ALTA -ALbuterol/budesonide Treatment in Acute Airway Obstruction

A randomized, double-blind, 2-period, cross-over study evaluating efficacy and safety of repeated doses of PT027 compared to PT007 in patients with asthma and acute airway obstruction induced by repeated mannitol challenges

ClinicalTrials.gov Identifier: NCT05555290

Clinical Study Protocol: version 4.0, dated 10 July 2023

Clinical Study Protocol

Study Intervention	PT027- Albuterol/Budesonide Sulfate
Study Code	D6930C00017
Version	4.0
Date	10 July 2023

ALTA - ALbuterol/budesonide Treatment in Acute Airway Obstruction

A randomized, double-blind, 2-period, cross-over study evaluating efficacy and safety of repeated doses of PT027 compared to PT007 in patients with asthma and acute airway obstruction induced by repeated mannitol challenges

Sponsor Name: AstraZeneca AB

Legal Registered Address: AstraZeneca AB, Södertälje, Sweden SE-15185

Manufacturer: AstraZeneca

Regulatory Agency Identifier Number IND 136213

This protocol has been subject to a peer review according to AstraZeneca standard procedures. The protocol is publicly registered, and the results are disclosed and/or published according to the AstraZeneca Global Standard - Bioethics and in compliance with prevailing laws and regulations.

Protocol Number: D6930C00017

Amendment Number: Amendment 3.0

Study Intervention: PT027 (albuterol/budesonide metered dose inhaler 90/80 µg); PT007 (albuterol metered dose inhaler 90 µg)

Brief Title: PT027 compared to PT007 in patients with asthma with mannitol-induced acute airway obstruction

Study Phase: IIIb

Acronym: ALTA - ALbuterol/budesonide Treatment in Acute airway obstruction

Study Physician Name and Contact Information will be provided separately

National Coordinating Investigator

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SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
CSP version 4.0 - Amendment 3	10 July 2023
CSP version 3.0 - Amendment 2,	03 February 2023
CSP version 2.0 - Amendment 1	02 August 2022
CSP version 1.0 - Original Protocol	25 May 2022

CSP Version 4.0, Amendment 3, 10 July 2023

Overall Rationale for the Modifications:

This protocol amendment is based on recommendations of an AstraZeneca Internal Advisory Board (IAB) to modify dosing and increase the sample size for Part 2 to enhance the likelihood of showing superiority of PT027 over PT007 for the secondary endpoint, peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, predose (pre-final dose of rescue/reliever).

The ALTA study was designed to have a Pilot Study (Part 1) that would be assessed by an unblinded IAB and based on the recommendations, an updated Part 2 protocol would be devised, if needed. The recommendations of the IAB are based on their role to assess the operational issues of the study, evaluate endpoint variability that could lead to reordering or modification of primary or secondary endpoints, and determine appropriateness of sample size to optimize success for the non-inferiority and superiority of the primary and secondary endpoints.

At the time of this amendment, the study team recommended additional exploratory endpoint sampling for Visit 1 and a shift in the time of dosing PT007, in order to provide control values against which the genomics and cyclic AMP values can be assessed.

Summary of Changes:

Substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 3 Objectives and Estimands Section 4.1 Overall Design Section 8.2 Efficacy Assessments Section 9.1 Statistical Hypotheses	The timing of challenge 2 for Visits 2 and 3 was moved from 420 min to 480 min.	To help minimize the risk of refractoriness due to mannitol challenge 1 prior to initiating mannitol challenge 2.

Section Number and Name	Description of Change	Brief Rationale
Section 9.2 Sample Size Determination Section 9.4.1 General Considerations Section 9.4.3.2 Secondary Endpoints Section 9.4.3.3 Exploratory Endpoints Section 9.4.4 Safety		
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 4.1 Overall Design Section 8.9.1.2 Serum cAMP, cAMP Mobilizing Analytes, and Additional Exploratory Blood Inflammatory Biomarkers Section 8.9.1.3 Beta Receptor and Corticosteroid Receptor Expression Genetics	Additional blood work was added to Visit 1.	In order to have a control by which to analyze the cAMP and genomics, blood for the cAMP and analytes will be obtained at time 0, 30, and 60 min and genomics at 0 and 60 minutes in Visit 1.
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 4.1 Overall Design	Visit 1 dosing of albuterol – PT007, was moved from time 0 to time 35 min post-mannitol challenge 1.	To allow for a cAMP control value showing impact of mannitol alone.
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 4.1 Overall Design	For Visits 2 and 3, the dose of investigational product at time 120 min was removed.	Most participants are above baseline at this point in time, thus a dose of 120 would not mimic real world use of rescue medication. This also allows for washout of any residual albuterol effects prior to mannitol challenge 2.
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 2.3.1 Risk Assessment	A 3 min spirometry timepoint was added following all mannitol challenges in Visits 1, 2, and 3.	Recovery from mannitol occurs quickly and this timepoint for spirometry was added to assure the onset of effect is accurately measured.
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 3 Objectives and Estimands Section 4.1 Overall Design Section 8.2 Efficacy Assessments Section 9.1. Statistical Hypotheses	Change from mannitol baseline in FEV_1 at 7 hours post-mannitol challenge 1, was changed to at 480 min post-mannitol challenge 1.	To help minimize the potential for residual refractoriness to mannitol and residual albuterol effects of repetitive dosing at challenge 2, challenge 2 will occur at 480 min instead of 420 min.

Section Number and Name	Description of Change	Brief Rationale
Section 9.2 Sample Size Determination Section 9.4.1 General Considerations Section 9.4.3 Efficacy Section 9.4.3.2 Secondary Endpoints Section 9.4.4 Safety		
Section 1.1 Synopsis Section 3 Objectives and Estimands Section 8.2 Efficacy Assessments Section 9.1 Statistical Hypotheses Section 9.2 Sample Size Determination Section 9.4.1 General Considerations Section 9.4.3.2 Secondary Endpoints	Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever, was reordered from the 4th to the 5th non-inferiority endpoint.	As per the recommendation of the IAB, this endpoint has moved down 1 in the non-inferiority ordering.
Section 1.1 Synopsis Section 3 Objectives and Estimands Section 8.2 Efficacy Assessments Section 9.1 Statistical Hypotheses Section 9.4.2 Adjustment for Multiple Comparisons Section 9.4.3.2 Secondary Endpoints	Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2 was moved to the first superiority endpoint.	As per the recommendation of the IAB.
Section 1.1 Synopsis Section 3 Objectives and Estimands Section 8.2 Efficacy Assessments Section 9.1 Statistical Hypotheses	Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever has been moved to the 2nd superiority endpoint.	As per the recommendations of the IAB.
Section 1.1 Synopsis Section 3 Objectives and Estimands Section 8.2 Efficacy Assessments Section 9.1 Statistical Hypotheses Section 9.4.3.2 Secondary Endpoints	A secondary endpoint to be tested for superiority was added to assess the protective effect of repetitive albuterol-budesonide vs albuterol: Peak fall from time 480 min FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever.	To understand treatment effects more fully.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis Section 3 Objectives and Estimands Section 8.2 Efficacy Assessments Section 9.1 Statistical Hypotheses Section 9.2 Sample Size Determination Section 9.4.1 General Considerations Section 9.4.3.2 Secondary Endpoints Section 9.4.3.3 Exploratory Endpoints	For the superiority endpoint Change from mannitol baseline in FEV_1 at 7 hours post-mannitol challenge 1, the timepoint was updated to 480 min.	To help minimize the risk of refractoriness due to mannitol challenge 1 prior to initiating mannitol challenge 2.
Section 1.1 Synopsis Section 3 Objectives and Estimands Section 4.1 Overall Design Section 9.2 Sample Size Determination	Sample size was increased from 50 completers to 74 completers for Part 2, and the sample size determination was updated.	IAB recommended to increase the sample size for Part 2 to enhance the likelihood of showing superiority of PT027 over PT007 for the secondary endpoint peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever.
Section 1.1 Synopsis Section 4.1 Overall Design Section 9.2 Sample Size Determination	Evaluable participants were defined as those who receive all 5 doses of the study medication.	Text updated due to removal of 120 min dose.
Section 1.3 Schedules of Activities	Urine sample for safety assessments will be taken at only one timepoint (predose).	Surplus sampling was removed as it was deemed unnecessary with regards to participant safety.
Section 1.1 Synopsis Section 1.3 Schedules of Activities Section 8.9.1.2 Serum cAMP, cAMP Mobilizing Analytes, and Additional Exploratory Blood Inflammatory Biomarkers	Blood for genomics and cAMP will be obtained at visit 1.	To evaluate control value showing impact of mannitol alone without drug on genomic and non-genomic (cAMP) exploratory indices.
Section 1.1 Synopsis Section 4.1 Overall Design	The time between mannitol challenges was changed from 7 hours to ~8 hours.	To help minimize the risk of refractoriness due to mannitol challenge 1 prior to initiating mannitol challenge 2.
Section 5.1 Inclusion Criteria Section 8.2.8 COVID-19 Testing (section deleted) Appendix F (appendix deleted)	All text regarding continuing the study during active COVID-19 periods was removed.	Current alert level does not warrant inclusion of these sections.

Non-Substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Section 1.3 Schedules of Activities	Added: Prior to challenge 2 participants should empty their bladder even though there will be no sample analysis. This is to optimize the accuracy of subsequent urinary leukotriene analyses.	Mirrors pre-mannitol challenge routine.
Section 1.3 Schedules of Activities	The Schedules of Activities tables were updated.	To align with the changes made in this amendment.
Throughout the document	The document was aligned with CSP template v8.0.	To align with current document template.
Section 9.2 Sample Size Determination	The nominal power testing for non-inferiority for the primary and secondary endpoints presented in Table 12 was updated	Based on emergent data from Part 1.
Section 9.2 Sample Size Determination	The testing hierarchy to control the type I error outlined in Figure 5 was updated.	To align with the changes made in this amendment.
Section 3 Objectives and Estimands Section 9.4.1 General Considerations Section 9.4.3.3 Exploratory Endpoints	Added percentage fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever as an exploratory endpoint.	Per recommendation of AstraZeneca IAB.
Section 9.3 Populations for Analyses	Redefined the randomized analysis set to the modified randomized analysis set, defined as all participants who are randomized to any of the 2 treatment sequences, A/B or B/A and receive at least one dose of randomized study treatment.	To define a new analysis population that comprises all randomized participants who also meet the mannitol challenge 1 positive reactivity criteria at the randomization visit.
Section 1.1 Synopsis Section 9 Statistical Considerations Section 9.4.3.1 Primary Endpoint	The linear mixed model was updated to include in the linear predictor also the average of the two periods mannitol baseline FEV_1 values.	To avoid cross-level bias.
Summary of Changes	Previous version (CSP v3 Amendment 2) Summary of Changes moved to Appendix G.	Per template.

Section Number and Name	Description of Change	Brief Rationale
Throughout the document	Converted all the timepoints given in hours to minutes for consistency throughout the protocol.	Consistency and clarity.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
AAFA	Asthma and Allergy Foundation of America
AE	Adverse event
AIRQ®	Asthma impairment and risk questionnaire
ALP	alkaline phosphatase
ALT	alanine aminotransferase/transaminase
ANOVA	Analysis of Variance
AST	aspartate aminotransferase/transaminase
AUC	Area under curve
ATS/ERS	American Thoracic Society/European Respiratory Society
bEOS	Blood eosinophil
β-2AR	β-2 adrenergic receptor
cAMP	Cyclic adenosine monophosphate
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence interval
COVID-19	Corona Virus disease 2019
CRO	Contract Research Organization
CSP	Clinical Study Protocol
CSR	Clinical Study Report
DES	Data Entry Site
DILI	Drug Induced Liver Injury
DNA	Deoxyribonucleic acid
DUS	Disease Under Study
ECG	Electrocardiogram
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in the first second
FVC	Forced vital capacity
GCP	Good Clinical Practice
GDPR	General Data Protections Agency
GINA	Global Initiative for Asthma

Abbreviation or special term	Explanation
HL	Hy's Law
IAB	Internal Advisory Board
IATA	International Airline Transportation Association
IB	Investigator's Brochure
ICE	Intercurrent event
ICF	Informed consent form
ICH	International Council for Harmonisation
ICS	Inhaled corticosteroids
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
IND	Investigational New Drug
IRB	Institutional Review Board
IxRS	Interactive Voice/Web Response System
LABA	Long-acting β2-agonist
LTE4	Leukotriene E4
MAR	Missing at random
MDI	Metered dose inhaler
mRS	Modified randomized analysis set
NAEPP	National Asthma Education and Prevention Program
NIMP	Non-Investigational Medicinal Product
PD	Provoking dose
PD15	Provoking dose (PD) that results in a 15% drop in FEV_1
PHL	Potential Hy's Law
PI	Principal Investigator
pMDI	Pressurized metered dose inhaler
POE	Perceived Onset of Effect
PP	Per Protocol analysis set
PRO	Patient-Reported Outcome
Q1	First quartile
Q3	Third quartile
QTcB	QT correction for heart rate with Bazett formula
QTcF	QT corrected for heart rate by Fridericia's cube root formula
RS	Randomized analysis set
SABA	Short-acting $beta_2$-agonists
SAE	Serious adverse event

Abbreviation or special term	Explanation
SAF	Safety analysis set
SAP	Statistical Analysis Plan
SD	Standard deviation
SoA	Schedule of Activities
TBL	Total Bilirubin
ULN	Upper Limit of Normal
US	United States
USPI	United States prescribing information
WOCBP	Women of childbearing potential

1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A randomized, double-blind, 2-period, cross-over study evaluating efficacy and safety of repeated doses of PT027 compared to PT007 in patients with asthma and acute airway obstruction induced by repeated mannitol challenges

Brief Title: PT027 compared to PT007 in patients with asthma with mannitol-induced acute airway obstruction

Regulatory Agency Identifier Number: IND 136213

Rationale: Asthma exacerbations typically follow exposure to environmental triggers. Inhaled corticosteroids, such as budesonide, treat inflammation and there is evidence of a 'window of opportunity' during periods of worsening symptoms in which the timely administration of ICS can prevent symptoms developing into an exacerbation. A combination of a SABA and an ICS as an as-needed rescue/reliever therapy will provide rapid bronchodilation and symptom relief and reduce the underlying airway inflammation, thereby mitigating the risk of a severe asthma exacerbation. PT027 utilizes an innovative Co-suspension Delivery Technology™ formulation containing the SABA albuterol and the ICS budesonide in a single pressurized metered dose inhaler. The ALTA study aims to demonstrate that PT027 (albuterol/budesonide) has a non-inferior or better efficacy profile compared to PT007 (albuterol) in treating acute airway obstruction when used repetitively.

Objectives and Estimands

Objectives	Estimands [a]
Primary	
• To assess the efficacy of repeated dosing of PT027 relative to PT007, on post-dose lung function, when used by participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• **Endpoint:** Change from mannitol baseline [b] FEV_1 AUC(0-60 min) post-mannitol challenge 1. • **Population:** Adult participants with asthma on SABA as-needed treatment only, and who are sensitive to mannitol. • **Summary measure:** Difference in the adjusted means of the endpoint for the treatment comparison of PT027 versus PT007. • **Treatment condition:** PT027 and PT007. • **Strategy for ICE:** A hypothetical strategy will be implemented. This estimand targets the hypothetical scenario in which the ICE did not occur and as such outcomes for participants

Objectives	Estimands [a]
	without an ICE are as observed and for those with an ICE will be set to missing from the timepoint at which the ICE occurs.
Secondary	
• To establish the efficacy of PT027 after a single dose compared with PT007 in reversal of acute airway obstruction, when used by participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• **Endpoint**: Change from mannitol baseline [b] FEV_1 AUC(0-15 min) post-mannitol challenge 1. • **Strategy for ICE**: A hypothetical strategy will be implemented. This estimand targets the hypothetical scenario in which the ICE did not occur and as such outcomes for participants without an ICE are as observed and for those with an ICE will be set to missing from the timepoint at which the ICE occurs. Population, summary measure, and treatment condition are the same as for the primary objective.
• To establish the efficacy of PT027 compared with PT007 in the sustainability of effect of reversal of acute airway obstruction post-mannitol challenge 1 in participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• **Endpoint**: Change from mannitol baseline [b] in FEV_1 at 480 min post-mannitol challenge 1. Population, summary measure, and treatment condition are the same as for the primary objective. Strategy for ICEs will be while-on-treatment.
• To establish the efficacy of a single dose of PT027 compared with PT007 on post-dose speed of recovery of lung function following a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• **Endpoint**: Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2, pre-final dose of rescue/reliever. • **Summary measure**: Difference in the adjusted medians of the participant-level outcomes for the treatment comparison of PT027 versus PT007. Population and treatment condition are the same as for the primary objective. Strategy for ICEs will be while-on-treatment.
• To establish the protective efficacy of prior repetitive doses of PT027 compared with PT007 on lung function fall in response to a recurring trigger of acute airway obstruction in participants with asthma on SABA as needed treatment only.	• **Endpoint**: Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever. Population, summary measure, treatment condition, and strategy for ICEs are the same as for the primary objective.
	• **Endpoint**: Peak fall from 480 min FEV_1 to post- mannitol challenge 2, pre-final dose of rescue/reliever.

Objectives	Estimands [a]
	Population, summary measure, treatment condition, and strategy for ICEs are the same as for the primary objective.
Safety	
• To assess the safety and tolerability of repeated dosing of PT027 as compared to PT007 in participants with asthma on SABA as-needed treatment only.	• **Incidence** of AEs and clinical abnormalities related to 12-lead ECG, clinical laboratory tests, and/or vital signs.

[a] Estimands are precise descriptions of the treatment effect reflecting the clinical question posed by the trial objective. They summarize at a population-level what the outcomes would be in the same participants under different treatment conditions being compared (ICH E9 (R1) Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials).

[b] Unless specified otherwise, baseline is the value taken pre-mannitol challenge 1 (-30 min) at Visit 2 and at Visit 3. Mannitol baseline is defined as the FEV_1 result where a positive response to mannitol is observed prior to dosing of study drug for challenge 1 in Visit 2 and in Visit 3 (time 0). A positive response is defined as a $\geq 15\%$ decrease in FEV_1 from the 0 mg FEV_1 value.

Abbreviations: AE=adverse events; AUC=area under the curve; ECG=electrocardiogram; FEV_1=Forced Expiratory Volume in the first second; ICE=intercurrent event; SABA=Short-Acting $Beta_2$-Agonist.

Overall Design Synopsis

This is a multi-center, randomized, double-blind, 2-period, cross-over clinical study. Part 1 will enroll a small cohort of participants and will be used as a pilot study. The data obtained from Part 1 were assessed by an internal AstraZeneca advisory board (IAB), and suggested changes were made to Part 2 of the study. The study will enroll participants with asthma using SABA as their only asthma treatment and with mannitol-induced acute airway obstruction. The study will be conducted at approximately 10 sites in the United States.

Disclosure Statement: This is a randomized, double-blind, 2-period cross-over study evaluating efficacy and safety of repeated doses of PT027 (albuterol/budesonide pMDI) compared to PT007 (albuterol pMDI), in participants with asthma and with acute airway obstruction induced by two mannitol challenges ~8 hours apart. PT027 180/160 µg will be given as 2 actuations of 90 µg albuterol/80 µg budesonide per inhalation (Treatment A) and PT007 180 µg will be given as 2 actuations of 90 µg albuterol per inhalation (Treatment B).

Number of Participants:

In Part 1 of the study, 17 participants were randomized to 1 of 2 treatment sequences (A/B or B/A) and received at least one dose, which yielded 15 evaluable participants who completed the study. In Part 2, approximately 88 participants will be randomized to the treatment sequences to ensure a minimum of 74 evaluable participants (37 per treatment sequence) complete the study. An evaluable participant is defined as a participant who is enrolled, randomized, and has received all five doses of the study intervention and completed both treatment periods.

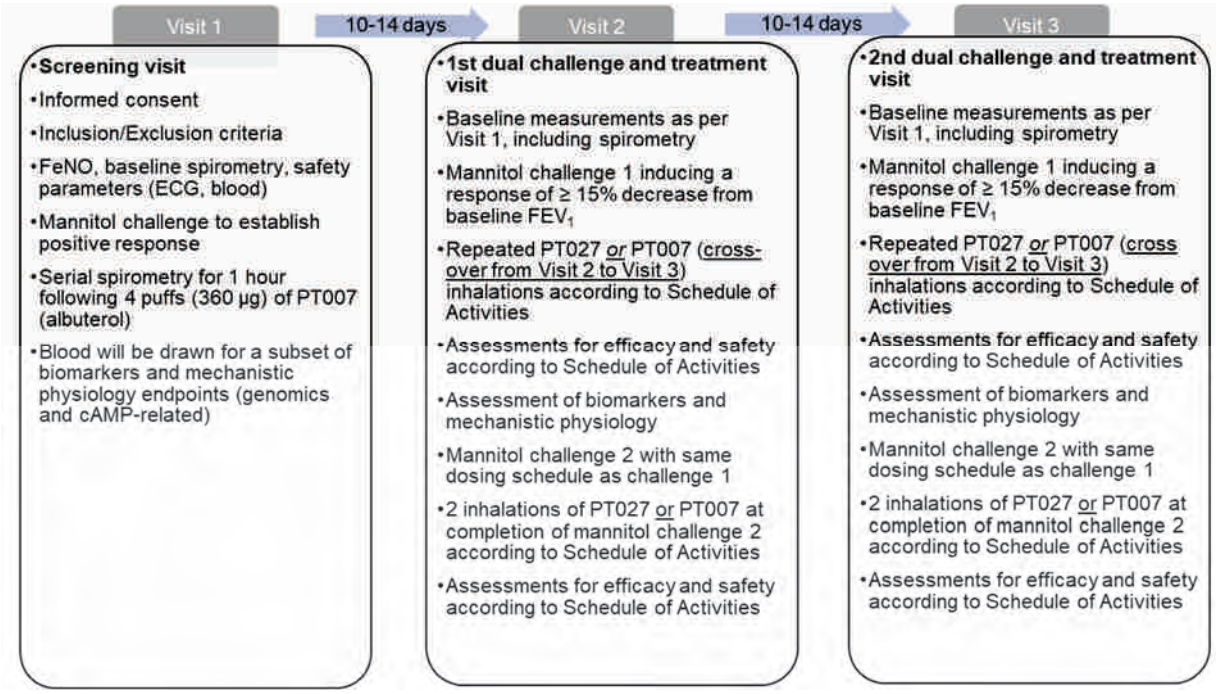
Intervention Groups and Duration:

Each participant will participate in either Part 1 or Part 2 of this study for approximately 6 weeks, from the time when informed consent is obtained through to end of study, inclusive of a screening visit, two treatment visits, and a follow-up telephone call 7 (-2/+3) days after the last treatment visit. The duration of each visit is as follows: screening visit approximately 5 hours, each treatment visit approximately 11 to 13 hours, and the follow-up telephone call approximately 30 minutes. Participants should remain confined in the study unit until completion of all assessments to ensure participant safety based on Investigator clinical judgement. There will be a 10- to 14-day washout period between each visit. The SoA will be altered for Part 2 based on IAB recommendation to: extend the time period between mannitol challenges from 7 to ~8 hours (the first challenge is at time -10. - in Part 1, the second challenge started at time 420 minutes (7 hours), but for Part 2, the second challenge will start at time 480 minutes [~8 hours]), eliminate the 120 minute-dosing, add 3-minute post-mannitol challenge spirometry, dyspnea and POE endpoints, add blood draws for Visit 1, and modify the timing of the open-label-albuterol (PT007) delivery for Visit 1. Data from Part 1 have been assessed by an internal AstraZeneca advisory board and suggested changes are now being made to Part 2, as per this protocol amendment. The data from Part 1 will not be included in the analysis of Part 2 for the evaluation of the primary and secondary endpoints. Safety endpoints will be reported for both Part 1 and Part 2.

All participants will be randomized to one of 2 treatment sequences, A/B or B/A, where treatment A is PT027 180/160 µg (given as 2 actuations of 90 µg albuterol / 80 µg budesonide per inhalation) and treatment B is PT007 180 µg (given as 2 actuations of 90 µg albuterol per inhalation).

Figure 1 represents a sequence of visits and high-level visit overview.

Figure 1 High-Level Overview of Study Visits



Abbreviations: cAMP=cyclic adenosine monophosphate; ECG=electrocardiogram; FeNO=Fractional exhaled Nitric Oxide; FEV_1=Forced Expiratory Volume in the first second.

Data Monitoring Committee: An AstraZeneca Internal Advisory Board (IAB) made recommendations to amend Part 2, based on Part 1 study data.

Statistical Methods

The primary endpoint for this study is the change from mannitol baseline in FEV_1 AUC(0-60 min) post-mannitol challenge 1. The primary efficacy comparison of non-inferiority of PT027 versus PT007 will evaluate the hypothetical estimand in the Per protocol (PP) set and will be based on a 1-sided hypothesis testing approach with a non-inferiority margin of -150 mL.

The change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1 will be analyzed using a linear mixed model with a random participant effect. The fixed effects in the model will include center, treatment, treatment sequence, mannitol baseline FEV_1, the average of the two periods mannitol baseline FEV_1 values, and period. Point estimates of the estimated adjusted treatment means, standard errors, and 95% confidence intervals (CIs) will be presented. The point estimate of the difference in treatment means for the comparison of PT027 to PT007 with associated 2-sided 95% CI will be used to evaluate non-inferiority, such that non-inferiority of PT027 compared with PT007 is established if the lower 95% confidence limit of the point estimate is greater than the non-inferiority margin of -150 mL.

