Statistical Analysis Plan Amendment 2

Study ID: 223166

Official Title: A Randomized, Non-inferiority, Double-blind, Controlled, Single-dose, 2-way Cross-over Study to Assess the Potential for Airway Sensitivity Reactions With Propellants HFA-152a (Test) and HFA-134a (Reference) Administered Via Pressurized Metered Dose Inhalers in Adults Aged 18-45 With Mild Asthma

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TITLE PAGE

Protocol Title:	A randomized, non-inferiority, double-blind, controlled, single-dose, 2-way cross-over study to assess the potential for airway sensitivity reactions with propellants HFA-152a (Test) and HFA-134a (Reference) administered via pressurized metered dose inhalers in adults aged 18-45 with mild asthma.
Study Number:	223166
Compound Number:	AH3365 – Salbutamol

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	22 November 2025	Protocol 08 October 2024(v3.0)	Not Applicable	Original version
SAP amendment 1	25 March 2025	Protocol 08 October 2024(v3.0)	• 4.2.1.1: Added derivation of FEV1 AUC • 6.1.4: Added ACT Classification • 6.3.2: Updated Post-Treatment Definition	Decided to perform FEV1 AUC derivation in-house.
SAP amendment 2	23 Jun 2025	Protocol 08 October 2024(v3.0)	• 4.6.2.3: Participant experience questionnaire has been removed • 4.8: Updated to reflect the removal of section 4.6.2.3. • 6.3.4: Updated to reflect the prioritization of scheduled visits in assessments.	The questionnaire is not study specific, as it gathers general clinical study feedback independentl y.

1. INTRODUCTION

The purpose of this SAP is to describe the planned analyses to be included in the CSR for Study 223166. 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 evaluate the potential for bronchoconstriction in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants	• Percentage change from baseline in FEV1 (at 15 mins) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants
Secondary	
To evaluate variables linked to the potential for HFA-152a to cause bronchoconstriction when administered via a pMDI containing HFA-152a (Test) and HFA-134a (Reference) propellants.	• FEV1 AUC0-15min after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants. • Percentage change from baseline in FEV1 (at timepoints, 5, 60 and 180 minutes) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants. • Participants with a percentage change from baseline in FEV1 < -15% (at timepoints 5, 15, 60 and 180 minutes) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants
To assess the safety and tolerability of single doses of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.	• Incidence of AEs and SAEs

AE = Adverse event; FEV1 = Forced expiratory volume in 1 second; FEV1 AUC0-15 mins = Area under the forced expiratory volume in 1 second-time curve from zero to 15 minutes; HFA-134a = 1,1,1,2-Tetrafluoroethane (Reference); HFA-152a = 1,1-Difluoroethane (Test); pMDI = Pressurized metered dose inhalers; SAE = Serious adverse event.

Primary estimand

The primary clinical question of interest is:

What is the adjusted mean difference in percentage change from baseline in FEV1 (at 15 mins post-dose) in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, in participants aged between 18 and 45 years with mild asthma who completed the study treatments without the use of rescue medication or making an incorrect number of actuations?

The estimand is described by the following attributes:

- Population:

  Male or female participants aged 18 to 45 years with mild asthma.

- Treatment condition:

  Administration of a single dose of propellant HFA-152a (Test) or HFA-134a (Reference) administered via a pMDI.

- Variable / endpoint:

  Percentage change from baseline in FEV1 (at 15 mins) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

- Summary measure:

  Adjusted mean difference in percentage change from baseline in FEV1 (at 15 mins post-dose) between pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, with 95% CI.

- Intercurrent events:

  1. Treatment discontinuation due to any reasons (Principal stratum strategy)
  2. Use of rescue medication (Principal stratum strategy)
  3. Incorrect number of actuations (Principal stratum strategy)

For all ICEs, interest lies in evaluating the primary parameter and comparison of the mean percentage change from baseline in FEV1 (at 15 mins) after administration of a single dose of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants using a principal stratum strategy for participants who completed the study without the use of rescue medication or incorrect number of actuations.

Rationale for estimand: The Test propellant is being developed with the aim to have comparable percentage change in FEV1 (at 15 mins post-dose) as the Reference propellant, under the scenario where participants are required to have the desired levels of exposure to study intervention. Interest lies in participants who adhere to the treatment sequence. The estimation will be based on the set of participants who received treatment in both periods, given the small sample size and the low likelihood of participants failing to complete the treatment sequence.

