TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A randomised double blind (sponsor unblinded), single and repeat ascending dose First Time in Human study in healthy subjects, cold urticaria and chronic spontaneous urticaria subjects to investigate safety, tolerability, pharmacodynamics and pharmacokinetics of topically applied GSK2646264.

Compound Number: GSK2646264

Development Phase: I

Effective Date: 13-OCT-2016

Protocol Amendment Number: 05

Author (s): PPD (II TAU), PPD (II TAU), PPD (CPSSO), PPD (QSci) PPD (II TAU), PPD (CPMS), PPD (CPSSO), PPD (GCSP), PPD (DMPK).

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2013N167482_00	2014-APR-16	Original
2013N167482_01	2014-SEP-05	Amendment No. 1
Reason for Amendment 01: Changes have been made to safety related study specific dose adjustment criteria following MHRA review. An update has been made to the exclusion criteria of the protocol Section 4.2.2. and a change has been made to Section 10.2 Regulatory and Ethical considerations following German (Berlin) Ethics Committee review.		
2013N167482_02	2014-DEC-10	Amendment No. 2
Reason for amendment 02: Changes have been made to list all of the challenge agents to be used in the study following guidance from German Regulatory Agency (BfArM). A table of the list of challenge agents has been added to the study treatment section. An update to the skin prick test procedure and the correct name of one of the challenge agents has been added to the inclusion criteria.		
2013N167482_03	2015-JUN-07	Amendment No. 3
Part B and C have changed from a bilateral to a placebo-controlled randomised parallel design 3:1 active to placebo. An informal review of data from the first 4 patients of Part B will be performed before starting part C The number of subjects in Part C has increased (from n=12 up to n=16); and a 2-week PK, safety and biomarker endpoints sampling period for parts B & C has been implemented		
2013N167482_04	2016-APR-11	Amendment No. 4
Updates have been made following the interim analysis of the first 4 subjects in Part B, related to the dosing in Parts B and C. A change has been made to the inclusion and exclusion criteria, related to use of contraception for women of child bearing potential.		

Changes have been made to the PK sampling time points for Part C.		
2013N167482_05	2016-OCT-13	Amendment No. 5
Administrative amendment to account for the change in primary medical monitor, as documented in the Medical Monitor and Sponsor Contact Information table. In addition, the following corrections have been made that were previously captured in a note to file format. The following file note is hereby superseded: • File note dated 26th April, 2016 concerning two clarifications of typographic/edits errors: Timing of tolerability assessment, and timing of the complete physical exam. These have been updated in Section 6.1.4		

SPONSOR SIGNATORY

PPD

13th October 2016

Caroline Savage, FRCP, FMedSci
VP and Head, Experimental Medicine Unit
ImmunoInflammation TA

PPD

SPONSOR/MEDICAL MONITOR INFORMATION PAGE

Medical Monitor and Sponsor Contact Information:

Role	Name	Day Time Phone Number	After-hours Phone/Cell / Pager Number	Fax Number	GSK Address
Primary Medical Monitor	PPD			n/a	Experimental Medicine Unit, Immuno-inflammation Therapy Unit GlaxoSmithKline Medicines Stevenage, Hertfordshire
Secondary Medical Monitor	PPD				Experimental Medicine Unit, Immuno-inflammation Therapy Unit GlaxoSmithKline Medicines Stevenage, Hertfordshire
SAE fax number				XXX	

Sponsor Legal Registered Address:

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

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

Regulatory Agency Identifying Number(s): EudraCT number: 2014-001015-39

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number 200196

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

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

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

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

TABLE OF CONTENTS

PAGE

LIST OF ABBREVIATIONS

AAS	Angioedema Activity Score
AE	Adverse Event
AUC	Area Under the Curve
BCR	B Cell Receptor
BHR	Basophil Histamine Release
BMI	Body Mass Index
BPM	Beats Per Minute
BSA	Body Surface Area
CLbq	Blood Clearance
Cmax	Peak plasma concentration of a drug after administration.
CPMS	Clinical Pharmacokinetics Modelling and Simulation
CsU	Chronic Spontaneous Urticaria
CTT	Critical Temperature Threshold
CU	Cold urticaria
CuQ2oL	Chronic Urticaria Quality of Life Questionnaire
DLQI	Dematology Life Quality Index
DMPK	Drug Metabolism and Pharmacokinetics
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FcεR1	Fc Epsilon receptor
FSH	Follicle Stimulating Hormone
GCSP	Global Clinical Safety and Pharmacovigilance
HBsAg	Hepatitis B surface Antigen
hCG	Human Chorionic Gonadotrophin
HIV	Human Immunodeficiency Virus
HRT	Hormone Replacement Therapy
IB	Investigator's Brochure
IgE	Immunoglobulin E
IUD	Intrauterine Device
IUS	Intrauterine System
IV	Intra venous
MABEL	Minimum Anticipated Biological Effect Level
MSDS	Material Safety Data Sheet
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
PD	Pharmacodynamic
PK	Pharmacokinetic
PUVA	Psoralen combined with Ultraviolet A
QOL	Quality of Life
QTcF	QTc interval Friderica's
RAP	Reporting Analysis Plan
SAE	Serious Adverse Event
SAE	Serious Adverse event
SMC	Safety Monitoring Commitee
SPM	Study Procedures Manual
SPT	Skin Prick Test

SYK	Spleen Tyrosine Kinase
Tmax	The time of Cmax occurring
TSH	Thyroid Stimulating Hormone
UAS7	Urticaria Activity Score
VAS	Visual Analog Scale
Vss	Volume of distribution at Steady State

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Chiron RIBA
Dapsone
Inform
Phoenix (includes WinNonlin)
Zaditen

1. INTRODUCTION

1.1. Study Rationale

This First Time in Human (FTIH) study, which will be performed in three parts is designed to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics after single and repeat topical applications of up to 2 strengths of GSK2646264 and corresponding placebo, in:

- healthy adult subjects (Part A)
- subjects with cold urticaria (CU, Part B)
- subjects with chronic spontaneous urticaria (CsU, Part C)

The study will also measure short term effects of GSK2646264 on the number and size of weals in subjects with CsU, the area and volume of weals following an allergen skin prick test in healthy volunteers and patients and a disease specific challenge (cold temperature test) for comparison of mechanism with the allergen skin prick test in the cold urticaria subjects.

1.2. Brief Background

Spleen tyrosine kinase (SYK) is an intracellular protein tyrosine kinase involved in the downstream signalling events of several immunoreceptors in a variety of cell types, notably the B-cell receptor (BCR), the IgG receptors (FcgRI and III) and the Fc epsilon receptor (FcεR1). These immunoreceptors including the FcεR1, the high affinity receptor for IgE, are important in contributing to the pathology of IgE mediated diseases such as chronic urticaria, and thus pharmacological inhibition of these kinases could be beneficial in these predominantly mast cell driven diseases.

Chronic urticaria is a common skin disease with up to 1% of the population affected (Maurer, 2011a). It is characterised by daily or almost daily itchy weals on the skin, with or without angioedema, that last for greater than 6 weeks. The classification of chronic urticaria includes six distinct patterns of which two will be investigated in this study.

Cold urticaria, a form of inducible urticaria, characterised by local skin reactions consisting of itchy weal and flare responses following cold exposures. These reactions can be induced on discrete areas of the skin using an electronic device called a TempTest thus allowing a critical temperature threshold (CTT) to be established. CTT and changes in CTT values can be correlated with disease severity and changes in disease activity respectively (Młynek, 2010).

Chronic spontaneous urticaria characterised by itchy weals for greater than 6 weeks is thought in part to be mediated by auto-antibodies against the FcεR1 or IgE which bind to the FcεR1 on mast cells and basophils leading to the degranulation and release of pro-inflammatory mediators from these cells (Greaves, 2000).

Chronic urticaria can be difficult to treat, with patients demonstrating a variable response to first-line therapies including oral H1 antihistamines. Second and third line treatments

include leukotrienes, cyclosporine and short term treatment with oral corticosteroids if no efficacy is obtained after increased dosage of antihistamines.

SYK inhibition, following IgE activation of the FcεR1, will reduce three main mast cell functions: the release of preformed mediators such as histamine, the secretion of cytokines including TNF alpha and the production of leukotrienes and prostaglandins. The majority of drugs currently used to treat IgE-mediated disorders target a single mediator e.g. antihistamines or leukotriene modifiers. The SYK inhibitor GSK2646264 should stabilise the mast cells and prevent multiple mediators from being released. The dose-dependent inhibition of histamine release with GSK2646264, after anti-IgE stimulation in ex-vivo human skin, supports the potential for the use of this compound as a treatment for chronic urticarias.

To further support this rationale, recent Phase 3 clinical trials of Omalizumab, a humanised monoclonal anti-IgE antibody which binds to free IgE preventing the interaction between IgE and mast cells or basophils, was shown to reduce clinical signs and symptoms in patients with chronic spontaneous urticaria who had not responded to H1 antihistamine therapy (Saini, 2011), (Maurer, 2011a). This data has supported an EMEA approval in adult and adolescent (12 years and above) patients with inadequate response to H1 antihistamine treatment.

Additionally, Omalizumab has shown benefit in a small population of cold urticaria patients and Phase 2 trials for this condition (Clinical Trials for Cold urticaria http://clinicaltrials.gov/ct2/show/ NCT01580592) are underway.

As SYK is downstream of the FcεR1, a similar inhibition of this pathway is likely to occur in the presence of a SYK inhibitor suggesting that GSK2646264 may be beneficial in chronic spontaneous urticaria, cold urticaria and other skin disease where omalizumab shows improvement in disease activity.

2. OBJECTIVE(S) AND ENDPOINT(S)

2.1. Primary objectives and endpoints for all study parts (A, B & C)

Objectives	Endpoints
Safety and Tolerability To investigate the safety and tolerability of topically applied GSK2646264 cream and its placebo in healthy subjects[1], subjects with cold urticaria (CU[1]) and subjects with chronic spontaneous urticaria (CsU).	**Safety and Tolerability** • Number, severity and frequency of AEs and serious AEs (local and systemic) • heart rate • blood pressure • 12- lead ECG • clinical laboratory safety tests • assessment of the local tolerability of the study medication

1. All Healthy subjects and the Cold urticaria subjects should be screened for reactivity to one of the allergens used in skin prick test. (grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander)

2.2. Secondary objectives and endpoints for all study parts (A, B & C)

Objectives	Endpoints
Pharmacokinetics in plasma To evaluate the plasma concentrations of GSK2646264 in Healthy, CU and CsU subjects	**Pharmacokinetics in plasma** • Plasma concentrations of GSK2646264 and pharmacokinetic parameters, including AUC, C_{max}, T_{max}, $t_{1/2}$ if data allows

2.3. Exploratory Objectives and Endpoints

2.3.1. Part A: Healthy Subjects

Objectives	Endpoints
Exploratory	
Pharmacodynamic measure of Allergen Challenge Assess measure of weal and flare size and erythema, in healthy subjects after allergen challenge (Skin Prick test, positive control (histamine) and negative control (saline)). • Duration of response in healthy subjects	**Pharmacodynamic measure of Allergen Challenge** • Percent inhibition on days 1,3, 4 and 6 will be derived for: o The longest weal diameter (measured by ruler). o Perpendicular weal length (measured by ruler). o Weal area calculated using weal diameter and length assuming ellipse area. • Weal Volume as measured by: • Quantitative volumetric morphometry[1] Flare erythema as measured by • Mexameter[2] Percent of inhibition at 24 hours post final dose, will be derived for the endpoints above

1. Measured using digital camera using PRIMOS 5.075D instrument software produced by GMF
2. Measures erythema and melanin index values using narrow band spectrophotometry

2.3.2. Part B: Cold urticaria Subjects

Objectives	Endpoints
Exploratory	
Pharmacodynamic measure of Cold Provocation Assess change in Cold Temperature Test values	**Pharmacodynamic measure of Cold Provocation** • Change from placebo of Cold Temperature Test[3] values at day 3
Pharmacodynamic measure of Allergen Challenge Assess measure of weal and flare size, in subjects with Cold urticaria after allergen challenge (Skin Prick test, positive control (histamine) and negative control (saline)).	**Pharmacodynamic measure of Allergen Challenge** • Percent of inhibition on day 3 will be derived for: o The longest weal diameter (measured by ruler). o Perpendicular weal length (measured by ruler). o Weal area calculated using weal diameter and length assuming ellipse area. • Weal Volume as measured by; • Quantitative volumetric morphometry[1] Flare Erythema as measured by; • Mexameter[2]
Systemic measure of target pharmacology Determine CD69 expression in an ex vivo blood sample following CD69 stimulation	**Systemic measure of target pharmacology** • Percent inhibition of CD69 expressing cells in blood samples stimulated with anti-IgM .

1. Measured using digital camera using PRIMOS 5.075D instrument software produced by GMF
2. Measures erythema and melanin index values using narrow band spectrophotometry
3. TEMPTest 4.0

2.3.3. Part C: Chronic Spontaneous Urticaria Subjects

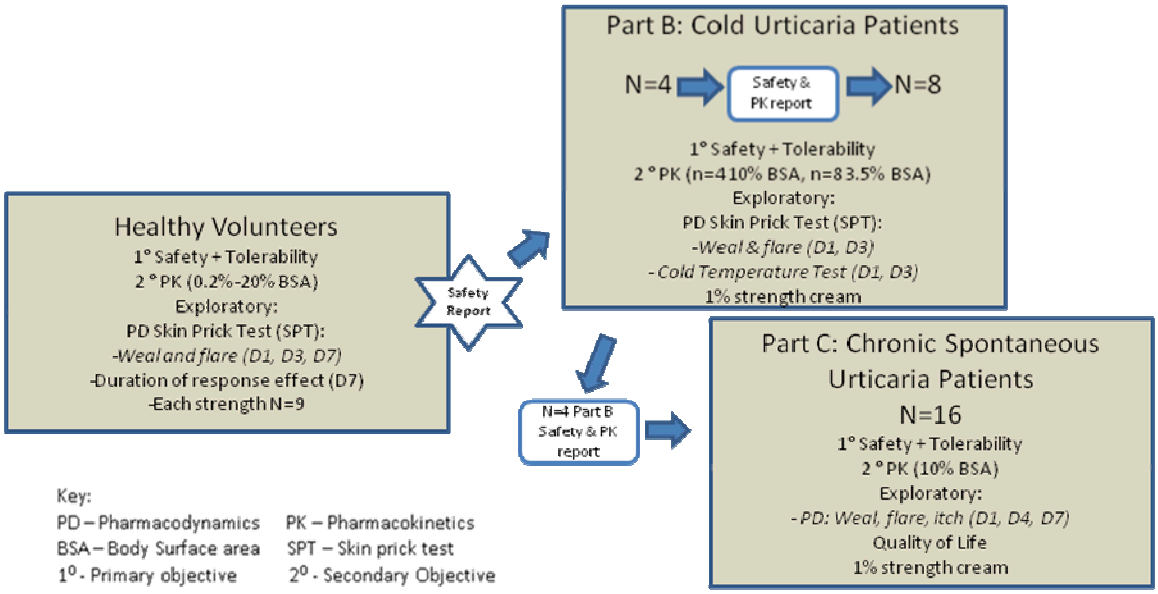
Objectives	Endpoints
Exploratory	
Pharmacodynamics as assessed by the Urticaria activity score Urticaria Activity Score: Assess measures of weal characterisation in CsU subjects within treated area only	**Pharmacodynamics as assessed by the Urticaria activity score** • Urticaria Activity Score, a composite score using key urticaria symptoms (number of weal, itch/pruritus), to derive change from baseline for the 7 days after dosing
To measure of Quality of Life	• Dermatology Life Quality Index (DLQI) questionnaire
To measure disease activity	• Angioedema Activity Score (AAS)
Investigate relationships between (basophil histamine release) BHR test and weal characteristics	• Relationship of positive BHR test with reduction in number or size of weals
Systemic measure of target pharmacology Determine CD69 expression in an ex vivo blood sample following CD69 stimulation	**Systemic measure of target pharmacology** • Percent inhibition of CD69 expressing cells in blood samples stimulated with anti-IgM.

3. STUDY DESIGN

3.1. Study Design

This study will be conducted in three parts: Part A (healthy subject cohort, inpatient), Part B (cold urticaria subject cohort, inpatient) and Part C (chronic spontaneous urticaria subject cohort, outpatient) (Figure 1). Subjects will participate in the study if they fulfil the eligibility criteria at the time of screening and prior to randomisation for the relevant cohort for which they are assessed.

Figure 1 Study Schematic Showing all 3 Cohorts



The study will enrol 1 sentinel subject for:

- Part A dosing group1
- Part A dosing group 2

Each sentinel subject will be dosed at least 1 day before any other subjects of their dosing group or cohort. Please refer to Section 5.5 to Section 5.7 for dose adjustments and stopping criteria.

The cream will be applied at 10 mg/cm^2 for all doses but the dose escalation is based on both increase in cream strength (0.5 % strength for Group 1 Part A; and if data permits 1.0% strength for Group 2 Part A), and increase in surface area of the body on which the cream will be applied. The escalation based on body surface area (BSA) starts at 0.2% BSA, then 1%, then 5% BSA, and up to 10% BSA. For the study, the total BSA has been chosen as 1.8 m^2 and will not be measured individually, so the surface area on which the cream is applied is similar for all subjects as follows, regardless of their weight and height. All weights of cream are nominal and any one dose will not exceed 40 g :

Nominal % BSA	**Actual surface to spread the active or placebo cream on:**	**Weight of active or placebo cream to be applied**
0.2% BSA	36 cm^2	0.36g
1% BSA	180 cm^2	1.8g
3.5% BSA	630 cm^2	6.3g
5% BSA	900 cm^2	9g
10% BSA	1800 cm^2	18g
20%	3600 cm^2	36g

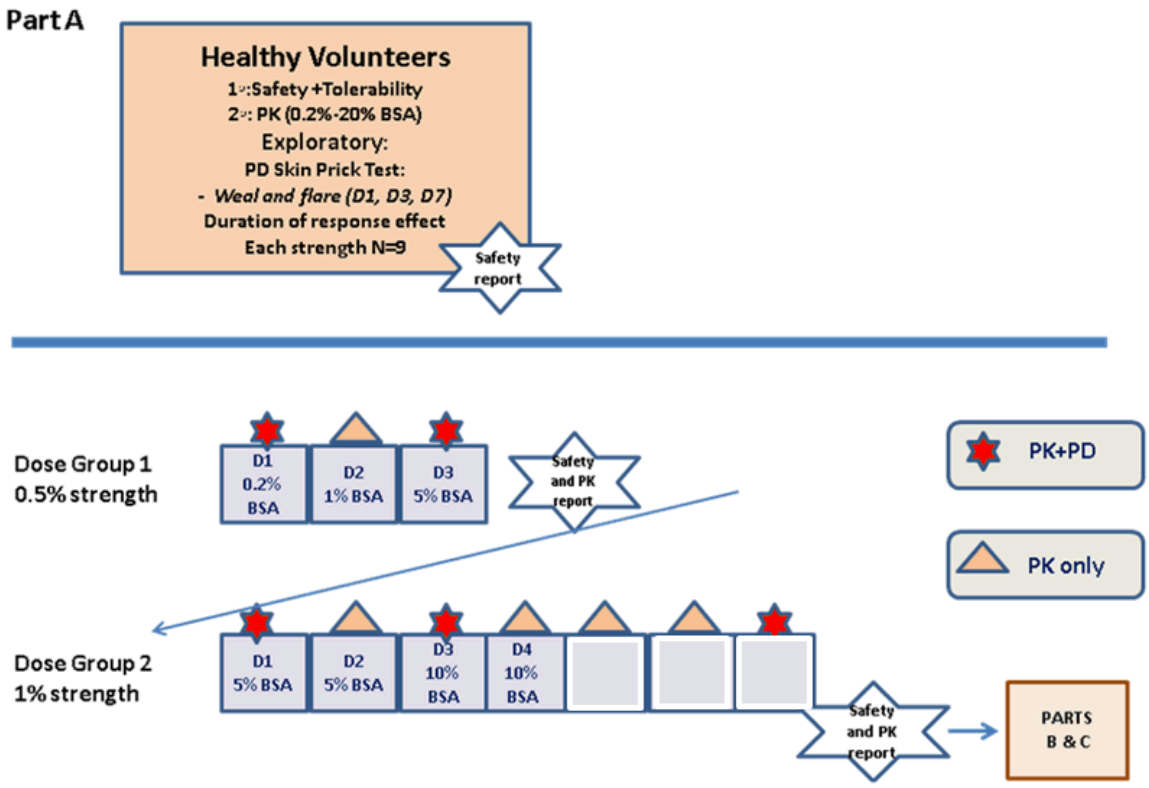
Specific assessments for each study Part A, B and C, are shown in the Time and Events Tables in Section 6.1.
In Part A, randomisation will assign active and placebo to specified areas on left or right side of the body. **The left or right side assignment of treatment always refers to the subject's left or right**. Each bottle of cream will be for single use only.

In Parts B and C, which will be a parallel design, randomisation will assign subjects to either active **or** placebo treatment. This will be a 3:1 ratio active to placebo respectively.

Each bottle of cream will be for single use only.

3.1.1. Part A Healthy Subjects Schematic

Figure 2 Schematic showing Part A: ascending dose periods and increasing body surface area within periods in healthy volunteers



Key:

PD - Pharmacodynamics	SPT - Skin Prick Test
PK - Pharmacokinetics	BSA - Body Surface Area

3.1.2. Part A: Dose group 1: A double-blind (sponsor unblinded) placebo-controlled, randomised to bilateral dose ascending treatments, single dose continuing to three days repeat dosing period, in healthy subjects

This healthy subject cohort will be run in 2 dose groups, where 1 and 2 will be administered an increasing dose strength of GSK2646264 and body surface area. Both groups will remain in-house at a phase 1 clinical pharmacology unit (hospital based) for the complete dose period and completion of all post dose assessments.

For day 1 in dose group 1, subjects will be treated with active and placebo on an area of approximately 12 x 3 cm on the volar aspect of the arm which approximates to 0.2% total BSA, on each arm. On days 2 and 3 subjects will receive active treatment and placebo on the same arms as on day 1, with the percentage BSA being 1% on day 2 and 5% on day 3. The area covered on day 1 will be included in the total area covered on days 2 and 3 and this will be the area where the skin prick test assessment will be performed.

Initially, a single dose of active GSK2646264 (0.5% strength) and placebo will be applied on one sentinel subject on the morning of day 1 and following a safety and tolerability assessment further subjects will be dosed. See Table 1, Figure 2 and Section 5.5.

The timing of and the assessments to be completed at each visit are provided in the Time and Events table (Section 6.1.2).

Safety, tolerability and PK data will be reviewed at the end of the dosing period (See Section 5.5) and used to decide on progression to dose group 2.

Table 1 Treatment applied to specified areas per day on Part A; healthy subjects, n=9, dose group 1

	Day 1	Day 2	Day 3
Area	**Morning**	**Morning**	**Morning**
Left Arm (L)	0.36g of 0.5% GSK2646264 or placebo cream applied to 36cm^2 (~0.2% BSA)	1.8g of 0.5% GSK2646264 or placebo cream applied to 180cm^2 (~1% BSA)	9g of 0.5% GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)
Right Arm (R)	0.36g of 0.5% GSK2646264 or placebo cream applied to 36cm^2 (~0.2% BSA)	1.8g of 0.5% GSK2646264 or placebo cream applied to 180cm^2 (~1% BSA)	9g of 0.5% GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)

3.1.3. Part A: Dose group 2: A double blind (sponsor unblinded) placebo-controlled, randomised to bilateral dose ascending treatments, single dose continuing to seven days repeat dosing period, in healthy subjects

Initially, a single dose of active and placebo will be applied to subjects on the morning of day 1 starting at the final % BSA dosed at day 3 in group 1 which is anticipated to be 5% BSA following a safety and tolerability assessment further subjects will be dosed. (See Table 2)

In dose group 2, the starting BSA will increase to 10% at day 3. If the 1% strength is not tolerated after single or repeat application, then a dose of 0.5% dose strength would be administered instead and the complete regime would be followed as in Table 2. Administration of the evening (PM) dose will be dependent on the data from Part A dose group 1.

The timing of and the assessments to be completed at each visit are provided in the Time and Events table (Section 6.1.2).

Table 2 Treatment applied to specified areas per day on Part A; healthy subjects, n=9, dose group 2

	Day 1-2		Day 3-4		Day 5-6		Day 7 (Morning Dose Only)
Area	**AM**	**PM[a]**	**AM**	**PM[a]**	**AM**	**PM[a]**	**AM**
Left Arm	0.36g of 1.0%[b] GSK2646264 or placebo cream applied to 36cm^2 (~0.2% BSA)						0.36g of 1.0% [b] GSK2646264 or placebo applied to 36cm^2 (~0.2% BSA)
Right Arm	0.36g of 1.0%[b] GSK2646264 or placebo cream applied to 36cm^2 (~0.2% BSA)						0.36g of 1.0% [b] GSK2646264 or placebo applied to 36cm^2 (~0.2% BSA)
Left Front Torso	9g of 1.0% [b] GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)
Right Front Torso	9g of 1.0% [b] GSK2646264 or placebo applied to 900cm^2 (~5% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)
Right Leg					18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)
Left Leg					18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)		18g of 1.0% [b] GSK2646264 or placebo applied to 1800cm^2 (~10% BSA)

a. AM dosing only if data for Part A, group 1 and/or group 2 supports
b. 0.5% strength may be used if data for Part A and/or group 2 supports

Criteria to enable progression and dose escalation (single to repeat and increasing BSA) are detailed in Section 5.5.

Safety and PK from this cohort will enable the dosing of Part B and Part C. Criteria to enable this are detailed in Section 3.2.2 and Section 5.5.

3.1.3.1. Changes after review of preliminary data of Part A Dosing Group 1 by the Safety Monitoring Committee

Following the review by the Safety Monitoring Committee (see Section 5.5), changes were made to Part A Group 2 dosing as shown in Table 3.

Table 3 Treatment applied to specified areas per day on Part A; healthy subjects, n=9, dose group 2

	Day 1-2	Day 3-4	Day 5 to 7
Area	**AM[a]**	**AM[a]**	
Left Arm	0.36g of 1.0%[b] GSK2646264 or placebo cream applied to 36cm^2 (~0.2% BSA)		NO DOSING
Right Arm	0.36g of 1.0%[b] GSK2646264 or placebo cream applied to 36cm^2 (~0.2% BSA)		NO DOSING
Left Torso	9g of 1.0% [b] GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	9g of 1.0% [b] GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	NO DOSING
Right Torso	9g of 1.0% [b] GSK2646264 or placebo applied to 900cm^2 (~5% BSA)	9g of 1.0% [b] GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	NO DOSING

a. AM dosing only
b. 0.5% strength may be used if data for Part A and/or group 2 supports

3.1.4. Part B: A double blind (sponsor unblinded) placebo-controlled, randomised to active or placebo treatment, three day repeat dose study, in cold urticaria subjects

Subjects will be randomised in a 3:1 ratio to receive either active treatment or placebo applied to defined areas once a day for 3 days. The first 4 subjects of Part B will be randomised so that one of the four subjects will receive placebo and the three other subjects will receive GSK2646264. An interim review of the preliminary data from the first 4 subjects, after they have completed the study, will be conducted before dosing the rest of the patients in Part B (n=12 total). PK, safety and tolerability preliminary data up to and including the follow-up visit, will be reviewed by the SMC (See Section 5.5). See Figure 3 for a visual representation.

If preliminary data indicates it is appropriate to review additional PD data, this may then be conducted, either in parallel to or before the remainder of Part B subjects are enrolled.

Criteria to choose dose strength and dose regime in Part B are detailed in Section 5.5. Further dose adjustments may be made as a result of reviewing preliminary data from the first n=4 subjects in Part B.

This cohort will remain in-house for the whole dosing period (until completion of the final dose on the morning of day 3) and completion of subsequent study assessments on day 4.

The timing of the assessments to be completed at each visit are provided in the Time and Event table (Section 6.1.3).

Table 4 Part B: Cold urticaria treatment (first n=4 subjects)

	Days 1-3
Area	**Morning dosing**
Both Arms	9g of 1% strength GSK2646264 or placebo applied to 900cm^2 (5% BSA) on each arm and the volar aspect of each arm must be included. = Total BSA = 10% (18g)

3.1.4.1. Changes after review of preliminary data of n=4 from Part B by the Safety Monitoring Committee

Following the review by the Safety Monitoring Committee (see Section 5.5), changes were made to Part B for the remaining n=8 subjects, as shown in Table 5.

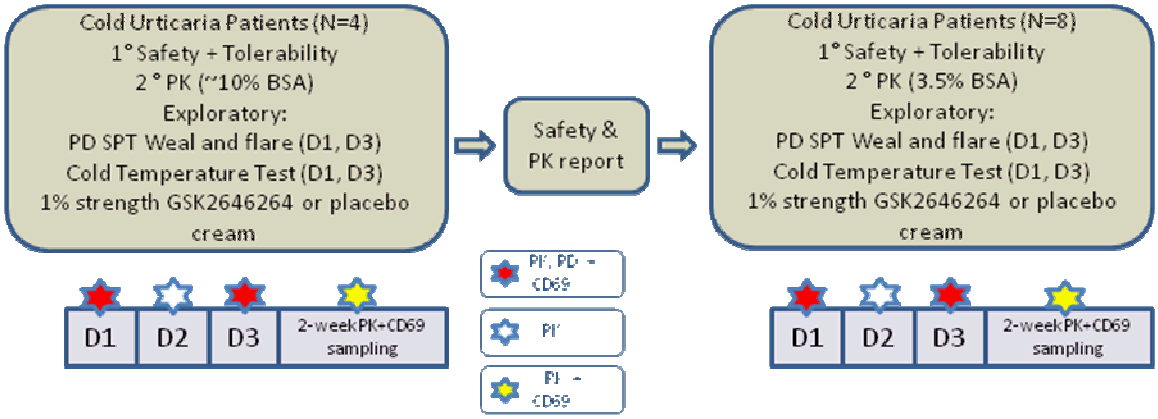
Table 5 Part B: Cold urticaria treatment (remaining n=8 subjects)

	Days 1-3
Area	**Morning dosing**
Both Arms	6.3g of 1% strength GSK2646264 or placebo to be applied to a total area of 630cm^2 (3.5% BSA), spread over the 2 arms, and the volar aspect must be included. The investigator should use their discretion as to how much cream is applied to each arm, but it must be equivalent to 3.5% BSA in total.

Following inclusion subjects will be randomised to either active or placebo. The investigator will use his judgement and discretion to decide on which area to apply the study treatment within requirements given in Table 4.

Subjects will receive either 1% strength GSK2646264 cream or placebo cream. The skin prick test will be performed on the volar aspect of the arm only in subjects who have a positive allergen challenge test at screening, as per inclusion criteria.