If non-inferiority is demonstrated for the primary endpoint and for all of the four secondary endpoints included in the non-inferiority testing hierarchy, then comparisons will be made to establish the superiority of PT027 versus PT007 and the hypothetical estimand in the mRS population will be evaluated.

Efficacy comparisons testing superiority of PT027 versus PT007 will be based on a 2-sided hypothesis testing approach.

The primary endpoint of change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1 will be analyzed using the specified linear mixed model as above for the non-inferiority comparison. The estimated mean treatment difference, as well as 2-sided 95% CI, and 2-sided p-value will be presented.

For secondary endpoints:

- Change from mannitol baseline FEV_1 AUC(0-15 min) post-mannitol challenge 1.
- Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1.
- Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2 pre-final dose of rescue/reliever.
- Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever.

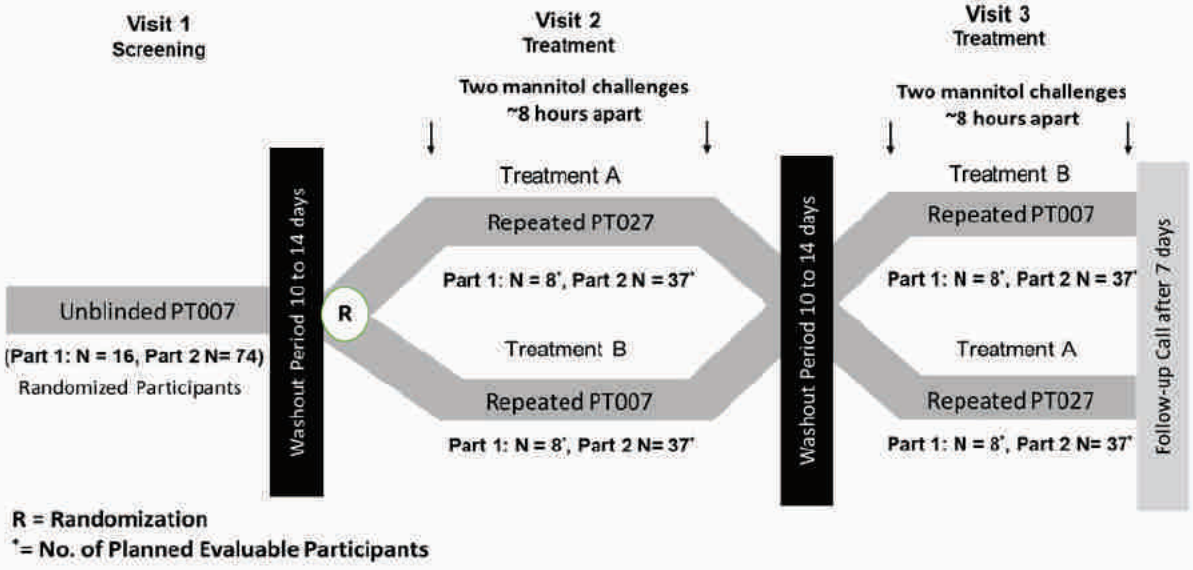
The comparison of non-inferiority will be evaluated using the same approach as for the primary endpoint. If all secondary endpoints demonstrate non-inferiority, the comparisons of superiority for these endpoints, and the secondary endpoint, peak fall in FEV_1 from time 480 min FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever will be evaluated using the same approach as that for the primary endpoint.

Similarly, time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2 will be based on the while-on-treatment estimand for the non-inferiority and superiority assessments. Return to baseline FEV_1 will be achieved at the first timepoint the FEV_1 value is within 5% of baseline FEV_1 (baseline FEV_1 * 0.95). Linear interpolation will be used to estimate the FEV_1 between spirometry timepoints. Median differences and 95% CIs will be provided using the Hodges-Lehmann estimate based on the Wilcoxon signed-rank statistic. If more than 5% of participants do not return to within 5% of baseline FEV_1 post-mannitol challenge 2, then an alternative Kaplan-Meier analysis will be considered.

Multiplicity will be controlled using a hierarchical testing strategy, see Section 9.4.2 for specific details.

1.2 Schema

Figure 2 Study Design



1.3 Schedules of Activities

Table 1 Schedule of Activities (Part 1 and Part 2)

Procedure	Screening	Treatment Period		Follow-up TC (7 [-2/+3] days after Visit 3/ after ED)	Details in CSP Section or Appendix
Visit	**1** [a]	**2** [b]	**3** [c]	**NA**	
Informed consent	X				Section 5.1
Inclusion and exclusion criteria	X				Sections 5.1 and 5.2
Check for criteria of participant discontinuation from study		X	X		Section 7
Medical history and surgical history (including asthma history) [c]	X				Sections 5.1 and 5.2
Severe asthma exacerbation history (within last 12 months)	X				Sections 8.2.2 and 8.2.3
Estimated date/time of last SABA administration	X	X	X		Section 5.1
Demography	X				Sections 5 and 5.1
Physical examination including height and weight	X	X	X		Section 8.3.1
Drug/substance abuse and smoking history	X				Section 5.2

Procedure	Screening	Treatment Period		Follow-up TC (7 [-2/+3] days after Visit 3/ after ED)	Details in CSP Section or Appendix
Visit	**1** [a]	**2** [b]	**3** [c]	**NA**	
Mannitol challenge [e]	X	X [f]	X [f]		Section 8.2.5
AIRQ®	X				Section 8.2.2
Severity per NAEPP	X				Section 8.2.3
Spirometry	X	X	X		Sections 8.2.1 and 8.3.3
Perceived onset of effect		X	X		Section 8.2.6
Symptoms: Borg scale		X	X		Section 8.2.4
Training and assessment on MDI technique	X	X	X		Section 6.2.1
FeNO	X	X	X		Section 8.3.6
bEOS measurement	X	X	X		Section 8.3.7
Blood sample for cAMP measurement	X	X	X		Section 8.9.1.2
Blood sample for cAMP mobilizing analytes	X	X	X		Section 8.9.1.2
Blood sample for exploratory inflammatory biomarkers	X	X	X		Section 8.9.1.2
Blood sample for biomarker assessment beta receptor, corticosteroid receptor expression genetics, and genomics	X	X	X		Section 8.9.1.3
Nasal scrape sample for beta receptor and corticosteroid receptor expression genetics	X	X	X		Section 8.9.1.3
Urine sample for leukotrienes including LTE4 biomarker assessment		X	X		Section 8.9.1.1
Urine pregnancy test (WOCBP only)	X	X	X		Section 5.1
Clinical safety laboratory assessments	X	X	X		Section 8.3.5
12-lead ECG	X	X	X		Section 8.3.4
Vital signs	X	X	X		Section 8.3.2
Randomization		X			Section 6.3

Procedure	Screening	Treatment Period		Follow-up TC (7 [-2/+3] days after Visit 3/ after ED)	Details in CSP Section or Appendix
Visit	**1** [a]	**2** [b]	**3** [c]	**NA**	
Study intervention (PT027 or PT007) [d, g]	X	X	X		Section 6.1
Optional Genomics Initiative sample		X			Section 8.8 and Appendix D
AEs and asthma exacerbations	X	X	X	X	Section 8.4
Concomitant medication	X	X	X	X	Section 6.5
Distribute Thank you Cards		X			

[a] Assessment windows for spirometry are: ± 2 minutes for timepoints 0 to 60 min and ± 5 minutes for all other timepoints.

[b] At timepoints of −30 and 60 min, the assessments that take priority are: perceived onset of effect, Borg scale, and spirometry, where applicable. Other assessments at timepoints of −30 and 60 will have a time window of +10 minutes after the planned timepoint. For Visit 1, participants must complete spirometry at all timepoints of 3, 5, 10, 15, 35, 45, and 60 minutes post-mannitol challenge, even if they reach 10% of baseline FEV_1 sooner. (Note, "baseline" FEV_1 here refers to the FEV_1 at the pre-mannitol challenge spirometry at timepoint -30 min).

[c] At timepoints that have multiple assessments planned, the general order of assessments will be: vital signs, ECG, blood sampling, perceived onset of effect, Borg scale, FeNO, and spirometry. However, spirometry assessment will always take priority.

[d] Spirometry to be performed just before treatment administration. For Timepoint 0, this is equivalent to the last spirometry performed in the mannitol challenge (when PD15 is identified). For Timepoint 0, please note that study drug treatment should be administered at the time of completion of a positive mannitol challenge (ie, as soon as spirometry for time 0 has been completed, and within 2 minutes) of the delivery of the dose of mannitol which produces a $\geq$ 15% decrease in FEV_1 from the 0 mg dose).

[e] For spirometry occurring immediately prior to the mannitol challenge (at timepoints -30 and +480 minutes), a maximum of 8 maneuvers may be performed, until 3 technically adequate maneuvers are achieved. See Section 8.2.1 for details. Once this spirometry is complete, the participant should begin the mannitol challenge.

[f] For each post-mannitol challenge spirometry, at timepoints corresponding to 3, 5, 10, and 15 minutes, a maximum of 8 maneuvers may be performed until 1 technically adequate maneuver is achieved.

[g] For other spirometry assessments (at timepoints 35, 45, and 60 min), unless specified, a maximum of 8 maneuvers may be performed until 2 technically adequate maneuvers are achieved. See Section 8.2.1 for details.

Abbreviations: AE=adverse event; AIRQ®=Asthma Impairment and Risk Questionnaire; bEOS=blood eosinophil; cAMP=cyclic adenosine monophosphate; ECG=electrocardiogram; FeNO=Fractional exhaled Nitric Oxide. FEV_1=Forced Expiratory Volume in the first second; ICS=inhaled corticosteroids; LTE4=Leukotriene E4; MDI=metered dose inhaler; NAEPP=National Asthma Education and Prevention Program; SABA=Short-Acting $Beta_2$-Agonists; TC=telephone call; WOCBP=women of child-bearing potential.

Table 2 Screening Visit 1 Spirometry, Mannitol Challenge, Dosing, Biomarkers, Safety Blood Samples

	Walk-in Baseline		*Mannitol Baseline*	POST-SCREENING MANNITOL CHALLENGE											
Minutes	-30	-10	0	3	5	10	15	20	25	30	35	40	45	50	55	60
Spirometry [a, b, c, d, e, f, g]	X		X	X	X	X	X				X		X			X
Mannitol [h, i]		X														
Dose: 4 puffs PT007 post-spiro [d]											X					
FeNO [c]	X															
cAMP [X], β-1, and GC receptors [Y] [c]	X,Y [j]									X						X, Y [i]
bEOS measurement (local lab) [c]	X															
Vitals [c, k]	X									X						X
12-lead ECG [c]	X															X
Safety Labs [l]	X									X						X

[a] Assessment windows for spirometry are: ± 2 minutes for timepoints 0 to 60 min, and ± 5 minutes for all other timepoints.

[b] At timepoints of −30 and 60 min, the assessments that take priority are: spirometry, where applicable. Other assessments at timepoints of −30 and 60 will have a time window of +10 minutes after the planned timepoint. For Visit 1, participants must complete spirometry at all timepoints of 3, 5, 10, 15, 35, 45, and 60 minutes post-mannitol challenge, even if they reach 10% of baseline FEV_1 sooner. (Note, "baseline" FEV_1 here refers to the FEV_1 at the pre-mannitol challenge spirometry at timepoint -30 min).

[c] At timepoints that have multiple assessments planned, the general order of assessments will be: vital signs, ECG, blood sampling, FeNO, and spirometry. However, spirometry assessment will always take priority.

[d] Spirometry to be performed just before treatment administration. For Timepoint 0, this is equivalent to the last spirometry performed in the mannitol challenge (when PD15 is identified).

[e] For spirometry occurring immediately prior to the mannitol challenge (at timepoint -30 min), a maximum of 8 maneuvers may be performed, until 3 technically adequate maneuvers are achieved. See Section 8.2.1 for details. Once this spirometry is complete, the participant should begin the mannitol challenge.

[f] For each post-mannitol challenge spirometry, at timepoints corresponding to 3, 5, 10, and 15 minutes, as indicated in the Schedule of Assessments, a maximum of 8 maneuvers may be performed until 1 technically adequate maneuver is achieved.

[g] For other spirometry assessments (at timepoints 35, 45, and 60 min), unless specified, a maximum of 8 maneuvers may be performed until 2 technically adequate maneuvers are achieved. See Section 8.2.1 for details.

[h] Mannitol challenge inducing a response of ≥ 15% decrease in FEV_1 from the 0 mg mannitol FEV_1 value.

[i] Vital signs assessments, blood sampling, FeNO, and spirometry are to be performed just before mannitol challenge. During the mannitol challenge, the next mannitol dose should be administered as soon as possible following spirometry, and delays between doses should be avoided.

[j] "Y" indicates blood and nasal scrape.

[k] Vital signs (pulse, blood pressure, pulse oximetry and respiratory rate) are to be recorded at the timepoints mentioned in this table and additionally at the discretion of the investigator to ensure participant safety. At the investigator's discretion, pulse oximetry may be measured continuously or outside of these timepoints as well.

[l] Chemistry, hematology, and urinalysis are only taken at -30 min timepoint.

Abbreviations: bEOS= blood eosinophil; cAMP= cyclic adenosine monophosphate; ECG=electrocardiogram; FeNO=Fractional exhaled Nitric Oxide; spiro=spirometry.

Table 3 Schedule of Activities: Mannitol Challenge 1 and Mannitol Challenge 2

Mannitol Challenge 1

Walk-in Baseline			*Mannitol Baseline*			POST-MANNITOL CHALLENGE 1																
↓			↓																			
Spirometry, Mannitol Challenges, Dosing, Biomarkers																						
Minutes	**-30**	**-10**	**0**	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**90**	**120**	**180**	**240**	**300**	**360**	**480**
Spirometry [a, b, c, d, e, f, g]	X		X	X	X	X	X				X		X			X	X	X	X	X	X	X	X
Mannitol [h, i]		X																					X
Study intervention dose			X					X				X				X							
FeNO and bEOS [c]	X																						X
Urine for leukotrienes including LTE4 [j]	X																	X					
cAMP [X], β-1, and GC receptors [Y] [c]	X Y									X						X	X Y						X
Symptoms and Perception of Effect																							
Minutes	**-30**	**-10**	**0**	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**90**	**120**	**180**	**240**	**300**	**360**	**480**
POE [b, c, k]				X	X	X	X	X	X	X	X	X	X	X	X	X							
Borg Scale [b, c]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Characteristics, Safety Measures, and Labs																							
Minutes	**-30**	**-10**	**0**	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**90**	**120**	**180**	**240**	**300**	**360**	**480**
Vitals, O_2, ECG [c]	X									X						X			X				X
Chemistry panel (Table 7) [c, k]	X															X			X				

Walk-in Baseline			*Mannitol Baseline*	POST-MANNITOL CHALLENGE 1																		
↓			↓																			
Urinalysis and hematology [c, l]	X																					
Serum glucose and potassium [c, m]	X														X			X				
Minutes	**-30**	**-10**	**0**	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**90**	**120**	**180**	**240**	**300**	**360**	**480**
Adverse events	X															X		X					

Mannitol Challenge 2

Post-treatment Baseline	*Mannitol 2 FEV_1 response*	POST-MANNITOL CHALLENGE 2													
↓	↓														
Spirometry, Mannitol Challenges, Dosing, Biomarkers (cont.)															
	480	**490**	**493**	**495**	**500**	**515**	**520**	**525**	**530**	**535**	**540**	**545**	**550**	**555**	**560**
Spirometry [a, b, c, d, e, f, g]	X	X	X	X	X	X			X			X			X
Mannitol [h, i]	X														
Dose [d]		X													
FeNO and bEOS [c]	X														X
Urine for leukotrienes including LTE4 [j]															X
cAMP [X], β-1, and GC receptors [Y] [c]	X								X						X Y
Symptoms and Perception of Effectccc															
	480	**490**	**493**	**495**	**500**	**515**	**520**	**525**	**530**	**535**	**540**	**545**	**550**	**555**	**560**
POE [b, c, d]			X	X	X	X	X	X	X	X	X	X	X	X	X
Borg Scale [b, c]	X		X	X	X	X	X	X	X	X	X	X	X	X	X

Post-treatment Baseline	*Mannitol 2 FEV_1 response*	POST-MANNITOL CHALLENGE 2													
↓	↓														
Clinical Characteristics, Safety Measures, and Labs															
	480	**490**	**493**	**495**	**500**	**515**	**520**	**525**	**530**	**535**	**540**	**545**	**550**	**555**	**560**
Vitals, O_2, ECG [c,k]															X
Chemistry panel (Table 7) [c,l]															X
	480	**490**	**493**	**495**	**500**	**515**	**520**	**525**	**530**	**535**	**540**	**545**	**550**	**555**	**560**
Urinalysis and hematology [c,m]															
Serum glucose and potassium [c,n]															X
Adverse events															X

[a] Assessment windows for spirometry are: ± 2 minutes for timepoints 0 to 60 min and 480 to 560 min, and ± 5 minutes for all other timepoints.

[b] At timepoints of −30, 60, 90, 490, and 560, the assessments that take priority are: perceived onset of effect, Borg scale, and spirometry, where applicable. Other assessments at timepoints of −30, 60, 90, 490, and 560 will have a time window of +10 minutes after the planned timepoint.

[c] At timepoints that have multiple assessments planned, the general order of assessments will be: vital signs, ECG, blood sampling, perceived onset of effect, Borg scale, FeNO, and spirometry. However, spirometry assessment will always take priority.

[d] Spirometry to be performed just before treatment administration. For timepoint 0 and +490, this is equivalent to the last spirometry performed in mannitol challenge 1 (when PD15 is identified) and mannitol challenge 2 (when the same final cumulative dose as mannitol challenge 1 is reached). For timepoint 0 and +490, please note that study drug treatment should be administered at the time of completion of a positive mannitol challenge (ie, as soon as spirometry for time 0 and +490 min has been completed, and within 2 minutes) of the delivery of the dose of mannitol which produces a ≥ 15% decrease in FEV_1 from the 0 mg dose).

[e] For spirometry occurring immediately prior to the mannitol challenge (at timepoints -30 and +480), a maximum of 8 maneuvers may be performed, until 3 technically adequate maneuvers are achieved. See Section 8.2.1 for details. Once this spirometry is complete, the participant should begin the mannitol challenge.

[f] For each post-mannitol challenge spirometry, at timepoints corresponding to 3, 5, 10, 15, 493, 495, 500, and 515 minutes, as indicated in the Schedule of Assessments, a maximum of 8 maneuvers may be performed until 1 technically adequate maneuver is achieved.

[g] For other spirometry assessments (at timepoints 35, 45, 60, 90, 120, 180, 240, 300, 360,530, 545, 560 min), unless specified, a maximum of 8 maneuvers may be performed until 2 technically adequate maneuvers are achieved. See Section 8.2.1 for details.

[h] Mannitol challenge 1 inducing a response of ≥ 15% decrease in FEV_1 from the 0 mg mannitol FEV_1 value.

[i] Mannitol challenge 2 should proceed to have the same final cumulative dose as the first challenge, even if the participant achieves a ≥ 15% decrease sooner than in the first challenge. However, every investigator should use his/her own discretion, and if they notice anything of concern, they should reach out to the Medical Monitor. Once Mannitol challenge 2 is completed and treatment is administered, the clock essentially "resets" at timepoint 430 and subsequent timing of assessments should be based off of this "new time 0".

[j] Collection of urine samples for biomarker analysis will have a time window of ± 15 minutes at timepoints 120 and 560 min. Prior to Mannitol Challenge 2, participants should empty their bladder to optimize accuracy of subsequent urinary leukotriene analyses.

[k] Vital signs (pulse, blood pressure, pulse oximetry and respiratory rate) are to be recorded at the timepoints mentioned in this table and additionally at the discretion of the investigator to ensure participant safety. At the investigator's discretion, pulse oximetry may be measured continuously or outside of these timepoints as well.

[l] Chemistry panel only taken at -30 min and last timepoint
[m] Hematology and urinalysis only taken at -30 min.
[n] Serum glucose and potassium will be taken at -30, 60, 180, and 560 timepoints
Abbreviations: cAMP= cyclic adenosine monophosphate; FeNO=Fractional exhaled Nitric Oxide; POE=perception of effect.

2 INTRODUCTION

Asthma manifests as a heterogenous disease, characterized by chronic airway inflammation (GINA 2021). Short-acting beta$_2$-agonists such as albuterol (salbutamol) induce smooth muscle airway relaxation and provide quick relief of asthma symptoms, but do not address the underlying airway inflammation. Reliance on SABA, and inadequate utilization of ICS, is linked to increased risk of exacerbations.

Asthma exacerbations typically follow exposure to environmental triggers. Inhaled corticosteroids, such as budesonide, treat inflammation and there is evidence of a 'window of opportunity' during periods of worsening symptoms in which the timely administration of ICS can prevent symptoms developing into an exacerbation (Tattersfield et al 1999).

PT027 is being developed as a novel as-needed rescue/reliever therapy for the treatment of asthma. It is a combination of a SABA and an ICS and would not only provide rapid bronchodilation and symptom relief but would also treat flare-ups of the underlying airway inflammation when required, thereby reducing the risk of a severe asthma exacerbation. PT027 is a first-in-class albuterol/ICS rescue/reliever therapy, superseding SABA across all asthma severities to become the foundation of asthma care and has been approved in the US for the as-needed treatment or prevention of bronchoconstriction and to reduce the risk of exacerbations in patients with asthma 18 years of age and older.

PT027 contains a fixed-dose combination of a SABA (albuterol) and an ICS (budesonide) delivered in an innovative Co-suspension Delivery Technology™ in a single pressurized metered dose inhaler (pMDI). PT027 was developed in two strengths; 180/80 µg (given as 2 actuations of 90 µg albuterol / 40 µg budesonide / puff) as-needed and 180/160 µg (given as 2 actuations of 90 µg albuterol / 80 µg budesonide / puff) as-needed (considered the target dose in adults and adolescents). Currently PT027 is approved only at the higher dose for patients 18 years and above to treat and prevent bronchoconstriction and reduce the risk of exacerbations.

Albuterol is a SABA, inducing airway smooth muscle relaxation and reducing or preventing bronchoconstriction. Albuterol is approved in many countries in multiple formulations for treatment or prevention of bronchoconstriction and is also known under the generic name of salbutamol. In clinical practice, albuterol is used as rescue/reliever therapy on an as-needed basis (NAEPP 2007).

Budesonide is a well-established anti-inflammatory corticosteroid that exhibits potent glucocorticoid and weak mineralocorticoid activity and is approved worldwide in inhaled formulations for the treatment of asthma and chronic obstructive pulmonary disease both as a mono-product and in combination with a LABA, eg, formoterol.

Currently the approved albuterol products have not been studied for safety and efficacy for use in home-based asthma action plans to treat an acute worsening of symptoms and/or lung function (multiple repetitive doses of albuterol over several hours).

The ALTA study aims to demonstrate PT027 to be safe and effective when used repetitively over short periods of time to treat acute worsening of lung function as is recommended for home management.

As-needed albuterol/ICS will over time allow the genomic anti-inflammatory effects of budesonide to combat the variability in airway inflammation that leads to exacerbations. However, there are circumstances in which type-2 inflammation may not be the predominant cause of an acute deterioration of asthma (eg, viral infections, environmental triggers, exercise) and where the non-genomic effects of an ICS (vasoconstriction to mitigate airway mucosal edema, enhanced beta-agonist smooth muscle relaxation) may contribute to relief of airway obstruction and asthma symptoms within minutes. There is therefore a potential opportunity for PT027 to be superior compared to albuterol alone in the ALTA study.

The ALTA study will evaluate the efficacy and safety of repeated doses of PT027 (2 puffs / dose of 90 µg albuterol / 80 µg budesonide pMDI) vs PT007 (2 puffs /dose of 90 µg albuterol pMDI) to treat acute airway obstruction induced by a repeated airway challenge bronchoconstriction model using mannitol. Mannitol indirectly produces acute airway obstruction by inducing mast cell degranulation, the mechanism by which the early response to allergen, exercise, cold air, weather changes, strong emotions, odors, air pollution, and viral infections induce airway obstruction (Sverrild et al 2021).

2.1 Study Rationale

The ALTA study aims to demonstrate that PT027 (albuterol/budesonide) has a non-inferior or better efficacy profile compared to PT007 (albuterol) in treating acute airway obstruction when used repetitively. The efficacy evaluation will assess the magnitude and duration/sustainability of bronchodilatation and symptom relief, time to onset of effect, and protection against repeated stimuli of bronchoconstriction and symptoms.

2.2 Background

PT027 is a combination of albuterol, a beta$_2$-adrenergic agonist and budesonide, a corticosteroid, indicated for the as-needed treatment or prevention of bronchoconstriction and to reduce the risk of exacerbations in patients with asthma 18 years of age and older.

A detailed description of the chemistry, pharmacology, efficacy, and safety of PT027 is provided in the IB.