Estimands Supporting Secondary Objectives

1. FEV1 AUC0-15mins

The key secondary clinical question of interest is:

What is the adjusted mean difference in FEV1 AUC0-15min, in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, in participants aged between 18 and 45 years with mild asthma who completed the study treatments without the use of rescue medication or making an incorrect number of actuations?

This secondary 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:

FEV1 AUC0-15mins after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

- Population-level summary:

Adjusted mean difference in FEV1 AUC0-15min between pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, with 95% CI.

2. Percentage Changes from Baseline in FEV1

The secondary clinical question of interest is:

What is the adjusted mean difference in percentage change from baseline in FEV1 (at 5, 60 and 180 minutes post-dose) for pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, in participants aged between 18 and 45 years with mild asthma who completed the study treatments without the use of rescue medication or making an incorrect number of actuations?

This secondary 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:

Percentage change from baseline in FEV1 (at timepoints 5, 60 and 180 minutes) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

- Population-level summary:

Adjusted mean difference in percentage change from baseline in FEV1 (at 5, 60 and 180 minutes post-dose) between pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, with 95% CI.

3. Participants with a Percentage Change from Baseline in FEV1 <-15%

The secondary clinical question of interest is:

What is the proportion of participants with a percentage change from baseline in FEV1 <-15% (at timepoints 5, 15, 60 and 180 minutes), in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, in participants aged between 18 and 45 years with mild asthma who completed the study treatments without the use of rescue medication or making an incorrect number of actuations?

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

- Endpoint:

Participants with a percentage change from baseline in FEV1 <-15% (at timepoints 5, 15, 60 and 180 minutes) after administration of a single dose of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

- Population-level summary:

Number and percentage of participants with a percentage change from baseline in FEV1 <-15% (at timepoints 5, 15, 60 and 180 minutes) after administration of a single dose of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

Estimands Supporting Secondary Safety Objectives

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

What is the safety and tolerability of single doses of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants in participants aged between 18 and 45 years with mild asthma, regardless of whether they completed the study treatments, used rescue medication, or made incorrect number of actuations?

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:

1. Treatment discontinuation due to any reasons – (Treatment policy strategy)
2. Use of rescue medication (Treatment policy strategy)
3. Incorrect number of actuations (Treatment policy strategy)

Population-level summary:

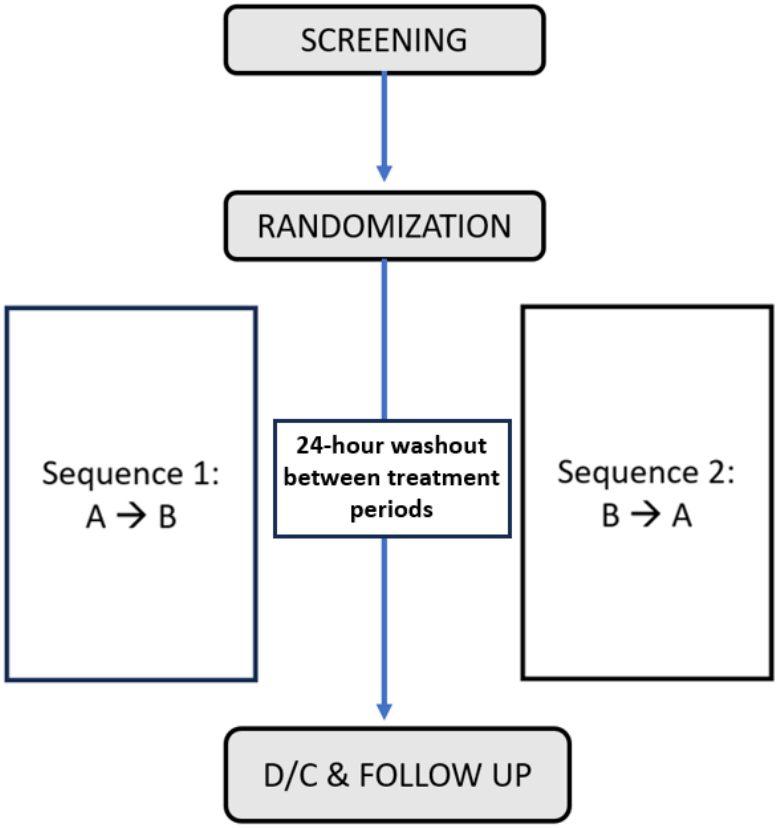
Number and percentages for incidence of AEs and SAEs

Rationale for estimand:

This is a study in mild asthma patients 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 clinical study.

