Information Type:	Statistical Analysis Plan (SAP)

TITLE PAGE

Protocol Title:	A randomized, double-blind, single-site, two-way crossover Phase 1 study to assess the effect of repeated doses of Test propellant (HFA-152a) on mucociliary clearance as compared to Reference propellant (HFA-134a) in healthy male and female participants.
Study Number:	221781
Compound Number:	AH3365 – Salbutamol
Abbreviated Title:	PH I, Study of the effect of HFA-152a and HFA-134a propellants on mucociliary clearance in healthy participants

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

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

VERSION HISTORY

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
SAP	19 Jul 2024	Protocol 14 May 2024 (v2.0)	Not Applicable	Original version

1. INTRODUCTION

The purpose of this SAP is to describe the planned analyses to be included in the CSR for Study 221781. Details of the final analyses are provided. It describes the rules and conventions to be used in the analysis and presentation of data, the data to be summarised and analysed, including specificities of the statistical analyses to be performed.

Any post-hoc, or unplanned, analyses not identified in this SAP performed will be clearly identified in the respective CSR.

1.1. Objectives, Estimands and Endpoints

Objectives	Endpoints
Primary	
• To assess mucociliary clearance (MCC) expressed as integrated measurements of retained radioactivity in the right lung up to 4 h after 7-day administration of Test and Reference propellants to healthy male and female participants	• AUC0-4h after nebulized ^{99m}Tc inhalation on Day 7
Secondary	
• (MCC) To assess MCC expressed as retained radioactivity in the right lung at individual times after 7-day administration of Test and Reference propellants to healthy male and female participants	• Percent radiolabelled particle retention at 1 h after nebulized 99mTc inhalation on Day 7 • Percent radiolabelled particle retention at 1.5 h after nebulized 99mTc inhalation on Day 7 • Percent radiolabelled particle retention at 3 h after nebulized 99mTc inhalation on Day 7
• (Safety) To assess the safety and tolerability after 7-day administration of Test and Reference propellants in healthy male and female participants.	• Incidence of AEs and SAEs

Primary estimand

What is the Geometric Mean Ratio (GMR) in primary parameter AUC0-4h after repeated dosing for 7 days of HFA-152a, aka 1 – Difluoroethane (Test propellant) and HFA-134a, aka 1,1,1,2 – Tetrafluoroethane (Reference propellant) in healthy participants?

The estimand is described by the following attributes:

- *Population:*

  Healthy male or female participants aged 30 to 55 years.

- *Treatment condition:*

  Administration of repeated doses of Test propellant (HFA-152a) or Reference propellant (HFA-134a) for 6 days, given as 4 inhalations BID and last administration on the morning of Day 7.

- *Variable / endpoint:*

  AUC(0-4h) in the right lung after nebulized 99mTc inhalation on Day 7.

- *Summary measure:*

  Ratio of adjusted GM for AUC(0-4h) (for logarithmic transformed values) following repeated dosing of the 2 formulations (HFA-152a and HFA-134a), with 90% CI.

- *Intercurrent events:*
  - Treatment discontinuation due to any reasons (Principal stratum strategy)
  - Participants unable to take all doses as prescribed (Principal stratum strategy)
  - Dosing error in propellants (Principal stratum strategy)

The principal stratum strategy is a commonly accepted strategy for MCC studies. For all ICEs, interest lies in evaluating primary parameter and comparison of the ratio of 2 formulations for AUC(0-4h) after nebulized 99mTc inhalation in a principal stratum strategy for participants completed the study and who achieved 100% compliance with no dosing error.

Rationale for estimand: The Test formulation is being developed with the aim to have comparable AUC(0-4h) after nebulized ^{99m}Tc inhalation characteristics as the reference formulation, under the scenario where participants are required to have the desired levels of exposure to study intervention. Interest lies in the AUC(0-4h) values obtained in the scenarios had the participant been exposed to correct dose as prescribed, completed the study and 100% compliance.

Estimands Supporting Secondary MCC Objectives

The secondary clinical question of interest is: What is the mean difference in percent radiolabeled particle retention at 1, 1.5, and 3 hours after nebulized 99mTc inhalation, after repeated dosing for 7 days of HFA-152a , aka 1 – Difluoroethane (Test propellant) and HFA-134a, aka 1,1,1,2 – Tetrafluoroethane (Reference propellant) in healthy participants?

This secondary MCC estimand has the same estimand attributes as the primary estimand (population, treatment condition, ICEs strategy, rationale for the estimand), except for the following attributes:

- *Endpoint:*
  - Right lung percent radiolabeled particle retention at 1 h after nebulized 99mTc inhalation on Day 7
  - Right lung percent radiolabeled particle retention at 1.5 h after nebulized 99mTc inhalation on Day 7
  - Right lung percent radiolabeled particle retention at 3 h after nebulized 99mTc inhalation on Day 7
- *Population-level summary:*

  Adjusted mean difference in percent radiolabeled particle retention at 1, 1.5 and 3 h following repeated dosing of the 2 formulations (HFA-152a and HFA-134a), with 90% CI.