Figure 3 Schematic showing Part B Dosing in Cold urticaria patients



Key:
PD - Pharmacodynamics
PK - Pharmacokinetics
1° - Primary Objective
SPT - Skin Prick Test
BSA - Body Surface Area
2° - Secondary Objective

3.1.5. Part C : A double-blind (sponsor unblinded) placebo-controlled, randomised to active or placebo treatment repeat dose study (3 doses over 7 days), in subjects with mild to moderate Chronic Spontaneous Urticaria (CsU)

Subjects will be randomised to a 3:1 ratio to receive active treatment or placebo applied to areas of the body chosen by the investigator once a day every 3 days, over a total period of 7 days. Dosing will occur on days 1, 4 and 7. The chosen area where cream is applied will remain the same throughout the dosing period. Dosing in Part C will only commence once relevant data from the first four subjects in Part B have been reviewed, to allow greater confidence in predictions of the pharmacokinetics in Part C. The BSA over which study treatment will be administered in Part C will be 10%, but is subject to change, if appropriate, based on preliminary data from the first 4 subjects in Part C However, the maximum BSA will not exceed 20% (see Figure 4 for a visual representation of the dosing schedule).

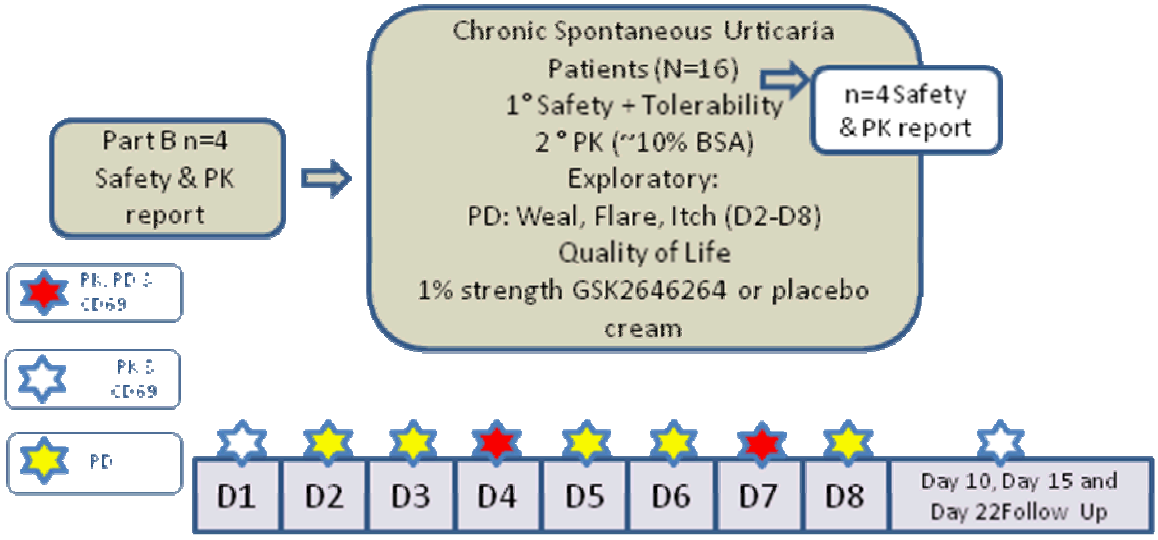
The first 4 subjects of Part C will be randomised so that one of the 4 subjects will receive placebo and the three other subjects will receive GSK2646264. An informal review of the preliminary data from the first 4 subjects may be conducted after they have completed the study. PK, safety, and tolerability, up to and including the follow-up visit, will be reviewed by the Sponsor (See Section 5.5). If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held. Recruitment of the full planned cohort (n=16) may continue during any data review.

The timing and the assessments to be completed at each visit are provided in the Time and Event tables (Section 6.1.4). Early termination of this patient group may occur due to feasibility of recruitment and stopping criteria see (Section 5.5 and Section 5.6). Following inclusion in the study, subjects will be randomised. 10 mg/cm^2 of 1%

GSK2646264 cream or placebo cream will be applied to a given BSA once per day, every 3 days, on days 1, 4and 7, on areas of the body chosen by the investigator. The areas where cream is applied will remain the same for each day of dosing. The 10 % BSA can be distributed between the arms, legs and torso for an individual subject and will be decided by the investigator prior to randomisation. This pattern of cream application will remain the same for the duration of dosing. The maximum % BSA and the frequency of dosing will be decided after Part A and n=4 in Part B of the study but will not exceed 20% BSA.

The subjects will be dosed in the unit for the first dose on day 1 and then discharged from the clinic. Subjects will return to the clinic prior to the dose and specified assessments on days 4 and 7.. Subjects will be asked to complete a daily diary over the 7 days. . Details of the diary will be provided in the Study Procedures Manual (SPM)

Figure 4 Schematic showing Part C dosing in Chronic Spontaneous Urticaria patients



Key:

PK - Pharmacokinetics
PD – Pharmacodynamics (UAS-7 and AAS)

AAS – Angioedema Activity Score
UAS7 – Urticaria activity score - 7

1^0 - Primary Objective
BSA - Body Surface Area

3.1.6. Progression Criteria for dose escalation in the study

The steps for decisions on dose adjustments and progression criteria to the next stage are presented in Section 5.5.

3.2. Discussion of Study Design

3.2.1. Design Rationale

This study is designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of single and repeat ascending dose of GSK2646264 in healthy subjects (First Time in Human study), cold urticaria and chronic spontaneous urticaria subjects.

The proposed bilateral design for Part A is well established and most efficient study design in absence of any effect. In this design, each subject in Part A will be his/her own control. The subject's left and right side of the defined study body surface area will be randomised to either GSK2646264 or Placebo.

Parts B & C will be a placebo controlled randomised parallel design randomisation where subjects will receive either active or placebo study treatment, not both (unlike Part A of the study).

3.2.1.1. Part A: Healthy Subject Cohort

Healthy male and female subjects (female subjects who are of non-child bearing potential will be included in Part A of the study) will be assessed for safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) after once or twice daily dosing of GSK2646264. Safety assessments over the day 1 dosing period will enable a repeat dose period with increasing body surface and safety assessment from days 1 through to day 3 in group 1. Also, based on the current understanding of SYK kinase inhibition, it is anticipated that target engagement will be determined by the pharmacodynamic endpoint of allergen challenge.

Safety and tolerability in these subjects will also enable covering an increase in strength and increasing body surface area from days 1 through to day 4 of the dosing period in group 2. In addition, PK will drive the decision of whether group 2 will be dosed once or twice daily. Safety, tolerability and systemic exposure data including any effect on IgM stimulated CD69 generated in Part A will be used to provide margins for the strength of GSK2646264 applied and also the body surface area (BSA) covered in cold urticaria subjects in Part B and in chronic spontaneous urticaria subjects in Part C.

3.2.1.2. Part B: Cold urticaria Subjects

This cohort will provide safety, tolerability and PK over 3 days of dosing (with an additional 2 weeks follow-up post last dose for PK, safety and biomarker endpoints) in this population and will enable further clinical studies. Also, based on the current understanding of SYK kinase inhibition, it is anticipated that target engagement will be determined by the pharmacodynamic endpoints of allergen challenge and critical temperature threshold testing.

3.2.1.3. Part C: Chronic Spontaneous Urticaria Subjects

This part of the study will provide safety, tolerability, PK and PD from dosing every 3 days, over a total period of 7 days (with an additional 2 weeks follow-up post last dose for PK, safety and biomarker endpoints) to enable further clinical studies in this population.

3.2.2. Dose Rationale

GSK2646264 will be administered topically as a cream at two strengths of 0.5% and 1% (w/w). A corresponding placebo cream will also be applied.

Previous to Part A of this protocol GSK2646264 had not been applied to humans therefore dose rationale is based on pre-clinical data.

Summary

- The proposed starting dose applied to the smallest surface areas (0.5 % strength on 0.2 % BSA e.g, 36 cm^2) is unlikely to result in quantifiable plasma concentrations, however application to body surface areas from 5% BSA up to 10% BSA are likely to show quantifiable plasma concentrations.
- Based on the integrated data from the human *in vitro* dermal penetration and histamine release at 4 and 24h after topical administration both strengths of cream administered at 10mg/cm^2 are predicted to lead to >90% inhibition of histamine release from the skin mast cells.
- The maximum topically applied dose will be 10mg/cm^2 of cream over 3600 cm^2 (20% BSA 360mg of free base drug) with based on pre-clinical predictions,:-
  - 6 fold systemic margin, compared to the C_{max} at the NOEL (based on thyroid finding in rat IV)
  - 3.5 fold systemic margin compared to the $AUC_{(0-t)}$ at the NOAEL and 3 to 6 fold local tolerability margin based on the applied dose to the skin. (based on lack of thyroid or skin findings in rat dermal study).
- The recommended starting dose for this trial is predicted to deliver exposure lower than the systemic MABEL dose. At a dose of 0.5% strength, no significant adverse events are expected as per the results from preclinical toxicology studies.
- The protocol allows for some alteration from the currently outlined dosing schedule as described in Section 5.5, Section 5.6 and Section 5.7.

3.2.2.1. Comparison of predicted human plasma exposures to animal safety study exposures

The *in vitro* skin penetration study performed over 24 hours provided quantifiable levels of GSK2646264 in the receiving fluid thus allowing the determination of the maximum observed permeation rate (flux) for all of the cream strengths tested. The flux determined for the 0.5 and 1% creams and the predicted human PK parameters (CLb: 13 mL/min/kg, Vss: 0.8 L/kg) were used to predict the maximum expected plasma concentrations and

$AUC_{0\text{-}24h}$ when applying the creams to the different BSA in the clinical study. The calculated values are displayed in Appendix 6.

As the maximum suggested dose to be applied topically in Part A group 1 is 45 mg of free base drug, the predicted maximum systemic exposure achieved is expected to be ~50 fold lower than the C_{max} NOEL achieved (~16ng/ml).

In Part A and C the maximally applied dose of 360 mg of free drug will lead to systemic exposures that are expected to be 6 fold lower than C_{max} NOEL and ~ 1,882 and 447 fold lower than the systemic concentration expected to inhibit SYK activity (MABEL) by 80% (IC_{80} of 4835 ng/mL) and 20% (IC_{20} of 1150 ng/mL) respectively.

Further details are available in the Investigator's Brochure (IB) [GlaxosmithKline Document Number 2013N182566_00].

3.2.2.2. Additional preliminary data from Part A

Preliminary human PK data from Part A (up to 1% strength, up to 10% BSA) demonstrated a geometric mean Cmax after 4 days dosing of 5.59 ng/ml, providing a margin of 2.8 in comparison to the C_{max} NOEL achieved (~16ng/ml) . After 4 days of dosing the geometric mean AUC was 103.8 ng·h/ml, allowing a 2.1 fold systemic margin compared to the $AUC_{(0\text{-}t)}$ at the NOAEL.

This allows 3 days dosing at 1% strength, 10% BSA for Part B. For further progression of dosing in parts B and C refer to Section 5.5.3.3.

Further predictive modeling using the data from Part A, but with no inclusion of elimination rate, suggests that coverage of 10% BSA using the 1% cream over 7 days of dosing would lead to an estimated Cmax of 10.12ng/ml and an AUC of 219.8ng·h/ml.

The information in the above paragraph is superseded by the information in Section 3.2.2.3.

3.2.2.3. Additional preliminary data from Part B

A population pharmacokinetic model integrating all plasma data from part A and the preliminary data from n=4 in part B lead to an estimation of the half life of 57 hours for GSK2646264. This model shows that in order to maintain a 1.8 fold margin with the AUC NOAEL to allow women of child bearing potential in the trial the doses of 180mg (10% BSA) should be administered every three days, over a total 7 day duration, rather than every day.

3.2.2.4. GSK2646264 strength for Part B and C

Parts B and C of the study will recruit cold urticaria and chronic spontaneous urticaria patients, respectively. The drug strength (1%) selected for Parts B and C will be the highest strength tested in Part A that was not associated with clinically significant skin irritation and/or systemic adverse effects.

Preliminary data from Part A (up to 1% strength cream, up to 4 days dosing, and up to 10% BSA) showed that the 1% cream was well tolerated and did not identify clinically significant safety issues. Therefore the 1% strength cream will be used in Parts B and C.

3.2.3. Clinical Experience

This is the first clinical application for GSK2646264 in any formulation and therefore, the first exposure will be in an in-patient setting with healthy volunteers (Part A) followed by cold urticaria patients (Part B) and Chronic Spontaneous Urticaria patients (Part C) with monitoring of routine safety parameters including systematic review of signs and symptoms, physical examination, AEs, vital signs and clinical laboratory results.

Preliminary data from Part A showed that dosing with up to 1% GSK2646264 cream, up to 4 days and up to 10% BSA in healthy volunteers was well tolerated and did not identify clinically significant safety issues. Clinically significant safety issues were also not observed in n=4 subjects in Part B (cold urticaria subjects).

Table 6 Summary of Key Issues, Their Impact and Strategy to Mitigate Risk

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
Hypersensitivity	There may be potential for hypersensitivity reactions from the procedures used in the study or if subjects are allergic to ingredients in the study medications. There is no previous experience of GSK2646264 or of the matched placebo in humans	Subjects will be asked at screening if they are allergic to any of the ingredients – if known, Subjects will be excluded from the study if they are allergic to any ingredient of the study medications or if they have previously experienced an anaphylactic reaction.	Subjects will undergo regular medical assessment and instructed what action to take in the event of experiencing an allergic reaction.
Skin prick	There have been rare reports of systemic allergic reactions following skin prick testing (Heinzerling, 2013)	Subjects will be excluded from the study if have previously experienced an anaphylactic reaction. Subjects will be excluded from the study if the subject has a reaction which contraindicates to the study	Skin prick testing will be performed in a facility with appropriate emergency equipment available in case of the need for treatment for systemic allergic reaction.
Pregnancy and lactation	There is no data available on the effect of GSK 2646264 in pregnancy and lactation in humans. Fetal malformations were seen after IV dosing to pregnant rabbits with a NOAEL that is expected to exceed the highest expected human exposure by >4 fold. No similar changes were seen in the rat after substantially higher exposure	Female subjects of non child bearing potential will be included in Part A of the study. Female subjects that are of child-bearing potential will be required to follow the contraceptive requirements that are outlined in the protocol Section 4.2.1 inclusion criteria for Part B and C. A decision to allow females of child-bearing potential in Parts B and C will be made based on the safety margin from the dosing in Part A of the study.	Female subjects that are of child-bearing potential will undergo regular pregnancy testing and treatment stopped immediately if a subject is found to be pregnant during the study. In part B, these subjects must be on oral contraceptives 28 days before screening begins. They will undergo serum pregnancy testing at day -28, day -7 to -4, a serum or urine pregnancy test the day of the start of the study prior to dosing, then testing on an approximately weekly basis and a serum pregnancy test at the follow up visit. In Part C, a serum pregnancy test will be

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
		Nursing females will be excluded from participating in the study.	performed on the day of screening. If this is between Days -13 and -7, a further pregnancy test will not be required until Day 1. However, if the day of screening is between Day -28 and -14, an additional serum pregnancy test will be required between Days -7 and -4. A urine pregnancy test will be required prior to dosing on Day 1
Immune cell effects, including neutropenia	SYK is involved in immunoreceptor signalling in a number of cell types, including mast cells, basophils, B cells, neutrophils and macrophages (Riccaboni, 2010) Studies with R788 in rheumatoid arthritis identified an increased risk of neutropenia in subjects treated with the oral SYK inhibitor compared to placebo (placebo 0.7%, R788 150mg OD 6.6%, R788 100mg BD 5.9%) (Weinblatt, 2010)	Subjects with co-morbid conditions that would put them at risk or laboratory values outside the reference range at screening would be excluded from participating in the study.	The product is being investigated in a topical formulation, which should limit potential systemic exposure. Increasing exposure will be performed gradually. Subjects will undergo regular haematological assessments, including white blood cell counts during the study. Treatment would be stopped if laboratory test results are identified that would put the safety of the subject at risk.
Thyroid	Minimal thyroid follicular epithelial hypertrophy was observed in all doses following iv administration of GSK2646264 in rats for 4 weeks. Thyroid function test (TSH, free and total T3 and T4) at single timepoints 12 hours post last dose were within normal range.	Requirement for normal TSH in all subjects in Part A, and normal TSH, free T4, T4 and T3 in Parts B and C.. Exclusion of subjects with history of Graves' disease or any thyroid cancer	The risk of thyroid effect in this study is considered not to be significant. Monitoring will be based on signs and symptoms and serum markers of thyroid function will be measured in patients in Parts B and C.

4. STUDY POPULATION

4.1. Number of Subjects

The aim is to achieve approximately 18 randomised evaluable subjects in Part A (9 subjects in dosing group 1 and 9 subjects in dosing group 2), approximately 12 randomised evaluable subjects who complete the study in Part B and approximately 16 subjects in Part C. In Parts A and B, it is anticipated that a proportion of subjects will not demonstrate weal and flare at the screening skin prick test (See Section 4.2.1.2 and Section 4.2.1.3). However these proportions are not known, so enough subjects will be enrolled to achieve the target study population.

4.2. Eligibility Criteria

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

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

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

For a subject to be included in the study they must fulfil all requirements for both the 'All subjects' Inclusion and Exclusion Criteria and then specific inclusion and exclusion for either healthy subjects, Cold urticaria subjects or Chronic Spontaneous subjects as shown in Section 4.2.

For those criteria where an investigator's decision will be required to it will be documented on every occasion.

4.2.1. Inclusion Criteria

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

4.2.1.1. Inclusion criteria for all subjects in Parts A, B and C

1. Male or female subject aged at least 18 years at the time of signing the informed consent. The upper age limit of subjects is defined in the specific inclusion criteria for each cohort.
2. All subjects must be free from scarring or skin markings (e.g. tattoos or piercings) and open wounds on the defined areas of the body that cream will be applied onto, unless in the opinion of the investigator it will not compromise the subjects' safety and quality of data.

3. Able to refrain from exposure to extended and direct sunlight during the study period, from screening until follow up, especially the area that is under treatment during the study.
4. Able to refrain from shaving and waxing the areas on which the study cream will be applied during the duration of the study from screening to follow up.
5. Male subjects with female partners of child-bearing potential must agree to use one of the contraception methods listed in Section 4.3.1. This criterion must be followed from the time of the first dose of study medication until the follow up visit or 12 days, whichever is the latter.
6. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form. Willing, committed and able to return for all clinic visits and complete all study-related procedures. Able to read, understand and complete study- related questionnaires.

4.2.1.2. Inclusion criteria specific for healthy subjects (Part A)

Subjects will be included in the healthy subject cohort if all the following criteria apply:

1. The subject is aged between 18 and 55 years of age inclusive, at the time of signing the informed consent.
2. Body weight ≥ 50 kg and BMI within the range 19 to 30 kg/m2 (inclusive).
3. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring. A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical monitor if required, agrees and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.
4. Demonstration of a positive weal and flare reaction (≥ 3mm in diameter relative to negative control) to at least one allergen from a battery of allergens (**Mixed** grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander) on skin prick testing at screening.
5. Subjects must be free from any past or present benign or malignant skin conditions and disease, unless in the opinion of the investigator it will not compromise the subjects' safety and quality of data.
6. Non –smokers or if the subject is a tobacco smoker: smokes less than 5 cigarettes per day and commits to not smoke tobacco for the duration of the in-house stay, and commits to stable and moderate use (as determined by the Investigator) of tobacco or nicotine-containing products, including nicotine patches/gum, during the course of the study, as long as the patches do not interfere with the study procedures.
7. A female subject is eligible to participate if she is of:

- Non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy for this definition, "documented" refers to the outcome of the investigator's/designee's review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records; or postmenopausal defined as 12 months of spontaneous amenorrhea in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40 MlU/ml and estradiol < 40 pg/ml (<147 pmol/L) is confirmatory.
- Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods described if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment. For most forms of HRT, at least 2-4 weeks will elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT. Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.

4.2.1.3. Additional Inclusion criteria specific for subjects with cold urticaria (Part B)

Subject will be included in the cold urticaria subject cohort if all the following criteria apply:

1. Diagnosed with cold urticaria for more than six weeks as confirmed by medical history and with a positive cold stimulation test assessed by TEMPTest 4.0 prior to first dose.
2. The subject is aged between 18 and 70 years of age inclusive, at the time of signing the informed consent.
3. Body weight ≥ 50 kg and BMI within the range 19 to 35 kg/m2 (inclusive).
4. Other than a diagnosis of cold urticaria, the subject should have no other co-morbidities which would introduce additional risk factors and will not interfere with the study procedures, as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring.
5. A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical monitor if required, agrees and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedure.
6. In addition, the following criterion will apply to a minimum of 4 patients in Part B:

Demonstration of a positive weal and flare reaction (≥3 mm relative to negative control) to at least one allergen from a battery of allergens (**mixed** grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander) on skin prick testing at screening,

7. Subjects must be free from any past or present benign or malignant skin conditions and disease, other than the specified condition required for eligibility of subjects as defined in the specific inclusion criteria for CU cohort unless in the opinion of the investigator it will not compromise the subjects safety and quality of data.
8. Non–smokers or if the subject is a tobacco smoker: smokes less than 5 cigarettes per day and commits to not smoke tobacco for the duration of the in-house stay, and commits to stable and moderate use (as determined by the Investigator) of tobacco or nicotine-containing products, including nicotine patches/gum, during the course of the study, as long as the patches do not interfere with the study procedures.
9. Female subjects of child bearing potential must have been using one of the contraception methods listed in Section 4.3.1 at least 28 days before their screening visit and agree to continue with these methods until the follow up visit or 12 days after the last dose of study treatment, whichever is the longer.

If a patient who has been screened and is eligible for the study becomes unavailable to do the study at the initially planned dates due to exceptional circumstances, the subject may be re-screened for the study; in total a patient may be screened 2 times. This will only be at the discretion of the Investigator.

4.2.1.4. Additional Inclusion criteria specific for subjects with chronic spontaneous urticaria (Part C)

Subjects will be included in the chronic spontaneous urticaria cohort if all the following criteria apply

1. The subject is aged between 18 and 70 years of age inclusive, at the time of signing the informed consent.
2. Body weight ≥ 50 kg and BMI within the range 19 to 35 kg/m2 (inclusive)
3. Subjects who have a score of >14 on the UAS7 questionnaire with between 4-20 weals observed in a defined area of the body will be included in this study. This area must include either arms, legs or torso for 7 consecutive days during the screening period, prior to the Day 1 visit. If a subject has not completed 7 consecutive days of UAS questionnaire prior to dosing on Day 1 due to exceptional circumstances, the screening period may be extended until the subject has completed 7 consecutive days of UAS questionnaire. This will only be at the discretion of the Investigator.
4. No other aetiology identified for chronic urticaria such as drug-related or inducible urticaria as determined by history, physical examination and laboratory studies.
5. Subjects must be free from any past or present benign or malignant skin conditions and disease, other than the specified condition required for eligibility of subjects as defined in the specific inclusion criteria for CsU cohort unless in the opinion of the investigator it will not compromise the subjects safety and quality of data.

6. Other than a diagnosis of chronic spontaneous urticaria the subject should have no other co-morbidities which would introduce additional risk factors and will not interfere with the study procedures, as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring.
7. A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the Investigator, in consultation with the GSK Medical monitor if required, agrees and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.
8. Female subjects of child bearing potential must have been using one of the contraception methods listed in Section 4.3.1 at least 28 days before their screening visit and agree to continue with these methods until the follow up visit or 12 days after the last dose of study treatment, whichever is the longer.

If a patient who has been screened and is eligible for the study becomes unavailable to do the study at initially planned dates due to exceptional circumstances, the subject may be re-screened for the study; in total a patient may be screened 2 times. This will only be at the discretion of the Investigator.

4.2.2. All Cohorts Exclusion Criteria

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

1. TSH levels outside the normal range.
2. Subjects with a history of Graves disease.
3. Subjects with a history of any thyroid cancer.
4. Unable or unwilling to avoid use of topical creams/lotions at sites where medication will be applied. Washing with soap and water will be permitted.
5. Based on averaged QTcF values of triplicate ECGs obtained over a brief recording period:
    - QTc(F) > 450 msec; or QTc(F) > 480 msec in subjects with Bundle Branch Block.
6. ALT, alkaline phosphatase and bilirubin ≥ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
7. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones or previous history of uncomplicated cholecystectomy).
8. History of regular alcohol consumption within 6 months of the study defined as:
    - An average weekly intake of >21 units for males or >14 units for females. One unit is equivalent to 8 g of alcohol: a half-pint (~240 ml) of beer, 1 glass (125 ml) of wine or 1 (25 ml) measure of spirits.

9. History of sensitivity to any of the study medications, or components thereof, history of anaphylaxis or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical monitor, contraindicates their participation.
10. Unable to refrain from vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half lives (whichever is longer) prior to the screening visit until the completion of the follow-up assessments, unless in the opinion of the Investigator, in consultation with the GSK Medical monitor if required, the medication will not interfere with the study procedures or compromise subject safety.
11. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening.
12. A positive test for HIV antibody.
13. A positive drug screen at screening or Day -1 (parts A & B only). For Part B patients, a positive drug screen due to concomitant medication may be acceptable for inclusion in the study based on the Investigator's opinion. The Investigator's opinion must be based on interview of the subject, documented in the medical history and the Investigator is asked to document that the positive test can be explained by a relevant and permitted medication (see Section 5.15.1.) and that the medication will not interfere with the study procedures or compromise subject safety. A drug screen will not be performed in Part C.
14. Lactating females.
15. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56 day period.
16. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
17. Exposure to more than four new chemical entities within 12 months prior to the first dosing day
18. Use of topical steroids or calcinurin inhibitors is prohibited during the study from screening to follow up (See Section 5.12).
19. Exclusion related to prior drug treatments:
    a) Intake of oral corticosteroids within 7 days >10mg/day prior to first screening visit.
    b) Use of depot corticosteroids within 7 days prior to first screening visit.
    c) Subjects who are taking anticoagulants (e.g. warfarin) must not be on warfarin within 21 days prior to screening. (See Section 5.15).
    d) Subjects who are having psoralen combined with ultraviolet A (PUVA) treatment must not be using PUVA treatment within 21 days prior to screening.

20. Subjects who work for the Sponsor, Contract Research Organisation (CRO), or one of the study centres.

4.2.2.1. Country Specific Exclusion criteria wording for Germany that applies to Part A, Part B and Part C

1. Subjects who live in detention on court order or on regulatory action, see §40 subsection 1 sentence 3 no. 4 AMG. (Arzneimittelgesetz) [MEDICINAL PRODUCTS ACT, 2015].

4.2.2.2. Additional Exclusion for Part A – Healthy Subjects

2. Use of H1 antihistamines within 3 days prior to first screening visit

4.2.2.3. Additional Exclusion for Part B – Cold urticaria Subjects

1. fT4, T4, T3 outside the normal range.
2. Exclusion related to prior drug treatments:

- Use of zaditen (Ketotifen) within 14 days prior to first screening visit
- Use of doxepin AZU and other tricyclic antidepressants with antihistaminergic properties within 14 days prior to first screening visit
- Use of H2 antihistamines within 7 days prior to first screening visit
- Use of H1 antihistamines within 7 days prior to first screening visit
- Use of monteleukast or any other leukotriene antagonist within 7 days prior to first screening visit
- Use of biologicals including omalizumab within 5 months prior to first screening visit

4.2.2.4. Additional Exclusion for Part C- Chronic Spontaneous Urticaria patients

1. fT4, T4, T3 outside the normal range.
2. Exclusion related to prior drug treatments

- Intake of cyclosporin within 10 days prior to first screening visit
- Intake of other immunosuppressant drugs within 28 days of first screening visit
- Use of monteleukast or any other leukotriene antagonist within 7 days prior to first screening visit
- Use of dapsone within 7 days prior to first screening visit
- Use of zaditen (Ketotifen) within 14 days prior to first screening visit
- Use of doxepin AZU and other tricyclic antidepressants with antihistaminergic properties within 14 days prior to first screening visit
- Use of H2 antihistamines within 7 days prior first screening visit

- Use of biologicals including omalizumab within 5 months prior to first screening visit
- Use of H1 antihistamines above the licensed dose within 3 days prior to first screening visit

4.3. Lifestyle and/or Dietary Restrictions

4.3.1. Contraception Requirements

4.3.1.1. Female Subjects

In Part A: Female subjects of non-childbearing potential will be recruited onto the study.

In Parts B and C: Female subjects of child bearing potential may be recruited onto the study, and must not become pregnant and so must be sexually inactive by abstinence or use contraceptive methods with a failure rate of < 1%. Female subjects of childbearing potential with same sex partners are not required to be abstinent or to use contraception. Subjects in parts B and C will need to be on established contraceptive as listed in Section 4.2.1 inclusion criteria at the time of screening (day -28) and will have regular serum/urine pregnancy testing throughout the duration of the study as listed in the Time and Events Table Section 6.1. Contraceptive use shall be continuous through the pre-study, study and follow-up periods of the study.

Abstinence

Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Contraceptive Methods with a Failure Rate of < 1%

- Combined oral contraceptive
- Injectable progestogen
- Implants of etonogestrel or levonorgestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches
- Intrauterine device (IUD) or intrauterine system (IUS) that meets the <1% failure rate as stated in the product label
- **Documented** male partner sterilization prior to **the female subject's entry** into the study, and this male is the sole partner for that subject. For this definition, "documented" refers to the outcome of the investigator's/designee's review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records.
- Male condom **combined with a female** diaphragm, either with or without a vaginal spermicidea (foam, gel, film, cream, or suppository) (Hatcher, 2007)

NOTE: 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 subjects understand how to properly use these methods of contraception.

4.3.1.2. Male Subjects

Male subjects with female partners of child-bearing potential must use one of the following contraceptive methods after the first dose of study treatment and until the follow-up contact.

- Condom plus partner use of a highly effective contraceptive (see list in Section 4.3.1.1).
- Abstinence, defined as sexual inactivity consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception

4.3.2. Meals and Restrictions

4.3.2.1. Caffeine, Alcohol, and Tobacco

Healthy volunteers (Part A)

- Will abstain from ingesting caffeine- or xanthine-containing products (e.g. coffee, tea, cola drinks, chocolate) and alcohol for 24 hours prior to the start of dosing until collection of the final pharmacokinetic and/or pharmacodynamic sample

Subjects with Cold urticaria (Part B)

- Will abstain from ingesting caffeine- or xanthine-containing products (e.g. coffee, tea, cola drinks, chocolate) and alcohol for 24 hours prior to the start of dosing until discharge from the research unit.

Subjects with Chronic spontaneous urticaria (Part C)

- Will be allowed alcohol (an average weekly intake of <21 units for males or <14 units for females).
- There are no caffeine, or tobacco restrictions for patients with chronic spontaneous urticaria.

4.3.2.2. Fasting

- Subjects will be required to be fasted prior to the screening visit.

4.3.3. Activity and other restrictions during the study

Subjects will abstain from strenuous exercise for 48 hours prior to each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities during studies (e.g., watch television, read).

4.3.3.1. Showering/bathing during the study

In all dose cohorts, subjects will be allowed to shower/bathe approximately 2 hours prior to dosing and approximately 18-22 hours following dosing. During showering/bathing the subject should only use mild, unscented, and non-medicated soap A mild shampoo can be used for washing hair. The site of blood sampling will be protected (taped off and covered) if required, allowing the subjects to shower freely, including washing the application site(s). Subjects should refrain from rubbing the areas where the cream has been applied.

Timings of showering/bathing must be recorded (as detailed in the SPM).

Subjects must refrain from taking part in water based activities such as swimming and use of sauna/steam room for the duration of dosing until the follow-up visit.