1.2. Study Design

Overview of Study Design and Key Features



Treatment A = Propellant HFA-152a pMDI; Treatment B = Propellant HFA-134a pMDI; pMDI= Pressurized metered dose inhaler, D/C = Discharge.

Design Features	This is a randomized, non-inferiority, double-blind, controlled, single dose (8 puffs), 2-way cross-over study in mild asthmatic subjects with administration of pMDIs containing propellants HFA-152a (Test) and HFA-134a (Reference). A minimum of 16 participants (approximately n = 18 enrolled to account for potential ICEs) will be randomly assigned with 8 randomized to each treatment sequence. Participants will be randomized to 1 of the 2 treatment sequences AB or BA.

Overview of Study Design and Key Features	
Study intervention	• HFA-152a (Test) • HFA-134a (Reference)
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: AB Treatment Sequence 2: BA (where A is the Test propellant and B is the Reference). A single dose will be administered, to deliver 8 actuations of either Test or Reference propellant. Participants will be randomly assigned to one of 2 treatment sequences in 2 treatment periods using a cross-over design. There will be a 24-hour washout period (i.e., treatment with the propellants will be given on 2 consecutive days). Spirometry assessments will be recorded during the study treatment periods to assess for bronchoconstriction. ECG, clinical laboratory tests and vital signs will be recorded as safety assessments. The period between randomization and screening will be maximum of 28 days. Participants will arrive at the clinic on Day-1 and will remain confined to the clinic for TP1 and TP2, which are consecutive treatment days. Subjects will exit the study when deemed safe for discharge following all assessments on TP2 by the investigator.
Interim Analysis	Not Applicable

2. STATISTICAL HYPOTHESES

The primary objective of the study is to evaluate the potential for bronchoconstriction in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants in participants aged 18 to 45 with mild asthma. This is a non-inferiority study designed to test whether pMDIs containing HFA-152a (Test) propellant is not worse than HFA-134a (Reference) propellant. The primary endpoint of the study is percentage change from baseline in FEV1 (at 15 mins) after administration of a single dose of pMDIs. The study is designed to determine whether the lower limit of two-sided 95% confidence interval (CI) of the adjusted mean difference between Test and Reference propellants for the primary endpoint, greater than a predefined non-inferiority margin of -10%. Hence, the null and alternative hypotheses are as follows:

Null Hypothesis (H0):

$\mu T - \mu R \leq -10\%$

Alternative Hypothesis (H1):

$\mu T - \mu R > -10\%$

Where μT represents the mean change from baseline in the HFA-152a (Test) propellant and μR represents the mean change from baseline in the HFA-134a (Reference) propellant.

Here, the null hypothesis (H0) states that the adjusted mean difference between HFA 152a (Test) and HFA-134a (Reference) propellants falls below the non-inferiority margin of -10%. The alternative hypothesis (H1) states that the adjusted mean difference between HFA-152a (Test) and HFA-134a (Reference) propellants does not fall below the non-inferiority margin of -10%.

The non-inferiority margin for the study has been decided based on insights from a previous study [Salvadori, 2023], ensuring it reflects a predefined threshold below which the Test propellant is considered clinically non-inferior to the Reference propellant.

2.1. Multiplicity Adjustment

No multiplicity adjustments will be applied for this study.

3. ANALYSIS SETS

Analysis Set	Definition / Criteria	Analyses Evaluated
Screened	• All participants who were screened for eligibility.	• Study population
Enrolled	• All participants who entered the study (who were randomized or received study intervention or underwent a post screening study procedure). • 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
FAS	• All randomized participants who received at least a single dose of study intervention • Data will be reported according to the randomized study intervention.	• Study Population
Safety	• All participants who received at least one dose of study intervention.	• Safety

Analysis Set	Definition / Criteria	Analyses Evaluated
FCAS	• All participants who were able to adhere to the dose of study intervention as prescribed and completed the study	• Estimand for Primary and Secondary endpoints

FAS = Full Analysis Set; FCAS = Fully compliant analysis set.

4. STATISTICAL ANALYSES

4.1. General Considerations

The primary objective of this study is to demonstrate non-inferiority for bronchoconstriction in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants. The primary endpoint of the study is percentage change from baseline in FEV1 at 15 minutes post-dose. Secondary endpoints will evaluate FEV1 AUC0-15 minutes, safety variables linked to the potential for bronchoconstriction in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants as well as the general safety and tolerability of both propellants containing pMDIs.

