Protocol

Study ID: 213224

Official Title of Study: An open label, single centre, enabling study to investigate the optimum method for use of intradermal Substance P as a challenge agent in healthy participants

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TITLE PAGE

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Protocol Number: 213224

Compound Number or Name: None

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Short Title: Intradermal Substance P challenge in healthy participants

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SPONSOR SIGNATORY:

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PPD

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title: An open label single-centre, enabling study to investigate the optimum method for use of intradermal Substance P as a challenge agent in healthy participants

Short Title: Intradermal Substance P challenge in healthy participants

Rationale:

This study aims to establish a Substance P (SP) intradermal challenge model which will allow the future clinical evaluation of the pharmacological effects of antagonists to block or decrease the induced wheal and flare response.

Objectives and Endpoints:

Part 1

Objectives	Endpoints
Primary	
• To characterise the wheal response over time following skin challenge with ascending concentrations of SP on a single day	• Wheal response during the 2 h post-challenge period area under the curve (AUC) at each concentration of SP
Secondary	
• To characterise the wheal response over time following skin challenge with ascending concentrations of SP on a single day	• Wheal response during the 2 h post-challenge period at each concentration of SP (including maximum observed wheal response, time to maximum observed wheal response, time of complete wheal response disappearance)

Objectives	Endpoints
• To characterise the flare response over time following skin challenge with ascending concentrations of SP on a single day	• Flare response during the 2 h post-challenge period at each concentration of SP (including AUC, maximum observed flare response, time to maximum observed flare response, time of complete flare response disappearance)
• To evaluate the safety and tolerability of SP	• Incidence of Adverse Events (AE) and Serious Adverse Events (SAE) • Incidence of findings of clinical important changes in: o vital signs o laboratory safety data o 12-lead electrocardiogram (ECG)

Part 2

Objectives	Endpoints
Primary	
• To compare the wheal response over time following skin challenge with ascending concentrations of SP on 2 days	• Wheal response during the 2 h post-challenge period (AUC) at each concentration of SP across 2 challenge days
Secondary	
• To compare the wheal response over time following skin challenge with ascending concentrations of SP on 2 days	• Wheal response during the 2 h post-challenge period at each concentration of SP across 2 challenge days (including maximum observed wheal response, time to maximum observed wheal response, time of complete wheal response disappearance)

Objectives	Endpoints
• To compare the flare response over time following skin challenge with ascending concentrations of SP on 2 days	• Flare response during the 2 h post-challenge period at each concentration of SP across 2 challenge days (including AUC, maximum observed flare response, time to maximum observed flare response, time of complete flare response disappearance)

• To evaluate the safety and tolerability of SP	• Incidence of Adverse Events (AE) and Serious Adverse Events (SAE)
	• Incidence of findings of clinical important changes in:
	  ○ vital signs
	  ○ laboratory safety data
	  ○ 12-lead ECG

Overall Design:

This is an open label single-centre, 2-part, prospective enabling study of SP intradermal challenge in healthy participants. Parts 1 and 2 will run sequentially. A schematic of the overall study design is provided in Figure 1.

Disclosure Statement: This is a 2-part, single arm, sequential study with no masking.

Number of Participants:

Part 1 will enrol a sufficient number of participants to achieve up to 12 participants who have completed the challenge visit. Once Part 1 is complete, SP concentrations to be given in Part 2 will be confirmed. Part 2 will occur only if Part 1 is successful.

Part 2 will enrol a sufficient number of participants to achieve up to 20 participants who have completed the 2 challenge visits. The study may be stopped after review of data from about the first 10 participants.

Participants who withdraw or are withdrawn from the study may be replaced. In Part 2, following screening, replacement participants must start at Challenge Visit 1.

Participants may be enrolled into Part 1 or Part 2, not both. Therefore, the anticipated sample size is up to 32 participants (not including replacements).

Intervention Groups and Duration:

All participants will attend the clinical unit for a screening visit (V0) within 28 days before their first or only challenge visit.

In Part 1, participants will attend the unit as outpatients for 1 challenge visit. The duration of Part 1, including screening, is not expected to exceed 4 weeks for each participant.

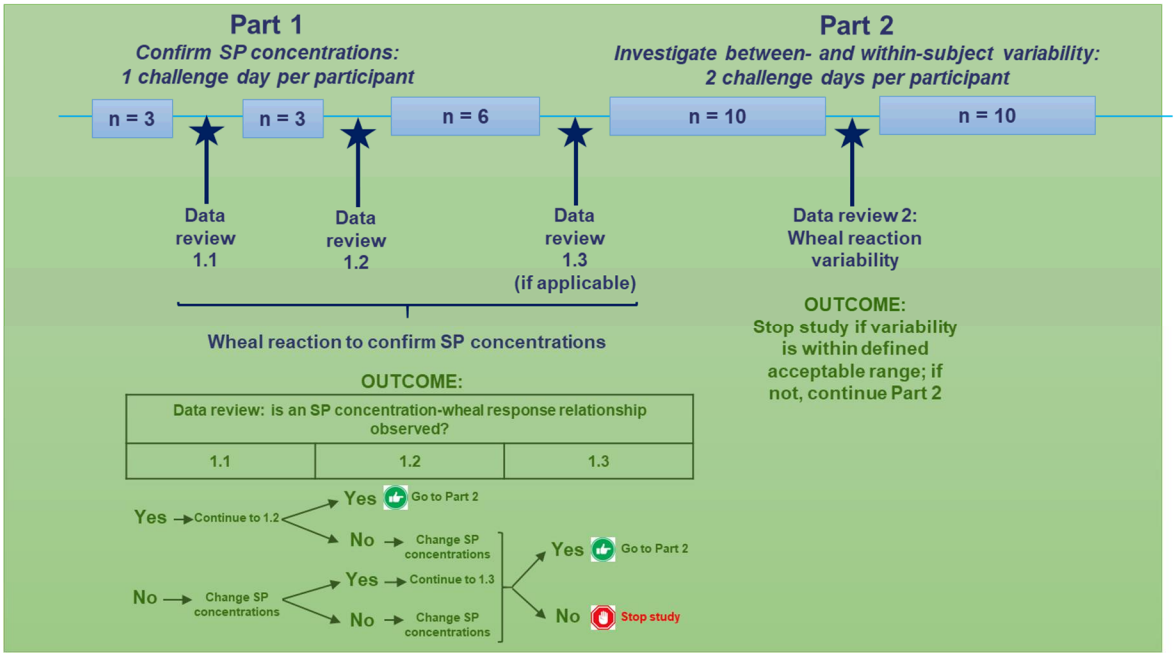
In Part 2, participants will attend the unit as outpatients for 2 challenge visits and have 1 follow--up phone call; the timing of visits will be decided based on emerging data. The duration of Part 2, including screening and follow-up is not expected to exceed 7 weeks for each participant.

