Statistical Analysis Plan

Study ID: 213224

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

NCT ID: NCT04676763

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TITLE PAGE

Protocol Title:	An open label, single center, enabling study to investigate the optimum method for use of intradermal Substance P as a challenge agent in healthy participants
Study Number:	*213224*
Compound Number:	*None*
Abbreviated Title:	Intradermal Substance P challenge in healthy participants

Sponsor Name: GlaxoSmithKline Research & Development Limited

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Version history

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
SAP	01 Jul 2021	03-NOV-2020	Not Applicable	Original version

1. INTRODUCTION

- The purpose of this SAP is to describe the planned analyses and output to be included in the Clinical Study Report for study 213224 (Protocol 2019N419175_00).

1.1. Objectives and Endpoints

Part 1

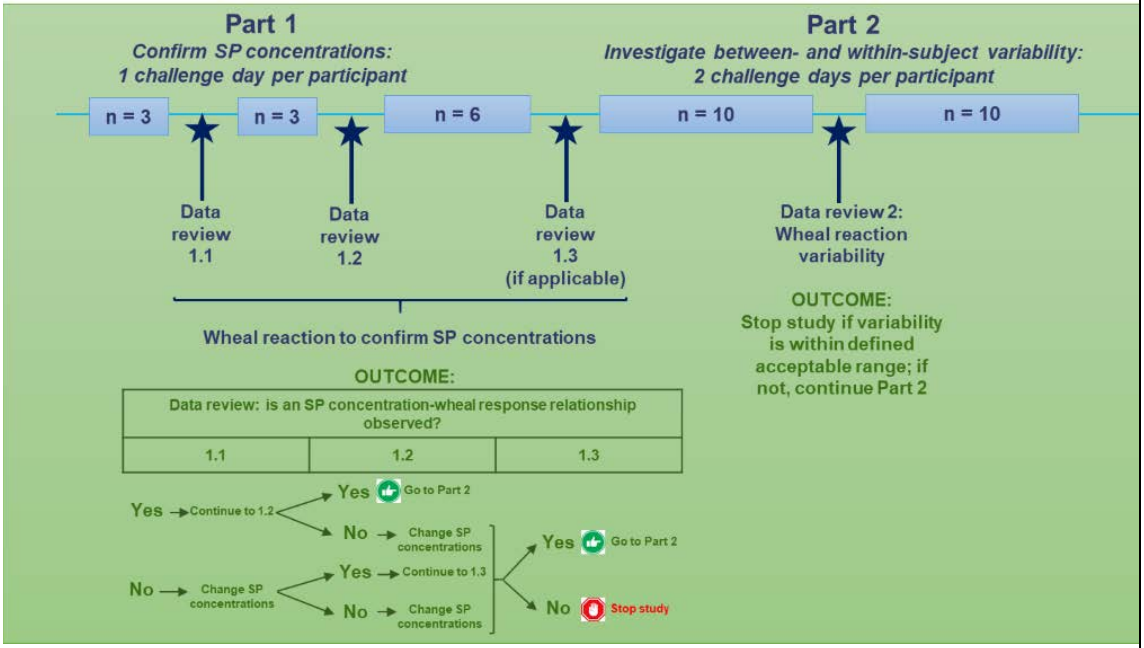
Objectives	Endpoints
Primary	
• To characterise the wheal response over time following skin challenge with ascending concentrations of SP on a single day	• Wheal response during the 2 h post-challenge period area under the curve (AUC) at each concentration of SP
Secondary	
• To characterise the wheal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

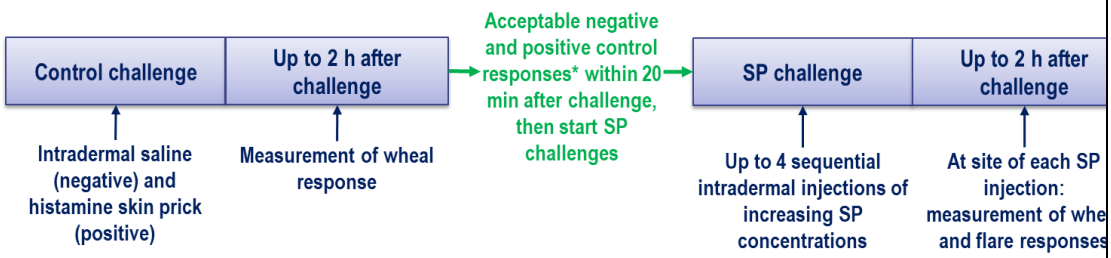
1.2. Study Design

Overview of Study Design and Key Features



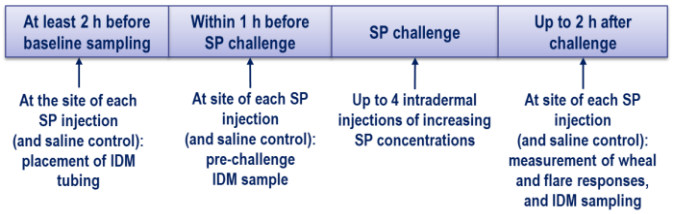
	Challenge Visit 1	**Challenge Visit 2**
Part 1	Wheal and flare post SP challenges (Panel A) only	Not applicable
Part 2	Wheal and flare post SP challenges (Panel A); and IDM during SP challenges (Panel B)	Wheal and flare post SP challenges (Panel A) IDM during SP challenges (Panel B) Biopsy post challenges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