Among the previous studies conducted, the MANDALA study evaluated efficacy and safety of as-needed PT027 as rescue/reliever therapy versus as-needed PT007 in patients with moderate-to-severe asthma receiving maintenance therapy. The study demonstrated that PT027 produced statistically significant and clinically meaningful reductions in the risk of severe exacerbations compared to PT007, when used as a rescue/reliever medicine (Chipps et al 2021, Papi et al 2022).

2.3 Benefit/Risk Assessment

The study will be conducted in accordance with ICH guidelines. Permission from the research IEC will be sought and the study will start only after authorization.

To mitigate any potential risks, all participants will be closely monitored to ensure participant safety. At study visits, participants will only be discharged from the clinic after satisfactory lung function based on the Investigator's clinical judgment. Safety will be further monitored during the follow-up telephone call 7 (-2/+3) days after the last treatment visit. Additional non-investigational rescue/reliever medication may be allowed at the Investigator's discretion prior to discharge from a study visit and/or during a study visit for persistent low lung function and/or severe or unresolving symptoms.

When considering and assessing all non-clinical and clinical data available for study treatments, the Sponsor considers the risk and benefit profile of the ALTA study to be acceptable.

More detailed information about the known and expected benefits and potential risks of PT027 and PT007 may be found in the IB.

2.3.1 Risk Assessment

Table 4 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
Study interventions (PT027 and PT007)		
Clinical risk of PT027 and PT007	PT027 has been approved in the US for the as-needed treatment or prevention of bronchoconstriction and to reduce the risk of exacerbations in patients with asthma 18 years of age and older. The main risks for PT027 during a brief treatment period resemble the risks of PT007. These risks include paradoxical	Participants will be closely monitored by site staff during the treatment visit days (including electrocardiogram, repeated spirometry, vital signs, etc).

Table 4 Risk Assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation strategy
	bronchospasm, immediate hypersensitivity reactions, and cardiovascular events.	
Other (Mannitol Challenge)		
Risks Associated with Mannitol Challenge (Aridol ™).	**Severe bronchospasm**: Mannitol may cause severe bronchospasm in susceptible patients. Administer by trained professionals under the supervision of a physician. **Participants with co-morbid conditions**: may increase sensitivity to the broncho-constricting or other potential effects of Mannitol such as: severe cough, ventilatory impairment, unstable angina, or active upper or lower respiratory tract infection that may worsen with use of a bronchial irritant.	Participants will be closely monitored by site staff during the treatment visit days (including electrocardiogram, repeated spirometry, vital signs [including blood pressure, heart rate, pulse oximetry, and respiratory rate], etc). Medications and equipment to treat severe bronchospasm will be present in the testing area.

2.3.2 Benefit Assessment

There will be no direct clinical benefit to the participants from the study intervention, but the data collected during the study may help to improve asthma rescue/reliever treatment for patients in future.

2.3.3 Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to participants participating in this study, the potential risks identified in association with the mannitol challenge, PT027, and PT007 are expected to be managed effectively.

More detailed information about the known and expected benefits and potential risks of PT007 and PT027 will be found in the IB. Expected benefits and potential risks of mannitol challenge will be found in the Aridol™ USPI.

3 OBJECTIVES AND ESTIMANDS

Table 5 Objectives and Estimands

Objectives	Estimands [a]
Primary	
• To assess the efficacy of repeated dosing of PT027 relative to PT007, on post-dose lung function, when used by participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• **Endpoint**: Change from mannitol baseline [b] FEV_1 AUC(0-60 min) post-mannitol challenge 1. • **Population**: Adult participants with asthma on SABA as-needed treatment only, and who are sensitive to mannitol. • **Summary measure**: Difference in the adjusted means of the endpoint for the treatment comparison of PT027 versus PT007. • **Treatment condition**: PT027 and PT007. • **Strategy for ICE**: A hypothetical strategy will be implemented. This estimand targets the hypothetical scenario in which the ICE did not occur and as such outcomes for participants without an ICE are as observed and for those with an ICE will be set to missing from the timepoint at which the ICE occurs.
Secondary	
• To establish the efficacy of PT027 after a single dose compared with PT007 in reversal of acute airway obstruction, when used by participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• **Endpoint**: Change from mannitol baseline [b] FEV_1 AUC(0-15 min) post-mannitol challenge 1. • **Strategy for ICE**: A hypothetical strategy will be implemented. This estimand targets the hypothetical scenario in which the ICE did not occur and as such outcomes for participants without an ICE are as observed and for those with an ICE will be set to missing from the timepoint at which the ICE occurs. Population, summary measure, and treatment condition are the same as for the primary objective.
• To establish the efficacy of PT027 compared with PT007 in the sustainability of effect of reversal of acute airway obstruction post-mannitol challenge 1 in participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• **Endpoint**: Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1. Population, summary measure, and treatment condition are the same as for the primary objective. Strategy for ICEs will be while-on-treatment.

Objectives	Estimands [a]
• To establish the efficacy of a single dose of PT027 compared with PT007 on post-dose speed of recovery of lung function following a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• **Endpoint**: Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2, pre-final dose of rescue/reliever. • **Summary measure**: Difference in the adjusted medians of the participant-level outcomes for the treatment comparison of PT027 versus PT007. Population and treatment condition are the same as for the primary objective. Strategy for ICEs will be while-on-treatment.
• To establish the protective efficacy of prior repetitive doses of PT027 compared with PT007 on lung function fall in response to a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• **Endpoint**: Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever. Population, summary measure, treatment condition, and strategy for ICEs are the same as for the primary objective.
• To assess the protective efficacy of prior repetitive doses of PT027 compared with PT007 on lung function fall in response to a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• **Endpoint**: Peak fall from 480 min FEV_1 to post- mannitol challenge 2, pre-final dose of rescue/reliever. Population, summary measure, treatment condition, and strategy for ICEs are the same as for the primary objective.
Safety	
• To assess the safety and tolerability of repeated dosing of PT027 as compared to PT007 in participants with asthma on SABA as-needed treatment only.	• **Incidence** of AEs and clinical abnormalities related to 12-lead ECG, clinical laboratory tests, and/or vital signs.
Exploratory	
• To explore the efficacy after a single dose of PT027 compared with PT007 on post-dose lung function following a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• Change from baseline (-30 min) FEV1 AUC(0-15 min) post-mannitol challenge 2.
• To assess the perception of relief of dyspnea from PT027 as compared to PT007.	• Change from baseline in Borg dyspnea scale post-mannitol challenge.
• To assess the perception of onset of effect of PT027 as compared to PT007.	• Time to perceived onset of effect of study medication working post-mannitol challenge.
• To assess the impact of repetitive PT027 as compared to PT007 on airway inflammation.	• Change from baseline FeNO and eosinophils over time, post-mannitol challenge.

Objectives	Estimands [a]
• To assess the mechanistic etiology of PT027 as compared to PT007 on reversal of acute airway obstruction and maintenance of bronchodilation.	• Urinary leukotrienes. • Serum cAMP. • cAMP mobilizing analytes. • Exploratory blood markers of inflammation. • Beta receptor and corticosteroid receptor expression genetics.
• To assess the protective effect of PT027 relative to PT007.	• The proportion of participants who are non-responders to mannitol challenge 2. A non-responder is defined as a < 15% fall in FEV_1 relative to baseline FEV_1.
• To assess the lung function efficacy and sustainability of effect reversal of acute airway obstruction of PT027 compared to PT007.	• Change from mannitol baseline in FEV_1 and percentage change from mannitol baseline in FEV_1, post-mannitol challenge 1 at each measured timepoint up to and including 480 min.
• To assess the protective effect of PT207 relative to PT007 in response to a repetitive trigger of bronchoconstriction.	• Percentage fall in FEV_1 from baseline FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever. This will be derived as 100*(FEV_1 at baseline – FEV_1 at 490 min)/FEV_1 at baseline (-30 min).

[a] Estimands are precise descriptions of the treatment effect reflecting the clinical question posed by the trial objective. They summarize at a population-level what the outcomes would be in the same participants under different treatment conditions being compared (ICH E9 (R1) Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials).

[b] Unless specified otherwise, baseline FEV_1 is the value taken pre-mannitol challenge 1 at Visit 2 and at Visit 3. The mannitol baseline is defined as the FEV_1 result where a positive response to mannitol is observed prior to dosing of study drug for challenge 1 in Visit 2 and in Visit 3 (time 0). A positive response is defined as a ≥15% decrease in FEV_1 from the 0 mg FEV_1 value. Refer to Section 9.4.1 for further definitions of baseline and mannitol baseline and associated endpoints.

Abbreviations: AE=adverse events; AUC=area under the curve; cAMP=cyclic adenine monophosphate; ECG=electrocardiogram; FEV_1=Forced Expiratory Volume in the first second; ICE=intercurrent event; FeNO=Fractional exhaled Nitric Oxide; SABA=Short-Acting Beta2-Agonists.

4 STUDY DESIGN

4.1 Overall Design

This is a multi-center, randomized, double-blind, 2-period, cross-over study evaluating efficacy and safety of repeated doses of PT027 (albuterol/budesonide pMDI) compared to PT007 (albuterol pMDI) in participants with asthma and acute airway obstruction induced by 2 mannitol challenges ~8 hours apart.

The study will be conducted in male and female participants ≥ 18 years old with asthma using as-needed SABA as the only asthma treatment at approximately 10 sites in the United States.

It is a two-period study, where Part 1 will be used as a pilot study to understand the treatment effect, operational feasibility, and variability estimates for the secondary efficacy endpoints in Part 2.

Each participant will participate in either Part 1 or Part 2 of this study for approximately 6 weeks, from the time when informed consent is obtained through to end of study, inclusive of a screening visit, two treatment visits, and a follow-up telephone call 7 (-2/+3) days after the last treatment visit. The duration of each visit is as follows: screening visit of approximately 5 hours, each treatment visit of approximately 10 to 12 hours in Part 1 and 11 to 13 hours in Part 2, and a follow-up telephone call of approximately 30 minutes. Participants should remain confined in the study unit until completion of all assessments to ensure participant safety based on Investigator clinical judgment. There will be a 10-to-14-day washout period between each visit. The visit schedule was altered for Part 2 relative to Part 1, the screening Visit 1 will have exploratory biomarkers and genomics assessment performed and the post-challenge treatment with PT007 will be given at time 35 min instead of at time 0; all mannitol challenges will include a 3-minute post-completion FEV_1 measure, there will be no 120 minute treatment dose for Visits 2 and 3, and the timing of the second mannitol challenge will be extended from time 420 to 480 min. Data from Part 1 were assessed by an internal AstraZeneca advisory board, and they suggested these changes be made to Part 2. The data from Part 1 will not be included in the analysis of Part 2 for the evaluation of primary and secondary endpoints.

The following is the sequence of study visits:

- Visit 1 (V1) screening
- Visit 2 (V2) 10 to 14 days after Visit 1 assessments; 1st dual challenge and treatment visit
- Visit 3 (V3) 10 to 14 days after Visit 2; 2nd dual challenge and treatment visit

At Visit 1, all participants will be subjected to a single mannitol challenge to establish a positive response (defined as a ≥15% decrease in FEV_1 from the 0 mg mannitol FEV_1 value) and would receive 4 puffs of open-label PT007 (total 360 µg of albuterol).

At Visit 2, all participants will be randomized to one of 2 treatment sequences, A/B or B/A, where treatments A and B are defined as:

Treatment A = PT027 180/160 µg (180/160 µg given as 2 actuations of 90/80 µg per dose)

Treatment B = PT007 180 µg (180 µg given as 2 actuations of 90 µg per dose)

Participants will receive repeated doses of either PT027 or PT007 during the treatment visits as per schedule in Table 3

Participants should refrain from using SABAs for at least 12 hours before all visits.

Refer to Table 1 for further information on the SoA to be performed throughout the study.

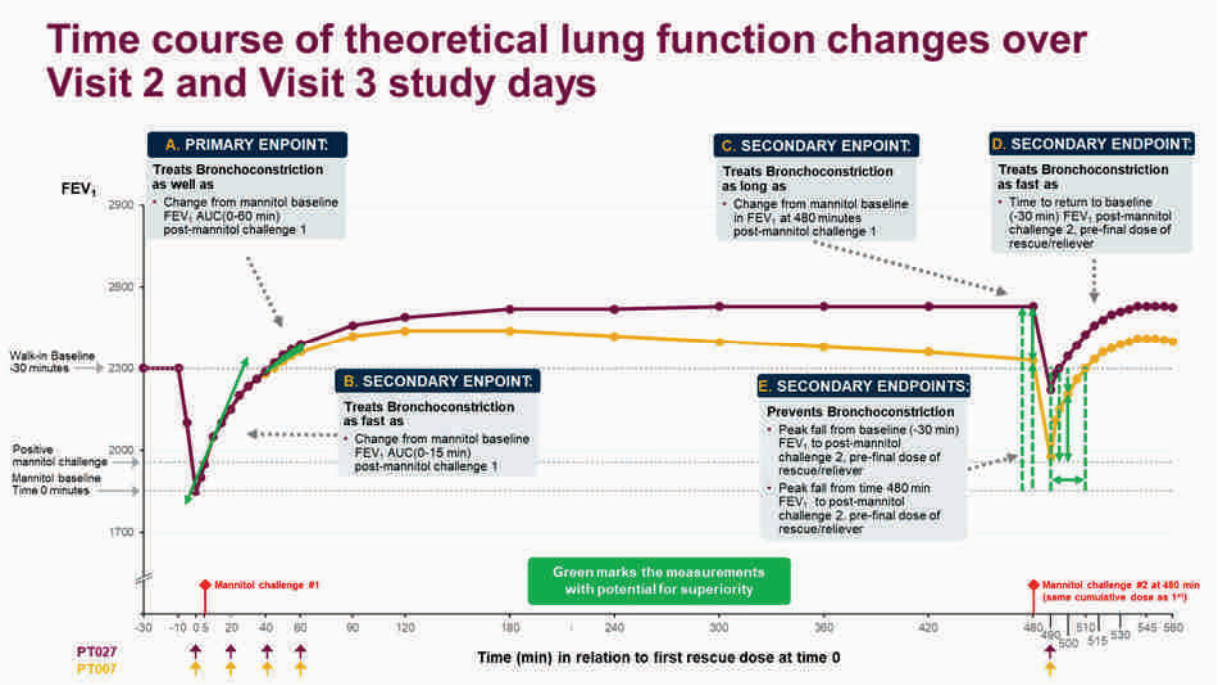
In Part 1 of the study approximately 17 participants were randomized to one of 2 treatment sequences (A/B or B/A) and received at least one randomized dose that yielded 15 evaluable participants who completed all 3 study visits. In Part 2 approximately 88 participants will be randomized to treatment sequences to ensure a minimum of 74 evaluable participants (37 per treatment sequence) complete the study. An evaluable participant is defined as a participant who is enrolled, randomized, and has received all five doses of the study intervention and completed both treatment periods.

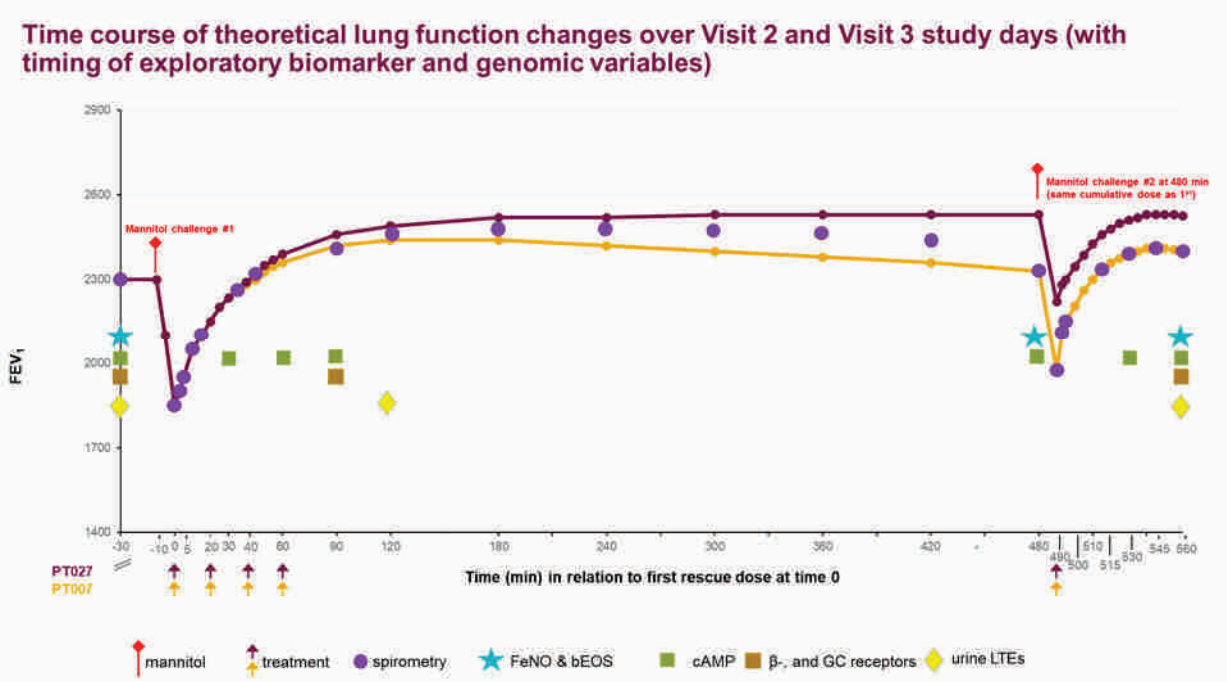
4.2 Scientific Rationale for Study Design

The ALTA study will evaluate the efficacy and safety of repeated doses of PT027 (2 puffs / dose of 90 µg albuterol / 80 µg budesonide pMDI) vs PT007 (2 puffs /dose of 90 µg albuterol pMDI) to treat acute airway obstruction induced by a repeated airway challenge bronchoconstriction model (mannitol, which indirectly produces acute airway obstruction by inducing mast cell degranulation, the mechanism by which the early response to allergen, exercise, cold air, weather changes, strong emotions, odors, air pollution, and viral infections induce airway obstruction). The aim is to demonstrate that PT027 is non-inferior or better than albuterol as rescue/reliever medication with regards to efficacy and safety parameters including magnitude and duration/sustainability of bronchodilatation and symptom relief, time to onset of effect, and protection against repeated stimuli of bronchoconstriction and symptoms.

Figure 3 is an illustration of some efficacy aspects of FEV_1 and symptoms that will be evaluated during Visits 2 and 3 to support study objectives. Note that the FEV_1 curves in this figure are hypothetical for illustration only.

Figure 3 Illustration of the Aspects of FEV_1 and Symptoms That may be Evaluated to Support Objectives (During Visits 2 and 3)





Note: FEV_1 curves are hypothetical, for illustration only.
Abbreviations: AUC=area under the curve; FEV_1=Forced Expiratory Volume in the first second; min=minutes.

4.2.1 Participant Input into Design

The ALTA study design was reviewed by patients with asthma according to standard patient engagement processes. Their insights were applied to study participant materials resulting in less technical language and more clearly described observations. The potential impact of these changes could improve understanding of the study, better accommodate participant needs, and improve recruitment and retention.

4.3 Justification for Dose

In the ALTA study, the term "dose" refers to the amount of PT027 or PT007 administered via its device to the participant at Visit 2 and Visit 3. In participants 12 years of age and older, clear dose ordering was apparent across all efficacy endpoints in the Phase III program, with PT027 180/160 µg demonstrating consistently larger treatment effects compared with PT027 180/80 µg. Based on these results, the higher dose, 180/160 µg, has been selected for the ALTA study. In this study, in a cross-over design, participants will be inhaling PT027 (given as 2 actuations of 90 µg albuterol/80 µg budesonide per occasion) or PT007 (2 actuations of 90 µg albuterol per occasion) repeatedly as per schedule, simulating standardized rescue/reliever use according to common asthma action plans ie, 2 puffs of SABA every 20 minutes for an hour (4 total doses) and an additional 2 puffs 1 hour later, if needed (eg, NAEPP https://www.nhlbi.nih.gov/files/docs/public/lung/asthma_actplan.pdf). Based on the results of Part 1 and the recommendations of the IAB, this latter dose will not be employed in Part 2, as with the prior 4 doses, most patients had recovered at least to their -30 min FEV_1 baseline and would not require an additional dose.

4.4 End-of-Study Definition

A participant is considered to have completed the study if he/she has completed Visit 3 of the study, shown in Table 1 the SoA.

The end of the study is defined as the date of the last visit of the last participant in the study or last scheduled procedure shown in the SoA Table 1 for the last participant in the study globally.

5 STUDY POPULATION

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

Adult participants with asthma with as-needed SABA as only asthma treatment and a positive mannitol challenge (performed at Visit 1, to confirm asthma diagnosis and suitability for study) will be eligible for recruitment. The study will aim to include approximately 15% African American and approximately 15% Latin American participants as a reflection of the population with asthma in the United States (AAFA 2020).

5.1 Inclusion Criteria

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

Age

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

Type of Participant and Disease Characteristics

2 Participants who have been diagnosed with asthma > 6 months before Visit 1 by a physician.

3 Participants must have been prescribed and using as-needed SABA as only asthma treatment for at least 4 weeks before screening visit.

4 Participants should have pre-bronchodilator $FEV_1 \geq 1.5$ L and $FEV_1 \geq 60\%$ to < 90% predicted normal (Hankinson et al 1999) at Visit 1.

5 Participants should have a positive response to mannitol challenge performed at Visit 1 (a decrease in FEV_1 by at least 15% [PD15] at ≤ 635 mg) (Hallstrand et al 2018).

6 Participants should return to within 10% of baseline FEV_1 (≥ 90% of baseline FEV_1), within 1 hour after positive mannitol challenge and 4 inhalations of PT007, performed at Visit 1.

7 Participants should be able to adhere to study procedures in the judgment of the Investigator.

Sex

8 Male or female

9 Women of childbearing potential must have a negative urine pregnancy test at each study visit. They must agree to use at least one effective method of birth control throughout the study from enrolment till at least 14 days after last dose of study intervention as defined below:

Effective birth control methods include:

(a) vasectomized partner,

(b) Implanon® or equivalent,

(c) bilateral tubal occlusion,

(d) intrauterine device/levonorgestrel intrauterine system,

(e) Depo-Provera™ or equivalent injections,

(f) oral contraceptive,

(g) Evra Patch™ or equivalent,

(h) Xulane™ or equivalent,

(i) or NuvaRing® or equivalent,

(j) or Double-barrier birth control (ie, a combination of male condom with either cap, diaphragm, or sponge with spermicide).

Sexual abstinence (periodic abstinence eg, calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.

Women not of childbearing potential are defined as women who are either permanently sterilized (hysterectomy, bilateral oophorectomy, or bilateral salpingectomy), or who are post-menopausal. Women will be considered post-menopausal if they have been amenorrhoeic for 12 months prior to the planned date of randomization without an alternative medical cause. The following age-specific requirements apply:

- Women < 50 years old would be considered post-menopausal if they have been amenorrhoeic for 12 months or more following cessation of exogenous hormonal treatment and follicle stimulating hormone levels in the post-menopausal range.
- Women ≥ 50 years old would be considered post-menopausal if they have been amenorrhoeic for 12 months or more following cessation of all exogenous hormonal treatment.

Informed Consent

10 Capable of giving signed informed consent as described in Appendix A which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

11 Provision of signed and dated written Optional Genetic Research Information informed consent prior to collection of samples for optional genetic research that supports Genomic Initiative.

5.2 Exclusion Criteria

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

Medical Conditions

1 As judged by the Investigator, any evidence of clinically significant lung disease other than asthma which in the Investigator's opinion makes it undesirable for the participant to participate in the study.

2 If the participant has had any face-to-face unscheduled or urgent visit for asthma worsening within the last 4 weeks.

3 Any significant disease or disorder, or evidence of drug/substance abuse which in the Investigator's opinion would pose a risk to participant safety, interfere with the conduct of study, have an impact on the study results, or make it undesirable for the participant to participate in the study.

Prior/Concomitant Therapy

4 If participants have used ICS within 1 month prior to enrolment (Visit 1).

5 If they have used immunosuppressive medication (including but not limited to methotrexate, troleandomycin, cyclosporine, azathioprine, systemic corticosteroids including regular treatment with oral corticosteroids or intramuscular long-acting depot corticosteroids, or any experimental anti-inflammatory therapy) within 3 months prior to enrolment (Visit 1) or plan on starting immunosuppressive medications during the study.

6 If they have used allergen-specific immunotherapy (desensitization) within 3 months prior to enrolment (Visit 1).

7 If they have used systemic corticosteroids (including oral and injected) within 3 months prior to enrolment (Visit 1).

Prior/Concurrent Clinical Study Experience

8 If they have received any marketed or investigational biologic within 4 months or 5 half-lives prior to enrolment (whichever is longer) or received any investigational nonbiologic agent within 30 days or 5 half-lives prior to enrolment (whichever is longer).

9 Participants with a known hypersensitivity to beta$_2$-agonists, ICS, mannitol, or any of the excipients of the product.

Diagnostic Assessments

10 Any clinically significant abnormal findings in physical examination, vital signs, ECG (eg, participants with QTcF > 500 ms), hematology, clinical chemistry, or urinalysis, which in the opinion of the Investigator, may put the participant at risk because of his/her

participation in the study, or may influence the results of the study, or the participant's ability to complete entire duration of the study.