4.3.3.2. Clothing during the study

Subjects will be allowed to use their normal clothing for the duration of the dosing period as long as it is not tight fitting. Subjects will not be allowed to put clothing over dosed areas until 60 minutes after application of the cream.

4.4. Screen and Baseline Failures

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

4.4.1. Subjects exceeding screening visit window

Subjects in Part B and Part C that are not randomized within the allotted screening window may be re-screened once, as per Section 4.2.1. If re-screening is performed, it is essential that subjects are assigned a different unique subject ID number for the additional screening attempt. See the SPM for specific details.

4.5. Withdrawal Criteria and Procedures

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.

Refer to Section 5.5 for planned dose adjustment/stopping criteria and Section 5.7 for subject specific dose adaptation/stopping criteria including Liver Chemistry and QTcF.

Liver chemistry threshold stopping criteria have been designed to assure subject safety and to evaluate liver event aetiology (in alignment with the FDA premarketing clinical liver safety guidance). See Section 5.7.1 for details.

4.6. Subject Completion

A completed subject is one who has completed all visits of the study including the follow-up visit.

The end of the study is defined as the last subject's last visit which is a follow up visit.

5. STUDY TREATMENT

5.1. Investigational Product and Other Study Treatment

Table 7 Study treatment and Challenge agent table

	Study Treatment		
Product name:	0.5% GSK2646264	1% GSK2646264	Placebo
Formulation description:	White to off white aqueous cream	White to off white aqueous cream	White to off white aqueous cream
Dosage form:	Topical	Topical	Topical
Unit dose strength(s)/Dosage level(s):	0.5% (w/w)	1% (w/w)	NA
Route/ Administration/ Duration:	Topical; ; SINGLE USE BOTTLES ONLY	Topical; ; SINGLE USE BOTTLES ONLY	Topical; ; SINGLE USE BOTTLES ONLY
Dosing instructions:	Should be applied topically as directed.	Should be applied topically as directed.	Should be applied topically as directed.
Physical description:	White-to-off-white aqueous cream stored in amber glass jars	White-to-off-white aqueous cream stored in amber glass jars	White-to-off-white aqueous cream stored in amber glass jars
Manufacturer/ source of procurement:	Medpharm Guildford	Medpharm Guildford	Medpharm Guildford

Each bottle of cream is for single use only.

	Challenge Agent Treatments						
Product name:	**Dermatophagoides pteronyssinus**	**Timothy Grass Pollen**	**Cat dander**	**Birch pollen**	**Positive Control**	**Negative Control**	**Mixed Grass Pollen**
Formulation description:	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**
Dosage form:	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**
Unit dose strength(s)/Dosage level(s):	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**
Route/ Administration/ Duration:	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**
Dosing instructions:	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**
Physical description:	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**
Manufacturer/ source of procurement:	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**

5.2. Challenge Agents

5.2.1. Skin prick test allergens

A solution of each challenge agent will be applied using the skin prick method as described in The Global Allergy and Asthma European Network (GA2LEN) guidelines (Heinzerling, 2013). This is a widely accepted method used in allergy clinics to test for histamine and allergen sensitivity; causing pruritus, skin flare and a skin wheal. The skin prick method involves placing a drop of histamine or allergen solution onto the skin and using a lancet to gently pierce the superficial layer of the skin. The excess histamine or allergen solution is then wiped away.

The following challenge agents will be used as allergens as part of the skin prick test. Full details are listed in the table in Section 5.1

- **mixed** grass pollen,
- Dermatophagoides pteronyssinus,
- birch pollen,
- cat dander
- **positive control – histamine**
- **negative control – saline**

5.3. Medical Devices

Not applicable.

5.4. Treatment Assignment

Subjects will be assigned to treatment in accordance with the randomization schedule generated by Parexel, prior to the start of the study. Randomisation will occur within each cohort of study. Subjects will be randomised to 1:1 to receive bilateral treatment of GSK2646264 or placebo in Part A. For Parts B & C, subjects will be randomised 3:1 active:placebo respectively, to receive either GSK2646264 or placebo.

5.5. Planned Dose Adjustments/Progression and Stopping criteria

The study will enrol 1 sentinel subject for Part A dosing group 1 and, Part A dosing group 2. Each sentinel subject will be dosed 1 day at least before any other subjects of their dosing group or cohort. Please refer to Section 5.6 and Section 5.7 for dose adjustments and stopping criteria.

This protocol allows some alteration from the currently outlined dosing schedule, but the the maximum topically applied dose at any one time will be 10mg/cm^2 of cream (360mg of free base drug) over 3600 cm^2 (20% BSA) with, based on preclinical modeling, a 6 fold cover compared to the predicted C_{max} at the NOEL (16 ng/mL) and 3.5 fold cover

compared to the predicted AUC at the NOAEL (220 ng.hr/mL), or the dose which will provide this Cmax and this AUC (See Section 3.2.2.).

The GSK review team will be unblinded to the randomisation code and will usually include the GSK pharmacokineticist, GSK statistician, GSK data management, GSK medical monitor, GSK study team leader, GSK Global Clinical Safety and Pharmacovigilance and GSK Clinical Matrix Team leader. Other GSK employees may be included in discussions around dose adjustments and progression if it is deemed necessary and relevant by the above mentioned team members.

The Investigator or designee, study co-ordinators and any other unit staff who have been involved in assessing subjects will remain blinded during the study; however, they will attend the start of each review meeting to update the GSK team on safety and tolerability summary from each cohort; these team members will not be part of the unblinded dose escalation discussions. The investigator and/or designee should send the relevant data to the GSK review team prior to the meeting of the Safety Monitoring Committee (SMC). The study monitor will remain blinded.

The SMC will consist of the GSK review team and the Investigator and/or designee. The SMC will:

- Approve escalation from dosing group 1 to dosing group 2 in Part A (See Section 5.5.3)

- Approve the selection of dosing regimen to be used in Parts B and C (See Section 5.5.3.2)

- Evaluate and confirm potential dose-limiting toxicities

- Pause enrolment and/or request safety-related changes to the trial protocol.

5.5.1. Dose-Limiting Toxicity

Toxicity is defined as dose-limiting toxicity if it meets the following criteria:

- Occurs within 6 hours after any dose for Parts A & B, or within 24 hours after any dose for Part C.

- and is considered by the Investigator to be possibly or probably related to treatment

- and, for AE or laboratory abnormality, is severe

- and, for tolerability,

  - is of Grade 3 or higher for “dermal response scoring (i)”, and
  - is Grade C, F, G, or H according to the “other effects scoring (ii)” (Section 6.3.4.1)

5.5.2. Progression of dosing from sentinel subject to the rest of the dosing group (Part A)

The study design allows for sentinel dosing prior to dose administration to the rest of the cohorts, in part A of the study. Guidance is given below for progression of dosing following dosing of the sentinel subjects, in Part A.

Sentinel subjects in Part A should be under constant supervision after dosing. If dose limiting toxicity develop at any other time than described in the guidance below, the Investigator should contact the sponsor at the earliest convenience on the day when the observation is made.

Table 8 Guidance for progression criteria for sentinel subjects to the rest of dosing group A

Day of Dosing:	Action:	Observation:	Result:
On **Day 1** of the dosing of the sentinel subject, at 6 hours post-dose (immediately prior to the skin prick)	The Investigator will review the available safety data from this subject.	If NO dose limiting toxicity is observed, the skin prick test may be conducted at the Investigator's discretion, according to protocol, and dosing may proceed for the rest of the dosing group. The Investigator is required to document this decision and to inform the sponsor in writing at the earliest convenience. This time should not exceed 24 hours post dose .	If dose limiting toxicity is observed, the PI or designee should immediately contact the medical monitor or designee (on the day they observe issues). GSK will then decide whether it is necessary for the SMC to be convened immediately or not, and will advise on dose progression or not.
On **Day 2** of the dosing of the sentinel subject, and at 6 hours post-dose of Day 1* of the rest of the subjects.	The Investigator reviews the available data of sentinel subject's Day 1 and Day 2 AND available data of 3 subjects at least from the rest of the subjects' Day 1 up to that point.	If NO dose limiting toxicity is observed, the skin prick test may be conducted at the Investigator's discretion, according to protocol, in the rest of the subjects. Dosing may proceed with Day 3 of the sentinel subject, and Day 2 of the rest of the dosing group.	If dose limiting toxicity is observed, the Investigator or designee should immediately contact the medical monitor or designee (on the day they observe issues). GSK will then decide whether it is necessary for the SMC to be convened immediately or not, and will advise on

Day of Dosing:	Action:	Observation:	Result:
		The Investigator is required to document this decision and to inform the sponsor in writing at the earliest convenience.	dose progression or not.
On **Day 3** of the dosing of the sentinel subject, and at 6 hours post-dose of Day 2* of the rest of the subjects.	The Investigator reviews the available data of sentinel subject's Day 2 and Day 3 AND available data of 3 subjects at least from the rest of the subjects' Day 2 up to that point.	If NO dose limiting toxicity is observed, the skin prick test may be conducted at the Investigator's discretion, according to protocol, in the rest of the subjects. Dosing may proceed with Day 3 of the rest of the dosing group. The Investigator is required to document this decision and to inform the sponsor in writing at the earliest convenience.	If dose limiting toxicity is observed, the Investigator or designee should immediately contact the medical monitor or designee (on the day they observe issues). GSK will then decide whether it is necessary for the SMC to be convened immediately or not, and will advise on dose progression or not.

* Assuming the sentinel subject is dosed 1 day before the rest of the subjects.

5.5.3. Progression from dosing group 1 to dosing group 2 in Part A, and from Part A to Parts B and C

5.5.3.1. Progression from dosing group 1 to dosing group 2 in Part A

At the end of dosing group 1 in Part A, the decision to proceed to the next higher strength and dose for dosing group 2 will be made by the SMC based on a safety report from the PI or designee, on the clinical safety, tolerability, up to and including the follow up visit, and pharmacokinetic exposure up to 24 hours after the last dose, in at least 6 subjects at the highest dose level.

These dose adjustments may involve either an increase or a decrease in the planned dose in terms of strength of cream used (0.5% or 1%) and/or extent of body surface area and the decision on whether to dose once or twice daily in Parts B and C.

5.5.3.2. Progression from Part A to Parts B and C

At the end of Part A, the decision to proceed to the dosing of patients in Parts B and C will be made by the SMC based on a safety report from the PI or designee on the clinical safety, tolerability, pharmacokinetic exposure and effect on systemic biomarker (CD69 expression), of all subjects in Part A. A decision will also be made whether the data support progressing with females of child-bearing potential in Parts B and C of the study.

A decision may be made for the dose to take forward to Parts B and C only if data are available from dosing group 1 and dosing group 2 of Part A for at least 6 completed subjects in Group 2. All the other data available for subjects who may not have completed the study should also be presented in the safety report from the PI or designee.

The actual doses to be administered may be adjusted based on safety, tolerability and preliminary pharmacokinetic data at previous dose levels; these dose adjustments may involve either an increase or a decrease in the planned dose.

The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety, pharmacokinetic findings at a given dose level, or to add cohorts to evaluate up to 1% strength and up to 20% BSA. The study procedures for these additional subject(s) or cohort(s) will be the same as that described for other study subjects.

5.5.3.3. Progression in Part B and from Part B to Part C

In Parts B, the first 4 subjects will be randomised so that one of the 4 subjects will receive placebo and the three other subjects will receive GSK2646264. An interim review of the preliminary data from the first 4 subjects, after they have completed the study, will be conducted before dosing the rest of the patients in Part B (n=12 total). Preliminary PK, safety, and tolerability data up to and including the follow-up visit, will be reviewed by the SMC (See Section 5.5). In addition, further modeling of PK parameters after 3 days of dosing in these first 4 cold urticaria patients focusing on the elimination of the compound, will be performed to aid the decision to move forward to the rest of the subjects in Part B and to Part C, taking into account the pre-determined safety margins (See Appendix 6).

5.5.3.4. Progression within Part C

Dosing in Part C will only commence once relevant data from the first four subjects in Part B have been reviewed, (see Section 5.5.3.3) to allow greater confidence in predictions of the pharmacokinetics in Part C. The BSA over which study treatment will be administered in Part C is anticipated to be 10%, but is subject to change if appropriate based on preliminary data from Part B. However, the maximum BSA will not exceed 20%.

The first four subjects of Part C will be randomised so that one of the four subjects will receive placebo and the three other subjects will receive GSK2646264. An informal review of the preliminary data from the first 4 subjects may be conducted after they have completed the study. PK, safety and tolerability data up to and including the follow-up visit, will be reviewed by the Sponsor. If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held. Recruitment of the full planned cohort (n=16) may continue during any data review. The dosing regimen or study population may be adjusted as appropriate after this interim review for the rest of the subjects in Part C..

5.6. Safety Related Study Specific Dose Adjustment Criteria

If AEs which are of severe intensity and are similar across subjects in the group, or if unacceptable pharmacological effects, reasonably attributable in the opinion of the investigator to dosing with GSK2646264, or if dose limiting toxicity (see Section 5.5.1), are observed in 2 or more subjects in dosing group 1 or dosing group 2 of part A, the dose escalation will be stopped. Relevant reporting and discussion with the GSK medical monitor and the SMC will take place. A substantial amendment will be submitted to the Ethics committees and Regulatory authorities for approval prior to any resumption of dosing.

If AEs which are of severe intensity and are similar across subjects in either Part B or C, or if unacceptable pharmacological effects, reasonably attributable in the opinion of the investigator to dosing with GSK2646264, or if dose limiting toxicity (see Section 5.5.1), are observed in 2 or more subjects in either Part B or C, relevant reporting and discussion with the GSK medical monitor and the SMC will take place.

If a Serious Adverse Event (SAE) occurs in 1 subject and is deemed to be drug related, the dose escalation in Part A, or the study if in Part B or Part C,will be stopped. Relevant reporting and discussion with the GSK medical monitor and the SMC will take place. A substantial amendment will be submitted to the Ethics committees and Regulatory authorities for approval prior to any resumption of dosing.

The above criteria will apply regardless of whether pharmacokinetics are less than the above mentioned PK stopping criteria and every effort will be made to take a blood sample at the time of the event for pharmacokinetics analysis in the presence of any of the above events.

5.7. Stopping Criteria for a subject

5.7.1. Liver Chemistry Stopping Criteria

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

Study treatment will be stopped **for a subject** if the following liver chemistry stopping criteria is met:

- ALT ≥ 3xULN

NOTE: Refer to Appendix 1 for details of the required assessments if a subject meets the above criteria.

5.7.2. QTcF Withdrawal Criteria

A subject that meets either criterion below will be withdrawn from the study. The same QT correction formula (e.g QTcF) should be used to determine inclusion and discontinuation for any individual subject throughout the study.

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

If a subject in Part B or Part C has underlying bundle branch block the following withdrawal criteria should be used instead:

Baseline QTcF value (with underlying bundle branch	QTcF withdrawal criteria
<450 msec	>500 msec
450-480 msec	≥530 msec

Withdrawal of subjects is to be based on an average QTcF value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, then obtain 2 more ECGs over a brief period of time and then use the averaged QTcF values of the 3 ECGs to determine whether the subject should be discontinued from the study

5.7.3. Safety related Stopping criteria for an individual subject

A subject may be withdrawn from the study if they present dose limiting toxicity (as defined in Section 5.5.1; also refer to tolerability assessment definition in Section 6.3.4.1.). If dose limiting toxicity is observed in an individual, the Investigator or designee may use their medical judgment and stop dosing the individual subject, and is required to immediately inform the GSK medical monitor or designee. They will then decide whether it is necessary for the SMC to be convened immediately or not, for review of the data and decision on dose adjustment or withdrawal of the individual subject.

A subject may be withdrawn from the study if they present a Serious Adverse Event (SAE), regardless of its severity, and reasonably attributable in the opinion of the investigator to dosing with GSK2646264.

A subject will be withdrawn if they require concomitant medications listed in Section 5.15.

Dosing to an individual may be stopped due to unacceptable local tolerability as evidenced by dose limiting toxicity (as defined in Section 5.5.1).

Tolerability assessments on treated skin areas will be made using the criteria in Section 6.3.4.1.

In the presence of any of the above events, every effort will be made to take a blood sample at the time of the event for pharmacokinetics analysis.

5.8. Dose Adjustment/Stopping Pharmacokinetic Criteria

Available pharmacokinetic, safety and tolerability data for at least 6 subjects in Part A, and 4 subjects in Part B following administration of each dose will be reviewed prior to progressing to the subsequent dose group or cohort in the study as described in Section 5.5.2 and Section 5.5.3. The decision to proceed will be made by the Principal Investigator/ Physician designee together with the Medical monitor following discussion

at the SMC. If deemed necessary at any time, the dose escalation will be temporarily halted and no further subject will be dosed and/or the planned number of doses of study medication received by a subject and/or amount of dose applied may be adjusted as appropriate to be lower or higher than currently proposed.

After the first 4 subjects in Part C, available pharmacokinetic data may be reviewed by the sponsor (see Section 5.5.3.4). If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held.

5.8.1. Adjusting dose/ stopping dose escalation

If (cohort's average) predicted or observed C_{max}or $AUC_{(0\text{-}24\ h\ after\ last\ dose)}$of GSK264624 is greater than 16 ng/mL or 220 ng.hr/mL, respectively, doses for subsequent cohorts may be adjusted based on tox cover and will need to be decided by the SMC but will not exceed agreed margins.

5.9. Blinding

This will be a randomised double blind (sponsor unblinded), single, bilateral (Part A) and parallel (Parts B and C) repeat ascending dose First Time in Human study in healthy subjects, cold urticaria and chronic spontaneous urticaria subjects study.

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 contact the GSK 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 appropriate data collection tool.

A subject will be withdrawn if the subject's treatment code is unblinded by the investigator or treating physician. The primary reason for discontinuation (the event or condition which led to the unblinding) will be recorded in the eCRF.

GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to clinical investigators in accordance with local regulations and/or GSK policy.

5.10. Packaging and Labelling

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

5.11. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for preparation of GSK2646264 is given below. More details will be available for sites to use in the SPM. All bottles are for single use only.

5.11.1. Part A and Part B

Study treatment must be dispensed or administered according to procedures described herein. Only subjects enrolled in the study may receive study treatment. Only authorised site staff may supply or administer study treatment. All study treatment must be stored in a secure area with access limited to the investigator and authorized site staff. Study treatment is to be stored at 2-8°C and protected from light. Maintenance of a temperature log (manual or automated) is required.

5.11.2. Part C

Study treatment must be dispensed or administered according to procedures described herein. Only subjects enrolled in the study may receive study treatment. Only authorised site staff may supply or administer study treatment. . All study treatment must be stored in a secure area with access limited to authorized staff. Study treatment is to be stored at 2-8°C and protected from light. Maintenance of a temperature log (manual or automated) is required.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance. The investigator or the head of the medical institution (where applicable), or designated site staff (e.g., storage manager, where applicable) must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of study treatment received from and returned to GSK and the amount supplied and/or administered to and/or returned by subjects. The required accountability unit for this study will be a bottle. Discrepancies are to be reconciled or resolved. Procedures for final disposition of unused study treatment are listed in the SPM.

Investigational product is not expected to pose significant occupational safety risk to site staff under normal conditions of use and administration. Gloves should be used during the application of this product to the body. 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 study manager. 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 investigational product. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions will be provided to the investigator for the API (GSK2646264).

The formulation used in this study has been developed specifically for assessment in the FTIH and is unlikely to be the final formulation.

5.12. Assessment of Compliance

When subjects are dosed at the study unit, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and start and end times of each dose administration in the clinic will be recorded in the source documents and transcribed into the InForm eCRF. The dose of study treatment and study participant 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.

5.13. Treatment of Study Treatment Overdose

For this study, any dose of GSK2646264 that is above that intended dose is considered an overdose.

GSK does not recommend specific treatment for an overdose. The investigator or physician in charge of the subject at the time will use clinical judgment to treat any overdose.

5.14. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because OR the indication being studied is not life threatening or seriously debilitating and/or other treatment options are available.

The investigator is responsible for ensuring that consideration has been given to the post-study care of the patient's medical condition, whether or not GSK is providing specific post-study treatment.

5.15. Concomitant Medications and Non-Drug Therapies

Concomitant medication must be recorded (as detailed in the SPM).

5.15.1. Permitted Medications

5.15.1.1. Parts A, B and C

Paracetamol, at doses of up to 2 grams/day is permitted for use any time during the study.

5.15.1.2. Part C only

Any non-sedating, short-acting antihistamine at licensed dose is permitted for use as rescue medication any time during the study.

Other concomitant medication may be considered on a case by case basis by the investigator in consultation with the GSK Medical monitor if required.

5.15.2. Prohibited Medications and Non-Drug Therapies

Subjects must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements), within 7 days (or 14 days if the drug is a potential enzyme inducer), or 5 half-lives (whichever is longer or as specified in the inclusion / exclusion Section 4.1), or as indicated for specific medications, from screening until completion of the follow-up visit, unless in the opinion of the Investigator and sponsor if required, the medication will not interfere with the study, in which case the investigator is required to document the rationale for their opinion.

Table 9 List of Prohibited Medications and Non-Drug Therapies

Medication	**Examples**
Use of anticoagulants	e.g. warfarin
Use of systemic immunosupressants/immunomodulators	e.g.ciclosporin A, dapsone, methotrexate, mycophenolate, chloroquine, and comparable drugs
Use of oral, depot, or topical corticosteroids	e.g. prednisolone
Use of topical calcinurin inhibitors	e.g. tacrolimus, pimecrolimus
Use of tricyclic antidepressants with antihistaminergic properties	e.g. doxepin
Use of UV therapy	including PUVA.
Use of Ketotifen,	
Use of H2 antihistamines	e.g. ranitidine, cimetadine
Use of Leukotriene antagonists	e.g. montelukast
Part A and Part B only: Use of H1 antihistamines	e.g. cetirizine, loratadine
Part C Use of H1 antihistamines above the licensed dose	e.g. cetirizine, loratadine

6. STUDY ASSESSMENTS AND PROCEDURES

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 6.1. Whenever vital signs, 12-lead ECGs and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: 12-lead ECG, vital signs, blood draws. The timing of the assessments should allow the blood draw to occur at the exact nominal time.

The timing and number of planned study assessments, including safety, pharmacokinetic, pharmacodynamic/biomarker assessments may be altered during the course of the study based on newly available data (e.g. to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form. No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

Photographs may be taken of some of the procedures, tests, medical conditions or reactions which may occur during the study.

6.1. Time and Events Table

6.1.1. Part A: Healthy Volunteer Time and Event Table Dose group 1

Day:	Screening	-1	1	2	3	4	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7						Day 5 to Day 7	
Admission to Unit		X						
Informed Consent	X							
Demographics	X							
Complete physical	X						X	
Body weight (kg)	X							
Height (cm) without shoes	X							
Brief physical		X						
Medical/medication/drug/ alcohol history	X							
12-lead ECG[1]	X	X				X	X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X	X	X	X	X	X	
Urine drug/alcohol screen	X	X						
HIV, Hep B and Hep C screen	X							
Clinical Laboratory Tests	X	X				X	X	
Allergen Challenge- Skin Prick Test	X[2,3]		X[2,4]		X[2,4]	X[5]		2. SPT- Skin Prick Test analysis will be performed at ~15-20mins post challenge. 3. SPT with all 4 specified allergens 4. SPT using one allergen will be performed 6 hours post dose (see Section 6.4.1) 5. SPT using one allergen will be performed 24 hrs after the last dose
TSH[6]	X							6. TSH test only at screening only
Randomisation		X						

Day:	**Screening**	**-1**	**1**	**2**	**3**	**4**	**Follow-up**	**Notes**
Visit Window (relative to Day 1)	-28 to -7						Day 5 to Day 7	
Tolerability Assessment			X[7] ←----------→					7. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		X	←----------→				X	
Concomitant Medication		X	←----------→				X	
Pharmacokinetic Blood Sample[8]			X	X	X	X[9]		8.PK blood sample taken at pre dose, 1 ,2, 4, 8, 12 and 24hr post dose 9.PK refers to sample 24 hours post dose
Study Treatment Dosing			X	X	X			
Discharge from unit						X		
Outpatient visit	X						X	

6.1.2. Part A: Healthy Volunteer Time and Event Table Dose group 2

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										Day 9 – Day 11	
Admission to Unit		X										
Informed Consent	X											
Demographics	X											
Complete physical	X										X	
Body weight (kg)	X											
Height (cm) without shoes	X											
Brief physical		X										
Medical/medication/drug /alcohol history	X											
12-lead ECG[1]	X	X								X	X	1. Triplicate ECG at screening and CV risk factors
Vital signs	X	X	X	X	X	X	X	X	X	X	X	
Urine drug/alcohol screen	X	X										
Urine for metabolite analysis			X[10]			X[22]						10. Pre dose and pool samples taken 0-12 and 12-24 hours post dose 22. Pool samples taken 0-12 and 12-24 hours post
HIV, Hep B and Hep C screen	X											
Clinical Laboratory Tests	X[17]	X[17]		X[9]		X[9]	X[17]		X[17]		X[17]	9. Pre-dose AST, ALT, Alkaline Phosphatase, Total Bilirubin Only- : results to be communicated to the GSK Medical monitor on the same day 17.Clinical lab test as per Section 6.3.4 of the protocol

Day:	**Screening**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Follow-up**	**Notes**
Visit Window (relative to Day 1)	-28 to -7										Day 9 – Day 11	
Allergen Challenge- Skin Prick Test	X[3,4]		X[3,5]		X[3,5]	X[3,5]		X [3,7]				3. SPT- Skin Prick Test measurements will be performed ~15-20 mins post challenge. 4. SPT with all 4 specified allergens 5. SPT will be performed 6 hours post dose 7. SPT will be performed 48 hours post dose of Day 4 (on the morning of Day 6)
TSH[2]	X											2. TSH test only at screening only
Randomisation		X										
Tolerability Assessment			X[11] ←----------→ only on dosing days 1 to 4									11. Tolerability assessment at pre – and ~6 hours post-dose on dosing days
AE assessment		X	←----------→								X	
Concomitant Medication		X	←----------→								X	
Pharmacokinetic Blood Sample			X[6]	X[6]	X[6]	X[6]	X[18]	X[19]	X[20]	X[21]		6. PK blood sample taken at pre dose, 1, 2, 4, 8, 12 and 24hours post previous dose 18. PK blood sample taken at 30, 36, 48 hours, post dose of Day 4 19. PK blood sample taken at 54, 60, 72 hours, post dose of Day 4 20. PK blood sample taken at 78, 84 hours post dose of Day 4 21. PK blood sample taken at 96 hours post dose of Day 4

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										Day 9 – Day 11	
Blood Sample for CD69 expression			X[12]			X[13]	X[14]		X[15]	X[16]		12. Predose 13. 4 hours post dose of Day 4 14. 24 hours post dose of Day 4 (sample taken in the morning of day 5) 15. 72 hours post dose of Day 4 16. 96 hours post dose of Day 4
Study Treatment Dosing[8]			X	X	X	X						8. This will be once daily dosing using fresh bottles at 1% strength in the morning
Discharge from unit										X		
Outpatient visit	X										X	

6.1.3. Part B: Cold urticaria Subjects Time and Event table

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
Visit Window (relative to Day 1)	-28 to -7 days[1]								1. Screening can occur on any day between 28 days and 7 days prior to Day 1 dosing.
Admission to Unit		X							
Informed Consent	X								
Demographics	X								
Complete physical	X							X	
Body weight (kg)	X								
Height (cm) without shoes	X								
Brief physical		X							
Medical/medication/ drug/alcohol history	X								
12-lead ECG	X[2]	X			X			X	2. Triplicate ECG at screening and CV risk factors
Vital signs[3]	X	X	X	X	X		X	X	3. Should be performed at the same time points as PK samples are taken
Urine drug/alcohol screen	X	X							
Pregnancy Test (women)[4]	X(S)		X(S/U)				X (S)	X(S)	4. Pregnancy test will be performed 3 times during screening, serum on day -28, day -7 to -4, and a serum or urine pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test between day 10-12 (to coincide with a PK sample) and at the follow up visit.
HIV, Hep B and Hep C screen	X								
Clinical Laboratory Tests	X	X			X			X	

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
Allergen Challenge-SPT	X[5,6]		X[5,8]		X[5,7]	X[5]			5. SPT- Skin Prick Test measurements will be performed ~15-20mins post challenge 6. SPT using all 4 specified allergens 7. SPT will be performed at 6 hours post dose (see Section 6.4.1), and at 24 hours post last dose on day 3 8. SPT will be performed pre-dose on Day 1
TSH, free T4, T4, T3	X					x	X[9]	x	9. One sample between day 10-12 to coincide with PK sample
Cold Temp Test (Temp Test 4.0)	X		X10		X[11]	X[12]			10. Pre-dose 11. 6 hours post dose 12. 24 hours post last dose on Day 3
Randomisation		X							
Tolerability Assessment			←------X[13]-----→						13. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		←----------X----------→						X	
Concomitant Medication		X	←----------X----------→					X	
Pharmacokinetic Blood Sample			X[14]	X[14]	X[14]	X[15]	X[16]	X	14. PK blood sample taken at pre dose,1, 4, 8, 12hr post dose 15. One PK sample to be taken 24 hours post last dose on Day 3 16. One PK blood sample per day will be taken as follows:. PK samples will be obtained on the following days (± 1 day): Day 6, 9, 12, & 15.
Study Treatment Dosing			X	X	X				
Discharge from unit						X			
Outpatient visit	X						X	X	
Blood Sample for CD69 expression			X[17]		X[18]		X[19]	X[19]	17. Pre-dose 18. 4 hrs post-dose 19. Sample collection should coincide with PK sample collection.

6.1.4. Part C: Chronic Spontaneous Urticaria Cohort

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Visit Window (relative to Day 1)	-28 to -7[1]												1. Screening can occur on any day between 28 days and 7 days prior to Day 1 dosing.
Informed Consent	X												
Demographics	X												
Complete physical	X											X	
Body weight (kg)	X												
Height (cm) without shoes	X												
Brief physical		X											
Medical/medication/drug/alcohol history	X												
12-lead ECG	X2	X						X				X	2. Triplicate ECG at screening and CV risk factors
Vital signs	X	X			X			X		X[3]	X[3]	X[3]	3. To be performed on same day as PK samples
Urine alcohol screen	X	X											
Pregnancy Test (Women of child bearing potential)4	X(S)[4]	X (U)[5]						X(U)[5]			X (S)	X(S)	4. A serum (S) pregnancy test will be performed on the day of screening. If the day of screening is between Days -13 and -7, a further pregnancy test will not be required until Day 1. However, if the day of screening is between Days -28 and -14, an additional serum pregnancy test will be required between Days -7 and -4.