Panel A: Wheal and Flare measurement

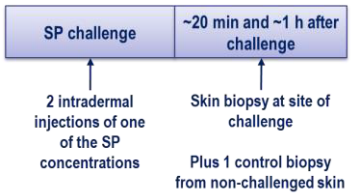


Overview of Study Design and Key Features

Panel B: IDM measurement



Panel C: Biopsy sample



Design Features	• 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.
	• Wheal and flare responses will be assessed after each challenge (parts 1 and 2)
	• In part 2, 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.

Overview of Study Design and Key Features	
Study intervention	**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. o If the wheal responses immediately after the 20 min post challenge period do not meet the acceptable saline and histamine responses (as defined in the SRM), the participant will not receive SP. o If it happens in Part 1 or on Challenge Visit 1 in Part 2, the participant will be withdrawn from the study. o 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
Study intervention Assignment	• No randomization is planned • Participants will receive up to 4 ascending SP concentrations
Data reviews	• See Table 1

2. 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.

2.1. Multiplicity Adjustment

No multiplicity adjustment is planned here.

3. ANALYSIS SETS

Analysis Set	Definition / Criteria	Analyses Evaluated
Screened	• All participants who signed the ICF and were screened for eligibility.	• Screen Failure
Enrolled	• All participants in the Screened analysis set who entered the study. Note: screening failures (who never passed screening even if rescreened) and participants screened but never enrolled into the study (Met eligibility but not needed) are excluded from the Enrolled analysis set as they did not enter the study.	• Study Population
Safety	• All participants in the Enrolled analysis set who received at least one challenge-related study procedure on their (first) challenge day	• Safety • PD

4. STATISTICAL ANALYSES

The objectives of this enabling study are to characterise the wheal and flare responses over time following skin challenges with ascending concentrations of SP.

4.1. General Considerations

4.1.1. General Methodology

Unless otherwise specified, continuous data will be summarized using descriptive statistics: n, mean, standard deviation (SD), median, interquartile range, minimum and maximum. Categorical data will be summarized as the number and percentage of participants in each category.

4.1.1.1. Bayesian Inference

All the main statistical analyses will be carried out in the Bayesian framework. Models will be fitted by MCMC simulations. Vague priors will be used for modelling parameters and will be specified in the following sections. Sensitivity analyses on priors may be performed. Covariance structures may also be investigated..

The following summaries of the posterior distribution will be reported: posterior median, posterior standard deviation (SD), 95% equal-tailed credible interval (95% CrI), posterior probabilities of the true difference being greater than pre-specified thresholds.

The inference will be carried out as follows:

- 2 chains will be run, with different (over-dispersed) initial values in order to assess convergence. The posterior summaries will use only 1 chain.
- A minimum burn-in period of 10,000 MCMC samples (for each chain) will be used.

- A minimum of 10,000 MCMC samples (for each chain) will be run to generate samples of the posterior distribution.
- A thinning sample may be used to improve convergence. If k is the thinning ratio, the number of MCMC samples will be minimum k*10,000.

To assess convergence (MCMC), the following will be used:

- Ratio Monte Carlo error /posterior SD should be as small as to ensure that only a fraction of the posterior variability is due to simulation error.
  The final MCMC samples will be such that this ratio for all the parameters in the model is ≤ 0.01.
- Gelman & Rubin diagnostic is based on the ratio of the pooled variance across the chains over the overall within-chain variance. Values close to 1 indicate that each of the subsets of MCMC samples is close to the target posterior distribution.
  A cut-off of 1.1 will be used.
- Diagnostic plots and visual inspection
  - Trace plots (the distribution of points should not change as the chain progresses and the posterior mean and variance are relatively constant)
  - Autocorrelation plots (provide information on how slow or fast the Markov chain converges)

To assess the model adequacy, diagnostic plots of the median predicted value vs median residual (should not show evidence of any patterns) will be used.

All the diagnostic outputs and alternative models fitted (where applicable) will be stored in the refdata folder in HARP (production) in the relevant reporting efforts.

4.1.2. Baseline Definition

For part 1, there will be 1 baseline value (baseline visit 1). For part 2, there will be 2 baseline values, one for each visit (baseline visit 1 and visit 2).

For the PD endpoints (wheal and flare responses), the baseline value will be the saline control non-missing value pre-SP challenge at challenge visit 1 and challenge visit 2 (parts 1 and 2). No missing baseline values will be allowed as part of the inclusion/exclusion criteria.