Estimands Supporting Secondary Safety Objectives

The clinical question of interest for the safety secondary objective is:

What is the safety and tolerability profile of HFA-152a (Test propellant) compared to HFA-134a (Reference propellant) in healthy participants?

This secondary safety estimand has the same estimand attributes as the primary estimand (population, treatment condition), except for the following attributes:

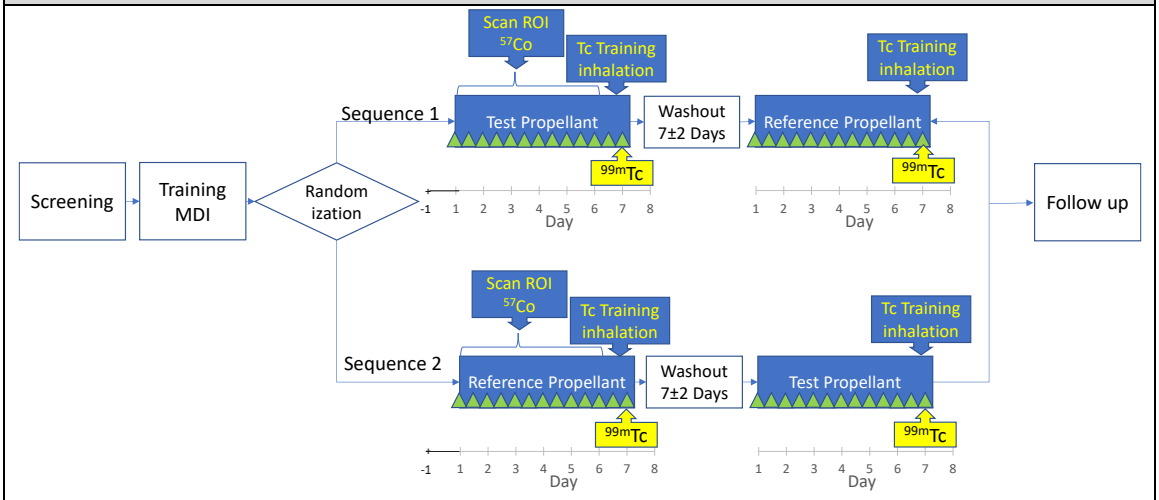
- *Endpoint*:

  Incidence of AE and SAEs.
- *ICEs and estimand strategies*:
  - Treatment discontinuation due to any reasons – (Treatment policy strategy)
  - Participants unable to take all doses as prescribed (Treatment policy strategy)
  - Dosing error in propellants (Treatment policy strategy)
- *Population-level summary*:

  Number and percentages for incidence of AEs and SAEs

  Rationale for estimand: This is a study in healthy volunteers with short follow-up duration. Therefore, all AEs and SAEs data are of interest, regardless of ICEs to support completeness of reporting and transparency of the study.

1.2. Study Design

Overview of Study Design and Key Features	
	
Design Features	This is a randomized, double-blind, single-site, 2-way crossover study to assess the effect of repeated doses of Test propellant (HFA-152a, aka 1 – Difluoroethane) on MCC as compared to Reference propellant (HFA-134a, aka 1,1,1,2 – Tetrafluoroethane) in healthy male and female participants. The study has planned to enrol a total of 24 healthy participants to ensure at least 20 participants are included in the principal stratum analysis set with at least 10 participants randomized to each treatment sequence.
Study intervention	• HFA-152a, aka 1 – Difluoroethane • HFA-134a, aka 1,1,1,2 – Tetrafluoroethane
Study intervention Assignment	Participants will be randomly assigned 1:1 to 1 of 2 treatment sequences in 2 treatment periods using a cross-over design. The following treatment sequences will be tested: • Treatment Sequence 1: T-R • Treatment Sequence 2: R-T Where T is HFA-152a, aka 1 – Difluoroethane (Test) and R is HFA-134a, aka 1,1,1,2 – Tetrafluoroethane (Reference). In each study period, Test or Reference propellants will be administered by oral inhalation BID on Days 1-6 and as a single dose on the morning of Day 7. MCC will be determined using gamma-scintigraphy following the administration of nebulized colloidal 99mTc-sulphur colloid by inhalation after the final dose of the

Overview of Study Design and Key Features	
	propellant on Day 7. The study will comprise a Screening Period of up to 21 days prior to first dosing; Two TPs of 8 days each, with a 7±2 days Washout Period between the 2 TPs; and a final safety Follow-up Visit up to 8 ± 2 days after the final dose administration in TP2 (maximal duration 56 days).
Interim Analysis	Not Applicable

2. STATISTICAL HYPOTHESES

2.1. Multiplicity Adjustment

The primary or secondary endpoints will be analyzed in a descriptive manner with no formal hypothesis testing and therefore no multiplicity adjustments will be applied.