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
													5. A urine pregnancy test is required prior to dosing on Day 1 & Day 7
HIV, Hep B and Hep C screen	X												
Clinical Laboratory Tests	X	X						X				X	
TSH, free T4, T4, T3	X							X		X6	X6	X6	6. Sample to be taken on same day as PK sample
Blood Sample for CD69 expression		X[7]			X[8]			X[8]		X[9]	X[9]	X[9]	7. Pre-dose 8. 4 hours post dose 9. Sample to be taken on same day as PK sample
Randomisation		X											
UAS-7 Diary	X[10]		X[11]	X[11]	X[11]	X[11]	X[11]	X[11]	X				10. Screening UAS7 diary will be completed for the 7 successive days before Randomisation (Day 1). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 11. The UAS-7 diary should be completed pre-dose on those days where dosing occurs
QOL (DLQI)	X	X[12]							X				12. The baseline DLQI assessment on Day 1, looking back over the previous 7 days prior to dosing, should be completed pre-dose

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
AAS	X[13]		X[14]	X[14]	X[14]	X[14]	X[14]	X[14]	X				13. Screening AAS will be completed for the 7 successive days before Randomisation (Day 1) (on the same days as the UAS7). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 14. The AAS assessment should be completed pre-dose on those days where dosing occurs
AE assessment		←----------X----------→										x	
Tolerability Assessment at pre-dose		X[15]			X[15]			X[15]					15. Tolerability assessment at 1 to 2 hours post-dose
BHR- Basophil Histamine Release Test		X[16]											16. Two samples to be taken pre-dose
Pharmacokinetic Blood Sample		X[17]			X[17]			X[17]		X[18]	X[18]	X[18]	17. PK blood sample taken at pre-dose and 4hr post dose 18. Sample to be taken on same day as CD69 sample, TSH, free T4, T4, T3 samples and vital signs
Study Treatment Dosing		X			X			X					
Con Meds		←----------X----------→										X	
Outpatient visit	X	X			X			X		x	X	X	

6.2. Demographic/Medical History Assessments

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

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 4.2. Cardiovascular medical history/risk factors including height, weight, blood pressure, smoking history, medical conditions will also be assessed at baseline.

6.3. Safety

Planned timepoints for all safety assessments are listed in the Time and Events Table (Section 6.1). Additional time points for safety tests such as vital signs, physical exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring. This would not constitute a protocol amendment.

6.3.1. Physical Exams

- A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities. Height and weight will also be measured and recorded.
- A brief physical examination will include assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

6.3.2. Vital Signs

- Vital sign measurements to be measured in semi-supine position after 10 minutes rest will include systolic and diastolic blood pressure and pulse rate.

6.3.3. Electrocardiogram (ECG)

- Triplicate 12-lead ECGs will be obtained at screening and then during the study single ECGs will be taken. At each time point during the study ECG will be taken using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals. Refer to Section 5.7.2 for QTcF withdrawal criteria and additional QTcF readings that may be necessary.
- A CV risk factors questionnaire will also be completed at screening

6.3.4. Clinical Laboratory Assessments

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed below. Details for the preparation and shipment of samples will be provided by the local laboratory. Reference ranges for all safety parameters will be provided to the site by the laboratory.

If additional non-protocol specified laboratory assessments are performed at the site's local laboratory and result in a change in subject management or are considered clinical significant by the Investigator (for example SAE or AE or dose modification) the results must be captured and sent to GSK along with other study data as defined in Section 7 Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed below:

Haematology

Platelet Count	*RBC Indices*:	*Automated WBC Differential:*
RBC Count	MCV	Neutrophils
WBC Count (absolute)	MCH	Lymphocytes
Hemoglobin	MCHC	Monocytes
Hematocrit		Eosinophils
		Basophils

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Albumin
Glucose, (fasting at screening only)	Calcium	GGT	Total Protein
Sodium	Phosphate	Alkaline phosphatase	
TSH , free T4, T4, T3			

NOTE: Details of Liver Chemistry Stopping Criteria and Follow-Up Procedures are given in Section 5.7.1.

Routine Urinalysis

Specific gravity
pH, glucose, protein, blood and ketones by dipstick
Microscopic examination (if blood or protein is abnormal)

Other screening tests

HIV
Hepatitis B (HBsAg)
Hepatitis C (Hep C antibody -- if second generation Hepatitis C antibody positive, a hepatitis C antibody Chiron RIBA immunoblot assay (or other third generation immunoassay) should be reflexively performed **on the same sample** to confirm the result)
FSH and estradiol (as needed in women of non-child bearing potential only)
Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines).

6.3.4.1. Tolerability

Tolerability will be assessed with the following skin irritation scoring system, where the score consists of a numeric score according to the dermal response scoring i), and a letter according to the other effects scoring ii), as follows:

i) Dermal response scoring:

0 = no evidence of irritation

1 = minimal erythema, barely perceptible (pink)

2 = moderate erythema (definite redness), readily visible; minimal edema or minimal papular response

3 = strong erythema (intense redness), or erythema and papules

4 = definite edema

5 = erythema, edema, and papules

6 = vesicular eruption

7 = strong reaction spreading beyond test site

ii) Other effects:

Z = no other effect

A = slight glazed appearance

B = marked glazing

C = glazing with peeling and cracking

F = glazing with fissures

G = film of dried serous exudate covering all or part of the patch site

H = small petechial erosions and/or scabs

6.4. Study Assessments

6.4.1. Skin Prick Test (SPT)

At screening a solution of each allergen (**mixed** grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander **along with the positive and negative controls)** will be applied to the volar aspect of the subjects forearm using the skin prick method as described in The Global Allergy and Asthma European Network (GA2LEN) guidelines (Heinzerling, 2013). The skin prick method involves placing a drop of histamine or allergen solution onto the skin and using a lancet to gently pierce the superficial layer of the skin. The excess histamine or allergen solution is then wiped away. Study assessment will commence 15-20 mins after the skin has been pricked.

During the dosing period only one allergen positive at screening and the positive control will be used as described in the SPM.

6.4.2. TempTest

The Temptest otherwise known as the cold stimulation test is an electronic testing device consisting of metallic cooling elements that can maintain a precise pre-set temperature from below 5°C to above 40°C. The device will be applied for a short period of time to the forearm or leg of the subject. This allows the determination of whether Cold urticaria is present and the evaluation of the Cold Temperature Test values including critical temperature thresholds.

6.4.3. Blood and Urine Sample Collection

6.4.3.1. PK samples

Blood samples for pharmacokinetic analysis of GSK2646264 will be collected at the time points indicated in Section 6.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

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

6.4.3.2. Systemic biomarker samples

Blood samples to identify the effect of GSK2646264 on anti-IgM stimulated CD69 (and potentially other biomarkers of interest) will be collected at the time points indicated in Section 6.1, Time and Events Table. This assay was used to define the systemic concentration expected to inhibit SYK activity (MABEL) as detailed in Section 3.2. The timing of samples may be altered and/or samples may be obtained at additional time points depending on the PK data obtained.

Blood samples to test for donor basophil histamine release in-vitro will be taken in Part C at the time points indicated in Section 6.1, Time and Events Table.

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

6.4.3.3. Urine Sample Collection

Urine samples will be collected for analysis of any metabolite(s) from subjects in Part A, Group 2 at predose, and dosing Days 1 and 7 Results will be reported under a separate DMPK protocol.

Following review of PK in dose group 1 urine samples were collected for analysis of any metabolite(s) from subjects in Part A, Group 2 at predose, and dosing Days 1 and 4.

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

6.4.4. Sample Analysis

Plasma analysis will be performed under the control of PTS-DMPK/Scinovo, GlaxoSmithKline, the details of which will be included in the Study Procedures Manual. Concentrations of GSK2646264 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SPM).

Once the plasma has been analysed for GSK2646264 any remaining plasma and urine may be analysed for other compound-related metabolites and the results reported under a separate PTS-DMPK, GlaxoSmithKline protocol.

6.5. Pharmacodynamic Measures

6.5.1. Part A & B

The following PD measures will be assessed:

- Longest weal diameter (measured by ruler).
- Perpendicular weal length (measured by ruler).
- Weal area (calculated using ellipse shape).
- Weal volume (measured by quantitative volumetric morphometry)
- Flare erythema (measured by Mexameter).

For Part B only, the following PD measure will be assessed

- Change in critical temperature thresholds..

6.5.2. Part C

6.5.2.1. Urticaria Activity Score (UAS7)

For the chronic spontaneous urticaria subjects in Part C, Urticaria activity score (UAS7), a composite endpoint, will be derived using key urticaria symptoms (number of weal, itch/pruritus):

UAS7 components will be captured using a daily diary for 7 days. The daily UAS7 score will be the sum of each key component score. A detail of UAS7 daily diary is provided in Appendix 3. The days on which to record in the UAS7 diary are captured in the T&E Table (Section 6.1.4). On days where dosing occurs, the UAS7 diary will be completed pre-dose.

6.5.2.2. The Angioedema Activity Score (AAS)

For the chronic spontaneous urticaria subjects in Part C, the Angioedema Activity Score (AAS) will be captured during Screening, and between Day 2 and Day 8. Details of the questionnaire are provided in Appendix 7. The days on which to record the AAS are

captured in the T&E Table (Section 6.1.4). On days where dosing occurs, the AAS will be completed pre-dose.

6.6. Health Outcomes

6.6.1. Dermatology Life Quality Index

For the chronic spontaneous urticaria subjects in Part C, the health outcome measure of Quality of Life will be captured at Screening, Baseline and Day 8, using a 10-item Dermatology Life Quality Index (DLQI) (Finlay, 1994) questionnaire. An overall score will be calculated as well as the following domains: Symptoms and Feelings, Daily Activities, Leisure, Work and School, Personal Relationships, and Treatment. A detail of questionnaire is provided in Appendix 4. The days on which to record the DLQI are captured in the T&E Table (Section 6.1.4). On days where dosing occurs (Day 1 only), it will be completed pre-dose.

7. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS, PREGNANCY AND MEDICAL DEVICE INCIDENTS

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

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

7.1.1. Time period for collecting AE and SAE information

AEs will be collected from the start of study treatment and until the follow-up contact (as detailed in the SPM). 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 CRF.

SAEs will be collected over the same time period as stated above for AEs. However, any SAEs assessed as related to study participation (e.g., 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. All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Appendix 2.

7.1.2. 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?"

7.1.3. Definition of Adverse Events

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 meeting the definition of an AE **include**:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae.).
- When skin tolerability is assessed, a “dermal scoring” of 2 or above, and a score of C, F, G or H in “other effects scoring (ii)”, will be reported as adverse event.

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

- 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.

7.1.4. Definition of Serious Adverse Events

If an event is not an AE per Section 7.1.3, 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).

An SAE is any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

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

c. Requires hospitalization or prolongation of existing hospitalization

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

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

d. Results in disability/incapacity, or

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

e. Is a congenital anomaly/birth defect

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

g. Is associated with liver injury **and** impaired liver function defined as:

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

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

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

- Refer to Appendix 1 for the required liver chemistry follow-up instructions.

7.1.5. Cardiovascular Events

Investigators or designees will be required to fill out event specific data collection tools 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 thrombosis
- Deep Venous Thrombosis
- Revascularization

This information should be recorded within one week of when the AE/SAE(s) are first reported.

7.1.6. Death Events

In addition, all deaths, whether or not they are considered SAEs, will require a specific death data collection tool to be completed. The death data collection tool includes questions regarding cardiovascular (including sudden cardiac death) and non-cardiovascular death.

This information should be recorded within one week of when the death is first reported.

7.1.7. Prompt Reporting of SAEs to GSK

Once the investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to GSK within 24 hours. Any follow-up information on a previously reported SAE will also be reported to GSK within 24 hours.

If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying GSK of the event and completing the

appropriate data collection tool. The investigator will always provide an assessment of causality at the time of the initial report as described in Appendix 2.

7.1.8. Regulatory Reporting Requirements for SAEs

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

GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will comply with country specific regulatory requirements relating to safety reporting to regulatory authorities, IRBs/IECs 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 an SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

7.2. Pregnancy

7.2.1. Time period for collecting pregnancy information

All pregnancies in female subjects and/or female partners of male subjects will be collected after the start of dosing and until follow up.

7.2.2. Action to be taken if pregnancy occurs

The investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of a subject's pregnancy. The subject will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported as such. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Section 7.1.7. 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 and be withdrawn from the study.

7.2.3. Action to be taken if pregnancy occurs in a female partner of a male study subject

The 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 written informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy. The partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

8. DATA MANAGEMENT

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

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

9. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

9.1. Hypotheses and Treatment Comparisons

The primary focus of the statistical analysis is to describe safety and characterize the preliminary pharmacokinetics of GSK2646264 through estimates of parameters and their variability. Thus, an estimation approach will be taken to provide a plausible range of values, where appropriate, with no formal hypothesis testing.

9.1.1. Precision Estimation

This study is designed to estimate the effect of GSK2646264 relative to Placebo on comparison of interest. No formal hypothesis will be tested. Point estimates and corresponding 95% confidence intervals will be constructed.

9.2. Sample Size Considerations & Statistical Analyses

There are no formal hypotheses to be tested. An estimation approach will be taken and 95% confidence intervals will be constructed to provide a range of plausible values for the comparisons of interest.

Approximately 42 subjects will be randomised in total, consisting of 18 subjects in Part A (9 subjects on first dose group and 9 subjects in second dose group), 12 subjects in Part B and 16 subjects in Part C of the study. The sample size is based on feasibility and not driven by any formal hypothesis testing.

9.2.1. Sample Size Sensitivity

For Part A, based on a sample size of 9 randomised subjects, it is estimated that the 95% confidence interval for the percent of inhibition effect will be within 18.5% of the estimate for the weal size assuming standard deviation of 24, within 8.5% of the estimate for the flare size assuming standard deviation of 11 and within 17.7% of the estimate for the weal volume assuming standard deviation of 23 (Schoepke, 2011).

Table 10 Results of the sensitivity analyses for part A

	Percent of Inhibition Weal Size		Percent of Inhibition Flare Size		Percent of Inhibition Weal Volume	
N	Precision around estimate	SD	Precision around estimate	SD	Precision around estimate	SD
9	15.4	20	6.9	9	15.4	20
9	16.9	22	7.7	10	16.1	21
9	18.5	24	8.5	11	17.7	23
9	20.0	26	10	13	19.2	25
9	21.5	28	11.5	15	20.8	27

9.2.2. Sample Size Re-estimation

No sample size re-estimation will be performed.

No sample size re-estimation is currently planned for this study. However, if during the course of the study, new information becomes available about clinically meaningful differences or variability estimates, a blinded sample size re-estimation may be conducted. Full details of the procedure used would be specified in the RAP, and any subsequent change to the target sample size would be documented in a protocol amendment.

9.3. Data Analysis Considerations

9.3.1. Interim Analysis

No formal interim analyses are planned.

9.3.1.1. Summary of safety reporting from Part A

A summary of the safety data from Part A will be performed once all subjects are recruited and completed all visits. This summary will include PK and PD data from Part A of study cohort. All safety data including adverse events, laboratory tests, ECGs and vital signs will be reviewed. The decision to proceed to higher dose strengths will be made by the investigator and the GSK SMC review team based on assessment of safety and pharmacokinetic data of the studied doses.

9.3.1.2. Summary of exposure and safety reporting from Part B: first 4 subjects

The safety, tolerability, and preliminary exposure data from Part B (first 4 subjects) will be reviewed by the SMC once those subjects have completed the study up to and including the follow-up visit. The decision for the dosing regimen and study population for the remainder of Part B, and Part C will be made by the SMC review based on assessment of safety, and pharmacokinetic data of the studied doses.

This study is double blind (sponsor open), where the subject, investigator and site staff will remain blinded to the treatment allocation.

9.3.1.3. Summary of exposure and safety reporting from Part C: first 4 subjects

Preliminary safety and tolerability, and potentially biomarkers of interest), and preliminary exposure data from Part C (first 4 subjects) may be reviewed by the sponsor once those subjects have completed the study up to and including the follow-up visit. The decision for the potential adjustment of the dosing regimen and/or study population for the rest of Part C will be made by the sponsor review based on assessment of safety and pharmacokinetic data of the studied doses. If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held.

Other experts from CPMS and DMPK, at GSK, may be consulted to review blinded placebo/active data (safety, PK and any available PD) during the study for further relevant input. In-stream review of the data may also take place at any time at SRT (Safety Review Team) meetings.

9.3.2. Final Analyses

9.3.2.1. Safety Analyses

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

No formal statistical analysis of the safety data will be conducted. The following safety assessments will be listed and summarized where appropriate: extent of systemic

exposure, local skin reactions, adverse events, clinical laboratory evaluations, concurrent medications, vital signs, ECG interval data, and other relevant safety assessments. Adverse events, local skin reaction assessments, and liver chemistry will be summarized by doses strength (where possible) and BSA.

9.3.2.2. Pharmacokinetic Analyses

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modelling and Simulation Department, CPMS, GlaxoSmithKline and Parexel. Plasma concentration-time data will be recorded in tabular and/or graphical form. From GSK2646264 concentration-time data, the following pharmacokinetic parameters will be determined using the currently approved and validated software, as data permit: maximum plasma concentration (C_{max}); time of maximum plasma concentration (T_{max}); area under the plasma concentration-time curve (AUC) and apparent terminal elimination half-life ($t_{1/2}$), if data permit.

Pharmacokinetic data will be presented in graphical and/or tabular form and descriptively summarized. Pharmacokinetic data will be stored in the Archive, GlaxoSmithKline.

9.3.2.3. Pharmacodynamic/Biomarker Analyses

9.3.2.3.1. Part A & B: Healthy and Cold urticaria Subjects

Raw data and percent of inhibition for each PD parameter (i.e. longest weal diameter, perpendicular weal length, weal area, weal volume, and flare erythema assessments) will be provided by strength, BSA, and day. Descriptive summary statistics (i.e. n, minimum, arithmetic mean, standard error, median, maximum) for both raw and percent of inhibition for Part B will be calculated by dose strength, BSA, and day. Additionally, figures will be produced for PD parameters. No formal statistical testing of the PD data will be conducted.

Descriptive summary statistics for percent of inhibition of systemic CD69 biomarker will be provided by strength, BSA and day.

9.3.2.3.2. Part C: Chronic Spontaneous Urticaria Subjects

UAS7

PD assessment in CsU subjects will be measured using a composite score UAS7.

The UAS7 score will be calculated using the 7 consecutive days sum. A score will be derived for the screening visits and for the post dose visits In the presence of one or more missing daily UAS7 scores, the following algorithm will be applied:

- If a patient has at least 4 non-missing daily UAS7 scores within the 7 days, the UAS7 score will be calculated as the sum of the available eDiary UAS7 scores in that week, divided by the number of days that have a non-missing diary UAS7 score, multiplied by 7.

- If there are less than 4 non-missing daily UAS7 scores, then the UAS7 score will be missing.

Change from baseline in UAS7 will be derived as post dose UAS7 total score minus the Baseline UAS7 total score (screening). The mean and standard deviation of the change from baseline in UAS7 for each treatment group will be presented. The change from baseline in UAS7 will be analysed using the ANCOVA model including treatment, baseline UAS7 and location of treatment. Estimates of the means and treatment difference and associated 95% confidence intervals will be presented. Analysis of each component will be performed similar to UAS7 analysis.

Summary statistics for each component of UAS7 questionaire will be provided by visit.

For CsU Subjects (Part C), descriptive summary statistics for percent of inhibition of CD69 biomarker will be provided by strength, BSA and day if samples are taken in Part C.

More details will be provided in the RAP.

The Angioedema Activity Score

Each AAS item will be scored between 0 to 3 and daily total score AAS score will range between 0 and 15. The daily AAS score will summed to compute a 7-day total scores for screening (baseline) and post dose. Change from baseline, the 7 day total and individual item scores will be calculated. The mean and standard deviation of the change from baseline in total and individual item score for each treatment group will be presented.. The change from baseline will be analysed using the ANCOVA model including treatment, baseline and location of treatment as covariates. Point estimates and 95% confidence intervals will be presented. Missing values will not be imputed. More details will be provided in RAP.

9.3.2.4. Health Outcomes Analyses

For CsU subjects, quality of life will be measured using the Dermatology Life Quality Index (DLQI) questionnaire.

9.3.2.4.1. Dermatology Life Quality Index

A total score and its six individual domains (symptoms and feelings, daily activities, leisure, work and school, personal relationships, and treatment) will be calculated. Change in DLQI score from baseline in total and individual domain scores will be calculated. The mean and standard deviation of the change from baseline in total and individual domain score will be presented. The change from baseline will be analysed using the ANCOVA model including treatment, baseline and location of treatment as covariates.Point Estimates and 95% confidence intervals will be presented. More details on scoring method will be provided in 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 study site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

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

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and, the guiding principles of the **1996** Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favourable opinion/approval to conduct the study and of any subsequent relevant amended documents
- Written informed consent (and any amendments) to be obtained for each subject before participation in the study
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)

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

10.2.1. Urgent Safety Measures

If an event occurs that is related to the conduct of the study or the development of the study treatment, and this new event is likely to affect the safety of subjects, the sponsor and the investigator will take appropriate urgent safety measures to protect subjects against any immediate hazard.

The sponsor will work with the investigator to ensure the IEC/IRB is notified.

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 CRF will serve as the source document.

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

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK procedures.

In addition, 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 this with the investigator or the head of the medical institution (where applicable), including the reasons for taking such action. When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action prior to it taking effect.

If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform investigators or the head of the medical institution (where applicable) and the 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 by someone else, in a safe and secure location. The records

must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and 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 assure that all reproductions are legible and are a true and accurate copy of the originals, 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, or GSK standards/procedures; otherwise, the retention period will default to 15 years.

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 leaves 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 results summary will be posted to the Clinical Study Register no later than eight months after the final primary completion date, the date that the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome. In addition, a manuscript will be submitted to a peer reviewed journal for publication no later than 18 months after the last subject's last visit (LSLV). When manuscript publication in a peer reviewed journal is not feasible, a statement will be added to the register to explain the reason for not publishing.

11. REFERENCES

Center for Drug Evaluation and Research (CDER). December 1999. Skin Irritation *and Sensitization. Testing of Generic Transdermal Drug Products. (http://www.fda.gov/ohrms/dockets/98fr/990236Gd.pdf, accessed 02 Dec 2013)

Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994 May;19(3):210-6.

Greaves MW Chronic Urticaria J allergy Clin Immunol 2000 105: 664-72

GSK2646264 Investigator's Brochure GlaxoSmithKline Document Number 2013N182566_00 15 Apr 2014

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

Heinzerling. The skin prick test – European standards. Clinical and Translational Allergy 2013; 3:3

Maurer M, M.D., Karin Rosén, M.D., Ph.D., Hsin-Ju Hsieh, Ph.D., Sarbjit Saini, M.D., Clive Grattan, M.D., Ana Gimenéz-Arnau, M.D., Ph.D., Sunil Agarwal, M.D., Ramona Doyle, M.D., Janice Canvin, M.D., Allen Kaplan, M.D., and Thomas Casale, M.D.Omalizumab for the Treatment of Chronic Idiopathic or Spontaneous Urticaria N Engl J Med 2013; 368:924-935

Maurer M, MD, Sabine Altrichter, MD, Thomas Bieber, MD, Tilo Biedermann, MD, Matthias Brautigam, PhD,Stefan Seyfried, PhD, Randolf Brehler, MD, J€urgen Grabbe, MD,Nicolas Hunzelmann, MD, Thilo Jakob, MD, Andreas Jung, MD, J€org Kleine-Tebbe, MD, Martin Mempel, MD, Michael Meurer, MD, Kristian Reich, MD, Franziska Ru€eff, MD, Knut Sch€akel, MD, Kaushik Sengupta, BDS, MScD, Christian Sieder, MSc, Jan C. Simon, MD Bettina Wedi, MD,Torsten Zuberbier, MD, Vera Mahler, MD, and Petra Staubach, MD Efficacy and safety of omalizumab in patients with chronicurticaria who exhibit IgE against thyroperoxidase J Allergy Clin Immune 2011a 128(1) 202 -209

Maurer M, Weller, C. Bindslev-Jensen, A. Giménez-Arnau, P. J. Bousquet, J. Bousquet, G. W. Canonica, M. K. Church, K. V. Godse,C. E. H. Grattan, M. W. Greaves, M. Hide, D. Kalogeromitros, A. P. Kaplan, S. S. Saini, X. J. Zhu andT. Zuberbier Unmet clinical needs in chronic spontaneous urticaria. A GA2LEN task force report1 Allergy 2011b 66:317-330

MEDICINAL PRODUCTS ACT (THE DRUG LAW) (ARZNEIMITTELGESETZ – AMG) of the FEDERAL REPUBLIC OF GERMANY. http://www.gesetze-im-internet.de/englisch_amg/. 2015.

Młynek A.,Magerl M.,Siebenhaar F.,Weller K.,Vieira R. Vieira dos Santos, Zuberbier T., Zalewska-Janowska A. and Maurer M. Results and relevance of critical temperature

threshold testing in patients with acquired cold urticaria British Journal of Dermatology 2010, 162: 198-200

Obach RS[1], Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance DJ, Wastall P.The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. J Pharmacol Exp Ther. 1997 Oct;283(1):46-58.

Riccaboni M. Spleen tyrosine kinase: biology, therapeutic targets and drugs. Drug Discovery Today. 2010; 15: 517-530

Saini S, Rosen KE, Hsieh HJ, et al. A randomized, placebo-controlled, dose-ranging study of single-dose omalizumab in patients with H1-antihistamine-refractory chronic idiopathic urticaria. J Allergy Clin Immunol 2011; 128: 567-73.

Schoepke N, Church, MK and Maurer, M; Acta Derm Venereol 2013; 93: 286–293Lukas Kofler,[1] Hanno Ulmer,[2] and Heinz Kofler Histamine 50-Skin-Prick Test: A Tool to Diagnose Histamine Intolerance ISRN Allergy Volume 2011 (2011), Article ID 353045

Siraganian RP. Mast cell signalling: The role of protein tyrosine kinase SYK, its activation and screening methods for new pathway participants.Current Opinion in Immunology 2003, 15:639–646

U.S. Department of Health and Human Services, Food and Drug Administration

Weinblatt M, Kavanaugh, Arthur M.D., Genovese, Mark C. M.D., Musser, Theresa K. B.A.,. Grossbard, Elliott B M.D., and Magilavy, Daniel B. M.D. An oral spleen tyrosine kinase (SYK) inhibitor for rheumatoid arthritis. NEJM 2010; 363: 1303-12

12. APPENDICES

12.1. Appendix 1: Liver Safety Process

The procedures listed below are to be followed if a subject meets the liver chemistry stopping criteria defined in Section 5.7.1:

- Immediately withdraw the subject from study treatment
- Notify the GSK medical monitor within 24 hours of learning of the abnormality to confirm the subject's study treatment cessation and follow-up.
- Complete the "Safety Follow-Up Procedures" listed below.
- Complete the liver event case report forms. If the event also meets the criteria of an SAE (see Section 7.1.4), the SAE data collection tool will be completed separately with the relevant details.
- Upon completion of the safety follow-up withdraw the subject from the study unless further safety follow up is required
- Do not restart investigational product
- Refer to the Flow chart for a visual presentation of the procedures listed below.

Safety Follow-Up Procedures for subjects with ALT ≥ 3xULN:

- Monitor subjects weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

Safety Follow-Up Procedures for subjects with ALT ≥3xULN and total bilirubin ≥2xULN (>35% direct bilirubin); or ALT ≥ 3xULN and INR[1] > 1.5:

- This event is considered an SAE (see Section 7.1.4). Serum bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).
- Make every reasonable attempt to have subjects return to the clinic within 24 hours for repeat liver chemistries, additional testing, and close monitoring (with specialist or hepatology consultation recommended).
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

In addition, for all subjects with ALT ≥ 3xULN, every attempt must be made to also obtain the following:

- Viral hepatitis serology including:

[1] INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants.

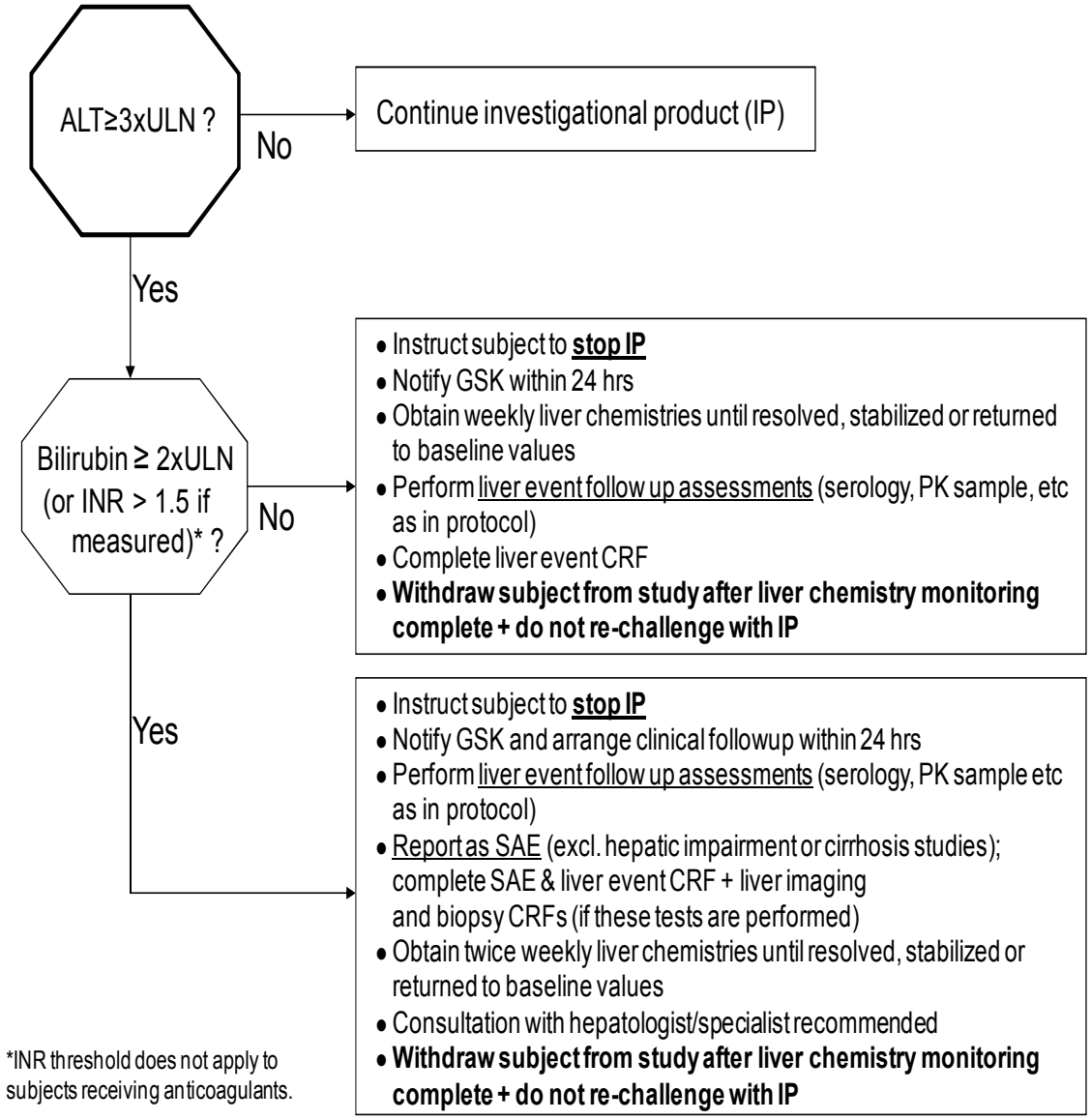
- Hepatitis A IgM antibody.
- Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM).
- Hepatitis C RNA.
- Cytomegalovirus IgM antibody.
- Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
- Hepatitis E IgM antibody.