For the other endpoints collected at challenge visits 1 and 2, the baseline value will be the latest non-missing pre-1st control challenge assessment value at challenge visit 1 and challenge visit 2 (parts 1 and 2), including those from unscheduled visits.

For Part 2, the Baseline for Visit 2 will be the latest assessment performed after Visit 1 visits (such as Unscheduled) but before the first Visit 2 challenge.

Participants with a missing baseline value will be excluded from the change from baseline analyses.

The mean of replicate assessments at any given time point will be used as the value for that time point.

If baseline data are missing no derivation will be performed and baseline will be set to missing.

4.2. Primary Pharmacodynamic Analyses

4.2.1. Definition of endpoint

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 (challenge visit 1) and 2 days (challenge visits 1 and 2), respectively, expressed as mm^2 /min (see OPS).

The wheal response variable will be the area in millimetres squared (mm^2) and will be calculated using the following formula for area of an ellipse (see OPS), using the longest and orthogonal diameters measured by the calliper method.

The wheal response variable will be calculated for each ascending SP concentration and each control challenge (saline and histamine) and at each timepoint during the 2h post-challenge period at challenge visit 1 (parts 1 and 2) and challenge visit 2 (part 2).

The same as above will be repeated for flare.

4.2.2. Main analytical approach

4.2.2.1. Summary Measure

The summary measure is the difference in wheal response AUC between 2 consecutive SP concentrations at challenge visit 1 (parts 1 and 2) and at challenge visit 2 (part 2).

4.2.2.2. Population of interest

The primary pharmacodynamics analyses will be based on the Safety population and on participants who have completed the challenge visits (complete case analysis).

Statistical analyses

Unless otherwise specified, endpoints / variables defined in Section 4.2.1 will be summarised using descriptive statistics, graphically presented and listed (where appropriate).

Statistical Methodology Specification for Part 1

Endpoint / Variables	
• SP-induced wheal response-time AUC at challenge visit 1	
Model Specification	
• Bayesian analysis of covariance (ANCOVA)	
Model	$y_i \sim Normal(\mu_i, \sigma)$ $\mu_i = \boldsymbol{X}_i \boldsymbol{\beta}$
Response	y_i = AUC for participant *i*
Prior for σ	σ = between-participant SD ~ $Gamma\left(\text{shape} = 2, \text{rate} = \frac{1}{A}\right)$ with A reasonably large e.g. A=10 (Chung et al, 2013)
Population-level coefficients (**β**)	**β** = parameters associated to predictors $\beta_k \sim Normal(0, sd = 1e6)$
Predictors (**X**)	• SP = SP concentration (categorical) • Saline-induced (Baseline) wheal response-time AUC (continuous)
Model Checking & Diagnostics	
See Section 4.1.1.1	
Model Results Presentation	
• Summaries of the posterior distributions of true AUC for each SP challenge and the true difference in AUC using the mean baseline to calculate the marginal estimates • Probabilities that the true difference in AUC is greater than 0	

Statistical Methodology Specification for Part 2

Endpoint / Variables	
• SP-induced wheal response-time AUC at challenge visits 1 and 2 (using only data when no IDM intervention was done)	
Model Specification	
• Bayesian analysis of covariance (ANCOVA) with visit effects	
Model	**Y** ~ MVN(**μ**, **Σ**) **μ** = **Xβ** + **Zγ**
Outcome	y_{ij} = SP-induced AUC for participant *i* at visit *j*

Prior for **Σ** (covariance matrix)	$\Sigma_{ij} = Cov[Y_i, Y_j]$ assumed to be unstructured Σ ~ Inverse Wishart (J, R), with J = 2 (visits), R = empirical covariance matrix
Population-level coefficients (**β**)	**β** = parameters associated to predictors $\beta_k \sim Normal(0, sd = 1e6)$ β_0 = intercept, Vague: $Normal(0, sd = 1e6)$
Predictors (**X**)	• SP = SP concentration (categorical) • Visit = challenge visit (categorical) • SP*visit = SP by-visit interactions (categorical) • Saline_AUC_base = average baseline defined as the mean of the 2 saline-induced AUCs for each participant (continuous) • Saline_AUC_visit = Deviation of each visit saline-induced baseline from average baseline defined as saline-induced AUC - Saline_AUC_base • Saline_AUC_visit*visit = Saline_AUC_visit by-visit interactions
Subject-level effects (**γ**)	• $\boldsymbol{Z}_i$ = design matrix for the subject-level effects • **γ**~ Normal (**0,** σ^2**I)**
Model Checking & Diagnostics	
See Section 4.1.1.1	
Model Results Presentation	
• Summaries of the posterior distributions of true AUC for each SP challenge and the true difference in AUC using the mean baseline to calculate the marginal estimates • Probabilities that the true difference in AUC is greater than 0	

4.2.3. Sensitivity analyses

Sensitivity analyses on the priors (in particular the prior for the between-participant and within pa SD) will be carried out.