3. ANALYSIS SETS

Analysis Set	Definition / Criteria	Analyses Evaluated
Screened	• *All participants who were screened for eligibility*	All Screened, Reason for Screen Failures
Enrolled	*All participants who screen passed and entered in the study (who were randomized).* • *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*
Full Analysis Set (FAS)	• All randomized participants who received at least a single dose period of study intervention • Data will be reported according to the randomized study intervention.	• Study Population
MCC (Principal Stratum)	• All participants who were able to adhere to all doses of study intervention as prescribed and completed the study	Estimand for primary and secondary objective of MCC parameters
Safety	• All participants who received at least one dose of study intervention • single dose = 1 puff / actuation	Estimand for secondary safety objective

4. STATISTICAL ANALYSES

4.1. General Considerations

The purpose of this study is to investigate the effect of the new propellant HFA-152a (Test) on MCC in comparison to that of the current propellant HFA-134b (Reference) in healthy male and female participants using the percent radiolabeled particle retention following inhalation of a radiolabeled compound (colloidal 99mTc sulphur) after a short, repeated exposure to the Test and Reference propellants.

No formal hypothesis testing will be performed for the primary and secondary endpoints. This study is designed to estimate MCC of orally inhaled HFA-152a MDI (Test propellant) and HFA-134a MDI (Reference propellant). For this aim, the GMR will be calculated by taking the ratio of the GMs of the Test (T) to the Reference propellant (R) for primary endpoint, area under the percent radiolabeled particle retention -time curve up to 4 hours(AUC_{0-4h}), and 90% Confidence Interval of GMR (T/R) will also be calculated.

4.1.1. General Methodology

Participants who prematurely withdraw from study will not be replaced.

Calculations will be based on the actual sampling times recorded during the study. From the retained radioactivity data, the following parameters will be estimated: retained radioactivity at all different timepoints and AUC0-4h of the retained radioactivity.

The MCC parameters data will be summarized using descriptive statistics (N, n, arithmetic mean, 95% CI of arithmetic mean, GM, 95% CI of GM, SD, SD (log_e) median, CV_b in %, minimum and maximum), and will be listed and summarized in tabular and/or graphical form. Where appropriate, listings and graphical presentation will also be produced for MCC parameters endpoints. Discrete data (safety) will be summarized by counts and percentages as appropriate.

4.1.2. Baseline Definition

For the MCC endpoints, the baseline value will be the measurement of retained radioactivity performed immediately (scintigraphic time=”0”) after nebulized 99mTc administration.

For the other (safety) endpoints, the baseline value will be the latest pre-dose assessment with a non-missing value, including those from unscheduled visits. If time is not collected, Day 1 assessments are assumed to be taken prior to first dose and used as baseline.

4.2. Primary Endpoint(s) Analyses

Primary estimands (refer Section 1.1) for primary endpoints will be implemented for the primary analysis. Primary endpoint analysis will be based on MCC analysis set.

4.2.1. Definition of endpoints

The Primary endpoints are defined in Section 1.1.

The definitions of MCC parameters are described in the following table:

Table 1 MCC parameters

Parameter	Description
AUC0-4h (primary)	Area under the right lung percent radiolabeled particle retention -time curve up to 4 h after dosing with ^{99m}Tc on Day 7
R1h (secondary)	Right lung percent radiolabeled particle retention at 1 h after dosing with ^{99m}Tc on Day 7
R1.5h (secondary)	Right lung percent radiolabeled particle retention at1.5 h after dosing with ^{99m}Tc on Day 7
R3h (secondary)	Right lung percent radiolabeled particle retention at 3 h after dosing with ^{99m}Tc on Day 7

4.2.2. Main analytical approach

The primary MCC endpoint will be analysed using MCC analysis set.

For the $\log_e$ transformed data of AUC(0-4h), descriptive statistics (N, n, GM, 95% CI of GM, SD ($\log_e$), between- subject coefficient of variation [CV_b in %]) will be presented, where $\%CV_b=100*(SQRT\ (EXP(SD^2)-1))$ and SD is the standard deviation on the $\log_e$ scale.

For the untransformed data of AUC(0-4h), descriptive statistics (N, n, AM, 95% CI of AM, SD median, minimum and maximum) will be computed.

Following $\log_e$-transformation, AUC(0-4h) will be analyzed using mixed effect model approach with fixed effect terms for period and treatment group. Participants will be treated as random effects in the model. Unstructured (UN) variance and covariance structures will be assumed. If the above model fails to converge, other covariance structures (e.g. CS, AR(1) etc.) might be investigated. The point estimates and their associated 90% CI will then be back-transformed to provide point estimates and 90% CI for the ratios (GMR) of HFA-152a (test)/HFA-134a (reference).

If an intercurrent event (ICE) (as described in Section 1.1) happens during a specific treatment period, and this event affects the measurement of individual MCC data, then the data points for those affected individuals in the treatment period will not be used for the analysis.

In cases where the measurement is not performed, it will be treated as missing data.