- Blood sample for pharmacokinetic (PK) analysis, obtained within 24 hours of last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of 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 included in the SPM.
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Fractionate bilirubin, if total bilirubin ≥ 2xULN.
- Assess eosinophilia
- Record the appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia) on the AE CRF.
- Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins on the Concomitant Medications CRF.
- Record alcohol use on the Liver Events CRF.

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

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies.
- Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.
- The Liver Imaging and/or Liver Biopsy CRFs are also to be completed if these tests are performed.

Refer to the diagram below for a visual presentation of the procedures listed above.



12.2. Appendix 2: Procedures for Detection, Evaluation, Follow-Up and Reporting of Adverse Events and Medical Device Incidents

Recording of AEs and SAEs

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 appropriate data collection tool.

It is not acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE data collection tool. However, there may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

Subject-completed health outcomes questionnaires and the collection of AE data are independent components of the study. Responses to each question in the health outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer. The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

Evaluating AEs and SAEs

Assessment of Intensity

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

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities.

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

Assessment of Causality

The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE. A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated. The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment.

For each AE/SAE the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK.** The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals. If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded in the originally completed data collection tool. The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

Reporting of SAEs to GSK

The primary mechanism for reporting SAEs to GSK will be the electronic data collection tool.. If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the GSK Medical monitor. Then the site

will enter the serious adverse event data into the electronic system as soon as it becomes available.

After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data. If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to their GSK protocol contact by telephone.

GSK contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical monitor Contact Information page.

12.3. Appendix 3: Urticaria symptoms questionnaire and activity Score (UAS7)

Final Protocol Amendment 1 - 27 MAY 15
Final - 15 DEC 14

Urticaria Activity Score - 7 (UAS-7) Diary

Confidential

200196

UK/English

☐ **Screening:**	☐ **Dosing Period:**
☐ Day 1	☐ Day 1
☐ Day 2	☐ Day 2
☐ Day 3	☐ Day 3
☐ Day 4	☐ Day 4
☐ Day 5	☐ Day 5
☐ Day 6	☐ Day 6
☐ Day 7	☐ Day 7

UK:ENG (United Kingdom/English)

GlaxoSmithKline CONFIDENTIAL

Final Protocol Amendment 1 - 27 MAY 15
Final - 15 DEC 14

Protocol Identifier	Subject Identifier	
200196		

Date and time of assessment ☐☐ Day ☐☐☐ Month ☐☐ Year ☐☐:☐☐ Hr:Min(00:00-23:59)

URTICARIA ACTIVITY SCORE - 7 (UAS-7) DIARY

		How many wheals/ erythemas have appeared during the last 24 hours?	What average size did these wheals/ erythemas have during the last 24 hours?	What was the size of the largest wheal/ erythema during the last 24 hours?	For how many hours did you have wheals/ erythemas during the last 24 hours?	For how many hours were the longest-lasting wheals/ erythemas present during the last 24 hours?	Were there large confluent areas of wheals during the last 24 hours?
1. **Wheals**	☐ yes →	☐ <20	☐ <1 cm	☐ <1 cm	☐ <3 hrs	☐ <3 hrs	☐ yes
		☐ 20-50	☐ 1-5 cm	☐ 1-5 cm	☐ 3-12 hrs	☐ 3-24 hrs	☐ no
	☐ no	☐ >50	☐ >5 cm	☐ >5 cm	☐ >12 hrs	☐ >24 hrs	
2. **Erythema**	☐ yes →	☐ <20	☐ <1 cm	☐ <1 cm	☐ <3 hrs	☐ <3 hrs	
		☐ 20-50	☐ 1-5 cm	☐ 1-5 cm	☐ 3-12 hrs	☐ 3-24 hrs	
	☐ no	☐ >50	☐ >5 cm	☐ >5 cm	☐ >12 hrs	☐ >24 hrs	

		Please choose the category that best describes your angioedema(s) during the last 24 hours?	How severe were the complaints caused by edema during the last 24 hours?
3. **Angioedema**			
☐ yes →		☐ small / mild	☐ mild
		☐ moderate	☐ moderate
☐ no		☐ large / severe	☐ severe

	How many hours did you experience itch during the last 24 hours?	How severe was the itch during the last 24 hours?
4. **Itch/ Pruritus**		
☐ yes →	☐ <3 hrs	☐ mild
	☐ 3-12 hrs	☐ moderate
☐ no	☐ >12 hrs	☐ severe

UK:ENG (United Kingdom/English)

Protocol Identifier	Subject Identifier	
200196		

Date and time of assessment ☐☐ ☐☐☐ ☐☐ ☐☐:☐☐
Day Month Year Hr:Min(00:00-23:59)

URTICARIA ACTIVITY SCORE - 7 (UAS-7) DIARY (Continued)

5. **Please rate the overall severity of your urticaria complaints:**

☐ no complaints ☐ mild complaints ☐ moderate complaints ☐ severe complaints

6. **Intake of antihistamines:**

☐ no
☐ yes, because of >50 wheals and severe pruritus
☐ yes, because of angioedema
☐ yes, because of other reasons: _____

drug (name, dosage in mg), number of tablets, time of day:

_____

_____

7. **Please note possible triggers of your urticaria symptoms:**

☐ Food
☐ Stress
☐ Exercise
☐ Drugs (NSAIDs)
☐ Other, specify _____ _____

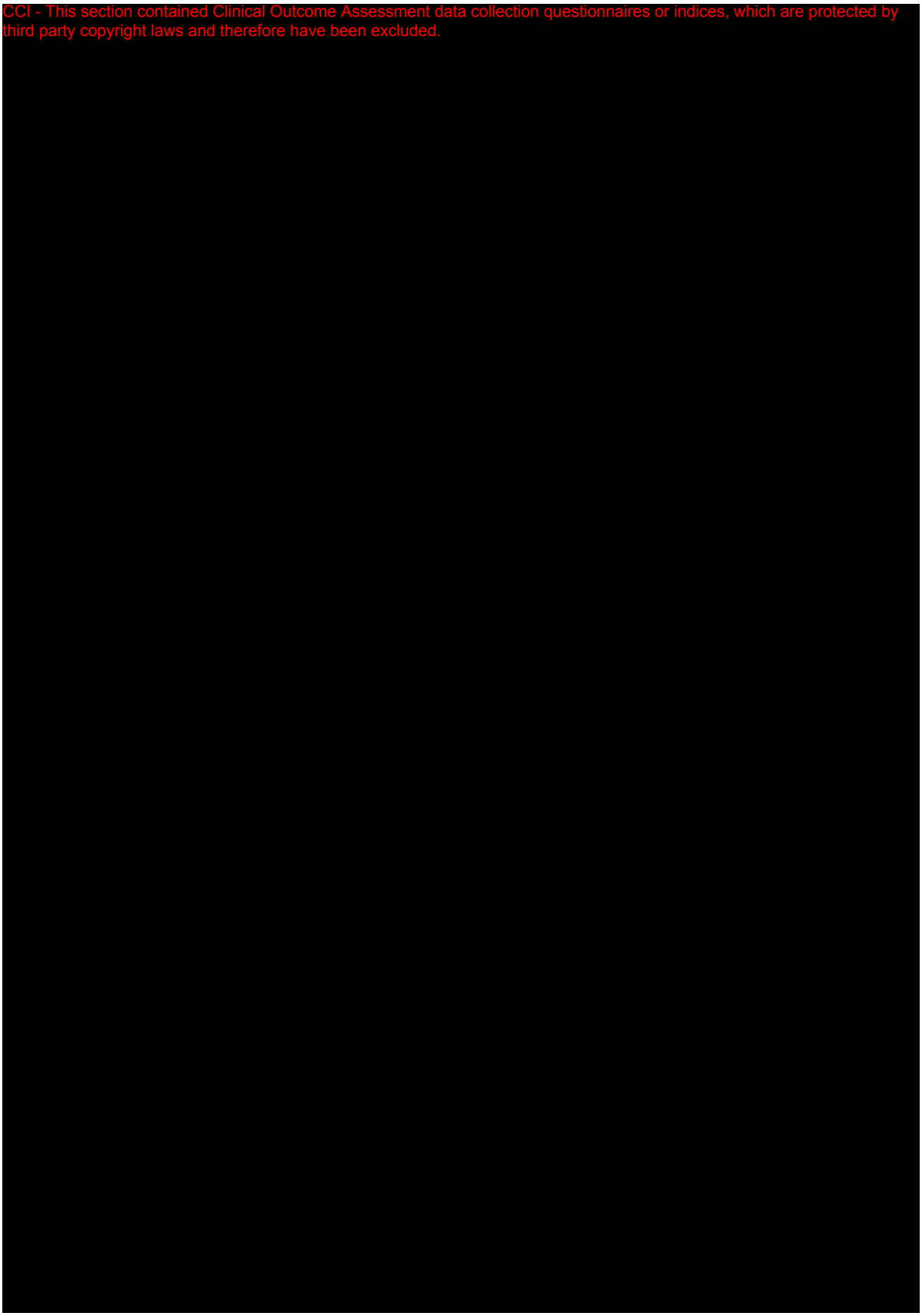
_____

_____

UK:ENG (United Kingdom/English)

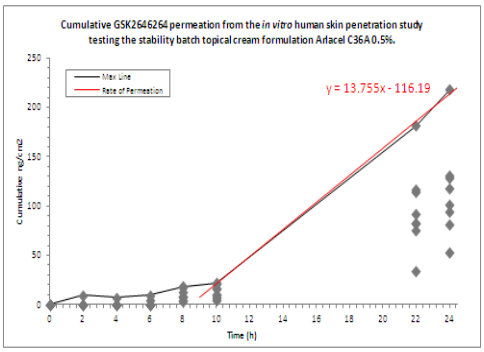
12.4. Appendix 4: Dermatology Life Quality Index (DLQI)

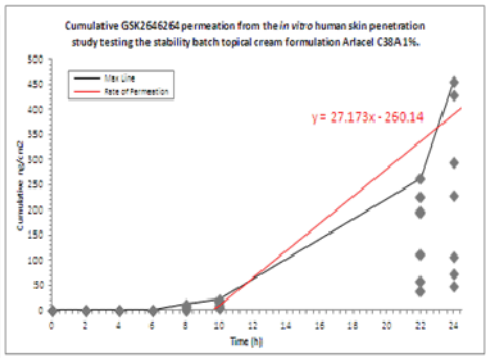
CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.

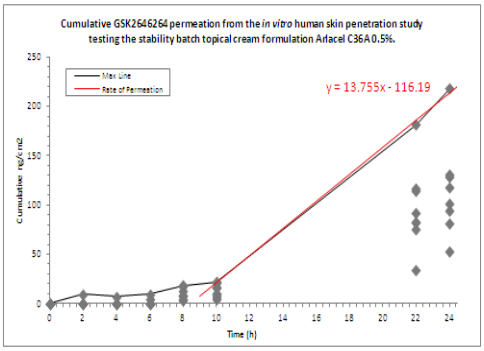


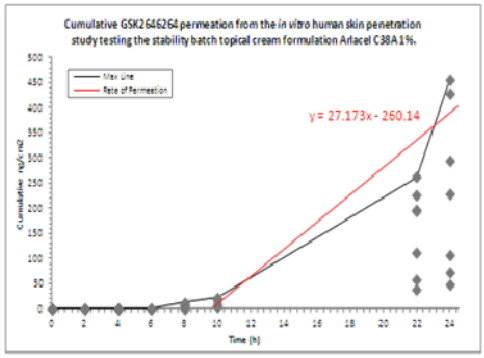
12.5. Appendix 5: Calculation of predicted human exposure

Dermal concentrations achieved:









The GSK2646264 dermal recovery data generated by Medpharm (Report No. 007-1311-01R) was taken and converted into a dermal concentration assuming the following skin section dimensions; 0.6 cm^2 surface area, 0.03 cm depth equating to a volume of 0.018 cm^3 (1 mL equivalent to 1 cm^3). Dermal concentration was then converted to µM and the mean concentration calculated from the replicate experiments from 3 donors.

Arlacel Cream Formulation Strength (%)	24 h Dermal Concentration (µM)
1	63
0.5	49

Estimation of the maximum penetration rates:

The cumulative receiving fluid concentration data generated by Medpharm (Report No. 007-1311-01R) was taken and plotted as individual values on a graph of cumulative concentration (ng/mL) against time (h). The maximum observed concentration at each

time point was then taken as the maximum observed penetration and the rate of penetration equates to the slope of this line (See examples and table below).

Arlacel Cream Formulation Strength (%)	**Max observed penetration rate (ng/cm^2/h)**
1	27.17
0.5	13.76

Calculation of Predicted human exposure

The rate of penetration values are in effect the dose of GSK2646264 which reaches the systemic circulation per cm^2 per hour. We assumed that the rate of penetration remains constant over a 24 hour period and thereby acts like a constant infusion into the blood.

The dose in 24 h is therefore reliant on the surface area covered by the cream formulation. We have assumed 10% body surface area coverage on a 70 kg human (1.8 m^2) which is therefore 1800 cm^2.

Dose in 24 h = Penetration Rate * 24 * 1800

The flux determined for the creams and the predicted human PK parameters (CLb: 13 mL/min/kg, Vss: 0.8 L/kg) were used to predict the Cmax and $AUC_{0\text{-}24h}$ when applying the creams to the different BSA in the clinical study. Predictions were performed in WinNonlin (Phoenix v6.3.0.395) using an IV infusion model.

The derived Cmax and AUC were converted to plasma using the human blood:plasma ratio of 0.7 and also displayed corrected for fraction unbound using a human Fup of 1.05%. The calculated values are displayed below and in Section 3.2.2.1 of the protocol.

Formulation strength	BSA %	Plasma Cmax (ng/mL)	Plasma AUC (ng.h/mL)
Arlacel - 0.5%	0.2	0.01	0.31
	1	0.06	1.56
	5	**0.32**	**7.79**
	10	0.64	15.57
	20	1.29	31.14
Arlacel - 1%	0.2	0.03	0.61
	1	0.13	3.07
	5	0.64	15.36
	10	1.29	30.71
	20	2.57	61.43
Fraction unbound corrected			
Arlacel - 0.5%	0.2	0.000	0.00
	1	0.001	0.02
	5	0.003	0.08
	10	0.007	0.16
	20	0.013	0.33
Arlacel - 1%	0.2	0.000	0.01
	1	0.001	0.03
	5	0.007	0.16
	10	0.013	0.32
	20	0.027	0.64

Formulation strength	BSA %	Plasma Cmax (ng/mL)	Plasma AUC (ng.h/mL)
Arlacel - 0.5%	0.2	0.01	0.31
	1	0.06	1.56
	5	**0.32**	**7.79**
	10	0.64	15.57
	20	1.29	31.14
Arlacel - 1%	0.2	0.03	0.61
	1	0.13	3.07
	5	0.64	15.36
	10	1.29	30.71
	20	2.57	61.43
Fraction unbound corrected			
Arlacel - 0.5%	0.2	0.000	0.00
	1	0.001	0.02
	5	0.003	0.08
	10	0.007	0.16
	20	0.013	0.33
Arlacel - 1%	0.2	0.000	0.01
	1	0.001	0.03
	5	0.007	0.16
	10	0.013	0.32
	20	0.027	0.64

12.6. Appendix 6: Comparison Of Predicted Human Plasma Exposures To Animal Safety Studies

	MABEL	**Human WB CD69 IC_{20}: 1150 ng/mL**	**Exposure with 1% cream (20% BSA) expected 447- fold lower than the IC_{20}.**
Human predicted PK parameters	Clearance (*in vivo* CLb)	13 mL/min/kg (60% LBF)	IVIVE
	Volume (Vss in human)	0.8 L/kg	Single species scaling. Rat Vss corrected for species Fub diff. (Obach. 1997)
	Half-life	0.7h	Estimated from CLb and Vss

	Cmax (ng/mL)	**AUC (ng.h/mL)**	**Dose (mg/cm^2) [mg of free drug]**	**%BSA [cm^2]**	**Predicted margin to NOEL Cmax (thyroid finding)**	**Predicted margin to NOAEL AUC $_{(0-t)}$ 3% dermal**	**Predicted margin MABEL IC_{20}**	**Predicted margin to Cmax NOAEL (EFD findings)**	**Predicted margin to AUC NOAEL (EFD findings)**
Rat (0.5% strength)	*1.4 - 6.6*	*15.3 - 57*	*20-44* *[4.1-9.0]*	*10%*	*NA*	*NA*	*NA*	*NA*	*NA*
Pig (0.5% strength)	*1.9 - 47*	*20 - 118*	*43* *[8.7]*	*10%*	*NA*	*NA*	*NA*	*NA*	*NA*
Human Starting dose 0.5% strength (predicted)	0.01	0.31	10 [1.8]	0.2% [36]	**1600**	**700**	**115,000**	**16,700**	**719**
Human Highest dose group 1 and CU 0.5% strength (predicted)	0.32	7.8	10 [45]	5% [900]	**50**	**30**	**3600**	**521**	**28.5**

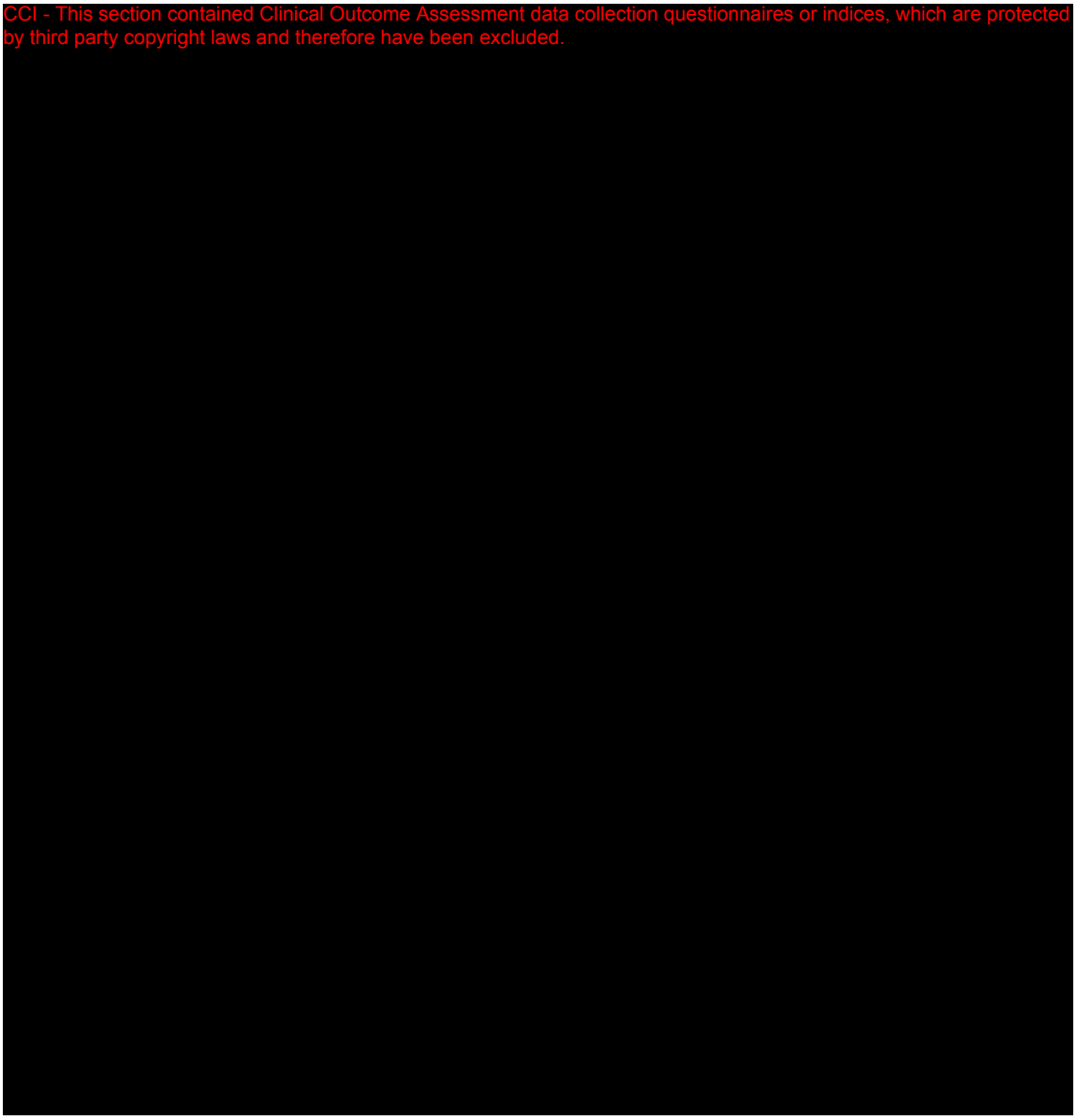
	Cmax (ng/mL)	AUC (ng.h/mL)	Dose (mg/cm^2) [mg of free drug]	%BSA [cm^2]	Predicted margin to NOEL Cmax (thyroid finding)	Predicted margin to NOAEL AUC $_{(0\text{-}t)}$ 3% dermal	Predicted margin MABEL IC_{20}	Predicted margin to Cmax NOAEL (EFD findings)	Predicted margin to AUC NOAEL (EFD findings)	
Human Highest Dose Group 2 and CsU 1% strength (predicted)	2.57	61.4	10 [360]	20% [3600]	**6**	**3.5**	**447**	**65**	**3.6**	
Human Highest Dose Group 2 and CsU 1% strength dib (predicted)	2.57	61.4	10 [720]	20% [3600]	**3**	**1.75**	**224**	**32.5**	**1.8**	
Systemic levels in human predicted using the maximum observed penetration rates for each cream strength (0.5 and 1%) and predicted human PK parameters. (IVIVE: *in vitro-in vivo* extrapolation).										
Application of the 1% and 0.5% creams (10 mg/cm^2) are expected to produce dermal concentrations above the IC90 as determined by the *in vitro* skin penetration and *ex vivo* histamine release assays.										

12.7. Appendix 7: Angioedema Activity Score

AAS

(Angioedema Activity Score)

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



12.8. Appendix 8: Protocol Amendment Changes

AMENDMENT 1

Summary of Amendment Changes with Rationale

Changes have been made to safety related study specific dose adjustment criteria following MHRA review.

List of Specific Changes

Section 5.6 Safety Related Study Specific Dose Adjustment Criteria

PREVIOUS TEXT

If AEs which are of severe intensity and are similar across subjects in the group, or if unacceptable pharmacological effects, reasonably attributable in the opinion of the investigator to dosing with GSK2646264, or if dose limiting toxicity (see Section 5.5.1), are observed in 2 or more subjects in dosing group 1 or dosing group 2, the dose escalation will be temporarily halted and no further subject will be dosed until a full safety review of the study has taken place. Relevant reporting and discussion with the GSK medical monitor and the SMC, and with the Ethics Committee and regulatory authorities according to local regulations and processes, will then take place prior to any resumption of dosing.

If a similar Serious Adverse Event (SAE) occurs in 2 or more subjects and is deemed to be drug related, the dose escalation will be temporarily halted and no further subject will be dosed until a full safety review of the data has taken place. Relevant reporting and discussion with the GSK medical monitor and SMC, relevant GSK personnel, and with the Ethics Committee and regulatory authorities according to local regulations and processes, will then take place prior to any resumption of dosing.

The above criteria will apply regardless of whether pharmacokinetics are less than the above mentioned PK stopping criteria and every effort will be made to take a blood sample at the time of the event for pharmacokinetics analysis in the presence of any of the above events.

REVISED TEXT

If AEs which are of severe intensity and are similar across subjects in the group, or if unacceptable pharmacological effects, reasonably attributable in the opinion of the investigator to dosing with GSK2646264, or if dose limiting toxicity (see Section 5.5.1), **are observed in 2 or more subjects in dosing group 1 or dosing group 2, the dose escalation will be stopped. Relevant reporting and discussion with the GSK medical monitor and the SMC will take place. A substantial amendment will be submitted to the Ethics committees and Regulatory authorities for approval prior to any resumption of dosing.**

If a Serious Adverse Event (SAE) occurs in 1 subject and is deemed to be drug related, the dose escalation will be stopped. Relevant reporting and discussion with the GSK medical monitor and the SMC will take place. A substantial amendment will be submitted to the Ethics committees and Regulatory authorities for approval prior to any resumption of dosing.

The above criteria will apply regardless of whether pharmacokinetics are less than the above mentioned PK stopping criteria and every effort will be made to take a blood sample at the time of the event for pharmacokinetics analysis in the presence of any of the above events.

Summary of Amendment Changes with Rationale

An update has been made to the exclusion criteria of the protocol Section 4.2.2. and a change has been made to Section 10.2 Regulatory and Ethical considerations following German (Berlin) Ethics Committee review.

List of Specific Changes

Section 4.2.2.

PREVIOUS TEXT

4.2.2 All Cohorts Exclusion Criteria

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

1. TSH levels outside normal range.
2. Subjects with a history of Graves disease.
3. Subjects with a history of any thyroid cancer.
4. Unable or unwilling to avoid use of topical creams/lotions at sites where medication will be applied. Washing with soap and water will be permitted.
5. Based on averaged QTcF values of triplicate ECGs obtained over a brief recording period:
    - QTc(F) > 450 msec; or QTc(F) > 480 msec in subjects with Bundle Branch Block.
6. ALT, alkaline phosphatase and bilirubin ≥ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
7. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) or previous history of uncomplicated cholecystectomy.
8. History of regular alcohol consumption within 6 months of the study defined as:

- An average weekly intake of >21 units for males or >14 units for females. One unit is equivalent to 8 g of alcohol: a half-pint (~240 ml) of beer, 1 glass (125 ml) of wine or 1 (25 ml) measure of spirits.

9. History of sensitivity to any of the study medications, or components thereof, history of anaphylaxis or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical monitor, contraindicates their participation.
10. Unable to refrain from vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half lives (whichever is longer) prior to the screening visit until the completion of the follow-up assessments, unless in the opinion of the Investigator, in consultation with the GSK Medical monitor if required, the medication will not interfere with the study procedures or compromise subject safety.
11. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening.
12. A positive test for HIV antibody.
13. Lactating females.
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56 day period.
15. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
16. Exposure to more than four new chemical entities within 12 months prior to the first dosing day
17. Use of topical steroids or calcinurin inhibitors are prohibited during the study from screening to follow up (See Section 5.12).
18. Exclusion related to prior drug treatments:
    a) Intake of oral corticosteroids within 7 days >10mg/day prior to first screening visit.
    b) Use of depot corticosteroids within 7 days prior to first screening visit.
    c) Subjects who are taking anticoagulants (e.g. warfarin) must not be on warfarin within 21 days prior to screening. (See Section 5.12).
    d) Subjects who are having psoralen combined with ultraviolet A (PUVA) treatment must not be using PUVA treatment within 21 days prior to screening.

4.2.2.1 Additional Exclusion for Part A – Healthy Subjects

1. Use of H1 antihistamines within 3 days prior to first screening visit

4.2.2.2 Additional Exclusion for Part B – Cold urticaria Subjects

1. Exclusion related to prior drug treatments:

- Use of Zaditen (Ketotifen) within 14 days prior to first screening visit
- Use of Doxepin AZU and other tricyclic antidepressants with antihistaminergic properties within 14 days prior to first screening visit
- Use of H2 antihistamines within 7 days prior to first screening visit
- Use of H1 antihistamines within 7 days prior to first screening visit
- Use of monteleukast or any other leukotriene antagonist within 7 days prior to first screening visit
- Use of biologicals including omalizumab within 5 months prior to first screening visit

4.2.2.3 Additional Exclusion for Part C- Chronic Spontaneous Urticaria patients

3. Exclusion related to prior drug treatments

- Intake of cyclosporin within 10 days prior to first screening visit
- Intake of other immunosuppressant drugs within 28 days of first screening visit
- Use of monteleukast or any other leukotriene antagonist within 7 days prior to first screening visit
- Use of Dapsone within 7 days prior to first screening visit
- Use of Zaditen (Ketotifen) within 14 days prior to first screening visit
- Use of Doxepin AZU and other tricyclic antidepressants with antihistaminergic properties within 14 days prior to first screening visit
- Use of H2 antihistamines within 7 days prior first screening visit
- Use of biologicals including omalizumab within 5 months prior to first screening visit
- Use of H1 antihistamines above the licensed dose within 3 days prior to first screening visit

REVISED TEXT

4.2.2 All Cohorts Exclusion Criteria

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

1. TSH levels outside normal range.
2. Subjects with a history of Graves disease.
3. Subjects with a history of any thyroid cancer.
4. Unable or unwilling to avoid use of topical creams/lotions at sites where medication will be applied. Washing with soap and water will be permitted.

5. Based on averaged QTcF values of triplicate ECGs obtained over a brief recording period:

   - QTc(F) > 450 msec; or QTc(F) > 480 msec in subjects with Bundle Branch Block.

6. ALT, alkaline phosphatase and bilirubin ≥ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
7. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) or previous history of uncomplicated cholecystectomy.
8. History of regular alcohol consumption within 6 months of the study defined as:

   - An average weekly intake of >21 units for males or >14 units for females. One unit is equivalent to 8 g of alcohol: a half-pint (~240 ml) of beer, 1 glass (125 ml) of wine or 1 (25 ml) measure of spirits.

9. History of sensitivity to any of the study medications, or components thereof, history of anaphylaxis or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical monitor, contraindicates their participation.
10. Unable to refrain from vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half lives (whichever is longer) prior to the screening visit until the completion of the follow-up assessments, unless in the opinion of the Investigator, in consultation with the GSK Medical monitor if required, the medication will not interfere with the study procedures or compromise subject safety.
11. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening.
12. A positive test for HIV antibody.
13. Lactating females.
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56 day period.
15. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
16. Exposure to more than four new chemical entities within 12 months prior to the first dosing day
17. Use of topical steroids or calcinurin inhibitors are prohibited during the study from screening to follow up (See Section 5.12).
18. Exclusion related to prior drug treatments:

    a) Intake of oral corticosteroids within 7 days >10mg/day prior to first screening visit.

b) Use of depot corticosteroids within 7 days prior to first screening visit.

c) Subjects who are taking anticoagulants (e.g. warfarin) must not be on warfarin within 21 days prior to screening. (See Section 5.12).

d) Subjects who are having psoralen combined with ultraviolet A (PUVA) treatment must not be using PUVA treatment within 21 days prior to screening.

19. <u>Subjects who work for the Sponsor, CRO, or one of the study centres.</u>

4.2.2.1.1. Country Specific Exclusion criteria wording for Germany that applies to Part A, Part B and Part C

2. Subjects who live in detention on court order or on regulatory action, see §40 subsection 1 sentence 3 no. 4 AMG. (Arzneimittelgesetz).

List of Specific Changes

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

PREVIOUS TEXT

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

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

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and, the guiding principles of the 2008 Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval to conduct the study and of any subsequent relevant amended documents
- Written informed consent (and any amendments) to be obtained for each subject before participation in the study
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)

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

REVISED TEXT

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

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

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and, the guiding principles of the **1996** Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval to conduct the study and of any subsequent relevant amended documents
- Written informed consent (and any amendments) to be obtained for each subject before participation in the study
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)

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

AMENDMENT 2

Summary of Amendment Changes with Rationale

Changes have been made to list all of the challenge agents to be used in the study following guidance from German Regulatory Agency (BfArM).

