Statistical Analysis Plan

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A Double-Blind, Randomized, Parallel Group, Placebo-Controlled Multi-Centre Study to Evaluate the Effect of Benralizumab on Allergen-Induced Inflammation in Mild, Atopic Asthmatics

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF ABBREVIATIONS

The following abbreviations and special terms are used in this statistical analysis plan.

List of Abbreviations

Abbreviation or special term	Explanation
AE	Adverse Event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BMI	Body mass index
BUN	Blood urea nitrogen
[redacted]	[redacted]
CFU	Colony Forming Units
CO2	Carbon dioxide
CRF	Case Report Form (electronic/paper)
CSR	Clinical Study Report
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DAE	Discontinuation of Investigational Product due to Adverse Event
EAR	Early Allergic Response
ECG	Electrocardiogram
ECP	Eosinophil cationic protein
EDN	Eosinophil-derived neurotoxin
EoP	Eosinophil Progenitor
EOT	End of Treatment
ER	Emergency Room
FEV_1	Forced Expiratory Volume in 1 Second
GGT	Gamma-glutamyl transpeptidase
IL-3	Interleukin-3
IL-5	Interleukin-5

List of Abbreviations

Abbreviation or special term	Explanation
IL-5Rα	Interleukin-5 Receptor alpha
ILC2	Type 2 Innate Lymphoid Cell
IP	Investigational Product
IPD	Important Protocol Deviation
ITT	Intent-to-Treat
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LAR	Late Allergic Response
LTRA	Leukotriene Receptor Antagonist
MBP	Major Basic Protein
MCh	Methacholine
MedDRA	Medical Dictionary for Regulatory Activities
MCV	Mean Corpuscular Volume
MMRM	Mixed models for repeated measures
NSAID	Non-Steroidal Anti-Inflammatory Drug
PT	Preferred Term
RBC	Red blood cell
SAE	Serious adverse event
SABA	Short-acting Beta Agonist
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SC	Subcutaneous
SD	Standard Deviation
SOC	System Organ Class
TBL	Total bilirubin
ULN	Upper Limit of Normal
WBC	White blood cell
WHO	World Health Organization

AMENDMENT HISTORY

Category*: Change refers to	Date	Description of change	In line with the CSP?	Rationale
N/A	12/January/2018	V1.0 Initial Version	Y, CSP V3.0	N/A
Primary endpoints	22/October/2019 V2.0	Sensitivity analysis on primary endpoints to exclude subjects with allergen challenge post-screening doses that differ from Screening Allergen Challenge doses as well as subjects with IPD likely to affect interpretation of efficacy.	Y, CSP V4.0	Review of subject data during BDR1
Primary and secondary endpoints	22/October/2019 V2.0	Primary efficacy analysis population was added to allow evaluation of the allergen-induced change endpoints (including the two primary endpoints) excluding subjects with a higher allergen-challenge dose post-screening.	N, CSP V4.0	Subjects with higher allergen-challenge dose post-screening represent an IPD that may affect the interpretation of allergen-induced change endpoints.
Other	22/October/2019 V2.0	Editorial updates are not listed; updates were made to ensure consistent use of terminology, reduce redundancies, and consolidate text related to definitions of baseline and change from baseline throughout the document	Y, CSP V4.0	To add clarity and improve readability
Other	22/October/2019 V2.0	Additional detail added for exploratory endpoints to align with the CSP.	Y, CSP V4.0	Exploratory endpoints and corresponding analyses were not adequately addressed in V1.0 of the SAP.
Other	22/October/2019 V2.0	Updated definition of important protocol deviations and added Appendix 8.2 to provide clarity on derivations	Y, CSP V4.0	To align with current AZ guidelines for IPD identification and reporting

Data presentation	22/October/2019 V2.0	Added Appendix 8.3 to map efficacy endpoints based on laboratory data to laboratory test codes used for analysis and to clarify the test names/labels to be used in reporting	Y, CSP V4.0	To provide additional clarification for analysis based on data transfer specifications

* Pre-specified categories are Primary or secondary endpoints; Statistical analysis method for the primary or secondary endpoints; Derivation of primary or secondary endpoints; Multiple Testing Procedure; Data presentations; Other

1. STUDY DETAILS

This statistical analysis plan (SAP) outlines the analysis to be generated for the global clinical study report (CSR).