Graphs for MCC parameters -

- Individual percent radiolabeled particle retention - time plots (linear and semi-logarithmic) by subjects
- Individual percent radiolabeled particle retention - time plots (linear and semi-logarithmic) by treatment group
- Median (range) percent radiolabeled particle retention - time plot (linear and semi-logarithmic) by treatment group
- Mean (+ SD) percent radiolabeled particle retention - time plots (linear and semi-logarithmic) by treatment group
- Percent radiolabeled particle retention – time (box plots) by treatment group

Listing for primary endpoint will be generated using MCC analysis set.

4.2.3. Sensitivity analyses

If missing data is more than 10% then a sensitivity analysis will be conducted by using $AUC_{0\text{-}4h}$ estimated by linear extrapolation method of the log-transformed data.

The same analytical method (as described in Section 4.2.2) will be used with the $AUC_{0\text{-}4h}$ estimated data.

4.2.4. Additional estimands

Not Applicable.

4.3. Secondary Endpoint(s) Analyses

Secondary MCC endpoints will be analysed using the secondary estimand (refer to Section 1.1). Secondary endpoint analysis will be based on MCC analysis set.

4.3.1. Secondary MCC endpoints

The secondary MCC endpoints are as follows:

- Right lung percent radiolabeled particle retention at 1 h after dosing with ^{99m}Tc on day 7
- Right lung percent radiolabeled particle retention at 1.5 h after dosing with ^{99m}Tc on day 7
- Right lung percent radiolabeled particle retention at 3 h after dosing with ^{99m}Tc on day 7

4.3.1.1. Definition of secondary MCC endpoints

For the definitions of secondary MCC parameters, refer Section 4.2.1.

4.3.1.2. Main analytical approach

For the untransformed secondary endpoints data, descriptive statistics (N, n, AM, 95% CI of AM, SD) median, minimum and maximum) will be computed.

Secondary endpoints will be analyzed using mixed effect model approach with fixed effect such as period, treatment group and participants as random effects. The point estimates of mean difference and their associated 90% CI will be provided for HFA-152a (Test) and HFA-134a (Reference).

ICEs will be managed in the same way as primary endpoint.

4.3.1.3. Sensitivity analyses

If the distribution of the secondary endpoints' (percent radiolabeled particle retention at 1, 1.5, 3 h after dosing with ^{99m}Tc on day 7) data does not follow normal distribution, then log$_e$ transformation will be applied.

Log$_e$ transformed data of secondary endpoints will be analyzed using mixed effect model approach with fixed effect such as period, treatment group and participants as random effects. The point estimates and their associated 90% CI will then be back-transformed to provide point estimates and 90% CI for the ratio (GMR) of HFA-152a (Test) / HFA-134a (Reference).

If missing data of percent radiolabeled particle retention at either timepoint (1h, 1.5h and 3h) is > 10% then sensitivity analysis will be performed using data obtained using log-linear interpolation/extrapolation imputation of available measurements

4.3.1.4. Additional estimands

Not applicable

4.3.2. Supportive secondary endpoint(s)

Not applicable.

4.4. Tertiary/Exploratory Endpoint(s) Analyses

Not applicable.

4.5. Safety Analyses

Safety analysis set will be used for the analysis of safety endpoints. Secondary estimand to analyses safety endpoints (refer Section 1.1) will be implemented. No formal statistical comparisons will be made for safety data. All safety data will be reported according to the actual treatment the subject received.

The tables will use the "safety" population unless otherwise specified.

4.5.1. Extent of Exposure

The total exposure on study (propellant HFA-152a (Test) or HFA-134a MDI (Reference) is calculated as the date of last dose of study treatment minus first dose of study treatment plus one. Subjects who entered the study treatment phase but did not report any treatment dates will be categorized as having zero days of exposure.For each treatment and period, dose administration and exposure data for all participants will be generated in the listing for MCC.

4.5.2. Adverse Events

The applicable definition of an AE and SAE are in the study protocol Sections 8.4 and 10.3. All reported AEs will be coded using the standard GSK dictionary, (MedDRA ≥ 26.1 version).

An AE is considered study treatment emergent (on-treatment) if the AE onset date is on or after study treatment start date and on or before study treatment stop date plus 1 day (inclusive) follow-up or worsens (change in AE severity from lower severity to higher severity category) relative to the pre-study treatment state.

AEs occurring following dosing in a specific period but before dosing in the next period will be attributed to that specific period. If the time is missing for an AE on a dosing day then the AE will be attributed to the treatment given on that day.

Overview of AEs:

The following points will be presented in a summary table for overview of AEs in overall (on and post-treatment) and on-treatment period.

- Total number of AEs reported with the number of participants who experienced at least 1 AE
- AE by maximum severity intensity

- Drug-related AE and SAE, fatal SAE, drug-related fatal SAE, non-fatal SAE, drug-related non-fatal SAE
- AE leading to discontinuation of study treatment and AE leading to withdrawal from study

AEs and SAEs by SOC and PT:

The below summaries (counts and percentages) will be produced for AEs and SAEs by system organ classes (SOC) and (PT) for overall (on- and post-treatment) and on-treatment period.

