STATISTICAL ANALYSIS PLAN

Study Code	AZ-RU-00004
Edition Number	3.0
Date	6-Aug-2024

A Phase III, Multicentre, Randomized, Double-blind, Single-Dose, 2-Arm, 2-Period, Crossover Study to Investigate the Efficacy of PT027 Compared with Placebo on Exercise-Induced Bronchoconstriction in Adult Patients with Asthma (BREATH)

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LIST OF ABBREVIATIONS

List abbreviations and definitions of specialized or unusual terms, measurements, or units. Examples are provided below. These can be modified at study level.

Abbreviation or Specialized Term	Definition
AE	Adverse Event
ANCOVA	Analysis of Covariance
AR(1)	Autoregressive order 1 covariance matrix
AUC	Area Under Curve
BMI	Body Mass Index
CI	Confidence Interval
CRF	Case Report Form
CS	Clinically Significant or Compound Symmetric covariance matrix
CSP	Clinical Study Protocol
CSR	Clinical Study Report
ECG	Electrocardiogram
ECT	Exercise Challenge Test
FAS	Full Analysis Set
FEV_1	Forced Expiratory Volume in 1 second
FU	Follow Up
GAM	Generalized Additive Model
GCP	Good Clinical Practice
HR	Heart Rate
ICE	Intercurrent Event
ICH	International Council for Harmonisation
ICS	Inhaled Corticosteroid
IP	Investigational Product
IPD	Important Protocol Deviation
KM	Kaplan-Meier
LSMD	Least Squares Mean Difference
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model with Repeated Measures
NCS	Not Clinically Significant
PD	Protocol Deviation
PE	Physical Examination
PK	Pharmacokinetics
PT	Preferred Term
SAE	Serious Adverse Event

Abbreviation or Specialized Term	Definition
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SOC	System Organ Class
TC	Telephone Call
TOEP	Toeplitz covariance matrix
UN	Unstructured covariance matrix
VS	Vital Signs
WHO Drug	World Health Organization Drug dictionary

AMENDMENT HISTORY

CATEGORY Change refers to:	Date	Description of change	In line with CSP?	Rationale
N/A	11/2/2023	Initial approved SAP	N/A	N/A
Analysis sets	31/07/2024	Name of enrolled/screened participants set is clarified	Yes	Clarification
Baseline characteristics	31/07/2024	Desription of analysis of smoking history is added	Yes	Clarification
Prohibited medications	31/07/2024	Method of classification is added	Yes	Clarification after meeting with Study Clinical Lead
Subgroup analysis	31/07/2024	Wording is updated. GAM models are excluded	Yes	In order to be in line with the global study
Adverse events	31/07/2024	Some clarifications in analysis.	Yes	Clarification after meeting with Study Clinical Lead
Other laboratory data	31/07/2024	Weight, height and BMI will be presented only in listings	Yes	Clarification after meeting with Study Clinical Lead
Prior and concomitant medications	31/07/2024	Definitions and presentation of prior and concomitant medications was clarified	Yes	Clarification after meeting with Lead Statistician
Intercurrent event	06/08/2024	Intercurrent event strategy was clarified	Yes	Clarification after meeting with Lead Statistician

1 INTRODUCTION

The purpose of the current statistical analysis plan (SAP) is a detailed description of basic principles of statistical analysis, description of methods used to analyse the primary and secondary endpoints, and other data obtained during the study.

This plan provides for compliance of the planned and conducted statistical analysis with the clinical study protocol (CSP), including the definition of datasets for analysis, transformations and calculations for endpoints, the required number of observations, etc. All discrepancies with the CSP should be clarified.

The current statistical analysis plan is prepared in compliance with:

- ICH harmonised tripartite guideline. Statistical principles for clinical trials (E9). Step 5 finalized guideline February 1998
- ICH harmonised tripartite guideline. Structure and Content of Clinical Study Reports (E3). Step 5 finalized guideline dated November 1995
- Good Clinical Practice of the Eurasian Economic Union, adapted by the decision of the Council of Eurasian Economic Commission on 3 November 2016, N 79
- Recommendation of the Board of the Eurasian Economic Commission No. 19 "On Guidelines for the Application of Biostatistics principles in Clinical trials of Medicinal Products", dated 3 November 2020

This SAP is based on version 1.0 of the CSP dated 29 August 2023. In case of future amendments to the protocol, the SAP may be modified to account for changes relevant to the statistical analysis.

2 CHANGES TO PROTOCOL PLANNED ANALYSES

This SAP is written in line with the current version of the CSP. All planned analyses correspond to the statistical section of the CSP.

3 DATA ANALYSIS CONSIDERATIONS

3.1 Timing of Analyses

It is planned to conduct only final analysis. It will be performed when all participants completed the study.

3.2 Analysis Populations

The following populations are defined:

Table 1. Populations for Analysis

Population/analysis set	Description
Enrolled/screened participants set	All participants who sign the ICF.
Full analysis set (FAS)	All participants who are randomized to treatment and have at least one postdose, pre-exercise baseline and at least one corresponding post-dose postexercise FEV_1 measure at Visit 3 and/or Visit 4.
Safety analysis set	All participants who have received any amount of the study intervention.

3.3 General Considerations

The data analyses will be conducted using SAS® System (SAS Institute Inc., Cary, NC), version 9.4 or higher.