1.1 Study objectives

1.1.1 Primary objective

Table 1 Primary study objectives

Objective	Endpoint
To evaluate the effect of benralizumab on allergen-induced increases in eosinophils in induced sputum	Change in percent of eosinophils in sputum 7 hours post-allergen challenge
To evaluate the effect of benralizumab on the allergen-induced late (3 – 7 hrs post-challenge) asthmatic response (LAR)	Primary measure: Maximum percentage decrease in Forced Expiratory Volume in 1 Second (FEV_1) 3-7 hours post-allergen challenge ($LAR_{3\text{-}7\ hr}$) Supportive measure: Area under the curve (AUC) of time adjusted percent decrease in FEV_1 curve in late asthmatic response ($LAR_{3\text{-}7}$ hr)

AUC = Area Under the Curve, FEV_1 = Forced Expiratory Volume in 1 second, LAR = Late Allergic Response

1.1.2 Secondary objectives

Table 2 Secondary study objectives

Objective	Endpoint
To assess the effect of benralizumab on allergen-induced increases in number of eosinophils in induced sputum	Change in percent of eosinophils in sputum 24 hours post-allergen challenge
To assess the effect of benralizumab on allergen-induced increases in number of basophils in induced sputum	Change in percent of basophil numbers by toluidine blue staining

Table 2 Secondary study objectives

Objective	Endpoint
To assess the effect of benralizumab on allergen-induced early (within 2 hrs post-allergen challenge) asthmatic response (EAR)	Maximum percentage decrease in FEV_1 0 – 2 hours post-allergen challenge ($EAR_{0\text{-}2\ hr}$) AUC of time adjusted percent decrease in FEV_1 curve in early asthmatic response ($EAR_{0\text{-}2\ hr}$)
To assess the effect of benralizumab on allergen-induced increases in number of eosinophils and basophils in lung tissue biopsies	Change in eosinophil and basophil numbers in endobronchial biopsies from Visit 8 pre-challenge to 24 hours post-allergen challenge (in a sub-set of subjects)
To assess the effect of benralizumab on allergen-induced increases in eosinophils, eosinophil progenitor cells and basophils in bone marrow aspirates	Change in eosinophils by smears, eosinophil progenitor cells and basophils by flow cytometry in bone marrow aspirates, from pre-challenge to 24 hours post-allergen challenge (in a sub-set of subjects)
To assess the effect of benralizumab on allergen-induced increases in eosinophils and basophils in blood	Change in eosinophil counts (by central lab count) and basophil counts (by flow cytometry) in blood from pre-challenge to 24 hours post-allergen challenge
To assess the effect of benralizumab on baseline levels of eosinophil and basophil inflammation in sputum, blood, bone marrow aspirates and lung tissue prior to allergen challenge	Change in percent of eosinophils in cytospins and basophils by toluidine blue staining from baseline to Visit 10 in induced sputum. Change in eosinophil counts (by central lab count) and basophil counts (by flow cytometry) from baseline to Visit 10 in blood Change in eosinophils by smears, eosinophil progenitor cells and basophils measured by flow cytometry from baseline to Visit 10 in bone marrow aspirates (in a sub-set of subjects) Change in eosinophil and basophil numbers from baseline to Visit 8 in endobronchial biopsies (in a sub-set of subjects)
To evaluate the effect of benralizumab on airway hyper-responsiveness post-allergen challenge	Methacholine (MCh) PC20 (concentration of inhaled MCh that produces a 20% fall in FEV_1)

AUC = Area Under the Curve, EAR = Early Allergic Response, FEV_1 = Forced Expiratory Volume in 1 second, LAR = Late Allergic Response, MCh = Methacholine

1.1.3 Safety objectives

Table 3 Safety objectives

Objective	Endpoint
To assess the safety and tolerability of benralizumab in subjects with mild atopic asthma	Adverse Events (AEs) and Serious adverse events (SAEs) Vital signs Electrocardiogram (ECG) Clinical chemistry/hematology/urinalysis Physical examination

AE = Adverse Events, ECG = Electrocardiogram, SAE = Serious adverse event

1.1.4 Exploratory objectives

Table 4 Exploratory objectives

Objective	Endpoint
[redacted]	[redacted]

CSR = Clinical Study Report, IL-5 = Interleukin-5, ILC2 = Type 2 Innate Lymphoid Cell

1.2 Study Design

This randomized, double-blind, parallel group, placebo-controlled study will evaluate the effect of a fixed 30 mg dose of benralizumab administered subcutaneous (SC) every 4 weeks for 3 doses on allergen-induced inflammation in subjects with mild atopic asthma challenged with an inhaled allergen.