- Summary of AEs by SOC and PT
- Summary of SAEs by SOC and PT
- Summary of SAEs by SOC and PT with number of subjects and occurrences

Common AEs:

The following summaries (counts and percentages) will be displayed for common (≥10% incidence, before rounding) AEs for overall (on- and post-treatment) and on-treatment period.

- Summary of common (>=10%) AEs by PTs
- Summary of common (>=10%) Non-SAEs by SOCs and PTs with number of subjects and occurrences
- Summary of Non-SAEs (0-5%) by SOCs and PTs with number of subjects and occurrences
- Summary of common (>=10%) SAEs by SOCs and PTs with number of subjects and occurrences

AEs and SAEs related to study treatment:

Relationship to study treatment, as indicated by the investigator, is classified as “not related” or “related”. AEs with a missing relationship to study treatment will be recorded as “related” to study treatment. If a participant reports the same AE more than once within an SOCs/PTs, the AE with the worst-case relationship to study treatment will be used in the corresponding relationship summaries.

The following summaries (counts and percentages) will be presented for drug-related AEs and SAEs for on-treatment period

- Summary of drug-related (Propellant) AEs by overall frequency (SOC and PT), by test /reference propellant.
- Summary of drug-related (Propellant) SAEs by overall frequency (SOC and PT),by test /reference propellant.
- Summary of serious fatal and non-fatal drug-related adverse events (Propellant) will be created by overall frequency(SOC and PT).,by test /reference propellant.

- Summary of drug-related (Propellant) non-serious AEs by SOC and PT will also be presented , by test /reference propellant.
- Summary of drug-related (99mTc-sulphur colloid inhalation) AEs by overall frequency (SOC and PT)
- Summary of drug-related (99mTc-sulphur colloid inhalation) SAEs by overall frequency (SOC and PT)

AEs and SAEs by severity:

Adverse event severity is categorised as mild, moderate, severe. Adverse events starting after the first dose of study treatment with a missing severity will be classified as severe. If a participant reports an AE more than once within an SOCs/PTs, the AE with the worst-case (maximum) severity will be used in the corresponding severity summaries.

The following summaries (counts and percentages) will be produced for severity of AEs and SAEs by SOCs, PTs, and maximum severity in overall (on- and post-treatment) and on-treatment period.

- Summary of AEs by SOC and PT and maximum severity
- Summary of SAEs by SOC and PT and maximum severity

AEs and SAEs leading to permanent discontinuation of study treatment

The below summaries (counts and percentages) for AEs and SAEs leading to permanent discontinuation of study treatment by SOCs and PTs and maximum severity will be generated in overall (on- and post-treatment) and on-treatment period.

- Summary of AEs leading to permanent discontinuation of study treatment by SOC and PT
- Summary of SAEs leading to permanent discontinuation of study treatment by SOC and PT

Adverse events analyses including the analysis of AEs and SAEs will be based on GSK Core Data Standards(IDSL).

Separate listings will be produced of all AEs for each treatment and period in FAS. Summary of death will be summarized and participants profile for death will also be listed using enrolled participants.

In summary tables, SOCs will be sorted in descending order of the total incidence then alphabetically, PTs will be sorted in descending order of the total incidence then alphabetically within the SOC.

For completely missing or partial missing AE start date or end date, imputation rules will be applied following Appendix 2 Section 6.3.5.

Details of the planned displays for AEs and SAEs are provided in Output programming specifications (OPS) and will be based on GSK Data Standards and statistical principles.

4.5.2.1. Adverse Events of Special Interest

No adverse events of special interest (AESI) are identified for the study therefore no statistical analysis will be performed for AESI.

4.5.3. Additional Safety Assessments (if applicable)

4.5.3.1. Laboratory Data

Laboratory evaluations including the analyses of chemistry laboratory tests, hematology laboratory tests and urinalysis will be based on GSK Core Data Standards.

Table 2 Parameters of laboratory tests assessments

Laboratory Tests	Parameters	
Hematology	• **Platelet count**	
	• **Red blood cell (RBC) count**	
	RBC indices	• Mean corpuscular volume (MCV) • Mean corpuscular hemoglobin (MCH) • %Reticulocytes
	Absolute WBC count with differential:	• Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils
	• **Hemoglobin**	
	• **Hematocrit**	
Clinical chemistry[1]	• Urea • Potassium • Creatinine* • Sodium • Calcium • Glucose fasting • Creatine phosphokinase (CPK)	• Aspartate aminotransferase (AST)/serum glutamic-oxaloacetic transaminase (SGOT)[1] • Alanine aminotransferase (ALT)/serum glutamic-pyruvic transaminase (SGPT)[1] • Alkaline phosphatase[2] • Total bilirubin[1] • Direct bilirubin, only if total bilirubin is elevated • Total protein
Routine urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, [bilirubin, urobilinogen, nitrite, leukocyte esterase] by dipstick • Microscopic examination. Please note, this will only be performed if there is an abnormality in accordance with Clinical Laboratory standard procedures at investigator discretion. • Culture at investigator discretion (if positive: specify pathogen)	

Laboratory Tests	Parameters
Hematology	• **Platelet count**
	• **Red blood cell (RBC) count**
Other screening tests	• FSH and estradiol (as needed in WONCBP only) • Cotinine, alcohol and drug screen (to include at minimum: amphetamines [including XTC], methadone, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serology [(HIV antibody 1/2 antibody test, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)] • Testing for SARS-CoV-2 may be performed at the discretion of the physician and based on local guidelines.