A formal hypothesis testing will be performed on the primary endpoint to demonstrate non-inferiority for bronchoconstriction in pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants. Non-inferiority will be demonstrated if the lower limit of the 95% confidence interval for the difference in percentage change from baseline in FEV1 between the pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants exceeds -10% and if the upper limit of the 95% CI exceeds 0%.

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.

The Primary and secondary endpoint 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 primary and secondary endpoints.

Discrete data (safety) will be summarized by counts and percentages as appropriate.

4.1.2. Baseline Definition

For all 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.

Unless otherwise stated, if baseline data is missing no derivation will be performed and baseline will be set to missing.

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 FCAS analysis set.

4.2.1. Definition of endpoints

The definitions of parameters are described in Table 1.

Table 1 Definition of endpoints

Parameter	Description
Percentage change from baseline in FEV1 (at 15 mins post dose) (primary)	Percentage change from baseline in FEV1 (at 15 mins) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants
FEV1 AUC0-15min (secondary)	Area under the FEV1-time curve from time zero to 15 minutes after administration of a single dose(8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants
Percentage change from baseline in FEV1 (at 5, 60 and 180 mins post dose) (secondary)	Percentage changes from baseline in FEV1 (at timepoints 5, 60 and 180 minutes) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants
Participants with a Percentage change from baseline in FEV1 <-15% (at timepoints pre-dose,5, 15, 60 and 180 minutes) (secondary)	Participants with a Percentage change from baseline in FEV1 <-15% (at timepoints 5, 15, 60 and 180 minutes) after administration of a single dose (8 puffs) of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants

FEV1 = Forced expiratory volume in 1 second; FEV1 AUC0-15 mins = Area under the forced expiratory volume in 1 second-time curve from zero to 15 minutes; pMDI = Pressurized metered dose inhalers.

4.2.1.1. Derivation of FEV1 AUC 0-15 min Endpoint

The FEV1 AUC 0-15 min endpoint will be calculated as the area under the curve (AUC) using the trapezoidal rule. The 0-hour value will be the pre-dose measurement and actual times will be used for all other measurements in the calculation of the AUC.

The AUC will be calculated from the first non-missing timepoint to the 15-minute time point. If observation is missing between two non-missing observations, the value will be linearly interpolated between the two non-missing values. The 0 and 15 min FEV1 values must be present for AUC 0-15 min to be calculated.

The AUC 0-15 mins will be calculated as follows:

$$\text{AUC 0-15 min} = {}^{1}/_{2} \sum_{i=0}^{L-1} (C_i + C_{i+1})(t_{i+1} - t_i)$$

Where,

i = collected measurement,

L = last collected measurement within 15 minutes,

C_i = result of collected measurement I,

t_i = actual time of assessment for collected measurement i.

4.2.2. Main analytical approach

The primary analysis will evaluate the primary estimand in the FCAS analysis set.

For the primary endpoint data of percentage change from baseline in FEV1 (at 15 mins post dose), descriptive statistics (N, n, AM, 95% CI of AM, SD) median, minimum and maximum) will be computed.

The primary endpoint will be analysed using mixed effect model approach with the percent change from baseline in FEV1 as the response variable. The model will include fixed effect for FEV1 at baseline, period, treatment group, timepoint, and interaction terms for treatment group and timepoint, FEV1 at baseline and period, FEV1 at baseline and timepoint. Participants will be included as random effects. Unstructured (UN) variance and covariance structures will be assumed. If the above model fails to converge, other covariance structures (e.g. VC, CS, AR(1) etc.) might be investigated. The point estimates of adjusted mean difference and their associated 95% CI will be provided for pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

Non-inferiority will be demonstrated if the lower limit of the 95% confidence interval for the difference in percentage change from Baseline in FEV1 between the pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants exceeds -10%.

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 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 primary parameter -

- Individual percentage change from baseline in FEV1 - time plots (linear and semi-logarithmic) by subjects
- Individual percentage change from baseline in FEV1 - time plots (linear and semi-logarithmic) by treatment group
- Median (range) percentage change from baseline in FEV1 - time plot (linear and semi-logarithmic) by treatment group

- Mean (+ SD) percentage change from baseline in FEV1 - time plots (linear and semi-logarithmic) by treatment group
- Percentage change from baseline in FEV1 – time (box plots) by treatment group

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

4.2.3. Sensitivity analyses

No sensitivity analyses planned.

4.2.4. Additional estimands

Not Applicable.