Approximately 38 non-smoking men and women (18 – 65 years of age) corticosteroid-free (oral and inhaled) mild, atopic asthmatics who have demonstrated a dual (early and late) asthmatic response to inhaled allergen challenge at screening will be recruited to complete the study.

This study will include 6 centers in [redacted] Canada. All centers will participate in the characterization of the early and late asthmatic response and induced sputum collections. A subset of 5 centers will conduct the

endobronchial biopsy collections, and bone marrow aspirates will be conducted at one center only.

After enrolment and confirmation of entry criteria, subjects will enter a 4-week run-in period during which their suitability for randomization will be confirmed and to allow resolution of airway inflammation induced from the allergen challenge screening assessments. Subjects who meet all the eligibility criteria will return to the clinic for collection of tissue samples according to the sub-group they are assigned to (sputum, bone marrow aspirates, lung biopsies and blood) for assessment of baseline inflammation. This will be conducted at 2 visits over a 3-day period.

Subjects will then be randomized to receive either benralizumab 30 mg subcutaneous (SC) or matching placebo (1:1). Subjects will receive 2 additional doses of investigational product (IP) every 4 weeks for a total of 3 doses.

Twenty-eight days following the first dose, subjects will return to the clinic for assessment of dosing effects on airway hyperresponsiveness (MCh PC20) and collection of a blood sample for eosinophil and serum biomarkers measurements and a sputum sample for eosinophil and biomarker measurements.

Twenty-eight days following the second dose, subjects will return to the clinic for assessment of dosing effects on baseline inflammation in the lung biopsies (only subjects participating in the endobronchial biopsy collections) and collection of a blood sample (all sites) for blood eosinophil measurements. Subjects will then receive a third dose of IP and return to the clinic no less than 7 days later for the first allergen challenge period.

The first allergen challenge will assess effects of benralizumab on the primary endpoint of allergen–induced eosinophils in induced sputum and the LAR. The first allergen challenge and associated assessments will take place over 3 consecutive days. At least twenty-one days and no more than 28 days following the first allergen challenge, subjects participating in the endobronchial biopsy collections will return to the clinic for a second allergen challenge. The second allergen challenge will assess effects of benralizumab on the secondary endpoint of allergen–induced eosinophils and basophils in lung tissue. Subjects will return to the clinic 8 weeks and 12 weeks following the last dose of benralizumab for end of treatment (EOT) and follow-up assessments, respectively.

1.3 Number of subjects

It is expected that approximately 38 subjects in total and 19 subjects in each treatment group are needed for this study. This is based on two primary endpoints: 1) the allergen-induced change in percentage of eosinophils in induced sputum at 7-hr post-allergen challenge during Allergen Challenge 1, and 2) the maximum percent decrease in FEV_1 in late asthmatic response ($LAR_{3\text{-}7\ hr}$), also during Allergen Challenge 1. These endpoints will be tested using a hierarchical fixed sequence approach (first assessing the effect on percentage of eosinophils in sputum) as described in Protocol section 8.5.

For the percent eosinophils in induced sputum, it is assumed the mean of placebo group and the standard deviation (SD) are similar to sputum eosinophil data from a previously conducted study (mean of 20.8, and a SD of 17.3 at 7-hr post-challenge in placebo group). A sample size of approximately 19 subjects per treatment group (38 total) will be needed to achieve 80% power of detecting an 80% reduction in the benralizumab group versus placebo, assuming mean of 20.8 in placebo group, and a SD of 17.3. This calculation assumed a two-sided 5% alpha level.

For the maximum percent decrease in FEV_1 $LAR_{3\text{-}7hr}$, a sample size of approximately 19 subjects per treatment group (38 total) will be needed to achieve 80% power of detecting a 11% absolute difference between benralizumab group and placebo, assuming a SD of 12% (similar to the variability estimated in Gauvreau et al 2014

. This calculation assumed a two-sided 5% alpha level.

Based on previous studies with bone marrow aspirate collections, it is estimated that approximately 22 subjects are required to be able to detect meaningful differences between placebo and active treatment.

Although formal powering has been used to estimate the sample size required for the primary endpoint, it is expected to be sufficient for all endpoints using an estimation type approach that has been used for most studies of this type.

2. ANALYSIS SETS

2.1 Definition of analysis sets

2.1.1 All subjects analysis set

This analysis set will comprise all subjects screened for the study and will be used for reporting of disposition and screening failures.