A schematic of each challenge day is provided in Figure 2.

Data Monitoring or Other Committee: No

1.2. Schema

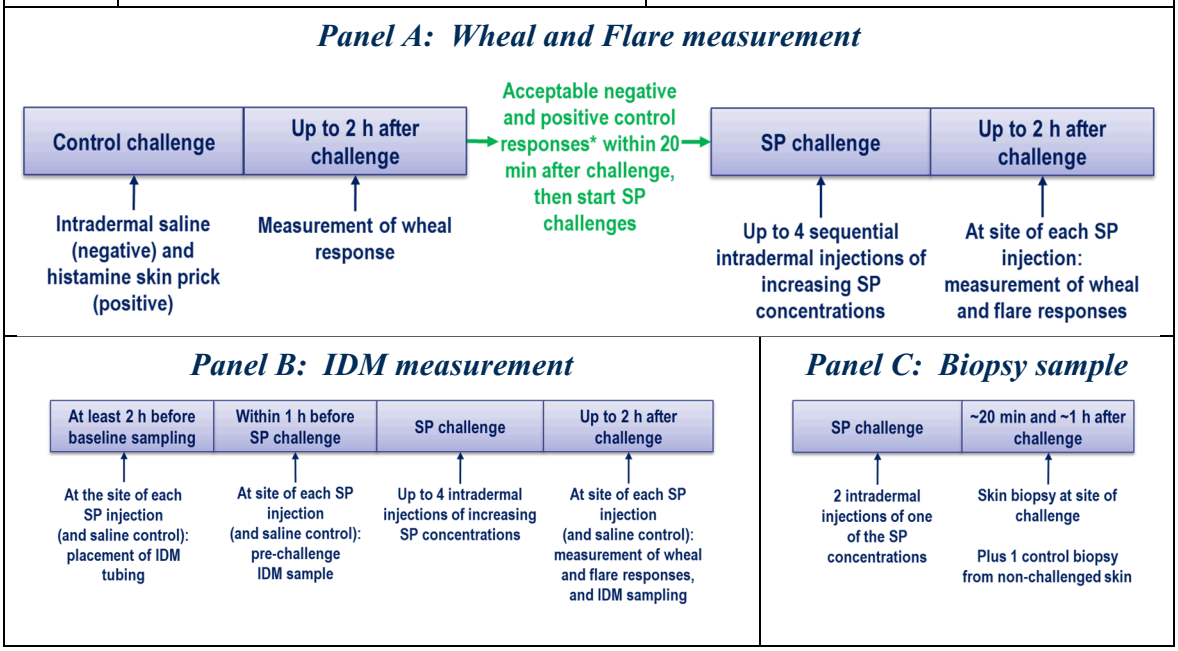
Figure 1 Overall study design schematic



Abbreviations: SP = Substance P.

Figure 2 Schematics of challenge days

	Challenge Visit 1	Challenge Visit 2
Part 1	• Wheal and flare post SP challenges (Panel A) only	Not applicable
Part 2	• Wheal and flare post SP challenges (Panel A); and • IDM during SP challenges (Panel B)	• Wheal and flare post SP challenges (Panel A) • IDM during SP challenges (Panel B) • Biopsy post challenges (Panel C) • Before first and after last SP challenge: biomarker blood sample^



Abbreviations: IDM = intradermal microdialysis; SP = Substance P.
^ To coincide with safety laboratory blood sampling.
* Acceptable saline and histamine responses are defined in the Study Reference Manual (SRM).

1.3. Schedule of Activities (SoA)

Protocol waivers or exemptions are not allowed. Therefore, adherence to the study design requirements, including those specified in the SOA, are essential and required for study conduct. This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the SOA.

1.3.1. Screening

Visit (V)	**V0**	**Notes**
Procedure	**Screening** (up to 28 days before Day 1)	
Informed consent	X	
Inclusion and exclusion criteria	X	
Demography	X	
Complete physical examination including height and weight	X	
Medical /medication/drug/alcohol/tobacco history	X	Includes Substance usage and current medical condition
Drug/alcohol/cotinine test	X	As per standard local practice
HIV, Hepatitis B and C screening	X	If test otherwise performed within 3 months before first challenge day, testing at screening is not required.
Serum pregnancy test (WCBP only)	X	
FSH and estradiol (in WNCBP)	X	As required, if postmenopausal state in women is in doubt.
12-lead ECG	X	In triplicate. Mean of 3 measure used to confirm eligibility.
Vital signs	X	Blood pressure and heart rate, in triplicate. Mean of 3 measure used to confirm eligibility. Single measurement of temperature.
Control challenge (histamine/saline)	X	
Clinical chemistry/Haemtology and urinalysis	X	
SAE review	X	From signing consent

Abbreviations: ECG = electrocardiogram; HIV = human immunodeficiency virus; SAE = serious adverse event; WCBP = women of childbearing potential; WNCBP = women of non childbearing potential.

1.3.2. Part 1 & Part 2 Challenge Visits

Visit (V)	V1, V2 (Part 2 only)					Follow-up phone call (5-7 days post last challenge day)	Early withdrawal	Notes
Procedure	Pre-challenge	Within 1 h before first control challenge	At least 20 min before first SP challenge	SP challenges	2 h after last SP challenge			
Clinical chemistry/ Haematology and urinalysis		X			X*		X	*In Part 2, 2 h post last SP challenge at last challenge visit only.
12-lead ECG		X			X		X	Triplicate measurements.
Vital signs		X			X		X	Refer to Section 8.2.2.
Brief PE		X						
Urine pregnancy test (WCBP only)	X							
Visual check of planned challenge sites	X							Prior to any other study procedures.
Saline and Histamine challenges			X					Histamine to be administered at least 20 cm from any IDM sampling
SP challenge				X				Part 1: Up to 4 SP injections: increasing concentrations of SP at a minimum of 20-min intervals. Part 2: Up to 10 SP injections: • up to 4 increasing concentrations of SP at a minimum of 20-min intervals, in duplicate

Visit (V)	**V1, V2 (Part 2 only)**					**Follow-up phone call (5-7 days post last challenge day)**	**Early withdrawal**	**Notes**
Procedure	**Pre-challenge**	**Within 1 h before first control challenge**	**At least 20 min before first SP challenge**	**SP challenges**	**2 h after last SP challenge**			
								• a further 2 SP injections at one concentration may also be given, if ongoing data suggest biopsy samples are required
Wheal and flare assessment		See Section 1.3.3						
Blood sample for exploratory biomarkers		X			X			Part 2, V2 only.
IDM		See Section 1.3.3						Part 2 only
Skin punch biopsy		See Section 1.3.3						Part 2, V2 only. May or may not be performed based on ongoing data.
AE review	□	←==========→					X	
SAE review	□	←==========→					X	
Concomitant medication review		X						Review of medications since last visit, if applicable.
Follow-up on possible reactions at sites of IDM/biopsy						X		Part 2 only.