In general, all data will be listed, sorted by treatment sequence (i.e. PT027/Placebo and Placebo/PT027) and participant, and when appropriate by visit number within participant.

Unless otherwise noted, data will be presented in tables by treatment sequence using descriptive statistics.

3.3.1 General Study Level Definitions

The below-mentioned general principles will be followed throughout the study:

- Descriptive statistics will be used for all variables, as appropriate.
  - Continuous variables will be summarised by the number of observations, mean, standard deviation, median, upper, and lower quartiles where indicated, minimum, and maximum by timepoint as appropriate.
  - Categorical variables will be summarised by frequency counts and percentages for each category. Unless otherwise stated, percentages will be calculated based on the analysis set population total by timepoint as appropriate.
- For continuous data, the mean, standard deviation and median are rounded to one additional decimal place compared to the original data. Minimum and maximum are displayed with the same accuracy as the original data.
- Time-to-event variables will be presented using the Kaplan-Meier (KM) methodology, including median time calculated from the KM curves.
- For categorical data, percentages are rounded to one decimal place with the exception of 100% which is presented as a whole number.

Only acceptable or usable spirometry values will be recorded in eCRF and included in the analyses and presented in tables, listings and figures. Values marked as usable will be utilizied only in case if acceptable values are unavailable.

3.3.1.1 Period specific post-dose pre-exercise baseline

Post-dose pre-exercise baseline is defined as the FEV_1 result taken post-dose of randomized treatment and prior to the exercise challenge test conducted at Visit 3 and Visit 4 (approximately occurring 30 minutes post dose of IP, see CSP, Table 4).

The post-dose pre-exercise baseline is period specific and therefore will be calculated at each Visit 3 and Visit 4.

The post-dose pre-exercise baseline will be used to calculate the primary and secondary endpoints of changes from baseline.

3.3.1.2 Period specific pre-dose baseline

The period specific pre-dose baseline is defined as the spirometry measurement taken prior to dosing of randomized treatment, which should occur at 5 minutes pre-dose of IP (Table 4). The period specific pre-dose baseline will be calculated separately at Visit 3 and Visit 4.

The period specific pre-dose baseline will be included as a covariate in parametric analyses of the primary and secondary endpoints.

The period specific pre-dose baseline will additionally be used to calculate changes from baseline in safety endpoints of vital sign parameters and ECG parameters.

3.3.1.3 Average pre-dose baseline

The average pre-dose baseline will be calculated as the average of the non-missing period specific pre-dose baseline result for each subject. The average pre-dose baseline will be included as a covariate in parametric analyses of the primary and secondary endpoints.

3.3.1.4 Absolute and percent change from baseline

Absolute change from baseline outcome variables is computed as:

Absolute change from baseline = (post-baseline value – baseline value).

Percent change from baseline is computed as:

*Percentage change from baseline = [(post-baseline value – baseline value) / baseline value] * 100%.*

If either the post-baseline value or the baseline value is missing, then the absolute/percent change from baseline value will also be set to missing.

3.3.1.5 Percentage fall from post-dose pre-exercise baseline FEV_1

Percentage fall from post-dose pre-exercise baseline FEV_1 will be used to calculate primary and secondary lung function endpoints and is defined as:

Percentage fall from post-dose pre-exercise baseline = [(post-dose pre-exercise baseline value – post-exercise value) / post-dose pre-exercise baseline value] × 100%.

The percentage fall from post-dose pre-exercise FEV_1 will be calculated at each post-exercise timepoint at Visits 2, 3 and 4.

If either the post-baseline value or the baseline value is missing, then the percentage fall from baseline value will also be set to missing.

3.3.1.6 Crossover difference in maximum percentage fall of FEV_1

Crossover difference in maximum percentage fall of FEV_1 will be calculated for each participant as difference of maximum percentage fall of FEV_1 registered in period of PT027 administration and maximum percentage fall of FEV_1 registered in period of Placebo administration.

3.3.2 Visit Window

Due to the design of this trial, there will be no visit windowing applied other than the identification of baseline.

3.3.3 Handling of Unscheduled Visits

Retest and reassessment of spirometry will be registered as unscheduled visits. If some retests will be registered after Visit 3 and before Visit 4 then the latest result will be included in the analysis as result of Visit 3. If some retests will be registered after Visit 4 then the latest result will be included in the analysis as result of Visit 4. All other results will be presented only in listings. Other unscheduled and early discontinuation measurements will not be included in by-visit summaries.

3.3.4 Multiplicity/Multiple Comparisons

For purposes to demonstrate statistically significant superiority of the IMP, the following comparison sequence will be conducted:

1. Primary endpoint: the maximum percentage fall from post-dose, pre-exercise baseline in forced expiratory volume in 1 second (FEV_1) observed up to 60 minutes post-exercise challenge
2. Secondary endpoint: the percentages of subjects with a maximum percentage fall in FEV_1 post-exercise challenge of <10%

If 1st comparison is significant ie obtained 2-sided p-value < 0.05, then 2nd comparison will be reported as significant if corresponding obtained 2-sided p-value < 0.05.

3.3.5 Handling of Protocol Deviations in Study Analysis

Protocol deviations will be collected, reviewed, and reconciled throughout the study. Important protocol deviations (IPDs) will be identified from the complete set of protocol deviations. IPDs are those which may significantly impact the completeness,

accuracy, or reliability of the study data or that may significantly affect a participant's rights, safety, or wellbeing.

