years. 9. History of regular alcohol consumption exceeding 21 units/week for men and

14 units/week for females (1 unit = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of spirits) within 6 months of screening. 10. All subjects who are currently or in the last month have worked night-shifts are excluded from the study.
CONTRAINDICATIONS
11. **Previous Participation:** Exposure to more than four new chemical entities within 12 months prior to the first dosing day or received an investigational product within 30 days of study start, or 5 half-lives of study drug if that is longer.
DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
12. **Other concurrent Diseases/Abnormalities:** A subject has any clinically significant, uncontrolled condition or disease state that, in the opinion of the investigator, would put the safety of the subject at risk through study participation or would confound the interpretation of the study results if the condition/disease exacerbated during the study. **(e.g. stroke or myocardial infarction within 3 months, uncontrolled hypertension, congestive heart failure, uncontrolled diabetes mellitus.)**
13. Evidence of cancer or history of cancer in the past 5 years other than adequately treated basal or squamous cell carcinoma of the skin or adequately treated in situ carcinoma of the cervix.
14. **Pregnancy and Lactating Females**: • Pregnant females as determined by positive urine HCG test at screening or by positive urine HCG test prior to dosing. • Lactating females.
15. Subjects with active or chronic infections including hepatitis B or C, human immunodeficiency virus (HIV). A positive serology at screening.
16. **Allergies:** • **Milk Protein Allergy:** History of severe milk protein allergy. • **Drug Allergy:** Any adverse reaction including immediate or delayed hypersensitivity to intranasal, inhaled, or systemic corticosteroid therapy. Known or suspected sensitivity to the constituents of FF, FP or BUD (i.e., lactose or magnesium stearate, etc). • **Historical Allergy:** History of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.
17. **12-Lead electrocardiogram (ECG) abnormality:** Significant abnormality in the 12-lead ECG performed at screening. The mean of the three individual ECGs will be taken. Mean QT interval corrected for heart rate (QTc) > 450 msec for males or QTc > 470 msec for female subjects and > 480 in subjects with bundle branch block. The QTc is the QT interval corrected for heart rate according to Fridericia's formula (QTcF).

5.3. Screening/Baseline/Run-in Failures

Subjects who fail the initial screening visit may be rescreened once, after discussion with the sponsor. In order to be rescreened, subjects must have demonstrated AMP sensitivity and meet the spirometry and respiratory test results as described in inclusion criterion 3. Subjects must not have met any of the exclusion criteria. If AMP sensitivity was not established at screening, subjects known to be insensitive to AMP will not be rescreened.

Screen failures are defined as subjects who consent to participate in the clinical trial but are never subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials publishing requirements, and respond to queries from regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.3.1.5).

5.4. Withdrawal/ Stopping Criteria

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

- The site must attempt to contact the subject and re-schedule the missed visit as soon as possible.
- The site must counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- In cases where the subject is deemed 'lost to follow-up', the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and if necessary a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to follow-up".
- Subjects who are not eligible to perform an AMP challenge at any of the specified time during the treatment periods (i.e. for reasons of safety) will be withdrawn from the study. One repeat attempt is permitted within 1-3 days.

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.

Worsening asthma: The following criteria will be used to aid the investigator in determining asthma stability. A randomized subject who meets any of the following stopping criteria will be withdrawn from the study:

- Clinic FEV_1 > 35% below the FEV_1 value calculated at pre-treatment Day 1, treatment period 1.

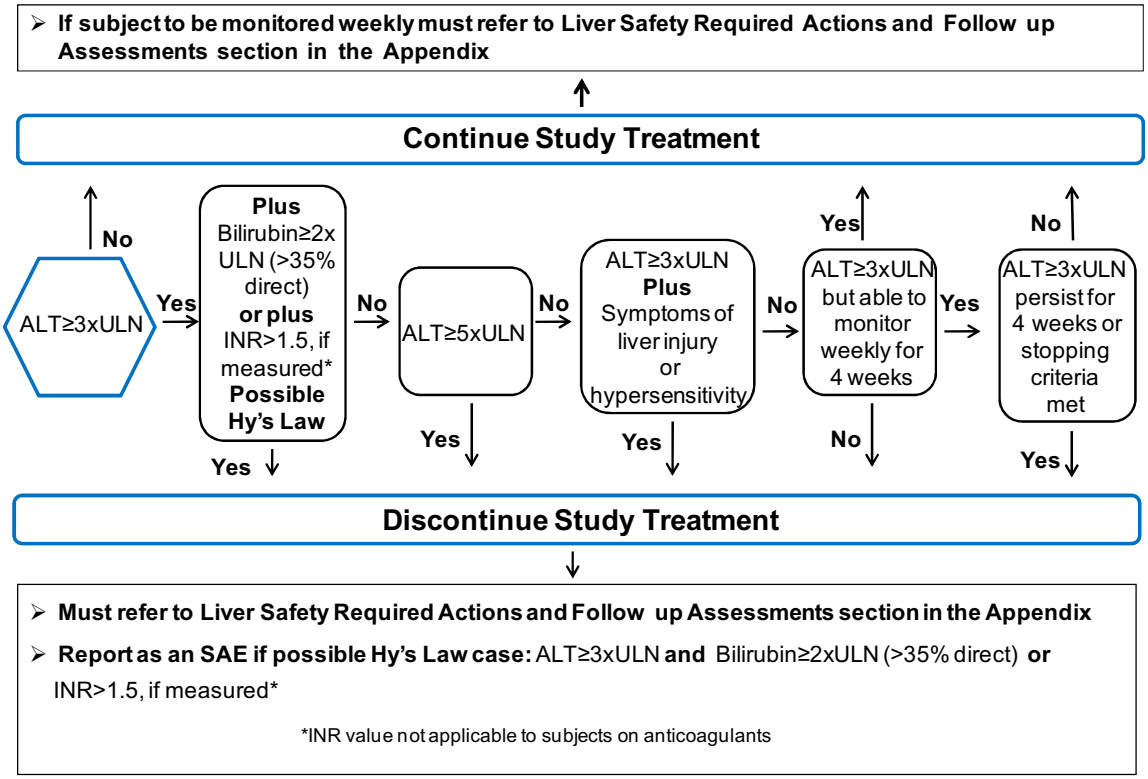
During the study, the subject experienced:

- At least 3 consecutive days in which ≥12 inhalations/day of salbutamol were used.
- Subjects who experience a protocol-defined severe exacerbation, defined as deterioration of asthma requiring the use of systemic corticosteroids (tablets, suspension, or injection) or an in-patient hospitalization or emergency department visit due to asthma that required systemic corticosteroids.
- Clinical asthma worsening which in the opinion of the investigator requires additional asthma treatment other than study medication or salbutamol.

5.4.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the Food and Drug Administration premarketing clinical liver safety guidance) [FDA, 2009].

5.4.1.1. Liver Chemistry Stopping and Increased Monitoring Algorithm



AST- Aspartate aminotransferase; ALT-Alanine transaminase; INR- International normalized ratio; ULN-Upper limit of normal.

Liver Safety Required Actions and Follow-up Assessments Section can be found in Appendix 2.

5.4.1.2. 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.

5.4.2. QTc Stopping Criteria

The ECGs are only recorded at screening thus any QTc stopping criteria are not applicable.

5.5. Subject and Study Completion

A completed subject is one who has completed the number of treatment periods they consented to, 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 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.

Table 8 Study treatments

	Study Treatment			
Product name:	FF (GW685698) 25 µg, 100 µg, and 200 µg Dry Powder Inhaler	FP (CCI18781) 50 µg, 100 µg, 250 µg, and 500 µg Dry Powder Inhaler	Placebo	BUD Turbuhaler
Formulation description:	Dry Powder Inhaler	Dry Powder Inhaler	Dry Powder Inhaler	See label for details.
Dosage form:	Dry Powder Inhaler	Dry Powder Inhaler	Dry Powder Inhaler	Dry Powder Inhaler
Unit dose strength(s)/Dosage level(s):	25, 100, and 200 µg per blister	50, 100, 250, and 500 µg per blister	Lactose	100, 200 and 400 µg per dose
Route of Administration	Inhaled	Inhaled	Inhaled	Inhaled
Dosing instructions:	Per Protocol	Per Protocol	Per Protocol	Per Protocol
Physical description:	Dry White Powder	Dry White Powder	Dry White Powder	White to off-white rounded granules, which disintegrate to a fine powder upon slight pressure
Storage conditions	Store up to 25°C (77°F)	Store at 2-25°C (36-77F)	ELLIPTA™: Store up to 25°C (77°F) DISKUS™: Store at 2-25°C (36-77°F)	Per commercial Label: Do not store above 30°C or See label for details
Device:	ELLIPTA™	DISKUS™	ELLIPTA™ and DISKUS™	Turbuhaler
Method for individualizing dosage:	Inhalation (oral)	Inhalation (oral)	Inhalation (oral)	Inhalation (oral)

BUD- Budesonide; FF – Fluticasone furoate; FP – Fluticasone propionate

6.2. Treatment Assignment

Subjects will be assigned to one of the treatment sequences in accordance with the randomization schedule, generated by Clinical Statistics prior to the start of the study, using validated internal software.

Randomization numbers will be assigned to subjects using the randomization schedule. Subjects should be randomized before they receive their first dose of study medications on the same day (Day 1).

A description of each regimen is provided in Table 3.

Withdrawn subjects may be replaced. Replacement subjects will be assigned to the same treatment sequence as the subject they are replacing.

For subjects completing two treatment periods:

- Each subject will receive 2 different treatments, one in treatment period 1, and one in treatment period 2; the possible treatment sequences are pre-defined in Table 4. Two subjects will be randomized to each of the 12 treatment sequences (Table 4).

- If a subject withdraws during treatment period 1, the replacement subject will start from the beginning of period 1 of the same treatment sequence. If a subject withdraws after period 1 or during period 2, the replacement subject may start from period 2; a maximum of 2 subjects can be replaced from the start of period 2. If more than 2 subjects withdraw after period 1 or during period 2, they will be replaced from the beginning of period 1.

For subjects completing one treatment period:

- Each subject will receive one treatment, as defined in Table 5. Six subjects will be randomized to each treatment, including placebo overall (Table 5).

- Replacements for withdrawn subjects will start from the beginning of period 1 of the same treatment.

6.3. Planned Dose Adjustments

No dose adjustments are planned for this study.

6.4. Blinding

The study is not double-blinded or double-dummy but a partially blinded study by the use of DISKUS™ placebo and ELLIPTA™ placebo, but no Turbuhaler placebo will be used.

In addition to the subjects, the investigators, nurses, technicians and other staff that interact with the subjects will also be blinded to the treatment designations. The subjects will be provided with a dry powder inhaler for each dose escalation phase either active

treatment or placebo. The subjects will not be informed which are the active or placebo treatments although no Turbuhaler placebo will be used in the study.

The following will also 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.

- Selected sponsor study team members may be unblinded to perform the in-stream analysis. This may include the study statistician, study programmer (and delegates) and study DMPK scientist. Access to unblinded data will be kept to the minimum set of individuals required to implement any in-stream or 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.

6.5. Packaging and Labeling

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

All supplies will be Packaged and Labelled in an Open-Label fashion. An unblinded site pharmacist will blind the supplies before dispensing them to the subjects.

6.6. Preparation/Handling/ Storage/Accountability

No special preparation of study treatment is required. An unblinded site pharmacist will blind the supplies (by over-labelling) before dispensing them to the subjects.

- 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 labeled 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.

Precaution will be taken to avoid direct contact with the study treatment. A MSDS describing occupational hazards and recommended handling precautions will be provided to the investigator. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.

6.7. Compliance with Study Treatment Administration

When subjects are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

When subjects self-administer study treatments at home, compliance with FF or FP or BUD or placebo will be assessed through querying the subject during the site visits regarding the completion of subject's diary and then this can be transcribed into electronic case report form (eCRF). A record of the number of FF or FP or BUD or placebo dry powder inhalers dispensed to and taken by each subject must be maintained and reconciled with study treatment and compliance records.

6.8. Treatment of Study Treatment Overdose

For this study, any dose of FF or FP or BUD above the highest doses defined in the protocol within a 24 hour time period will be considered an overdose.

In the event of an overdose the investigator should:

1. Inform the Medical Monitor(s).
2. Monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities as considered appropriate by the investigator. Further medical management of overdose cases is at the discretion of investigator.
3. Document the quantity of the excess dose as well as the duration of the overdosing in the eCRF.

6.9. Treatment After the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because other treatment options are available.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

- Refrain from consumption of chocolate and chocolate drinks, fish, Seville oranges, products containing St John's Wort, grapefruit or grapefruit juice or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until after the final dose.

6.10.2. Caffeine, Alcohol, and Tobacco

- During each dosing session, subjects will abstain from ingesting caffeine-or xanthine-containing products (e.g., coffee, tea and cola drinks) for 24 hours prior to and during all visits.
- During each dosing session, subjects will abstain from alcohol for 24 hours prior to and during all visits.
- Light smokers may continue to use inhaled or non-inhaled nicotine containing products as long as they do not exceed 20 cigarettes or equivalent per week from screening until after the final follow-up visit. To ensure subjects maintain the same degree of AMP sensitivity, they must not intend to increase, reduce or stop the amount they smoke during the course of the study. Subjects must refrain from smoking for at least 1 hour before lung function tests.

6.10.3. Activity

Subjects will abstain from strenuous exercise for 48 hours prior to each blood collection for clinical laboratory tests. Subjects will abstain from vigorous exercise at least 30 minutes before lung function tests.

6.11. Concomitant Medications and Non-Drug Therapies

6.11.1. Permitted Medications and Non-Drug Therapies

The following medications are allowed during the study:

- Short-Acting Beta2-Agonists (SABA) (except within 8 hours prior to spirometry).
- Paracetamol (except within 24hours of metabolomics plasma sample).
- Non-steroidal anti-inflammatory drugs (except within 24 hours of metabolomics plasma sample).
- Use of antihistamines is allowed, however subjects should not take antihistamines within 96 hours of each AMP challenge.
- HRT, hormonal contraception with highly effective contraception methods (see inclusion criterion 6- Section 5.1).
- Any medication, herbal therapy or vitamin which is not listed as prohibited is permitted at the discretion of the investigator.