4.3. Secondary Endpoint(s) Analyses

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

4.3.1. Secondary endpoints

The secondary endpoints are as follows:

- FEV1 AUC0-15min after administration of a single dose of pMDIs
- Percentage changes from baseline in FEV1 (at timepoints 5, 60 and 180 minutes) after administration of a single dose of pMDIs
- Participants with a percentage change from baseline in FEV1 <-15% (at timepoints 5, 15, 60 and 180 minutes) after administration of a single dose of pMDIs

4.3.1.1. Main analytical approach

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

The key secondary endpoint, FEV1 AUC0-15min, will be analyzed using mixed effect model approach with fixed effect period, treatment group and participants as random effects. Unstructured (UN) variance and covariance structures will be assumed. If the above model fails to converge, other covariance structures (e.g. VC, CS, AR(1) etc.) might be investigated. The point estimates of mean difference and their associated 95% CI will be provided for of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants.

The secondary endpoint, percentage change from baseline in FEV1 at the timepoints 5, 60, and 180 minutes post-dose, will be analysed using the same mixed effects model as the primary analysis. Point estimates of adjusted mean differences and their associated 95% confidence intervals at these timepoints will be extracted from the primary analysis model results.

The endpoint, proportion of participants with a Percentage change from baseline in FEV1 <-15% (at timepoints 5, 15, 60 and 180 minutes) after administration of a single dose of pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, will be summarized descriptively.

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

4.3.1.2. Sensitivity analyses

No sensitivity analyses planned.

4.3.1.3. 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 Safety.

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 common ((>=5%) Non-SAEs 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 propellant-related AEs by overall frequency (SOC and PT), by test /reference propellant.
- Summary of propellant-related SAEs by overall frequency (SOC and PT),by test /reference propellant.
- Summary of serious fatal and non-fatal propellant-related adverse events will be created by overall frequency(SOC and PT).,by test /reference propellant.
- Summary of propellant-related non-serious AEs by SOC and PT will also be presented , by test /reference propellant.

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 6.3.6

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	
	• RBC count	
	RBC indices	• MCV • MCH • %Reticulocytes
	Absolute WBC count with differential:	• Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils
	• **Hemoglobin**	
	• **Hematocrit**	
Clinical chemistry[1]	• BUN/Urea • Potassium • Creatinine* • Sodium • Calcium • Glucose fasting • CPK	• AST/SGOT • ALT/SGPT • Alkaline phosphatase • Total bilirubin • Direct bilirubin • 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.	
Pregnancy testing	• Highly sensitive serum or urine hCG pregnancy test [2]	
Other tests conducted at screening/prior to treatment	• FSH and estradiol • 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, 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	

ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; BUN = Blood urea nitrogen; CPK = Creatine phosphokinase; eGFR = Estimated glomerular filtration rate; FSH = Follicle stimulating hormone; HBsAg = Hepatitis B surface antigen; hCG = Human chorionic gonadotropin; HCV = Hepatitis C virus; HIV = Human immunodeficiency virus ;IEC = International ethics committee; INR = International normalized ratio; IRB = Institutional review board; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; RBC = Red blood cell; SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2; SGOT = Serum glutamic-oxaloacetic transaminase; SGPT = Serum glutamic-pyruvic transaminase; ULN = Upper limit of normal; WBC = White blood cell.

1 Details of liver event stopping criteria and required actions and follow-up are given in Protocol Section 7.1.1Liver event stopping criteria and Protocol Section 10.6. Liver safety requirements and 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 24 hours (excluding studies of hepatic impairment or cirrhosis).

2 Local serum testing will be done at sceening and urine testing thereafter.

* To assess the kidney function, use eGFR 2021 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 screening, 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 screening, 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, Day -1, and post-dose TP2 (or early discontinuation). 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 screening, baseline, and absolute and change from baseline for HR, QTc and other ECG parameters at each assessed visit for 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. 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

4.6.2.1. ACQ-6 Score

The ACQ-6 measures 6 attributes of asthma control [Juniper, 1999]. Six attributes are measured with a patient-completed questionnaire, and the questions are designed to be self-completed by the participant. Participants will complete the ACQ at specified study visits.

Descriptive statistics (n, mean, standard deviation (SD), median, minimum value (min), and maximum value (max)) will be presented for ACQ-6 score at each assessed visits for overall and by treatment period.

4.6.2.2. Spirometry

Spirometry will be performed at the study site to assess FEV1 as per protocol section 8.2.2. Spirometry will be conducted at screening, Day-1 and during the study intervention period as detailed in the protocol.