Abbreviations: AE = adverse event; ECG = electrocardiogram; IDM = intradermal microdialysis; SAE = serious adverse event; SP = substance P.

1.3.3. Each challenge

Procedure	Min in relation to time of each intradermal injection of each SP or saline, or the time of skin prick administration of histamine											Notes
	Pre-challenge	0	5	10	15	20	30	40	60	90	120	
Challenge		X										
Wheal and flare assessment			X	X	X	X		X	X	X	X	See Section 8.5.2 for methods.
IDM (SP and saline challenged sites, as indicated in Figure 2)	X	←==========→										Part 2 only (see Figure 2). Tubing inserted at least 2 h before baseline sampling. A 30-min pre-challenge sample will be taken within 1 h before challenge. Post-challenge samples will be collected in 10-min interval from 0–120 min post-challenge.
Skin punch biopsy						X			X			Part 2, Challenge Visit 2 only (see Figure 2). 20-min and 60-min samples to be taken from different SP challenged sites; a third biopsy to be taken from a non-challenged area of skin to be collected at approximately the same time as the 20-min SP biopsy. Biopsy sampling may or may not be included in the Part 2 based on ongoing data.

Abbreviations: IDM = intradermal microdialysis; SP = substance P.

- The timing and number of planned study assessments, including the challenge or sampling and assessment time points in Part 2 (safety, wheal and flare, biomarker samples [including IDM and skin punch biopsy]) may be altered during the course of the study based on newly available data.
- Any changes to the timing or addition of time points for any planned study assessments in Part 2 as the result of emerging data from Part 1 of this study must be documented and approved by the relevant study team member and then archived in the sponsor and site study files but will not constitute a protocol amendment. The Competent Authority (CA) and ethics committee (EC) will be informed of any safety issues that constitute a substantial amendment and require alteration of the safety monitoring scheme or amendment of the informed consent form (ICF). The changes will be approved by the EC before implementation.
- Acceptable time windows around the nominal time points for specific assessments will be included in the Study Reference Manual (SRM) and assessments performed within these time windows will not constitute a protocol deviation.

2. INTRODUCTION

2.1. Study Rationale

This study aims to establish and validate a Substance P (SP) intradermal challenge model which will allow the future clinical evaluation of the pharmacological effects of antagonists to block or decrease the induced wheal and flare response.

2.2. Background

SP is a neuropeptide with an important role in neurogenic inflammation [Mashaghi, 2016]. When it is injected intradermally it causes wheal and flare responses, which increase in size with increased concentrations of SP [Borici-Mazi, 1999; Grouzmann, 2011; Sabroe, 1997; Zirbs, 2013]. While wheal and flare response to SP has been previously studied, the methods used to determine effect size differ between published reports and variability is difficult to establish. In addition, previous studies did not investigate possible modulation of pro-inflammatory markers and immune cells within the skin following SP challenge. This knowledge will aid the understanding of the mechanistic pathways downstream of SP activation.

Techniques such as intradermal microdialysis (IDM) can be used to take samples of interstitial fluid [Holmgaard, 2012; Baumann, 2019]. IDM involves insertion of dialysis membranes into the dermis, which is then perfused at a low speed with a physiological solution (the perfusate). Endogenous or exogenous molecules soluble in the extracellular fluid diffuse into the membrane and are collected in small vials for analysis. The technique has not previously been used to take samples from SP challenged skin.

This will be a 2-part study: Part 1 will aid understanding of the wheal and flare responses following intradermal SP Part 2 will investigate the variability of the responses, including the release of locally acting mediators and may include immune cells markers that can be detected in the skin after challenge..

2.3. Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of SP can be found in the Participant Information Leaflet.

2.3.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Intradermal Substance P (SP) Challenge Agent		
Substance P intradermal challenge	Transient local and limited regional skin flushing and local urticarial-like oedema has been observed with **intradermal administration**. One study with total doses of 5575 pmol intradermally in a single day had no systemic effects noted [Zirbs, 2013]. A second study administered a total dose of 5555 pmol [Grouzmann, 2011] with only minor flushing and no blood pressure effects. Transient flushing, tachycardia, dizziness, and decreased blood pressure have been observed with **intravenous** dosing at >16 pmol/min for 6 minutes (96 pmol). [Newby , 1997].	Only small doses of Substance P will be administered intradermally. For Part 1 the planned doses will total 220 pmol and Part 2 the planned doses will total 740 pmol resulting in a 25x and 7.5x coverage from the previously safe doses administered in reported publications [Zirbs, 2013; Grouzmann, 2011]. There is a provision to administer up to 500 pmol should no wheal and flare occur at lower doses. This top allowed dose would result in a Part 1 total dose of 715 pmol and a Part 2 total dose of 2430 pmol, still resulting in a 7.8x and 2.3x coverage from the previously safe doses administered in reported publications [Zirbs, 2013; Grouzmann, 2011]. The participants will be closely monitored during administration of SP challenge. The clinical site will have appropriate precautions and treatments in place during

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		administration and post-administration observation.
Skin prick test-Histamine Control Agent		
Histamine intradermal challenge	Highly sensitised patients may experience stronger local reactions. In extremely rare cases generalised side reactions and even severe systemic reactions (anaphylactic shock) may occur. Mild systemic adverse reactions include rhinitis, conjunctivitis, asthma, generalized exanthema, or urticaria. More severe adverse reactions include itching of tongue, throat, palms or soles. The reaction may progress to cyanosis, tachycardia, dizziness, headache, bronchial constriction, urticaria, asthma, marked hypotension or hypertension, vomiting, urination or defecation.	Histamine challenge is an established proinflammation/allergy test. Only small concentrations will be administered via skin prick. One drop (~50 µL) of 1.7 mg/mL solution) is applied to the skin and a prick needle is passed through to introduce a small amount intradermally. This dose and technique are commonly used in histamine skin challenge test. The participants will be closely monitored during administration of histamine challenge. The clinical site will have appropriate precautions and treatments in place, per the product label, during administration and post-administration observation.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Intradermal Microdialysis	Mild pain, bruising and irritation at dialysis site	Site staff under supervision of experienced staff will follow local standard approaches to perform the procedure.
Phlebotomy	Fainting, mild pain, bruising, irritation or redness at a phlebotomy site may be associated with blood draws.	Experienced site staff will follow local standard approaches for managing events related to blood draws. Participants are instructed to visually inspect phlebotomy sites after clinical visit are informed what signs of inflammation/infection to watch for and contact the investigator with any concerns.
Skin Biopsy	Complications of punch skin biopsy are uncommon but may include bleeding, infection, and scarring. Scarring risk is increased in individuals with a history of scarring and in individuals with darker skin.	Exclusion criterion #3: History of risk for or actual experience of complications from skin biopsy including excess bleeding, infection, or scarring/keloid formation (Participants with darker skin (Fitzpatrick>2) will be excluded). Observation of the subject while in the clinical unit and at follow-up after discharge from the unit.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		Participants are instructed to visually inspect skin biopsy sites after clinical visit and are informed what signs of inflammation/infection to watch for and contact the investigator with any concerns.