6.11.2. Prohibited Medications and Non-Drug Therapies

Prohibited medications and non-drug therapies are as follows:

- The subject should be able to abstain from short-acting inhaled β-agonists, for 8 hours prior to spirometry and AMP challenges.
- Use of anti-depressant drugs for 1 week prior to screening and throughout the course of the study.
- Subjects undergoing de-sensitization therapy.
- Use of LABA, LAMA, LTRA therapy for 3 months prior to the start of the study and use of ICS, LABA, LAMA, LTRA therapy throughout the course of the study. Subjects currently receiving low-dose ICS will be consented and perform screening visit 1, then if the subject is eligible for the study they will undergo a 4-week washout period for ICS. If the subject's condition remains stable they will attend screening visit 2 and progress to randomization, if eligible.
- Use of biological therapies for at least 6 months prior to start of the study and throughout the course of 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:

- 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)/Independent Ethics Committee (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

Table 9 Screening and Follow-up Visit

Procedure	Screening Visit 1	Screening Visit 2	Treatment Period 1	Washout[d]	Treatment Period 2[d]	FU
Day	Day -42 to Day -4[b]	Day -14 to Day -4[c]	Assessments during treatment period are detailed in Table 10	Washout will be a minimum of 25 days to maximum of 42 days	Assessments during treatment period are detailed in Table 10	7 to 14 days[g]
Outpatient visit	X	X				X
Informed consent[a]	X					
Inclusion and exclusion criteria	X	X				
Demography	X					
Full physical examination including height and weight	X					
Urine drugs of abuse and alcohol breath test	X					
Brief physical						X
Medical history (includes substance usage)	X					
Urine pregnancy test (all female subjects)	X					X
HIV, Hep B and Hep C screen	X					
Safety labs: chemistry, hematology and urinalysis	X					X
12-lead ECG	X					
Vital signs	X					
PEFR	X[b]					
AMP challenge		X[f]				
Spirometry (FEV_1 and FVC)	X	X				X
AE/SAE[e]	X	X				X
Concomitant medication[e]	X	X				X

AE – Adverse event; AMP- Adenosine 5 monophosphate; ECG – Electrocardiogram; FEV1 – Forced expiratory volume in 1 second; FVC – Forced vital capacity; FU – Follow-up; HIV- Human immunodeficiency virus; Hep B –Hepatitis B; Hep C – Hepatitis C; PEFR –Peak expiratory flow rate; SAE – Serious adverse event;.

[a] Informed consent may be obtained on a separate visit prior to screening visit 1.

[b] Baseline PEFR values will be captured for all subjects at screening visit 1. Subjects who are receiving low-dose ICS may take part after a 4-week washout and screening procedures will be completed in two screening visits. For these subjects, screening visit 2 will be at least 28 days after screening visit 1. During the 4-week ICS washout subjects will keep a diary (from screening visit 1 to randomization) to record twice daily PEFR (subject to contact site if there is a 20% decrease) and SABA use (subject to contact site if SABA intake is increased). Salbutamol will be dispensed for rescue use. The reference PEFR value will be obtained at screening visit 1 and an alert value recorded in subject diary by site staff.

[c] Subjects who do not require an ICS washout may have only one screening visit, these procedures may then be done at screening visit 1. Screening visit 1 procedures may be repeated at screening visit 2 as deemed necessary by the Investigator or delegate.

[d] Subjects who consent to only 1 treatment period will not undergo the washout period or treatment period 2.

[e] AEs and concomitant medication will be documented on all days throughout the study, but review will only occur when the subject is in the unit.

[f] The screening visit 2 AMP challenge can be repeated only once; at least 72h after the first attempt.
[g] 7 to 14 days after the last dose of study treatment.

Table 10 Time and Events Treatment Period 1 and 2

	Treatment period 1 and 2								
	Day 1, pre-dose[a]	**Day 1 dosing and post-dose, Days 8, 15, 22, 29**	**Days 2, 9, 16, 23, 30**	**Days 3, 10, 17, 24, 31**	**Days 4, 11, 18, 25, 32**	**Days 5, 12, 19, 26, 33**	**Days 6, 13, 20, 27, 34**	**Days 7, 14, 21, 28, 35**	**Day 36[b]**
Study treatment dispensing[c]	X	X						X	
Outpatient visit[d]	X	X							
Confinement[e]							X	X	
Randomization[f]	X								
Urine pregnancy test (all female subjects)	X	X							X
Vital signs[g]	X								X
Use of inhaler training[h]	X								
Peak expiratory flow rate[i]		X	X	X	X	X	X	X	
Study Treatment[j]		X	X	X	X	X	X	X	
Patient diary[k]		X	X	X	X	X	X	X	
AMP challenge[l]		X							X
Spirometry (FEV_1 and FVC)	X								
Blood sampling for plasma cortisol[m]	X						X	X	
Metabolomics sample[n]	X	X							X
AE/SAE[o]	X	X	X	X	X	X	X	X	X
Concomitant medication[o]	X	X	X	X	X	X	X	X	X

AE – Adverse event; AMP- Adenosine monophosphate; FEV_1 – Forced expiratory volume in 1 second; FVC – Forced vital capacity; SAE – Serious adverse event.
[a] Day 1 pre-dose completed at start of treatment period 1 and 2, respectively. Urine pregnancy test (where applicable), Vital signs, Spirometry (FEV_1 and FVC), blood sampling for plasma cortisol, metabolomics sample, AE/SAE review, restriction checks and concomitant medication review will be performed on the morning of Day 1 (Pre-dose) of each treatment

period. Use of inhaler training, randomization (treatment period 1 only), AE/SAE review, restriction checks, concomitant medication review and study treatment dispensing will be performed in the evening of Day 1, prior to study treatment administration.

[b] Day 36 completed following completion of treatment period 1 and 2, respectively.

[c] Subjects are dispensed study treatments for the first dose level on Day 1 prior to evening dose. Study treatments for the subsequent 4 dose levels are dispensed prior to release after completion of the AMP challenge on Days 8, 15, 22, and 29.

[d] Subjects receive first dose for treatment Period 1 and 2 during the outpatient visit.

[e] Subject will be admitted to unit prior to evening dose on Days 6, 13, 20, 27 and 34 of each treatment period. Subjects will be confined in the unit until they have recovered from the AMP challenge on Days 8, 15, 22, 29, and 36, but may leave the unit on the evenings of Days 7, 14, 21, 28, and 35 after their last plasma cortisol sample and return the following morning, if they prefer.

[f] Prior to first dose of treatment period 1 only.

[g] Performed on Days 1 and 36.

[h] Reinforcement by the investigator on the proper use of the inhaler.

[i] Peak expiratory flow reading must be taken before each dose of study treatment at home. Three PEFR manoeuvres should be performed in each session. The highest PEFR of the 3 efforts from any session will be the one that will be recorded in a paper diary.

[j] Subjects will take doses of study treatment in the evening only (for all ELLIPTA FF/placebo doses, and the first escalation phase for FP [50 mcg dose] and BUD [100 mcg dose]) or in the morning and evening, 12 hours apart (for all other dose regimens). There will be no dosing in the mornings of Days 1, 8, 15, 22 and 29. When subjects are not at the unit, they will take their study treatments at home.

[k] On Days 1, 8, 15, 22, and 29, subjects will receive a patient diary to document day and time of each dose, the peak expiratory flow rate (PEFR) recording and any AEs or concomitant medication including the number of SABA doses. The subject will enter their data in a paper diary.

[l] The AMP challenges will be done in the morning of Days 8, 15, 22, 29, 36 and 12 hours after the preceding evening dose. If subjects cannot perform the AMP challenge, this may be rescheduled 1-3 days later, in this case; the subjects will continue dosing at the same level until the rescheduled AMP challenge and the study schedule will be shifted.

[m] Blood samples for plasma cortisol will be taken on Day 1 pre-dose (baseline) and at the following time points related to evening study drug administration on Days 6, 13, 20, 27, 34: pre-dose and at 1, 2, 3, 5, 10, 12 (before morning dose), 14, 16, 18 and 24 hours post-dose.

[n] The plasma metabolomics will be taken on Day 1 pre-dose (baseline) and on Days 8, 15, 22, 29 and 36, at the end of each dose escalation phase, at 12 hour after the last evening dose (before AMP challenge procedure).

[o] AEs and concomitant medication will be documented on all days throughout the study, but review will only occur when the subject is in the unit.

7.2. Screening and Critical Baseline Assessments

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

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.

Subjects who are receiving low-dose ICS may take part after a 4-week ICS washout. For these subjects, screening visit 2 will be at least 28 days after screening visit 1. During the 4-week ICS washout and up to randomization, subjects will keep a diary to record twice daily PEFR measurements and SABA use. The site will also contact the subjects weekly to monitor their asthma symptoms and may ask the subject to attend the site if necessary. The subject must contact the site if the PEFR measurement is below the 20% decrease value calculated by the sites, if there is an increase in SABA use, or if they experience an exacerbation.

Salbutamol will be dispensed to all subjects for rescue use from screening and throughout the study as required.

7.3. Safety

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

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

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

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

7.3.1.1. 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 consent until the follow-up contact (see Section 7.3.1.3), 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 eCRF.

- All SAEs will be recorded and reported to PAREXEL within 24 hours, as indicated in Appendix 3.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify PAREXEL.

NOTE: The method of recording, evaluating, and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to PAREXEL are provided in Appendix 3.

7.3.1.2. 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?"

7.3.1.3. 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 3.

7.3.1.4. Cardiovascular and Death Events

For any cardiovascular events detailed in Appendix 3 and all deaths, whether or not they are considered SAEs, specific cardiovascular (CV) and Death sections of the eCRF will be required to be completed. These sections include questions regarding cardiovascular (including sudden cardiac death) and non-cardiovascular death.

The CV eCRFs are presented as queries in response to reporting of certain CV Medical Dictionary for Regulatory Activities (MedDRA) terms. The CV information should be recorded in the specific cardiovascular section of the eCRF within one week of receipt of a CV event data query prompting its completion.

The Death eCRF is provided immediately after the occurrence or outcome of death is reported. Initial and follow-up reports regarding death must be completed within one week of when the death is reported.

7.3.1.5. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to PAREXEL 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 by GSK.

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.

7.3.2. Pregnancy

- Details of all pregnancies in female subjects and female partners of male subjects will be collected after the start of dosing and until follow-up contact that is 7-14 days after the final dose of the study.
- If a pregnancy is reported, then the investigator should inform PAREXEL within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4.

7.3.3. Physical Examinations

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

7.3.4. Vital Signs

Vital signs will be measured after subjects have been rested in supine position for at least 5 minutes and will include temperature, systolic and diastolic blood pressure, pulse rate and respiratory rate. The measurement may be repeated twice if out of range.

7.3.5. Electrocardiogram

- A triplicate 12-lead ECGs will be obtained at screening using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals. The triplicate ECGs will be recorded within a period of 10 minutes. Refer to Section 5.2 (see exclusion criterion 17).
- The ECG measurements will be made with the subject in a supine position having rested in this position for at least 5 minutes.

7.3.6. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 11, must be conducted in accordance with the SRM, and Protocol Time and Events Schedule. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the SRM. 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 eCRF.

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

All study-required laboratory assessments will be performed by a local laboratory. The result of each test has to enter into the eCRF.

Hematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 11.

Table 11 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet Count	*RBC Indices*:	*WBC count with Differential:*	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit		Monocytes	
			Eosinophils	
			Basophils	
Clinical Chemistry [1]	Blood urea nitrogen (BUN)	Potassium	Aspartate aminotransferase (AST or serum glutamic-oxaloacetic transaminase)	Total and direct bilirubin

<table>
<tr><th>Laboratory Assessments</th><th>Parameters</th></tr>
<tr><td></td><td>
<table>
<tr><td>Creatinine</td><td>Sodium</td><td>Alanine aminotransferase (ALT or serum glutamic-pyruvic transaminase)</td><td>Total Protein</td></tr>
<tr><td>Glucose</td><td>Calcium</td><td>Alkaline phosphatase</td><td>Albumin</td></tr>
<tr><td>BUN</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>Routine Urinalysis</td><td>Specific gravity.
pH, glucose, protein, blood and ketones by dipstick.
Microscopic examination (if blood or protein is abnormal) for casts (cellular, granular, hyaline); WBC and RBC.</td></tr>
<tr><td>Other Screening Tests</td><td>HIV
Hepatitis B (HbsAg)
Hepatitis C (Hep C antibody)
FSH and estradiol (if needed to confirm postmenopausal status in women of non-child bearing potential only)
Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines).
Urine HCG Pregnancy test (all female subjects) [2]</td></tr>
<tr><td colspan="2">NOTES :
[1]Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.4.1 and Appendix 2 (Section 12.2).
[2]Local urine pregnancy testing will be standard for the protocol unless serum testing is required by local regulation or ethics committee.</td></tr>
</table>

MCH-Mean cell hemoglobin; MCV- Mean corpuscular volume; RBC- Red blood cell count; WBC – White blood cell count.

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

7.3.7. Inhaler Training

Inhaler training using paper-training materials will be performed at Day 1, with reinforcement of proper technique at subsequent visits. At each subsequent visit, the correct inhalation technique will be reinforced by the investigator prior to dose administration.

7.3.8. Spirometry

Spirometry will be performed using a spirometer calibrated/maintained in accordance with the manufacturer's guidance, which meets American Thoracic Society (ATS)/European Respiratory Society (ERS) standards.

Spirometry (FEV_1 and FVC) will be measured with subjects in a sitting position using a calibrated spirometer in accordance with ATS guidelines using ERS guidelines for predicted values [Quanjer, 2012]. Measurements will be made at the screening visit, on Day 1, and follow-up visit and as a part of the AMP challenge procedures.

7.3.8.1. Screening Baseline Lung Function

Measurements will be made in triplicate and the best recordings (i.e. the highest FEV_1 and the highest FVC) from 3 technically acceptable maneuvers will be recorded in the eCRF. To fulfil the entry criteria, FEV_1 should be ≥ 65% of predicted. This measurement will be the screening baseline value.

7.3.8.2. Pre-dose Baseline Lung Function

Baseline measurements of trough FEV_1 will be made prior to dosing on Day 1 of the treatment period(s). These measures should be made at the same time of day during the treatment period(s). Three technically acceptable measures will be obtained and the highest of these three measurements will be recorded in the eCRF and will constitute the pre-dose baseline value. This pre-dose baseline FEV_1 should not have changed significantly in the opinion of the investigator from the screening baseline value and ≥ 65% predicted for the subject to continue.

7.3.8.3. Peak Expiratory Flow Rate (PEFR) Measurements

Baseline PEFR values will be captured for all subjects at screening visit 1. Subjects who undergo low-dose ICS washout will be provided with a peak flow meter at screening visit 1 for use at home, until randomization. Subjects will also be provided with a diary to record their measurements and symptoms, and will be instructed to contact the site if their PEFR measurement is below the 20% decrease value calculated by the sites, if there is an increase in SABA use, or if they experience an exacerbation.

During each treatment period, subjects will be given a peak flow meter for use at home to measure PEFR. The paper diary will have a place for subjects to record their PEFR measurements that must be taken before each dose. Three PEFR maneuvers should be performed in each session. The highest PEFR of the 3 efforts from any session will be the one that will be recorded in a paper diary.

All PEFR measurements should be taken before subjects take their study drug. Subjects should not, whenever possible, use SABA reliever therapies for 6 hours before performing PEFR.

Subjects who consented to 2 treatment periods will also be provided with a peak flow meter and a patient diary to record their asthma symptoms at home during the washout period. Subjects will be instructed to contact the site if their PEFR measurement is below the 20% decrease value calculated by the sites, if there is an increase in SABA use, or if they experience an exacerbation.

7.3.8.4. Diary Assessments

Subjects who wash-out from low-dose ICS prior to screening visit 2 will be provided with a patient diary to document the following until randomization:

- Any AEs
- Any medical problems the subject may have experienced and any medications used to treat those medical problems
- PEFR measurements twice daily
- Number of occasions rescue salbutamol used over the previous 24 hours

Subjects who wash-out from low-dose ICS prior to screening visit 2 will be instructed to perform PEFR measurements twice daily and to contact the site if there is a 20% fall in PEFR, if there is an increase in SABA use, or if they experience an exacerbation at any point during the study.

Subjects who participate in two treatment periods will be provided with a patient diary during the wash-out period to document:

- Any AEs
- Any medical problems the subject may have experienced and any medications used to treat those medical problems.
- Number of occasions rescue salbutamol used over the previous 24 hours.
- PEFR measurements if their asthma symptoms are getting worse or if they are using their salbutamol more than usual.