A table of the list of challenge agents has been added to the study treatment section. An update to the skin prick test procedure and the correct name of one of the challenge agents has been added to the inclusion criteria.

List of Specific Changes

Section 4.2.1.2 Inclusion criteria specific to healthy subjects (Part A) – Criteria number 4.

PREVIOUS TEXT

> **4.** Demonstration of a positive weal and flare reaction (≥ 3mm in diameter relative to negative control) to at least one allergen from a battery of allergens (grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander) on skin prick testing at screening.

REVISED TEXT

> **4.** Demonstration of a positive weal and flare reaction (≥ 3mm in diameter relative to negative control) to at least one allergen from a battery of allergens **(Mixed** grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander) on skin prick testing at screening.

List of Specific Changes

Section 4.2.1.3 Additional Inclusion criteria specific for subjects with cold urticaria (Part B) – Criteria number 6.

PREVIOUS TEXT

6. In addition, the following criterion will apply to a minimum of 4 patients in Part B:
    a) Demonstration of a positive weal and flare reaction (≥3 mm relative to negative control) to at least one allergen from a battery of allergens (grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander) on skin prick testing at screening,

REVISED TEXT

6. In addition, the following criterion will apply to a minimum of 4 patients in Part B:
    a) Demonstration of a positive weal and flare reaction (≥3 mm relative to negative control) to at least one allergen from a battery of allergens (**Mixed** grass pollen, Dermatophagoides pteronyssinus, birch pollen and cat dander) on skin prick testing at screening,

List of Specific Changes

Section 5.1 Investigational Product and Other Study Treatment

PREVIOUS TEXT

5.1 Investigational Product and Other Study Treatment

Table 6 Study treatment table

	Study Treatment		
Product name:	0.5% GSK2646264	1% GSK2646264	Placebo
Formulation description:	White to off white aqueous cream	White to off white aqueous cream	White to off white aqueous cream
Dosage form:	Topical	Topical	Topical
Unit dose strength(s)/Dosage level(s):	0.5% (w/w)	1% (w/w)	NA
Route/ Administration/ Duration:	Topical	Topical	Topical
Dosing instructions:	Should be applied topically as directed	Should be applied topically as directed	Should be applied topically as directed
Physical description:	White-to-off-white aqueous cream stored in amber glass jars	White-to-off-white aqueous cream stored in amber glass jars	White-to-off-white aqueous cream stored in amber glass jars
Manufacturer/ source of procurement:	Medpharm Guildford	Medpharm Guildford	Medpharm Guildford

REVISED TEXT

Table 6 Study treatment table

	Study Treatment		
Product name:	0.5% GSK2646264	1% GSK2646264	Placebo
Formulation description:	White to off white aqueous cream	White to off white aqueous cream	White to off white aqueous cream
Dosage form:	Topical	Topical	Topical
Unit dose strength(s)/Dosage level(s):	0.5% (w/w)	1% (w/w)	NA
Route/ Administration/ Duration:	Topical	Topical	Topical
Dosing instructions:	Should be applied topically as directed	Should be applied topically as directed	Should be applied topically as directed
Physical description:	White-to-off-white aqueous cream stored in amber glass jars	White-to-off-white aqueous cream stored in amber glass jars	White-to-off-white aqueous cream stored in amber glass jars
Manufacturer/ source of procurement:	Medpharm Guildford	Medpharm Guildford	Medpharm Guildford

	Challenge Agent Treatments						
Product name:	**Dermatophagoides pteronyssinus**	**Timothy Grass Pollen**	**Cat dander**	**Birch pollen**	**Positive Control**	**Negative Control**	**Mixed Grass Pollen**
Formulation description:	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**	**Solution for skin prick test**
Dosage form:	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**
Unit dose strength(s)/Dosage level(s):	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**	**0.003 microlitres**
Route/ Administration/ Duration:	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**	**Cutaneous**
Dosing instructions:	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**	**Superficial layer of skin is pierced with an ALK lancet perpendicular to the skin.**
Physical description:	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**	**Solution for skin prick testing**
Manufacturer/ source of procurement:	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**	**ALK-Abello A/S**

List of Specific Changes

Section 5.2.1 Skin prick test allergens

PREVIOUS TEXT

5.2.1 Skin prick test allergens

A solution of each challenge agent will be applied using the skin prick method as described in The Global Allergy and Asthma European Network (GA2LEN) guidelines (Heinzerling, 2013). This is a widely accepted method used in allergy clinics to test for histamine and allergen sensitivity; causing pruritus, skin flare and a skin wheal. The skin prick method involves placing a drop of histamine or allergen solution onto the skin and using a lancet to gently pierce the superficial layer of the skin. The excess histamine or allergen solution is then wiped away.

- histamine
- grass pollen,
- Dermatophagoides pteronyssinus,
- birch pollen,
- cat dander

REVISED TEXT

5.2.1 Skin prick test allergens

A solution of each challenge agent will be applied using the skin prick method as described in The Global Allergy and Asthma European Network (GA2LEN) guidelines (Heinzerling, 2013). This is a widely accepted method used in allergy clinics to test for histamine and allergen sensitivity; causing pruritus, skin flare and a skin wheal. The skin prick method involves placing a drop of histamine or allergen solution onto the skin and using a lancet to gently pierce the superficial layer of the skin. The excess histamine or allergen solution is then wiped away.

The following challenge agents will be used as allergens as part of the skin prick test. Full details are listed in the table in Section 5.1

- **mixed** grass pollen**,**
- dDermatophagoides pteronyssinus,
- birch pollen,
- cat dander
- **positive control – histamine**
- **negative control - saline**

List of Specific Changes

Section 6.4.1 Skin Prick Test (SPT)

PREVIOUS TEXT

6.4.1 Skin Prick Test (SPT)

At screening a solution of each allergen (mixed grass pollen, dDermatophagoides pteronyssinus, birch pollen and cat dander) will be applied to the volar aspect of the subjects forearm using the skin prick method as described in The Global Allergy and Asthma European Network (GA2LEN) guidelines (Heinzerling, 2013). The skin prick method involves placing a drop of histamine or allergen solution onto the skin and using a lancet to gently pierce the superficial layer of the skin. The excess histamine or allergen solution is then wiped away. Study assessment will commence 15-20 mins after the skin has been pricked.

REVISED TEXT

6.4.1 Skin Prick Test (SPT)

At screening a solution of each allergen (**mixed** grass pollen**,** dDermatophagoides pteronyssinus, birch pollen and cat dander **along with the positive and negative controls)** will be applied to the volar aspect of the subjects forearm using the skin prick method as described in The Global Allergy and Asthma European Network (GA2LEN) guidelines (Heinzerling, 2013). The skin prick method involves placing a drop of histamine or allergen solution onto the skin and using a lancet to gently pierce the superficial layer of the skin. The excess histamine or allergen solution is then wiped away. Study assessment will commence 15-20 mins after the skin has been pricked.

During the dosing period only one allergen positive at screening and the positive control will be used as described in the SPM.

AMENDMENT 3

Summary of Amendment Changes with Rationale

As a result of an informal preliminary data review from Part A of this study, several changes have been made to the design of Parts B and C of this study. The changes represent a more conservative strategy (staggering part C start relative to Part B), and reflect the necessity to fully understand the terminal elimination phase of GSK2646264 via the dermal route.

Becuase the terminal elimination phase was longer than anticipated it is proposed that thyroid function will be monitored via blood sample testing for the duration of exposure until the follow-up visit in parts B and C. The risk benefit with regards to thyroid function has not changed.

Changes are as follows:

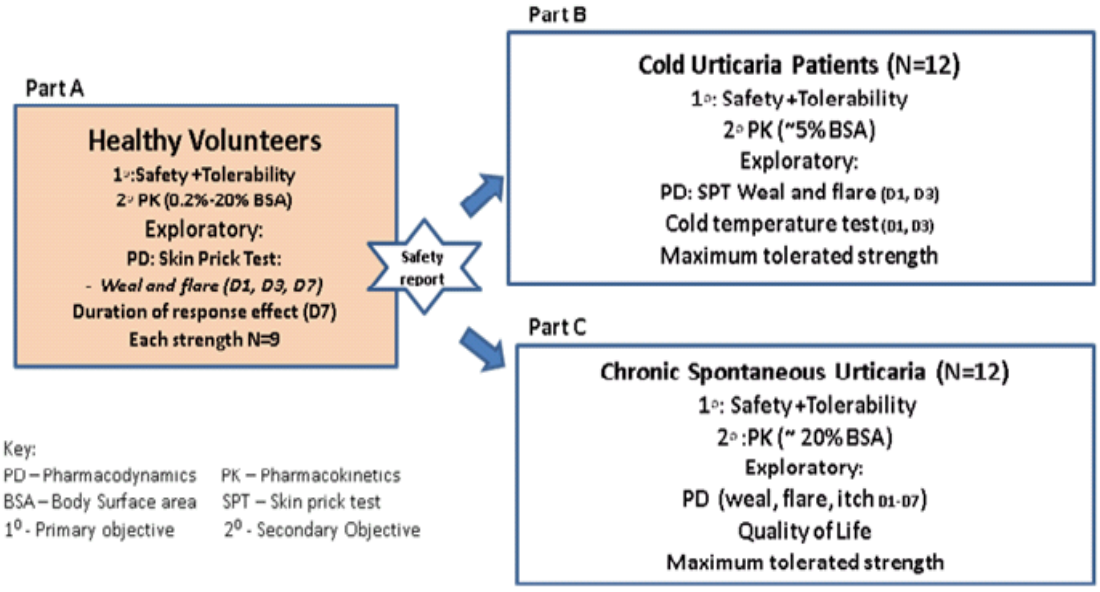
- Part B and C have changed from a bilateral to a placebo-controlled randomised parallel design 3:1 active to placebo.
- An informal review of data from the first 4 patients of Part B will be performed before starting part C.
- The number of subjects in Part C has increased (from n=12 up to n=16); and a 2-week PK/PD sampling period for parts B & C has been implemented.
- Free T4, T4 and T3 monitoring has been included for parts B and C at intervals until the follow-up visit.

List of Specific Changes

Section 3.1

PREVIOUS TEXT

Figure 2 **Study Schematic Showing all 3 Cohorts**



PREVIOUS TEXT

Randomisation will assign active and placebo to specified areas on left or right side of the body.

REVISED TEXT

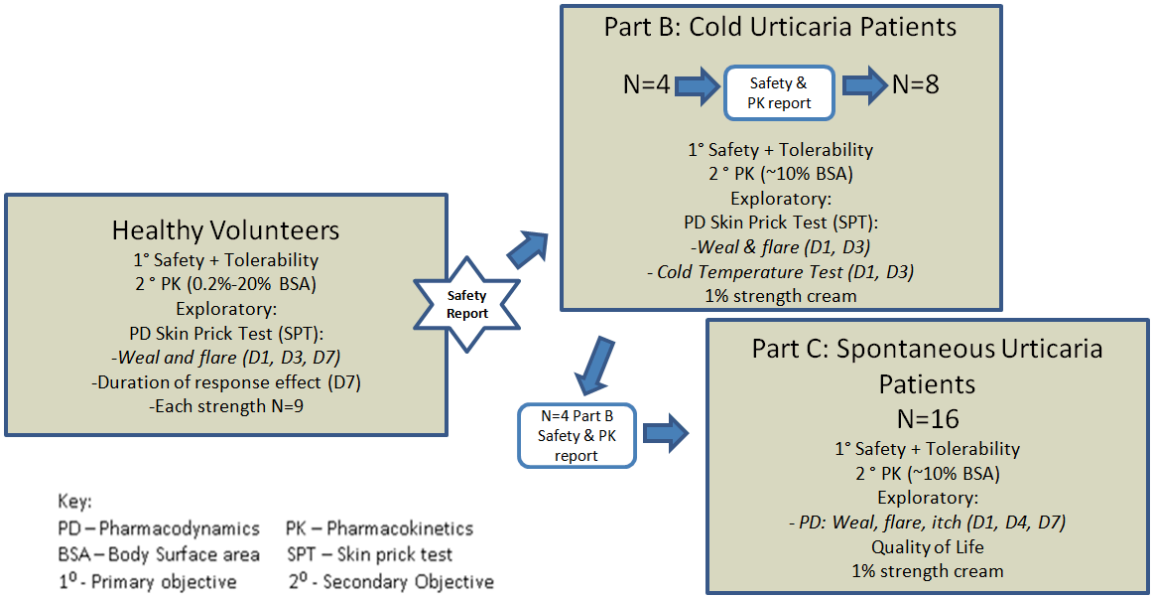
In Part A, randomisation will assign active and placebo to specified areas on left or right side of the body.

NEW TEXT

In Parts B and C, which will be a parallel design, randomisation will assign subjects to either active or placebo treatment. This will be a 3:1 ratio active to placebo respectively.

REVISED TEXT

Figure 1 Study Schematic Showing all 3 Cohorts



Section 3.1.4

PREVIOUS TEXT

Initially, a single dose of 1 active treatment and placebo will be applied to defined areas on subjects on the morning of day 1 and following a safety and tolerability assessment further subjects will be dosed; this will enable a second dose in the evening of day 1 and will enable the twice daily repeat dose period on days 2 unless Part A indicates once daily dosing is acceptable in which case once daily dosing will apply (see Table 4 and Figure 3).

Criteria to choose dose strength and dose regime in Part B are detailed in Section 5.5.

This cohort will remain in-house for the whole dosing period and until completion of the final dose on the morning of day 3 and completion of subsequent study assessments on day 4.

REVISED TEXT

Subjects will be randomised in a 3:1 ratio to receive either active treatment or placebo applied to defined areas once a day for 3 days. The first four subjects of Part B will be randomised so that one of the four subjects will receive placebo and the three other subjects will receive GSK2646264. An interim review of the preliminary data from the first 4 subjects, after they have completed the study, will be conducted before dosing the rest of the patients in Part B (n=12 total). PK, safety and tolerability preliminary data up to and including the follow-up visit, will be reviewed by the SMC (See Section 5.5). See Figure 3 for a visual representation.

If preliminary data indicates it is appropriate to review additional PD data, this may then be conducted, either in parallel to or before the remainder of Part B subjects are enrolled.

Criteria to choose dose strength and dose regime in Part B are detailed in Section 5.5. **Further dose adjustments may be made as a result of reviewing preliminary data from the first n=4 subjects in Part B.**

This cohort will remain in-house for the whole dosing period (until completion of the final dose on the morning of day 3) and completion of subsequent study assessments on day 4.

PREVIOUS TEXT

Table 3 Part B: Cold Urticaria treatment

	Days 1+2		Day 3
Area	**AM**	**PM[a]**	**AM**
Left Arm	0.36g maximum tolerated dose of GSK2646264 or placebo applied to 36 cm^2 (~0.2% BSA)		0.36g maximum tolerated dose of GSK2646264 or placebo applied to 36 cm^2 (~0.2% BSA)
Right Arm	0.36g maximum tolerated dose of GSK2646264 or placebo applied to 36 cm^2 (~0.2% BSA)		0.36g maximum tolerated dose of GSK2646264 or placebo applied to 36 cm^2 (~0.2% BSA)
Left Leg	9g maximum tolerated dose of GSK2646264 or placebo applied to 900 cm^2 (~5% BSA)		9g maximum tolerated dose of GSK2646264 or placebo applied to 900 cm^2 (~5% BSA)
Right Leg	9g maximum tolerated dose of GSK2646264 or placebo applied to 900 cm^2 (~5% BSA)		9g maximum tolerated dose of GSK2646264 or placebo applied to 900 cm^2 (~5% BSA)

a. AM dosing only if data for Part A, group 1 and/or group 2 supports

REVISED TEXT

Table 4 Part B: Cold Urticaria treatment

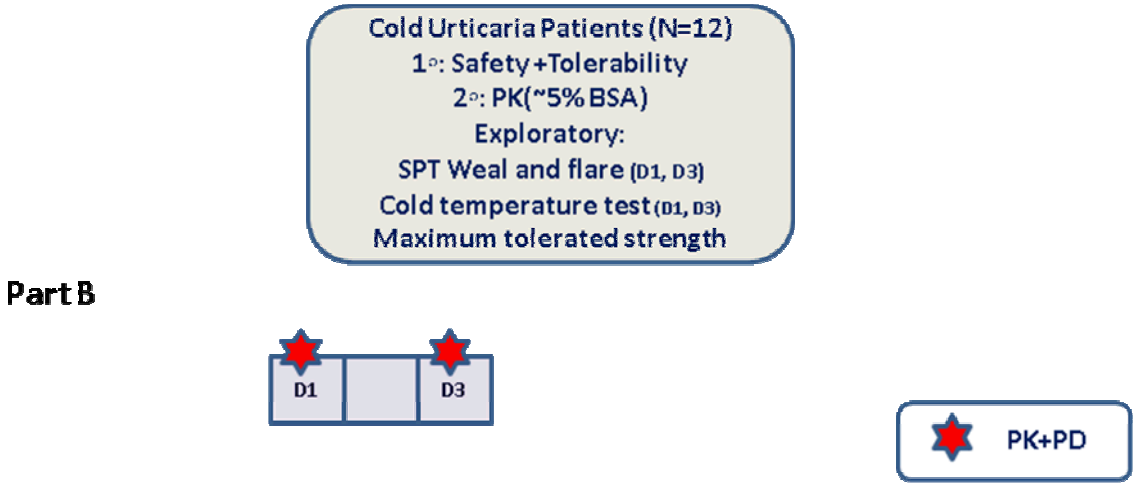
	Days 1-3
Area	**Morning dosing**
Both Arms	9g of 1% strength GSK2646264 or placebo applied to 900cm^2 (5% BSA) on each arm and the volar aspect of each arm must be included. = Total BSA = 10% (18g)

Following inclusion subjects will be randomised to either active or placebo. The investigator will use his judgement and discretion to decide on which area to apply the study treatment within requirements given in Table 4.

Subjects will receive either 1% strength GSK2646264 cream or placebo cream. The skin prick test will be performed on the volar aspect of the arm only in subjects who have a positive allergen challenge test at screening, as per inclusion criteria.

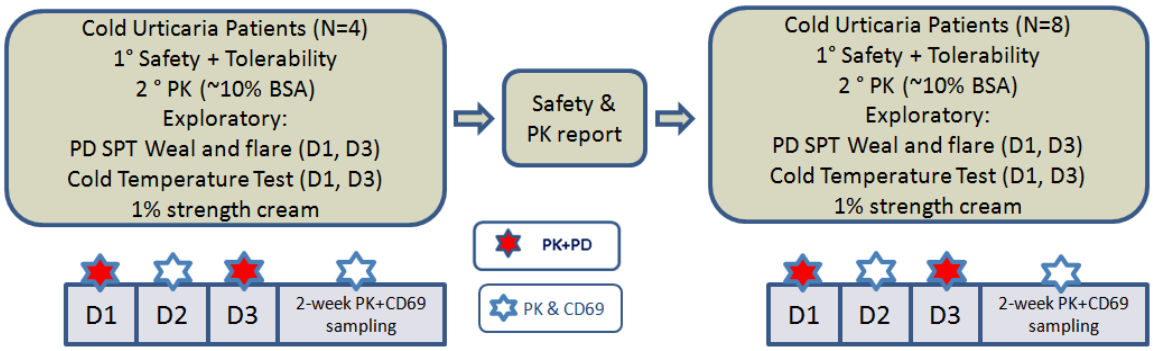
PREVIOUS TEXT

Figure 3 Schematic showing Part B Dosing in Cold Urticaria patients



REVISED TEXT

Figure 3 Schematic showing Part B Dosing in Cold Urticaria patients



Section 3.1.5

PREVIOUS TEXT

Initially, a single dose of the maximum tolerated strength from Part A and placebo will be applied to defined areas on subjects on the morning of day 1 and following a safety and tolerability assessment further subjects will be dosed. This will enable a second dose in the evening of day 1. In turn this will enable the twice daily repeat dose period from days 2 through to day 7. Once daily dosing may occur if supported by data from Part A of the study.

The timing and the assessments to be completed at each visit are provided in the Time and Event tables (Section 6.1.4). Early termination of this patient group may occur due to feasibility of recruitment and stopping criteria see (Section 5.5 and Section 5.6).

Following inclusion in the study subjects will be randomised. 10 mg/cm^2 of either the 0.5% or 1% and placebo will be applied to a given BSA (up to a maximum of 20% BSA) morning and evening on days 1 to 7. The total % BSA for an individual subject will be decided by the investigator prior to randomisation. The maximum % BSA and the frequency of dosing will be decided after part A of the study.

The subjects will be dosed in the unit for the first dose on day 1. Following completion of all day 1 assessments, the subjects will be discharged from the clinic at the clinical investigator's discretion. Subjects will return to the unit prior to the morning dose and specified assessments on days 4 and 7. Dosing will occur at home on days 2, 3, 5 and 6. Subjects will be asked to complete a daily diary over the 7 days. Details of the diary will be provided in the Study procedures manual (SPM).

Table 4 Part C: Chronic Spontaneous Urticaria treatment

	Days 1-7	
Area	**AM**	**PM[a]**
Left Arm	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)
Right Arm	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)
Left Leg	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA
Right Leg	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)	9g of maximum tolerated dose of GSK2646264 or placebo cream applied to 900cm^2 (~5% BSA)
Left Front Torso	18g of maximum tolerated dose of GSK2646264 or placebo cream applied to 1800cm^2 (~10% BSA)	18g of maximum tolerated dose of GSK2646264 or placebo cream applied to 1800cm^2 (~10% BSA)
Right Front Torso	18g of maximum tolerated dose of GSK2646264 or placebo cream applied to 1800cm^2 (~10% BSA)	18g of maximum tolerated dose of GSK2646264 or placebo cream applied to 1800cm^2 (~10% BSA)

a. AM dosing only if supported by data from Part A

REVISED TEXT

Subjects will be randomised to 3:1 ratio to receive active treatment or placebo applied to areas of the body chosen by the investigator once a day for 7 days. The chosen area where cream is applied will remain the same throughout the dosing period. Dosing in Part C will only commence once relevant data from the first four subjects in Part B have been reviewed, to allow greater confidence in predictions of the pharmacokinetics in Part C. The BSA over which study treatment will be administered in Part C is anticipated to be 10%, but is subject to change, if appropriate, based on preliminary data from Part B (see Figure 4 for a visual representation). However, the maximum BSA will not exceed 20%.

The first four subjects of Part C will be randomised so that one of the four subjects will receive placebo and the three other subjects will receive GSK2646264. An informal review of the preliminary data from the first 4 subjects will be conducted after they have completed the study. PK, safety, and tolerability, up to and including the follow-up visit, will be reviewed by the Sponsor (See Section 5.5). If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held. Recruitment of the full planned cohort (n=16) may continue during any data review.

Following inclusion in the study, subjects will be randomised. 10 mg/cm^2 of 1% active cream or placebo **cream** will be applied to a given BSA once per day on days 1 to 7, inclusive, **on areas of the body chosen by the investigator once a day for 7 days. The**

areas where cream is applied will remain the same for each day of dosing. The **10** % BSA can be distributed between the arms, legs and torso for an individual subjectwill be decided by the investigator prior to randomisation. **This pattern of cream application will remain the same for the duration of dosing.** The maximum % BSA and the frequency of dosing will be decided after part A of the study but will not exceed 20% BSA.

Section 4.2.1.3

NEW TEXT

If a patient who has been screened and is eligible for the study becomes unavailable to do the study at the initially planned dates due to exceptional circumstances, the subject may be re-screened for the study; in total a patient may be screened 2 times. This will only be at the discretion of the Investigator.

Section 4.2.1.4

NEW TEXT

If a patient who has been screened and is eligible for the study becomes unavailable to do the study at the initially planned dates due to exceptional circumstances, the subject may be re-screened for the study; in total a patient may be screened 2 times. This will only be at the discretion of the Investigator.

Section 4.2.2.3

NEW TEXT

Additional Exclusion for Part B – Cold urticaria Subjects

1. **fT4, T4, T3** levels outside normal range.

Section 4.2.2.4

NEW TEXT

Additional Exclusion for Part C – Chronic Spontaneous Urticaria patients

1. **fT4, T4, T3** levels outside normal range.

Section 4.3.2.1

NEW TEXT

Subjects with Cold Urticaria (Part B)

will abstain from ingesting caffeine- or xanthine-containing products (e.g. coffee, tea, cola drinks, chocolate) and alcohol for 24 hours prior to the start of dosing until discharge from the research unit.

Section 4.4.1

NEW TEXT

Subjects exceeding screening visit window

Subjects in Part B and Part C that are not randomized within the allotted screening window may be re-screened once, as per Section 4.2.1. If re-screening is performed, it is essential that subjects are assigned a different unique subject ID number for the additional screening attempt. See the SPM for specific details.

Section 5.4 Treatment Assignment

PREVIOUS TEXT

Subjects will be assigned to treatment in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software, RandAll NG. Randomisation will occur within each cohort of study. Subject will be randomised to 1:1 to receive bilateral treatment of GSK2646264 or placebo.

REVISED TEXT

Subjects will be assigned to treatment in accordance with the randomization schedule generated by **Parexel**, prior to the start of the study. Randomisation will occur within each cohort of study. Subjects will be randomised to 1:1 to receive bilateral treatment of GSK2646264 or placebo **in Part A**. **For Parts B & C, subjects will be randomised 3:1 active:placebo respectively, to receive either GSK2646264 or placebo.**

Section 5.5.1

PREVIOUS TEXT

Toxicity is defined as dose-limiting toxicity if it meets the following criteria:

- Occurs within 6 hours after any dose.

REVISED TEXT

Toxicity is defined as dose-limiting toxicity if it meets the following criteria:

- Occurs within 6 hours after any dose **for Parts A & B, or within 24 hours after any dose for Part C.**

Section 5.5.3.3

NEW TEXT

In Parts B,the first four 4subjects will be randomised so that one of the four 4 subjects will receive placebo and the three other subjects will receive GSK2646264. An interim review of the preliminary data from the first 4 subjects, after they have completed the study, will be conducted before dosing the rest of the patients in Part

B (n=12 total). PK, safety, and tolerabilitytolerabilityand systemic CD69 preliminary data up to and including the follow-up visit, will be reviewed by the SMC (See Section 5.5). In addition, further modeling of PK parameters after 4 days of dosing in these first 4 cold urticaria patients focusing on the elimination of the compound, will be performed to aid the decision to move forward to the rest of the subjects in Part B and to Part C, taking into account the pre-determined safety margins (See Appendix 6).

Section 5.5.3.4

NEW TEXT

Dosing in part C will only commence once relevant data from the first four subjects in Part B have been reviewed (see Section 5.5.3.3.) to allow greater confidence in predictions of the pharmacokinetics in Part C. The BSA over which study treatment will be administered in Part C is anticipated to be 10%, but is subject to change if appropriate based on preliminary data from Part B. However, the maximum BSA will not exceed 20%.

The first four subjects of Part C will be randomised so that one of the four subjects will receive placebo and the three other subjects will receive GSK2646264. An informal review of the preliminary data from the first 4 subjects may be conducted after they have completed the study. PK, safety and tolerability data up to and including the follow-up visit, will be reviewed by the Sponsor. If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held. Recruitment of the full planned cohort (n=16) may continue during any data review. The dosing regimen or study population may be adjusted as appropriate after this interim review for the rest of the subjects in Part C.

Section 5.6

NEW TEXT

If AEs which are of severe intensity and are similar across subjects in either Part B or C, or if unacceptable pharmacological effects, reasonably attributable in the opinion of the investigator to dosing with GSK2646264, or if dose limiting toxicity (see Section 5.5.1), are observed in 2 or more subjects in either Part B or C, relevant reporting and discussion with the GSK medical monitor and the SMC will take place.

Section 5.8

NEW TEXT

After the first 4 subjects in Part C, available pharmacokinetic data will be reviewed by the sponsor (see Section 5.5.3.4). If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held.

If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held.

Section 5.8.1

PREVIOUS TEXT

If (cohort's average) predicted or observed C_{max} or $AUC_{(0-\infty t)}$ of GSK264624 is greater than 16 ng/mL or 220 ng.hr/mL, doses for subsequent cohorts will need to be decided by the SMC.

REVISED TEXT

If (cohort's average) predicted or observed C_{max} or $AUC_{(0\text{-}24\ h\ after\ last\ dose)}$ of GSK264624 is greater than 16 ng/mL or 220 ng.hr/mL, **respectively**, doses for subsequent cohorts **may be adjusted based on tox cover and** will need to be decided by the SMC **but will not exceed agreed margins**.

Section 5.9

PREVIOUS TEXT

This will be a randomised double blind (sponsor unblinded), single, bilateral and repeat ascending dose First Time in Human study in healthy subjects, cold urticaria and chronic spontaneous urticaria subjects study.

REVISED TEXT

This will be a randomised double blind (sponsor unblinded), single, bilateral **(Part A) and paralled (Parts B and C)**repeat ascending dose First Time in Human study in healthy subjects, cold urticaria and chronic spontaneous urticaria subjects study.