Other Exclusions

11 If they are current smokers or participants with smoking history ≥ 10 pack years including the use of vaping products, such as electronic cigarettes, and water pipes. If they are former smokers with a smoking history of <10 pack years, including former vaping or water pipe users, smoking must have stopped for at least 6 months prior to Visit 1 to be eligible. Calculator for pack years here: https://www.smokingpackyears.com/.

12 Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and its affiliates and/or staff at the study site and their immediate relative(s)).

13 Judgment by the Investigator that the participant should not participate in the study if the participant is unlikely to comply with study procedures, restrictions, and requirements.

14 Breast feeding, pregnancy or intention to become pregnant during the course of the study.

15 Previous randomization in the present study.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Participants should avoid eating a large meal for at least 2 hours prior to study visits.

5.3.2 Caffeine, Alcohol, and Tobacco

1 Ingestion of significant (as per Investigator judgment) quantities of coffee, tea, cola drinks, chocolate or other food containing caffeine should be avoided for 12 hours prior to and during the day long visits (ie, on-site meal should not contain these things).

2 Participants should abstain from alcohol for 12 hours prior to and on visit days, including during the visit.

3 Participants should abstain from tobacco for 6 months prior to and the duration of the study.

4 Participants should abstain from smoking cannabis for 6 months prior to and the duration of the study.

5.3.3 Activity

Exercise should be avoided for 12 hours prior to study visits. Participants may participate in light recreational activities during studies (eg, watching television and reading).

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently assigned to randomized study intervention (ie, at Visit 2). Screen failures

do not include participants who failed to meet eligibility criteria at Visit 2 (ie, failure to achieve positive mannitol challenge at Visit 2 or failure to demonstrate FEV_1 of ≥ 60% to < 90% predicted at mannitol challenge 1 of Visit 2); these participants should instead be identified with the participant status of Early Termination. A minimal amount of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened only once after discussion and approval by the designated Study Physician. Participants who screen fail due to negative mannitol challenge at Visit 1 may NOT be rescreened. Rescreening should be documented so that its effect on study results, if any, can be assessed. Participants who are rescreened are required to sign a new ICF and will be assigned a new Participant Identity (ID).

6 STUDY INTERVENTION(S) AND CONCOMITANT MEDICATIONS

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

The terms 'study intervention' and 'investigational medicinal product' are used interchangeably in this document for PT027 and PT007.

6.1 Study Intervention(s) Administered

6.1.1 Investigational Products

PT027 (albuterol/budesonide pMDI) is formulated (Table 6) as both micronized budesonide and micronized albuterol co-suspended with spray-dried porous particles in an hydrofluoroalkane propellant. The co-suspension formulation ensures that participants receive a consistent delivery of the drug from each actuation of the pMDI.

PT007 formulated as a suspension with micronized albuterol crystals co-suspended with spray-dried porous particles in an hydrofluoroalkane propellant.

Table 6 Investigational Products

Arm name	Part 1 and 2	Part 1 and 2
Intervention name	PT027	PT007
Type	Combination product	Combination product
Dose formulation	Pressurized Inhalation Suspension	Pressurized Inhalation Suspension
Unit dose strengths	80/90 µg ie, budesonide/albuterol per actuation	90 µg albuterol per actuation
Dosage levels	Each dose taken as 2 actuations	Each dose taken as 2 actuations
Route of administration	Oral inhalation	Oral inhalation
Use	experimental	active comparator
IMP and NIMP	IMP	IMP
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor

Packaging and labeling	Study Intervention will be provided in an MDI. Each MDI will be labeled as required per country requirement. It will be provided as blinded MDI.	Study Intervention will be provided in an MDI. Each MDI will be labeled as required per country requirement. For Visit 1 it will be provided as an open-label MDI while for Visits 2 and 3 it will be provided as blinded MDI
Current/former names or aliases	Budesonide and Albuterol Sulfate Pressurized Inhalation Suspension	Albuterol Sulfate Pressurized Inhalation Suspension

Abbreviations: IMP=investigational medicinal product; NIMP=non-investigational medicinal product; MDI=metered dose inhaler.

6.2 Preparation, Handling, Storage, and Accountability

1 The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit and storage for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

2 Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

3 The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

4 Further guidance and information for the final disposition of unused study interventions are provided in the “Instructions for Use” manual provided to the site.

6.2.1 Metered Dose Inhaler Handling

Detailed handling instructions will be provided to the site in the form of a “Instruction for Use” manual document, which will cover all aspects of the study with regard to IMP.

The importance of the device priming requirements should be emphasized. Priming of the IMP MDI must occur. Device priming should not be conducted in the same room as spirometry assessments are being conducted and should only be primed when the IMP is to be administered.

All sites will be provided with training devices (MDIs) without IMP for training and assessment on MDI technique, as detailed in the “Instructions for Use” (App) Training devices will be provided to the participants at Visits 1, 2, and 3.

Participants will be assessed each time before using the MDI for treatment as per SoA Table 1.

6.3 Assignment to Study Intervention

All participants will be assigned to open-label (ie, non-randomized) PT007 via IxRS during Visit 1, and assigned to randomized study intervention (PT007 and PT027) at Visit 2. Before the study is initiated, the telephone number and call-in directions for the IxRS and/or the log in information and directions for the IxRS will be provided to each site.

Study intervention will be dispensed at the study visits summarized in the SoA Table 1.

The IxRS will provide to the Investigator(s) or pharmacists the kit identification number to be allocated to the participant at the dispensing visit.

Routines for this will be described in the IxRS user manual that will be provided to each center.

The randomization code should not be broken except in medical emergencies when the appropriate management of the participant requires knowledge of the treatment randomization.

AstraZeneca retains the right to break the code for SAEs that are unexpected and are suspected to be causally related to an IMP and that potentially require expedited reporting to regulatory authorities. Randomization codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual participant have been made and documented. If a participant's intervention assignment is unblinded, the Sponsor must be notified within 24 hours after breaking the blind. The Investigator documents and reports the action to the Study Physician, without revealing the treatment given to participant to the AstraZeneca staff and affiliates.

The IxRS will be programmed with blind-breaking instructions. In case of an emergency, in which the knowledge of the specific blinded study intervention will affect the immediate management of the participant's condition, the Investigator has the sole responsibility for determining if unblinding of a participants' intervention assignment is warranted. Participant safety must always be the first consideration in making such a determination.

6.4 Study Intervention Compliance

When participants are dosed at the site, they will receive study intervention directly from the Investigator or designee, under medical supervision. The date, and time if applicable, of dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Dose Modification

Dose modifications will not be made during this study.

6.6 Continued Access to Study Intervention After the End of the Study

PT027 and PT007 will not be provided upon study completion or early study withdrawal.

6.7 Treatment of Overdose

For this study, any dose of study intervention greater than 12 puffs of PT027 90/80 µg (albuterol / budesonide) or PT007 90 µg (albuterol) within a 24-hour timeframe will be considered an overdose.

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the eCRF and on the Overdose eCRF module.
- An overdose without associated symptoms is only reported on the Overdose eCRF module.

If an overdose on an AstraZeneca study intervention occurs in the course of the study, the Investigator or other site personnel inform appropriate AstraZeneca representatives immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site **within one or 5 calendar days** for overdoses associated with an SAE (see Section 8.4.8) and **within 30 days** for all other overdoses.

6.8 Prior and Concomitant Therapy

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

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

Asthma therapies in the 12 months prior to the screening visit should also be recorded in the EDC.

The Study Physician should be contacted if there are any questions regarding concomitant or prior therapy.

Paracetamol/Acetaminophen, at doses of 2 grams/day, is permitted for use. Other concomitant medication may be considered on a case-by-case basis by the Investigator in consultation with the Study Physician if required.

6.8.1 Prohibited/Restricted Medications

Prohibited concomitant medications include the following, with specified timeframes where needed:

- Participants must not use any asthma medication other than as-needed SABA (as part of their usual treatment between study visits).
- Participants must refrain from using SABAs for at least 12 hours before visits and from using non-IMP SABA therapy during clinic visits unless as determined necessary by the Investigator.
- Leukotriene receptor antagonists (eg, montelukast sodium) must be avoided for 4 days before study visits.
- Antihistamines (eg, cetirizine, fexofenadine, loratadine, diphenydramine, etc.) must be avoided for 72 hours before study visits.
- Non-steroidal anti-inflammatory drugs should be avoided for 48 hours before study visits.
- Other medications which are necessary for the participant's well-being, and which do not affect the participation or results of the study are allowed at the Investigator's discretion. All such medication should be recorded in the participant's eCRF.
- Participants must not begin allergen-specific immunotherapy during the study.

6.8.2 Rescue/Reliever Medicine

Asthma rescue/reliever medication (eg, SABA) will not be provided in this study between study visits. Rescue medication for bronchoconstriction is administered as part of the study protocol. Additional non-investigational SABA may be administered during or at end of study visits per Investigator's discretion due to unresolved lung function impairment or symptoms.

7 DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

Procedures for early discontinuation follow-up should be followed as specified in SoA Table 1 and the participant will be withdrawn from the study.

Note that discontinuation from study intervention is NOT the same thing as a withdrawal from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix A.

If a participant has no baseline assessments which are successfully performed (spirometry) then the participant must be withdrawn from the study, procedures and study intervention should be discontinued.

7.1 Discontinuation of Study Intervention

It may be necessary for a participant to permanently discontinue study intervention. If study intervention is permanently discontinued at Visit 2 or Visit 3, the participant will be asked to attend the follow-up visits (Table 1).

7.2 Participant Withdrawal from the Study

Discontinuation of the participant from the study by the investigator:

- A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.

Voluntary withdrawal from the study by the participant

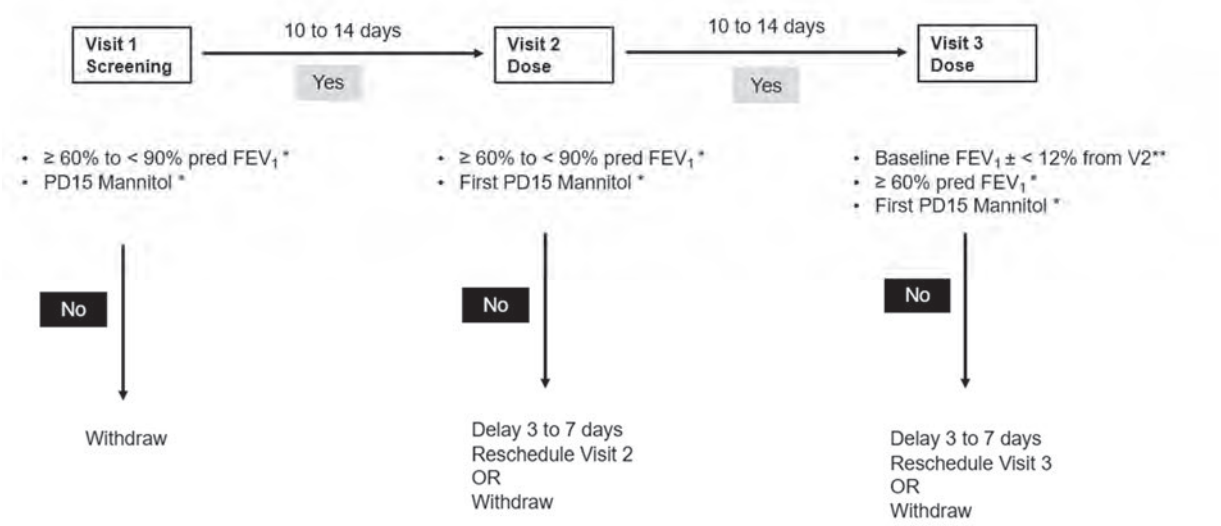
- A participant may withdraw from the study at any time at the participant's own request for any reason (or without providing any reason).
- A participant who considers withdrawing from the study must be informed by the Investigator about the follow-up telephone call after 7 days of Early Discontinuation.
- If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, it should be confirmed if he/she still agrees for existing samples to be used in line with the original consent. If he/she requests withdrawal of consent for use of samples, destruction of any samples taken and not tested should be carried out in line with what was stated in the informed consent and local regulation. The Investigator must document the decision on use of existing samples in the site study records and inform the Global Study Team.

- A participant must be discontinued from the study under the following conditions:
  - Has had any face-to-face unscheduled or urgent visit for asthma worsening
  - Use of systemic or inhaled corticosteroids
  - Pregnancy during study
  - Adverse events at the discretion of the treating physician for safety reason as judged by the study team
  - Positive COVID-19 assessment

7.2.1 FEV_1 Baseline, Mannitol Challenge Positive Reactivity, and FEV_1 Stability Criteria (Visit 3 only)

The baseline FEV_1 at Visit 3 needs to be < 12% above and < 12% below the absolute FEV_1 (mL) achieved at baseline in Visit 2. Participants who do not meet the FEV_1 requirements (at Visits 2 or 3), the mannitol challenge positive reactivity criteria (at Visits 2 or 3), or the FEV_1 stability criteria (at Visit 3) must be rescheduled as soon as is practical but within the protocol-specified wash-out window (10 to 14 days). Participants who fail to meet these criteria after 2 attempts (original visit and 1 rescheduled/repeat visit) for a treatment visit will be withdrawn from the study. See Figure 4 for schematic representation of the study stability criteria check.

Figure 4 High-level Schema of Study Stability Criteria FEV_1 and PD15 Mannitol



* Visit 1 and Visit 2 requirements are eligibility criteria. Participants need to achieve both to proceed with the visit. Please note, for Visit 2, the requirement to demonstrate FEV_1 of ≥ 60% to < 90% predicted FEV_1 applies only to Mannitol Challenge 1.

** The baseline FEV_1 for Visit 3 needs to be < 12% above and < 12% below the absolute baseline FEV_1 (in mL) achieved at Visit 2. Visit 3 baseline criteria are required to ensure stability between Visit 2 and Visit 3.

Note: All criteria are required to be met in order to proceed with each visit.

Abbreviations: FEV_1=Forced Expiratory Volume in the first second; PD15=provoking dose (PD) that results in a ≥ 15% drop in FEV_1.

7.3 Lost to Follow-up

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

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local

equivalent methods). These contact attempts should be documented in the participant's medical record.

- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarized in the SoA Table 1. Protocol waivers or exemptions are not allowed. Visit 2 should be performed 10-14 days after Visit 1. Time window may be extended to 21 days after Visit 1 after confirmation with the Study Physician. Visit 3 should be performed 10-14 days after Visit 2. Time window may be extended to 21 days after Visit 2 after confirmation with the Study Physician.

Immediate safety concerns should be discussed with the Study Physician immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA Table 1, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Before the first participant is entered into the study, a designated representative will review and discuss the requirements of the CSP and related documents with the investigational staff and also train them in any study specific procedures.

The Investigator(s) will ensure that appropriate training relevant to the study is given to all of the site staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Investigator(s) will maintain a record of all individuals involved in the study (medical, nursing, and other staff).

8.1 Administrative and General/Baseline Procedures

Baseline procedures are outlined in the SoA include medical history, (including asthma history), surgical history, physical exam including height and weight, demographics, severe asthma exacerbation history (within last 12 months), estimated date/time of last SABA administration, collection of blood for safety laboratory testing, drug/substance abuse and smoking history, and FeNO.

8.2 Efficacy Assessments

Efficacy measures have been selected to demonstrate the therapeutic benefit of PT027 compared with PT007.

- Early effect (first 60 minutes post-mannitol challenge 1) ("PT027 relieves symptoms and airway obstruction as well as albuterol", ie, the effect of PT027 is comparable to PT007 during the 60-minute acute phase), "A" in Figure 3 assessed by:
  - Change from mannitol baseline in FEV_1 AUC(0-60 min) post-mannitol challenge 1
  - Change from mannitol baseline in Borg dyspnea scale to 60 minutes post-mannitol challenge 1
- Onset of effect ("PT027 works as fast as albuterol in onset of action", ie, the onset of effect of PT027 is comparable to PT007), "B" in Figure 3 assessed by:
  - Change from mannitol baseline in FEV_1 AUC(0-15 min) post-mannitol challenge 1
  - Time to first "yes" response for POE post-mannitol challenge 1 post-dose
- Sustained effect ("PT027 maintains effect of reversal of acute airway obstruction as long as albuterol", ie, the effect of PT027 is comparable to PT007 measured just before the second mannitol challenge at 480 minutes, "C" in Figure 3 assessed by:
  - Change from mannitol baseline in FEV_1 at 480 minutes post-mannitol challenge 1
- Onset of effect ("PT027 works as fast as albuterol in onset of action", ie, the onset of effect of PT027 is comparable to PT007 following a second trigger of obstruction) in effects on lung function and symptom relief after a second challenge) "D" in Figure 3 assessed by:
  - Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2 (within 5%), pre-final dose of IMP
- Effect upon repeated trigger ("PT027 provides a protective effect upon repeated triggers comparable to albuterol", ie, the effect of PT027 is comparable to PT007 upon a second mannitol challenge (with same dose as challenge 1) in terms of magnitude of FEV_1 fall after a second challenge), "E" in Figure 3 assessed by:
  - Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever
  - Peak fall from time 480 min FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever dose
  - Percentage fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever

8.2.1 Lung Function Test: Spirometry

Lung function will be measured by spirometry at the study site using equipment provided by Clario. Spirometry will be performed according to ATS/ERS guidelines (Miller et al 2005). Spirometry calibration will be detailed in a separate spirometry procedures manual.

The vendor providing central spirometry services will be responsible for assuring that the spirometer used by each site meets ATS/ERS recommendations, and that the study site personnel who will be performing the testing are properly certified.

A site study personnel would oversee the spirometry procedure throughout the study to reach optimal performance and to enhance reproducibility. A centralized spirometry data collection system incorporating a quality control program will be used to reduce FEV_1 variability between and within participants and between study sites.

Spirometry should be performed as specified in Table 1, Table 2, and Table 3. The measurements are to be made with the participant seated in an upright position. During the breathing maneuvers, the thorax should be able to move freely; hence tight clothing should be loosened.

The participant should rest for at least 15 minutes prior to the initial test. Multiple maneuvers are necessary at specific timepoints.

For the spirometry assessment occurring immediately prior to the mannitol challenge, corresponding to timepoints -30 and +480, FEV_1 and forced vital capacity repeatability within 150 mL will be required according to ATS/ERS guidelines. A maximum of 8 maneuvers may be performed, until 3 technically adequate maneuvers are achieved (timepoints marked with footnote “e” in Table 3).

Any spirometry during the mannitol challenge requires only 1 technically adequate maneuver (which also includes spirometry at timepoint 0 and timepoint 490 [last spirometry in mannitol challenge 1 and mannitol challenge 2, respectively]).

For each post-mannitol challenge spirometry, at timepoints corresponding to +3,+5, +10, +15, and + 493, +495, +500, and +515 in Table 3, a maximum of 8 maneuvers may be performed until 1 technically adequate maneuver is achieved (timepoints marked with footnote “f” in Table 3).

For all other spirometry assessments, a maximum of 8 maneuvers may be performed until 2 technically adequate maneuvers are achieved (timepoints marked with footnote “g” in Table 3).

In case only 1 acceptable maneuver is achieved, that value will be used. In case no acceptable maneuver is achieved or 2 technically adequate maneuvers are achieved, the highest FEV_1 value obtained will be used.

For spirometry purposes, and based on current available research, participants will be classified in either of the following population groups:

- African-American: Hispanic and Black, Black
- Mexican-American: Mexican, Latin (Central American and Brazilian)
- Caucasian: White and/or Hispanic, Caribbean (Cuban, Dominican, Puerto Rican, and South American [except Brazilian]), Asians, and Native Americans

8.2.2 Asthma Impairment and Risk Questionnaire (AIRQ®)

The Asthma Impairment and Risk Questionnaire is a PRO control tool intended to identify participants 12 years and older whose health may be at risk because of uncontrolled asthma (Murphy et al 2020; Beuther et al 2021). The 12-month recall version of the AIRQ® will be used in this study. It has 10 questions that ask about respiratory symptoms, activity limitation, sleep, rescue/reliever medication use, social activities, exercise, difficulty controlling asthma, and exacerbations. All items have a yes/no response option, and the tool is scored by summing the total number of 'yes' responses. This sum score is used to assess level of asthma control where: 0-1 is well controlled, 2-4 is not well controlled, and 5-10 is very poorly controlled. Thus, a higher score indicates worse asthma control status.

Participants will complete the AIRQ® at the study center electronically on a handheld device, prior to the spirometry test and IMP administration at Visit 1.

8.2.3 Severity per National Asthma Education and Prevention Program

To assess asthma severity, each participant will complete the NAEPP severity assessment items on the handheld device. These items will encompass questions relating to frequency of daytime symptoms, nighttime awakenings, inference with normal activities, and use of rescue/reliever bronchodilators. (NAEPP 2007). The NAEPP severity items will be completed at the site, at Visit 1. Each participant will select the most appropriate option that best reflects their current condition. NOTE there are only 4 unique items to be captured from the participants, as the pre-bronchodilator lung function categorization will be the screening baseline value and the exacerbation history will be provided by the site Investigator.

8.2.4 Modified Borg Scale

Participants will be asked to report their perceived breathlessness on the modified Borg scale electronically using a handheld device, before spirometry as per SoA. This is a 0-10 scale that asks participants to rate the difficulty of breathing, where 0 = breathing is causing no difficulty at all and 10 = where your breathing difficulty is maximal. How much difficulty breathing is causing the participant at a given point is assessed.

8.2.5 Mannitol Challenge Test

The mannitol challenge test is an indirect bronchial provocation test widely used in clinical trials and in clinical practice for diagnosis of asthma with regulatory approval in the United States, Europe, and Australia (AAFA 2020).

The test is conducted according to a standardized protocol and test kit using a capsule-based dry powdered inhaler device that is used to deliver increasing doses of mannitol to the lower airways according to the instructions in the site manual.

Note that in clinical practice the test is stopped when there is a 15% decrease from baseline FEV_1 or a 10% decrease in FEV_1 between two consecutive doses. For the purpose of the ALTA study, the 10% decrease in FEV_1 between two consecutive doses is NOT applied.

8.2.6 Perceived Onset of Effect

Participants will be asked a single POE question "Can you feel your study medication working?" repeatedly (Kaiser et al 2018). The question will be repeated at timepoints as specified in the SoA Table 1 and Table 3 and will be repeated after each intervention administration only if the prior response has been "No" (ie, until the participant confirms "yes").

8.3 Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA Table 1, Table 2, and Table 3.

8.3.1 Physical Examinations

A complete physical examination will be performed at Visit 1 including the following assessments; general appearance, respiratory, cardiovascular, abdomen, skin, head and neck (including ears, eyes, nose, and throat), lymph nodes, thyroid, muscular-skeletal (including spine and extremities) and neurological systems.

A brief physical examination will be conducted at Visit 2 and 3, including at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

Physical examination will be performed at time-points as specified in the SoA Table 1.

Any evidence of clinically significant abnormalities identified during the physical examination at screening and during the study will be recorded in the eCRF. Clinically significant abnormalities identified during physical examinations during the study should also be reported per Section 8.4.4.

The participant's height and weight will be measured, and Body Mass Index will be calculated at Visit 1 (Screening).

8.3.2 Vital Signs

Vital signs (pulse, blood pressure, pulse oximetry, and respiratory rate) will be performed at timepoints specified in the SoA (Table 1). Measurements should be taken in the sitting position after at least 10 minutes of rest.

Any clinically significant abnormalities will be recorded in the eCRF and reported per Section 8.4.4.

8.3.3 Safety Spirometry

Pre-challenge spirometry will be performed at each treatment visit and will capture FEV_1 forced vital capacity (FVC), and FEV_1/FVC. Each Mannitol challenge will start with establishing a pre-challenge FEV_1 value. The FEV_1 at Visits 1 and 2 must be ≥ 60% to < 90% of the predicted value adjusting for age, height, gender, and race, as per the National Health and Nutrition Examination Survey III reference values.

8.3.4 Electrocardiograms

An ECG will be performed locally by sites at all visits as specified in the SoA Table 1.

A 12-lead ECG will be taken after the participant has been resting in semi-supine position for at least 10 minutes.

A standardized ECG machine should be used, and the participant should be examined using the same machine throughout the study.

ECGs will be interpreted for the rate, rhythm, and the presence of any conduction abnormalities (including QT interval [QTcB and QTcF] and QRS complex abnormalities).

The Investigator or authorized delegate will be responsible for the overall interpretation and determination of clinical significance of any potential ECG findings. The Investigator's interpretation should be noted on the printout and recorded in the eCRF. A copy of the ECG will be produced and kept in case of further need for re-evaluation.

Any clinically significant abnormalities will be reported per Section 8.4.4.

8.3.5 Clinical Safety Laboratory Assessments

Blood and urine samples for determination of clinical chemistry, hematology, and urinalysis will be taken at the visits indicated in the SoA Table 1, Table 2, and Table 3.

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 300 mL, or approximately 10 oz. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Additional safety samples may be collected if clinically indicated at the discretion of the Investigator. The date, time of collection and results (to be evaluated by local laboratory reference ranges) will be recorded on the appropriate eCRF.

The clinical chemistry and hematology will be performed at a local laboratory at or near to the Investigator site. Urinalysis will be performed at the Investigator site. Sample tubes and sample sizes may vary depending on laboratory method used and routine practice at the site.

The following laboratory variables will be measured.