Subjects should complete their daily diary pages during the treatment periods. On Days 1, 8, 15, 22, and 29, subjects will receive a patient diary to document:

- Day and time of each dose.
- PEFR measurements.
- Any AEs or medical problems the subject may have experienced and any medications used to treat those medical problems.
- Number of occasions rescue salbutamol used over the previous 24 hours.

Reminding or retraining of the subject on proper paper diary documentation will be conducted if there is a discrepancy between the device metrics and paper diary entries.

7.4. Pharmacodynamics Assessment

Pharmacodynamics assessments will be performed at the time points presented in the Time and Events Table (Section 7.1).

7.4.1. AMP Challenge

The AMP challenge agent to be used for the procedure is Good Manufacturing Practice (GMP)-grade product and will be sourced by the investigator site from Stockport Pharmaceuticals. A certificate of analysis will be provided for each batch of the AMP challenge agent to ensure its quality and safety.

After each 7-day dose escalation phase of each treatment period an AMP challenge [Joos, 1998] 12 hour post-evening dose on Day 7 will be performed.

All subjects will wear a nose-clip during the procedures. Three technically acceptable recordings of FEV_1 will be taken at 1-min intervals prior to administration of diluent. The highest of these three measurements will be used as the pre-diluent baseline. The challenge will not to take place if the subject demonstrates an $FEV_1 < 65\%$ predicted or has significant asthma symptoms of wheeze, chest tightness or cough. Following this baseline assessment, subjects will inhale five breaths of 0.9% diluent, nebulised from a breath-activated dosimeter of known output, by inspiring slowly from functional residual capacity to total lung capacity (i.e., at the beginning of deep inhalation) over 3 seconds and then breath holding for 6 seconds. Two FEV_1 measurements will be taken, the first at 60 seconds and the second at 180 seconds after inhalation of diluent. The higher of the two measurements will be used as the post-diluent FEV_1 to calculate the PC_{20} value (i.e., 80% of post-diluent FEV_1). In the event of a post-diluent fall in FEV_1 of more than 10% from baseline, subjects will be rested for at least 20 minutes before the diluent administration procedure is repeated. If, after three diluent administrations the subject still has a fall in FEV_1 of greater than 10% from the original baseline value, the procedure will be abandoned. If subjects cannot perform an on-treatment AMP challenge, this may be rescheduled 1-3 days later and the subjects will then continue dosing at the same level until the rescheduled AMP challenge and the study schedule will be shifted. If a subject fails to perform the repeated AMP challenge, the subject will be withdrawn from the study.

If post-diluent FEV_1 is equal to10% from pre-diluent, then subjects will inhale doubling increments of AMP until a $\geq 20\%$ fall in FEV_1 from the post-diluent value is achieved or the highest permitted dose of AMP is achieved (320 mg/mL). Doubling concentrations of AMP to be used in the challenge are: 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, 80.0, 160.0 and 320.0 mg/mL. For the screening visit, the maximum dose of AMP to be used for documenting AMP sensitivity is ≤ 80 mg/mL.

FEV_1 will be recorded 60 seconds and 180 seconds after each administration of AMP. If the best FEV_1 is still less than 20% below the post-saline FEV_1 reference subjects will progress immediately within 5 minutes of the previous dose to receiving the next highest concentration of AMP.

The normal bronchoconstrictor response to AMP disappears within 30 to 45 minutes after PC_{20} has been achieved. Subjects are to remain in the unit until their FEV_1 has returned to ≥90% of pre-diluent baseline with the use of salbutamol rescue as needed.

7.4.2. Plasma Cortisol Sample Collection

Blood samples (approximately 3 mL) for measurement of plasma cortisol will be taken at the time points indicated in Section 7 (Time and Events Table).

The actual date and time of each blood sample collection will be recorded. The timing of cortisol samples may be altered and/or cortisol samples may be obtained at additional time points to ensure thorough cortisol monitoring.

Processing, storage and shipping procedures are provided in the SRM.

7.4.3. Sample Analysis

Plasma cortisol analysis will be performed under the control of Platform Technology and Science - Drug Metabolism and Pharmacokinetics (PTS-DMPK)/Scinovo, GlaxoSmithKline, the details of which will be included in the SRM. Concentrations of plasma cortisol will be determined using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Once the plasma cortisol has been analyzed any remaining plasma may be analyzed for other cortisol-related metabolites and the results reported under a separate PTS-DMPK/Scinovo, GlaxoSmithKline protocol.

7.5. Biomarkers

7.5.1. Metabolomic Research

Biofluid [plasma] metabolome studies will be performed by mass spectrometry (LC-MS). This may include analysis of identified or uncharacterized metabolites and lipids that are known to be or emerge in the future as being important in the pathogenesis of asthma, or a related medical condition, the subject's response to study treatment or adverse events.

Subjects will abstain from eating or drinking (except water) for a minimum of 8 hours via overnight fasting prior to each blood collection for metabolomics analysis.

Blood samples (approximately 1 ml) for measurement of plasma metabolomics will be taken on Day 1 pre-dose (baseline) and on Days 8, 15, 22, 29 and 36, at the end of each dose escalation phase, at 12 hour after the last evening dose (before morning dose and AMP challenge procedure).

Plasma samples will be prepared and handled according to Metabolon Sample Preparation Guidelines as follows:

- Collect whole blood in tubes (e.g. Vacutainer or Vacuette) containing Ethylene di amine tetra acetic acid (EDTA) anti-coagulant and follow tube manufacturer's processing instructions. Immediately aliquot the collected plasma/serum/whole blood

into chilled, polypropylene tubes and flash-freeze. Store samples at approximately -80°C until shipment. For each sample 2 x 150 microL aliquots will be prepared and stored for analysis. If possible one or more back-up aliquots will also be prepared and stored at approximately -80°C.

8. DATA MANAGEMENT

- For this study, subject data will be entered into GSK defined eCRFs, 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 the Medical Dictionary for Regulatory Activities (MedDRA) and an internal validated medication dictionary, GSK Drug.
- 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 will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

9.1. Hypotheses

The main purpose of this study is to characterize the dose response and relative potency following repeat inhaled doses of FF, FP and BUD on AMP PC_{20} at 12 hours after the last dose on Day 7 and also to characterize the dose response and relative potency for FF, FP and BUD on 24 hour plasma cortisol suppression (pre-dose PM dose on Day 6 to pre-dose PM dose Day 7).

To achieve these objectives a dose response maximum effect (E_{max}) model will be fitted to the AMP PC_{20} data and the cortisol suppression 0-24 hour weighted mean data. There will be no formal hypothesis tested, but point estimates and corresponding 95% confidence intervals will be constructed for each of the three parameters for the E_{max} model (response at dose 0 [E_0], E_{max}, and ED_{50}).

9.2. Sample Size Considerations

Subjects will be recruited such that 48 subjects complete the study. Twenty-four subjects will be enrolled to complete 2 treatment periods; a further 24 subjects will be enrolled to complete 1 treatment period. This sample size is based on feasibility.

If subjects are replaced they will be assigned to the same treatment or treatment sequence as the subject they are replacing. If a subject withdraws during treatment period 1 then the replacement subject will start from the beginning of period 1 of the same treatment or treatment sequence. If a subject withdraws after period 1 or during period 2 then the

subject may be replaced just to do period 2. A maximum of 2 subjects can be replaced from the start of period 2; if more than 2 subjects on the study withdraw after period 1 or during period 2, the replacements must start from the beginning of period 1.

9.2.1. Sample Size Assumptions

The response to AMP (as measured by PC_{20}) has been measured for FP in study SIG103337 [GSK Document Number GM2005/00525/00] and the estimated placebo response rate has ranged from 0.8 to 1.8 Doubling Dose (DD). ED_{50} is assumed to be around the lowest dose being studied for each active treatment. The maximum effect observed for BUD in a previous study was 4DD [Phillips, 2004] and it is expected that the same maximum effect (E_{max}) will be observed for all active treatments. The Slope (γ=0.9) value is also kept as constant for all treatments this estimate has been considered from the study SIG103337 [GSK Document Number GM2005/00525/00] in which FP was studied. The results in this study are expected to be between the 2 hour and 14 hour data from the study SIG103337 [GSK Document Number GM2005/00525/00], hence, the selected slope of 0.9 which is between the two estimated slopes.

Variability estimates have been considered from in-house studies utilizing the AMP challenge model for inhaled steroids. These are shown in Table 12.

Table 12 Variability Estimates from Inhaled Steroid Studies with an AMP Challenge Model

Study	Compound	Analysis/ Model	Variability Estimate	
			Within Subject SD	Total between Subject SD
SIG102335	GW870086X	ANOVA	1.628	2.040
SIG103337	FP	E_{max}	1.151	2.343
SIG103337	FP	ANOVA	1.221	2.441
All AMP challenges at steady state All challenges at 2 hours post-dose				

ANOVA – Analysis of variance; FP – Fluticasone propionate; SD – Standard deviation

Based on these assumptions, four subjects being assigned to each of the sequences detailed in Table 4. Table 13 details the precision to which this study can estimate each of the three E_{max} model parameters, and thus ultimately the dose response curve for FP, FF and BUD. The below table was generated based on stimulations performed incorporating the variability estimates from the E_{max} model from study SIG103337 (see Table 12) and the initial assumptions on the parameters based on expert opinion and information on doubling doses and ED_{50} from reference studies.

Table 13 Expected Precision of Parameter Estimates for Cross-over and Parallel Design Combined; N=18 Subjects Overall for Each Treatment

Doses	Assumptions	Sample Size	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
FF	E0 = 0.8 Emax = 4 DD ED50 = 25 mcg Slope = 0.9	18	±0.51	±0.55	±15.7
FP	E0 = 0.8 Emax = 4 DD ED50 = 50 mcg Slope = 0.9	18	±0.51	±0.54	±30.3
BUD	E0 = 0.8 Emax = 4 DD ED50 = 100 mcg Slope = 0.9	18	±0.51	±0.55	±62.1

BUD- Budesonide; CI- Confidence interval; FF – Fluticasone furoate; FP – Fluticasone propionate

In previous studies the variability for the cortisol suppression endpoint was observed to be less than for the AMP challenge endpoint. Therefore it is assumed that the sample size deemed to provide sufficient precision estimates for AMP will also be sufficient for cortisol. The variability estimates of cortisol suppression endpoints are shown in Table 14.

Table 14 Variability Estimates from Cortisol Suppression Weighted Mean (0-24 hours) model

Study	Compound	Analysis/ Model	Variability Estimate
			Within Subject SD
FFA103096	FP/ GW685698X	ANOVA	0.385
HZA102940	GW685698X/ GW642444M	ANOVA	0.158
HZA105871	GW685698X/ GW642444M	ANOVA	0.120
HZA108799	GW685698X	ANOVA	0.129

ANOVA – Analysis of variance; SD – Standard deviation

9.2.2. Sample Size Sensitivity

The predicted widths of the confidence interval for the parameters of the model depend on the assumed model parameters. A sensitivity analysis was performed to assess the importance of the assumptions on the results. A range of values observed from the reference studies [GSK Document Number GM2005/00525/00, GSK Document Number GM2007/00123/00, GSK Document Number GM2005/00082/00] have been considered. The results in this study are expected to be somewhere between the 2 hour and 14 hour

data from the SIG10337 study [GSK Document Number GM2005/00525/00] hence, the selected slope of 0.9 which is between the two estimated slopes, and 1.5 DD for E_{max} which is also between the two time points.

The study will include 2 groups of subjects; 1 in which the subjects participate in 2 treatment periods in a cross-over design, and the other in which subjects complete only 1 treatment period in a parallel design. Precision estimates were calculated on the feasible sample size per study treatment, based on 12 subjects in a cross-over design and 6 subjects in a parallel design, and the combined estimates for 18 subjects in total per treatment (Table 15, and Table 16).

Table 15 Simulation Results for Cross-over Design; N=12 Subjects in Each Treatment Group

Doses	Assumptions	Sample Size	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
FF	E0 = 0.8 Emax = 4 DD ED50 = 25 mcg Slope = 0.9	12	±0.67	±0.61	±19.0
FP	E0 = 0.8 Emax = 4 DD ED50 = 50 mcg Slope = 0.9	12	±0.67	±0.59	±37.1
BUD	E0 = 0.8 Emax = 4 DD ED50 = 100 mcg Slope = 0.9	12	±0.67	±0.61	±72.6

Table 16 Simulation Results for Parallel Design; N=6 Subjects in Each Treatment Group

Doses	Assumptions	Sample Size	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
FF	E0 =0.8 Emax =4 DD ED50=25 mcg Slope =0.9	6	±0.61	±1.18	±37.9
FP	E0 =0.8 Emax =4 DD ED50=50 mcg Slope =0.9	6	±0.61	±1.15	±74.6
BUD	E0 =0.8 Emax =4 DD ED50=100 mcg Slope =0.9	6	±0.61	±1.19	±152.5

9.2.3. Sample Size Re-estimation or Adjustment

The need for sample size re-estimation will be reviewed by in-stream data review (see Section 4.4).

9.3. Data Analysis Considerations

9.3.1. Analysis Populations

All subjects' population: All subjects' population will consist of all subjects who are randomized and who receive at least one dose of trial medication.

Pharmacodynamic (PD) population: The PD population is defined as all subjects in All Subjects population who received at least one dose of study treatment and who also have at least one PD measurement.

9.3.2. Interim Analysis

The need for interim analysis will be reviewed by in-stream data review (see Section 4.4).

9.4. Key Elements of Analysis Plan

9.4.1. Pharmacodynamic Analyses

AMP PC_{20}

To characterize the dose response of FF, FP and BUD on AMP PC_{20} at 12 hours after the last dose on Day 7, a non-linear mixed model will be fitted. AMP PC_{20} will be log-transformed (base 2) and then the following model will be fitted.

$$\log_2(PC_{20})_{ijk} = E_0 + Period_j + I_{FF,ij} * \frac{FF\_E_{max} * Dose_{ijk}^{\gamma}}{Dose_{ijk}^{\gamma} + FF\_ED_{50}^{\gamma}} + I_{FP,ij} * \frac{FP\_E_{max} * Dose_{ijk}^{\gamma}}{Dose_{ijk}^{\gamma} + FP\_ED_{50}^{\gamma}} + I_{BUD,ij} * \frac{BUD\_E_{max} * Dose_{ijk}^{\gamma}}{Dose_{ijk}^{\gamma} + BUD\_ED_{50}^{\gamma}} + s_i + \varepsilon_{ijk}$$

where:

i= Subject (i=1 to N), j= Period (j=1 to 2), k= Escalation phase within jth period (k=1 to 5), $Dose_{ijk}$ = Dose received by ith subject in the jth period and kth escalation phase (if this corresponds to Placebo then set Dose to zero).
$I_{FF,ij}$, $I_{FP,ij}$ and $I_{BUD,ij}$ are indicator functions that take the value 1 if the ith subject in the jth period was on the associated study drug and zero otherwise.