All decisions on importance will be made throughout the study and finalised before clinical data base lock.

3.3.6 Missing Data

Safety assessments of the form of "<x" (ie, below the lower limit of quantification) or ">x" (ie, above the upper limit of quantification) are imputed as "x" in the calculation of summary statistics but are displayed as "<x" or ">x" in the listings.

For missing start dates for adverse events (AEs) and medications/procedures, the following rules are applied:

- Missing day: Impute the first of the month unless the month is the same as month of the first dose of study drug and the end date is on or after the first dose of study drug or ongoing then impute first dose date
- Missing day and month: Impute 01 January unless year is the same as first dose date and the end date is on or after the first dose of study drug or ongoing then impute first dose date.
- Completely missing date:
    - For AEs and treatment identified as prior to study start in the eCRF (i.e. end date before day of first dose): Impute the day before first dose date unless the end date is prior to this in which case impute the date the patient was enrolled.
    - For treatment not identified as prior to study start in the eCRF, no imputation will be performed. For an AE not identified as prior to study start, the date of first dose will be imputed.

An imputed start date of an AE must be prior to the end date of the AE.

For missing stop dates of AEs or medications/procedures, the following rules are applied:

- Missing day: Impute the last day of the month unless month is the same as month of study discontinuation, then impute as study discontinuation date. For prior medications impute date of informed consent if month is the same as month informed consent was provided.
- Missing day and month: Impute 31 December unless year is the same as year of study discontinuation then impute study discontinuation date. For prior medications impute date of informed consent if year is the same as month informed consent was provided.

- Completely missing: If an AE/medication has a completely missing end date then it is treated as ongoing unless the outcome is stated in the eCRF as recovered/resolved for AEs or treatment continues stated as "No" for medications.
    - If the AE/medication is not ongoing (as identified in the eCRF), then the stop date will be imputed by the date of study discontinuation.
    - For stopped medication, if the medication is marked as prior and start date is prior to first dose date, then impute the date of the day before first dose.
    - If the medication started on or after first dose date, then impute the date of study discontinuation.

The imputation of dates for AEs and medications are used to determine if an AE is treatment emergent and whether a medication is concomitant. Flags are retained in the database indicating where any programmatic imputation has been applied, and in such cases, any durations are not calculated.

For time to event endpoints, dropouts and missing data are handled according to the censoring rules detailed within the relevant sections for the endpoint.

Other rules for handling missing data are described under the derivation rules for that particular variable.

4 STATISTICAL ANALYSIS

This section provides information on definitions, derivation and analysis/data presentation per domain.

4.1 Study Population

The domain study population covers subject disposition, analysis sets, protocol deviations, demographics, baseline characteristics medical history, prior and concomitant medication and study drug compliance.

4.1.1 Subject Disposition and Completion Status

4.1.1.1 Definitions and Derivations

Participants disposition and completion status will be comprised of the following:

- Enrolled participants;
- Non-randomized participants (screen failures);
- Randomized participants;
- Participants who were randomized but no dosed with reasons;
- Participants who were randomized and treated on 1st period;
- Participants who were randomized and treated on 2nd period;

- Participants who were randomized and treated with PT027
- Participants who were randomized and treated with placebo
- Randomized participants who completed FU visit.
- Randomized participants who withdrew from the study with reasons

4.1.1.2 Presentation

Number and percentage of participants in each category will be reported by treatment sequence for Enrolled/Screened Participants Set.

4.1.2 Analysis Sets

4.1.2.1 Definitions and Derivations

Refer to Section 3.2 for definition of analysis sets:

- Enrolled/Screened Participants Set
- Full Analysis Set
- Safety Analysis Set

4.1.2.2 Presentation

The number of participants included in each analysis set will be reported by treatment sequence for Enrolled/Screened Participants Set. In addition, for each analysis set, the number of participants excluded, including the reasons, will be reported, if applicable.

4.1.3 Protocol Deviations

4.1.3.1 Definitions and Derivations

A detailed list of possible important protocol deviations (IPDs) and the process for reviewing them by the clinical study team will be outlined in the Protocol Deviation plan (PD plan).

The medical and statistical team members will review the protocol deviation categories in a blinded fashion before the clinical database lock and be classified as important per the PD plan.

At a minimum, the following deviations categories will be included for review and classified as important for the evaluation and implementation of the study:

- Inclusion Criteria Deviations
- Exclusion Criteria Deviations
- Discontinuation Criteria for study product met but participant not withdrawn from study treatment
- Discontinuation Criteria for overall study withdrawal met but participant not withdrawn from study
- Investigational Product Deviation
- Excluded Medications taken

- Deviations to study procedure
- Other Important Protocol Deviations

4.1.3.2 Presentation

The number and percentage of participants with IPDs will be provided per protocol deviation category. The summary will be based on the Safety Analysis Set.

All important protocol deviations will be presented in a listing.