2.1.2 Full analysis set

The full analysis set consists of all randomized subjects who received at least one dose of investigational product (IP) irrespective of their protocol adherence and continued participation in the study. Subjects will be analyzed according to the randomized treatment assignment, irrespective of whether or not they have prematurely discontinued, according to the ITT principle. Subjects who withdraw consent to participate in the study will be included up to the date of their study termination. Subjects with data available for baseline and at least one post baseline assessment will be included in the corresponding analysis.

This analysis set will be used for the primary efficacy endpoints as well as the effect on inflammation endpoints. For consistency, demographic, baseline characteristics and safety objectives will be presented using the full analysis set.

2.1.3 Primary efficacy analysis set

The primary efficacy analysis set will be the same as the full analysis set, excluding subjects who received a higher allergen challenge dose post-screening compared to the allergen challenge dose given at the screening allergen challenge. This analysis set will be considered the primary analysis set for all allergen-induced change efficacy endpoints, including the co-primary efficacy endpoints.

2.1.4 Safety analysis set

The safety analysis set will be the same as the full analysis set. Any major deviations from the randomized treatment assignment will be listed and considered when interpreting the safety data. All safety summaries will be based on this analysis set.

2.2 Randomization

Subjects will be randomized in a 1:1 ratio to benralizumab or placebo.

In order to have balanced number of subjects for the outcomes from induced sputum, blood, bone marrow and bronchoscopy measures, randomization will be stratified by the following types of measurements: 1) bone marrow plus bronchoscopy measurements, 2) bronchoscopy measures only, and 3) neither bone marrow nor bronchoscopy measurements.

Randomization codes will be assigned strictly sequentially as subjects become eligible for randomization.

Specific information concerning the use of the Interactive Voice Response System (IVRS)/Interactive Web Response System (IWRS) will be provided in the separate manual.

Subjects who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be randomized or receive study medication. There can be no exceptions to this rule.

2.3 Blinding and Unblinding

Individual treatment codes, indicating the treatment randomization for each randomized subject, will be available to the Investigator(s) or pharmacists from the IVRS/IWRS. Routines for this will be described in the IVRS/IWRS user manual that will be provided to each center.

The treatment code should not be broken except in medical emergencies when the appropriate management of the subject requires knowledge of the treatment randomization. The Investigator documents and reports the action to AstraZeneca, without revealing the treatment given to the subject to the AstraZeneca staff.

AstraZeneca staff may decide to break the code for SAEs that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

2.4 Violations and deviations

Subjects who do not meet eligibility criteria but are still randomized will be analyzed according to the analysis sets described in Section 2.1.

2.4.1 Important protocol deviations (IPD)

Important protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being.

All protocol deviations will be reviewed by medical advisors and statisticians prior to database lock to determine those which are considered important deviations as outlined below, with additional details provided in Appendix 8.2:

- Subjects who did not meet key eligibility criteria (inclusion/exclusion criteria)
- Deviations from informed consent procedures
- Subjects who received prohibited/restricted concomitant medication(s)
- Subjects who developed IP discontinuation criteria during the study but were not withdrawn from IP
- IP management and administration
- Subjects with deviations from study procedures
- Other potential important deviations, such as allergen challenge dose differences between screening and post-baseline, and severe non-compliance with the protocol

Subjects who received incorrect IP will be identified after database lock and unblinding. These subjects will be classified according to the treatment groups they were randomized to for the full analysis set.

Only important protocol deviations will be summarized by treatment group and provided in listings.

For a discussion of IPDs that are exclusionary for the primary efficacy endpoint sensitivity analyses, refer to Section 3.2.

2.4.2 Visit window definitions

For all efficacy analyses, no windows will be applied. For laboratory, ECGs and vital signs, the nominal visit will be used for summaries by visit. For shift tables and tables that present the minimum or maximum value across visits, unscheduled visits will be considered.

2.4.3 The definition of baseline, change from baseline, percent change from baseline

For efficacy endpoints of allergen-induced change, the pre-challenge measurement at the corresponding challenge period will serve as baseline within that challenge period.

For efficacy endpoints of effect on baseline inflammation, baseline will be defined as the last recorded value (excluding post-challenge values) on or prior to the date of first IP (Visit 4). For efficacy parameters that were collected at both Visit 1b and Visit 4, if the value at Visit 4 is missing, then the value at Visit 1b will be used as baseline, unless otherwise specified. For endobronchial biopsy measures, baseline is defined as the value at Visit 5.