Section 6.1.2

PREVIOUS TEXT

Part A: Healthy Volunteer Time and Event Table Dose group 2

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										9-11 days	
Admission to Unit		X										
Informed Consent	X											
Demographics	X											
Complete physical	X										X	
Body weight (kg)	X											
Height (cm) without shoes	X											
Brief physical		X										
Medical/medication/drug /alcohol history	X											
12-lead ECG[1]	X	X								X	X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X	X	X	X	X	X	X	X	X	X	
Urine drug/alcohol screen	X	X										
Urine for metabolite analysis		X[15]							X[15]			15. Pre dose,and pool samples taken 0-12 and 12-24 hours post dose
HIV, Hep B and Hep C screen	X											
Clinical Laboratory Tests	X	X								X	X	

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										9-11 days	
Allergen Challenge- Skin Prick Test	X[3,4]		X[3,5]		X[3,5]				X[3,5]	X[7]		3. SPT- Skin Prick Test measurements will be performed ~15-20mins post challenge. 4. SPT with all 4 specificed allergens 5. SPT will be performed 6 hours post dose (see Section 6.4.1) 7. SPT will be performed 24 hours post dose
TSH[2]	X											2. TSH test only at screening only
Randomisation		X										
Tolerability Assessment			X[11] ←----------→									11. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		X	←----------→								X	
Concomitant Medication		X	←----------→								X	
Pharmacokinetic Blood Sample[6]			X	X	X	X	X	X	X	X[10]		6. PK blood sample taken at pre dose, 1, 2, 4, 8, 12 and 24hr post dose 10. PK refers to sample 24 hours post dose
Blood Sample for CD69 expression			X[12]			X[13]	X[14]		X[13]	X[14]		12. Predose 13. 4 hours after previous dose 14 24 hours after previous dose
Study Treatment Dosing[8]			X	X	X	X	X	X	X[9]			8. This could be once or twice daily dosing 9. Single AM dose on Day 7
Discharge from unit										X		
Outpatient visit	X										X	

Part B: Cold Urticaria Subjects Time and Event table

Day:	Screening	-1	1	2	3	4	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7 days						Day 5 to Day 7 after last dose	
Admission to Unit		X						
Informed Consent	X							
Demographics	X							
Complete physical	X						X	
Body weight (kg)	X							
Height (cm) without shoes	X							
Brief physical		X						
Medical/medication/ drug/alcohol history	X							
12-lead ECG[1]	X	X			X		X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X	X	X	X		X	
Urine drug/alcohol screen	X	X						
Pregnancy Test (women)[2]	X(S)		X(S/U)				X(S)	2. Pregnancy test will be performed 3 times during screening, serum on day -28, day -7 to -4, and a serum or urine pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test at the follow up visit
HIV, Hep B and Hep C screen	X							
Clinical Laboratory Tests	X	X			X		X	
Allergen Challenge-SPT	X[4,5]		X[4,6]		X[4,6]			4. SPT- Skin Prick Test measurements will be performed ~15-20mins post challenge 5. SPT using all 4 specified allergens 6. SPT will be performed at 6 hours post dose (see Section 6.4.1)
TSH[3]	X							3. TSH test only at screening only
Cold Temp Test (Temp Test 4.0)	X		X		X	X[7]		7. 24 hours after last dose
Review of eligibility criteria and medication prior to randomisation		X						

Day:	Screening	-1	1	2	3	4	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7 days						Day 5 to Day 7 after last dose	
Randomisation		X						
Tolerability Assessment			X[10] ←----------→					10. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		X	←----------→				X	
Concomitant Medication		X	←----------→				X	
Pharmacokinetic Blood Sample[8]			X		X	X[9]		8. PK blood sample taken at pre dose,1,2,4, 8, 12hr post dose 9. If only once daily dosing then a PK sample will be taken at 24h post dose
Study Treatment Dosing			X	X	X			
Discharge from unit						X		
Outpatient visit	X						X	

Part C: Chronic Spontaneous Urticaria Cohort

Day:	Screening	1	2	3	4	5	6	7	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7 days								9-14 days after last dose	
Informed Consent	X									
Demographics	X									
Complete physical	X								X	
Body weight (kg)	X									
Height (cm) without shoes	X									
Brief physical		X							X	
Medical/medication/ drug/alcohol history	X									
12-lead ECG[1]	X	X						X	X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X			X			X	X	
Urine drug/alcohol screen	X	X								
Pregnancy Test (women)[2]	X(S)	X (S/ U)							X(S)	2. Pregnancy test will be performed 3 times during screening, serum (S) on day -28, day -7 to -4, and a serum or urine (U) pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test at the follow up visit
HIV, Hep B and Hep C screen	X									
Clinical Laboratory Tests	X	X						X	X	
TSH[3]	X									3. TSH test only at screening only
Blood Sample for CD69 expression[11]		X[9]						X[10]		9. Predose 10. 4 hours post dose 11 A decision will be made after Part A on whether samples are taken in Part C

Day:	Screening	1	2	3	4	5	6	7	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7 days								9-14 days after last dose	
Randomisation		X								
UAS-7 Diary[4]	X	X	X	X	X	X	X	X		4. Screening UAS7 diary will be completed using 7 successive days before randomisation (day 1). If there is a lapse in any day prior to day 1 the subject must complete the full 7 days worth of diary entries again during the screening period.
QOL (DLQI)	X	X						X		
AAS	X	X	X	X	X	X	X	X		
AE assessment		X[8] ←----------→							X	8. Tolerability assessment at pre – and post-dose
BHR- Basophil Histamine Release Test		X[5]								5. Sample taken predose
Pharmacokinetic Blood Sample[6]		X			X			X		6. PK blood sample taken at pre-dose sample and 2 and 4hr post dose
Study Treatment Dosing		X	X[7]	X[7]	X	X[7]	X[7]	X		7. The nurse will visit the subject at home to apply the dose
Outpatient visit	X	X			X			X	X	

REVISED TEXT

Part A: Healthy Volunteer Time and Event Table Dose group 2

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										Day 9 – Day 11	
Admission to Unit		X										
Informed Consent	X											
Demographics	X											
Complete physical	X										X	
Body weight (kg)	X											
Height (cm) without shoes	X											
Brief physical		X										
Medical/medication/drug /alcohol history	X											
12-lead ECG[1]	X	X								X	X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X	X	X	X	X	X	X	X	X	X	
Urine drug/alcohol screen	X	X										
Urine for metabolite analysis			X[10]			X[22]						10. Pre dose and pool samples taken 0-12 and 12-24 hours post dose 22. Pool samples taken 0-12 and 12-24 hours post
HIV, Hep B and Hep C screen	X											
Clinical Laboratory Tests	X[17]	X[17]		X[9]		X[9]	X[17]		X[17]		X[17]	9. Pre-dose AST, ALT, Alkaline Phosphatase, Total Bilirubin Only- : results to be communicated to the GSK Medical monitor on the same day 17.Clinical lab test as per Section 6.3.4 of the protocol

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										Day 9 – Day 11	
Allergen Challenge- Skin Prick Test	X[3,4]		X[3,5]		X[3,5]	X[3, 5]		X[3,7]				3. SPT- Skin Prick Test measurements will be performed ~15-20 mins post challenge. 4. SPT with all 4 specified allergens 5. SPT will be performed 6 hours post dose 7. SPT will be performed 48 hours post dose of Day 4 (on the morning of Day 6)
TSH[2]	X											2. TSH test only at screening only
Randomisation		X										
Tolerability Assessment			X[11] ←----------→ only on dosing days 1 to 4									11. Tolerability assessment at pre – and ~6 hours post-dose on dosing days
AE assessment		X	←----------→								X	
Concomitant Medication		X	←----------→								X	
Pharmacokinetic Blood Sample			X[6]	X[6]	X[6]	X[6]	X[18]	X[19]	X[20]	X[21]		6. PK blood sample taken at pre dose, 1, 2, 4, 8, 12 and 24hours post previous dose 18. PK blood sample taken at 30, 36, 48 hours, post dose of Day 4 19. PK blood sample taken at 54, 60, 72 hours, post dose of Day 4 20. PK blood sample taken at 78, 84 hours post dose of Day 4 21. PK blood sample taken at 96 hours post dose of Day 4

Day:	Screening	-1	1	2	3	4	5	6	7	8	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7										Day 9 – Day 11	
Blood Sample for CD69 expression			X[12]			X[13]	X[14]		X[15]	X[16]		12. Predose 13. 4 hours post dose of Day 4 14. 24 hours post dose of Day 4 (sample taken in the morning of day 5) 15. 72 hours post dose of Day 4 16. 96 hours post dose of Day 4
Study Treatment Dosing[8]			X	X	X	X						8. This will be once daily dosing using fresh bottles at 1% strength in the morning
Discharge from unit										X		
Outpatient visit	X										X	

Part B: Cold urticaria Subjects Time and Event table

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
Visit Window (relative to Day 1)	-28 to -7 days								
Admission to Unit		X							
Informed Consent	X								
Demographics	X								
Complete physical	X							X	
Body weight (kg)	X								
Height (cm) without shoes	X								
Brief physical		X							
Medical/medication/ drug/alcohol history	X								
12-lead ECG[1]	X	X			X			X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X	X	X	X		X	X	
Urine drug/alcohol screen	X	X							
Pregnancy Test (women)[2]	X(S)		X(S/U)				X (S)	X(S)	2. Pregnancy test will be performed 3 times during screening, serum on day -28, day -7 to -4, and a serum or urine pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test between day 10-12 (to coincide with a PK sample) and at the follow up visit.
HIV, Hep B and Hep C screen	X								
Clinical Laboratory Tests	X	X			X			X	
Allergen Challenge-SPT	X[3,4]		X[3,6]		X[3,5]	X[9]			3. SPT- Skin Prick Test measurements will be performed ~15-20mins post challenge 4. SPT using all 4 specified allergens 5. SPT will be performed at 6 hours post dose (see Section 6.4.1) 6. SPT will be performed pre-dose
TSH, free T4, T4, T3	X					x	X[7]	x	7. One sample between day 10-12

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
Cold Temp Test (Temp Test 4.0)	X		X[8]		X[10]	X[9]			8. Pre-dose 9. 24 hours post dose 10. 6 hours post dose
Randomisation		X							
Tolerability Assessment			←----- X[11] -----→						11. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		X	←---------X--------→					X	
Concomitant Medication		X	←-------X----------→					X	
Pharmacokinetic Blood Sample			X[12]	X[12]	X[12]	X[12]	X[13]	X	12. PK blood sample taken at pre dose,1, 4, 8, 12hr post dose 13. One PK blood sample per day will be taken as follows:. PK samples will be obtained on the following days (± 1 day): Day 6, 9, 12, & 15.
Study Treatment Dosing			X	X	X				
Discharge from unit						X			
Outpatient visit	X						X	X	
Blood Sample for CD69 expression[12]			X[10]		X[11]		X	X	10. Pre-dose 11. 4 hrs post-dose 12. Sample collection should coincide with PK sample collection.

Part C: Chronic Spontaneous Urticaria Cohort

Day:	Screening	1	2	3	4	5	6	7	Day 10 to 19	Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7									21-23	
Informed Consent	X										
Demographics	X										
Complete physical	X									X	
Body weight (kg)	X										
Height (cm) without shoes	X										
Brief physical		X								X	
Medical/medication/ drug/alcohol history	X										
12-lead ECG[1]	X	X						X		X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X			X			X	X[13]	X	13. Only conducted if subjects is attending the clinical site.
Urine drug/alcohol screen	X	X									
Pregnancy Test (women)[2]	X(S)	X (S/U)							X(S)[14]	X(S)	2. Pregnancy test will be performed 3 times during screening, serum (S) on day -28, day -7 to -4, and a serum or urine (U) pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test between day 14-16 and at the follow up visit 14. Samples should be obtained at approximately 1 week intervals
HIV, Hep B and Hep C screen	X										

Day:	Screening	1	2	3	4	5	6	7	Day 10 to 19	Follow-up	Notes
Clinical Laboratory Tests	X	X						X		X	
TSH, free T4, T4, T3	X							X	X[3]	X	3. Between Days 14-16
Blood Sample for CD69 expression		X[4]			X[5]			X[5]	X[6]	X	4. Predose 5. 4 hours post dose 6. Sample to be taken every time a PK sample is taken
Randomisation		X									
UAS-7 Diary[7]	X	X	X	X	X	X	X	X			7. Screening UAS7 diary will be completed using 7 successive days before randomisation (day 1). If there is a lapse in any day prior to day 1 the subject must complete the full 7 days worth of diary entries again during the screening period.
QOL (DLQI)	X	X						X			
AAS	X[15]	X	X	X	X	X	X	X			15. Screening AAS will be completed using 7 successive days before randomisation (day 1) on the same days as the UAS7. If there is a lapse in any day prior to day 1 the subject must complete the full 7 days worth of diary entries again during the screening period.
AE assessment		X ←----------→								X	
Tolerability Assessment at pre-dose		X[8] ←----------→									8. Tolerability assessment at pre dose on each day.
BHR- Basophil Histamine Release Test		X[9]									9. Two samples to be taken predose
Pharmacokinetic		X[10]			X[10]			X[10]	X[11]	X	10. PK blood sample taken at pre-dose and 4hr post dose

Day:	Screening	1	2	3	4	5	6	7	Day 10 to 19	Follow-up	Notes
Blood Sample											11. One PK blood sample will be taken during this period at each of the following timepoints:. PK samples will be obtained on the following days (± 1 day): Day 10, 13, 16, 19 and 22, with each sample being no more than 3 days, apart.
Study Treatment Dosing		X	X[12]	X[12]	X	X[12]	X[12]	X			12. The subject may have study treatment applied at home by a nurse or they can travel to the clinic
Con Meds		X ←----------→									
Outpatient visit	X	X			X			X	x	X	

Section 6.3.4

PREVIOUS TEXT

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Albumin
Glucose, fasting	Calcium	GGT	Total Protein
Sodium	Phosphate	Alkaline phosphatase	

REVISED TEXT

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Albumin
Glucose, fasting	Calcium	GGT	Total Protein
Sodium	Phosphate	Alkaline phosphatase	
TSH , free T4, T4, T3			

Section 6.4.3.3

NEW TEXT

Following review of PK in dose group 1 urine samples were collected for analysis of any metabolite(s) from subjects in Part A, Group 2 at predose, and dosing Days 1 and 4.

Section 9.2.1

PREVIOUS TEXT

Table 9 Results of the sensitivity analyses

	Percent of Inhibition Weal Size		**Percent of Inhibition Flare Size**		**Percent of Inhibition Weal Volume**	
N	**Precision around estimate**	**SD**	**Precision around estimate**	**SD**	**Precision around estimate**	**SD**
9	15.4	20	6.9	9	15.4	20
9	16.9	22	7.7	10	16.1	21
9	18.5	24	8.5	11	17.7	23
9	20.0	26	10	13	19.2	25
9	21.5	28	11.5	15	20.8	27

REVISED TEXT

For Part A, based on a sample size of 9 randomised subjects, it is estimated that the 95% confidence interval for the percent of inhibition effect will be within 18.5% of the estimate for the weal size assuming standard deviation of 24, within 8.5% of the estimate for the flare size assuming standard deviation of 11 and within 17.7% of the estimate for the weal volume assuming standard deviation of 23 (Schoepke, 2013).

Table 9 Results of the sensitivity analyses for part A

	Percent of Inhibition Weal Size		Percent of Inhibition Flare Size		Percent of Inhibition Weal Volume	
N	Precision around estimate	SD	Precision around estimate	SD	Precision around estimate	SD
9	15.4	20	6.9	9	15.4	20
9	16.9	22	7.7	10	16.1	21
9	18.5	24	8.5	11	17.7	23
9	20.0	26	10	13	19.2	25
9	21.5	28	11.5	15	20.8	27

Section 9.3.1

NEW TEXT

Summary of exposure and safety reporting from Part B: first 4 subjects

The safety, tolerability, and preliminary exposure data from Part B (first 4 subjects) will be reviewed by the SMC once those subjects have completed the study up to and including the follow-up visit. The decision for the dosing regimen and study population for the remainder of Part B, and Part C will be made by the SMC review based on assessment of safety, and pharmacokinetic data of the studied doses.

This study is double blind (sponsor open), where the subject, investigator and site staff will remain blinded to the treatment allocation.

Summary of exposure and safety reporting from Part C: first 4 subjects

Preliminary safety and tolerability, and potentially biomarkers of interest), and preliminary exposure data from Part C (first 4 subjects) may be reviewed by the sponsor once those subjects have completed the study up to and including the follow-up visit. The decision for the potential adjustment of the dosing regimen and/or study population for

the rest of Part C will be made by the sponsor review based on assessment of safety and pharmacokinetic data of the studied doses. If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held.

Other experts from CPMS and DMPK, at GSK, may be consulted to review blinded placebo/active data (safety, PK and any available PD) during the study for further relevant input.

Section 9.3.2.3

Part A & B: Healthy and Cold Urticaria Subjects

PREVIOUS TEXT

Raw data and percent of inhibition for each PD parameter (i.e. weal area, weal volume, weal height and flare area and erythema assessments) will be provided by strength, BSA, and day. Descriptive summary statistics (i.e. n, minimum, arithmetic mean, standard error, median, maximum) for both raw and percent of inhibition and change from baseline values will be calculated by dose strength, BSA, and day. Additionally, figures will be produced for PD parameters. No formal statistical testing of the PD data will be conducted.

For Health Subjects (Part A), descriptive summary statistics for percent of inhibition of CD69 biomarker will be provided by strength, BSA and day.

PD assessment in CsU subjects will be measured using a composite score UAS7. Change from baseline in UAS7 at day 7 is the Day UAS7 minus the Baseline UAS7 score. The mean and standard deviation of the change from baseline in UAS7 for each treatment group will be presented. The change from baseline in UAS7 at day 7 will be analysed using the ANCOVA model including treatment, baseline UAS7and location of treatment. Estimates of the mean and treatment difference and associated 95% confidence intervals will be presented. Analysis of each component will be performed similar to UAS7 analysis.

The UAS7 score will be calculated using the 7 consecutive days sum. In presence of one or more missing daily UAS7 scores, the following algorithm will be applied:

- If a patient has at least 4 non-missing daily UAS7 scores within the 7 days, the UAS7 score will be calculated as the sum of the available eDiary UAS7 scores in that week, divided by the number of days that have a non-missing diary UAS7 score, multiplied by 7.
- If there are less than 4 non-missing daily UAS7 scores, then the UAS7 score will be missing.

Summary statistics for each component of UAS7 questionaire will be provided by visit.

For CsU Subjects (Part C), descriptive summary statistics for percent of inhibition of CD69 biomarker will be provided by strength, BSA and day if samples are taken in Part C.

More details will be provided in the RAP.

REVISED TEXT

Part A & B: Healthy and Cold Urticaria Subjects

Raw data and percent of inhibition for each PD parameter (i.e. longest weal diameter, perpendicular weal length, weal area, weal volume, and flare erythema assessments) will be provided by strength, BSA, and day. Descriptive summary statistics (i.e. n, minimum, arithmetic mean, standard error, median, maximum) for both raw and percent of inhibition for Part B will be calculated by dose strength, BSA, and day. Additionally, figures will be produced for PD parameters. No formal statistical testing of the PD data will be conducted.

Descriptive summary statistics for percent of inhibition of systemic CD69 biomarker will be provided by strength, BSA and day.

Part C: Chronic Spontaneous Urticaria Subjects

PD assessment in CsU subjects will be measured using a composite score UAS7.

The UAS7 score will be calculated using the 7 consecutive days sum. A score will be derived for the screening visits and for the post dose visits In presence of one or more missing daily UAS7 scores, the following algorithm will be applied:

- If a patient has at least 4 non-missing daily UAS7 scores within the 7 days, the UAS7 score will be calculated as the sum of the available eDiary UAS7 scores in that week, divided by the number of days that have a non-missing diary UAS7 score, multiplied by 7.
- If there are less than 4 non-missing daily UAS7 scores, then the UAS7 score will be missing.

Change from baseline in UAS7 will be derived as post dose UAS7 total score minus the Baseline UAS7 total score (screening). The mean and standard deviation of the change from baseline in UAS7 for each treatment group will be presented. The change from baseline in UAS7 will be analysed using the ANCOVA model including treatment, baseline UAS7 and location of treatment. Estimates of the means and treatment difference and associated 95% confidence intervals will be presented. Analysis of each component will be performed similar to UAS7 analysis.

Summary statistics for each component of UAS7 questionaire will be provided by visit.

For CsU Subjects (Part C), descriptive summary statistics for percent of inhibition of CD69 biomarker will be provided by strength, BSA and day if samples are taken in Part C.

More details will be provided in the RAP.

AMENDMENT 4

Summary of Amendment Changes with Rationale

After a data review from the first 4 subjects in Part B of this study, several changes have been made to the design of Parts B and C of this study. The major changes are to the dose level for Part B, the dosing regimen for Part C, and update of the contraception criteria for women of child bearing potential. Additional typographical errors have been corrected also.

List of specific changes:

PREVIOUS TEXT

2.3.2 Part B: Cold urticaria Subjects

Objectives	Endpoints
Exploratory	
Pharmacodynamic measure of Cold Provocation Assess change in Cold Temperature Test values	**Pharmacodynamic measure of Cold Provocation** • Change from placebo of Cold Temperature Test[3] values at day 3
Pharmacodynamic measure of Allergen Challenge Assess measure of weal and flare size, in subjects with Cold urticaria after allergen challenge (Skin Prick test, positive control (histamine) and negative control (saline)).	**Pharmacodynamic measure of Allergen Challenge** • Percent of inhibition on day 3 will be derived for: o The longest weal diameter (measured by ruler). o Perpendicular weal length (measured by ruler). o Weal area calculated using weal diameter and length assuming ellipse area. • Weal Volume as measured by; • Quantitative volumetric morphometry[1] Flare Erythema as measured by; • Mexameter[2]

REVISED TEXT

2.3.2 Part B: Cold urticaria Subjects

Objectives	Endpoints
Exploratory	
Pharmacodynamic measure of Cold Provocation Assess change in Cold Temperature Test values	**Pharmacodynamic measure of Cold Provocation** • Change from placebo of Cold Temperature Test[3] values at day 3
Pharmacodynamic measure of Allergen Challenge Assess measure of weal and flare size, in subjects with Cold urticaria after allergen challenge (Skin Prick test, positive control (histamine) and negative control (saline)).	**Pharmacodynamic measure of Allergen Challenge** • Percent of inhibition on day 3 will be derived for: o The longest weal diameter (measured by ruler). o Perpendicular weal length (measured by ruler). o Weal area calculated using weal diameter and length assuming ellipse area. • Weal Volume as measured by; • Quantitative volumetric morphometry[1] Flare Erythema as measured by; • Mexameter[2]
Systemic measure of target pharmacology Determine CD69 expression in an ex vivo blood sample following CD69 stimulation	**Systemic measure of target pharmacology** • Percent inhibition of CD69 expressing cells in blood samples stimulated with anti-IgM .

PREVIOUS TEXT

2.3.3 Part C: Chronic Spontaneous Urticaria Subjects

Objectives	**Endpoints**
Exploratory	
Pharmacodynamics as assessed by the Urticaria activity score Urticaria Activity Score: Assess measures of weal characterisation in CsU subjects within treated area only	**Pharmacodynamics as assessed by the Urticaria activity score** • Urticaria Activity Score, a composite score using key urticaria symptoms (number of weal, itch/pruritus), to derive change from baseline **for the 7 days after dosing**
To measure of Quality of Life	• Dermatology Life Quality Index (DLQI) questionnaire
To measure disease activity	• Angioedema Activity Score (AAS)
Investigate relationships between (basophil histamine release) BHR test and weal characteristics	• Relationship of positive BHR test with reduction in number or size of weals
Systemic measure of target pharmacology Determine CD69 expression in an ex vivo blood sample following CD69 stimulation	**Systemic measure of target pharmacology** • Percent inhibition of CD69 expressing cells in blood samples stimulated with anti-IgM.

PREVIOUS TEXT

Nominal % BSA	**Actual surface to spread the active or placebo cream on:**	**Weight of active or placebo cream to be applied**
0.2% BSA	36 cm^2	0.36g
1% BSA	180 cm^2	1.8g
5% BSA	900 cm^2	9g
10% BSA	1800 cm^2	18g
20%	3600 cm^2	36g

REVISED TEXT

Nominal % BSA	**Actual surface to spread the active or placebo cream on:**	**Weight of active or placebo cream to be applied**
0.2% BSA	36 cm^2	0.36g
1% BSA	180 cm^2	1.8g
3.5% BSA	**630 cm^2**	**6.3g**
5% BSA	900 cm^2	9g
10% BSA	1800 cm^2	18g
20%	3600 cm^2	36g

PREVIOUS TEXT

Table 6 Part B: Cold urticaria treatment

	Days 1-3
Area	**Morning dosing**
Both Arms	9g of 1% strength GSK2646264 or placebo applied to 900cm^2 (5% BSA) on each arm and the volar aspect of each arm must be included. = Total BSA = 10% (18g)

REVISED TEXT

Table 7 Part B: Cold urticaria treatment (first n=4 subjects)

	Days 1-3
Area	**Morning dosing**
Both Arms	9g of 1% strength GSK2646264 or placebo applied to 900cm^2 (5% BSA) on each arm and the volar aspect of each arm must be included. = Total BSA = 10% (18g)

12.8.1.1 Changes after review of preliminary data of n=4 from Part B by the Safety Monitoring Committee

Following the review by the Safety Monitoring Committee (see Section 5.5), changes were made to Part B for the remaining n=8 subjects, as shown in Table 5.

Table 8 Part B: Cold urticaria treatment (remaining n=8 subjects)

	Days 1-3
Area	**Morning dosing**
Both Arms	6.3g of 1% strength GSK2646264 or placebo to be applied to a total area of 630cm^2 (3.5% BSA), spread over the 2 arms, and the volar aspect must be included. The investigator should use their discretion as to how much cream is applied to each arm, but it must be equivalent to 3.5% BSA in total.

PREVIOUS TEXT

~~3.1.5 Part C :A double-blind (sponsor unblinded) placebo-controlled, randomised to active or placebo treatment 7 days repeat dose study, in subjects with mild to moderate Chronic Spontaneous Urticaria (CsU)~~

~~Subjects will be randomised to 3:1 ratio to receive active treatment or placebo applied to areas of the body chosen by the investigator once a day for 7 days. The chosen area where cream is applied will remain the same throughout the dosing period. Dosing in Part C will only commence once relevant data from the first four subjects in Part B have been reviewed, to allow greater confidence in predictions of the pharmacokinetics in Part C. The BSA over which study treatment will be administered in Part C is anticipated to be 10%, but is subject to change, if appropriate, based on preliminary data from Part B (see Figure 4 for a visual representation). However, the maximum BSA will not exceed 20%.~~

~~The first four subjects of Part C will be randomised so that one of the four subjects will receive placebo and the three other subjects will receive GSK2646264. An informal review of the preliminary data from the first 4 subjects may be conducted after they have completed the study. PK, safety, and tolerability, up to and including the follow-up visit, will be reviewed by the Sponsor (See Section 5.5). If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held. Recruitment of the full planned cohort (n=16) may continue during any data review.~~

~~The timing and the assessments to be completed at each visit are provided in the Time and Event tables (Section 6.1.4). Early termination of this patient group may occur due to feasibility of recruitment and stopping criteria see (Section 5.5 and Section 5.6). Following inclusion in the study, subjects will be randomised. 10 mg/cm^2 of 1% GSK2646264cream or placebo cream will be applied to a given BSA once per day on days 1 to 7, inclusive, on areas of the body chosen by the investigator once a day for 7 days. The areas where cream is applied will remain the same for each day of dosing.The 10 % BSA can be distributed between the arms, legs and torso for an individual subject and will be decided by the investigator prior to randomisation. This pattern of cream application will remain the same for the duration of dosing. The maximum % BSA and the frequency of dosing will be decided after part A of the study but will not exceed 20% BSA.~~

~~The subjects will be dosed in the unit for the first dose on day 1 and then discharged from the clinic. Doses and assessments on days 2 and 3 can occur either in the clinic or at home with the study nurse. Subjects will return to the clinic prior to the dose and specified assessments on days 4 and 7. Dosing can occur at home or at the clinic on days 5 and 6. Subjects will be asked to complete a daily diary over the 7 days. Details of the diary will be provided in the Study Procedures Manual (SPM)~~

~~18g of 1% GSK2646264 cream or placebo cream will be applied to 1800cm^2 (~10% BSA) on Day 1. The same 1800 cm^2 will be used for the 7 days duration of dosing. The cream can be applied to any of the arms, legs or torso and the location and size of each application documented but must be the same area every day.~~

REVISED TEXT

3.1.5 Part C : A double-blind (sponsor unblinded) placebo-controlled, randomised to active or placebo treatment repeat dose study (3 doses over 7 days), in subjects with mild to moderate Chronic Spontaneous Urticaria (CsU)

Subjects will be randomised to a 3:1 ratio to receive active treatment or placebo applied to areas of the body chosen by the investigator once a day every 3 days, over a total period of 7 days. Dosing will occur on days 1, 4 and 7. The chosen area where cream is applied will remain the same throughout the dosing period. Dosing in Part C will only commence once relevant data from the first four subjects in Part B have been reviewed, to allow greater confidence in predictions of the pharmacokinetics in Part C. The BSA over which study treatment will be administered in Part C will be 10%, but is subject to change, if appropriate, based on preliminary data from the first 4 subjects in Part C However, the maximum BSA will not exceed 20% (see Figure 4 for a visual representation of the dosing schedule).

The first 4 subjects of Part C will be randomised so that one of the 4 subjects will receive placebo and the three other subjects will receive GSK2646264. An informal review of the preliminary data from the first 4 subjects may be conducted after they have completed the study. PK, safety, and tolerability, up to and including the follow-up visit, will be reviewed by the Sponsor (See Section 5.5). If required, a discussion with the SMC regarding any changes or adjustments to the dosing regimen in Part C will be held. Recruitment of the full planned cohort (n=16) may continue during any data review.

The timing and the assessments to be completed at each visit are provided in the Time and Event tables (Section 6.1.4). Early termination of this patient group may occur due to feasibility of recruitment and stopping criteria see (Section 5.5 and Section 5.6). Following inclusion in the study, subjects will be randomised. 10 mg/cm^2 of 1% GSK2646264 cream or placebo cream will be applied to a given BSA once per day, every 3 days, on days 1, 4and 7, on areas of the body chosen by the investigator. The areas where cream is applied will remain the same for each day of dosing. The 10 % BSA can be distributed between the arms, legs and torso for an individual subject and will be decided by the investigator prior to randomisation. This pattern of cream application will remain the same for the duration of dosing. The maximum % BSA and the frequency of dosing will be decided after Part A and n=4 in Part B of the study but will not exceed 20% BSA.

The subjects will be dosed in the unit for the first dose on day 1 and then discharged from the clinic. Subjects will return to the clinic prior to the dose and specified assessments on days 4 and 7. Subjects will be asked to complete a daily diary over the 7 days. Details of the diary will be provided in the Study Procedures Manual (SPM).

PREVIOUS TEXT

3.2.1.3 Part C: Chronic Spontaneous Urticaria Subjects

This part of the study will provide safety, tolerability, PK and PD ~~over 7 days of dosing~~ (with an additional 2 weeks follow-up post last dose for PK, safety and biomarker endpoints) to enable further clinical studies in this population.

REVISED TEXT

3.2.1.3 Part C: Chronic Spontaneous Urticaria Subjects

This part of the study will provide safety, tolerability, PK and PD **from dosing every 3 days, over a total period of 7 days** (with an additional 2 weeks follow-up post last dose for PK, safety and biomarker endpoints) to enable further clinical studies in this population.

ADDITIONAL TEXT

3.2.2.2 Additional preliminary data from Part A

Preliminary human PK data from Part A (up to 1% strength, up to 10% BSA) demonstrated a geometric mean Cmax after 4 days dosing of 5.59 ng/ml, providing a margin of 2.8 in comparison to the C_{max} NOEL achieved (~16ng/ml) . After 4 days of dosing the geometric mean AUC was 103.8 ng·h/ml, allowing a 2.1 fold systemic margin compared to the $AUC_{(0-t)}$ at the NOAEL.

This allows 3 days dosing at 1% strength, 10% BSA for Part B. For further progression of dosing in parts B and C refer to Section 5.5.3.3

Further predictive modeling using the data from Part A, but with no inclusion of elimination rate, suggests that coverage of 10% BSA using the 1% cream over 7 days of dosing would lead to an estimated Cmax of 10.12ng/ml and an AUC of 219.8ng·h/ml.

The information in the above paragraph is superseded by the information in section 3.2.2.3.

3.2.2.3 Additional preliminary data from Part B

A population pharmacokinetic model integrating all plasma data from part A and the preliminary data from n=4 in part B lead to an estimation of the half life of 57 hours for GSK2646264. This model shows that in order to maintain a 1.8 fold margin with the AUC NOAEL to allow women of child bearing potential in the trial the doses should be administered every three days, over a total 7 day duration, rather than every day.