4.1.4 Demographics

4.1.4.1 Presentation

Following demographic characteristics will be summarized for all participants in the Safety Analysis Set:

- Age
- Age group
  - <65 years
  - ≥65 years
- Sex
- Race
- Ethnic group

4.1.5 Baseline Characteristics

4.1.5.1 Presentation

Following baseline characteristics will be summarized for all participants in the Safety Analysis Set:

- Weight (kg)
- Height (cm)
- Body mass index (BMI, kg/m^2)
- Maximal HR results during Exercise Test for visits 1 and 2
- Smoking history
- Results of spirometry on Visits 1 and 2
- Results of spirometry on Visit 3 prior randomization

4.1.6 Disease Characteristics

Disease characteristics will be presented for the Safety Analysis Set.

4.1.6.1 Definitions and Derivations

The following characteristics will be collected:

- Allergen asthma trigger
- Aspirin asthma trigger
- Exercise asthma trigger
- Other asthma trigger

The time since diagnosis of asthma (years) will be calculated as:

(Date of first dose of randomized treatment (Visit 3) - Date of diagnosis of asthma)/365.25.

Partial dates for the above calculations will be handled as per Section 3.3.6.

4.1.6.2 Presentation

Disease characteristics will be presented in a table with descriptive statistics and in a listing.

4.1.7 Medical History and Concomitant Disease

4.1.7.1 Definitions and Derivations

Medical history and relevant surgical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 26.1 or later. The version used will be indicated in the tables and listings.

Any medical history which is ongoing at time of informed consent will be considered as current medical history, otherwise it is considered as past medical history.

Clinically significant abnormal physical examination findings at screening will be recorded as adverse events

4.1.7.2 Presentation

Past and current medical history will be grouped by MedDRA System Organ Class (SOC) and Preferred Term (PT) and summarized in the Safety Analysis Set as number and percentage of participants in each treatment sequence.

Also past and current medical history will be presented in listings.

4.1.8 Prior and Concomitant Medications

4.1.8.1 Definitions and Derivations

Prior medication: all prescripted drugs, herbal products, vitamins, minerals, and over-the-counter medications with end date within 3 months before date of randomization.

Concomitant medications: all medications with start date on or after date of randomization, or with end date after date of randomization or ongoing at the time of the follow-up telephone call (TC).

If a medication has start date before date of randomization and end date after date of randomization or ongoing the follow-up telephone call (TC), so this medication will be considered as prior and as concomitant, therefore it will be presented in both corresponding tables.

Restricted Medications

As described in Table 8 of the CSP.

Prohibited Medications

As described in Table 8 of the CSP. Medications will be classified as prohibited or not on the medical review.

Rescue Medications

AstraZeneca will supply rescue medication – Ventolin® (salbutamol, SABA), 100 µg metered dose inhaler, to the study site.

For the purpose of inclusion in prior or concomitant mediation, incomplete medication start and stop dates will be imputed as detailed in Section 3.3.6.

4.1.8.2 Presentation

All medications will coded using World Health Organization Drug dictionary (WHO Drug) version Sep 2023 B3 or later. The version used will be indicated in the data summaries and listings. Medications will be presented in the Safety Analysis Set.

Prior and concomitant medications will be presented in separate outputs.

The number and percentage of participants receiving prior or concomitant medication (by ATC classification system codes and generic name) will be presented by treatment sequence.

Additionally, prior and concomitant medications will be listed. The listing of concomitant medications will include reason for use, start and end dates of administration, dosage information including dose, frequency and route.

Restricted medications also will be listed. The listing will include date and time of SABA administration and date and time of consequent planned FEV_1 testing procedures.

Events of prohibited medication administration will be registered and presented as protocol deviations (Section 4.1.3).

Data on rescue medication administrations will be listed. The listing will include date, time of administration, name and dosage regimen.

4.1.9 Study Drug Compliance

Not applicable for this study.

4.2 Endpoint Analyses

This section covers details related to the endpoint analyses such as primary, secondary, other endpoints including sensitivity and supportive analyses.

Descriptive statistics and analysis results (if applicable) will be presented by treatment, unless otherwise specified.

Examples of SAS code implemented efficacy analysis models presented in corresponding sections. These codes can be updated, for example, in order to obtain necessary statistics of a model.

Statistical category	Endpoint	Analysis Set	Intercurrent event strategy	Population level summary (analysis)	Details in section
Objective 1: To estimate the efficacy of PT027 (budesonide/albuterol) metered-dose inhaler as compared with placebo metered-dose inhaler after a single dose on exercise-induced bronchoconstriction in adult participants with asthma					
Primary	The maximum percentage fall from post-dose, pre-exercise baseline in forced expiratory volume in 1 second (FEV_1) observed up to 60 minutes post-exercise challenge	Full Analysis Set	Intercurrent event defined as rescue medication administration prior to the first spirometry reading. Only FEV_1 results prior to rescue therapy (within the study visit) will be included in the analyses. A while on treatment strategy will be used such that missing values will be assumed missing at random (MAR) and handled by the direct likelihood methods of the mixed effects model. Missing data not preceded by an intercurrent event, such as subjects who withdraw from study will be assumed to be MAR.	Difference of means between treatments	4.2.1
Objective 2: To further estimate the efficacy of PT027 (budesonide/albuterol) metered-dose inhaler as compared with placebo metered-dose inhaler after a single dose on exercise-induced bronchoconstriction in adult participants with asthma					
Secondary	The percentages of subjects with a maximum percentage fall in FEV_1 post-exercise challenge of <10% and <20%, respectively	Full Analysis Set	While on treatment strategy will be applied as a primary strategy: only FEV_1 results prior to administration of rescue medication (within	The percentage fall will be calculated relative to the pre-exercise FEV_1 assessment at	4.2.2