NOTES

1. Details of liver chemistry stopping criteria and required actions and follow-up are given in Section 10.6 of the protocol. Liver Safety: Suggested Actions and Follow-up Assessments [and Study Intervention Rechallenge Guidelines]. All events of ALT [or AST] ≥3 × ULN and total bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT [or AST] ≥3 × ULN and INR >1.5 (if INR measured), which may indicate severe liver injury (possible Hy's law), must be reported to [sponsor] in 24hours (excluding studies of hepatic impairment or cirrhosis).
2. If alkaline phosphatase is elevated, consider fractionating.

* To assess the kidney function, use eGFR 2009 calculator (CKD-Epi creatinine equation). eGFR (based on CKD-Epi) will be measured at all time points when creatinine is measured.

Descriptive statistics (n, mean, standard deviation (SD), median, minimum value (min), and maximum value (max)) will be presented for quantitative measurements of absolute value for continuous laboratory parameters at baseline and each assessed visit on-treatment period. Counts and percentages will be produced for qualitative measurements measure laboratory parameters.

Participants listing of all clinical laboratory data will be produced for each treatment and period in Safety.

If a laboratory value which is expected to have a numeric value for summary purposes, has a non-detectable level reported in the database, where the numeric value is missing, but typically a character value starting with '<=' or '=>' (or indicated as less than x or greater than x in the comment field) is present, the number of significant digits in the observed values will be used to determine how much to add or subtract in order to impute the corresponding numeric value.

- Example 1: 2 significant digits = '<= x' becomes x – 0.01
- Example 2: 1 significant digit = '=> x' becomes x + 0.1
- Example 3: 0 significant digits = '<=x' becomes x – 1

Details of the planned displays for clinical laboratory evaluations are provided in Output programming specifications (OPS) and will be based on GSK Data Standards and statistical principles.

4.5.3.2. Vital Signs

The analyses of vital signs parameters will be based on GSK Core Data Standards (IDSL).

The following vital signs measurements will be measured in a supine position after 5 minutes rest at baseline and each assessed visit.

- Systolic and Diastolic blood pressure
- Pulse rate

Descriptive statistics (n, mean, standard deviation (SD), median, minimum value (min), and maximum value (max)) will be presented at baseline, absolute value of vital signs parameters at each assessed visit for on-treatment period.

Participants listing for all vital signs data will be produced for each treatment and period in Safety.

Details of the planned displays for vital signs parameters are provided in Output programming specifications (OPS) and will be based on GSK Data Standards and statistical principles.

4.5.3.3. ECG

All ECGs will be performed as single assessments at the following times: during screening, upon admission, every morning on dosing days for each TP at pre-dose, 20±10 minutes post-dose, and at follow-up. In case of any QT abnormalities ECGs will be repeated in triplicate. ECG measurements will be recorded, including heart rate (HR), PR interval, QRS duration, QT interval, and QTc interval. QT intervals will be based on Fridericia's formula and reported as per captured in eCRF:

Descriptive statistics (n, mean, standard deviation (SD), median, minimum value (min), and maximum value (max)) will be presented at baseline, and absolute and change from baseline for HR, QTc and other ECG parameters at each assessed visit for overall (on and post treatment) and on-treatment period.

Participants listings will be provided for 12 lead ECGs values and findings including abnormal ECGs for each treatment sequence and period in Safety.

Details of the planned displays for ECG parameters are provided in Output programming specifications (OPS) and will be based on GSK Data Standards and statistical principles.

4.5.3.4. Pulmonary Tests

Spirometry will be performed at the study site to assess FEV1 and FVC.

At least 3 acceptable spirometry maneuvers (from a maximum of 8 attempts) should be achieved on each occasion that spirometry assessments are performed, in accordance with the American Thoracic Society/European Respiratory Society standards [Graham, 2019]. The highest of 3 technically acceptable measurements will be recorded at each visit. Global Lung Function Initiative equations will be used to determine predicted values. Predicted values will be corrected for gender, age, height and race.

Descriptive statistics (n, mean, standard deviation (SD), median, minimum value (min), and maximum value (max)) will be presented at baseline, and absolute and change from baseline for FEV1 and FVC parameters at each assessed visit for overall (on and post treatment) and on-treatment period.