Table 7 Laboratory Safety Variables

Hematology/Hemostasis (whole blood)	Clinical Chemistry (serum or plasma)
B-Hemoglobin (Hb)	S/P-Creatinine
B-Leukocyte count	S/P-Bilirubin, total
B-Leukocyte differential count (absolute count)	S/P-Alkaline phosphatase (ALP)
B-Platelet count	S/P-Aspartate transaminase (AST)
	S/P-Alanine transaminase (ALT)
Urinalysis (dipstick)	S/P-Albumin
U-Hb/Erythrocytes/Blood	S/P-Potassium
U-Protein/Albumin	S/P-Calcium, total
U-Glucose	S/P-Sodium
	S/P-Creatine kinase (CK)
	S/P-Glucose

Note. In case a participant shows an AST **or** ALT ≥ 3 × ULN together with total bilirubin ≥ 2 × ULN please refer to Appendix E. Actions required in cases of increases in liver biochemistry and evaluation of Hy's Law, for further instructions.

8.3.6 Fractional Exhaled Nitric Oxide Measurement

Fractional Exhaled Nitric Oxide will be measured before spirometry at the visits specified in the SoA (Table 1) and the timepoints specified in Table 3.

8.3.7 Blood Eosinophil Measurement

Blood samples for estimation of blood eosinophil will be collected at the visits specified in the SoA (Table 1) and the timepoints specified in Table 3.

8.4 Adverse Events, Serious Adverse Events, and Other Safety Reporting

The Principal Investigator (PI) is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

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

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

The Investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE.

8.4.1 Time Period and Frequency for Collecting AE and SAE Information

Adverse events will be collected from the time of signing of the ICF at Visit 1 throughout the treatment period and including the telephone follow-up last contact.

Serious adverse events will be recorded from the time of signing of the ICF.

If the Investigator becomes aware of a SAE with a suspected causal relationship to the IMP that occurs after the end of the clinical study in a participant treated by him or her, the Investigator shall, without undue delay, report the SAE to the Sponsor.

8.4.2 Follow-up of AEs and SAEs

Any AEs that are unresolved at the participant's last AE assessment in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca retains the right to request additional information for any participant with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Adverse Event Variables

'The following variables will be collected for each AE;

- AE (verbatim)
- The date and time when the AE started and stopped
- Maximum intensity
- Whether the AE is serious or not
- Investigator causality rating against the IMP(s) (yes or no)
- Action taken with regard to IMP(s)
- AE caused participant's withdrawal from study (yes or no)
- Outcome

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for SAE
- Date Investigator became aware of SAE
- AE is serious due to
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Causality assessment to other medication

8.4.3 Causality Collection

The Investigator should assess causal relationship between IMP and/or Investigational Medical devices and each AE and/or Incident, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the IMP?'

For SAEs and Serious Incidents, causal relationship should also be assessed for other medication and study procedures and/or medical devices. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix B.

8.4.4 Adverse Events Based on Examinations and Tests

The results from the CSP mandated laboratory tests and vital signs will be summarized in the clinical study report.

Deterioration as compared to baseline in protocol mandated laboratory values, vital signs, and spirometry (FEV_1) measurements should therefore only be reported as AEs if they fulfill any of the SAE criteria, are the reason for discontinuation of treatment with the IMP or are considered to be clinically relevant as judged by the Investigator (which may include but not limited to consideration as to whether treatment or non-planned visits were required or other action was taken with the study intervention, eg, dose adjustment or drug interruption).

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign

will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (eg, anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE unless unequivocally related to the disease under study.

8.4.5 Adverse Events Based on Signs and Symptoms

All AEs spontaneously reported by the participant or reported in response to the open question from the study site staff: 'Have you had any health problems since the previous visit/you were last asked?' or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

8.4.6 Hy's Law

Cases where a participant shows elevations in liver biochemistry may require further evaluation and occurrences of AST or ALT $\geq 3 \times$ ULN together with total bilirubin $\geq 2 \times$ ULN may need to be reported as SAEs. Please refer to Appendix E for further instruction on cases of increases in liver biochemistry and evaluation of Hy's Law.

8.4.7 Disease Under Study

Symptoms of DUS are those which might be expected to occur as a direct result of usual, intermittent variations in disease status or response to environmental stimuli (eg, allergens or weather) or routine pulmonary procedures (eg, spirometry). Events which are unequivocally due to DUS should not be reported as an AE during the study unless they meet SAE criteria or lead to discontinuation of the IMP or is a sign or symptom that is new to the participant or inconsistent with the participant's pre-existing asthma history as judged by the Investigator.

Asthma exacerbation should be recorded as an AE only if it fulfills any of the criteria for an SAE (see Section 8.4.8) or if the AE leads to IMP discontinuation.

8.4.8 Reporting of Serious Adverse Events

All SAEs have to be reported, whether or not considered causally related to the IMP, or to the study procedure(s). All SAEs will be recorded in the eCRF.

If any SAE occurs in the course of the study, Investigators or other site personnel will inform the appropriate AstraZeneca representatives within one day ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative will work with the Investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within one calendar day** of initial receipt for fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up will be undertaken immediately. Investigators or other site personnel will inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day, ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

Once the Investigators or other site personnel indicate an AE is serious in the EDC system, an automated email alert is sent to the designated AstraZeneca representative.

If the EDC system is not available, then the Investigator or other study site staff reports a SAE to the appropriate AstraZeneca representative by telephone.

The AstraZeneca representative will advise the Investigator/study site staff how to proceed.

For further guidance on the definition of a SAE, see Appendix B of the CSP.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug for the active comparator product (including any AstraZeneca comparator.).

8.4.9 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to AstraZeneca except for:

- Pregnancies discovered before study participant has received any study intervention.
- Pregnancies in the partner of male participants.

8.4.9.1 Maternal exposure

If a participant becomes pregnant during the study, the participant will be discontinued.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IMP under study may have interfered with the effectiveness of a contraceptive medication. Congenital anomalies/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal

birth or congenital anomaly/birth defect) should be followed up and documented even if the participant was discontinued from the study.

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel informs the appropriate AstraZeneca representatives within 1 day, ie, immediately but no later than 24 hours of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 or 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

The PREGREP module in the eCRF is used to report the pregnancy and the paper-based PREGOUT module is used to report the outcome of the pregnancy.

8.4.10 Medication Error, Drug Abuse, and Drug Misuse

8.4.10.1 Timelines

If an event of medication error, drug abuse, **or** drug misuse occurs in the course of the study, then the Investigator or other site personnel informs the appropriate AstraZeneca representatives within **1 calendar day**, ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is completed within **1** (Initial Fatal/Life-Threatening or follow-up Fatal/Life-Threatening) **or 5** (other serious initial and follow-up) **calendar days** if there is an SAE associated with the event of medication error, drug abuse, or misuse (see Section 8.4.8) and **within 30 days** for all other events.

8.4.10.2 Medication Error

For the purposes of this clinical study a medication error is an **unintended** failure or mistake in the treatment process for an IMP or AstraZeneca NIMP that either causes harm to the participant or has the potential to cause harm to the participant.

The full definition and examples of medication error can be found in Appendix B 4.

8.4.10.3 Drug Abuse

Drug abuse is the persistent or sporadic **intentional**, non-therapeutic excessive use of IMP or AstraZeneca NIMP for a perceived reward or desired non-therapeutic effect.

The full definition and examples of drug abuse can be found in Appendix B 4.

8.4.10.4 Drug Misuse

Drug misuse is the **intentional** and inappropriate use (by a study participant) of IMP or AstraZeneca NIMP for medicinal purposes outside of the authorized product information, or for unauthorized IMPs or AstraZeneca NIMPs, outside the intended use as specified in the protocol and includes deliberate administration of the product by the wrong route.

The full definition and examples of drug misuse can be found in Appendix B 4.

8.4.11 Reporting of Overdose

Refer to Section 6.7 for the definition and treatment of overdose.

- An overdose with associated AEs is recorded as the AE diagnoses/symptoms on the relevant AE modules in the eCRF and on the Overdose eCRF module.
- An overdose without associated symptoms is only reported on the Overdose eCRF module.

If an overdose on an IMP or AstraZeneca NIMP occurs in the course of the study, the investigator or other site personnel inform appropriate AstraZeneca representatives immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site **within 1 or 5 calendar days** for overdoses associated with an SAE (see Section 8.4.8) and **within 30 days** for all other overdoses.

8.4.12 Medical Device Deficiencies

An investigational device as a constituent part of a combination product is included in this study. Any AEs and/or any SAEs reported in this study will undergo causality assessment by the Investigator for causal relationship with the IMP, inclusive of both the drug and device constituents. If a causal relationship with the device is suspected of an AE and/or an SAE, it will be documented within the AE and/or SAE report. All SAE reports which meet the definition of an IND safety report will be submitted to health authorities according to local regulations.

8.5 Pharmacokinetics

Pharmacokinetic parameters will not be evaluated in this study.

8.6 Immunogenicity Assessments

Immunogenicity assessments will not be conducted in this study.

8.7 Pharmacodynamics

Pharmacodynamic parameters will not be evaluated in this study.

8.8 Optional Genomics Initiative

Collection of optional samples for Genomics Initiative research is also part of this study as specified in the SoA Table 1 and is subject to agreement in the ICF addendum.

Blood sample for DNA isolation will be collected from participants who have consented to participate in the genetic analysis component of the study at Visit 2 post randomization. Participation is optional. Participants who do not wish to participate in the genetic research may still participate in the study.

See Appendix D for information regarding the Genomics Initiative genetic sample. Details on processes for collection and shipment and destruction of these samples can be found either in the appendices or in the Laboratory Manual.

For storage and destruction of genetic samples see Appendix D.

8.9 Biomarkers

Biomarker data will be pooled for Part 1 and Part 2, and will be reported separately from the CSR.

8.9.1 Mandatory Biomarker Sample Collection

By consenting to participate in the study the participant consents to the mandatory research components of the study.

Blood, nasal scrape, and urine samples for biomarker research are required and will be collected from all participants in this study as specified in the SoA Table 1.

8.9.1.1 Urinary Leukotrienes including LTE4

Urine samples will be collected to determine the levels of urinary leukotrienes including LTE4, prior to and following mannitol challenges and in response to PT027 and PT007 administration. A total of 6 urine samples will be collected, 3 samples at Visit 2 and 3 samples at Visit 3. For timepoints of sample collection, see Table 3.

8.9.1.2 Serum cAMP, cAMP Mobilizing Analytes, and Additional Exploratory Blood Inflammatory Biomarkers

Blood samples will be collected to study the levels of cAMP, cAMP mobilizing analytes, and additional blood inflammatory biomarkers in serum in response to mannitol challenges and PT027 and PT007 administration. A total of 25 blood samples will be collected, 3 at Visit 1, 11 at Visit 2 and 11 at Visit 3. For timepoints of sample collection, see Table 3.

8.9.1.3 Beta Receptor and Corticosteroid Receptor Expression Genetics

Blood and nasal scrape samples may be collected to study the beta receptor and corticosteroid receptor expression genetics in response to PT027 and PT007 administration. A total of 8 blood samples and nasal scrape samples will be collected, 2 at Visit 1, 3 at Visit 2, and 3 at Visit 3. For timepoints of sample collection, see Table 3.

AstraZeneca or designated organizations will investigate the whole blood and nasal scrape sample for beta receptor and corticosteroid receptor expression genetics. This analysis is required to study the genetic variation at the receptor level and understand the inter-individual clinical differences in response to PT027 and PT007.

8.9.2 Optional Biomarker Sample Collection

No optional biomarker samples will be collected in this study.

8.9.3 Other Study-Related Biomarker Research

No other study-related biomarker research is proposed in this study.

For storage, reuse and destruction of biomarker samples see Appendix C.

8.10 Study Participant Feedback Questionnaire

Study participant feedback questionnaires will not be included in this study.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

For this study there are several hypotheses tested depending on the study objectives analyzed. The following Table 8 describes the hypothesis that will be tested for each of the study objective:

Table 8 Hypotheses Tested

Objectives	Hypothesis
Primary	
• To assess the efficacy of repeated dosing of PT027 relative to PT007, on post-dose lung function, when used by participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• To establish non-inferiority of the acute bronchodilatory effect of PT027 compared with PT007. Null 1: The mean change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1 for PT027 is inferior to PT007 by more than −150 mL. • To establish superiority of the acute bronchodilatory effect between PT027 and PT007, only if non-inferiority has been established for the primary endpoint and for each of the four secondary endpoints after rejecting their corresponding Null 1 hypotheses. • Change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1. Null 2: The difference in mean change from mannitol baseline in FEV_1 AUC(0-60 min) post-mannitol challenge 1 between PT027 and PT007 is equal to 0.
Secondary	
• To establish the efficacy of PT027 compared with PT007 in reversal of acute airway obstruction, when used by participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• To establish non-inferiority of PT027 compared with PT007 in reversal of acute airway obstruction. Null 1: The change from mannitol baseline in FEV_1 AUC(0-15 min) post-mannitol challenge 1, for PT027 is inferior to PT007 by more than -150 mL. • To establish the superiority of PT027 compared with PT007 in reversal of acute airway obstruction, only if non-inferiority has been established for the primary endpoint and for

Objectives	Hypothesis
	each of the four secondary endpoints after rejecting their corresponding Null 1 hypotheses. Null 2: The difference in mean change from mannitol baseline FEV_1 AUC(0-15 min) post-mannitol challenge 1 between PT027 and PT007 is equal to 0. • Change from mannitol baseline in FEV_1 AUC(0-15 min) post-mannitol challenge 1.
• To establish the efficacy of PT027 compared with PT007 in the sustainability of effect of reversal of acute airway obstruction post-mannitol challenge 1 in participants with asthma on SABA as-needed treatment only who are experiencing acute airway obstruction.	• To establish non-inferiority of the sustainability of effect of reversal of acute airway obstruction post-mannitol challenge 1 of PT027 compared with PT007. Null 1: The mean change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1 for PT027 is inferior to PT007, by more than −150mL. • To establish superiority of the sustainability of effect of reversal of acute airway obstruction post-mannitol challenge 1 of PT027 compared with PT007, only if non-inferiority has been established for the primary endpoint and for each of the four secondary endpoints after rejecting their corresponding Null 1 hypotheses. Null 2: The difference in mean change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1 between PT027 and PT007 is equal to 0. • Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1.
• To establish the efficacy of a single dose of PT027 compared with PT007 on post-dose speed of recovery of lung function following a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• To establish non-inferiority of the post-dose speed of recovery of lung function after repeated challenge, of PT027 compared with PT007. Null 1: The mean paired differences in time to return to baseline (-30 min) FEV_1 for PT027 is inferior to PT007 by more than 3.5 min. • To establish superiority of the acute bronchodilatory effect after repeated challenge, of PT027 compared to PT007, only if non-inferiority has been established for the primary endpoint and for each of the four secondary endpoints after rejecting their corresponding Null 1 hypotheses.

Objectives	Hypothesis
	Null 2: The mean paired difference in time to return to baseline FEV_1 post-mannitol challenge 2 between PT027 and PT007 is equal to 0. • Time to return to baseline (-30 min) FEV_1 (within 5%) post-mannitol challenge 2, pre-final dose of rescue/reliever.
• To establish the protective efficacy of prior repetitive doses of PT027 compared with PT007 on lung function fall in response to a recurring trigger of acute airway obstruction in participants with asthma on SABA as-needed treatment only.	• To establish non-inferiority of the protective effect of PT027 compared with PT007. Null 1: The mean fall in FEV_1 from baseline (-30 min) to post-mannitol challenge 2 for PT027 is inferior to PT007 by more than 150 mL. • To establish superiority of the protective effect of PT027 compared to PT007, only if inferiority has been established for the primary endpoint and for each of the four secondary endpoints after rejecting their corresponding Null 1 hypotheses. Null 2: The difference in mean peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2 between PT027 and PT007 is equal to 0. • Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever. • To establish superiority of the protective effect of PT027 compared to PT007, only if inferiority has been established for the primary endpoint and for each of the four secondary endpoints after rejecting their corresponding Null 1 hypotheses. Null 1: The difference in mean peak fall from time 480 min FEV_1 to post-mannitol challenge 2 between PT027 and PT007 is equal to 0. • Peak fall from time 480 min FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever.

Objectives	Hypothesis
Safety	
• To assess the safety and tolerability of repeated dosing of PT027 as compared to PT007 in participants with asthma on SABA as-needed treatment only.	• No formal hypothesis testing will be performed.

Abbreviations: AUC=area under the curve; FEV_1=Forced Expiratory Volume in the first second; SABA=Short-Acting Beta2-Agonists.

9.2 Sample Size Determination

For Part 1 (Pilot Study):

Part 1 will be comprised of approximately 16 randomized participants to the study to ensure that 14 participants complete the study. This is approximately 25% of the presently calculated total number of participants required to be randomized into the main study (Part 2).

Due to the uncertainty of the assumed variability and treatment effect size from the supporting lung function data, which mostly is not from a mannitol challenge setting, a sample size re-estimation was performed on completion of Part 1 of the study. Sample size determinations for the secondary endpoints in Part 2 of the study were re-calculated by the treatment effect sizes, variability estimates, and study operational changes for Part 2 that were obtained from the Part 1 data.

For Part 2:

Sample size calculations based on the secondary endpoint time to return to baseline (-30 min) FEV_1 (within 5%) post-mannitol challenge 2, since this endpoint has higher variability than the primary endpoint and will be tested using a non-parametric test. A sample size of 50 participants (25 participants per treatment sequence) provides this study with an ~80% power to detect non-inferiority using a 1-sided paired Wilcoxon signed-rank test at a 2.5% significance level assuming that the actual distribution of paired differences is logistic when the non-inferiority margin is 3.5 minutes, the actual mean of paired differences is 0.0, and the standard deviation of paired differences is 9.0. These calculations are supported by data from a mild to moderate asthma population in study PT007001 (ANTORA), which was a randomized, double-blind, single dose, dose-ranging, placebo-controlled, 5-period, 5-treatment, cross-over study that demonstrated the non-inferiority of lung function efficacy of PT007 compared to Proventil®. In this study, the mean (SD) of the paired differences in time to 15% improvement from baseline FEV_1 (a similar magnitude of recovery that will be expected following the second mannitol challenge), between Albuterol Sulfate MDI 180 µg and Proventil 180 µg was –3.25 minutes (9.48). Allowing for a drop-out rate of 15% to achieve the total sample size required is 60 participants (randomized).

Sample Size Re-estimation

Sample size rationale is based on the secondary endpoint Fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, predose (pre-final dose of rescue/reliever). A sample size of 74 participants (37 participants per treatment sequence) provides Part 2 with an ~80% power to show superiority of PT027 over PT007, assuming a difference in means of -75 mL, and assuming the standard deviation of differences is 228 mL using a two group t-test (Crossover ANOVA) with a 0.025 one-sided significance level. This calculation is based on assumptions obtained from Part 1 data, and an assumption that protocol modifications for Part 2 will increase the magnitude of the treatment effect. Allowing for a drop-out rate of 15% to achieve the total sample size required is 88 participants (randomized).

The sample size of 74 participants (37 participants per treatment sequence) will provide the following nominal probabilities to demonstrate the efficacy of PT027 compared to PT007 in the primary endpoint and secondary endpoints (Table 9).

Table 9 Nominal power for endpoints with a sample size of 74 participants

Endpoint	Assumptions	Nominal power (%)
Primary		
Change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1	Non-inferiority test of mean difference between PT027 and PT007, using a one-sided 2.5% equal variance t-test, with a non-inferiority margin of -150 mL, and assuming the true difference between means equals 0 and within participant standard deviation is 96 mL (Part 1 data).	~99%
Secondary		
Change from mannitol baseline FEV_1 AUC(0-15 min) post-mannitol challenge 1	Non-inferiority test of mean difference between PT027 and PT007, using a one-sided 2.5% equal variance t-test, with a non-inferiority margin of -150 mL, and assuming the true difference between means equals 0 and within participant standard deviation is 117 mL (Part 1 data).	~99%
Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1.	Non-inferiority test of mean difference between PT027 and PT007, using a one-sided 2.5% equal variance t-test, with a non-inferiority margin of -150 mL, assuming the true difference between means equals 0 and within participant standard deviation is 130 mL (Part 1 data).	~99%
Time to return to baseline (-30 min) FEV1 post-mannitol challenge 2, pre-final dose of rescue/reliever.	Non-inferiority test of mean of paired differences between PT027 and PT007, using a one-sided paired Wilcoxon signed-rank test at a 2.5% significance level, with a non-inferiority margin of 3.5 minutes, assuming that the actual mean of paired differences is 0.0 minutes, and the standard deviation of paired differences is 3.0 minutes (Part 1 data).	~99%
Peak fall from baseline (-30-min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever.	Non-inferiority test of mean difference between PT027 and PT007, using a one-sided 2.5% equal variance t-test, with a non-inferiority margin of 150 mL, assuming the true difference between means equals 0 and within participant standard deviation is 228 mL (Part 1 data).	~99%

Abbreviations: AUC, Area under the curve; FEV_1, Forced expiratory volume in the first second.

The non-inferiority limit of -150 mL for change from baseline in FEV_1 AUC(0-60 min) is based on supporting evidence in the literature that the minimum participant perceived improvement in FEV_1 is 230 mL (Santanello et al 1999) and results from the PT007 study PT007002 (ASPEN), that showed that the change from baseline in FEV_1 (mL) least square mean estimate and 90% CI for the comparison between Albuterol Sulfate MDI versus Proventil after 60 minutes cumulative dosing up to 720 µg was –31 (−80, 19) and 120 minutes of cumulative dosing up to 1440 µg, was −84 (−148, −21) and were compared with equivalence limits of ± 200 mL. The non-inferiority limit of −150 mL will be applied for all

change from baseline FEV_1 endpoints, ie, FEV_1 AUC(0-t) normalized by time and those at individual timepoints, with the exception of fall in FEV_1 from baseline (-30 min) to post-mannitol challenge 2, for which the non-inferiority limit will be 150 mL.

9.3 Populations for Analyses

The analyses populations are defined in Table 10:

Table 10 Populations for Analysis

Population/Analysis Set	Description
Enrolled analysis set (ES)	All participants who sign the ICF.
Modified randomized analysis set (mRS)	All participants who are randomized to any of the 2 treatment sequences, A/B or B/A and receive any amount of randomized study treatment. Treatments A and B are defined as: Treatment A = PT027 180/160 µg pMDI Treatment B = PT007 180 µg pMDI.
Per protocol analysis set (PP)	All participants who received all doses of study treatment following mannitol challenge 1, have baseline and post-treatment study evaluation (spirometry tests) and do not have any important protocol deviations.
Safety analysis set (SAF)	All participants randomly assigned to any of the 2 study treatment sequences and who take at least 1 dose of study treatment. Participants will be classified on the basis of treatment they actually received within each treatment period.

Abbreviations: AE=adverse event(s); pMDI=pressurized metered dose inhaler.

9.4 Statistical Analyses

The statistical analysis plan for each part of the study will be finalized prior to clinical database lock, and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and secondary endpoints.

9.4.1 General Considerations

All continuous variables will be summarized using n, means, standard deviations, and/or medians and inter-quartile ranges, depending on the distributions of the data unless otherwise stated. Mean baseline and changes from baseline in continuous variables will be reported. Categorical data will be summarized as the number and percentage among participants with non-missing data. The two-sided (2.5% significance level each side) hypothesis test will be presented with the corresponding 95% CIs, further detail can be found in the description of the analysis for each of the study endpoints and in the SAP.

For efficacy variables, specific baseline definitions will be provided in the SAP.

Deviations from the protocol will be assessed as "important" or "not-important". Deviations will be defined before database lock to determine the impact on any planned analyses. Important deviations will be defined in a separate protocol deviation document. All the important protocol deviations will be listed by participant for all enrolled participants. Further details will be described in the SAP.

Table 11 Baseline for Primary/Secondary Endpoints and Safety Parameters Definitions

Category/Assessment	Baseline Definition	Endpoints/Parameters
Baseline (-30 min) FEV_1	Baseline (-30 min) FEV_1 is defined as the best FEV_1 value (highest FEV_1 of the acceptable efforts or the highest FEV_1 if no acceptable efforts are obtained) taken pre-mannitol challenge at -30 min for Visit 2 and Visit 3.	• Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2, pre-final dose of rescue/reliever • Peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever
Mannitol baseline FEV_1	The mannitol baseline is defined as the FEV_1 result where a positive response to mannitol is observed prior to dosing of study drug for challenge 1 in Visit 2 and in Visit 3 (time 0). A positive response is defined as a ≥15% decrease in FEV_1 from the 0 mg mannitol FEV_1 value.	• Change from mannitol baseline FEV_1 to the normalized FEV_1 AUC(0-60 min) post-mannitol challenge 1 • Change from mannitol baseline FEV_1 to the normalized FEV_1 AUC(0-15 min) post-mannitol challenge 1 • Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1
Vital signs	Visit 2 and Visit 3 will have separate baseline results utilizing the -30 min time point. If the -30 value is missing, baseline will be the latest non-missing value prior to the mannitol challenge ie, prior to time -10.	Results and change from baseline for: • Pulse • Blood pressure • Pulse oximetry • Respiratory rate
12-lead ECG	Visit 2 and Visit 3 will have separate baseline results utilizing the -30 min time point. If the -30 value is missing, baseline will be the latest non-missing value prior to the mannitol challenge ie, prior to time -10.	Result and change from baseline for all scheduled timepoints at for all ECG parameters
Laboratory assessments	Visit 2 and Visit 3 will have separate baseline results utilizing the -30 min time point. If the -30 value is missing, baseline will be the latest non-missing value prior to the mannitol challenge ie, prior to time -10.	Result and change from baseline for all scheduled timepoints for Laboratory safety variables as listed in Table 7 of the protocol

Abbreviations: AUC=area under the curve; ECG=electrocardiogram; FEV_1=Forced Expiratory Volume in the first second.