Statistical category	Endpoint	Analysis Set	Intercurrent event strategy	Population level summary (analysis)	Details in section
			the study visit) will be analysed. Composite strategy will be used for purposes of a supplementary analysis: articipants who used rescue medication administration prior to the first spirometry reading will be analysed as non-responders	the respective visit. The measure of interest is the odds ratio between PT027 and Placebo for each threshold separately (<10% and <20%).	
Secondary	The percentage fall from post-dose, pre-exercise baseline in FEV_1 at each timepoint within 60 minutes post-exercise challenge	Full Analysis Set	Hypothetical strategy will be used for analysis of this endpoint: only FEV_1 results prior to administration of rescue medication (within the study visit) will be included in the analyses, missing values will be utilized in the analysis by using the mixed model with repeated measures (MMRM).	The measure of interest is the percentage fall from baseline in FEV_1 at each timepoint within 60 minutes post-exercise challenge.	4.2.3
Secondary	Post-exercise FEV1 area under the curve from 0 to 30 minutes ($AUC_{0\text{-}30min}$)	Full Analysis Set	While on treatment strategy will be applied: only the FEV_1 $AUC_{0\text{-}30min}$ measurements prior to administration of rescue medication will be included in the analyses.	The measure of interest is the difference between treatments in the post-exercise FEV_1 $AUC_{0\text{-}30min}$.	4.2.4
Secondary	Time to recovery, defined as the time from completionof the exercise challenge to the first measured post-exercise challenge FEV_1 value within 10% of the post-dose, pre-exercise challenge baseline FEV_1	Full Analysis Set	While on treatment strategy will be applied: only FEV_1 results prior to administration of rescue medication (within the study visit) will be included in the analyses	The measure of interest is the median of the time to recovery.	4.2.5
Objective 3: To assess the safety and tolerability of PT027 (budesonide/albuterol) metered-dose inhaler as compared with placebo metered-dose inhaler after a single dose in adult participants with asthma.					
Safety	Safety and tolerability will be evaluated in terms of: • AEs • SAEs • AEs lead to discontinuation.	Safety Analysis Set	All data will be analysed	The measure of interest is frequencies and percentages of participants with reported AEs	4.6.2

Statistical category	Endpoint	Analysis Set	Intercurrent event strategy	Population level summary (analysis)	Details in section
	VS, PE, ECGs will be analysed in terms of AE data.				

4.2.1 Primary Endpoint

4.2.1.1 Definition

The primary variable is the maximum percentage fall of FEV_1 available in the post-exercise 60-minute assessment period prior to the use of rescue medication, from FEV_1 value measured after study treatment and prior to the exercise. It will be calculated at Visit 3 and Visit 4.

4.2.1.2 Derivations

The fall from post-dose, pre-exercise baseline in FEV_1 will be calculated at each post-exercise FEV_1 assessment time point within Visits 3 and 4 as described in Section 3.3.1.

The post-dose, pre-exercise baseline FEV_1 assessment is expected to occur 30 minutes after administration of IP and 5 minutes prior to the exercise challenge within Visits 3 and 4.

The percentage fall in FEV_1 will be calculated as difference from to the post-exercise value to the pre-exercise value at each measurement during the 60-minute assessment period divided by the pre-exercise value and presented in percents within Visits 3 and 4.

The maximum percentage fall available in the 60-minute assessment period, prior to the use of rescue medication, will be calculated at Visit 3 and Visit 4.

4.2.1.3 Handling of Dropouts and Missing Data

Values of FEV_1 obtained after rescue medication and missing values will not be imputed.

4.2.1.4 Primary Analysis of Primary Endpoint

The primary analysis will be performed in the FAS. Only data prior to the occurrence of an intercurrent event (ICE) will be included in the analysis in accordance with the WoT strategy.

The primary efficacy endpoint will be analyzed with a mixed effect model including categorical fixed effects for treatment, treatment period and treatment sequence. Continuous covariates include period-specific pre-dose baseline FEV_1 and average pre-dose baseline FEV_1 (as defined in Section 3.3.1.3) in order to eliminate cross-level bias whilst using pre-treatment baselines. Also, a random subject within treatment sequence effect will be specified.

Post-dose, pre-exercise baseline FEV_1 will be defined as the 30-minute post-dose value, i.e., 5 minutes before exercise challenge, at each visit for the respective treatment. The period-specific pre-dose baseline FEV_1 will be calculated separately at Visit 3 and Visit 4 as the pre-dose result, approximately 5 minutes prior to dosing. The average pre-dose baseline FEV_1 will be calculated as the mean of the period-specific pre-dose FEV_1 baselines. Estimated treatment differences and 95% confidence intervals (CIs) will be provided.

Applied significance level (alpha): 0.05 (using a 2-sided test).

Additionally, least squares mean estimates, 95% confidence intervals and associated p-values of the treatment difference will be calculated for the subgroups of clinical interest by additionally adjusting for background therapy (ICS or no ICS) and background therapy*treatment group interaction in the mixed model.

A forest plot will be created showing the difference in least squares mean estimates and associated 95% confidence intervals for the Overall, ICS and non-ICS subgroups.

Model results as well as descriptive statistics will be presented in tables. Figure for adjusted means and 95% CIs will be presented.