Period	=Main effect of period
E_0	= Response at dose 0
FF_E_{max}	= Maximum effect for FF treatment
FF_ED_{50}	= Dose at which 50% of the maximum effect is reached for FF treatment

FP_E_{max} = Maximum effect for FP treatment
FP_ED_{50} = Dose at which 50% of the maximum effect is reached for FP treatment
BUD_E_{max} = Maximum effect for BUD treatment
BUD_ED_{50} = Dose at which 50% of the maximum effect is reached for BUD treatment
γ = Slope constant (fixed at 1 in the first instance)
s_i = Subject effect
ε_{ijk} = Residual error

The slope parameter gamma will be assumed to be 1 (i.e. first intent is to fit three parameter Emax models to each treatment, but four parameter E_{max} formulae are presented as gamma may be fitted as an additional model parameter as part of sensitivity analyses).

The subject term will be fitted as a random effect. This is equivalent to adding a random coefficient to the E_0 parameter. All other terms (except the residual error) in the model will be fitted as fixed effects. The dose will be set to zero for all placebo data. Random coefficients may also be fitted to the other parameters in the model (E_{max} and ED_{50}) if possible. The suitability of such models will be explored.

Prior to analysis, the AMP challenge results for placebo across the five dose escalation phases will be investigated. If there appears to be an effect of repeated AMP challenge on placebo (e.g. a linear trend through time, or evidence contradicting the assumption that each of the placebo responses in the five escalation phases are the same) then the dose response modelling may be performed on an adjusted version of the dataset (adjustments made to active data points to account for the varying placebo data points). Further details will be given in the Reporting and Analysis Plan (RAP).

From the analysis, the predicted value for the 3 parameters of interest (E_0, E_{max} and ED_{50}) for each of the treatments will be calculated and back transformed onto the original scale. The 95% confidence interval for each of the predicted values will also be calculated and back transformed.

Predicted AMP PC_{20} values and 95% confidence intervals will be plotted against dose for each of the active treatments. The potency of FF and BUD relative to FP will be assessed by comparing the estimates for ED_{50}.

The E_{max} model will also be used to address the exploratory endpoint to assess the dose of FF and BUD that gives the same AMP response as the FP doses administered in the study.

Details of the in-stream data review and analysis will be presented in the RAP.

Plasma Cortisol

Similar dose response E_{max} model as mentioned for the AMP PC_{20} data will be constructed to characterize the dose response of FF, FP and BUD on cortisol suppression 0-24 hours weighted mean after Day 6 dose. The cortisol 0-24 hours weighted mean will be log-transformed using natural logs (i.e. base e).

The E_{max} model will also be used to address the exploratory endpoint to assess the dose of FF and BUD that gives the same cortisol response as the FP doses administered in the study.

Therapeutic Index (TI)

E_{max} models as described above will also be used to obtain the ED_{20} 0-24 hr weighted mean cortisol suppression and the ED_{80} for AMP PC_{20}. The TI is then calculated as ED_{20} cortisol suppression /ED_{80} for AMP PC_{20}.

9.4.2. Safety Analyses

Safety data will be summarized descriptively according to GSK's Integrated Data Standards Library standards.

Further details regarding the safety analyses will be given in the RAP.

9.4.3. Biomarker Analyses

Further details regarding the metabolomic analyses will be given in the RAP.

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 enrolment of subjects begins.

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

Prior to initiation of a site, GSK will obtain favorable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with International Council for Harmonization (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.

Substantial amendments to the protocol will be approved by the regulatory agency and/or ethics committee, as applicable, before implementation.

The study will also be conducted in accordance with ICH 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 reported on the CRFs or 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.
- Definition of what constitutes source data can be found in the SRM.

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

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

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

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in

conjunction with assessment of the facility, supporting systems, and relevant site staff.

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

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

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

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

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

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

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
AMP	Adenosine-5-monophosphate
AMP PC20	Provocative concentration of AMP causing a 20% fall in forced expiratory volume in 1 second
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATS	American Thoracic Society
BMI	Body mass index
BUD	Budesonide
BUN	Blood urea nitrogen
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CPK	Creatinine phosphokinase
CRF	Case report form
CV	Cardiovascular
DD	Doubling dose
DHEA-S	Dehydroepiandrosterone sulphate
ECG	Electrocardiogram
eCRF	Electronic case report form
E_0	Response at dose 0
ED_{20}	Dose at which 20% of the maximum effect is reached
ED_{50}	Dose at which 50% of the maximum effect is reached
ED_{80}	Dose at which 80% of the maximum effect is reached
EDTA	Ethylene di amine tetra acetic acid
E_{max}	Maximum effect
ERS	European Respiratory Society
FF	Fluticasone furoate
FP	Fluticasone propionate
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FSH	Follicle stimulating hormone
FU	Follow-up
FVC	Forced vital capacity
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GPPG	Global Pharmacovigilance Processing Group
GR	Glucocorticoid receptor
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen

HCG	Human chorionic gonadotropin
Hep B	Hepatitis B
Hep C	Hepatitis C
HIV	Human immunodeficiency virus
HPA	Hypothalamic pituitary-adrenal
HRT	Hormone replacement therapy
IB	Investigator Brochure
ICH	International Conference on Harmonization
ICS	Inhaled corticosteroids
IEC	Independent ethics committee
IgM	Immunoglobulin M
INR	International normalized ratio
IRB	Institutional review board
ITT	Intention-to-treat
LABA	Long-acting beta2-agonist
LAMA	Long-acting muscarinic antagonist
LC-MS	Liquid chromatography-Mass spectroscopy
LDH	Lactate dehydrogenase
LS	Least square
LTRA	Leukotriene receptor antagonist
MedDRA	Medical Dictionary for Regulatory Activities
mcg	Microgram
MCH	Mean cell haemoglobin
MCV	Mean corpuscular volume
MS	Mass spectrometry
MSDS	Material safety data sheet
PC	Provocative concentration
PD	Pharmacodynamic
PEFR	Peak expiratory flow rate
PK	Pharmacokinetic
PTS-DMPK	Platform Technology and Science - Drug Metabolism and Pharmacokinetics
QTc	Corrected QT interval
QTcF	QT interval with Fridericia's correction
RAP	Reporting analysis plan
RBC	Red blood cell
SABA	Short-Acting Beta2-Agonists
SAE	Serious adverse event
SD	Standard deviation
SRM	Study reference manual
TDD	Total doubling dose
TI	Therapeutic index
ULN	Upper limit normal
UMEC	Umeclidinium bromide
VI	Vilanterol
WBC	White blood cell

WHO	World Health Organization

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
DISKUS
ELLIPTA

Trademarks not owned by the GlaxoSmithKline group of companies
Turbuhaler

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

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

Phase II liver chemistry stopping criteria and required follow-up assessments

Liver Chemistry Stopping Criteria – Liver Stopping Event	
ALT-absolute	ALT ≥ 5xULN
ALT Increase	ALT ≥ 3xULN persists for ≥4 weeks
Bilirubin[1, 2]	ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (> 35% direct bilirubin)
INR[2]	ALT ≥ 3xULN **and** INR> 1.5, if INR measured
Cannot Monitor	ALT ≥ 3xULN and cannot be monitored weekly for 4 weeks
Symptomatic[3]	ALT ≥ 3xULN associated with symptoms (new or worsening) believed to be related to liver injury or hypersensitivity

Required Actions and Follow-up Assessments following ANY Liver Stopping Event	
Actions	**Follow-Up Assessments**
• **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 chemistry event follow-up assessments • Monitor the subject until liver chemistries resolve, stabilize, or return to within baseline (see **MONITORING** below) • **Do not restart/rechallenge** subject with study treatment unless allowed per protocol and GSK Medical Governance approval **is** granted. • If restart/rechallenge **not allowed per protocol or not granted**, permanently discontinue study	• Viral hepatitis serology[4] • Blood sample for pharmacokinetic (PK) analysis, obtained up to 24h post-dose after last dose[5] • 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

treatment and may continue subject in the study for any protocol specified follow-up assessments **MONITORING:** **<u>For bilirubin or INR criteria:</u>** • 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, stabilize or return to within baseline • A specialist or hepatology consultation is recommended **<u>For All other criteria:</u>** • 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	form including acetaminophen, herbal remedies, other over the counter medications. • Record alcohol use on the liver event alcohol intake case report form **<u>For bilirubin or INR criteria:</u>** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG)or gamma globulins • Serum acetaminophen-adduct High performance liquid chromatography 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. New or worsening symptoms believed to be related to liver injury (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia).
4. 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.
5. 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.

Phase II liver chemistry increased monitoring criteria with continued therapy

Liver Chemistry Increased Monitoring Criteria – Liver Monitoring Event	
Criteria	**Actions**
ALT ≥ 3xULN and < 5xULN **and** bilirubin < 2xULN**, without** symptoms believed to be related to liver injury or hypersensitivity, **and** who can be monitored weekly for 4 weeks	• Notify the GSK medical monitor **within 24 hours** of learning of the abnormality to discuss subject safety • Subject can continue study treatment • Subject must return weekly for repeat liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) until they resolve, stabilize or return to within baseline • If at any time subject meets the liver chemistry stopping criteria, proceed as described above • If, after 4 weeks of monitoring, ALT < 3xULN and bilirubin < 2xULN, monitor subjects twice monthly until liver chemistries normalize or return to within baseline.

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: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.3.1. Definition of Adverse Events

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

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgment 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.

12.3.2. Definition of Serious Adverse Events

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

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

a. **Results in death**

b. **Is life-threatening**

NOTE:

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

c. **Requires hospitalization or prolongation of existing hospitalization**

NOTE:

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

d. **Results in disability/incapacity**

NOTE:

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

e. **Is a congenital anomaly/birth defect**

f. **Other situations:**

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

g. **Is associated with liver injury <u>and</u> impaired liver function defined as:**

- ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (> 35% direct), **or**
- ALT ≥ 3xULN and INR** > 1.5.

* Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE.

** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.

- Refer to Appendix 2 for the required liver chemistry follow-up instructions

12.3.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:

Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs:

- Myocardial infarction/unstable angina
- Congestive heart failure
- Arrhythmias
- Valvulopathy
- Pulmonary hypertension
- Cerebrovascular events/stroke and transient ischemic attack
- Peripheral arterial thromboembolism
- Deep venous thrombosis/pulmonary embolism
- Revascularization

12.3.4. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the 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 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.

12.3.5. Evaluating AEs and SAEs

Assessment of Intensity

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

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal

everyday activities.

- Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.
- The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment.
- For each AE/SAE the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to PAREXEL. 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 PAREXEL.**
- 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 PAREXEL and/or 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 PAREXEL 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 PAREXEL within the designated reporting time frames.

12.3.6. Reporting of SAEs

SAE reporting

- GSK has contracted PAREXEL GPPG to handle the collection and initial processing of SAEs.
- On discovery, all SAEs should be immediately reported (latest within 24 hours of knowledge of the event) to PAREXEL Global Medical Services (GMS) /GSK Medical Monitors by:

1-Completing the paper SAE report form which will be emailed/faxed/ ***(whatever is more convenient for the site, e-mail would be preferred)*** **to PAREXEL GPPG and to GSK medical monitor, for all sites globally via:**

E-mail to: PPD **and** PPD **and** PPD

Or FAX: PPD

AND

2-Entering the adverse event in the appropriate section of the eCRF, indicating that the event is considered serious and providing all the details per the eCRF completion guidelines at the same time the event is entered.

- Note: Email should be used preferentially. Fax to be used in case of email failure.
- The reporting of the serious adverse event should be done immediately in the e-CRF even though the paper SAE report is completed. Thus, one alert email will be triggered and the safety team will be aware that one SAE has been notified.
- In the event that the site is unable to complete the SAE report form, the investigators may report the SAE over the telephone via the SAE answering service, and then provide the completed SAE form via email/fax, (within the next 24 hours). If questions arise regarding the reporting procedures or the specifics of the reporting of an event, sites may notify PAREXEL utilizing the following numbers:

  Paris: Tel: PPD

Paris: Fax: PPD or PPD

- GPPG will check the eCRF/SAE form and source documents (if submitted) for validity, completeness, accuracy, legibility, signature, compliance with patient data protection, and investigator's causality assessment. GPPG may interact with the site, as needed, to clarify information and/or obtain missing or additional information.
- Follow-up SAE reports and support documents should be reported in the same manner as the initial report.

12.4. Appendix 4: Modified List of Highly Effective Methods for Avoiding Pregnancy in FRP and Collection of Pregnancy Information

12.4.1. Modified List of Highly Effective Methods for Avoiding Pregnancy in Females of Reproductive Potential (FRP)

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 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.

The definition of 'non-reproductive' for male subject's female partners is either postmenopausal, or 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 Female

12.4.2. 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 PAREXEL within 24 hours 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 PAREXEL. 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 that is considered reasonably related to the study treatment by the investigator will be reported to PAREXEL as described in Appendix 3. 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 randomized 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 PAREXEL within 24 hours 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 PAREXEL.
- 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.

12.4.3. References

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12.5. Appendix 5: Low, Medium, and High Daily Doses of Inhaled Corticosteroids

Low, medium, and high daily dosage of inhaled corticosteroids in adults and adolescents (12 years and older)			
Drug	Daily dose (mcg)		
	Low	Medium	High
Beclometasone dipropionate (CFC)	200-500	>500-1000	>1000
Beclometasone dipropionate (HFA)	100-200	>200-400	>400
Budesonide (DPI)	200-400	>400-800	>800
Ciclesonide (HFA)	80-160	>160-320	>320
Fluticasone furoate (DPI)	100	NA	200
Fluticasone propionate (DPI)	100-250	>250-500	>500
Fluticasone propionate (HFA)	100-250	>250-500	>500
Mometasone furoate	110-220	>220-440	>440
Triamicolone acetonide	400-1000	>1000-2000	>2000

Abbreviations: CFC: chlorofluorocarbon propellant, DPI: dry powder inhaler; HFA: hydrofluroalkane propellant; NA: not applicable.
Source: 2017 Global Initiative for Asthma (GINA 2017) Report

12.6. Appendix 6: Protocol Amendment Changes

12.6.1. Amendment 1

Summary of Amendment Changes with Rationale

This amendment is to justify the use of higher doses of budesonide and fluticasone furoate than the recommended doses. Specific sections of the protocol have also been updated to clarify that investigators have direct access to unblinding of treatment in an emergency, and to clarify that the amendments to the protocol will be approved by the regulatory agency and/or ethics committee, as applicable, before implementation. Those changes were requested by the UK MHRA. Text has also been updated to clarify blood sample instructions and time points, and to update the medical monitor contact details.

List of Specific Changes

CHANGE 1

Updated medical monitor contact details.

REVISED TEXT

Medical Monitor/SAE Contact Information:

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Site Address
Primary Medical Monitor ~~(Parexel) (urgent medical questions, subject eligibility questions, etc)~~	PPD			~~Herman Heijermansweg 20 1077 WL Amsterdam, The Netherlands~~ GlaxoSmithKline Stockley Park West, Uxbridge, Middlesex, UB11 1BT, UK

Secondary Medical Monitor	PPD			~~GlaxoSmithKline Stockley Park West, Uxbridge, Middlesex, UB11 1BT, UK~~ GSK, 709 Swedeland Rd, King of Prussia, PA 19406, USA
SAE contact information (PAREXEL)	PAREXEL Medical Services	PPD		NA
SAE contact information (GSK)	[~~Secondary~~Primary Medical monitor, details as above] Central Safety Department	FAX: PPD PPD EMAIL: PPD		

CHANGE 2

Updated Dose Justification Section to justify the use of higher doses of budesonide and fluticasone furoate than the recommended doses.