2.3.2. Benefit Assessment

There will be no benefit to the participant taking part in this study. However, participants will be contributing to the process of developing new methodologies to assess future medicines by taking part in the study.

2.3.3. Overall Benefit: Risk Conclusion

The measures taken to minimize the potential risks identified in association with intradermal administration of SP are considered sufficient to justify participation by healthy participants.

3. OBJECTIVES AND ENDPOINTS

Part 1

Objectives	Endpoints
Primary	
• To characterise the wheal response over time following skin challenge with ascending concentrations of SP on a single day	• Wheal response during the 2 h post-challenge period area under the curve (AUC) at each concentration of SP
Secondary	
• To characterise the wheal response over time following skin challenge with ascending concentrations of SP on a single day	• Wheal response during the 2 h post-challenge period at each concentration of SP (including maximum observed wheal response, time to maximum observed wheal response, time of complete wheal response disappearance)

• To characterise the flare response over time following skin challenge with ascending concentrations of SP on a single day	• Flare response during the 2 h post-challenge period at each concentration of SP (including AUC, maximum observed flare response, time to maximum observed flare response, time of complete flare response disappearance)
• To evaluate the safety and tolerability of SP	• Incidence of Adverse Events (AE) and Serious Adverse Events (SAE) • Incidence of findings of clinical important changes in: o vital signs o laboratory safety data o 12-lead electrocardiogram (ECG)

Part 2

Objectives	Endpoints
Primary	
• To compare the wheal response over time following skin challenge with ascending concentrations of SP on two days	• Wheal response during the 2 h post-challenge period (AUC) at each concentration of SP across 2 challenge days
Secondary	
• To compare the wheal response over time following skin challenge with ascending concentrations of SP on two days	• Wheal response during the 2 h post-challenge period at each concentration of SP across 2 challenge days (including maximum observed wheal response, time to maximum observed wheal response, time of complete wheal response disappearance)

Objectives	Endpoints
• To compare the flare response over time following skin challenge with ascending concentrations of SP on two days	• Flare response during the 2 h post-challenge period at each concentration of SP across 2 challenge days (including AUC, maximum observed flare response, time to maximum observed flare response, time of complete flare response disappearance)
• To evaluate the safety and tolerability of SP	• Incidence of Adverse Events (AE) and Serious Adverse Events (SAE) • Incidence of findings of clinical important changes in: o vital signs o laboratory safety data o 12-lead ECG
Exploratory	
• To investigate the effect of intradermal SP challenges on local exploratory biomarkers of mast cell activation.	• Change in levels of exploratory biomarkers in skin during up to 2hr post challenge time at each of the 2 challenge days.

4. STUDY DESIGN

4.1. Overall Design

This is an open label, single centre, 2-part, prospective enabling study of SP intradermal challenge in healthy participants. Parts 1 and 2 will run sequentially. Part 1 will include 1 challenge visit; Part 2 will include two challenge visits.

All participants will attend the clinical unit for a screening visit within 28 days before their first or only challenge visit. Eligible participants will be enrolled into Part 1 or Part 2, based on their availability.

- In Part 1, participants will attend the unit as outpatients for 1 challenge visit. The duration of Part 1, including screening, is not expected to exceed 4 weeks for each participant. .

- In Part 2, participants will attend the unit as outpatients for 2 challenge visits and have 1 follow-up phone call; the timing of visits will be decided based on emerging data. The duration of Part 2, including screening and follow-up is not expected to exceed 7 weeks for each participant.

At each challenge visit, up to 4 concentrations of SP will be assessed, as follows:

- In Parts 1 and 2:
  - Participants will first receive saline, by intradermal injection, and histamine by skin prick, as negative and positive controls.
    - If the wheal responses within the 20 min after each control challenge do not meet the acceptable saline and histamine responses (as defined in the SRM), the participant will not receive SP.
      - If it happens in Part 1 or on Challenge Visit 1 in Part 2, the participant will be withdrawn from the study.
      - If it happens on Challenge Visit 2 in Part 2 and if the participant marginally fails to meet the control criteria (as defined in the SRM), they may have the tests repeated. If they still fail to meet the control criteria, they will be withdrawn from the study.
  - Following confirmation of acceptable control responses, participants will receive up to 4 intradermal injections of SP at different concentrations; injections will be given sequentially from lowest to highest concentration; wheal and flare responses will be assessed after each challenge.

In Part 2 only:

- Participants will receive up to 6 additional SP intradermal injections for biomarker analysis:
  - IDM challenges: 4 increasing concentrations of SP (the same concentrations as those administered in the wheal and flare challenges); IDM samples will be taken at the site of each challenge, before and after challenge.
  - Biopsy challenges at Challenge Visit 2: 2 at the same concentration of SP; 3 biopsies would be taken – one from each challenged site and one from a non-challenge area of skin. (these challenges and biopsies may or may not be performed, based on review of ongoing data.)
- Biomarker blood samples will be taken before the first SP challenge and after the last SP challenge at Challenge Visit 2.

Wheal response data will be reviewed during the study:

- After about 3, 6 and 12 participants who have completed the challenge visit (as applicable) in Part 1 – to confirm SP concentrations to be given in Part 2
- After about 10 participants who have completed the two challenge visits in Part 2 – to determine whether enough data have been generated to understand the variability of the wheal response

4.2. Scientific Rationale for Study Design

The primary endpoint in this study is the wheal response, which directly links SP to inflammation. The wheal response is caused by accumulation of mast cell proteins in the dermis and presents as a raised oedema [Takahashi, 2004]. Flare, a secondary effect of degranulation, will also be measured. Mast cell degranulation stimulates neuropeptide release from neurons, which in turn causes superficial vasodilation presenting as a flare response, characterised by a reddened area of skin [Schmelz, 2000].

Exploratory biomarkers of mast cell activation and the resulting immune response may be measured, at the site of SP challenge and systemically, to aid understanding of the wheal and flare responses.

Wheal and flare responses to intradermal SP challenge peak and resolve over about 2 h in healthy people [Borici-Mazi, 1999]. Therefore, a 2 h sampling window following challenge is deemed appropriate. If review of wheal response data from Part 1 indicate that a longer or shorter sampling window is more appropriate, the sampling window will be changed accordingly; the maximum post challenge- sampling window will be 4 h.