All data will be listed.

4.2.1.5 Sensitivity Analyses of the Primary Endpoint

Missing results for maximum percentage fall in FEV_1 are unlikely (i.e., rescue medication prior to the collection of the 5 minutes measure) and will be assumed to be missing at random. Sensitivity analysis will be conducted to explore the robustness of the primary analysis with respect to this missing data assumption. This analysis will be conducted in supportive purposes.

Data that are missing will be imputed using reference-based imputation. Participants with a missing maximum percentage fall in the 60-minute assessment period, for any visit, will be imputed using the least squares mean estimate of the maximum percentage fall in the Placebo treatment from a mixed model as specified in Section 4.2.1.4, using the observed data. After imputation the dataset of complete data, will be analyzed using the same model as descripted in Section 4.2.1.4.

Model results as well as descriptive statistics will be presented in tables. Figure for adjusted means and 95% CIs will be presented.

All data will be listed.

4.2.2 Secondary Endpoint: the percentages of subjects with a maximum percentage fall in FEV_1 of <10% and <20%

4.2.2.1 Definition

Protection against EIB will be defined having a maximum percentage fall in FEV_1 post-exercise change under the corresponding threshold values (<10% and <20%)

4.2.2.2 Derivations

Derivation will be completely based on comparing primary efficacy variable (described in Section 4.2.1.1) and threshold values of 10% and 20%.

For each threshold value a participant will be considered as protected patient (responder) if their maximum percentage fall in FEV_1 post-exercise change was less than the threshold value. Therefore two different types of responders will be analysed.

4.2.2.3 Handling of Dropouts and Missing Data

Participants with missing maximum percentage fall will not be included in the primary analysis.

4.2.2.4 Primary Analysis of Secondary Endpoint

The odds of being protected against EIB will be analyzed using a generalized linear mixed model with logit link function to compare the treatments. Two separate models will be presented for each two types of responders (participants protected against EIB) as described in Section 4.2.2.2.

The models will be adjusted with fixed effects for treatment, treatment period and treatment sequence, period specific pre-dose baseline FEV_1 and average pre-dose baseline FEV_1 as continuous covariates, and a random subject within treatment sequence effect. The odds ratio and 95% CI will be reported for pairwise treatment comparisons.

Model results as well as descriptive statistics will be presented in tables and figures. All data will be listed.

4.2.2.5 Supplementary Analysis of Secondary Endpoint

The suplementary analysis of this endpoint will be performed using the same models as in the primary analysis but participants who didn’t withdraw the study prematurely and had missing maximum percentage fall will be analysed as non-responders.

Model results as well as descriptive statistics will be presented in tables and figures.All data will be listed.

4.2.3 Secondary Endpoint: the percentage fall in FEV_1 from post-dose, pre-exercise at each timepoint within 60 minutes post-exercise

4.2.3.1 Derivations

Described in Section 4.2.1.2.

4.2.3.2 Handling of Dropouts and Missing Data

Described in Section 4.2.1.2.

4.2.3.3 Primary Analysis of Secondary Endpoint

An analysis of percentage fall in FEV_1 post-exercise challenge will be conducted using methods as per the primary analysis, with an additional adjustment for planned time point in the repeated measures model. The covariance within subject-periods will be Unstructured (UN) over the time points. In case of impossible to build the model due to divergence, the following variants of the covariance matrix will be sequentially tested: Compound Symmetric (CS), Autoregressive, order 1 (AR(1)), Toeplitz (TOEP).

Only FEV_1 results prior to administration of rescue therapy (within the study visit) will be included in the analyses.

Model results as well as descriptive statistics will be presented in tables. Figures for adjusted means and 95% CIs as well as unadjusted means and 95% CIs will be presented.

All data will be listed.

4.2.4 Secondary Endpoint: post-exercise FEV_1 area under the curve from 0 to 30 minutes

4.2.4.1 Definition

Post-exercise FEV_1 area under the curve (AUC) from 0 to 30 minutes will be defined on the base of changes from baseline in FEV_1 as defined in Section 4.2.1.2 for time points planned up to 30 minutes after the test.

4.2.4.2 Derivations

Post-exercise FEV_1 area under the curve from 0 to 30 minutes (FEV_1 $AUC_{0\text{-}30min}$) will be derived for the changes from the post-dose, pre-exercise baseline using the trapezoidal rule and will be normalized by dividing by the actual time (in minutes) from the exercise challenge test to the last included measurement, scheduled at 30 minutes post-exercise challenge at each of Visits 3 and 4. Only FEV_1 results prior to the use of rescue medication will be considered when calculating FEV_1 $AUC_{0\text{-}30min}$.

$$AUC_{0-30\,minutes} = \frac{1}{t_N - t_0} \times \sum_{i=1}^{N} \frac{(y_{i-1} + y_i) \times (t_i - t_{i-1})}{2}$$

For FEV_1 change from baseline results y_i recorded t_i minutes after the exercise challenge test (actual clock time) at $i = 0, 1, 2, \ldots, N$ actual timepoints. If all post-exercise timepoints are available, then $N = 4$, t_0 corresponds to the end time of the exercise challenge test and $y_0 = 0$. Only FEV_1 results of 'acceptable' or 'borderline acceptable' (investigator assigned) quality will be used in the derivation of FEV_1 $AUC_{0–30min}$. If a FEV_1 result is missing or recorded in the database, but assigned an

'unacceptable' quality grade at the scheduled assessment, then the assessment will be excluded from the calculation of FEV_1 $AUC_{0\text{-}30min}$. To calculate FEV_1 $AUC_{0\text{-}30\ min}$, there must be at least 1 non-missing post-dose FEV_1 within 0 to 30 minutes post-dose. Missing FEV_1 post-dose measures will not be imputed.