4.5.3.5. LVEF

Not Applicable.

4.6. Other Analyses

4.6.1. Subgroup analyses

No subgroup analysis will be performed for the study.

4.6.2. Other variables and/or parameters

No other variables/parameters will be defined for the study.

4.6.3. Analyses to Support Regional Submission

No analysis will be performed for regional submission.

4.7. Interim Analyses

No planned interim analyses.

4.8. Changes to Protocol Defined Analyses

There was one change to the originally planned statistical analysis specified in the protocol (Dated: [14 May 2024]).

An additional FAS analysis set has been added to the Analysis set table.

5. SAMPLE SIZE DETERMINATION

Up to 24 healthy participants will be randomly assigned to ensure at least 20 participants are included in the MCC analysis set with 10 randomized to each treatment sequence.

The target number of participants is chosen to achieve half width (refer to Table 3) of the 90% CI, in the estimate of the GMR for the primary endpoint.

An MCC study [Del Donno, 1988] reported a within-participant coefficient of variation (CVw) of 8.5% for the $AUC_{0\text{-}6h}$ parameter. The within-participant CVw for the $AUC_{0\text{-}4h}$ parameter was thus assumed to range from 8.5 % to 11.5%.

Using a sample size 20 and CVw of 10.5% in $AUC_{0\text{-}4h}$, the half width of the upper bound of 90% CI for observed treatment ratio of $AUC_{0\text{-}4h}$ between two treatment arms is 5.93 % (Table 4). Considering for example, that the GMR between Test and Reference is 1.05 for n = 20 and CVw = 10.5%, then the upper bound of the 90% CI for observed treatment ratio is 1.05*(1+0.0593)=1.11 and the lower bound is 1.05/(1+0.0593)=0.99 (ii).

Table 3 Half-width Expected for Different Sample Size and Intra-Subjects Variability

Sample size	Intra subject variability SD (CVw%)	Half-width (%) of the upper bound of 90% CI for Observed Treatment Ratio
16	0.0849 (8.5%)	5.44
18		5.07
20		4.77
22		4.52
16	0.0948 (9.5%)	6.09
18		5.68
20		5.35
22		5.06
16	0.1047 (10.5%)	6.76
18		6.30
20		**5.93**
22		5.61
16	0.1146 (11.5%)	7.42
18		6.92
20		6.51
22		6.16

Abbreviations: CI=confidence interval; CVw=coefficient of variation within subject

Table 4 Lower and Upper Bound GMR for Different Values of GMR

GMR (T/R)	Lower and Upper bound for observed GMR (T/R)	
	Lower Bound	**Upper Bound**
0.9	0.85	0.95
0.95	0.90	1.01
1	0.94	1.06
1.05	**0.99**	**1.11**
1.1	1.04	1.17

Note: n = 20 & CVw = 10.5%
Abbreviations: CVw=coefficient of variation within subjects, GMR=geometric mean ratio, R=reference, T=test.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

The study population analyses will be based on the FAS and enrolled participants, unless otherwise specified. A summary of the number of participants in each of the participant level analysis set will be provided.

Study population analyses will be included in analyses of participant's disposition, protocol deviations, demographic and baseline characteristics, prior and concomitant medications etc. based on GSK Core Data Standards.

6.1.1. Participant Disposition

Participant disposition will be tabulated by each treatment sequence for all participants combined with the counts and percentages of participants who complete the study, prematurely withdraw, and the reason for study withdrawal for FAS and enrolled participants.

Summary of treatment status and reasons for discontinuation of study treatment will be produced for study treatment, with the number of participants who completed study treatment, or prematurely discontinued treatment, and the primary reasons for treatment discontinuation tabulated for the FAS.

Participant listing of the reasons for study withdrawal will be provided for each treatment sequence and period in enrolled participants.

Summary of screen failure (did not meet inclusion/exclusion criteria, AE, SAE and other) and its reason will be provided for overall (Total) screened participants.

Table will be generated for summarizing the number of participants in each analysis set for all participants who provided informed consent.

6.1.2. Demographic and Baseline Characteristics

Demographic characteristics and baseline characteristics such as age, age group, sex, race, ethnicity, childbearing potential, height, weight, and body mass index (BMI) will be summarized and tabulated by treatment sequence and overall (Total) using participants in FAS and enrolled participants. Listing for demographic characteristics will be produced in FAS for each treatment sequence.

Descriptive statistics will be presented for age, height, weight, and BMI. Counts and percentages will be presented for sex, race, ethnicity.

6.1.3. Protocol Deviations

Important protocol deviations will be summarized for FAS in overall (Total) participants. Listing of important protocol deviation will also be generated for each treatment and period in FAS.

A summary of inclusion and exclusion criteria will be produced for FAS in overall (Total) participants.

Listing for participants with inclusion/exclusion criteria deviations will be generated for each treatment sequence in FAS.