It is unknown whether SP responses could change upon repeated challenge – e.g. because of exhaustion of mast cells. Timing of challenge days in Part 2 of this study will be chosen to evaluate anticipated intervals between challenge days in subsequent studies of antagonists.

4.3. Justification for Substance P concentrations

Published data indicate that the wheal and flare responses to intradermal SP continue to increase with increasing concentrations, even up to 100 µmol/L [Zirbs, 2013]. However, at the cellular level in an in-vitro mast cell line, SP begins to exert effects at ~0.1 µmol/L and the effect plateaus at ~10 µmol/L. The probable reason for the result in human skin is as the concentration of SP increases, it diffuses to a larger area of the dermis, causing larger reactions by activating more mast cells. To avoid concentrations of SP that are well above maximum effectiveness, the human mast cell line data were used to determine the SP concentrations in this study. Concentrations of 0.1, 0.3, 1 and 3 µmol/L SP are expected to give a range up to ~95% of the maximum effect at the cellular level. Those concentrations have been shown to cause wheal and flare responses when given intradermally to healthy people [Borici-Mazi, 1999; Grouzmann, 2011; Sabroe, 1997; Zirbs, 2013].

To allow for possible differences between published data and our SP challenge model, the concentrations of SP may be changed during the study in order to elicit the desired wheal response. For example, if the wheal response following one or more SP concentrations was indistinguishable from the wheal response following saline, and/or the wheal response was indistinguishable between 2 concentrations of SP, the concentrations could be adjusted accordingly. The maximum amount of SP administered in a single injection will not exceed 50 µL of 10 µmol/L.

The SP concentrations administered in Part 2 will be selected to ensure that the lowest concentration gives a wheal response larger than the wheal response following saline, and that each increasing concentration gives a wheal response larger than that at the previous concentration.

4.4. End of Study Definition

A participant is considered to have completed the study if he/she has completed all phases of the study including the follow up phone call as shown in the Schedule of Activities.

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

5. STUDY POPULATION

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

5.1. Inclusion Criteria

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

Age

1. Participant must be 18 to 50 years of age inclusive, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participants who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests, ECGs and vital signs.
3. Participants who responded positive to histamine skin prick test and negative to saline injection at screening.
4. Participants with Fitzpatrick skin type I-II (Caucasian).

Weight

5. Body weight ≥50 kg and body mass index (BMI) within the range 19–29.9 kg/m^2 (inclusive).

Sex

Sex and Contraceptive/Barrier Requirements

6. Male participants are eligible to participate in the study

- A female participant is eligible to participate if she is not pregnant or breastfeeding, and one of the following conditions applies:
    - Is a woman of non-childbearing potential (WONCBP) as defined in Section 10.4 : Contraceptive and Barrier Guidance.

      OR

    - Is a WOCBP and using a contraceptive method that is highly effective, with a failure rate of <1%, as described in Section 10.4 during the study intervention period. The investigator should evaluate potential for contraceptive method failure (e.g., noncompliance, recently initiated) in relationship to the first challenge.
- A sensitive pregnancy test is required to be negative on the day of each challenge. This pregnancy test is able to detect a pregnancy near the threshold of implantation, correlating with the earliest point of potential exposure. Due to the short half-life of the interventions, a negative highly sensitive pregnancy test as scheduled in the protocol mitigates the risk of pregnancy exposure because exposure will be completely gone even with a worst case but unlikely scenario of conception occurring near the time of dosing. See Section 8.2.5 Pregnancy Testing
- If a urine test cannot be confirmed as negative (e.g., an ambiguous result), a serum pregnancy test is required. In such cases, the participant must be excluded from participation if the serum pregnancy result is positive.
- Additional requirements for pregnancy testing during and after study intervention is located in Section 8.2.5 .
- The investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy

Informed Consent

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

5.2. Exclusion Criteria

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

Medical Conditions

1. Significant history of or current, cardiovascular (including hypotension, severe hypertension, vasomotor instability), respiratory (including asthma), renal, gastrointestinal, endocrine, haematological, infectious or neurological disorders constituting a risk when taking part in the study or interfering with the interpretation of data.

2. History or presence of significant skin disorder (such as but not limited to chronic urticaria, atopic dermatitis, severe eczema, psoriasis or skin cancer).
3. History of risk for or actual experience of complications from skin biopsy including excess bleeding, infection, or scarring/keloid formation.
4. Abnormal blood pressure as determined by the investigator.
5. Alanine transaminase (ALT) >1.5x upper limit of normal (ULN).
6. Total bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if total 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).
8. QT interval corrected for heart rate according to Fridericia's formula (QTcF) >450 msec, based on the mean of triplicate ECGs .

   NOTE: The QTc is the QT interval corrected for heart rate according to Fridericia's formula (QTcF).

Prior/Concomitant Therapy

9. Use of any form of H1 or H2 antihistamine, tricyclic antidepressants, beta2 agonists, dopamine, or beta blocking agents within 14 days before the first challenge visit through final assessments.
10. Use of topical medications such as but not limited to retinoids, steroids, and transdermal hormone replacement therapies on or near the intended site of application within 8 weeks prior to dosing through treatment follow up. Use of other topical preparations such as those containing vitamins, supplements or herbal within 2 weeks prior to dosing through treatment follow up.
11. Past or intended use of any other non-topical over-the-counter or prescription medication, including herbal medications, within 7 days before the first challenge visit, unless, in the opinion of the investigator and GSK Medical Monitor, the medication will not constitute a risk when taking the study intervention or interfere with the interpretation of data.

Prior/Concurrent Clinical Study Experience

12. Participation in the study would result in loss of blood or blood products in excess of 500 mL within 3 months.
13. Current enrolment in any clinical study involving an investigational study intervention or any other type of medical research.
14. Current enrolment or past participation in this study.

Diagnostic assessments

15. Presence of Hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HbcAb) at screening or within 3 months before the first challenge day.
16. Positive Hepatitis C antibody test result at screening or within 3 months before the first challenge day.

NOTE: Subjects with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA test is obtained

17. Positive Hepatitis C RNA test result at screening or within 3 months before the first challenge day.

    NOTE: Test is optional and subjects with negative Hepatitis C antibody test are not required to also undergo Hepatitis C RNA testing

18. Positive pre-study drug/alcohol screen.
19. Positive human immunodeficiency virus (HIV) antibody test at screening or within 3 months before the first challenge day.
20. Current use of known drugs of abuse.

Other Exclusions

21. Participants who present with damaged skin including sunburn, scar tissue, moles, uneven skin tones and dark skin tone (Fitzpatrick>2), tattoos, body piercings, branding or other skin disfiguration on or near the intended site of application which could interfere with the assessments.
22. Regular alcohol consumption within 6 months before 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.
23. Smoking test result indicative of smoking, history or regular use of tobacco- or nicotine-containing products (e.g. nicotine patches or vaporizing devices) within 6 months prior to screening.
24. Sensitivity to any of the study interventions, or components thereof, or drug or other allergy that, in the opinion of the investigator or medical monitor, contraindicates participation in the study.
25. Unable to refrain from the use of topical medications from before the first to after the last challenge visit.