For safety endpoints, baseline will be defined as the last recorded value on or prior to the date of first IP (Visit 4).

Change from baseline for allergen-induced change is defined as the value post-challenge minus the value pre-challenge, for each triad. Change from baseline for effect on baseline inflammation and for safety endpoints is defined as the post-baseline value minus the baseline value.

Percent change from baseline is computed as ((visit value – baseline value)/baseline value) × 100%. If either a visit value or the baseline visit value is missing, the absolute change from baseline value and the percent change from baseline will also be set to missing.

2.4.4 Prior/concomitant medications

A medication will be regarded as prior if the start date is prior to the date of randomization and the stop date is on or prior to the date of randomization.

A medication will be regarded as concomitant if the start date is after the date of randomization, or if it started on or prior to the date of randomization and was ongoing after the date of randomization.

Partial concomitant medication start and end dates will be imputed as described in Appendix 8.1. Medications with missing start and end dates will be considered to be concomitant.

3. PRIMARY, SECONDARY, AND EXPLORATORY VARIABLES

This study has three allergen challenges: Screening (Visit 1b, Visit 2 and Visit 3), Allergen Challenge 1 (Visits 10-12) and Allergen Challenge 2 (Visits 13-15). Each allergen challenge has three time points (referred to as a triad): pre-challenge, 7-hours post-challenge and 24-hours post-challenge. Allergen-induced change will refer to change within an allergen challenge triad and will be calculated as defined in Section 2.4.3.

No imputations for missing primary and secondary variable values will be made.

3.1 Primary outcome variables

The study has two primary endpoints,

1. Allergen-induced change in percentage of eosinophils in induced sputum at 7-hr post-allergen challenge during Allergen Challenge 1,

2. Maximum percent decrease in FEV_1 in late asthmatic response $(LAR)_{3\text{-}7\ hr}$ during Allergen Challenge 1

3.1.1 Allergen-induced change in percentage of eosinophils in induced sputum post-allergen challenge during Allergen Challenge 1

There are 2 triads where percentage of eosinophils in induced sputum are collected in the study – at Screening (pre-IP treatment) and at Allergen Challenge 1 (post-IP treatment). Each allergen challenge triad consists of three measurements: pre-challenge, 7-hr and 24-hr post-challenge. Table 5 shows the timepoints for allergen-induced change in percentage of eosinophils in induced sputum.

Table 5 Timepoints for percentage of eosinophils in induced sputum during allergen challenge

Triad	Pre-challenge	7-hr post-challenge	24-hr post-challenge
Screening (pre-IP treatment)	Visit 1b	Visit 2	Visit 3
Allergen Challenge 1 (post-IP treatment)	Visit 10	Visit 11	Visit 12

IP = Investigational Product

The allergen-induced change at 7-hr and 24-hr post-challenge will be derived for each allergen challenge as defined in Section 2.4.3.

The allergen-induced change in percentage of eosinophils in induced sputum for Allergen Challenge 1 at 7 hours post-challenge will be the primary endpoint.

3.1.2 Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ during allergen challenge

Prior to beginning each inhaled allergen challenge, pre-challenge FEV_1 will be measured with 3 technically satisfactory readings. The highest value will be entered into the Case Report Form (CRF) and will be used for calculation of the percentage decrease in FEV_1. The FEV_1 will then be measured at regular intervals until 7 hours post-allergen inhalation: 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 minutes post-allergen inhalation. The minimum measurement between 180 minutes (3 hours post-challenge) and 420 minutes (7 hours post-challenge) will be used to calculate the maximum percent decrease in FEV_1 in late asthmatic response ($LAR_{3\text{-}7\ hr}$).

FEV_1 measurements will be taken at the following 3 visits: Visit 2 (Screening), Visit 11 (Allergen Challenge 1), and Visit 14 (Allergen Challenge 2). Table 6 shows the timepoints for maximum percent decrease in FEV_1 in LAR.

Table 6 Timepoints for maximum percent decrease in FEV_1 in LAR

Triad	Pre-challenge	Post-challenge $LAR_{3\text{-}7\ hr}$
Screening (pre-IP treatment)	Visit 2 pre-challenge	Visit 2 post-challenge
Allergen Challenge 1 (post-IP treatment)	Visit 11 pre-challenge	Visit 11 post-challenge
Allergen Challenge 2 (post-IP treatment)	Visit 14 pre-challenge	Visit 14 post-challenge

FEV_1 = Forced Expiratory Volume in 1 Second, IP = Investigational Product, LAR = Late Allergen Response

Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ for each allergen challenge will be calculated as defined in Section 2.4.3. The maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ for Allergen Challenge 1 (Visit 11) is considered the primary endpoint.