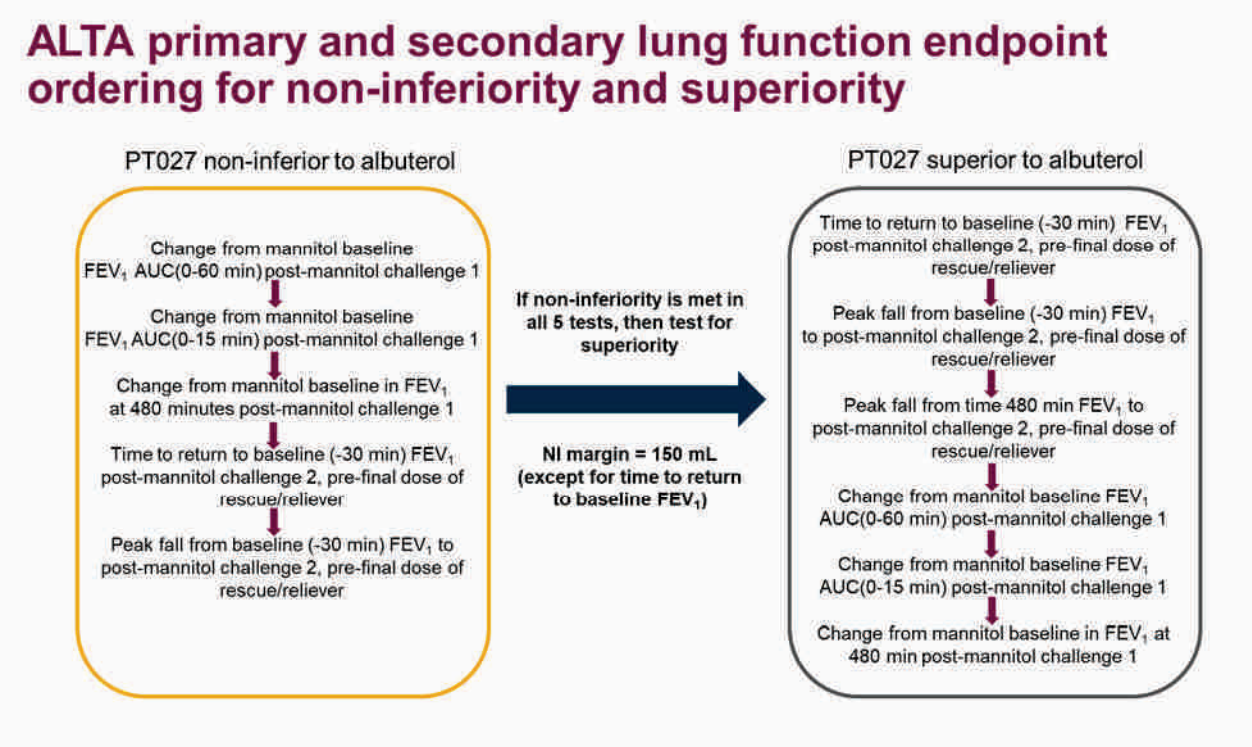
The statistical analyses will be performed by AstraZeneca or by designated third-party providers, under the direction of the Biostatistics Group, AstraZeneca. Further details will be provided in the SAP. All statistical analysis will be performed using the latest available version of SAS® (SAS Institute Inc., Cary, North Carolina, US), version 9.4 or higher. For the primary and secondary efficacy objectives, data from Part 1 will not be combined with data from Part 2. Study population data will be presented for the pool of Part 1 and Part 2, as well as being presented separately at the time for reporting of Part 2. Likewise, safety data are planned to be presented for pooled Part 1 and 2 data, and the same reporting strategy applies also to the exploratory objectives (except for the exploratory endpoint change from baseline FEV_1 AUC(0-15 min) post-mannitol challenge 2, which will be reported separately for Part 1 and Part 2, and for percentage fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever, and percentage fall in FEV_1 at time 480 min, which will be reported only for Part 2).

9.4.2 Adjustment for Multiple Comparisons

In order to account for the multiple tests across the primary and secondary endpoints, a hierarchical testing approach will be applied for the primary and secondary efficacy endpoints. Specifically, type 1 error control will be controlled by transferring alpha spending to subsequent endpoints in the testing hierarchy dependent on the statistical significance of the testing. .

Figure 5 shows the hierarchical testing approach, however, further details will be provided in the SAP.

Figure 5 Hierarchical Testing Structure



9.4.3 Efficacy

9.4.3.1 Primary Endpoint

The primary endpoint is the change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1. The mannitol baseline is defined as the FEV_1 result where a positive response to mannitol is observed prior to dosing of study drug for challenge 1 in Visit 2 and in Visit 3 (time 0). A positive response is defined as a $\geq$ 15% decrease in FEV_1 from the 0 mg mannitol FEV_1 value. Area under the curve will be calculated using the trapezoidal rule and will be normalized by dividing by the time in minutes from dosing to the last measurement included (typically 60 minutes). Only 1 non-missing, post- dose value, 0 to 30 minutes and 1 non-missing, post- dose value 30 to 60 minutes, is required for the calculation of AUC.

Non-inferiority comparisons

The primary efficacy comparison of non-inferiority will evaluate the hypothetical estimand in the PP set. The hypothetical estimand addresses the treatment effect under the scenario where intercurrent events in the treatment arms PT027 and PT007 do not occur. The following five attributes describe the estimand:

- Treatment condition = IMP within the randomized sequence: PT027 and PT007.
- Population = adult participants with asthma on SABA as-needed treatment only, and who are sensitive to mannitol.
- Participant-level outcome = Change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1.
- ICE handling = Intercurrent events are defined as discontinuation of study during a treatment period due to an asthma exacerbation and the taking of prohibited medications. A hypothetical strategy will be implemented. This estimand targets the hypothetical scenario in which the ICE did not occur and as such outcomes for participants without an ICE are as observed and for those with an ICE will be set to missing from the timepoint at which the ICE occurs.
- Summary measure = difference in the adjusted means of the participant-level outcomes for the treatment comparison of PT027 versus PT007.

Discontinuations from the study for any other reasons not defined as an ICE will be treated as MAR in the linear mixed effects model.

The primary efficacy comparison of non-inferiority will be based on a 1-sided hypothesis testing approach. The statistical null hypothesis for the PT027 versus PT007 comparison will be that the mean treatment difference shows that PT027 is inferior to PT007 by more than -150 mL. The null hypothesis is rejected if the lower limit of the 2–sided 95% CIs for the difference between treatments is greater than the non-inferiority limit of -150 mL.

The change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1, is the area under the curve for the changes from mannitol baseline FEV_1 calculated using the trapezoidal rule. To aid in interpretation, all AUC values will be normalized by dividing by the time from the first to the last non-missing value.

The change from mannitol baseline FEV_1 AUC(0-60 min) post-mannitol challenge 1, will be analyzed using a linear mixed model with a random participant effect. The fixed effects in the model will include center, treatment, treatment sequence, mannitol baseline FEV_1, the average of the two periods mannitol baseline FEV_1 values, and period. Taking the average of the two periods mannitol baseline FEV_1 values (ie, taking the average of the mannitol baseline FEV_1 values at Visit 2 and at Visit 3), and adding the average as a covariate to the model is to avoid cross-level bias (Kenward et al 2010). Contrasts will be used to obtain estimates of the treatment difference. Point estimates of the estimated adjusted treatment means, standard errors and 95% CIs will be presented. The point estimate of the difference in treatment means for the comparison of PT027 with PT007 with associated 2-sided 95% CI will be used to evaluate non-inferiority, such that non-inferiority of PT027 compared with PT007 is

established if the lower 95% confidence limit of the point estimate is greater than the non-inferiority margin of -150 mL.

Superiority comparison if first non-inferiority has been demonstrated

The primary efficacy comparison to establish the superiority of PT027 versus PT007 will evaluate the hypothetical estimand in the mRS.

The primary endpoint FEV_1 AUC(0-60 min) will be analyzed using the specified linear mixed model as detailed above. Point estimates of the estimated adjusted treatment means, standard errors and 95% CIs will be obtained. The estimated mean treatment difference as well as 95% CIs, and 2-sided p-value will be presented.

The primary efficacy comparison of superiority will be based on a 2-sided hypothesis testing approach.

9.4.3.2 Secondary Endpoints

For the peak fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever, and the change from mannitol baseline FEV_1 AUC(0-15 min) to post-mannitol challenge 1, the comparison of non-inferiority and the comparison of superiority if non-inferiority has been established, will evaluate the hypothetical estimand in the mRS following the same approach as for the primary endpoint. Likewise, the superiority test for peak fall from 480 min FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever, will follow the same analysis approach as for the primary endpoint, utilizing the mRS. Further details of these analyses will be provided in the SAP.

The non-inferiority test for the secondary endpoint of change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1, will evaluate the while-on-treatment estimand in the PP. Efficacy comparisons for superiority will evaluate the while-on-treatment estimand in the mRS population. The while-on-treatment estimand addresses the treatment effect where response to treatment prior to the occurrence of an intercurrent event is of interest. The following five attributes describe the estimand that will be used to define the treatment effect of interest for the primary analysis:

1 Treatment Condition = IMPs within the randomized sequence: PT027 and PT007.
2 Population = Adult participants with asthma on SABA as-needed treatment only, and who are sensitive to mannitol.
3 Participant-level outcome = Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1.
4 Handling ICEs: ICEs are defined as discontinuation of study during a treatment period due to an asthma exacerbation and the taking of prohibited medications. An on-treatment strategy for addressing intercurrent events will be implemented. This estimand targets the

treatment difference in a scenario, such that outcomes for participants without an ICE are as observed and outcomes for participants with an ICE are treated as MAR in the linear mixed effects model.

5 Summary measure = difference in adjusted means of participant-level outcome between PT027 and PT007.

Discontinuations from study for any other reasons not defined as an ICE will also be treated as MAR in the linear mixed effects model.

Change from mannitol baseline in FEV_1 at 480 min post-mannitol challenge 1 will be analyzed using the specified linear mixed model as detailed for the primary analysis above.

Time to return to baseline (-30 min) FEV_1 post-mannitol challenge 2, will be calculated using 95% of the baseline obtained prior to the first mannitol challenge and linear interpolation of actual timepoints rather than scheduled timepoints. This will reduce ties compared to using actual times because when using actual times, values will sometimes be interpolated as less than 0 and thus be left-censored at 0.

Median differences and 95% CIs will be provided using the Hodges-Lehmann estimate based on the Wilcoxon signed-rank statistic. Should 5% of participants not return to within 5% of the 480 minutes FEV_1 value post-mannitol challenge 2, then an alternative Kaplan-Meier analysis will be considered. Further details will be specified in the SAP.

Median differences and 95% CIs will be provided using the Hodges-Lehmann estimate based on the Wilcoxon signed-rank statistic. Should 5% of participants do not return to within 5% of baseline FEV_1 post-mannitol challenge 2, then an alternative Kaplan-Meier analysis will be considered. Further details will be specified in the SAP.

9.4.3.3 Exploratory Endpoints

The Exploratory Endpoints are: Percentage fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever; percentage fall from time 480 minutes FEV_1, the change from baseline FEV_1 AUC(0-15 min) post-mannitol challenge 2; the change from baseline in Borg dyspnea scale from baseline, post-mannitol challenge; the time to perceived onset of effect of study medication working, post-mannitol challenge; the change from baseline FeNO over time, post-mannitol challenge; the absolute and percent change from mannitol baseline in FEV_1 post-mannitol challenge 1 at each measured timepoint, up to and including 480 minutes; and, the proportion of participants who are non-responders to mannitol challenge 2. The exploratory endpoints will be analyzed to answer the exploratory objectives.

Change from baseline FEV_1 AUC(0-15 min) post-mannitol challenge 2 will be reported separately for Part 1 and Part 2 and will be analyzed by fitting the linear mixed model described for the primary endpoint in Section 9.4.3.1.

Percentage fall from baseline (-30 min) FEV_1 to post-mannitol challenge 2, pre-final dose of rescue/reliever, will be reported separately for Part 2 only, and will be analyzed fitting a linear mixed model adjusting for center, treatment, treatment sequence, baseline FEV_1, the average of the two periods baseline FEV_1 values, and period.

The remaining exploratory endpoints will be pooled for analyses, and details on how these study exploratory endpoints will be analyzed will be provided in the SAP for Part 2.

Serum cAMP and cAMP Mobilizing Analytes

It is hypothesized that levels of cAMP, a conventionally thought of intracellular second messenger, can serve as a new serum biomarker in non-severe asthma and correlates with the magnitude of the bronchodilator response. This study will further explore T2 high vs T2 low inflammation as a modulator of serum cAMP levels, the regulatory mechanisms of ABCC1, and their contributions to the pathophysiology of obstructive lung disease. We believe that increased cAMP levels may provide a new clinical variable for the phenotypic characterization of therapeutic responses, asthma phenotypes, and disease severity.

Urinary Leukotrienes and Exploratory Blood Inflammatory Biomarkers

Cysteinyl leukotrienes (LTB4, LTC4, LTD4, LTE4) play an important role in the pathogenesis of asthma. Leukotrienes are produced by mast cells, eosinophils, and other airway inflammatory cells and increase vascular permeability, constrict bronchial smooth muscle, and mediate bronchial hyperresponsiveness. The measurement of plasma and urinary Leukotrienes may include but are not limited to urinary LTE4, plasma LTB4 and plasma 5-oxo-ETE. Urinary LTE4 is of particular interest as it has been shown to correlate with airway obstruction and exacerbations in asthma.

Beta Receptor and Corticosteroid Receptor Gene Expression

Gene expression assays have been used to demonstrate a causative relationship between disease severity and treatment response. It has long been suggested that the β-2AR plays an important role in the development of asthma and it is identified as a candidate gene for asthma and its related phenotypes. Additionally, β_2-agonists are the mainstay of asthma therapy, but significant inter-individual clinical differences in response to these medications have been perhaps due to genetic variation at the receptor level. In this study, the expression of the β-2AR gene will be evaluated. Additionally, we will measure in this population of participants with asthma, the expression of the human glucocorticoid receptor gene previously demonstrating its regulation by pro-inflammatory cytokines and its downstream treatment response.

9.4.4 Safety

All safety analyses will be performed for the pooled Part 1 and Part 2 data, using the Safety analysis set.

Adverse events will be coded using the most recent version of the Medical Dictionary for Regulatory Activities that will have been released for execution at AstraZeneca/designee.

Safety data will be presented using descriptive statistics unless otherwise specified. Adverse events will be presented for each treatment group by system organ class and preferred term covering number and percentage of participants reporting at least one event and number of events where appropriate.

An overview of AEs will be presented for each treatment group: the number and percentage of participants with any AE, AEs with outcome of death, SAEs, and AEs leading to discontinuation of IMP. Separate AE tables will be provided taking into consideration relationship as assessed by the Investigator, intensity, seriousness, death, and events leading to discontinuation of IMP.

Key participant information will be presented for participants with AEs with outcome of death, serious AEs, and AEs leading to discontinuation of IMP. An AE listing for the safety analysis set will cover details for each individual AE.

Full details of AE analyses will be provided in the SAP for Part 2.

Laboratory parameters will be presented for each treatment group. Summary statistics for continuous variables cover n, mean, SD, minimum, Q1, median, Q3, and maximum. Frequency tables and shift tables cover number and percentage of participants in the respective category.

For each scheduled post-baseline visit, descriptive statistics for all clinical chemistry and hematology parameters will be presented for observed values and change from baseline. A shift table will present laboratory status including abnormality (eg, low, normal, high) from baseline to worst post-baseline. Elevation in liver parameters for assessment of Hy's Law will be done and reported appropriately if potential cases have been identified during the course of the study.

A frequency table for urinalysis presents number of participants reporting at least one treatment emergent increase in baseline category. A shift table for urinalysis will present the baseline assessment against the maximum on-treatment category.

Supportive laboratory listings will cover observed values and changes from baseline for each individual participant as well as abnormalities.

Details of laboratory analyses including definition of abnormality criteria will be provided in the SAP for Part 2.

Vital sign parameters will be presented for each treatment group. Summary statistics for continuous variables cover n, mean, SD, minimum, Q1, median, Q3, and maximum. Frequency tables and shift tables cover number and percentage of participants in the respective category.

For each scheduled post-baseline visit, descriptive statistics for all vital sign parameters will be presented for observed values and change from baseline.

A shift table will present vital sign status including abnormality (eg, low, normal, high; abnormal - clinically significant, abnormal clinically not significant) from baseline to maximum on-treatment value.

Supportive vital sign listings cover observed values and changes from baseline as well as abnormalities. Details of vital sign analyses including definition of abnormality criteria (eg, definition of low, normal, high; clinically significant) will be provided in the SAP for Part 2.

Electrocardiogram parameters will be presented for each treatment group. Summary statistics for continuous variables cover n, mean, SD, minimum, Q1, median, Q3, and maximum. Frequency tables and shift tables cover number and percentage of participants in the respective category. The (uncorrected) QT interval will be corrected according to the Fridericia's formula.

For each scheduled post-baseline assessment, descriptive statistics for all ECG parameters will be presented for observed values and change from baseline. For QTcF, a frequency table will present number of participants with values exceeding thresholds of 450 ms, 480 ms, and 500 ms at any time during the treatment and number of participants with changes from baseline in QTcF exceeding 30 ms, 60 ms, and 90 ms at any time during the treatment.

A table will present the interpretation of the ECG reading (normal, abnormal - clinically not significant, abnormal – clinically significant) at baseline and for each scheduled post-baseline visit, including shifts in interpretation as compared to baseline.

Supportive ECG listings will cover observed values for each individual participant.

9.4.5 Other Analyses

No other analyses are planned.

9.5 Interim Analyses

No interim analysis has been planned for this study.

However, unblinded results obtained after Part 1 of the study will be analyzed and utilized similar to a pilot study to inform the treatment effect size and variability estimates and endpoint ordering for the secondary efficacy endpoints in study Part 2. In this sense, data from Part 1 will be used to confirm the design of Part 2, to support the non-inferiority margins in Part 2 and drop-out rate where required and/or it will also be used to address operational difficulties.

Due to the uncertainty of the assumed variability and treatment effect size from the supporting lung function data, which mostly is not from a mannitol challenge setting, a sample size re-estimation may need to be performed on completion of Part 1 of the study. Sample size determinations for the secondary endpoints in Part 2 of the study might be re-calculated informed by the treatment effect sizes and variability estimates obtained from Part 1 data.

The data from Part 1 of the study will not be combined with the data from Part 2 in the final analyses of the primary and secondary endpoints.

Due to the study being double-blind, an independent team will be assigned to evaluate Part 1 data analyses, the results of which will be used to make recommendations for Part 2. A charter for Part 1 analysis will be created.

9.6 Data Monitoring Committees

9.6.1 AstraZeneca Internal Advisory Board

An AstraZeneca Internal Advisory Board (IAB) consisting of experts from AstraZeneca that are independent to the study team will review unblinded Part 1 study operational feasibility and efficacy and safety data once Part 1 is complete. The goal of the AstraZeneca IAB is to make recommendations to amend the study, based on Part 1 study data. Unblinded Part 1 study data will not be reported to study sites while the study is ongoing. Unblinding details are specified in the unblinding plan section of the SAP or in a separate unblinding plan document. Full details of the advisory board procedures and processes can be found in the AZ IAB Charter.

The safety of all AstraZeneca clinical studies is closely monitored on an ongoing basis by Parexel representatives in consultation with AstraZeneca Patient Safety.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

Appendix A Regulatory, Ethical, and Study Oversight Considerations

A 1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, revised protocol, ICF, IB, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any revised protocol will require IRB/IEC and applicable Regulatory Authority approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- AstraZeneca will be responsible for obtaining the required authorizations to conduct the study from the concerned Regulatory Authority. This responsibility may be delegated to a CRO, but the accountability remains with AstraZeneca.
- The Investigator will be responsible for providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European Regulation 536/2014 for clinical studies (if applicable), European Medical Device Regulation 2017/745 for clinical device research (if applicable), and all other applicable local regulations.

Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities toward the safety of participants and the safety of a study intervention under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.
- For all studies except those utilizing medical devices, Investigator safety reports must be prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- Adherence to European Medical Device Regulation 2017/745 for clinical device research (if applicable), and all other applicable local regulations.

- An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

Regulatory Reporting Requirements for Serious Breaches

- Prompt notification by the Investigator to AstraZeneca of any (potential) serious breach of the protocol or regulations is essential so that legal and ethical obligations are met.
  - A 'serious breach' means a breach likely to affect to a significant degree the safety and rights of a participant or the reliability and robustness of the data generated in the clinical study.
- If any (potential) serious breach occurs in the course of the study, Investigators or other site personnel will inform the appropriate AstraZeneca representatives immediately after he or she becomes aware of it.
- In certain regions/countries, AstraZeneca has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about such breaches.
  - AstraZeneca will comply with country-specific regulatory requirements relating to serious breach reporting to the regulatory authority, IRB/IEC, and Investigators.
- The Investigator should have a process in place to ensure that:
  - The site staff or service providers delegated by the Investigator/institution are able to identify the occurrence of a (potential) serious breach.
  - A (potential) serious breach is promptly reported to AstraZeneca or delegated party, through the contacts (email address or telephone number) provided by AstraZeneca.

A 2 Financial Disclosure

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

A 3 Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary, and they are free to refuse to participate and may withdraw their consent at any time and for any reason

during the study. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.

- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- If new information requires changes to the ICF, consider if participants must be re-consented and if so, this must be to the most current version of the ICF(s) during their participation in the study.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

Participants who are rescreened are required to sign a new ICF.

The ICF will contain a separate section that addresses and documents the collection and use of any mandatory and/or optional human biological samples. The Investigator or authorized designee will explain to each participant the objectives of the analysis to be done on the samples and any potential future use. Participants will be told that they are free to refuse to participate in any optional samples or the future use and may withdraw their consent at any time and for any reason during the retention period.

A 4 Data Protection

- Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure and use of their data must also be explained to the participant in the informed consent.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- The participant must be informed that data will be collected only for the business needs. We will only collect and use the minimum amount of personal data to support our business activities and will not make personal data available to anyone (including internal staff) who is not authorized or does not have a business need to know the information.

- The participant must be informed that in some cases their data may be pseudonymized. The General data Protection Regulation (GDPR) defines pseudonymization as the processing of personal data in such a way that the personal data can no longer be attributed to a specific individual without the use of additional information, provided that such additional information is kept separately and protected by technical and organizational measures to ensure that the personal data are not attributed to an identified or identifiable natural person.

A 5 Dissemination of Clinical Study Data

A description of this clinical study will be available on http://www.clinicaltrials.gov as will the summary of the study results when they are available. The clinical study and/or summary of study results may also be available on other websites according to the regulations of the countries in which the study is conducted.

A 6 Data Quality Assurance

- All participant data relating to the study will be recorded on eCRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of non-compliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (eg, Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 25 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

A 7 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes source data can be found in Source Document Declaration.

A 8 Study and Site Start and Closure

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

The first act of recruitment is the activation of the first site and will be the enrolment study start date.

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

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

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

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

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

A 9 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Appendix B Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

B 1 Definition of Adverse Events

An AE is the development of any untoward medical occurrence in a patient or clinical study participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom (for example nausea, chest pain), or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

The term AE is used to include both serious and non-serious AEs and can include a deterioration of a pre-existing medical occurrence. An AE may occur at any time, including run-in or wash-out periods, even if no study intervention has been administered.

B 2 Definition of Serious Adverse Events

An SAE is an AE occurring during any study phase (ie, run-in, treatment, wash-out, follow-up), that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-participant hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may jeopardize the participant or may require medical treatment to prevent one of the outcomes listed above.

Adverse Events for **malignant tumors** reported during a study should generally be assessed as **Serious AEs**. If no other seriousness criteria apply, the ‘Important Medical Event’ criterion should be used. In certain situations, however, medical judgment on an individual event basis should be applied to clarify that the malignant tumor event should be assessed and reported as a **non-serious AE**. For example, if the tumor is included as medical history and progression occurs during the study, but the progression does not change treatment and/or prognosis of the malignant tumor, the AE may not fulfill the attributes for being assessed as serious, although reporting of the progression of the malignant tumor as an AE is valid and should occur. Also, some types of malignant tumors, which do not spread remotely after a routine treatment that does not require hospitalization, may be assessed as non-serious; examples in adults include Stage 1 basal cell carcinoma and Stage 1A1 cervical cancer removed via cone biopsy.

Life-threatening

'Life-threatening' means that the participant was at immediate risk of death from the AE as it occurred, or it is suspected that use or continued use of the product would result in the participant's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalization

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal edema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the participant was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important Medical Event or Medical Treatment

Medical and scientific judgment should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalization, disability or incapacity but may jeopardize the participant or may require medical treatment to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgment must be used.