5.3. Lifestyle Considerations

Not applicable.

5.3.1. Caffeine, Alcohol, and Tobacco

- During each visit, participants will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate).
- Participants will abstain from alcohol for 24 hours before each visit.
- Only non-smokers are allowed to participate in this study, therefore the use of tobacco products will not be allowed during the study.

5.3.2. Activity

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

5.4. Screen Failures

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

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened only once after consultation and on approval of the GSK Medical Monitor. Rescreened participants should be assigned a new participant number. In the event of out -of -range results of safety results, the test maybe repeated once within the screening window without this being considered a rescreen. If a retest result is again outside the reference range and considered clinically significant by the investigator and GSK medical monitor, the participant will be considered a screen failure.

Individuals who meet the eligibility criteria and are reserve participants who are subsequently not required may also be rescreened for a start date later in the study.

6. STUDY INTERVENTION AND CONCOMITANT THERAPY

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

6.1. Study Intervention(s) Administered

Participants will receive saline and SP by intradermal injection, and histamine by skin prick.

Saline and histamine will be sourced by the site; histamine will be administered in accordance with manufacturer’s instructions.

SP drug substance will be supplied by GSK and prepared into drug product extemporaneously at the trial site prior to administration.

Intervention Name	Substance P
Type	Drug
Formulation	Solution for injection
Unit Strength(s)	3.0, 1.0, 0.3 and 0.1 µmol/L (with possible increase up to 10 µmol/L, if no wheal and flare response is observed)
Dosage Level(s)	50 µL of: 150, 50, 15 and 5 pmol (with possible increase up to 500 pmol if no wheal and flare response is observed)
Route of Administration	Intradermal injection
Use	Challenge agent
Sourcing	Drug substance provided centrally by sponsor then prepared to drug product locally by the trial site.
Packaging and Labelling	Study Intervention will be provided in vials. Each vial will be labelled as required per country requirement.

6.2. Preparation/Handling/Storage/Accountability

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

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

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

6.3. Measures to Minimize Bias: Randomization and Blinding

Not applicable.

6.4. Study Intervention Compliance

- When the individual SP concentration for a participant is prepared from a bulk supply, the preparation will be confirmed by a second member of the study site staff.
- When participants are administered SP at the site, they will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each administration in the clinic will be recorded in the source documents. The concentration of study intervention and study participant identification will be confirmed at the time of administration by a member of the study site staff other than the person administering the study intervention.

6.5. Modification of Substance P concentrations

Following data reviews at the data review committee (DRC) (see Section 10.1.5) in Part 1, higher/lower concentrations of SP might be administered to subsequent participants, depending on the wheal reactions observed (see Section 4.3). The maximum amount of SP administered in a single injection will not exceed 50 µL of 10 µmol/L, and up to a maximum of 10 injections of SP will be allowed per challenge visit.

6.6. Intervention after the End of the Study

There will be no intervention offered at the end of the study.

6.7. Treatment of Overdose

- Intradermal SP doses up to ~7.5-fold higher than those planned for this study and up to ~2.3-fold higher than those permitted in this study have been reported to show no important side effects [Grouzmann, 2011; Zirbs, 2013], and thus an overdose is not anticipated given the use and local delivery. In case of any overexposure to SP refer to the MSDS.

- Histamine dose selection is based on the product label for histamine injection as a positive skin test control. An overdose is not anticipated given the per-product label use and local delivery, refer to the product label.

  In case of any overexposure or evidence of systemic adverse reactions to histamine, please refer to the product label for appropriate treatment response including, but not limited to tourniquet application, epinephrine administration, and antihistamine administration.

In the event of an overdose, the investigator should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until stable or resolved.
3. Document the quantity of the excess concentration as well as the duration of the overdosing in the CRF.

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

6.8. Concomitant Therapy

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

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

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

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

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

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

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Study Stopping criteria

- If a SAE, considered reasonably attributable to study intervention (SP, histamine) in the opinion of the Investigator, occurs in one participant, then dosing will be temporarily halted, and no further participants will be enrolled or dosed until a full safety review of the data has taken place. Relevant reporting and discussion with the Medical Monitor, relevant site personnel, and the IRB/IEC will take place before resumption of enrolment or dosing.

- If the significant clinical or laboratory abnormality, considered reasonably attributable to study intervention (SP, histamine) in the opinion of the Investigator, occurs in more than 2 participants, then dosing will be temporarily halted, and no further participants will be enrolled or dosed until a full safety review of the data has taken place. Relevant reporting and discussion with the Medical Monitor, relevant site personnel, and the IRB/IEC will take place before resumption of enrolment or dosing.

7.2. Discontinuation of Study Intervention

In rare instances, it may be necessary for a participant to permanently discontinue study intervention. If study intervention is permanently discontinued, the participant will remain in the study until the end of that challenge visit to be evaluated for safety. See the SOA for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

7.3. Participant Discontinuation/Withdrawal from the Study

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

7.4. Lost to Follow Up

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

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

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

8.1. Efficacy Assessments

Not applicable.

8.2. Safety Assessments

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

8.2.1. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the Skin, Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height and weight will also be measured and recorded.

- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

8.2.2. Vital Signs

- Tympanic body temperature, pulse rate and blood pressure will be assessed as a single or triplicate measurement(s), as described below.
- Blood pressure and pulse measurements will be assessed in a semi-supine position with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).
- At screening, vital signs will consist of 1 temperature, and 3 pulse and blood pressure measurements. On Challenge days, vital signs will consist of 1 pulse and 3 blood pressure measurements. Vital signs will to be taken before blood collection for laboratory tests. Triplicate readings will consist of 3 consecutive pulse and/or blood pressure readings recorded at intervals of at least 1 minute.

8.2.3. Electrocardiograms

- Triplicate 12-lead ECG will be obtained as outlined in the SoA (see Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 7 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- At Screening where a triplicate ECG are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes.

8.2.4. Clinical Safety Laboratory Assessments

Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.

- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within one day after the last administration of study intervention should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal by the investigator or medical monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA.

8.2.5. Pregnancy Testing

- Refer to Section 5.1 Inclusion Criteria for pregnancy testing entry criteria.
- Pregnancy testing (urine or serum as required by local regulations) should be conducted prior to each challenge during the study intervention period.
- Pregnancy testing (urine or serum as required by local regulations) should be conducted at the end of relevant systemic exposure.
- Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the participant's participation in the study.