PREVIOUS TEXT

Potential risk	**Summary of data**	**Impact- eligibility criteria**	**Strategy- monitoring/stopping criteria**
Hypersensitivity	There may be potential for hypersensitivity reactions from the procedures used in the study or if subjects are allergic to ingredients in the study medications. There is no previous experience of GSK2646264 or of the matched placebo in humans	Subjects will be asked at screening if they are allergic to any of the ingredients – if known, Subjects will be excluded from the study if they are allergic to any ingredient of the study medications or if they have previously experienced an anaphylactic reaction.	Subjects will undergo regular medical assessment and instructed what action to take in the event of experiencing an allergic reaction.
Skin prick	There have been rare reports of systemic allergic reactions following skin prick testing (Heinzerling, 2013)	Subjects will be excluded from the study if have previously experienced an anaphylactic reaction. Subjects will be excluded from the study if the subject has a reaction which contraindicates to the study	Skin prick testing will be performed in a facility with appropriate emergency equipment available in case of the need for treatment for systemic allergic reaction.
Pregnancy and lactation	There is no data available on the effect of GSK 2646264 in pregnancy and lactation in humans. Fetal malformations were seen after IV dosing to pregnant rabbits with a NOAEL that is expected to exceed the highest expected human exposure by >4 fold. No similar changes were seen in the rat after substantially higher exposure	Female subjects of non child bearing potential will be included in Part A of the study. Female subjects that are of child-bearing potential will be required to follow the contraceptive requirements that are outlined in the protocol Section 4.2.1 inclusion criteria for Part B and C. A decision to allow females of child-bearing potential in Parts B and C will be made based on the safety margin from the dosing in Part	Female subjects that are of child-bearing potential will undergo regular pregnancy testing and treatment stopped immediately if a subject is found to be pregnant during the study. These subjects must be on oral contraceptives 28 days before screening begins. They will undergo serum pregnancy testing at day -28, day -7 to -4, a serum or urine pregnancy test the day of the start of the study prior to dosing, then testing on an approximately weekly basis and a serum pregnancy test at the follow up visit.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy- monitoring/stopping criteria
		A of the study. Nursing females will be excluded from participating in the study.	
Immune cell effects, including neutropenia	SYK is involved in immunoreceptor signalling in a number of cell types, including mast cells, basophils, B cells, neutrophils and macrophages (Riccaboni, 2010) Studies with R788 in rheumatoid arthritis identified an increased risk of neutropenia in subjects treated with the oral SYK inhibitor compared to placebo (placebo 0.7%, R788 150mg OD 6.6%, R788 100mg BD 5.9%) (Weinblatt, 2010)	Subjects with co-morbid conditions that would put them at risk or laboratory values outside the reference range at screening would be excluded from participating in the study.	The product is being investigated in a topical formulation, which should limit potential systemic exposure. Increasing exposure will be performed gradually. Subjects will undergo regular haematological assessments, including white blood cell counts during the study. Treatment would be stopped if laboratory test results are identified that would put the safety of the subject at risk.
Thyroid	Minimal thyroid follicular epithelial hypertrophy was observed in all doses following iv administration of GSK2646264 in rats for 4 weeks. Thyroid function test (TSH, free and total T3 and T4) at single timepoints 12 hours post last dose were within normal range.	Requirement for normal TSH in all subjects in Part A, and normal TSH, free T4, T4 and T3 in Parts B and C.. Exclusion of subjects with history of Graves' disease or any thyroid cancer	The risk of thyroid effect in this study is considered not to be significant. Monitoring will be based on signs and symptoms and serum markers of thyroid function will be measured in patients in Parts B and C.

REVISED TEXT

Potential risk	**Summary of data**	**Impact- eligibility criteria**	**Strategy- monitoring/stopping criteria**
Hypersensitivity	There may be potential for hypersensitivity reactions from the procedures used in the study or if subjects are allergic to ingredients in the study medications. There is no previous experience of GSK2646264 or of the matched placebo in humans	Subjects will be asked at screening if they are allergic to any of the ingredients – if known, Subjects will be excluded from the study if they are allergic to any ingredient of the study medications or if they have previously experienced an anaphylactic reaction.	Subjects will undergo regular medical assessment and instructed what action to take in the event of experiencing an allergic reaction.
Skin prick	There have been rare reports of systemic allergic reactions following skin prick testing (Heinzerling, 2013)	Subjects will be excluded from the study if have previously experienced an anaphylactic reaction. Subjects will be excluded from the study if the subject has a reaction which contraindicates to the study	Skin prick testing will be performed in a facility with appropriate emergency equipment available in case of the need for treatment for systemic allergic reaction.
Pregnancy and lactation	There is no data available on the effect of GSK 2646264 in pregnancy and lactation in humans. Fetal malformations were seen after IV dosing to pregnant rabbits with a NOAEL that is expected to exceed the highest expected human exposure by >4 fold. No similar changes were seen in the rat after substantially higher exposure	Female subjects of non child bearing potential will be included in Part A of the study. Female subjects that are of child-bearing potential will be required to follow the contraceptive requirements that are outlined in the protocol Section 4.2.1 inclusion criteria for Part B and C. A decision to allow females of child-bearing potential in Parts B and C will be made based on the safety margin from the dosing in Part	Female subjects that are of child-bearing potential will undergo regular pregnancy testing and treatment stopped immediately if a subject is found to be pregnant during the study. **In part B**, these subjects must be on oral contraceptives 28 days before screening begins. They will undergo serum pregnancy testing at day -28, day -7 to -4, a serum or urine pregnancy test the day of the start of the study prior to dosing, then testing on an approximately weekly basis and a serum pregnancy test at the follow up visit.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy- monitoring/stopping criteria
		A of the study. Nursing females will be excluded from participating in the study.	**In Part C, a serum pregnancy test will be performed on the day of screening. If this is between Days -13 and -7, a further pregnancy test will not be required until Day 1. However, if the day of screening is between Day -28 and -14, an additional serum pregnancy test will be required between Days -7 and -4. A urine pregnancy test will be required prior to dosing on Day 1**
Immune cell effects, including neutropenia	SYK is involved in immunoreceptor signalling in a number of cell types, including mast cells, basophils, B cells, neutrophils and macrophages (Riccaboni, 2010) Studies with R788 in rheumatoid arthritis identified an increased risk of neutropenia in subjects treated with the oral SYK inhibitor compared to placebo (placebo 0.7%, R788 150mg OD 6.6%, R788 100mg BD 5.9%) (Weinblatt, 2010)	Subjects with co-morbid conditions that would put them at risk or laboratory values outside the reference range at screening would be excluded from participating in the study.	The product is being investigated in a topical formulation, which should limit potential systemic exposure. Increasing exposure will be performed gradually. Subjects will undergo regular haematological assessments, including white blood cell counts during the study. Treatment would be stopped if laboratory test results are identified that would put the safety of the subject at risk.
Thyroid	Minimal thyroid follicular epithelial hypertrophy was observed in all doses following iv administration of GSK2646264 in rats for 4 weeks. Thyroid function test (TSH, free and total T3 and T4) at single	Requirement for normal TSH in all subjects in Part A, and normal TSH, free T4, T4 and T3 in Parts B and C.. Exclusion of subjects with history of Graves' disease or any thyroid cancer	The risk of thyroid effect in this study is considered not to be significant. Monitoring will be based on signs and symptoms and serum markers of thyroid function will be measured in patients in Parts B and C.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy- monitoring/stopping criteria
	timepoints 12 hours post last dose were within normal range.		

PREVIOUS TEXT

Section 4.2.1.3 and Section 4.2.1.4

9. Female subjects must agree to use one of the contraception methods listed in Section 4.3.1 28 days before their screening visit and until the followup visit or a time period that is 5 terminal half-live post-last dose which will be determined following Part A of the studylonger.

REVISED TEXT

Section 4.2.1.3 and Section 4.2.1.4

9. Female subjects **of child bearing potential** must have been using one of the contraception methods listed in Section 4.3.1 **at least** 28 days before their screening visit and **agree to continue with these methods** until the follow up visit or **12 days after the last dose of study treatment, whichever is the longer**.

Section 4.2.2

ADDITIONAL TEXT

13. A positive drug screen at screening or Day -1. For Part B and Part C patients, a positive drug screen due to concomitant medication may be acceptable for inclusion in the study based on the Investigator's opinion. The Investigator's opinion must be based on interview of the subject, documented in the medical history and the Investigator is asked to document that the positive test can be explained by a relevant and permitted medication (see Section 5.1.5.1.) and that the medication will not interfere with the study procedures or compromise subject safety.

Section 4.3.2.2

ADDITIONAL TEXT

Fasting

- Subjects will be required to be fasted prior to the screening visit.

Section 4.3.3.1

PREVIOUS TEXT

In all dose cohorts, subjects will be allowed to shower/bathe approximately 2 hours prior to dosing and approximately 18-22 hours following dosing. During showering/bathing the subject should ~~use their normal soap or shower gel (mild, unscented and non-medicated)~~. A mild shampoo can be used for washing hair. The site of blood sampling will be protected (taped off and covered) allowing the subjects to shower freely, including washing the application site(s).

Timings of showering/bathing will be recorded.

Subjects must refrain from taking part in water based activities such as swimming and use of sauna/steam room for the duration of dosing until the follow-up visit.

REVISED TEXT

In all dose cohorts, subjects will be allowed to shower/bathe approximately 2 hours prior to dosing and approximately 18-22 hours following dosing. During showering/bathing the subject should only use mild, unscented, and non-medicated soap A mild shampoo can be used for washing hair. The site of blood sampling will be protected (taped off and covered) if required, allowing the subjects to shower freely, including washing the application site(s). Subjects should refrain from rubbing the areas where the cream has been applied.

Timings of showering/bathing must be recorded (as detailed in the SPM).

Subjects must refrain from taking part in water based activities such as swimming and use of sauna/steam room for the duration of dosing until the follow-up visit.

Section 5.12 Assessment of Compliance

PREVIOUS TEXT

~~In Part A & B –~~ When subjects are dosed at the study unit, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and start and end times of each dose administration in the clinic will be recorded in the source documents and transcribed into the InForm eCRF. The dose of study treatment and study participant 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.

~~In Part C- Subjects will visit the unit on the days as listed in the Time and Events table. On days where they do not visit the unit a study nurse will transport the treatment in the bottles (of known weight) as required for the nominal dose to be applied, from the site pharmacy to the subject's home. The study nurse shall administer the study treatment to the subject in their home environment. The date, start and end times of each dose administration will be recorded on cource documents and transcribed into the InForm eCRF. After the study visit is completed, the study nurse will return the bottles to the site pharmacy and weigh the bottles.~~

REVISED TEXT

When subjects are dosed at the study unit, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and start and end times of each dose administration in the clinic will be recorded in the source documents and transcribed into the InForm eCRF. The dose of study treatment and study participant 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.

Section 6.1.3

PREVIOUS TEXT

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
Visit Window (relative to Day 1)	-28 to -7 days								
Admission to Unit		X							
Informed Consent	X								
Demographics	X								
Complete physical	X							X	
Body weight (kg)	X								
Height (cm) without shoes	X								
Brief physical		X							
Medical/medication/ drug/alcohol history	X								
12-lead ECG[1]	X	X			X			X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X	X	X	X		X	X	
Urine drug/alcohol screen	X	X							
Pregnancy Test (women)[2]	X(S)		X(S/U)				X (S)	X(S)	2. Pregnancy test will be performed 3 times during screening, serum on day -28, day -7 to -4, and a serum or urine pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test between day 10-12 (to coincide with a PK sample) and at the follow up visit.
HIV, Hep B and Hep C screen	X								
Clinical Laboratory Tests	X	X			X			X	
Allergen Challenge-SPT	X[3,4]		X[3,6]		X[3,5]	X[9]			3. SPT- Skin Prick Test measurements will be performed ~15-20mins post challenge **13.** 4. SPT using all 4 specified allergens

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
									14. 5. SPT will be performed at 6 hours post dose (see Section 6.4.1)
									6. SPT will be performed pre-dose
TSH, free T4, T4, T3	X					x	X[7]	x	7. One sample between day 10-12
Cold Temp Test (Temp Test 4.0)	X		X[8]		X[10]	X[9]			8. Pre-dose 9. 24 hours post dose 10. 6 hours post dose
Randomisation		X							
Tolerability Assessment			←----- X[11] -----→						11. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		X	←---------X--------→					X	
Concomitant Medication		X	←-------X----------→					X	
Pharmacokinetic Blood Sample			X[12]	X[12]	X[12]	X[12]	X[13]	X	12. PK blood sample taken at pre dose,1, 4, 8, 12hr post dose 13. One PK blood sample per day will be taken as follows:. PK samples will be obtained on the following days (± 1 day): Day 6, 9, 12, & 15.
Study Treatment Dosing			X	X	X				
Discharge from unit						X			
Outpatient visit	X						X	X	
Blood Sample for CD69 expression[12]			X[10]		X[11]		X	X	10. Pre-dose 11. 4 hrs post-dose 12. Sample collection should coincide with PK sample collection.

REVISED TEXT

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
Visit Window (relative to Day 1)	-28 to -7 days[1]								1. Screening can occur on any day between 28 days and 7 days prior to Day 1 dosing.
Admission to Unit		X							
Informed Consent	X								
Demographics	X								
Complete physical	X							X	
Body weight (kg)	X								
Height (cm) without shoes	X								
Brief physical		X							
Medical/medication/ drug/alcohol history	X								
12-lead ECG	X[2]	X			X			X	2. Triplicate ECG at screening and CV risk factors
Vital signs[3]	X	X	X	X	X		X	X	3. Should be performed at the same time points as PK samples are taken
Urine drug/alcohol screen	X	X							
Pregnancy Test (women)[4]	X(S)		X(S/U)				X (S)	X(S)	4. Pregnancy test will be performed 3 times during screening, serum on day -28, day -7 to -4, and a serum or urine pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test between day 10-12 (to coincide with a PK sample) and at the follow up visit.
HIV, Hep B and Hep C screen	X								
Clinical Laboratory Tests	X	X			X			X	
Allergen Challenge-SPT	X[5,6]		X[5,8]		X[5,7]	X[5]			5. SPT- Skin Prick Test measurements will be performed ~15-20mins post challenge 6. SPT using all 4 specified allergens 7. SPT will be performed at 6 hours post dose (see Section 6.4.1), and at 24 hours post last dose on day 3

Day:	Screening	-1	1	2	3	4	Day 6 to 15	Follow-up (Day 17-19)	Notes
									8. SPT will be performed pre-dose on Day 1
TSH, free T4, T4, T3	X					x	X[9]	x	9. One sample between day 10-12 to coincide with PK sample
Cold Temp Test (Temp Test 4.0)	X		X10		X[11]	X[12]			10. Pre-dose 11. 6 hours post dose 12. 24 hours post last dose on Day 3
Randomisation		X							
Tolerability Assessment			←------X[13]-----→						13. Tolerability assessment at pre – and ~6 hours post-dose
AE assessment		←----------X----------→						X	
Concomitant Medication		X	←----------X----------→					X	
Pharmacokinetic Blood Sample			X[14]	X[14]	X[14]	X[15]	X[16]	X	14. PK blood sample taken at pre dose,1, 4, 8, 12hr post dose 15. One PK sample to be taken 24 hours post last dose on Day 3 16. One PK blood sample per day will be taken as follows:. PK samples will be obtained on the following days (± 1 day): Day 6, 9, 12, & 15.
Study Treatment Dosing			X	X	X				
Discharge from unit						X			
Outpatient visit	X						X	X	
Blood Sample for CD69 expression			X[17]		X[18]		X[19]	X[19]	17. Pre-dose 18. 4 hrs post-dose 19. Sample collection should coincide with PK sample collection.

Section 6.1.4

PREVIOUS TEXT

Day:	Screening	1	2	3	4	5	6	7		Day 10		Follow-up	Notes
Visit Window (relative to Day 1)	-28 to -7											21-23	
Informed Consent	X												
Demographics	X												
Complete physical	X											X	
Body weight (kg)	X												
Height (cm) without shoes	X												
Brief physical		X										X	
Medical/medication/ drug/alcohol history	X												
12-lead ECG[1]	X	X						X				X	1. Triplicate ECG at screening and CV risk factors questionnaire
Vital signs	X	X			X			X		X[13]		X	13. nly conducted if subjects is attending the clinical site.
Urine drug/alcohol screen	X	X											
Pregnancy Test (women)[2]	X(S)	X (S/U)								X(S)[14]		X(S)	2. Pregnancy test will be performed 3 times during screening, serum (S) on day -28, day -7 to -4, and a serum or urine (U) pregnancy test the day of the start of the study prior to dosing and a serum pregnancy test between day 14-16 and at the follow up visit 14. Samples should be obtained at approximately 1 week intervals

Day:	Screening	1	2	3	4	5	6	7		Day 10		Follow-up	Notes
HIV, Hep B and Hep C screen	X												
Clinical Laboratory Tests	X	X						X				X	
TSH, free T4, T4, T3	X							X				X	3. Between Days 14-16
Blood Sample for CD69 expression		X[4]			X[5]			X[5]		X[6]		X	4. Predose 5. 4 hours post dose 6. Sample to be taken every time a PK sample is taken
Randomisation		X											
UAS-7 Diary[7]	X	X	X	X	X	X	X	X					7. Screening UAS7 diary will be completed using 7 successive days before randomisation (day 1). If there is a lapse in any day prior to day 1 the subject must complete the full 7 days worth of diary entries again during the screening period.
QOL (DLQI)	X	X						X					
AAS	X[15]	X	X	X	X	X	X	X					15. Screening AAS will be completed using 7 successive days before randomisation (day 1) on the same days as the UAS7. If there is a lapse in any day prior to day 1 the subject must complete the full 7 days worth of diary entries again during the screening period.
AE assessment		X ----------										X	

Day:	Screening	1	2	3	4	5	6	7		Day 10		Follow-up	Notes
Tolerability Assessment at pre-dose		X[8] ----------											8. Tolerability assessment at pre dose on each day.
BHR- Basophil Histamine Release Test		X[9]											9. Two samples to be taken predose
Pharmacokinetic Blood Sample		X[10]			X[10]			X[10]					
Study Treatment Dosing		X	X[12]	**14.1.** X[12]	X	**14.2.** X[12]	X[12]	X					12. The subject may have study treatment applied at home by a nurse or they can travel to the clinic
Con Meds		X ----------											
Outpatient visit	X	X			X			X		x		X	

REVISED TEXT

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Visit Window (relative to Day 1)	-28 to -7[1]												1. Screening can occur on any day between 28 days and 7 days prior to Day 1 dosing.
Informed Consent	X												
Demographics	X												
Complete physical	X											X	
Body weight (kg)	X												
Height (cm) without shoes	X												
Brief physical		X										X	

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Medical/medication/drug/alcohol history	X												
12-lead ECG	X2	X						X				X	2. Triplicate ECG at screening and CV risk factors
Vital signs	X	X			X			X		X[3]	X[3]	X[3]	3. To be performed on same day as PK samples
Urine alcohol screen	X	X											
Pregnancy Test (Women of child bearing potential)4	X(S)[4]	X (U)[5]						X(U)[5]			X (S)	X(S)	4. A serum (S) pregnancy test will be performed on the day of screening. If the day of screening is between Days -13 and -7, a further pregnancy test will not be required until Day 1. However, if the day of screening is between Days -28 and -14, an additional serum pregnancy test will be required between Days -7 and -4. 5. A urine pregnancy test is required prior to dosing on Day 1 & Day 7
HIV, Hep B and Hep C screen	X												
Clinical Laboratory Tests	X	X						X				X	
TSH, free T4, T4, T3	X							X		X6	X6	X6	6. Sample to be taken on same day as PK sample
Blood Sample for CD69 expression		X7			X8			X8		X9	X9	X9	7. Pre-dose 8. 4 hours post dose 9. Sample to be taken on same day as PK sample
Randomisation		X											

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
UAS-7 Diary	X[10]		X[11]	X[11]	X[11]	X[11]	X[11]	X[11]	X				10. Screening UAS7 diary will be completed for the 7 successive days before Randomisation (Day 1). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 11. The UAS-7 diary should be completed pre-dose on those days where dosing occurs
QOL (DLQI)	X	X[12]							X				12. The baseline DLQI assessment on Day 1, looking back over the previous 7 days prior to dosing, should be completed pre-dose
AAS	X[13]		X[14]	X[14]	X[14]	X[14]	X[14]	X[14]	X				13. Screening AAS will be completed for the 7 successive days before Randomisation (Day 1) (on the same days as the UAS7). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 14. The AAS assessment should be completed pre-dose on those days where dosing occurs
AE assessment		←----------X----------→										x	
Tolerability Assessment at pre-dose		X15			X15			X15					15. Tolerability assessment at pre-dose on each day.
BHR- Basophil Histamine Release Test		X16											16. Two samples to be taken pre-dose

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Pharmacokinetic Blood Sample		X[17]			X[17]			X[17]		X[18]	X[18]	X[18]	17. PK blood sample taken at pre-dose and 4hr post dose 18. Sample to be taken on same day as CD69 sample, TSH, free T4, T4, T3 samples and vital signs
Study Treatment Dosing		X			X			X					
Con Meds		←----------X----------→										X	
Outpatient visit	X	X			X			X		x	X	X	

Section 6.3.3 Electrocardiogram (ECG)

ADDITIONAL TEXT

- A CV risk factors questionnaire will also be completed at screening

Section 6.3.4 Clinical Laboratory Assessments

PREVIOUS TEXT

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Albumin
Glucose (fasting)	Calcium	GGT	Total Protein
Sodium	Phosphate	Alkaline phosphatase	
TSH , free T4, T4, T3			

REVISED TEXT

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Albumin
Glucose, (**fasting at screening only)**	Calcium	GGT	Total Protein
Sodium	Phosphate	Alkaline phosphatase	
TSH , free T4, T4, T3			

Section 6.5.2

PREVIOUS TEXT

Part C

For the chronic spontaneous urticaria subjects, Urticaria activity score (UAS7), a composite endpoint, will be derived using key urticaria symptoms (number of weal, itch/pruritus):

UAS7 components will be captured using a daily diary for 7 days. The daily UAS7 score will be the sum of each key component score. A detail of UAS7 daily diary is provided in Appendix 3.

The Angioedema Activity Score (AAS)

For the chronic spontaneous urticaria subjects, will be captured between baseline and Day 7 using the Angioedema Activity Score (AAS). Details of the questionnaire are

provided in Appendix 7.

Health Outcomes

Dermatology Life Quality Index

For the chronic spontaneous urticaria subjects, health outcome measure will be captured at baseline and Day 7, using a 10-item Dermatology Life Quality Index (DLQI) (Finlay, 1994) questionnaire. An overall score will be calculated as well as the following domains: Symptoms and Feelings, Daily Activities, Leisure, Work and School, Personal Relationships, and Treatment. A detail of questionnaire is provided in Appendix 4.

REVISED TEXT

Part C

Urticaria Activity Score (UAS7)

For the chronic spontaneous urticaria subjects **in Part C**, Urticaria activity score (UAS7), a composite endpoint, will be derived using key urticaria symptoms (number of weal, itch/pruritus):

UAS7 components will be captured using a daily diary for 7 days. The daily UAS7 score will be the sum of each key component score. A detail of UAS7 daily diary is provided in Appendix 3. **The days on which to record in the UAS7 diary are captured in the T&E Table (Section 6.1.4). On days where dosing occurs, the UAS7 diary will be completed pre-dose.**

The Angioedema Activity Score (AAS)

For the chronic spontaneous urticaria subjects in Part C, the Angioedema Activity Score (AAS) will be captured during Screening, and between Day 2 and Day 8. Details of the questionnaire are provided in Appendix 7. **The days on which to record the AAS are captured in the T&E Table (Section 6.1.4). On days where dosing occurs, the AAS will be completed pre-dose.**

6.6 Health Outcomes

6.6.1 Dermatology Life Quality Index

For the chronic spontaneous urticaria subjects **in Part C, the** health outcome measure **of Quality of Life** will be captured at **Screening, B**aseline and Day **8**, using a 10-item Dermatology Life Quality Index (DLQI) (Finlay, 1994) questionnaire. An overall score will be calculated as well as the following domains: Symptoms and Feelings, Daily Activities, Leisure, Work and School, Personal Relationships, and Treatment. A detail of questionnaire is provided in Appendix 4**. The days on which to record the DLQI are captured in the T&E Table (Section 6.1.4). On days where dosing occurs (Day 1 only), it will be completed pre-dose.**

AMEDMENT 5

Summary of Amendment Changes with Rationale

The reason for this amendment is to document an administrative change in GSK primary medical monitor. Minor corrections also included below for the Part C T&E table.

List of specific changes:

SPONSOR/MEDICAL MONITOR INFORMATION PAGE

Medical Monitor and Sponsor Contact Information:

PREVIOUS TEXT:

Role	Name	Day Time Phone Number	After-hours Phone/Cell / Pager Number	Fax Number	GSK Address
Primary Medical Monitor	PPD				GSK Clinical Unit Cambridge, Addenbrooke's Hospital, Box 128, ACCI Building, Hills Road, Cambridge CB2 0GG, UK
Secondary Medical Monitor					Experimental Medicine Unit, Immuno-inflammation Therapy Unit GlaxoSmithKline Medicines Stevenage, Hertfordshire
SAE fax number				XXX	

REVISED TEXT:

Role	Name	Day Time Phone Number	After-hours Phone/Cell / Pager Number	Fax Number	GSK Address
Primary Medical Monitor	PPD			n/a	Experimental Medicine Unit, Immuno-inflammation Therapy Unit GlaxoSmithKline Medicines Stevenage, Hertfordshire
Secondary Medical Monitor	PPD				Experimental Medicine Unit, Immuno-inflammation Therapy Unit GlaxoSmithKline Medicines Stevenage, Hertfordshire
SAE fax number				XXX	

Section 6.1.4, Part C T&E Table

PREVIOUS TEXT:

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Visit Window (relative to Day 1)	-28 to -7[1]												1. Screening can occur on any day between 28 days and 7 days prior to Day 1 dosing.
Informed Consent	X												
Demographics	X												
Complete physical	X											X	
Body weight (kg)	X												
Height (cm) without shoes	X												
Brief physical		X										X	
Medical/medication/drug/alcohol history	X												
12-lead ECG	X2	X						X				X	2. Triplicate ECG at screening and CV risk factors
Vital signs	X	X			X			X		X[3]	X[3]	X[3]	3. To be performed on same day as PK samples
Urine alcohol screen	X	X											

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Pregnancy Test (Women of child bearing potential)4	X(S)[4]	X (U)[5]						X(U)[5]			X (S)	X(S)	4. A serum (S) pregnancy test will be performed on the day of screening. If the day of screening is between Days -13 and -7, a further pregnancy test will not be required until Day 1. However, if the day of screening is between Days -28 and -14, an additional serum pregnancy test will be required between Days -7 and -4. 5. A urine pregnancy test is required prior to dosing on Day 1 & Day 7
HIV, Hep B and Hep C screen	X												
Clinical Laboratory Tests	X	X						X				X	
TSH, free T4, T4, T3	X							X		X6	X6	X6	6. Sample to be taken on same day as PK sample
Blood Sample for CD69 expression		X7			X8			X8		X9	X9	X9	7. Pre-dose 8. 4 hours post dose 9. Sample to be taken on same day as PK sample
Randomisation		X											
UAS-7 Diary	X[10]		X[11]	X[11]	X[11]	X[11]	X[11]	X[11]	X				10. Screening UAS7 diary will be completed for the 7 successive days before Randomisation (Day 1). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 11. The UAS-7 diary should be completed pre-dose on those days where dosing occurs

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
QOL (DLQI)	X	X[12]							X				12. The baseline DLQI assessment on Day 1, looking back over the previous 7 days prior to dosing, should be completed pre-dose
AAS	X[13]		X[14]	X[14]	X[14]	X[14]	X[14]	X[14]	X				13. Screening AAS will be completed for the 7 successive days before Randomisation (Day 1) (on the same days as the UAS7). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 14. The AAS assessment should be completed pre-dose on those days where dosing occurs
AE assessment		←----------X----------→										x	
Tolerability Assessment at pre-dose		X15			X15			X15					15. Tolerability assessment at pre-dose on each day
BHR- Basophil Histamine Release Test		X16											16. Two samples to be taken pre-dose
Pharmacokinetic Blood Sample		X[17]			X[17]			X[17]		X[18]	X[18]	X[18]	17. PK blood sample taken at pre-dose and 4hr post dose 18. Sample to be taken on same day as CD69 sample, TSH, free T4, T4, T3 samples and vital signs
Study Treatment Dosing		X			X			X					
Con Meds		←----------X----------→										X	
Outpatient visit	X	X			X			X		x	X	X	

REVISED TEXT:

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
Visit Window (relative to Day 1)	-28 to -7[1]												1. Screening can occur on any day between 28 days and 7 days prior to Day 1 dosing.
Informed Consent	X												
Demographics	X												
Complete physical	X											X	
Body weight (kg)	X												
Height (cm) without shoes	X												
Brief physical		X											
Medical/medication/drug/alcohol history	X												
12-lead ECG	X2	X						X				X	2. Triplicate ECG at screening and CV risk factors
Vital signs	X	X			X			X		X[3]	X[3]	X[3]	3. To be performed on same day as PK samples
Urine alcohol screen	X	X											
Pregnancy Test (Women of child bearing potential)4	X(S)[4]	X (U)[5]						X(U)[5]			X (S)	X(S)	4. A serum (S) pregnancy test will be performed on the day of screening. If the day of screening is between Days -13 and -7, a further pregnancy test will not be required until Day 1. However, if the day of screening is between Days -28 and -14, an additional serum pregnancy test will be required between Days -7 and -4.

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
													5. A urine pregnancy test is required prior to dosing on Day 1 & Day 7
HIV, Hep B and Hep C screen	X												
Clinical Laboratory Tests	X	X						X				X	
TSH, free T4, T4, T3	X							X		X6	X6	X6	6. Sample to be taken on same day as PK sample
Blood Sample for CD69 expression		X7			X8			X8		X9	X9	X9	7. Pre-dose 8. 4 hours post dose 9. Sample to be taken on same day as PK sample
Randomisation		X											
UAS-7 Diary	X[10]		X[11]	X[11]	X[11]	X[11]	X[11]	X[11]	X				10. Screening UAS7 diary will be completed for the 7 successive days before Randomisation (Day 1). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 11. The UAS-7 diary should be completed pre-dose on those days where dosing occurs
QOL (DLQI)	X	X[12]							X				12. The baseline DLQI assessment on Day 1, looking back over the previous 7 days prior to dosing, should be completed pre-dose

Day:	Screening	1	2	3	4	5	6	7	8	Day 10 (± 1 day)	Day 15 (± 1 day)	Follow-up Day 22 (± 1 day)	Notes
AAS	X[13]		X[14]	X[14]	X[14]	X[14]	X[14]	X[14]	X				13. Screening AAS will be completed for the 7 successive days before Randomisation (Day 1) (on the same days as the UAS7). If there is a lapse in any day prior to Day 1 the subject must complete the full 7 days worth of diary entries again during the screening period. 14. The AAS assessment should be completed pre-dose on those days where dosing occurs
AE assessment		←----------X----------→										x	
Tolerability Assessment at pre-dose		X15			X15			X15					15. Tolerability assessment at 1 to 2 hours post-dose
BHR- Basophil Histamine Release Test		X16											16. Two samples to be taken pre-dose
Pharmacokinetic Blood Sample		X[17]			X[17]			X[17]		X[18]	X[18]	X[18]	17. PK blood sample taken at pre-dose and 4hr post dose 18. Sample to be taken on same day as CD69 sample, TSH, free T4, T4, T3 samples and vital signs
Study Treatment Dosing		X			X			X					
Con Meds		←----------X----------→										X	
Outpatient visit	X	X			X			X		x	X	X	