Section 4.5, Dose Justification

REVISED TEXT

FF (100 and 200 µg per day), is a once daily ICS that is being developed for the treatment of asthma, and has been approved in the United States (Arnuity ELLIPTA Highlights of Prescribing Information, 2014) and 5 other countries (Australia, Canada, Chile, Singapore, and Switzerland) for the treatment of asthma in adults and adolescents aged 12 years and over. FF (100 and 200 µg per day) is also approved worldwide (including the European Union - Relvar ELLIPTA™) as the ICS component of a combination product for COPD and asthma, with VI (GW642444). In the current study, it is proposed to dose escalate FF, to a maximum daily dose of 800 µg. FF has been administered to healthy volunteers at doses of 100 to 1600 µg once daily (OD) for 7 days (Study HZA113477, see Section 5.2.2.2 of the FF Investigator Brochure and Study FFA103096 [GSK Document Number GM2005/00082/00]) and as FF/VI at doses up to 800/100 µg OD for 7 days (Study HZA102936 [Kempsford, 2014]). These doses were well tolerated. FF was investigated in dose ranging studies in asthmatic subjects over the range 25 to 800 µg once daily for 8 weeks and was shown to be safe and well tolerated [Bateman 2012, Bleecker 2012, Busse 2012].

FP is indicated in the UK for the treatment of asthma, at doses of up to 1000 µg twice per day, which also represents the maximum dose that subjects will be exposed to in this study.

BUD is indicated in the UK for the treatment of asthma, at doses up to 1600 µg in divided doses. In the current study, it is proposed to dose escalate BUD, to a maximum daily dose of 3200 µg. BUD has been administered to asthmatic patients at doses of up to 3200 µg for up to 6 weeks [Kaiser, 1999]. These doses were well tolerated.

For the AMP challenge quadrupling dose increments are expected to show a well-defined dose response [Phillips, 2004] for this reason the following total daily doses of 25 µg, 100 µg, 400 µg for FF; 50 µg, 200 µg, 1000 µg for FP and 100 µg, 400 µg, 1600 µg for BUD have been chosen. However, three doses are insufficient to define the dose response for both AMP challenge and cortisol suppression and include all the doses used therapeutically since there is little overlap in the doses needed [Daley-Yates, 2015; Möllmann, 2001; Study FFA103096 ~~[GSK Document Number GM2005/00082/00]~~]. Therefore, FF 200 µg/day, FP 500 µg/day, and 2000 µg/day and BUD 800 µg/day are included to ensure the dose responses are adequately defined. Likewise, an 800 µg/day FF dose and 3200 µg/day BUD dose will also be included to fully define the cortisol suppression dose response (Study FFA103096) [GSK Document Number GM2005/00082/00] and ensure the dose escalation increments are matched between the three ICS under investigation.

CHANGE 3

Updated Blinding Section to clarify that investigators have direct access to unblinding of treatment in case of an emergency.

Section 6.4, Blinding

ADDED TEXT

The following will also 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.

CHANGE 4

Updated footnote in Time and Events Table to reflect different timepoints for the collection of metabolomic sample.

REVISED TEXT

Section 7.1, Time and Events Table 8 Footnotes

Change made to Table 10 to clarify that the plasma metabolomic sample will be collected on Day 36.

Table 19 Time and Events Treatment Period 1 and 2

	Treatment period 1 and 2								
	Day 1, pre-dose[a]	**Day 1 dosing and post-dose, Days 8, 15, 22, 29**	**Days 2, 9, 16, 23, 30**	**Days 3, 10, 17, 24, 31**	**Days 4, 11, 18, 25, 32**	**Days 5, 12, 19, 26, 33**	**Days 6, 13, 20, 27, 34**	**Days 7, 14, 21, 28, 35**	**Day 36[b]**
Metabolomics sample[m]	X	X							X

m The plasma metabolomics will be taken on Day 1 pre-dose (baseline) and on Days 8, 15, 22, 29 and 36, at the end of each dose escalation phase, at 12 hour after the last evening dose (before morning dose and AMP challenge procedure) ~~pre-dose before the start of each period and on Day 8 at the end of each dose escalation phase at 12 hour after the last evening dose and prior to the AMP challenge procedure~~.

CHANGE 5

Corrected errors in the sample analysis section for plasma cortisol.

Section 7.4.3, Sample Analysis

REVISED TEXT

Plasma cortisol analysis will be performed under the control of Platform Technology and Science - Drug Metabolism and Pharmacokinetics (PTS-DMPK)/Scinovo, GlaxoSmithKline, the details of which will be included in the SRM. Concentrations of ~~FF, FP, and BUD~~ plasma cortisol will be determined ~~in plasma cortisol samples~~ using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Once the plasma cortisol has been analyzed ~~for FF, FP, and BUD~~ any remaining plasma ~~cortisol~~ may be analyzed for other ~~compound~~cortisol-related metabolites and the results reported under a separate PTS- DMPK/Scinovo, GlaxoSmithKline protocol.

CHANGE 6

Updated Metabolomic Research Section to reflect blood sample volume and different timepoints for the collection of metabolomic sample.

Section 7.5.1, Metabolomic Research

REVISED TEXT

Blood samples (approximately ~~0.5~~ 1 ml) for measurement of plasma metabolomics will be taken on Day 1 pre-dose (baseline) and on Days 8, 15, 22, 29 and 36, at the end of each dose escalation phase, at 12 hour after the last evening dose (before morning dose and AMP challenge procedure). ~~pre-dose before the start of each period and on Day 8 at the end of each dose escalation phase at 12 hour after the last evening dose and prior to the AMP challenge procedure.~~

CHANGE 7

Added the text to clarify that the amendments to the protocol will be approved by the regulatory agency and/or ethics committee, as applicable, before implementation.

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

ADDED TEXT

Substantial amendments to the protocol will be approved by the regulatory agency and/or ethics committee, as applicable, before implementation.

12.6.2. Amendment 2 (03-OCT-2017) from the Amendment 1 (16-JAN-2017)

Protocol Amendment 2 applies to all sites participating in the conduct of the study.

Summary of Amendment Changes with Rationale

This amendment is to improve subject recruitment by reducing the requirements and increasing the flexibility of the study for the subjects. The protocol is amended to allow subjects to complete either 1 or 2 treatment periods, who are currently taking low-dose ICS and/or are light smokers.

Changes laid down in previous MEMOs from protocol amendment 1 were also incorporated into this protocol amendment.

List of Specific Changes

CHANGE 1

Synopsis, Overall Design

Rationale for change: The subjects' consent to complete either 1 or 2 treatment periods.

REVISED TEXT

Subjects will ~~be required~~ consent to participate in either:

- one treatment period; or
- two treatment periods, separated by a washout period of 25-42 days.

Each treatment period will comprise five consecutive 7-day dosing phases with escalating doses of one of three ICS products or placebo. Each 7-day period is referred to as an escalation phase. In each 7-day ~~phase of the dose~~ escalation ~~period~~ phase, subjects will receive ~~investigational product~~ study treatment from the evening of Day 1 to the evening of Day 7. ~~Each treatment period will be followed by a washout period of 25-42 days. There will be at least 21 days between the last dose of the Treatment Period 1 and the baseline AMP challenge of Treatment Period 2.~~

Subjects who consent to completing 2 treatment periods will be assigned to one of the treatment sequences ~~in accordance with the~~. Subjects who consent to completing 1 treatment period will be randomized to treatment. The randomization ~~schedule~~ schedules will be generated by Clinical Statistics prior to the start of the study, using validated internal software.

CHANGE 2

Synopsis, Treatment Arms and Duration

Rationale for change: The study duration text was changed as the study duration will be shorter for subjects who consent to 1 treatment period compared to those who consent to 2 treatment periods.

REVISED TEXT

There will be 5 different study treatments administered for subjects who consent to 2 treatment periods, these treatments will be in 12 different treatment sequences with 2 treatments each. ~~Four~~ Two subjects will be randomized to each of the 12 treatment sequences. Subjects who consent to 1 treatment period will be randomized to treatment, 6 subjects will be randomized to each active treatment and 3 subjects to each placebo treatment.

Study days for subjects who consent to 1 treatment period:

- Screening: Day ~~-28~~42 to Day ~~-7~~4.
- One treatment period of at least 35 days (i.e. 5 escalating doses administered for 7 days each).
- ~~Run-in-AMP challenge~~Follow-up period: 7 to 14 days after the last dose of treatment period 1.

Study days for subjects who consent to 2 treatment periods:

- Screening: Day ~~-17~~ 42 to Day ~~-~~4 days.
- Treatment periods: two periods of at least 35 days (i.e. 5 escalating doses administered for 7 days each), separated by a washout period of between 25 to 42 days.
- ~~Wash-out AMP challenge: At least 21 days after the last dose in Treatment Period 1 and no later than 4 days prior to the start of Treatment Period 2.~~
- Follow-up period: 7 to 14 days after the last dose of treatment period 2.

The study duration for ~~each subject~~subjects who consent to 1 treatment period will range from 46 to 91 days. The study duration for subjects who consent to 2 treatment periods will range from 106 to 168 days.

CHANGE 3

Synopsis, Type and Number of Subjects

Rationale for change: The section was updated to clarify how many subjects will consent to 2 treatment periods and how many will consent to 1 treatment period.

REVISED TEXT

~~Forty-eight adult subjects with asthma will be enrolled into this study~~. Subjects will be recruited such that 48 subjects will complete the study Twenty-four subjects will be enrolled to complete two treatment periods; and a further 24 subjects will be enrolled to complete one treatment period. This sample size is based on feasibility. ~~If the number of withdrawals results in less than 18 subjects per treatment (considering placebo overall) then subjects may be replaced at the discretion of the investigator and key GSK study team members. If subjects are replaced they will be assigned to the same treatment sequence as the subject they are replacing~~.

CHANGE 4

Section 4.1, Study Design

Rationale for change: The subjects are now given the option to complete 2 or 1 treatment periods.

REVISED TEXT

~~A run-in AMP challenge will be conducted 4-17 days prior to first dose. There will be a minimum of 4 days between the run-in visit and the first treatment day. Subjects will be required to respond with a PC_{20} of ≤80 mg/mL AMP at screening and run-in AMP PC_{20} has to be within 1.25-doubling concentrations of the screening PC_{20}.~~

Screening visit 1 will be conducted 4 – 42 days prior to the first treatment day. Subjects who are taking low-dose ICS will have a 4-week ICS washout during which their asthma symptoms must remain stable as defined in Section 5.1. Screening visit 2 will be conducted 4 – 14 days prior to the first treatment day and at least 4 weeks after screening visit 1. Subjects must have an AMP challenge PC_{20} of ≤80 mg/mL at screening visit 2 to be eligible. Procedures from screening visit 1 may be repeated at screening visit 2 as deemed necessary by the investigator/delegate.

Subjects who do not have to undergo ICS washout may only have 1 screening visit, and undergo screening visit 2 procedures at screening visit 1.

Subjects will consent to participate in either:

- one treatment period; or
- two treatment periods; separated by a washout period of 25-42 days.

Each treatment period will comprise five consecutive 7-day dosing phases with escalating doses of one of three ICS products or placebo. Each 7-day period is referred to as an escalation phase. In each 7-day ~~phase of the dose~~ escalation ~~period~~ phase, subjects will receive ~~investigational product~~ study treatment from the evening of Day 1 to the evening of Day 7. ~~Each treatment period will be followed by a washout period of 25-42 days. Before the second treatment period a baseline AMP challenge will be conducted at least 21 days after the last dose of Treatment Period 1 and at least 4 days prior to the first day~~

~~of Treatment Period 2. The AMP PC_{20} has to be within 1.25 doubling concentrations of the Run-in PC_{20}.~~

A follow-up visit will take place within 7-14 days after the last dose of ~~the second~~ study treatment ~~period~~. Time and events tables for ~~Screening, Run-In~~screening and ~~Follow~~ follow-up ~~Visits~~ visits (~~Table 7~~ Table 9) and Treatment Periods (~~Table 8~~Table 10) are provided in Section 7.1.

CHANGE 5

Section 4.2 Treatment Arms and Duration

Rationale for change: The study duration text was changed as the study duration will be shorter for subjects who consent to 1 treatment period compared to those who consent to 2 treatment periods

REVISED TEXT

The treatments for each ~~part of the study~~ dose escalation phase are listed in Table 2 and regime to provide each dose in Table 3. Subjects who consent to completing 2 treatment periods will be randomized to order of treatments they are included in~~; the treatment sequences shown in Table 4~~. Subjects who consent to completing 1 treatment period will be randomized to treatment.

Twenty-four subjects completing 2 treatment periods will be randomized to one of the treatment sequences shown in Table 4.

Table 4 Treatments proposed per study period for subjects completing 2 treatment periods

Sequence	Period 1	Period 2	n	~~n [1]~~
1	A	B	~~4~~2	~~3~~
2	A	C	~~4~~2	~~3~~
3	A	D	~~4~~2	~~3~~
4	B	A	~~4~~2	~~3~~
5	B	C	~~4~~2	~~3~~
6	B	E	~~4~~2	~~3~~
7	C	A	~~4~~2	~~3~~
8	C	B	~~4~~2	~~3~~
9	C	E	~~4~~2	~~3~~
10	D	A	~~4~~2	~~3~~
11	E	B	~~4~~2	~~3~~
12	D	C	~~4~~2	~~3~~
Total subjects			~~48~~24	~~36~~
Subjects per treatment			~~24~~12	~~18~~

~~[1]: If there are considerable numbers of dropouts in the study then there should be at least 36 completers such that there are at least 18 subjects in each active treatment group and overall for Placebo. For a completely balanced design there would be 14 sequences however an assumption has been made that ELLIPTA™ and DISKUS™ placebo will have the same effect therefore two of the sequences including placebo (EC and CD) have been dropped to give an equal number of subjects on each treatment administered overall.~~

Twenty-four subjects completing 1 treatment period will be randomized to one of the treatments shown in Table 5.

Table 5 Treatments proposed for subjects completing 1 treatment period

Treatment	n
A	6
B	6
C	6
D	3
E	3
Total subjects	24
Subjects per treatment	6

Therefore, a total of 48 subjects will complete the study, 18 subjects in total per study treatment (for each active treatment and for placebo combined).

Study durations for subjects completing either 2 or 1 treatment periods are shown in Table 6 and Table 7.

Table ~~5~~6 Study Duration for subjects completing 2 treatment periods

Screening days	Period 1 days	Washout days	Period 2 days	Follow-Up days	Total days
~~7-28~~4-42	35	~~2~~125-42	35	7-14	~~105-154~~ 106-168

Table 7 Study Duration for subjects completing 1 treatment period

Screening days	Period 1 days	Follow-Up days	Total days
4-42	35	7-14	46-91

CHANGE 6

Section 4.3, Type and Number of Subjects

REVISED TEXT

Subjects will be recruited such that 48 subjects will complete the study. Twenty-four subjects will be enrolled to complete 2 treatment periods; and a further 24 subjects will be enrolled to complete 1 treatment period. ~~Forty-eight adult subjects with asthma will be enrolled into this study.~~

~~If the number of withdrawals results in less than 18 subjects per treatment (considering placebo overall) then subjects may be replaced at the discretion of the investigator, GSK clinical investigation leader and GSK statistician.~~ This sample size is based on feasibility.

If subjects are replaced they will be assigned to the same treatment or treatment sequence as the subject they are replacing. ~~If a subject withdraws during period 2 then the subject can be replaced just to do period 2, unless the withdrawal results in less than 2 subjects on the same sequence. If this scenario occurs then the subject will have to begin from period 1 of the same sequence~~. Refer to Section 6.2 for more details.