4.3. Secondary Pharmacodynamic Analyses

4.3.1. Definition of endpoint

Wheal response related endpoints (parts 1 and 2):

The following endpoints will be calculated at challenge visit 1 (parts 1 and 2) and challenge visit 2 (part 2) using the wheal area (mm^2) determined by the calliper method (see OPS) and for each of the skin challenges:

- Wheal response-time area under the curve (AUC) over the 2h post-challenge period
- Maximum observed wheal area (mm^2) during the 2h post-challenge period.
- Time (min) to maximum observed wheal area during the 2h post-challenge period

- Time (min) of complete wheal area disappearance during the 2h post-challenge period. The complete disappearance is defined as a wheal area of 0. If the complete disappearance does not occur within the 2h post-challenge period, the time will be set to >120min.

The longest and orthogonal diameters (mm) of the wheal, measured by the calliper method, will also be summarised by challenge visit and skin challenge in the same way:

- Maximum observed wheal longest/orthogonal diameter (mm) during the 2h post-challenge period.
- Time (min) to maximum observed wheal longest/orthogonal diameter during the 2h post-challenge period
- Time (min) of complete wheal longest/orthogonal diameter disappearance during the 2h post-challenge period. The complete disappearance is defined as a wheal longest/orthogonal diameter of 0. If the complete disappearance does not occur within the 2h post-challenge period, the time will be set to >120min.

Flare response related endpoints (part 1 and 2)

The following endpoints will be calculated at challenge visit 1 (parts 1 and 2) and challenge visit 2 (part 2) using the flare area (mm^2) as determined by the calliper method (see OPS).

- Flare response-time area under the curve (AUC) over the 2h post-challenge period
- Maximum observed flare area during the 2h post-challenge period.
- Time (min) to maximum observed flare area during the 2h post-challenge period
- Time (min) of complete flare area disappearance during the 2h post-challenge period. The complete disappearance is defined as a flare area of 0. If the complete disappearance does not occur within the 2h post-challenge, the time will be set to >120min.

The longest and orthogonal diameters (mm) of the flare, measured by the calliper method, will also be summarised by challenge visit and skin challenge in the same way:

- Maximum observed flare longest/orthogonal diameter (mm) during the 2h post-challenge period.
- Time (min) to maximum observed flare longest/orthogonal diameter during the 2h post-challenge period
- Time (min) of complete flare longest/orthogonal diameter disappearance during the 2h post-challenge period. The complete disappearance is defined as a flare longest/orthogonal diameter of 0. If the complete disappearance does not occur within the 2h post-challenge period, the time will be set to >120min.

4.3.2. Main Analytic approach

4.3.2.1. Summary Measure

The summary measure is the difference in flare response AUC between 2 consecutive SP concentrations at challenge visit 1 (parts 1 and 2) and at challenge visit 2 (part 2).

4.3.2.2. Population of interest

The secondary pharmacodynamics analyses will be based on the Safety population, unless otherwise specified.

4.3.2.3. Statistical Analyses

Endpoints / variables defined in Section 4.3.1 will be summarised using descriptive statistics, and graphically presented (where appropriate).

The proposed statistical analyses planned for the primary PD endpoint (Section 0) will be repeated for the flare response-time area under the curve (AUC) over the 2h post-challenge period for parts 1 and 2.

For the other endpoints, no statistical analyses will be carried out.

4.4. Exploratory Biomarker Analyses

The exploratory biomarker analyses will be based on the Safety population and will be reported for part 2 only.

No formal statistical testing will be performed on biomarkers data.

Histamine concentrations (ng/mL) from intradermal microdialysis during the 2h post-challenge will be summarised using descriptive statistics and graphically presented (where appropriate) at challenge visits 1 and 2 of part 2 in a similar way as the wheal and flare responses.

Additional biomarker analyses of the skin punch biopsies and blood samples (visit 2 only) may be conducted. These results will be the subject of a post hoc analysis.

4.5. Safety Analyses

The safety analyses will be based on the Safety population. No formal statistical testing will be performed on safety data.

Displays will be generated for parts 1 and 2, separately (but in the same file) and for the study intervention group “Skin Challenges”.

4.5.1. Extent of Exposure

A listing of exposure to skin challenges (intervention) will be produced for each subject based on location, laterality and directionality for the skin challenges, concentration (µmol/L), volume (µL) and dose (pmol)

For the SP challenges, the dose (pmol) will be derived from the concentration and volume (see OPS).

4.5.2. Adverse Events

An adverse event (AE) is defined as a study intervention emergent AE if it is an AE that started on or after the date/time of first study intervention

An AE is not a study intervention emergent AE if it started and ended before the date/time of first study intervention.

All AE summaries will be based on study intervention emergent events unless otherwise specified. SAE summaries will be based on all SAEs reported regardless of whether they meet the definition of study intervention emergent or not. All AE and SAE summaries will be by PT only unless otherwise specified.

Adverse events will be coded using the standard Medical Dictionary for Regulatory Affairs (MedDRA dictionary).