4.2.4.3 Handling of Dropouts and Missing Data

Values of FEV_1 obtained after rescue medication and missing values will not be imputed. Missing actual times for non-missing change from baseline values will be replaced with planned times.

4.2.4.4 Primary Analysis of Secondary Endpoint

The endpoint will be analyzed using the same approach as described in Section 4.2.1.4.

4.2.5 Secondary Endpoint: time to recovery

4.2.5.1 Definition

Recovery will be defined as return of post-exercise changes of the FEV_1 within 10% interval of the value recorded at the post-dose, pre-exercise baseline.

4.2.5.2 Derivations

Time to recovery at each of Visits 3 and 4 will be derived as the time (in minutes) post-exercise challenge in which the FEV_1 result returns to within 10% of the value recorded at the post-dose, pre-exercise baseline.

The post-dose, pre-exercise baseline FEV_1 assessment and percentage fall in FEV_1 will be calculated as described in Section 4.2.1.2, at Visit 3 and Visit 4 separately.

4.2.5.3 Handling of Dropouts and Missing Data

Missing values of FEV_1 and times of measurements will not be imputed.

4.2.5.4 Primary Analysis of Secondary Endpoint

The median time to recovery will be reported descriptively by treatment. P-values will be calculated using a Prescott's period-adjusted sign test, based on categorizing subjects into period preferences (Senn S 1993).

Censoring rules:

- If a participant has not observed a fall greater than 10% of the post-dose pre-exercise baseline in their post-exercise challenge test by 60 minutes, they will be left censored at 0 minutes.

- Participants who have any rescue medication administered during the post-dose assessments will be censored at the time of receiving rescue medication.

- Participants who do not recover to within 10% of the post-dose, pre-exercise baseline will be censored at their last assessment.

4.2.6 Other Endpoints

Not applicable.

4.2.7 Subgroup Analyses

The assessment of treatment effect will also be investigated for primary endpoint (described in Section 4.2.1) and first secondary endpoint (described in Section 4.2.2) in the other clinically important subgroups described in Table:

Table 3. Subgroup Analyses

Group	**Subgroup**
Sex	Male
	Female
Age group (years)	Adults: ≥ 18 - < 65
	Elderly: >= 65
BMI (kg/m2)	Median value cut-off[1]
Baseline FEV_1 %	Median value cut-off[1]

[1] Subgroup will be categorized into 2 groups defined by the median value observed at baseline. Baseline FEV1 % for grouping purposes here will be pre-dose baseline FEV1 % predicted normal at Visit 3.

For all subgroup analyses, if there are less than 10 subjects/events available within a subgroup and at least 5 subjects with data available per treatment group under comparison, or the model does not converge, then only descriptive (summary) statistics will be presented. Subgroup analyses will be further broken down into the overall population, and ICS and non-ICS populations. Subjects with insufficient data to be allocated to a subgroup category will be excluded from the subgroup analyses.

Model results as well as descriptive statistics will be presented in tables. Figures for adjusted means and 95% CIs will be presented.

Forest plots will be produced showing the least squares mean difference and associated 95% confidence intervals within each subgroup for the primary endpoint of maximum percentage fall from post-dose, pre-exercise baseline in FEV_1.

All data will be listed.

4.3 Pharmacodynamic Endpoint(s)

Not applicable

4.4 Pharmacokinetics

Not applicable

4.5 Immunogenicity

Not applicable

4.6 Safety Analyses (if not already covered as endpoint variables)

The domain safety covers exposure, adverse events, clinical laboratory, vital signs, and ECG.

Tables and listings will be provided for the Safety Analysis Set.

4.6.1 Exposure

4.6.1.1 Definitions and Derivations

Participants will have single administration of placebo at the screening period and single administrations of placebo or PT027 (depending of randomization) on each of both study periods.

4.6.1.2 Presentation

Number and percentage of participants who received both treatments and who didn't receive the second treatment (Visit 4) with reasons will be presented by treatment sequence. Placebo administration on Visit 2 will not be accounted.

All data on exposure will be listed.

4.6.2 Adverse Events

4.6.2.1 Definitions and Derivations

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT) using the latest version of the MedDRA dictionary.

4.6.2.2 Presentation

AEs started before time of study treatment on Visit 3 will be considered as pre-treatment AEs and will be presented in a separate listing.

AEs of participants from A/B treatment sequence registered in period from time of study treatment on Visit 3 to time of study treatment on Visit 4 and AEs of participants from B/A treatment sequence registered after time of study treatment on Visit 4 will be considered as AEs of PT027. If participant from A/B treatment sequence didn't receive study treatment on Visit 4, then all AEs occurred after time of study treatment on Visit 3 will be considered as AEs of PT027.

AEs of participants from B/A treatment sequence registered in period from time of study treatment on Visit 3 to time of study treatment on Visit 4 and AEs of participants from A/B treatment sequence registered after time of study treatment on Visit 4 will be considered as AEs of Placebo. If participant from B/A treatment sequence didn't receive study treatment on Visit 4, then all AEs occurred after time of study treatment on Visit 3 will be considered as AEs of placebo.