CHANGE 7

Section 4.4, Design Justification

Rationale for change: The design justification section was updated to make it clearer that the subjects may receive treatment in either 1 or 2 treatment periods. Text was also added to indicate that the sponsor could be unblinded to study treatment to allow sample size re-estimation.

REVISED TEXT

The study is not double-blinded or double-dummy but a partially blinded study with the following design features mitigated for this aspect. The endpoints are objective rather than subjective. Subjects will receive one or two of the four possible treatments not knowing which are active or placebo. When receiving placebo they will be assigned either a DISKUS™ or an ELLIPTA™ device. There will be no BUD Turbuhaler placebo. Subjects assigned to placebo will follow the same dose escalation scheme taking the same number of inhalations in each phase as the corresponding active treatments. Additionally, this will provide a measure of stability of the AMP and cortisol response over the 5 week period where repeated AMP challenges are performed. For a completely balanced design there would be 14 treatment sequences for subjects completing 2 treatment periods; however, an assumption has been made that ELLIPTA™ and DISKUS™ placebo will have the same effect therefore two of the sequences including placebo (EC and CD) have been dropped to give an equal number of subjects on each treatment administered including placebo overall. Similarly, for subjects completing one treatment period, 3 subjects will be randomized to ELLIPTA™ and DISKUS™ placebo so that 6 subjects in total receive placebo overall, which is equal to the subjects on each active treatment.

In addition to the subjects, the investigators, nurses, technicians and other staff that interact with the subjects will also be blinded to the treatment designations. The subjects will be provided with one of three different dry powder inhalers for each period either active treatment or placebo. The subjects will not be informed which are the active or placebo treatments although no Turbuhaler placebo will be used in the study. The study sponsor will be unblinded to study treatment to allow selected sponsor study team members to perform unblinded in-stream analysis of available data. These findings will inform on the need for a sample size re-estimation or interim analysis (see Section 9.2.3).

CHANGE 8

Section 4.5, Dose Justification

REVISED TEXT (4th paragraph)

FP is indicated in the UK and Germany for the treatment of asthma, at doses of up to 1000 μg twice per day, which also represents the maximum dose that subjects will be exposed to in this study.

BUD is indicated in the UK and Germany for the treatment of asthma, at doses up to 1600 μg in divided doses.

CHANGE 9

Section 4.6.1, Risk Assessment

Rationale for change: To add risk assessment for subjects who are withdrawn from low-dose ICS treatment.

ADDED TEXT

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Withdrawal of Inhaled Corticosteroids		
Subjects receiving low-dose ICS as defined in Appendix 5 may take part after a 4 week ICS washout prior to AMP challenge. There is a risk associated with withdrawing inhaled corticosteroids in these subjects who are currently taking them	Subject may experience worsening of asthma symptoms	Subjects washing out from ICS will be monitored via weekly calls from site, and maintenance of diary to capture PEFR and SABA use. Subjects are instructed to contact the PI/site for assessment if they have a deterioration of their asthma (refer to section 7.3.8.4.Diary Assessments) or if they have an exacerbation. Any subject whose asthma is defined as not stable will not be eligible to participate in the study.

CHANGE 10

Section 5.1, Inclusion Criteria

Rationale for change: Inclusion criterion 2 was updated to remove short acting β 2-agonist therapy by inhalation.

REVISED TEXT

2. **Asthma**: Documented history of bronchial asthma, first diagnosed at least 6 months prior to the screening visit ~~and being treated only with intermittent short-acting β2-agonist therapy by inhalation.~~

CHANGE 11

Section 5.1, Inclusion Criteria

Rationale for change: Clarification was given for the severity of disease in inclusion criterion 3.

REVISED TEXT

3. **Severity of disease:**

a) Pre-bronchodilator FEV_1 ≥65% of predicted at screening.

b) The pre-dose baseline FEV_1 should ~~be ± 15% of the~~ not have changed significantly in the opinion of the investigator from the screening baseline value and should be ≥ 65% predicted for the subject to continue.

c) Documented sensitivity to AMP with a provocative concentration of AMP resulting in a fall of ≥20% FEV_1 with a PC_{20} AMP ≤80 mg/mL at the screening visit.

d) ~~Demonstrated stable bronchoconstriction in response to inhaled AMP at the run-in visit. The run-in PC_{20} to be within 1.25 doubling concentration of the screening PC_{20}.~~

CHANGE 12

Section 5.1, Inclusion Criterion Number 4

Rationale for change: To allow recruitment of subjects currently taking low-dose ICS.

REVISED TEXT

- ~~No~~Subjects receiving low-dose ICS, as defined in Appendix 5 may take part after a 4-week ICS washout prior to AMP challenge. The subject's asthma symptoms must remain stable during this 4-week period as assessed at screening visit 2. Stable asthma is defined as:
  - No more than 2 consecutive days where ≥12 inhalations/day of salbutamol were used.
  - No severe asthma exacerbations requiring use of systemic corticosteroids (tablets, suspension, or injection) or an in-patient hospitalization or emergency department visit due to asthma that required systemic corticosteroids.

- No clinical asthma worsening which in the opinion of the investigator requires additional asthma treatment other than study medication or salbutamol.

- Subjects taking LABA, LAMA, leukotriene receptor antagonist (LTRA) therapy ~~for three~~ within 3 months prior to the start of the study are not eligible.
- ~~No~~ Subjects taking biological therapies ~~for at least~~ within 6 months prior to start of the study are not eligible.

CHANGE 13

Section 5.1, Inclusion Criterion Number 8

Rationale for change: The inclusion criteria were changed to allow light smokers

ADDED TEXT

Light smokers, who smoke ≤20 cigarettes per week or equivalent unit dose of other tobacco products or e-cigarettes, are eligible for the study. Smokers who intend to stop, reduce or increase their smoking habit during the study period are not eligible.

CHANGE 14

Section 5.2, Exclusion Criterion Number 8

Rationale for change: The exclusion criteria were changed to allow light smokers.

REVISED TEXT

Tobacco Use: Current smokers who smoke >20 cigarettes per week or the equivalent unit dose of other tobacco products or e-cigarettes, or smokers with a smoking history of ≥10 pack years. ~~A subject must not have used any inhaled tobacco products in the 12 month period preceding the screening visit.~~

CHANGE 15

Section 5.3, Screening/Baseline/Run-in Failures

Rationale for change: Text was added to allow subjects to rescreen after discussion with the sponsor.

ADDED TEXT

Subjects who fail the initial screening visit may be rescreened once, after discussion with the sponsor. In order to be rescreened, subjects must have demonstrated AMP sensitivity and meet the spirometry and respiratory test results as described in inclusion criterion 3. Subjects must not have met any of the exclusion criteria. If AMP sensitivity was not established at screening, subjects known to be insensitive to AMP will not be rescreened.

CHANGE 16

Section 5.4, Withdrawal/Stopping Criteria

Rationale for change: The period of time where a rescheduled AMP challenge could be performed was extended from 2 to 3 days.

REVISED TEXT

Subjects who are not eligible to perform an AMP challenge at any of the specified time during the treatment periods (i.e. for reasons of safety) will be withdrawn from the study. One repeat attempt is permitted within ~~2~~1-3 days.

CHANGE 17

Section 5.4, Withdrawal/Stopping Criteria

Rationale for change: There is no longer an AMP PC_{20} measurement during the washout period so the withdrawal criterion linked to this measurement was removed.

DELETED TEXT

- ~~Subjects who do not meet the AMP PC_{20} reproducibility criteria prior to Treatment Period 2 Day 1 will be withdrawn. One repeat attempt is permitted.~~

CHANGE 18

Section 5.4, Withdrawal/Stopping Criteria

Rationale for change: Clarification that salbutamol will not be supplied as a study medication. This is to incorporate a change from a MEMO dated 30 MAY 2017.

REVISED TEXT

- Clinical asthma worsening which in the opinion of the investigator requires additional asthma treatment other than study medication or ~~study supplied/~~salbutamol

CHANGE 19

Section 5.5 Subject and Study Completion

Rationale for change: The definition of a completed subject was updated.

REVISED TEXT

A completed subject is one who has completed ~~all phases of the study~~ the number of treatment periods they consented to, including the follow-up visit.

CHANGE 20

Section 6.2, Treatment Assignment

Rationale for change: The treatment assignment section was updated to include details for subjects who consent to 2 or 1 treatment periods.

DELETED TEXT

~~If the number of withdrawals results in less than 18 subjects per active treatment and placebo overall then subjects may be replaced at the discretion of the investigator, GSK clinical investigation leader and GSK statistician.~~

~~If subjects are replaced they will be assigned to the same treatment sequence as the subject they are replacing or during period 2 then the subject can be replaced just to do period 2, unless the withdrawal results in less than 2 subjects on the same sequence. If this scenario occurs then the subject will have to begin from period 1 of the same sequence. The replacement will be at the discretion of the investigator, GSK clinical investigation leader and GSK statistician~~.

~~Each subject will receive 2 different treatments, one in treatment period 1, and one in treatment period 2; the possible treatment sequences pre-defined in Table 4. Four subjects will be randomized to each of the 12 treatment sequences (Table 4).~~

~~The study statistician will monitor the withdrawals with regards to treatment sequence assigned and treatments administered. As it is standard procedure to keep the treatment schedule concealed within the randomisation system until DBF regardless of blinding status, the study statistician will request approval from the Clinical Statistics Head to access the treatment and sequence information early in order to monitor the withdrawals on an ongoing basis. The study statistician will alert the study team when the number of withdrawals results in less than 18 subjects on a treatment (considering placebo overall) or if the number of withdrawals is greater than two subjects on a sequence. Subjects will be replaced at the discretion of the key GSK study team members (including study statistician, medical monitor and CIL) along with the investigator. The investigator will remain blinded to the treatments the withdrawn subjects were assigned.~~

ADDED TEXT

<u>Withdrawn subjects may be replaced. Replacement subjects will be assigned to the same treatment sequence as the subject they are replacing.</u>

<u>For subjects completing two treatment periods:</u>

- <u>Each subject will receive 2 different treatments, one in treatment period 1, and one in treatment period 2; the possible treatment sequences are pre-defined in Table 4. Two subjects will be randomized to each of the 12 treatment sequences (Table 4).</u>

- <u>If a subject withdraws during treatment period 1, the replacement subject will start from the beginning of period 1 of the same treatment sequence. If a subject</u>

withdraws after period 1 or during period 2, the replacement subject may start from period 2; a maximum of 2 subjects can be replaced from the start of period 2. If more than 2 subjects withdraw after period 1 or during period 2, they will be replaced from the beginning of period 1.

For subjects completing one treatment period:

- Each subject will receive one treatment, as defined in Table 5. Six subjects will be randomized to each treatment, including placebo overall (Table 5).

Replacements for withdrawn subjects will start from the beginning of period 1 of the same treatment.

CHANGE 21

Section 6.4, Blinding

Rationale for change: Text was added to the blinding section to indicate that key sponsor team members can be unblinded to allow in-stream analysis.

ADDED TEXT

Selected sponsor study team members may be unblinded to perform the in-stream analysis. This may include the study statistician, study programmer (and delegates) and study DMPK scientist. Access to unblinded data will be kept to the minimum set of individuals required to implement any in-stream or 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.

CHANGE 22

Section 6.10.2, Caffeine, Alcohol, and Tobacco

Rationale for change: The restriction on the use of nicotine containing products was updated as the protocol now allows light smokers to join the study.

REVISED TEXT

- ~~Use of~~ Light smokers may continue to use inhaled or non-inhaled nicotine containing products ~~(including nicotine patches and electronic cigarettes)~~as long as they do not exceed 20 cigarettes)~~is not allowed~~ or equivalent per week from screening until after the final follow-up visit. To ensure subjects maintain the same degree of AMP sensitivity, they must not intend to increase the amount they smoke during the course of the study. Subjects must refrain from smoking for at least 1 hour before lung function tests.

CHANGE 23

Section 6.11.2, Prohibited Medications and Non-Drug Therapies

Rationale for change: The restriction on ICS was updated to allow subjects who were receiving low-dose ICS.

REVISED TEXT

- ~~No ICS,~~ Use of LABA, LAMA, LTRA therapy for ~~three~~ 3 months prior to the start of the study and use of ICS, LABA, LAMA, LTRA therapy throughout the course of the study. Subjects currently receiving low-dose ICS will be consented and perform screening visit 1, then if the subject is eligible for the study they will undergo a 4-week washout period for ICS. If the subject's condition remains stable they will attend screening visit 2 and progress to randomization, if eligible.
- ~~No~~ Use of biological therapies for at least 6 months prior to start of the study and throughout the course of the study.

CHANGE 24

Section 7.1, Time and Events Table

Rationale for change: The Time and Events Table was updated to remove the Run-in, to add a second screening visit to assess subjects who have a 4-week ICS washout, and to extend the screening period to -42 to -4 days prior to the first dose of study treatment.

REVISED TEXT

Table ~~7~~9 Screening~~, Run-in,~~ and Follow-up Visit

Procedure	Screening Visit 1	~~Run in visit~~Screening Visit 2	Treatment Period 1	Washout[d]	~~Baseline visit prior to Treatment Period 2~~	Treatment Period 2[d]	FU
Day	Day ~~-28~~ -42 to Day ~~-7~~ -4[b]	Day ~~-17~~ -14 to Day -4[c]	Assessments during treatment period are detailed in Table 10	Washout will be a minimum of 25 days to maximum of 42 days	~~At least 21 days after the last dose in TP 1 and no later than 4 days prior to the start of TP 2 Day 1~~	Assessments during treatment period are detailed in Table 10	7 to 14 days[dg]
Outpatient visit	X	X					X
Informed consent[a]	X						
Inclusion and exclusion criteria	X	X					
Demography	X						
Full physical examination including height and weight	X						
Urine drugs of abuse and alcohol breath test	X	~~X~~			~~X~~		
Brief physical							X
Medical history (includes substance usage)	X						
Urine pregnancy test (all female subjects)	X	~~X~~			~~X~~		X
HIV, Hep B and Hep C screen	X						
Safety labs: chemistry, hematology and urinalysis	X						X
12-lead ECG	X						
Vital signs	X						
Peak expiratory flow rate	X[b]	X[b]					
~~Baseline~~ AMP challenge		X[f]					
Spirometry (FEV_1 and FVC)	X				~~X~~		X
AE/SAE[eg]	X	X					X
Concomitant medication[eg]	X	X					X

AE – Adverse event; AMP- Adenosine 5 monophosphate; ECG – Electrocardiogram; FEV1 – Forced expiratory volume in 1 second; FVC – Forced vital capacity; FU – Follow-up; HIV- Human immunodeficiency virus; Hep B –Hepatitis B; Hep C – Hepatitis C; SAE – Serious adverse event;.

[a] Informed consent may be obtained on a separate visit prior to screening visit 1.

[b] ~~There will be a baseline AMP challenge during run-in and at least 21 days after the last dose of Treatment Period 1 and at least 4 days prior to the first day of Treatment Period 2~~ Subjects who are receiving low-dose ICS may take part after a 4-week washout. For these subjects, screening visit 2 will be at least 28 days after screening visit 1. During the 4-week ICS washout subjects will keep a diary (from screening visit 1 to pre-dose Day 1) to record twice daily peak expiratory flow (subject to contact site if there is a 20% decrease) and SABA use (subject to contact site if SABA intake is increased). Salbutamol will be dispensed for rescue use.