8.3. Adverse Events and Serious Adverse Events

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

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

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

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

- All SAEs will be collected from the signing of the informed consent form until 2 hrs after the last SP challenge, as specified in the SoA (Section 1.3).
- All AEs will be collected from the first challenge-related procedure until 2 hrs after the last SP challenge, as specified in the SoA (Section 1.3).
- Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

- Investigators are not obligated to actively seek AEs or SAEs after the conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.2. Method of Detecting AEs and SAEs

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

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is given in Appendix 3.

8.3.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with other relevant documents and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5. Pregnancy

- Details of all pregnancies in female participants will be collected after the start of study intervention and until end of last challenge visit. If a pregnancy is reported, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 24 hours of learning of the female participant pregnancy. 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 reported as an AE or SAE.

- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.
- The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor.
- Any post-study pregnancy-related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in Section 8.3.4 . While the investigator is not obligated to actively seek this information in former study participants she may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention or be withdrawn from the study.

8.4. Pharmacokinetics

Not applicable to this study.

8.5. Pharmacodynamics

8.5.1. Visual check of planned challenge sites and administration of challenge agents

At each challenge visit, before any challenge related procedures are performed, the investigator (or delegate) will visually inspect the planned challenge sites for any markings or reactions that could interfere with the interpretation of data (e.g. carryover effects from a previous challenge day).

Saline and SP will be delivered by intradermal injection and histamine will be delivered by skin prick, as described in the SRM.

8.5.2. Wheal and Flare Assessments

Callipers will be used to measure the area of the responses at the sites of saline, histamine and SP challenge. Responses will also be digitally photographed for confirmation of results. In addition, perfusion of each challenge site will be measured using laser speckle contrast imaging.

Further details are provided in the SRM.

8.6. Genetics

Not applicable to this study.

8.7. Biomarkers

At challenge visits defined in the SoA, the following samples will be collected to measure exploratory biomarkers:

- IDM probes will be inserted intradermally in the skin of the forearm – in total, approximately 450 µl of dialysate will be collected from each probe
- 3 punch biopsy samples of up to 3mm in diameter each will be collected
- Up to 56mL of blood will be collected via venipuncture or cannula

Details of the processing, storage and shipping procedures for all samples are provided in the SRM.

IDM samples will be collected to investigate the effect of intradermal SP challenge on local exploratory biomarkers of mast cell activation and their variability. Biomarkers may include but not be limited to histamine.

Skin biopsies may be collected to investigate the effect of intradermal SP challenge on local exploratory biomarkers of mast cell activation and their variability. Biomarkers will include tryptase and/or markers of cells including mast cells, eosinophils, basophils, T cells, macrophages and neutrophils.

Blood samples will be stored and analysis may be performed on biomarkers thought to play a role in mast cell activation or SP challenge response.

GSK may store samples for up to 15 years after the end of the study to achieve study objectives. Additionally, with participants consent, samples may be used for further research by GSK or others such as universities or other companies to contribute to the understanding of mast cell related or other diseases, the development of related or new treatments or research methods.

Exploratory biomarker results may be entered into the clinical study database or reported separately.

8.8. Immunogenicity Assessments

Not applicable to this study.

8.9. Health Economics

Not applicable to this study.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

The objectives of this enabling study are to characterise the wheal and flare responses over time following skin challenges with ascending concentrations of SP. No formal hypotheses will be tested.

9.2. Sample Size Determination

A sufficient number of participants will be screened in order to enrol approximatively 32 participants in the study (up to 12 in part 1 and up to 20 in part 2).

The sample size for part 1 and part 2 is not based on statistical considerations and is based on previous published data.

9.3. Populations for Analyses

The following populations are defined:

Population	Description
Screened	All participants who signed the ICF and were screened for eligibility.
Enrolled	All participants in the Screened population who entered the study
Safety	All participants in the Enrolled population who received at least one challenge-related study procedure on their (first) challenge day

9.4. Statistical Analyses

The reporting and analysis plan (RAP) will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.4.1. General Considerations

This is an exploratory enabling study which is primarily designed to define the relationship between SP concentrations and wheal and flare response, within a range of intradermal SP concentrations. There are no formal hypotheses to be tested.

All the statistical analyses will be carried out in the Bayesian framework. Models will be fitted by Markov chain Monte Carlo simulations. The following summaries of the posterior distribution will be reported: posterior mean, posterior standard deviation (SD), posterior median, 95% equal tailed credible interval, posterior probabilities of the true effect being greater than pre-specified thresholds. Vague prior distributions will be used.

For all endpoints, the baseline value will be the negative control value. No missing baseline will be allowed (as part of the inclusion/exclusion criteria).

9.4.2. Primary Endpoints

For parts 1 and 2, the primary endpoint is the wheal response-time area under the curve (AUC) over the 2h post-challenge period following skin challenge with ascending concentrations of SP on a single day and 2 days, respectively. The wheal response variable will be area in millimetres squared. Other methods may be investigated as outlined in Section 8.5.2. These variables may be adjusted for baseline response.

The primary analysis on the Safety population and on participants who have completed the challenge visits (complete case analysis) will assess the difference in wheal response-time area under the curve (AUC) over the 2h post-challenge period between the different challenges (saline, SP). The following statistical models may be used:

- Part 1: Analysis of covariance (ANCOVA)

- Part 2: Mixed effect model for repeat measurements (MMRM), including predictors and random effects

Alternative approaches may be investigated. Further details will be provided in the RAP.

9.4.3. Secondary Endpoints

For part 1 and 2, the following parameters related to the wheal response will be calculated (not limited to) and summarised:

- Maximum observed wheal response
- Time to maximum observed wheal response
- Time of complete wheal response disappearance

For parts 1 and 2, the secondary endpoints are the flare response over time (including but not limited to AUC, maximum observed flare response, time to maximum observed flare response, time of complete flare response disappearance) following skin challenge with ascending concentrations of SP on a single day and 2 days, respectively. The proposed statistical analyses planned for the primary endpoint (Section 9.4.2) will be repeated for the flare response-time area under the curve (AUC) over the 2h post-challenge period.

All safety analyses will be performed on the Safety population. Safety data will be presented in tabular and/or graphical format and summarized descriptively according to GSK's Integrated Data Standards Library (IDSL) standards. No formal statistical testing will be performed on safety data.

Further details will be provided in the RAP.

9.4.4. Tertiary/Exploratory Endpoints

Full details will be described in the RAP.

9.5. Interim Analyses

No formal interim analysis will be performed. The following data monitoring will be performed based on data collected during the study. At a minimum, all reviews will include available wheal response data after each injection of SP.

- Part 1 data for about 3 participants who have completed the challenge visit will be reviewed prior to enrolment of subsequent participants, to allow any concentration adjustment.

- Part 1 data for about 6 participants who have completed the challenge visit will be reviewed prior to enrolment of subsequent participants, to allow any concentration adjustment and/or to confirm if sufficient data is already available to progress to Part 2.

- Part 1 data will be reviewed prior to participants being enrolled into Part 2.