Summary tables will include the number of participants with the AE, the percentage of participants with the AE. The following AE categories will be provided:

- All adverse events by SOC, PT and maximum intensity
- Common non-serious AEs by SOC and PT (Number of Subjects and Occurrences):
    - For disclosure purposes, common is defined as AEs occurring in at least 5% of any intervention arm. Due to the small sample size of this study this table will include all non-serious AEs.
- AEs related to study intervention by SOC, PT and maximum intensity:
    - A study intervention-related AE is defined as an AE for which the investigator classifies the possible relationship to study interventions as “Yes”. A worst-case scenario approach will be taken to handle missing data, i.e. the summary table will include events with the relationship to study intervention as ‘Yes’ or missing.
    - The summary will be generated for the relationship to SP only, histamine only, and both SP and histamine, separately.
- SAE by SOC, PT and maximum intensity
- SAE by SOC and PT (Number of Subjects and Occurrences)
- Serious fatal and non-fatal study intervention related AEs by overall frequency

4.5.2.1. Adverse Events of Special Interest

Not applicable.

4.5.2.2. COVID-19 Assessment and COVID-19 AEs

It is the investigator who decides whether an AE is a COVID related Adverse event or not.

Listings will be produced for subjects with COVID-19 Adverse Events.

4.5.2.3. Impact of COVID-19 Pandemic on Safety Results

The impact of the COVID-19 pandemic on the safety results will be assessed, if any.

4.5.3. Additional Safety Assessments

4.5.3.1. Laboratory Data

Summary tables for haematology and chemistry laboratory tests as well as urine concentration parameters will be produced:

- Change from baseline in laboratory parameters (clinical chemistry and hematology) will be presented for continuous variables, with a summary of baseline values included in the tables.
- Summaries of worst-case results post-baseline relative to baseline will be provided for the following tests:
    - For lab tests with associated Potential Clinical Importance (PCI) criteria: the summary will include baseline data and worst-case post-baseline. The baseline categories are: Low, W/in Range and High. The change categories are: To Low, To w/in Range or No Change, To High. If a subject has a decrease to low and an increase to high during the same time interval, then the subject is counted in both the "to Low" and the "to High" categories.
    - For categorical urinalysis parameters

For part 2, change from baseline will be calculated for each visit, (using the visit 1 baseline for visit 1, and visit 2 baseline for visit 2).

Baseline and post-baseline data will be included in these tables. If a participant moves to both a low and a high clinical concern range during the study intervention period, then the participant is counted in both categories.

Summaries of hepatobiliary laboratory events including possible Hy's law cases will be provided in addition to what has been described above. Possible Hy's law cases are defined as any elevated alanine aminotransferase (ALT)>3×upper limit of normal (ULN), total bilirubin≥2×ULN and alkaline phosphatase (ALP)<3×ULN/missing. Total bilirubin≥2×ULN can be within 28 days following the ALT elevation and if direct bilirubin is available on the same day, it must be ≥ 35% of total bilirubin. ALP<3×ULN/missing means it is satisfied unless the ALP is ≥3xULN at the time of bilirubin elevation. The summary will be produced for worst case post baseline only.

4.5.3.2. Vital Signs

A summary of change from baseline vital sign values will be provided by visit and intervention group, including systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, height, weight and BMI, with a summary of baseline values included in the table.

The number and percentage of participants with clinical important changes for vital signs (systoloic blood pressure, diastolic blood pressure, heart rate) will be generated using the PCI criteria stated in Section 6.2.1.3.

The mean of replicate assessments will be used as baseline and as value at each timepoint for blood pressure and heart rate values collected in triplicates.

4.5.3.3. ECG

The QTc data analysis will use the collected values based on Fridericia formula. The number and percentage of participants with clinical important changes for 12-lead ECG will be generated using the PCI criteria stated in Section 6.2.1.2.

For all ECG analyses, baseline is taken to be the mean of the triplicate values collected pre-1st skin challenge.

The following summaries will be provided by visit:

- Summary of ECG findings (the clinical significance and interpretation of each planned ECG). In the case of triplicate ECG measurements, results will be presented separately by measurement number.

- Summary of change from baseline in ECG values. In the case of triplicate ECG measurements, summaries will use the average of the triplicate, listings will present all individual measurements (and the average).

The absolute and change from baseline ECG values will be rounded to the nearest integer before being categorized according to the clinical concern ranges. The following summaries of participants will be produced:

- A summary of QTcF absolute values will display the number and percentage of participants with a post-baseline increase to a value within each clinical concern range for the worst-case post-baseline only.

- A summary of change from baseline QTcF values will display the number and percentage of participants with a change within each range for the worst-case post-baseline. Participants with missing baseline values will be excluded from this summary.

4.6. Other Analyses

Not applicable.

4.6.1. Subgroup analyses

Not applicable

4.6.2. Other variables and/or parameters

Not applicable

4.7. Data reviews

All data reviews will be performed by the data review committee (DRC) (see Section 10.1.5 of the protocol) in accordance to the Dose Escalation Plan and are summarised in

Table 1. Study population and safety data will be generated by the site, wheal and flare data will be generated by S&P.