[c] Subjects who do not require an ICS washout may have only one screening visit, these procedures may then be done at screening visit 1. Screening visit 1 procedures may be repeated at screening visit 2 as deemed necessary by the Investigator or delegate.

[d] Subjects who consent to only 1 treatment period will not undergo the washout period or treatment period 2.

[eg] AEs and concomitant medication will be documented on all days throughout the study, but review will only occur when the subject is in the unit.

[f] The screening visit 2 AMP challenge can be repeated only once; at least 72h after the first attempt.

[dg] 7 to 14 days after the last dose of ~~Treatment Period 2~~ study treatment.

CHANGE 25

Section 7.1, Time and Events Table

Rationale for change: The Time and Events Table for treatment periods 1 and 2 was updated to include PEFR measurements and to allow subjects to leave the study site after the Day 7 study procedures and return on Day 8 rather than staying at the unit overnight.

The table was also updated to incorporate changes from a MEMO dated 04 APR 2017. The AMP challenge for Day 36 is now indicated and footnote ‘c’ has ‘and Day 36’ deleted as study drug is not dispensed on this day.

REVISED TEXT

Table ~~8~~10 Time and Events Treatment Period 1 and 2

	Treatment period 1 and 2								
	Day 1, pre-dose[a]	**Day 1 dosing and post-dose, Days 8, 15, 22, 29**	**Days 2, 9, 16, 23, 30**	**Days 3, 10, 17, 24, 31**	**Days 4, 11, 18, 25, 32**	**Days 5, 12, 19, 26, 33**	**Days 6, 13, 20, 27, 34**	**Days 7, 14, 21, 28, 35[o]**	**Day 36[b]**
Study ~~drug~~ treatment dispensing[c]	X	X						X	
Outpatient visit[d]	X	X							
Confinement[e]							X	~~X~~	
Randomization[f]	X								
Urine pregnancy test ~~prior to AMP challenge~~ (all female subjects)	X	X							X
Vital signs[g]	X								X
Use of inhaler training[h]	X						~~X~~		
Peak expiratory flow rate[i]		X	X	X	X	X	X	X	
Study treatment[ij]		X	X	X	X	X	X	X	
Patient diary[ijk]		X	X	X	X	X	X	X	
AMP challenge[kl]		X							X
Spirometry (FEV_1 and FVC)	X								
Blood sampling for plasma cortisol[lm]	X						X	X	
Metabolomics sample[mn]	X	X							X
AE/SAE[no]	X	X	X	X	X	X	X	X	X
Concomitant medication[no]	X	X	X	X	X	X	X	X	X

AE – Adverse event; AMP- Adenosine monophosphate; FEV_1 – Forced expiratory volume in 1 second; FVC – Forced vital capacity; SAE – Serious adverse event.

[a] Day 1 ~~Pre-~~dose completed at start of treatment period 1 and 2, respectively. Urine pregnancy test (where applicable), Vital signs, Spirometry (FEV1 and FVC), blood sampling for plasma cortisol, metabolomics sample, AE/SAE review, restriction checks and concomitant medication review will be performed on the morning of Day 1 (pre-dose) of each treatment period. Use of inhaler training, randomization (treatment period 1 only), AE/SAE review, restriction checks, concomitant medication review and study treatment dispensing will be performed in the evening of Day 1, prior to study treatment administration.

[b] Day 36 completed following completion of ~~Treatment Period~~ treatment period 1 and 2, respectively.

[c] Subjects are dispensed ~~drug~~ study treatments for the first dose level on Day 1 prior to evening dose. ~~Drug~~ Study treatments for the subsequent 4 dose levels are dispensed prior to release after completion of the AMP challenge on Days 8, 15, 22, and 29~~, and 36~~.

[d] Subjects receive~~s~~ first ~~treatment for first~~ dose for ~~each of~~ treatment period 1 and 2 during the outpatient visit.

[e] Subject will be admitted to unit prior to evening dose on Days 6, 13, 20, 27 and 34 of each treatment period. Subjects will be confined in the unit until they have recovered from the AMP challenge on Days 8, 15, 22, 29, and 36, but may leave the unit on the evenings of Days 7, 14, 21, 28, and 35 after their last plasma cortisol sample and return the following morning, if they prefer.

[f] Prior to first dose of ~~Treatment Period~~ treatment period 1 only.

[g] Performed on ~~first and last day of each~~ Days 1 and 36 ~~Treatment period~~.

[h] Reinforcement by the investigator on the proper use of the inhaler.

[i] Peak expiratory flow reading must be taken before each dose of study treatment at home. Three PEFR manoeuvres should be performed in each session. The highest PEFR of the 3 efforts from any session will be the one that will be recorded in a paper diary.

[ij] ~~On Days 1,8,15, 22, and 29 of the Treatment period, the subjects do not receive a morning dose. This does not apply to the following: all of the FF doses, the Ellipta placebo doses and the first week of the dose escalations for FP (50mcg dose) and bud (100mcg dose), since these are all PM only dosing regimens. On all other days where the subjects receive a morning and an evening dose of FP or BUD the doses will be administered 12 hours apart~~. Subjects will take doses of study treatment in the evening only (for all ELLIPTA FF/placebo doses, and the first escalation phase for FP [50 mcg dose] and BUD [100 mcg dose]) or in the morning and evening, 12 hours apart (for all other dose regimens). There will be no dosing in the mornings of Days 1, 8, 15, 22 and 29. When subjects are not at the unit, they will take their study treatments at home.

[ijk] On Days 1, 8, 15, 22, and 29, subjects will receive a patient diary to document day and time of each dose, the peak expiratory flow rate (PEFR) recording and ~~the~~ any AEs or concomitant medication including the number of SABA doses. ~~The subject will enter their data in a paper diary. Rescue medication will also be captured in the diary.~~

[kl] ~~There will be a baseline AMP challenge during the Run-in visit and at least 21 days after the last dose of Treatment Period 1 and prior to Treatment Period 2. A baseline AMP challenge can be repeated only once at least 72 hours after the initial challenge.~~ or concomitant medication including the number of SABA doses. The subject will enter their data in a paper diary.

The AMP challenges will be done in the morning of Days 8, 15, 22, 29, 36 and 12 hours after the preceding evening dose. If subjects cannot perform the AMP challenge, this may be ~~repeated~~ rescheduled 1-~~2~~3 days later; in this case, ~~and~~ the subjects will ~~then~~ continue dosing at the same level until the rescheduled AMP challenge and the study schedule will be shifted.

[lm] Blood samples for plasma cortisol will be taken on Day 1 pre-dose (baseline) and at the following time points related to evening study drug administration on Days 6, 13, 20, 27, 34: pre-dose and at 1, 2, 3, 5, 10, 12 (before morning dose), 14, 16, 18 and 24 hours post-dose.

[n] The plasma metabolomics will be taken on Day 1 pre-dose (baseline) and on Days 8, 15, 22, 29 and 36, at the end of each dose escalation phase, at 12 hour after the last evening dose (before ~~morning dose and~~ AMP challenge procedure).

º AEs and concomitant medication will be documented on all days throughout the study, but review will only occur when the subject is in the unit.

CHANGE 26

Section 7.2 Screening and Critical Baseline Assessments

Rationale for change: Text was added to the section to describe the 2 screening periods.

ADDED TEXT

Subjects who are receiving low-dose ICS may take part after a 4-week ICS washout. For these subjects, screening visit 2 will be at least 28 days after screening visit 1. During the 4-week ICS washout and up to randomization, subjects will keep a diary to record twice daily PEFR measurements and SABA use. The site will also contact the subjects weekly to monitor their asthma symptoms and may ask the subject to attend the site if necessary. The subject must contact the site if the PEFR measurement is below the 20% decrease value calculated by the sites, if there is an increase in SABA use, or if they experience an exacerbation.

Salbutamol will be dispensed all subjects for rescue use from screening and throughout the study as required.

CHANGE 27

Section 7.3.7, Inhaler training

Rationale for change: Paper training materials will be used instead of dummy/placebo devices for inhaler training. This is to incorporate a change from a MEMO dated 04 APR 2017.

REVISED TEXT

Inhaler training using ~~dummy/placebo devices~~ paper training materials will be performed at Day 1, with reinforcement of proper technique at subsequent visits. At each subsequent visit, the correct inhalation technique will be reinforced by the investigator prior to dose administration.

CHANGE 28

Section 7.3.8.2, Pre-dose Baseline Lung Function

Rationale for change: The method for obtaining the pre-dose lung function was updated for clarity.

REVISED TEXT

Baseline measurements of trough FEV_1 will be made prior to dosing on Day 1 of ~~each~~the treatment period(s). These measures should be made at the same time of day ~~for all two periods~~ during the treatment period(s). Three technically acceptable measures will be

obtained and the highest of these three measurements will be recorded in the eCRF and will constitute the pre-dose baseline value. This pre-dose baseline FEV_1 should ~~be ± 15%~~ not have changed significantly in the opinion of the investigator from of the screening baseline value and ≥65% predicted for the subject to continue. ~~The baseline for period 2 should be within 15% of the baseline for period 1 and ≥ 65% predicted for the subject to continue.~~

CHANGE 29

Section 7.3.8.3, Peak expiratory flow rate (PEFR) measurements

Rationale for change: The frequency of PEFR measurements was updated to remove 'twice daily'. On days where the subjects receive one dose of study drug this statement is not true. This is to incorporate a change from a MEMO dated 04 APR 2017. The text was also updated to reflect the PEFR measurements that will be made during the ICS washout and PEFR were added to the wash-out period between periods 1 and 2.

REVISED TEXT

Subjects who undergo low-dose ICS washout, will be provided with a peak flow meter at Screening Visit 1, for use at home, until randomization. Subjects will also be provided with a diary to record their measurements and symptoms, and will be instructed to contact the site if their PEFR measurement is below the 20% decrease value calculated by the sites, if there is an increase in SABA use, or if they experience an exacerbation.

During each treatment period, ~~S~~subjects will be given a peak flow meter for use at home to measure PEFR. The paper diary will have a place for ~~patients~~subjects to record their PEFR measurements that must be taken before each dose. Three PEFR maneuvers should be performed in each session~~, twice daily~~. The highest PEFR of the 3 efforts from any session will be the one that will be recorded in a paper diary.

All PEFR measurements should be taken before subjects take their study drug. ~~Patients~~ Subjects should not, whenever possible, use SABA reliever therapies for 6 hours before performing PEFR.

Subjects ~~should complete~~who consented to 2 treatment periods will also be provided with a peak flow meter and a patient diary to record their asthma symptoms at home during the washout period. Subjects will be instructed to contact the site if their PEFR measurement is below the 20% decrease value calculated by the sites, if there is an increase in SABA use, or if they experience an exacerbation.

CHANGE 30

Section 7.3.8.4, Diary Assessments

Rationale for change: The diary assessments section was updated to include the patient diary for subjects who undergo wash-out from low-dose ICS.

ADDED TEXT

Subjects who wash-out from low-dose ICS prior to screening visit 2 will be provided with a patient diary to document the following until randomization:

- Any AEs.
- Any medical problems the subject may have experienced and any medications used to treat those medical problems
- PEFR measurements twice daily
- Number of occasions rescue salbutamol used over the previous 24 hours

Subjects who wash-out from low-dose ICS prior to screening visit 2 will be instructed to perform PEFR measurements twice daily and to contact the site if there is a 20% fall in PEFR if there is an increase in SABA use, or if they experience an exacerbation at any point during the study.

Subjects who participate in two treatment periods will be provided with a patient diary during the wash-out period to document:

- Any AEs
- Any medical problems the subject may have experienced and any medications used to treat those medical problems.
- Number of occasions rescue salbutamol used over the previous 24 hours.
- PEFR measurements if their asthma symptoms are getting worse or if they are using their salbutamol more than usual.

Subjects should complete their daily diary pages during the treatment periods. On Days 1, 8, 15, 22, and 29, subjects will receive a patient diary to document:

- Day and time of each dose.
- PEFR measurements.
- Any AEs or medical problems the subject may have experienced and any medications used to treat those medical problems.
- Number of occasions rescue salbutamol used over the previous 24 hours.

Reminding or retraining of the subject on proper paper diary documentation will be conducted if there is a discrepancy between the device metrics and paper diary entries.

CHANGE 31

Protocol Section 7.4.1, AMP Challenge

Rationale for change: Clarifications to the AMP dose to be used during AMP challenge at screening visit. This is to incorporate a change from a MEMO dated 30 MAY 2017. The text was also revised to extend the period of time where a rescheduled AMP challenge could be performed.

ADDED TEXT, 4th paragraph 3rd sentence

For the screening visit, the maximum dose of AMP to be used for documenting AMP sensitivity is ≤80 mg/mL.

REVISED TEXT

If subjects cannot perform an on-treatment AMP challenge, this may be ~~repeated~~ rescheduled 1-~~2~~3 days later and the subjects will then continue dosing at the same level until the rescheduled AMP challenge and the study schedule will be shifted.

CHANGE 32

Section 9.2, Sample Size Considerations

Rationale for change: The sample size considerations section was updated to include the number of patients who can consent to 2 treatment periods and 1 treatment period as well as clarifying that subjects who withdraw after treatment period 1 may be replaced.

REVISED TEXT

Subjects will be recruited such that 48 ~~Forty eight~~ subjects will ~~be recruited into this~~ complete the study. Twenty-four subjects will be enrolled to complete 2 treatment periods; a further 24 subjects will be enrolled to complete 1 treatment period. This sample size is based on feasibility. ~~If the number of withdrawals results in less than 18 subjects per active treatment and placebo overall then subjects may be replaced at the discretion of the investigator and key GSK study team members.~~

If subjects are replaced they will be assigned to the same treatment or treatment sequence as the subject they are replacing . If a subject withdraws during treatment period 1 then the replacement subject will start from the beginning of period 1 of the same treatment sequence. ~~i.e, if~~ If a subject withdraws after period 1 or during period 2 then the subject ~~can~~ may be replaced just to do period 2~~, unless the withdrawal results in more than 2 subjects from the same sequence. If this scenario occurs then the subject will have to begin from period 1 of the same sequence~~. A maximum of 2 subjects can be replaced from the start of period 2; if more than 2 subjects on the study withdraw after period 1 or during period 2, the replacements must start from the beginning of period 1.

CHANGE 33

Section 9.2.1, Sample Size Assumptions

Rationale for change: The expected precision of parameter estimates was updated to reflect a sample size of 18 subjects per study treatment.

REVISED TEXT

Table ~~11~~13 Expected Precision of Parameter Estimates for Cross-over and Parallel Design Combined; N=18 Subjects Overall for Each Treatment

Doses	Assumptions	Sample Size	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
FF	E0 = 0.8 Emax = 4 DD ED50 = 25 mcg Slope = 0.9	~~48~~18	±~~0.47~~0.51	±~~0.43~~0.55	±~~13.3~~15.7
FP	E0 = 0.8 Emax = 4 DD ED50 = 50 mcg Slope = 0.9	~~48~~18	±~~0.47~~0.51	±~~0.42~~0.54	±~~26.1~~30.3
BUD	E0 = 0.8 Emax = 4 DD ED50 = 100 mcg Slope = 0.9	~~48~~18	±~~0.47~~0.51	±~~0.43~~0.55	±~~53.2~~62.1

BUD- Budesonide; CI- Confidence interval; FF – Fluticasone furoate; FP – Fluticasone propionate

CHANGE 34

Section 9.2.2, Sample Size Sensitivity

Rationale for change: Precision estimates were updated based on a sample size per study treatment of 12 subjects in a cross-over design and 6 subjects in a parallel design, and the combined estimates for 18 subjects.