The following tables by IP will be presented:

- Overall table for AEs
- AEs by SOC and PT
- SAEs by SOC and PT
- AEs related to study intervention by SOC and PT
- SAEs related to study intervention by SOC and PT
- AEs by SOC and PT, by severity
- AEs by SOC and PT, by outcome
- SAEs by SOC and PT, by outcome
- AEs by SOC and PT, by action taken with IMP
- SAEs by SOC and PT, by action taken with IMP
- AEs leading to discontinuation of study intervention, by SOC and PT
- AEs by SOC and PT caused participant to withdraw from the study

The following lisitngs will be provided:

- Pre-treatment AEs
- AEs (excluding the pre-treatment AEs)
- SAEs (excluding the pre-treatment AEs)
- AEs with fatal outcome

4.6.3 Clinical Laboratory, Blood Sample

4.6.3.1 Definitions and Derivations

Blood samples for determination of clinical chemistry and hematology will be taken only at Screening (Visit 1). Additional safety samples may be collected if clinically indicated at the discretion of the investigator. Detailed list of parameters is presented in the CSP, Section 8.2.5.

4.6.3.2 Presentations

Measured values at Screening will be presented with descriptive statistics as planned in Section 3.3.1 by treatment sequence in tables.

All data on blood samples will be presented in listings.

4.6.4 Clinical Laboratory, Urinalysis

4.6.4.1 Definitions and Derivations

Urine samples for urinalysis will be taken only at Screening (Visit 1). Detailed list of parameters is presented in the CSP, Section 8.2.5.

4.6.4.2 Presentations

Results of urinalysis will be presented in a listing.

4.6.5 Other Laboratory Evaluations

4.6.5.1 Definitions and Derivations

A serum pregnancy test (β-human chorionic gonadotropin [β-hCG]) will be performed at Visit1, 4 and PDV; urine β-hCG test will be performed at Visit 3 (for women of childbearing potential only).

Urine samples will be taken during Visits 1-4 for drugs of abuse and cotinine testing. The analysis will be carried out using test strips. The list of psychoactive agents for testing includes marijuana, benzodiazepines, barbiturates, opiates, methadone, phencyclidine, cocaine, amphetamines.

Also, during Visits 1-4 alcohol detection test will be performed using breathalyser.

4.6.5.2 Presentations

All data of these additional laboratory tests will be presented in listings.

4.6.6 Vital Signs

4.6.6.1 Definitions and Derivations

Vital sings will be collected according to the Schedule of Activities: CSP, Section 1.3.

4.6.6.2 Presentations

SBP, DBP and HR will be presented with descriptive statistics in tables using the following approach:

For Visit 1: measured values and changes for values on 20, 40 and 60 minutes after the test from values on 50 minutes before the test. Grouping by treatment sequence.

For Visit 2: measured values and changes for values on 20, 40 and 60 minutes after the dosing from values on 50 minutes before the dosing. Grouping by treatment sequence.

For visits 3 and 4: measured values and changes for values on 20, 40 and 60 minutes after the dosing from values on 50 minutes before the dosing. Grouping by treatment.

For visits 3 and 4: maximal HR during ECT. Grouping by treatment.

Treatments will be summarized using the same algorithm as described for AEs in Section 4.6.2.2.

Also all data on vital signs, including weight, height and BMI, will be presented in a listing.

4.6.7 Electrocardiogram

4.6.7.1 Definitions and Derivations

Electrocardiogram will be performed according to the Schedule of Activities: CSP, Section 1.3.

4.6.7.2 Presentations

Continuous parameters of ECG will be presented using the following approach:

For each visit: measured values and changes for values on 45 minutes after the test from values on 50 minutes before the test.

Interpretation of ECG as nominal parameter will be presented in shift tables for values on 50 minutes before the test and for values on 45 minutes after the test. Number and percentages of participants with values: Normal, Borderline, Abnormal NCS and Abnormal CS will be presented.

For visits 1 and 2, for continuous parameters and interpretation grouping will be performed by treatment sequence.

For visits 3 and 4, for continuous parameters and interpretation grouping will be performed by treatment. Treatments will be summarized using the same algorithm as described for AEs in Section 4.6.2.2.

Also all data on vital signs will be presented in a listing.

4.6.8 Other Safety Assessments

Not Applicable

5 INTERIM ANALYSIS

Not Applicable

6 REFERENCES

- ICH harmonised tripartite guideline. Statistical principles for clinical trials (E9). Step 5 finalized guideline February 1998;
- ICH harmonised tripartite guideline. Structure and Content of Clinical Study Reports (E3). Step 5 finalized guideline dated November 1995;
- Good Clinical Practice of the Eurasian Economic Union, adapted by the decision of the Council of Eurasian Economic Commission on 3 November 2016, N 79.
- Senn S, Cross-over Trials in Clinical Research. Wiley, Chichester.1993.

7 APPENDIX

Not Applicable

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Approve: Document Level Task Verdict: Approved	PPD val 06-Aug-2024 08:06:20 GMT+0000

Approve: Document Level Task Verdict: Approved	PPD Approval 06-Aug-2024 08:12:54 GMT+0000

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