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anemia requiring blood transfusion, etc) or convulsions that do not result in hospitalization
- Development of drug dependency or drug abuse

Intensity Rating Scale:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Appendix B 2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE unless it meets the criteria shown in Appendix B 2. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE when it satisfies the criteria shown in Appendix B 2.

B 3 A Guide to Interpreting the Causality Question

When making an assessment of causality consider the following factors when deciding if there is a 'reasonable possibility' that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the participant actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?
- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? Or could the AE be anticipated from its pharmacological properties?
- De-challenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- No alternative cause. The AE cannot be reasonably explained by another etiology such as the underlying disease, other drugs, other host or environmental factors.
- Re-challenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a re-challenge.
- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship.

In difficult cases, other factors could be considered such as:

- Is this a recognized feature of overdose of the drug?
- Is there a known mechanism?

Causality of 'related' is made if following a review of the relevant data, there is evidence for a 'reasonable possibility' of a causal relationship for the individual case. The expression 'reasonable possibility' of a causal relationship is meant to convey, in general, that there are facts (evidence) or arguments to suggest a causal relationship.

The causality assessment is performed based on the available data including enough information to make an informed judgment. With no available facts or arguments to suggest a causal relationship, the event(s) will be assessed as 'not related'.

Causal relationship in cases where the DUS has deteriorated due to lack of effect should be classified as no reasonable possibility.

B 4 Medication Error, Drug Abuse, and Drug Misuse

Medication Error

For the purposes of this clinical study a medication error is an unintended failure or mistake in the treatment process for an AstraZeneca study intervention that either causes harm to the participant or has the potential to cause harm to the participant.

A medication error is not lack of efficacy of the drug, but rather a human or process related failure while the drug is in control of the study site staff or participant.

Medication error includes situations where an error.

- Occurred
- **Was identified and** intercepted before the participant received the drug
- Did not occur, but circumstances were recognized that could have led to an error

Examples of events to be reported in clinical studies as medication errors:

- Drug name confusion
- Dispensing error eg, medication prepared incorrectly, even if it was not actually given to the participant
- Drug not administered as indicated, for example, wrong route or wrong site of administration
- Drug not taken as indicated eg, tablet dissolved in water when it should be taken as a solid tablet
- Drug not stored as instructed eg, kept in the refrigerator when it should be at room temperature
- Wrong participant received the medication (excluding IxRS errors)
- Wrong drug administered to participant (excluding IxRS errors)

Examples of events that **do not** require reporting as medication errors in clinical studies:

- Errors related to or resulting from IxRS - including those which lead to one of the above listed events that would otherwise have been a medication error
- Participant accidentally missed drug dose(s) eg, forgot to take medication
- Accidental overdose (will be captured as an overdose)
- Participant failed to return unused medication or empty packaging
- Errors related to background and rescue/reliever medication, or standard of care medication in open-label studies, even if an AstraZeneca product

Medication errors are not regarded as AEs, but AEs may occur as a consequence of the medication error.

Drug Abuse

For the purpose of this study, drug abuse is defined as the persistent or sporadic intentional, non-therapeutic excessive use of IMP or AstraZeneca NIMP for a perceived reward or desired non-therapeutic effect.

Any events of drug abuse, with or without associated AEs, are to be captured and forwarded to the Data Entry Site (DES) using the Drug Abuse Report Form. This form should be used both if the drug abuse happened in a study participant or if the drug abuse involves a person not enrolled in the study (such as a relative of the study participant).

Examples of drug abuse include but are not limited to:

- The drug is used with the intent of getting a perceived reward (by the study participant or a person not enrolled in the study)
- The drug in the form of a tablet is crushed and injected or snorted with the intent of getting high

Drug Misuse

Drug misuse is the intentional and inappropriate use (by a study participant) of IMP or AstraZeneca NIMP for medicinal purposes outside of the authorized product information, or for unauthorized IMPs or AstraZeneca NIMPs, outside the intended use as specified in the protocol and includes deliberate administration of the product by the wrong route.

Events of drug misuse, with or without associated AEs, are to be captured and forwarded to the DES using the Drug Misuse Report Form. This form should be used both if the drug misuse happened in a study participant or if the drug misuse regards a person not enrolled in the study (such as a relative of the study participant).

Examples of drug misuse include but are not limited to:

- The drug is used with the intention to cause an effect in another person
- The drug is sold to other people for recreational purposes
- The drug is used to facilitate assault in another person
- The drug is deliberately administered by the wrong route
- The drug is split in half because it is easier to swallow, when it is stated in the protocol that it must be swallowed whole
- Only half the dose is taken because the study participant feels that he/she is feeling better when not taking the whole dose
- Someone who is not enrolled in the study intentionally takes the drug

Appendix C Handling of Human Biological Samples

C 1 Chain of Custody

A full chain of custody is maintained for all samples throughout their lifecycle.

The Investigator at each center keeps full traceability of collected biological samples from the participants while in storage at the center until shipment or disposal (where appropriate) and records relevant processing information related to the samples while at site.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps record of receipt of arrival and onward shipment or disposal.

AstraZeneca or delegated representatives will keep oversight of the entire life cycle through internal procedures, monitoring of study sites, auditing or process checks, and contractual requirements of external laboratory providers

Samples retained for further use will be stored in the AstraZeneca-assigned biobanks or other sample archive facilities and will be tracked by the appropriate AstraZeneca Team during for the remainder of the sample life cycle.

If required, AstraZeneca will ensure that remaining biological samples are returned to the site according to local regulations or at the end of the retention period, whichever is the sooner.

C 2 Withdrawal of Informed Consent for Donated Biological Samples

If a participant withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed/repatriated, and the action documented. If samples are already analyzed, AstraZeneca is not obliged to destroy the results of this research.

Following withdrawal of consent for biological samples, further study participation should be considered in relation to the withdrawal processes outlined in the informed consent.

The Investigator:

- Ensures participant's withdrawal of informed consent to the use of donated samples is highlighted immediately to AstraZeneca or delegate.
- Ensures that relevant human biological samples from that participant, if stored at the study site, are immediately identified, disposed of as appropriate, and the action documented.
- Ensures that the participant and AstraZeneca are informed about the sample disposal.

AstraZeneca ensures the organization(s) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of or repatriated as appropriate, and the action is documented, and study site is notified.

C 3 International Airlines Transportation Association 6.2 Guidance Document

LABELING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) (**https://www.iata.org/whatwedo/cargo/dgr/Pages/download.aspx**) classifies infectious substances into 3 categories: Category A, Category B or Exempt

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.
Category A Pathogens are, eg, Ebola, Lassa fever virus. Infectious substances meeting these criteria which cause disease in humans or both in humans and animals must be assigned to UN 2814. Infectious substances which cause disease only in animals must be assigned to UN 2900.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are, eg, Hepatitis A, C, D, and E viruses. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN 3373 and IATA 650

Exempt - Substances which do not contain infectious substances or substances which are unlikely to cause disease in humans or animals are not subject to these regulations unless they meet the criteria for inclusion in another class.

- Clinical study samples will fall into Category B or exempt under IATA regulations
- Clinical study samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging (https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR-60-EN-PI650.pdf)
- Biological samples transported in dry-ice require additional dangerous goods specification for the dry-ice content

Appendix D Optional Genomics Initiative Sample

D 1 Use/Analysis of DNA

- AstraZeneca intends to collect and store DNA for genetic research to explore how genetic variations may affect clinical parameters, risk and prognosis of diseases, and the response to medications. This genetic research may lead to better understanding of diseases, better diagnosis of diseases or other improvements in health care and to the discovery of new diagnostics, treatments or medications. Therefore, where local regulations and IRB/IEC allow, a swab sample will be collected for DNA analysis from consenting participants.
- This optional genetic research may consist of the analysis of the structure of the participant´s DNA, ie, the entire genome.
- The results of genetic analyses may be reported in a separate study summary.
- The Sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.

D 2 Genetic Research Plan and Procedures

Selection of Genetic Research Population

All participants will be asked to participate in this genetic research. Participation is voluntary and if a participant declines to participate there will be no penalty or loss of benefit. The participant will not be excluded from any aspect of the main study.

Inclusion Criteria

For inclusion in this genetic research, participants must fulfill all of the inclusion criteria described in the main body of the CSP and: Provide informed consent for the Genomics Initiative sampling and analyses.

Exclusion Criteria

- Exclusion from this genetic research may be for any of the exclusion criteria specified in the main study or any of the following:
  - Previous allogeneic bone marrow transplant
  - Non-leukocyte depleted whole blood transfusion in 120 days of genetic sample collection
  - Healthy Volunteers and pediatric patient samples will not be collected for the Genomics Initiative.

Withdrawal of Consent for Genetic Research

Participants may withdraw from this genetic research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary withdrawal will not prejudice further treatment. Procedures for withdrawal are outlined in Section 7.2 of the main CSP.

Collection of Samples for Genetic Research

The blood sample for this genetic research will be obtained from the participants at Visit 2 after randomization before dose administration. Although DNA is stable, early sample collection is preferred to avoid introducing bias through excluding participants who may withdraw due to an AE. If for any reason the sample is not drawn at Visit 2, it may be taken at any Visit 3. Only one sample should be collected per participant for genetics during the study.

Coding and Storage of DNA Samples

- The processes adopted for the coding and storage of samples for genetic analysis are important to maintain participant confidentiality. Samples will be stored for a maximum of 15 years, from the date of last participant last visit, after which they will be destroyed. DNA is a finite resource that is used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.
- An additional second code will be assigned to the sample either before or at the time of DNA extraction replacing the information on the sample tube. Thereafter, the sample will be identifiable only by the second, unique number. This number is used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated organization. No personal details identifying the individual will be available to any person (AstraZeneca employee or designated organizations working with the DNA).
- The link between the participant enrolment/randomization code and the second number will be maintained and stored in a secure environment, with restricted access at AstraZeneca or designated organizations. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit, and permit tracing of samples for destruction in the case of withdrawal of consent.

Ethical and Regulatory Requirements

The principles for ethical and regulatory requirements for the study, including this genetics research component, are outlined in Appendix A.

Informed Consent

The genetic component of this study is optional, and the participant may participate in other components of the main study without participating in this genetic component. To participate

in the genetic component of the study the participant must sign and date both the consent form for the main study and the addendum for the Genomics Initiative component of the study. Copies of both signed and dated consent forms must be given to the participant and the original filed at the study center. The PI(s) is responsible for ensuring that consent is given freely, and that the participant understands that they may freely withdrawal from the genetic aspect of the study at any time.

Participant Data Protection

AstraZeneca will not provide individual genotype results to participants, any insurance company, any employer, their family members, general physician unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the participant. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a participant. For example, in the case of a medical emergency, an AstraZeneca Physician or an Investigator might know a participant's identity and also have access to his or her genetic data. Regulatory authorities may require access to the relevant files, though the participant's medical information and the genetic files would remain physically separate.

Data Management

Any genetic data generated in this study will be stored at a secure system at AstraZeneca and/or designated organizations to analyze the samples.

AstraZeneca and its designated organizations may share summary results (such as genetic differences from groups of individuals with a disease) from this genetic research with other researchers, such as hospitals, academic organizations or health insurance companies. This can be done by placing the results in scientific databases, where they can be combined with the results of similar studies to learn even more about health and disease. The researchers can only use this information for health-related research purposes. Researchers may see summary results, but they will not be able to see individual participant data or any personal identifiers. Some or all of the clinical datasets from the main study may be merged with the genetic data in a suitable secure environment separate from the clinical database.

Appendix E Actions Required in Cases of Increases in Liver Biochemistry and Evaluation of Hy's Law

E 1 Introduction

This Appendix describes the process to be followed in order to identify and appropriately report PHL cases and HL cases. It is not intended to be a comprehensive guide to the management of elevated liver biochemistries.

During the course of the study the Investigator will remain vigilant for increases in liver biochemistry. The Investigator is responsible for determining whether a participant meets potential PHL criteria at any point during the study.

All sources of laboratory data are appropriate for the determination of PHL and HL events; this includes samples taken at scheduled study visits and other visits including central and all local laboratory evaluations even if collected outside of the study visits; for example, PHL criteria could be met by an elevated ALT from a central laboratory **and/or** elevated TBL from a local laboratory.

The Investigator will also review AE data (for example, for AEs that may indicate elevations in liver biochemistry) for possible PHL events.

The Investigator participates, together with AstraZeneca clinical project representatives, in review and assessment of cases meeting PHL criteria to agree whether HL criteria are met. HL criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than DILI caused by the IMP.

The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting SAEs and AEs according to the outcome of the review and assessment in line with standard safety reporting processes.

E 2 Definitions

Potential Hy's Law
Aspartate Aminotransferase or ALT ≥ 3 × ULN **together with** TBL ≥ 2× ULN at any point during the study following the start of study medication irrespective of an increase in ALP.

Hy's Law
AST or ALT ≥ 3× ULN **together with** TBL ≥ 2× ULN, where no other reason, other than the IMP, can be found to explain the combination of increases, eg, elevated ALP indicating cholestasis, viral hepatitis, another drug.

For PHL and HL the elevation in transaminases must precede or be coincident with (ie, on the same day) the elevation in TBL, but there is no specified timeframe within which the elevations in transaminases and TBL must occur.

E 3 Identification of Potential Hy's Law Cases

In order to identify cases of PHL it is important to perform a comprehensive review of laboratory data for any participant who meets any of the following identification criteria in isolation or in combination:

- ALT ≥ 3 × ULN
- AST ≥ 3 × ULN
- TBL ≥ 2 × ULN

Central Laboratories Being Used:

When a participant meets any of the PHL identification criteria, in isolation or in combination, the central laboratory will immediately send an alert to the Investigator (also sent to AstraZeneca representative).

The Investigator will also remain vigilant for any local laboratory reports where the PHL identification criteria are met, where this is the case the Investigator will:

- Notify the AstraZeneca representative
- Request a repeat of the test (new blood draw) by the central laboratory without delay
- Complete the appropriate unscheduled laboratory eCRF module(s) with the original local laboratory test result

When the identification criteria are met from central or local laboratory results the Investigator will without delay:

- Determine whether the participant meets PHL criteria (see Section E 2 for definition) by reviewing laboratory reports from all previous visits (including both central and local laboratory results)

E 4 Investigator Follow-up

E 4.1 Potential Hy's Law Criteria not met

If the participant does not meet PHL criteria the Investigator will:

- Inform the AstraZeneca representative that the participant has not met PHL criteria.
- Perform follow-up on subsequent laboratory results according to the guidance provided in the CSP.

E 4.2 Potential Hy's Law Criteria met

If the participant does meet PHL criteria the Investigator will:

- Notify the AstraZeneca representative who will then inform the central Study Team
- Within 1 day of PHL criteria being met, the Investigator will report the case as an SAE of Potential Hy's Law; serious criteria 'Important medical event' and causality assessment 'yes/related' according to CSP process for SAE reporting.
- For participants that met PHL criteria prior to starting IMP, the Investigator is not required to submit a PHL SAE unless there is a significant change[#] in the participant's condition
- The Study Physician contacts the Investigator, to provide guidance, discuss and agree an approach for the study participants' follow-up (including any further laboratory testing) and the continuous review of data
- Subsequent to this contact the Investigator will:
  - Monitor the participant until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated. Completes follow-up SAE Form as required.
  - Investigate the etiology of the event and perform diagnostic investigations as discussed with the Study Physician. This includes deciding which the tests available in the Hy's law lab kit should be used.
  - Complete the three Liver eCRF Modules as information becomes available

[#]**A 'significant' change** in the participant's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or TBL) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

E 5 Review and Assessment of Potential Hy's Law Cases

The instructions in this Section should be followed for all cases where PHL criteria are met.

As soon as possible after the biochemistry abnormality was initially detected, the Study Physician contacts the Investigator in order to review available data and agree on whether there is an alternative explanation for meeting PHL criteria other than DILI caused by the IMP, to ensure timely analysis and reporting to health authorities within 15 calendar days from date PHL criteria was met. The AstraZeneca Global Clinical Lead or equivalent and Global Safety Physician will also be involved in this review together with other subject matter experts as appropriate.

According to the outcome of the review and assessment, the Investigator will follow the instructions below.

Where there is an agreed alternative explanation for the ALT or AST and TBL elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for a SAE:

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate eCRF
- If the alternative explanation is an AE/SAE: update the previously submitted Potential Hy's Law SAE and AE eCRFs accordingly with the new information (reassessing event term; causality and seriousness criteria) following the AstraZeneca standard processes.

If it is agreed that there is **no** explanation that would explain the ALT or AST and TBL elevations other than the IMP:

- Send updated SAE (report term 'Hy's Law') according to AstraZeneca standard processes.
  - The 'Medically Important' serious criterion should be used if no other serious criteria apply
  - As there is no alternative explanation for the HL case, a causality assessment of 'related' should be assigned.

If, there is an unavoidable delay, of over 15 calendar days in obtaining the information necessary to assess whether or not the case meets the criteria for HL, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Provides any further update to the previously submitted SAE of Potential Hy's Law, (report term now 'Hy's Law case') ensuring causality assessment is related to IMP and seriousness criteria is medically important, according to CSP process for SAE reporting.
- Continue follow-up and review according to agreed plan. Once the necessary supplementary information is obtained, repeat the review and assessment to determine whether HL criteria are still met. Update the previously submitted PHL SAE report following CSP process for SAE reporting, according to the outcome of the review and amending the reported term if an alternative explanation for the liver biochemistry elevations is determined.

E 6 Laboratory Tests

Hy's Law Lab Kit for Central Laboratories

Additional standard chemistry and coagulation tests	GGT LDH Prothrombin time INR
Viral hepatitis	IgM anti-HAV HBsAg IgM and IgG anti-HBc HBV DNA [a] IgG anti-HCV HCV RNA [b] IgM anti-HEV HEV RNA
Other viral infections	IgM & IgG anti-CMV IgM & IgG anti-HSV IgM & IgG anti-EBV
Alcoholic hepatitis	Carbohydrate deficient transferrin (CD-transferrin) [c]
Autoimmune hepatitis	Antinuclear antibody (ANA) Anti-Liver/Kidney Microsomal Ab (Anti-LKM) Anti-Smooth Muscle Ab (ASMA)
Metabolic diseases	alpha-1-antitrypsin Ceruloplasmin Iron Ferritin Transferrin [c] Transferrin saturation

[a]HBV DNA is only recommended when IgG anti-HBc is positive
[b]HCV RNA is only recommended when IgG anti-HCV is positive or inconclusive
[c]CD-transferrin and Transferrin are not available in China. Study teams should amend this list accordingly

E 7 References

Aithal et al 2011
Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa, et al. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther. 2011;89(6):806-15.

FDA Guidance for Industry, July 2009
US Food and Drug Administration [internet]. FDA guidance for industry. Drug-induced liver injury: premarketing clinical evaluation. Available from; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/drug-induced-liver-injury-premarketing-clinical-evaluation.

Appendix F Instructions for Handling and Use of Oral Inhaler Device

F 1 Instructions For Use - Clinic

PT027 (budesonide and albuterol sulfate)
PT007 (albuterol sulfate)
Demo Device
Inhalation Aerosol, For Oral Inhalation Only

Please read these instructions prior to use.
The inhaler may be different from inhalers you have used before.

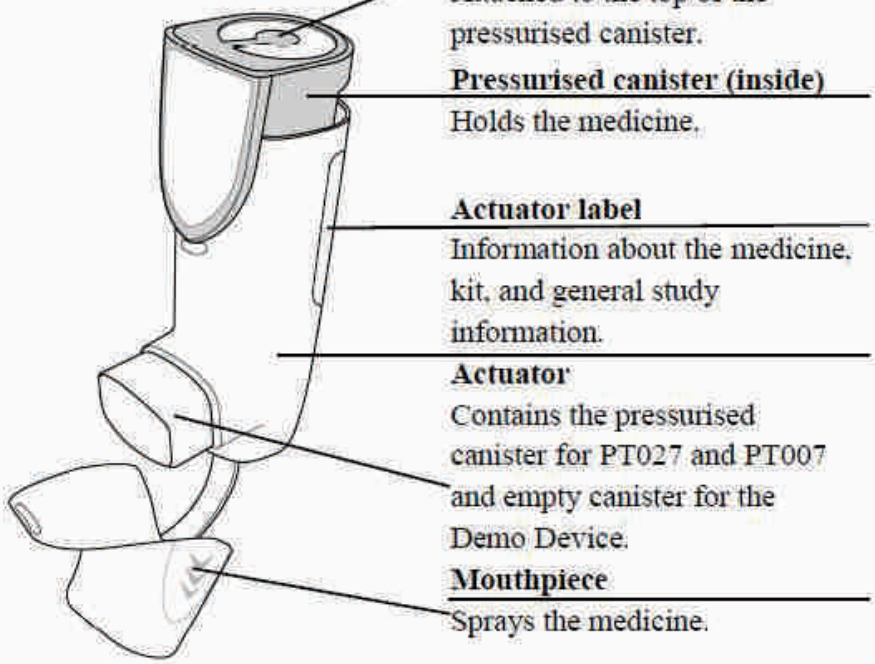
Important information

- For oral inhalation use only.
- Dispose of the patient's inhaler when finished.
- Prepare the patient's inhaler before they use it for the first time by priming it.
- Prime the inhaler so that the patient will get the right amount of medicine when they use it.
- Collect the patient's inhaler after use and securely dispose of it.

Storing your inhaler

- Store unopened inhalers at room temperature between 68°F to 86°F (20°C to 30°C).
- **Do not store in a humid environment, such as a bathroom.**
- Keep inhalers and all medicines out of the reach of children.

Parts of your inhaler



When to give the patient a new inhaler

Give the patient a new inhaler:

- when the pointer on the puff indicator is in the yellow or red zone.
- if the inhaler has been damaged.

Dispose of inhalers

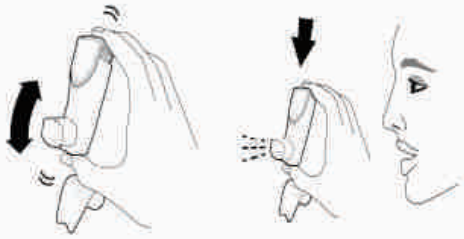
Collect and dispose of inhalers following supplier quality agreement when either:

- The patient has finished using the inhaler at the study visit.
  or
- 12 months after the inhaler has been removed from the foil pouch
  or
- after the expiry date on the carton and canister
  or
- puff indicator shows 0,
- whichever comes first.

Do not reuse or use the actuator with medicine canisters from other inhalers.
Do not puncture or throw the canister into a fire or incinerator.

Before First Use – Setting up and priming the patient's inhaler

- Before your patient uses the inhaler for the **first time, Shake and Spray 4 times** to prime.

Prime the patient's inhaler before first use	
Take the cover off the mouthpiece.	Shake well and spray **4 Test-puffs** away from you, shaking before each Test-puff.
	**Shake before spray. Repeat both actions 4 times.** 
Extra puffs are provided for priming. **Do not skip priming.**	

When and how to Re-prime the patient's inhaler

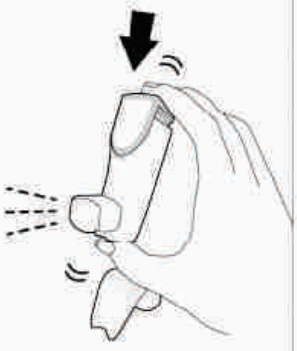
When to Re-prime your inhaler:

- after rinsing white actuator
- if dropped

How to Re-prime your inhaler:

Take the cover off the mouthpiece and spray **2 Test-puffs**, shaking before each Test-puff.

Shake before spray. Repeat both actions 2 times.



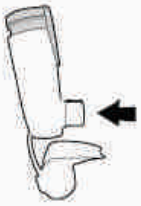
Extra puffs are provided for priming. **Do not skip priming.**

How To Use Your Inhaler – Inhaling your medicine

- Take **2 puffs (= 1 dose)** as needed. **Do not** use more than 12 puffs in 24 hours.
- After use, rinse your mouth if water is available, without swallowing, to prevent oral fungal infection.

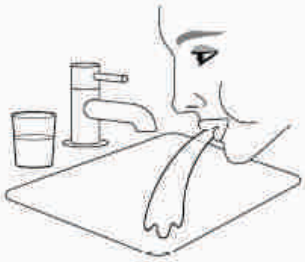
1. Check

Take the cover off the mouthpiece. **Check the mouthpiece for foreign objects and remove objects before use.**



Check inside mouthpiece.

2. Shake and Inhale				
Shake well.	Breathe out fully.	Place mouthpiece into mouth and close lips around the mouthpiece.	Start to breathe in deeply and slowly while spraying **1 puff**. Continue breathing in until you cannot any more.	Remove mouthpiece from mouth. Hold breath for as long as you can or up to 10 seconds.
				

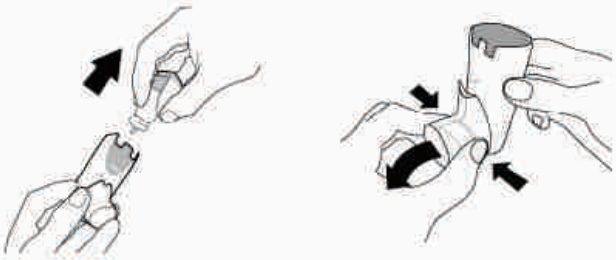
3. Repeat	4. Place cover back on	5. Rinse
Immediately repeat the Shake and Inhale step to take a second puff.  **2 puffs = 1 dose.**	Place mouthpiece cover back on. 	Rinse mouth if water is available **(do not swallow)** to prevent oral fungal infection. 