- Part 2 data for about 10 participants who have completed the 2 challenge visits will be reviewed; enrolment into Part 2 may continue during the review.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

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

10.1.1. Regulatory and Ethical Considerations

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

10.1.2. Financial Disclosure

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

10.1.3. Informed Consent Process

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

A participant who is rescreened is not required to sign another ICF if the rescreening occurs within (28) days from the previous ICF signature date.

GSK (alone or working with others) may use participant's coded study data and samples and other information to carry out this study; understand the results of this study; learn more about SP challenge or about the disease related to mast cells; publish the results of these research efforts; work with government agencies or insurers to have study interventions approved for medical use or approved for payment coverage.

The ICF contains a separate section that addresses the use of participant data and remaining samples for optional further research. The investigator or authorised designee will inform each participant of the possibility of further research not related to the study/disease. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate tick box will be required to document a participant's agreement to allow any participant data and/or remaining leftover samples to be used for further research not related to the study/disease. Participants who decline further research will tick the corresponding "No" box.

10.1.4. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of

disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5. Committees Structure

A data review committee (DRC) will review data during the study, at time points described in Section 9.5. At a minimum, available wheal response data after each SP challenge will be reviewed. Other data, such flare response, safety and biomarker data may also be reviewed, if applicable. The DRC will consist of the following people (or their delegate): GSK Medical Monitor, GSK Clinical Science Lead, GSK Clinical Delivery Lead, GSK Clinical Pharmacology Modelling and Simulation (CPMS) representative, a GSK Global Clinical Safety and Pharmacovigilance (GCSP) representative and GSK Statistician representative. Other study team members may attend, as needed. The DRC may decide to change the concentrations of SP to be administered to subsequent participants, as described in Section 6.5.

10.1.6. Dissemination of Clinical Study Data

- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.
- GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.
- The procedures and timing for public disclosure of the protocol and results summary and for development of a manuscript for publication for this study will be in accordance with GSK Policy.
- GSK intends to make anonymized patient-level data from this trial available to external researchers for scientific analyses or to conduct further research that can help advance medical science or improve patient care. This helps ensure the data provided by trial participants are used to maximum effect in the creation of knowledge and understanding
- A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge. Specifications about the data and publication are incorporated into the clinical trial agreement.

10.1.7. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- Guidance on completion of CRFs will be provided in the InForm portal
- Quality tolerance limits (QTLs) will be pre-defined in the QTL plan to identify systematic issues that can impact participant safety and/or reliability of study results. These pre-defined parameters will be monitored during and at the end of the study and all deviations from the QTLs and remedial actions taken will be summarized in the clinical study report.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy including definition of study critical data items and processes (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the monitoring plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8. Source Documents

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

- Definition of what constitutes source data can be found in a study specific source document agreement.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.9. Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants.

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

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

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

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

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up

10.1.10. Publication Policy

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

- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not

as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

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

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 1 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.
- Pregnancy Testing
- Refer to Section 5.1 Inclusion Criteria for screening pregnancy criteria and Section 1.3 Schedule of Activities (SoA).
- Pregnancy testing (urine or serum as required by local regulations) should be conducted prior to each SP challenge visit.
- Pregnancy testing (urine or serum as required by local regulations) should be conducted at the end of relevant systemic exposure.
- Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.

Table 1 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**			
Hematology	Platelet Count RBC Count Hemoglobin Hematocrit	RBC Indices: MCV MCH %Reticulocytes		WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Clinical Chemistry[1]	BUN	Potassium	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	Total Protein
	Glucose [non-fasting]	Calcium	Alkaline phosphatase	
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• Follicle-stimulating hormone and estradiol (as needed in women of non-childbearing potential only) • Breath alcohol test (as per standard local practice) • Drug screen to include at a minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Smoking test – breath test or urine cotinine, according to local procedures. • Highly sensitive serum or urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)[2] • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)			

NOTES :

1. All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).
2. Serum pregnancy testing at screening, at other visits urine testing.

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

10.3.1. Definition of AE

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

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

• The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of SAE

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

A SAE is defined as any untoward medical occurrence that, at any dose:
○ Results in death
○ Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
Requires inpatient hospitalization or prolongation of existing hospitalization • In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are 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.
Results in persistent or significant disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea,

influenza, and accidental trauma (e.g. sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
Is a congenital anomaly/birth defect
Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. • Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3. Recording and Follow-Up of AE and SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) related to the event. • The investigator will then record all relevant AE/SAE information in the CRF. • It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories: • Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.

- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

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

Assessment of Causality

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

Follow-up of AE and SAE

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

- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

10.3.4. Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

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

SAE Reporting to GSK via Paper CRF

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

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

10.4.1. Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first administration of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP

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

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

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

10.4.2. Contraception Guidance:

• **CONTRACEPTIVES[a] ALLOWED DURING THE STUDY INCLUDE:**
• **Highly Effective Methods[b] That Have Low User Dependency** *Failure rate of <1% per year when used consistently and correctly.*
• Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c]
• Intrauterine device (IUD)
• Intrauterine hormone-releasing system (IUS)[c]
• Bilateral tubal occlusion
• Azoospermic partner (vasectomized or due to a medical cause) Azoospermia is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days. Note: documentation of azoospermia for a male participant can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
• **Highly Effective Methods[b] That Are User Dependent** *Failure rate of <1% per year when used consistently and correctly.*
• Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[c] • oral • intravaginal • transdermal • injectable
• Progestogen-only hormone contraception associated with inhibition of ovulation[c] • oral • injectable
• Sexual abstinence • *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.*
a. Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies. b. Failure rate of <1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly. c. [Male condoms must be used in addition to hormonal contraception]. If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action. Note: Periodic abstinence (calendar, sympto-thermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception. Male condom and female condom should not be used together (due to risk of failure from friction)

10.5. Appendix 5: Abbreviations and Trademarks

LIST OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
AUC	Area under the curve
BMI	Body mass index
CA	Competent Authority
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CPMS	Clinical Pharmacology Modelling and Simulation
CSR	Clinical Study Report
DRC	Data review committee
EC	Ethics committee
ECG	Electrocardiogram
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
ICF	Informed consent form
IDM	Intradermal microdialysis
IDSL	Integrated Data Standards Library
IEC	Independent Ethics Committees
IRB	Institutional Review Boards
MMRM	Mixed effect model for repeat measurements
QTcF	QT interval corrected for heart rate according to Fridericia's formula
RAP	Reporting and analysis plan
SAE	Serious adverse event
SD	Standard deviation (SD
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SoA	Schedule of Activities
SP	Substance P
SRM	Study Reference Manual
ULN	Upper limit of normal
WCBP	Women of childbearing potential
WNCBP	Women of non-childbearing potential.

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
None

Trademarks not owned by the GlaxoSmithKline group of companies
None

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