REVISED TEXT

The predicted widths of the confidence interval for the parameters of the model depend on the assumed model parameters. A sensitivity analysis was performed to assess the importance of the assumptions on the results. A range of values observed from the reference studies (SIG103337, SIG102335 and FFA103096) [GSK Document Number GM2005/00525/00, GSK Document Number GM2007/00123/00 and GSK Document Number GM2005/00082/00, respectively] have been considered ~~for a sample size of 48 subjects~~. The results in this study are expected to be somewhere between the 2 hour and 14 hour data from the SIG103337 study [GSK Document Number GM2005/00525/00], hence, the selected slope of 0.9 which is between the two estimated slopes, and 1.5 DD

for Emax which is also between the two time points. ~~Table 13 gives the estimate for different scenarios with an Emax 1.5DD to 4DD.~~

The study will include 2 groups of subjects; 1 in which the subjects participate in 2 treatment periods in a cross-over design, and the other in which subjects complete only 1 treatment period in a parallel design. Precision estimates were calculated on the feasible sample size per study treatment, based on 12 subjects in a cross-over design and 6 subjects in a parallel design, and the combined estimates for 18 subjects in total per treatment (Table 16 and Table 17).

Table 15 Simulation Results for Cross-over Design; N=12 Subjects in Each Treatment Group

Doses	**Assumptions**	**Sample Size**	**Predicted Width of 95% CI**		
			E_0(DD)	**E_{max}(DD)**	**ED_{50} (mcg)**
FF	E0 =0.8 Emax =4 DD ED50=25 mcg Slope =0.9	12	±0.67	±0.61	±19.0
FP	E0 =0.8 Emax =4 DD ED50=50 mcg Slope =0.9	12	±0.67	±0.59	±37.1
BUD	E0 =0.8 Emax =4 DD ED50=100 mcg Slope =0.9	12	±0.67	±0.61	±72.6

Table 16 Simulation Results for Parallel Design; N=6 Subjects in Each Treatment Group

Doses	**Assumptions**	**Sample Size**	**Predicted Width of 95% CI**		
			E_0(DD)	**E_{max}(DD)**	**ED_{50} (mcg)**
FF	E0 =0.8 Emax =4 DD ED50=25 mcg Slope =0.9	6	±0.61	±1.18	±37.9
FP	E0 =0.8 Emax =4 DD ED50=50 mcg Slope =0.9	6	±0.61	±1.15	±74.6
BUD	E0 =0.8 Emax =4 DD ED50=100 mcg Slope =0.9	6	±0.61	±1.19	±152.5

Table 13 Sample Size Sensitivity Analysis (N=48)

Doses	Parameters	n	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
FF	E_0 =0.8 E_{max} =1.5 DD ED_{50}=25 mcg γ =0.9	48	±0.47	±0.43	±35.3
	E_0 =1.8 E_{max} =4 DD ED_{50}=25 mcg γ =0.9	48	±0.47	±0.43	±13.3
	E_0 =1.8 E_{max} =1.5 DD ED_{50}=25 mcg γ =0.9	48	±0.47	±0.43	±34.7
FP	E_0 =0.8 E_{max} =1.5 DD ED_{50}=50 mcg γ =0.9	48	±0.47	±0.42	± 67.7
	E_0 =1.8 E_{max} =4 DD ED_{50}=50 mcg γ =0.9	48	±0.47	±0.42	±26.0
	E_0 =1.8 E_{max} =1.5 DD ED_{50}=50 mcg γ =0.9	48	±0.47	±0.42	±67.9
BUD	E_0 =0.8 E_{max} =1.5 DD ED_{50}=100 mcg γ =0.9	48	±0.47	±0.43	±140.3
	E_0 =1.8	48	±0.47	±0.43	±53.3

Doses	Parameters	n	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
	E_{max} =4 DD ED_{50}=100 mcg				
	γ =0.9				
	E_0 =1.8 E_{max} =1.5 DD ED_{50}=100 mcg γ =0.9	48	±0.47	±0.43	±136.2

BUD- Budesonide; DD- Doubling dose; FF – Fluticasone furoate; FP – Fluticasone propionate; mcg–microgram

To assess the impact of withdrawals sample size sensitivity has also been performed based on a sample size of 36 subjects. The same assumptions have been used as for N=48 for the scenarios where E0=0.8. The E0=0.8 scenarios give the best representation of how the predicted width of the ED50 95% confidence interval is expected to vary, whereas the E0 and Emax predicted confidence intervals remained consistent.

Table 14 Sample Size Sensitivity Analysis (N=36)

Doses	Parameters	n	Predicted Width of 95% CI		
			E_0(DD)	E_{max}(DD)	ED_{50} (mcg)
FF	E_0 =0.8 E_{max} =4 DD ED_{50}=25 mcg	36	±0.54	±0.50	±15.5
	γ =0.9				
	E_0 =0.8 E_{max} =1.5 DD ED_{50}=25 mcg	36	±0.54	±0.50	±40.2
	γ =0.9				
FP	E_0 =0.8 E_{max} =4 DD ED_{50}=50 mcg	36	±0.54	±0.48	±30.1
	γ =0.9				
	E_0 =0.8 E_{max} =1.5 DD	36	±0.54	±0.48	±77.0

~~Doses~~	~~Parameters~~	~~n~~	~~Predicted Width of 95% CI~~		
			~~E_0(DD)~~	~~E_{max}(DD)~~	~~ED_{50} (mcg)~~
	~~ED_{50}=50 mcg~~				
	~~γ =0.9~~				
~~BUD~~	~~E_0 =0.8~~	~~36~~	~~±0.54~~	~~±0.50~~	~~±61.5~~
	~~E_{max} =4 DD~~				
	~~ED_{50}=100 mcg~~				
	~~γ =0.9~~				
	~~E_0 =0.8~~	~~36~~	~~±0.54~~	~~±0.50~~	~~±157.2~~
	~~E_{max} =1.5 DD~~				
	~~ED_{50}=100 mcg~~				
	~~γ..=0.9~~				

CHANGE 35

Section 9.2.3, Sample Size Re-estimation or Adjustment

Rationale for change: The sample size re-estimation or adjustment section was updated to indicate that the sample size can be re-estimated.

REVISED TEXT

~~Sample~~ The need for sample size re-estimation ~~is not planned.~~ will be reviewed by in-stream data review (see Section 4.4).

CHANGE 36

Section 9.3.2, Interim Analysis

Rationale for change: The possibility of an interim analysis was included.

REVISED TEXT

The need for interim analysis will be reviewed by in-stream data review (see Section 4.4). ~~No interim analysis is planned for this study.~~

CHANGE 37

Section 9.4.1, Pharmacodynamic Analyses

Rationale for change: The analysis plan for the in-stream data review was added.

ADDED TEXT, 8th paragraph

Details of the in-stream data review and analysis will be presented in the RAP.

CHANGE 38

Section 12.4.1, Contraceptive requirements for male subjects with female partners of reproductive potential (when applicable)

Rationale for change: A definition of 'non-reproductive' for male subject's female partners was added to Appendix 12.4.1. This is to incorporate a change from a MEMO dated 30 MAY 2017.

ADDED TEXT

The definition of 'non-reproductive' for male subject's female partners is either postmenopausal or 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 Female

CHANGE 39

Section 12.5, Appendix 5: Low, Medium and High Daily Doses of Inhaled Corticosteroids

Rationale for change: An Appendix was added from the GINA report 2017 to present equivalent dose for low-dose ICS.

ADDED TEXT

Low, medium, and high daily dosage of inhaled corticosteroids in adults and adolescents (12 years and older)			
Drug	Daily dose (mcg)		
	Low	Medium	High
Beclometasone dipropionate (CFC)	200-500	>500-1000	>1000
Beclometasone dipropionate (HFA)	100-200	>200-400	>400

Low, medium, and high daily dosage of inhaled corticosteroids in adults and adolescents (12 years and older)			
Drug	Daily dose (mcg)		
	Low	Medium	High
Budesonide (DPI)	200-400	>400-800	>800
Ciclesonide (HFA)	80-160	>160-320	>320
Fluticasone furoate (DPI)	100	NA	200
Fluticasone propionate (DPI)	100-250	>250-500	>500
Fluticasone propionate (HFA)	100-250	>250-500	>500
Mometasone furoate	110-220	>220-440	>440
Triamicolone acetonide	400-1000	>1000-2000	>2000

Abbreviations: CFC: chlorofluorocarbon propellant, DPI: dry powder inhaler; HFA: hydrofluroalkane propellant; NA: not applicable.
Source: 2017 Global Initiative for Asthma (GINA 2017) Report

12.6.3. Amendment 3 (26-APR-2018) from the Amendment 2 (03-OCT-2017)

Protocol Amendment 3 applies to all sites participating in the conduct of the study.

Summary of Amendment Changes with Rationale

This amendment is being issued to update the SAE contact information, as well as to include an administrative change clarifying the screening PEFR procedure.

List of Specific Changes

CHANGE 1

Medical Monitor/SAE Contact Information

Rationale for change: Contact information has been updated.

REVISED TEXT

Role	Name	Day Time Phone Number and email address	After-hours Phone/Cell/ Pager Number	Site Address
SAE contact information (PAREXEL Global Pharmacovigilance Processing Group (GPPG)	PAREXEL Medical Services	Email: PPD PPD and PPD PPD and PPD Or Fax: PPD	NA PPD PPD	NA
Primary Medical Monitor	PPD			GSK, Stockley Park West, Uxbridge, Middlesex, UB11 1BT, UK
Secondary Medical Monitor				GSK, 709 Swedeland Rd, King of Prussia, PA 19406, USA

~~SAE contact information (GSK)~~	~~[Primary Medical monitor, details as above]~~ ~~Central Safety Department~~	~~FAX:~~ PPD PPD ~~EMAIL:~~ PPD		

CHANGE 2

SAE Reporting and Processing Information

Rationale for change: SAE Management Plan has been updated.

REVISED TEXT

- SAE processing information was updated for alignment with the SAE management plan. It was clarified that PAREXEL GPPG will handle the collection and initial processing of SAEs. In Section 7.3.1.1, Section 7.3.1.5 and Section 12.3.5, GSK has been replaced by PAREXEL.

CHANGE 3

Section 12.3.6 Reporting of SAEs

Rationale for change: SAE Management Plan has been updated.

REVISED TEXT

SAE reporting
• ~~Within~~GSK has contracted PAREXEL GPPG to handle the collection and initial processing of SAEs. • On discovery, all SAEs should be immediately reported (latest within 24 hours of ~~being notified of the SAE,~~ knowledge of the event) to PAREXEL Global Medical Services (GMS) /GSK Medical Monitors by: **1-Completing the paper SAE report form which will be emailed/faxed/** ***(whatever is more convenient for the site***(s) ~~will contact the Parexel Safety Group either by phone or email, and they must also contact the~~ ***, e-mail would be preferred)*** **to PAREXEL GPPG and to GSK medical monitor,** ~~the GSK Study Manager and the GSK Central Safety Department.~~**for all sites globally via:** • ~~Paper SAE data collection forms can be used and faxed to the GSK Central Safety Department, the GSK Medical Monitor and the GSK Study Manager.~~ • ~~The site(s) must also enter the serious adverse event data into the Parexel electronic~~

~~data capture system.~~

- ~~After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.~~
- ~~If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site must report this information on a paper SAE form to the Parexel Safety Group and also to the GSK Central Safety Department, the GSK Medical Monitor and GSK Study Manager.~~

~~Contacts for SAE receipt for both PAREXEL and GSK can be found at the beginning of this protocol on the sponsor/Medical Monitor Contact Information page.~~E-mail to:

PPD **and** PPD **and**

PPD

Or FAX: PPD

AND

2-Entering the adverse event in the appropriate section of the eCRF, indicating that the event is considered serious and providing all the details per the eCRF completion guidelines at the same time the event is entered.

- Note: Email should be used preferentially. Fax to be used in case of email failure.
- The reporting of the serious adverse event should be done immediately in the e-CRF even though the paper SAE report is completed. Thus, one alert email will be triggered and the safety team will be aware that one SAE has been notified.
- In the event that the site is unable to complete the SAE report form, the investigators may report the SAE over the telephone via the SAE answering service, and then provide the completed SAE form via email/fax, (within the next 24 hours). If questions arise regarding the reporting procedures or the specifics of the reporting of an event, sites may notify PAREXEL utilizing the following numbers:

  Paris: Tel: PPD

  Paris: Fax: PPD or PPD

- GPPG will check the eCRF/SAE form and source documents (if submitted) for validity, completeness, accuracy, legibility, signature, compliance with patient data protection, and investigator's causality assessment. GPPG may interact with the site, as needed, to clarify information and/or obtain missing or additional information.
- Follow-up SAE reports and support documents should be reported in the same manner as the initial report.

CHANGE 4

Section 7.3.2 Pregnancy

Rationale for change: Change in reporting pregnancy information within 24 hours.

REVISED TEXT

• If a pregnancy is reported, then the investigator should inform ~~GSK~~PAREXEL within ~~2 weeks~~24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4.

CHANGE 5

Section 12.4.2 Collection of Pregnancy Information

Rationale for change: Change in reporting pregnancy information within 24 hours.

REVISED TEXT

- Information will be recorded on the appropriate form and submitted to PAREXEL within ~~2 weeks~~24 hours of learning of a subject's pregnancy.

CHANGE 6

Time and Events, Table 9 Screening and Follow-up Visit

Rationale for change: Administrative change clarifying that baseline PEFR will be captured for all subjects and that PEFR will not be performed at screening visit 2.

REVISED TEXT

The Time and Events Table was updated to reflect that PEFR will not be performed at screening visit 2. In addition, table footnote b provides further detail as follows:

Table footnote b
Baseline PEFR values will be captured for all subjects at screening visit 1. Subjects who are receiving low-dose ICS may take part after a 4-week washout and screening procedures will be completed in two screening visits. For these subjects, screening visit 2 will be at least 28 days after screening visit 1. During the 4 week ICS washout subjects will keep a diary (from screening visit 1 to randomization) to record twice daily PEFR (subject to contact site if there is a 20% decrease) and SABA use (subject to contact site if SABA intake is increased). Salbutamol will be dispensed for rescue use. The reference PEFR value will be obtained at screening visit 1 and an alert value recorded in subject diary by site staff.

CHANGE 7

Section 7.3.8.3- Peak Expiratory Flow Rate (PEFR) Measurements

Rationale for change: Change clarifying that baseline PEFR will be captured for all subjects at screening visit 1.

REVISED TEXT

Baseline PEFR values will be captured for all subjects at screening visit 1. Subjects who undergo low-dose ICS washout will be provided with a peak flow meter at screening visit 1 for use at home, until randomization.

CHANGE 8

Section 11 References

Rationale for change: Added one reference and cited in Section 7.4.1

REVISED TEXT

Joos GF, O´Connor B, Anderson SD, et al. Indirect airway challenges. *Eur Respir J.* 1998;21:1050-1068.

12.7. Appendix 7: Country-Specific Requirements

No country-specific requirements exist.