Cleaning – Rinsing your actuator if needed

- Rinse white actuator if it gets dirty/soiled or if a plume is not seen during an actuation.
- **Do not allow the canister to get wet.**
- Re-prime after rinsing.

1. Remove

Remove canister and set aside. Take the cover off the mouthpiece.

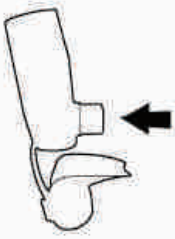


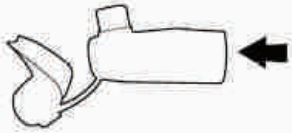
2. Rinse through both ends

Rinse with warm water 30 seconds each end of the actuator. Shake off as much water as you can.
Do not use soap. Do not dry with a towel or tissue.

60 seconds total

Look into the mouthpiece and actuator to make sure any medicine build-up has been completely washed away. If there is any build-up, rinse again.

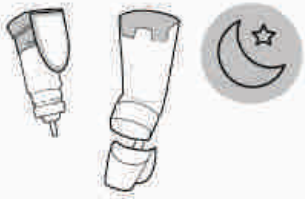




Check inside mouthpiece and actuator.

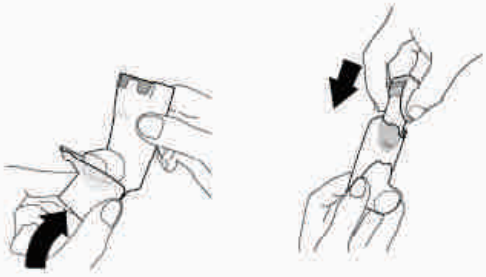
3. Air-dry

Air-dry as long as possible. See "Emergency inhaler use when wet" section and be sure to re-prime the device after rinsing.



4. Re-assemble and Re-prime

First, place mouthpiece cover back on. After the mouthpiece cover is on, gently press canister down into actuator. **Do not reassemble until inhaler is fully dry. Do not insert canister while mouthpiece cover is off.** Re-prime the inhaler by following the Re-prime steps above.



Emergency inhaler use when wet

If your patient needs to use the inhaler before it is dry:

- shake off excess water
- insert canister
- Shake and Spray twice into air away from face
- take prescribed dose
- rinse inhaler again following Rinse steps.

F 2 Instructions For Use – Participant

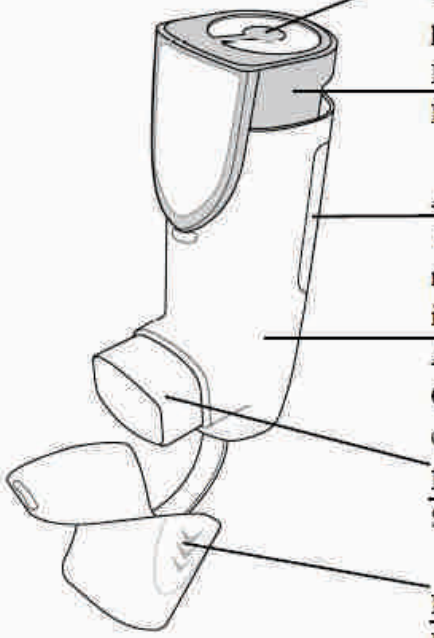
Inhalation Aerosol. For Oral Inhalation Only

Please read these instructions prior to use.
Your inhaler may be different from inhalers you have used before.

Important information

- For oral inhalation use only.
- Before first use, the clinic staff will prime the inhaler for you. There is no need to prime it under normal circumstances.
- Return your inhaler to the clinic staff when finished.

Parts of your inhaler



Puff indicator
Attached to the top of the pressurised canister.

Pressurised canister (inside)
Holds the medicine.

Actuator label
Information about the medicine, kit, and general study information.

Actuator
Contains the pressurised canister.

Mouthpiece
Sprays the medicine.

Mouthpiece cover
Protects the mouthpiece when the inhaler is not in use.

When to obtain a new inhaler

Obtain a new inhaler from the clinic staff:

- when the pointer on the puff indicator is in the yellow zone.
- if the inhaler has been damaged.

Disposal of your inhaler

At the end of your clinic visit, return the inhaler to the clinic staff. Do not take the inhaler with you when you leave.

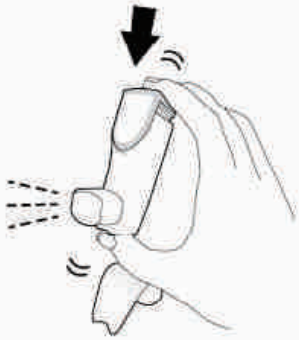
Do not reuse or use the actuator with medicine canisters from other inhalers.
Do not puncture or throw the canister into a fire or incinerator.

When and how to Re-prime your inhaler

When to Re-prime your inhaler:

- after rinsing white actuator
- if dropped

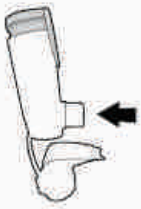
The clinic staff will re-prime your inhaler, if needed



Extra puffs are provided for priming. **The clinic staff should not skip priming.**

How To Use Your Inhaler – Inhaling your medicine

- Take **2 puffs (= 1 dose)** as needed. **Do not** use more than 12 puffs in 24 hours.
- After use, rinse your mouth if water is available, without swallowing, to prevent oral fungal infection.

1. Check
Take the cover off the mouthpiece. **Check the mouthpiece for foreign objects and remove objects before use.**  Check inside mouthpiece.

2. Shake and Inhale				
Shake well.	Breathe out fully.	Place mouthpiece into mouth and close lips around the mouthpiece.	Start to breathe in deeply and slowly while spraying **1 puff**. Continue breathing in until you cannot any more.	Remove mouthpiece from mouth. Hold breath for as long as you can or up to 10 seconds.
				

3. Repeat	4. Place cover back on	5. Rinse
Immediately repeat the Shake and Inhale step to take a second puff.  **2 puffs = 1 dose.**	Place mouthpiece cover back on. 	Rinse mouth if water is available **(do not swallow)** to prevent oral fungal infection. 

Cleaning – Rinsing your actuator

- The clinic staff will rinse the white actuator if it gets dirty/soiled or if a plume is not seen during an actuation.
- The clinic staff will re-prime the inhaler after rinsing.

Emergency inhaler use when wet

If you need to use the inhaler before it is dry:

- shake off excess water
- insert canister
- Shake and Spray twice into air away from face
- take prescribed dose
- rinse inhaler again following Rinse steps.

Appendix G Protocol Version History

The Summary of Changes Table for the current amendment is located directly before the Table of Contents.

CSP Version 3.0, Amendment 2: (03 February 2023)

Overall Rationale for the Modification:

The overall aim for this amendment was to add the pooling of data for Part 1 and Part 2 for safety analyses, to provide further clarity and updates in terms of ambiguity in baseline values, and to clarify timing of assessments, as detailed below.

Summary of Changes:

List of Substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis: Objectives and Endpoints Statistical methods Section 3 Objectives and Endpoints Section 8.1 Efficacy Assessments Section 9.1 Statistical Hypotheses Section 9.2 Sample Size Determination Section 9.4.1 General Considerations Section 9.4.2 Adjustment for multiple comparisons (. Figure 5) Section 9.4.3.2 Secondary Endpoints	Updated endpoint for secondary objective to establish efficacy in the sustainability of effect of reversal of acute airway obstruction post-mannitol challenge 1 to use mannitol baseline in FEV_1 as starting point instead of baseline in FEV_1.	The mannitol baseline in FEV_1 is the baseline relevant to airway obstruction measurements.
Section 1.1 Synopsis: Objectives and Endpoints Statistical Methods Section 3 Objectives and Endpoints Section 8.1 Efficacy Assessments Section 9.1 Statistical Hypotheses	Endpoint of change from baseline FEV_1 AUC(0-15 min) post mannitol challenge 2 to measure efficacy post-dose lung function to be an exploratory endpoint.	Due to the uncertainty in this endpoint, endpoint is removed as a secondary endpoint and added as an exploratory endpoint as it is better suited being hypothesis generating.

Section Number and Name	Description of Change	Brief Rationale
Section 9.2 Sample Size Determination Section 9.4.1 General Considerations Section 9.4.2 Adjustment for multiple comparisons (. Figure 5) Section 9.4.3.2 Secondary Endpoints Section 9.4.3.3 Exploratory Endpoints		
Section 1.1 Synopsis: Intervention Groups and Duration Section 4.1 Overall Design Section 9.4.1 General Considerations Section 9.5 Interim Analyses	Added clarification that the data from Part 1 will not be pooled with that of Part 2 for the analysis of the primary and secondary endpoints.	The Part 1 and Part 2 lung function data for primary and secondary analyses will not be pooled because the Part 1 data will be unblinded and thus should not be included with Part 2 analysis. Part 1 is not considered as an interim analysis, but rather a Pilot study.
Section 8.9 Human Biological Samples Section 9.4.5 Exploratory Endpoints	Added that data from Part 1 and Part 2 will be pooled for exploratory endpoints and details on how exploratory endpoints will be analyzed, will be provided in the SAP and may be reported separate from the CSR.	Exploratory biomarker data and other exploratory endpoints relative to baseline participant status, dyspnea score, onset of effect are not relevant to the analyses needed for Part 1 to inform Part 2 and thus will not be delivered to or assessed by the unblinded team. To avoid losing this information, the data will be pooled with Part 2.
Section 9.4.1 General Considerations Section 9.4.5 Exploratory Endpoints	Added reporting strategy for pooled data.	The reporting strategy for the pooled Part 1 and Part 2 data not previously included in the CSP.

Section Number and Name	Description of Change	Brief Rationale
Section 9.4.4 Safety	Added that safety analyses will be performed for the pooled Part 1 and Part 2 data.	All safety data from both Part 1 and Part 2 will be analyzed to make meaningful and as correct as possible interpretations and conclusions about safety at the end of the study.

Abbreviations: AE=adverse event; CSR=Clinical Study Report; FEV_1=Forced Expiratory Volume in the first second; SAP=Statistical Analysis Plan.

List of Non-Substantial Modifications

Section Number and Name	Description of Change	Brief Rationale
Throughout	Minor editorial updates.	For consistency within the document and compliance with Style Guide.
Summary of Changes Table Amendment 1, 02 August 2022	Updated description of change for spirometry in Section 1.3, Table 2, and Section 8.1.1 from assessment at 15, 20, and 25 minutes to 5, 10, and 15 minutes post-mannitol challenge 1 and 2 instead of at the start of the mannitol challenge.	Description of change incorrect.
Section 1.1 Synopsis: Statistical Methods	Removed the sentence “The model will not include treatment sequence unless that term is determined to be important (p◦<◦0.10)".	For consistency with Section 9.4.3.
Section 1.1 Synopsis: Intervention Groups and Duration Section 4.1 Overall Design	Added that participants should remain confined in the study unit until completion of all assessments to ensure participant safety based on Investigator clinical judgement.	For clarity on timing of when participants can leave.
Section 1.1 Synopsis: Objectives and Endpoints Statistical Methods Section 3 Objectives and Endpoints Section 4.2 Scientific Rationale for Study Design (Figure 3) Section 8.1 Efficacy Assessments Section 9.1 Statistical Hypotheses Section 9.2 Sample Size Determination	Clarified that the fall in FEV_1 from baseline FEV_1 will be measured at the completion of mannitol challenge 2.	For clarification of when the assessment will be done.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1 Synopsis: Objectives and Endpoints Section 3 Objectives and Endpoints	Clinical abnormalities related to safety assessments added as endpoint along with AEs.	For clarification of what assessments will be done for safety.
Section 1.1 Synopsis: Objectives and Endpoints Section 3 Objectives and Endpoints Section 9.4.1 General Considerations Section 9.4.3.1 Primary Endpoint	Added clarification of the mannitol baseline and positive response.	Updated for further clarification of mannitol baseline definition.
Section 1.1 Synopsis: Number of Participants Intervention Groups and Duration Section 4.1 Overall Design Section 9.3 Population for Analyses	Updated descriptions of treatment sequence from A/B and B/A to A/B or B/A. Clarified that participants will be randomized to one of 2 treatment sequences.	For clarification/accuracy as participants will be randomized to either sequence, not both.
Section 1.1 Synopsis: Statistical Methods Section 9.4.3.1 Primary Endpoint	Replaced average baseline FEV_1 with baseline FEV_1.	Model statement updated to reflect the updated baseline definition.
Section 1.1 Synopsis: Intervention Groups and Duration Section 1.3 Schedule of Activities Table 1 Section 2.3 Benefit/Risk Assessment Section 4.1 Overall Design	Added a window for -2/+3 days at the follow up telephone call, 7 days after Visit 3.	Participant safety is not affected by including a visit time window, while allowing some flexibility for the visit.
Section 1.3 Schedule of Activities Table 1	Added distribution of Thank you Cards at Visit 3 and footnote i to add clarification of when Thank you Cards can be distributed.	Omitted from previous version.
Section 1.3 Schedule of Activities Table 1	Footnote “a” added to state that Visit 1 assessments should preferably be done on the same day of signing the ICF.	To ensure a consistent 10-to-14-day washout period between each visit as the wash-out time period begins once Visit 1 assessments are performed.
Section 1.3 Schedule of Activities Table 1	Clarified in footnote b when Visit 2 should happen in reference to after the assessments are done at Visit 1.	To clarify timing.

Section Number and Name	Description of Change	Brief Rationale
Section 1.3 Schedule of Activities Table 1	Added in footnote e the requirements for mannitol challenges 1 and 2, and added it is at the Investigator's discretion.	For clarification.
Section 1.3 Timed Assessments at Visit 2 and Visit 3 Table 3	Added that bEOS will be done at a local lab	Updated for clarity.
Section 1.3 Timed Assessments at Visit 2 and Visit 3 Table 3	Updated footnote a and e to j to clarify timepoints of assessments.	Updated for clarity.
Section 1.3 Timed Assessments at Visit 2 and Visit 3 Table 3	Added specification to footnote c that the following dose should be administered as soon as possible following spirometry between both mannitol challenges.	Updated for clarity.
Section 1.3 Timed Assessments at Visit 2 and Visit 3 Table 3	Split footnote d into 2 so information for mannitol challenge 1 and mannitol challenge 2 is separate and added clarification on the timing of assessments post-mannitol challenge 2.	Updated for clarity.
Section 1.3 Timed Assessments at Visit 2 and Visit 3 Table 3	Changed assessment of Perceived onset of effect from 430 minutes to 435 minutes.	Minute 430 is at the time of administration. To give sufficient time after dose received, assessment should be done at 435 minutes, 5 minutes after dose received.
Section 2 Introduction Section 2.2 Background Section 2.3.1 Risk Assessment	Updated text to indicate PT027 is approved.	PT027 (Airsupra) has now been approved in the US for the as-needed treatment or prevention of bronchoconstriction and to reduce the risk of exacerbations in patients with asthma 18 years of age and older (astrazeneca.com).
Synopsis: Objectives and Endpoints Section 3 Objectives and Endpoints Section 4.1 Overall Design	Clarified that a positive mannitol challenge response is defined as a ≥15% decrease in FEV_1 from the 0 mg FEV_1 value.	Updated for clarity.
Section 5.1 Inclusion Criteria	Added wording to inclusion criterion 6 to clarify that return to within 10% of baseline FEV_1 is ≥ 90% of baseline FEV_1.	Updated for clarity and consistency.
Section 5.2 Exclusion Criteria	Added text to exclusion criterion 5 to clarify that participants should not plan on starting immunotherapy during the study.	Updated for clarity.

Section Number and Name	Description of Change	Brief Rationale
Section 5.4 Screen Failures	Added clarification on what will define a screen failure.	Updated definition of screen failures for clarity.
Section 6.2.1 Metered Dose Inhaler Handling	Added text stating device should only be primed when the IMP is to be administered.	To have the IMP available for administration immediately after the completion of the positive mannitol challenge.
Section 7.2.1 FEV_1 Baseline, Mannitol Challenge Positive Reactivity, and FEV_1 Stability Criteria (Visit 3 only); formerly FEV_1 Baseline and Mannitol Stability Criteria	Added and clarified visits and requirements/criteria that participants must meet, or they will be withdrawn.	Added for clarity.
Section 7.2.1 FEV_1 Baseline, Mannitol Challenge Positive Reactivity, and FEV_1 Stability Criteria (Visit 3 only); formerly FEV_1 Baseline and Mannitol Stability Criteria Figure 4	Reordered bullet points for Visit 3 so the reference to the baseline 12% range is the first bullet.	To clarify the FEV_1 requirement at Visit 3 to proceed with the mannitol challenge.
	Added text to footnote to clarify that for Visit 2, the requirement to demonstrate FEV_1 of ≥ 60% to < 90% of the predicted value for FEV_1 applies only to Mannitol Challenge 1.	Updated for clarity and consistency.
Section 8.1 Efficacy Assessments	Updated and added detail of assessments.	Updated for clarity.
Section 8.2.1 Lung Function Test: Spirometry	Added specific timepoints for spirometry assessments to be done.	Updated for clarity.
Section 8.2.1 Lung Function Test: Spirometry	Added wording to clarify ethnic background groupings for spirometry purposes based on current available research.	NHANES III references values for spirometry classification are currently only validated for individuals of African American, Caucasian, and Mexican American background.
Section 8.3.3 Safety Spirometry	Clarified that FEV_1 criteria that apply to Visit 1 also apply to Visit 2.	For clarity and since it is safety criteria that will apply to both Visit 1 and 2.
Section 8.3.5 Clinical Safety Laboratory Assessments	Clarified that urinalysis will be performed at the Investigator site.	Updated for clarity.
Section 9.1 Statistical Hypotheses	Updated time to return to baseline FEV_1 for PT027 to PT007 from 3 minutes to 3.5 minutes.	For consistency with subsequent sections.

Section Number and Name	Description of Change	Brief Rationale
Section 9.2 Sample Size Determination	Updated Part 1 size from the first 16 randomized participants to approximately 16 participants.	For consistency with other sections in the document.
Section 9.3 Population for Analyses	Updated definition of safety analysis set, ie, delete participants "for whom any post dose data are available" and delete description of how occurrences of safety events will be summarized.	To align with safety analysis set definition per Therapeutic Area Statistical guidance.
Section 9.4 Statistical Analysis	Updated that the SAP will be finalized prior clinical database lock, instead of prior to review of any potential treatment-revealing data for the corresponding part of the study is undertaken or 90 days after the First Participant In.	Updated to text that is a more accurate description as a finalized SAP is not required until prior to clinical database lock and unblinding.
Section 9.4.1 General Considerations	Updated baseline definitions for primary and secondary endpoints.	Updated for further clarification of baseline definitions.
Section 9.4.3.1 Primary Endpoint	Deleted sentence: Baseline FEV_1 will be the average baseline FEV_1 defined as the mean of the period-specific baseline values taken pre-challenge 1 at Visit 2 and 3.	Baseline definitions updated, as per Table 11, and not accurate anymore, as well as being a repetition of definition already provided in table.
Section 9.4.4 Safety	Added specification that additional details will be available in the SAP for Part 2.	For clarity.
Section 9.5 Interim Analysis	Added that that Part 1 analyses will inform the treatment effect size, variability estimates, and "endpoint ordering" for the secondary efficacy endpoints in study Part 2.	For clarity.
Section 9.6.1 AstraZeneca Internal Advisory Board	Updated safety monitoring from AstraZeneca representative to Parexel representative.	Parexel representative will monitor the safety of the study on an ongoing basis in consultation with AstraZeneca Patient Safety.
Appendix A 1	Added regulatory reporting requirements for serious breaches	Requirement added per updated Sponsor template.
Appendix A 3	Added that participants must be re-consented if new information requires changes to the ICF.	Requirement added per updated Sponsor template.
Appendix A 4	Added regulatory text on Data Protection that data will be collected for business needs and data will be pseudonymized.	Requirement added per updated Sponsor template.

Abbreviations: CSR=Clinical Study Report; FEV_1=Forced Expiratory Volume in the first second; ICF=Informed Consent Form; IMP=investigational medicinal product; SAP, Statistical Analysis Plan.

CSP Version 2.0, Amendment 1: (02 August 2022)

Overall Rationale for the Amendment:

The overall aim for this amendment was to provide further clarity in areas of ambiguity, adjust study procedures to make these more participant-centric, and to remove surplus maneuvers and samplings, as detailed below.

Section # and Name	Description of Change	Brief Rationale	Substantial/ Non-substantial
Section 1.1 Synopsis and Section 4.1 Overall Design	Evaluable participants are defined as those who receive all 6 doses of the study medication.	Text updated for clarity.	Non-substantial
Section 1.3, Table 1 Schedule of Activities (Part 1 and Part 2)	Buccal swab sampling was removed, and "nasal swab" was reworded as "nasal scrape".	Nasal sampling was considered sufficient for collecting the required study data. Hence, to maintain participant-centricity, buccal swabs sampling was removed. The terminology was changed to "nasal scrape" as this was considered a more appropriate description.	Non-substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Non-substantial
Section 1.3 Table 3 Timed Assessments at Visit 2 and Visit 3	Time windows permitted for various assessments were specified.	To improve clarity.	Non-substantial
	Urine sample for safety assessments will be taken at only one timepoint (predose).	Surplus sampling was removed as it was deemed unnecessary with regards to participant safety.	Non-substantial
	Blood glucose and potassium will be measured in samples collected at specified timepoints of predose, 60, 90, and 490 minutes with respect to study treatment administration.	Sampling timepoints were adjusted to allow for better operational comfort for the participant. It was clarified that laboratory assessments of the chemistry panel for hematology are to be done predose and at 490 minutes post dose with respect to study treatment administration. In addition, samples will be collected at 60 and 90 minutes with respect to study treatment administration to measure blood glucose and potassium.	Non-substantial
	Vital signs monitoring will not be continuous but at the specified timepoints.	For the sake of participant-centricity and to be compliant with the standard of care during mannitol challenges where continuous vital signs monitoring is not included in routine clinical practice.	Non-substantial
	Footnotes were updated and re-ordered.	To be consistent with all changes made in this protocol amendment and to improve clarity.	Non-substantial
Section 5.2 Exclusion Criteria	Exclusion criteria regarding ICS use is now presented under the heading, “Prior/Concomitant Therapy”.	For the sake of correctness.	Non-substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Non-substantial
Section 5.4 Screen Failures	Text updated to clarify that a participant may be rescreened only once.	To improve clarity.	Non-substantial
Section 7 Discontinuation of Study Intervention and Participant Discontinuation/Withdrawal	Criteria for a participant to discontinue study treatment were included.	Changes made with a view to participant-centricity and safety.	Non-substantial
Section 7.2.1, Figure 4 High-level Schema of Study Stability Criteria FEV_1 and PD15 Mannitol	Figure was updated to correct the FEV_1 value requirements at Visits 1, 2, and Visit 3. A footnote was added for further information.	To correct previous errors and for better clarity.	Non-substantial
Section 1.3 and Section 8.2.1, Lung Function Test: Spirometry	Spirometry procedure was clarified as follows. • For spirometry occurring immediately prior to the mannitol challenge, a maximum of 8 maneuvers may be performed, until 3 technically adequate maneuvers are achieved. • For each post-mannitol challenge spirometry, at timepoints corresponding to 5, 10, and 15 minutes after completion of mannitol challenge 1 and mannitol challenge 2, a maximum of 8 maneuvers may be performed until 1 technically adequate maneuver is achieved. • For all other spirometry assessments, unless specified, a maximum	To be compliant with the standard practice.	Non-substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Non-substantial
	of 8 maneuvers may be performed until 2 technically adequate maneuvers are achieved.		
Section 9.1 Table 8 Hypotheses Tested	Text corrections.	Text was corrected to specify the mannitol challenge that is being referred to (mannitol challenge 1). Text on one of the endpoints was corrected to match the objective.	Non-substantial
Section 9.2 Sample Size Determination and Section 11 References	A cross-reference was corrected, and the correct reference was added in the References.	Correction of an error.	Non-substantial
Section 9.5 Interim Analyses	Text on the Part 1 analyses evaluation prior to Part 2 was updated.	Minor language changes.	Non-substantial
Appendix F 1 Instructions For Use - Clinic Appendix F 2 Instructions For Use – Participant	Parts of the oral inhaler users' manual that are not applicable to this study were deleted.	For the sake of correctness.	Non-substantial
Throughout	Updated terminologies per AZ guidance and standards, eg, changed "patient" to participant" wherever applicable.	To be consistent across the document and with AZ standards.	Non-substantial
	Minor editorial and document formatting revisions.	Minor, therefore, have not been summarized.	Non-substantial

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Document Name: d6930c00017-csp-v4		
Document Title:	D6930C00017 Clinical Study Protocol version 4	
Document ID:	Doc ID-004813887	
Version Label:	4.0 CURRENT LATEST APPROVED	
Server Date **(dd-MMM-yyyy HH:mm 'UTC'Z)**	**Signed by**	**Meaning of Signature**
12-Jul-2023 14:30 UTC	PPD	Management Approval
12-Jul-2023 16:35 UTC	PPD	Content Approval
12-Jul-2023 14:41 UTC	PPD	Management Approval

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