Table 1 Timing, purpose, outcomes and data to be reviewed at each data review

Data review number	Timing	Purpose	Potential outcomes	Data to be reviewed
1.1	~3 participants who have completed the part 1 challenge visit	Review safety data Review PD data	Stop/pause study or recommend alterations to study design or conduct for safety Modify SP concentrations for the next participants to be enrolled in part 1 Continue recruitment to part 1 at the same SP concentrations	Study population Safety Wheal response (plots)
1.2	~6 participants who have completed the part 1 challenge visit	Review safety data Review PD data	Stop/pause study or recommend alterations to study design or conduct for safety Modify SP concentrations for the next participants to be enrolled in part 1 Stop recruitment to part 1 and open up part 2 Continue recruitment to	Study population Safety Wheal response (plots)

Data review number	Timing	Purpose	Potential outcomes	Data to be reviewed
			part 1 at the same SP concentrations	
1.3	~12 participants who have completed the part 1 challenge visit	Review safety data Review PD data	Stop/pause study or recommend alterations to study design or conduct for safety Confirm SP concentrations for part 2 Stop the study	Study population Safety Wheal response (plots, summary tables) Flare response (plots, summary tables)
2	~10 participants who have completed the part 2 challenge visit 2	Review safety data Review PD data	Stop/pause study or recommend alterations to study design or conduct for safety Continue recruitment to part 2 Stop the study	Study population Safety Wheal response (plots, summary tables, stats analyses) Histamine concentrations (plots, summary tables) if available

4.8. Changes to Protocol Defined Analyses

There were no changes or deviations to the originally planned statistical analysis specified in the protocol 2019N419175_00 (Dated: 03-NOV-2020).

5. SAMPLE SIZE DETERMINATION

A sufficient number of participants will be screened in order to enroll 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.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

The study population analyses will be based on the Screened (Section 6.1.1) and Enrolled (Section 6.1.1, Section 6.1.2, Section 6.1.3, Section 6.1.4) populations.

Study population analyses including analyses of participant's disposition, protocol deviations, demographic and baseline characteristics, prior and concomitant medications, and study intervention will be provided.

6.1.1. Participant Disposition

A summary of the number and percentage of participants who completed the study as well as those who prematurely withdrew from the study will be provided. Primary reasons for study withdrawal will be summarized.

A summary of study intervention status will be provided. This display will show the number and percentage of participants who have completed the scheduled study intervention, or have discontinued study intervention prematurely, as well as primary reasons for discontinuation of study intervention.

6.1.2. Demographic and Baseline Characteristics

The demographic characteristics including age, race, ethnicity, sex, height, screening body weight and Body Mass Index (BMI) will be summarized with descriptive statistics.

In addition, the following age categories will be summarized: 18-64, 65-84 and >=85.

Summary of race and racial combinations will also be summarised.

Medical conditions collected at screening will be summarized by past and current.

6.1.3. Protocol Deviations

Important protocol deviations (including deviations related to study inclusion/exclusion criteria, conduct of the trial, participant management or participant assessment) will be summarized.

Protocol deviations will be tracked by the study team throughout the conduct of the study. These protocol deviations will be reviewed to identify those considered as important as follows:

- Data will be reviewed prior to freezing the database to ensure all important deviations are captured and categorised on the protocol deviations dataset.
- This dataset will be the basis for the summaries of protocol deviations.

6.1.4. Prior and Concomitant Medications

Concomitant medications will be coded and summarized using the GSK Drug dictionary.

A summary of the number and percentage of subjects with concomitant medications will be displayed using the Generic Term without regard to ATC classifications. This summary will present single ingredient medications and multi-ingredient medications according to the combination of the ingredients. Multi-ingredient medications will be labelled according to the sum of their ingredients, i.e., Generic Term, e.g., “Tylenol Cold and Flu” would appear as “CHLORPHENAMINE MALEATE + DEXTROMETHORPHAN HYDROBROMIDE + PARACETAMOL + PSEUDOEPHEDRINE HYDROCHLORIDE”.

Concomitant medications include any medication that was taken at some point during the on-intervention period as defined in Section 6.2.2.1.

6.1.5. Study Intervention Compliance

Not Applicable.

6.1.6. Additional Analyses Due to the COVID-19 Pandemic

Not Applicable.