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 at each assessed visit for on-treatment period.

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

The Participant Experience Questionnaire will not be summarized as outlined in the protocol. This questionnaire is part of an independent initiative aimed at engaging in participants to gather insights about their overall experience in clinical studies. It is not specific to this study.

All other originally planned statistical analysis specified in the protocol (Dated: [08 October 2024]) remain unchanged.

5. SAMPLE SIZE DETERMINATION

A minimum of 16 participants (approximately n = 18 enrolled to account for potential ICEs) will be randomly assigned with 8 randomized to each treatment sequence.

The sample size for the primary endpoint was calculated using the 'Non-Inferiority Tests for the Difference Between Two Means in a 2x2 Crossover Design' module within PASS 19 software (Table 3). It was based on an assumption of no difference in percentage change from baseline in FEV1 between the pMDIs containing HFA-152a (Test) and HFA-134a (Reference) propellants, with a non-inferiority margin set at -10%, within-participant variability (Sw) of 8% (conservative approach, as the previous crossover study showed only 4% Sw), 90% power, and a one-sided alpha of 2.5% (total alpha = 5%).

Design assumptions were informed by a previous cross-over study conducted on HFA-152a. A 10% reduction in change in baseline FEV1 at 15 minutes was considered to be a clinically relevant and aligned to the NI margin pre-specified in the prior study. Primary endpoint results reported on percentage change in baseline FEV1 were reported to be 1.86% (95% CI -0.48; 4.20) [Salvadori, 2023].

Table 3 Total Sample Size and Expected 95% Confidence Intervals for Different Assumed Differences (T-R) and Within-Participant Variability

Assumed Difference (T-R)	Sw (Within-Participant Variability)	Total Sample Size	Expected 95% CI
0	7%	14	(-0.0519, 0.0519)
0	8%	16	(-0.0554, 0.0554)
0	9%	20	(-0.0558, 0.0558)
0.019	7%	10	(-0.0424, 0.0804)
0.019	8%	12	(-0.0450, 0.0830)
0.019	9%	16	(-0.0434, 0.0814)

CI = Confidence interval; Sw = within-participant variability.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

Unless otherwise specified, the study population analyses will be based on the FAS and enrolled. 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

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 for FAS. Reasons for study withdrawal will be summarized. For those who have neither completed nor withdrawn, they will be categorized as on study intervention or in follow up.

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

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, 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. 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 intervention details) are captured and categorised in the protocol deviations dataset.
- This dataset will be the basis for the summaries of important protocol deviations.
- Data will be reviewed prior to [unblinding and] freezing the database to ensure all deviations leading to analysis population exclusions are captured and categorised in the protocol deviations ADaM dataset (note these exclusions are not captured in the SDTM dataset).

6.1.4. Prior and Concomitant Medications

Concomitant medications will be coded using WHO Drug dictionaries. However, the summary will be based on GSK Drug dictionary only.

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-intervention period as defined in Section 6.9 of the protocol.

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

6.1.5. Study Intervention Compliance

Not applicable as this is a single dose study.

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 is defined as time prior to the first dose of study treatment.

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

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 1 of Period 2) or early withdrawal + 1 days.

Post-Treatment is defined as any time post On-Treatment window.

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

Please refer Section 6.3.6 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 Data ≥ 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.

If there are multiple assessments, including both scheduled and unscheduled visits, within the same analysis timepoint window, the scheduled visit will take precedence over unscheduled visits for inclusion in the analysis. In cases where multiple records of an assessment, regardless of whether they are from scheduled or unscheduled visits, exist within the same analysis timepoint window, the record closest to the target day will be selected for analysis. If multiple records are equidistant from the target day, the later record will be used in the analysis.

6.3.5. Multiple measurements at One Analysis Time Point

If triplicate ECG 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, the test from a central lab will be taken over the test from a local lab. If multiple assessments are taken from the same type of lab, the worst case will be used.

6.3.6. Handling of 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

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td></td><td>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
– 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:
• 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 end 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 end day and month</td><td>No Imputation</td></tr>
<tr><td>Completely missing start/end date</td><td>No imputation</td></tr>
</table></td></tr>
<tr><td>Concomitant Medications/Medical History</td><td>• Partial dates for any concomitant medications recorded in the CRF will be imputed using the following convention:
<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
– 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.</td></tr>
</table></td></tr>
</table>

Element	Reporting Detail	
		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	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	

6.3.7. Trademarks

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