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 (where possible without knowing the study treatment details) are captured and categorized in the protocol deviations dataset.
- This dataset will be the basis for the summaries of important protocol deviations.
- Data will be reviewed prior to freezing the database to ensure all deviations leading to analysis population exclusions are captured and categorized in the protocol deviations ADaM dataset (note these exclusions are not captured in the SDTM dataset).

6.1.4. Prior and Concomitant Medications

Prior and Concomitant medications will be coded using WHO Drug dictionary version on or after Global B3 Sept 2023.

Multi-ingredient term concomitant medications will be presented according to their combination ATC classification rather than the classifications of the preferred term.

A concomitant medication will be summarized (number and percentage) by ATC 1 and ingredient for FAS in each period (pre/on/post) in which it was taken, so a concomitant medication that was started in the screening and stopped during active treatment will appear in both the pre-treatment and the on-treatment tables.

Concomitant medications include any medication that was taken at some point during the on-treatment period as defined in Section 6.9 of the protocol.

Participants listing of concomitant medication will also be generated for FAS.

6.1.5. Medical History and Current Medical Conditions

Listings for medical history and current medical conditions will be presented for each body system of participants forFAS.

6.2. Appendix 2 Electronic Clinical Outcome Assessment (eCOA) Compliance

Not applicable

6.3. Appendix 3 Data Derivations Rule

Not applicable

6.3.1. Criteria for Potential Clinical Importance

Not applicable

6.3.2. Study Period

Assessments and events will be classified according to the time of occurrence relative to the study treatment period (days).

Pre-Treatment : For the presentation of serious adverse events (SAEs), concomitant medications, and other assessments in pre-treatment is defined as -

Date of assessment < Study treatment start date of period 1 (Day 1).

On-Treatment : For the presentation of adverse events (AEs), serious adverse events (SAEs), concomitant medications, 12-lead ECG, vital signs, and other assessments in on-treatment is defined as -

Study treatment start date of period 1 (Day 1 of Period 1) <= Date of assessment < Study treatment stop date of period 2 (Day 8of Period 2) or early withdrawal + 1 days

If time of assessment or study treatment is not collected (missed), the following assessment on the first dose date will be assumed to be taken prior to the first dose and therefore considered pre-treatment .

Post-Treatment : For the presentation of adverse events (AEs), serious adverse events (SAEs), concomitant medications,12-lead ECG, vital signs and laboratory assessments in post-treatment is defined as

> Date of assessment > Study treatment stop date of period 2 (Day 8 of Period 2) or early withdrawal + 1 days.

Please refer Section 6.3.5 for handling of missing and partial dates of assessments.

6.3.3. Study Day and Reference Dates

The safety reference date is the study intervention start date and will be used to calculate study day for safety measures.

The efficacy reference date is [the date of randomization OR the study intervention start date] and will be used to calculate study day for efficacy measures and baseline characteristics, as well as efficacy durations.

The study day is calculated as below:

- Assessment Date = Missing → Study Day = Missing
- Assessment Date < Reference Date → Study Day = Assessment Date – Ref Date
- Assessment Date ≥ Reference Date → Study Day = Assessment Date – Ref Date + 1

6.3.4. Assessment Window

Protocol assessment window as defined in protocol Section 1.3 will be followed for the statistical analysis.

6.3.5. Handling of Partial Dates

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td>General</td><td>• 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.</td></tr>
<tr><td>Adverse Events</td><td>• 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:
• If month and year of start date = month and year of study intervention start date, then</td></tr></table></td></tr>
</table>

Element	Reporting Detail	
		– *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.*
	Missing start day and month	*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:* • *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.*
	Missing end day	*A '28/29/30/31' will be used for the day (dependent on the month and year).*
	Missing end day and month	*No Imputation*
	Completely missing start/end date	*No imputation*
Concomitant Medications/Medical History	• Partial dates for any concomitant medications recorded in the CRF will be imputed using the following convention:	
	Missing start day	*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:* • *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.*
	Missing start day and month	*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:* • *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.*

Element	Reporting Detail	
	Missing end day	*A '28/29/30/31' will be used for the day (dependent on the month and year).*
	Missing end day and month	*A '31' will be used for the day and 'Dec' will be used for the month.*
	Completely missing start/end date	*No imputation*
Age	Age is derived using the date of first dose. When first dose date is missing, the ICF date is used. Only year of birth is collected so Day and Month of birth are imputed as 30 June. Formula for deriving age is the integer component of: (First Dose Date – 30 Jun of collected birth year +1)/365.25	

6.3.6. Trademarks

Trademarks of the [GlaxoSmithKline / ViiV Healthcare] Group of Companies
ADVAIR

Trademarks not owned by the [GlaxoSmithKline / ViiV Healthcare] Group of Companies
NONMEM
SAS
WinNonlin

7. REFERENCES

Del Donno M, Pavia D, Agnew JE, et al. Variability and reproducibility in the measurement of tracheobronchial clearance in healthy subjects and patients with different obstructive lung diseases. Eur Respir J. 1988;1(7):613-20.