6.2. Appendix 2 Data Derivations Rule

6.2.1. Criteria for Potential Clinical Importance

The following criteria will be used to flag potential clinical importance:

6.2.1.1. Laboratory Values

Haematology				
Laboratory Parameter	**Units**	**Category**	**Clinical Concern Range**	
			Low Flag (< x)	**High Flag (>x)**
Haematocrit	Ratio of 1	Male		0.54
		Female		0.54
		Δ from BL	↓ 0.075	
Haemoglobin	g/L	Male		180
		Female		180
		Δ from BL	↓ 25	
Platelet Count	$x10^9$/ L		100	550
While Blood Cell (WBC)				
Absolute Count	$x10^9$/ L		3	20
Neutrophil Count	$x10^9$/ L		1.5	
Lymphocytes	$x10^9$/ L		0.8	

Clinical Chemistry				
Laboratory Parameter	**Units**	**Category**	**Clinical Concern Range**	
			Low Flag (< x)	**High Flag (>x)**
Albumin	mmol/L		30	
Calcium	mmol/L		2	2.75
Creatinine	mmol/L	Δ from BL		↑ 44.2
Glucose (Fasting)	mmol/L		3	9
Potassium	mmol/L		3	5.5
Sodium	mmol/L		130	150
Total CO2	mmol/L		18	32

Liver Function			
Test Analyte	**Units**	**Category**	**Clinical Concern Range**
ALT/SGPT	U/L	High	≥ 2x ULN
AST/SGOT	U/L	High	≥ 2x ULN
AlkPhos	U/L	High	≥ 2x ULN
T Bilirubin	µmol/L	High	≥ 1.5xULN
T. Bilirubin + ALT	µmol/L U/L	High	1.5xULN T. Bilirubin + ≥ 2x ULN ALT

6.2.1.2. ECG

ECG Parameter	Units	Clinical Concern Range	
		Lower	Upper
Absolute			
Absolute QTc Interval	Msec		> 450
Absolute PR Interval	Msec	< 110	> 220
Absolute QRS Interval	Msec	< 75	> 110
Change from Baseline			
Increase from Baseline QTc	Msec		> 60

6.2.1.3. Vital Signs

Vital Sign Parameter (Absolute)	Units	Clinical Concern Range	
		Lower	Upper
Systolic Blood Pressure	mmHg	< 85	> 160
Diastolic Blood Pressure	mmHg	< 45	> 100
Heart Rate	bpm	< 40	> 110

Vital Sign Parameter (Change from Baseline)	Units	Clinical Concern Range	
		Decrease	Increase
Systolic Blood Pressure	mmHg	≥ 20	≥ 20
Diastolic Blood Pressure	mmHg	≥ 10	≥ 10
Heart Rate	bpm	≥ 15	≥ 15

6.2.2. Study Phase

6.2.2.1. Study phases for concomitant medication

Study Phase	Definition
Prior	If medication end date is not missing and is before 28 days prior to the most recent screening visit assessment
Concomitant	Any medication that is not a prior

Notes:

Please refer to Appendix Section 6.2.7.1: Handling of partial dates for concomitant medication. Use the rules in this table if concomitant medication date is completely missing.

6.2.3. Study Day and Reference Dates

Study day is calculated as the number of days from Date of First skin challenge done at Visit 1:

- Ref Date = Missing → Study Day = Missing
- Ref Date < First skin challenge Date → Study Day = Ref Date – Date of first Visit 1 Skin Challenge
- Ref Date ≥ First skin challenge Date → Study Day = Ref Date – (Date of First Skin Challenge) + 1

6.2.4. Assessment Window

No Assessment Windows will be defined for analysis, and all (except Safety) summaries and analyses will be based on nominal visits. Safety summaries and analyses will use the remapped visits. Please see OPS for further details.

Unscheduled and withdrawal visits will not be slotted to an analysis timepoint, but they will be considered for worse case and maximum displays. If multiple assessments qualify for worse case or maximum, the earlier one will be used.

Acceptable windows are included in the SRM and assessments performed within these windows are not protocol deviations.

6.2.5. Multiple measurements at One Analysis Time Point

When triplicate ECG/Vital Signs assessments are taken, mean of the measurement will be calculated first and summary statistics will be based on the calculated mean. This will apply to both baseline and post baseline assessments.

For lab tests on a study day, if more than one assessment is taken on the same day from the same type of local lab, the worst case will be used.

6.2.6. Premature Withdrawals

Element	Reporting Detail
General	• Participant study completion (i.e. as specified in the protocol) was defined as having completed phases of the study including the follow up phone call as shown in the Schedule of Activities (see Protocol) • All available data from participants who were withdrawn from the study will be listed and all available planned data will be included in summary tables and figures, unless otherwise specified.

6.2.7. Handling of Missing data

Element	Reporting Detail
General	• Missing data occurs when any requested data is not provided, leading to blank fields on the collection instrument: o These data will be indicated by the use of a 'blank' in participant listing displays. Unless all data for a specific visit are missing in which case the data is excluded from the table. o Answers such as 'Not applicable' and 'Not evaluable' are not considered to be missing data and should be displayed as such.
Wheal and flare	• No imputation methods will be used for missing value of the longest and orthogonal diameters
AUC	• If there are single non-consecutive missing time points, or 2 consecutive missing time points, AUC will be calculated using trapezoidal rule (using non missing time points) • If 3 or more consecutive time points are missing, AUC will be set to missing • If 50% or more time points are missing, AUC will be set to missing • If at least 50% points are non-missing, and the last 2 timepoints are missing, the AUC will be calculated using non-missing timepoints up to N-2
Biomarkers (IDM)	• No imputation methods will be used for missing value.
AE	• A worst-case scenario approach will be taken to handle missing data: if the relationship to study intervention is missing, the relationship will be "yes", if severity is missing the severity will be set to 'severe', if seriousness is missing the seriousness will be set to 'yes'

6.2.7.1. Handling of Missing & Partial dates

Element	Reporting Detail
General	• Partial dates will be displayed as captured in participant listing displays. • However, where necessary, display macros may impute dates as temporary variables for sorting data in listings only. In addition, partial dates may be imputed for 'slotting' data to study phases or for specific analysis purposes as outlined below. • Imputed partial dates will not be used to derive study day, time to onset or duration (e.g., time to onset or duration of adverse events), or elapsed time variables (e.g., time since diagnosis). In addition, imputed dates are not used for deriving the last contact date in overall survival analysis dataset.

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td>Adverse Events</td><td>

Imputations in the adverse events dataset are used for slotting events to the appropriate study time periods and for sorting in data listings
Partial dates for AE recorded in the CRF will be imputed using the following conventions:

<table>
<tr><td>Missing start day</td><td>If study intervention start date is missing (i.e. participant did not start study intervention), then set start date = 1st of month.

Else if study intervention start date is not missing:

o If month and year of start date = month and year of study intervention start date, then
▪ If stop date contains a full date and stop date is earlier than study intervention start date, then set start date= 1st of month.
▪ Else set start date = study intervention start date.
Else set start date = 1st of month.</td></tr>
<tr><td>Missing start day and month</td><td>If study intervention start date is missing (i.e. participant did not start study intervention), then set start date = January 1.

Else if study intervention start date is not missing:

o If year of start date = year of study intervention start date, then
▪ If stop date contains a full date and stop date is earlier than study intervention start date, then set start date = January 1.
▪ Else set start date = study intervention start date.
Else set start date = January 1.</td></tr>
<tr><td>Missing stop day</td><td>A '28/29/30/31' will be used for the day (dependent on the month and year)</td></tr>
<tr><td>Missing stop day and month</td><td>No Imputation</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Element</th><th colspan="2">Reporting Detail</th></tr>
<tr><td></td><td>Completely missing start/stop date</td><td>No imputation</td></tr>
<tr><td>Concomitant Medications/Medical History</td><td colspan="2">• Partial dates for any concomitant medications recorded in the CRF will be imputed using the following convention:</td></tr>
<tr><td></td><td>Missing start day</td><td>If study intervention start date is missing (i.e. participant did not start study Intervention), then set start date = 1st of month.

Else if study intervention start date is not missing:

o If month and year of start date = month and year of study intervention start date, then

▪ If stop date contains a full date and stop date is earlier than study intervention start date, then set start date= 1st of month.
▪ Else set start date = study intervention start date.
Else set start date = 1st of month.</td></tr>
<tr><td></td><td>Missing start day and month</td><td>If study intervention start date is missing (i.e. participant did not start study Intervention), then set start date = January 1.

Else if study intervention start date is not missing:

o If year of start date = year of study intervention start date, then

▪ If stop date contains a full date and stop date is earlier than study intervention start date, then set start date = January 1.
▪ Else set start date = study intervention start date.

Else set start date = January 1.</td></tr>
<tr><td></td><td>Missing end day</td><td>A '28/29/30/31' will be used for the day (dependent on the month and year).</td></tr>
</table>

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td></td><td><table>
<tr><td>Missing end day and month</td><td>A '31' will be used for the day and 'Dec' will be used for the month.</td></tr>
<tr><td>Completely missing start/end date</td><td>No imputation</td></tr>
</table>
• The recorded partial date will be displayed in listings.</td></tr>
<tr><td>Age</td><td>GSK standard IDSL algorithms will be used for calculating Age.
• Birth date will be presented in listings as ‘YYYY’.
• Birth date and month will be imputed as ‘30th June’ for calculating age since only birth year is collected.
The reference day for age calculation will be screening visit date (Screening date should be used to derive age as per programming note in Demo table).</td></tr>
</table>

7. TRADEMARKS & ABBREVIATIONS

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SAS

Abbreviation	Description
AE	Adverse Event
GSK	GlaxoSmithKline
IDM	Intradermal Micro dialysis
IDSL	Integrated Data Standards Library (GSK Standards Library)
PCI	Potential Clinical Importance
SOC	System Organ Class
BMI	Body Mass Index
DRC	Data Review Committee
PT	Preferred Term
OPS	Output &Programming Specification
PD	Pharmacodynamic
QTcF	Frederica's QT Interval Corrected for Heart Rate
SD	Standard Deviation
SP	Substance P
ECG	Electrocardiogram
MCMC	Monte Carlo Markov Chain

8. REFERENCES

Chung, Y., Rabe-Hesketh, S., Dorie, V., Gelman, A., Liu, J. (2013). A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. Psychometrika, 78, 685–709.