It is worth noting that subjects at sites not participating in the endobronchial biopsy collection will not participate in Allergen Challenge 2 (i.e. no Visit 14 FEV_1 assessments). Therefore, the number of subjects contributing to the analysis of maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ for Allergen Challenge 2 is expected to be smaller.

3.2 Sensitivity Analyses

The primary endpoint analyses, allergen-induced change in percentage of eosinophils in induced sputum for Allergen Challenge 1 at 7 hours post-challenge and maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ for Allergen Challenge 1, will be repeated on the primary efficacy analysis set, excluding any subjects where the allergen challenge dose was lower than the Screening Allergen Challenge dose as well as any subjects with an IPD of use of a systemic corticosteroid within 1 week prior to the allergen challenge study visit, as a sensitivity analysis. Any additional exclusionary IPDs (i.e. having the potential to impact on the interpretation of efficacy) for this sensitivity analysis will be identified and documented in the trial master file prior to database lock. There will be no imputations for missing data.

3.3 Supportive analysis to primary outcome variables

3.3.1 AUC of time adjusted percent decrease in FEV_1 curve in late asthmatic response $(LAR)_{3\text{-}7\ hr}$ during allergen challenge

The total AUC (L*Hour) for percent decrease in FEV_1 (L) in $LAR_{3\text{-}7\ hr}$ will be estimated using the linear trapezoidal rule using all available FEV_1 data between and inclusive of the 3 to 7 hours post-allergen challenge timepoints. Measurements at 180, 240, 300, 360 and 420 minutes post-dose will be used in the calculation. At each timepoint after 180 minutes, the AUC from the previous timepoint is calculated by taking the mean of the two FEV_1 scores

multiplied by the number of hours between measurements. In order to account for missing data, the time adjusted total AUC for percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ is calculated as total AUC/ (the length of time (hr) the total AUC is calculated). To calculate the length of time, the latest LAR measurement timepoint up to 420 minutes minus the earliest LAR measurement for a subject will be divided by 60 to determine the length of time in hours. For example, if the latest timepoint for a subject is at the 420 minute timepoint, 180 minutes (the first timepoint) will be subtracted from 420 minutes and divided by 60, resulting in a 4 hour length of time.

3.4 Secondary efficacy outcome variables

3.4.1 Percentage of eosinophils in induced sputum for the effect on baseline inflammation

Eosinophils in induced sputum are collected at Visit 4 (pre-IP dose), and at Visit 7 and Visit 10. The change from baseline (as defined in Section 2.4.3) of percent eosinophils to each post-baseline visit will be used in the analysis.

3.4.2 Basophil count in induced sputum for both allergen challenge and baseline inflammation

Similar to change from baseline of percent eosinophils in induced sputum, there are 2 periods of measurements for basophil count in induced sputum:

1. allergen-induced change at 7-hr and 24-hr post-allergen challenge to evaluate allergen response at Screening and Allergen Challenge 1, and

2. measurements for effect on baseline inflammation at Visit 4 (baseline), Visit 7 and Visit 10.

Basophil count in sputum will be obtained by two types of readings: [redacted] and cytospins with toluidine blue staining (a secondary endpoint). Refer to Table 7 below for the scheduling of readings for basophil counts in induced sputum during allergen challenge and to Table 8 below for basophil counts in induced sputum for baseline inflammation.

Table 7 Timepoints for basophil count in induced sputum during allergen challenge

	Pre-challenge	7-hr post	24-hr post
Screening	cytospins with toluidine blue staining	cytospins with toluidine blue staining	cytospins with toluidine blue staining
Allergen Challenge 1	flow cytometry; cytospins with toluidine blue staining	cytospins with toluidine blue staining	flow cytometry; cytospins with toluidine blue staining

Table 8 Timepoints for basophil count in induced sputum for baseline inflammation

Visit 4 (baseline)	Visit 7	Visit 10
flow cytometry; cytospins with toluidine blue staining	cytospins with toluidine blue staining	flow cytometry; cytospins with toluidine blue staining

The allergen-induced change and change from baseline (as defined in Section 2.4.3) for basophils will be used in the analysis.

3.4.3 Maximum percent decrease in FEV_1 in early asthmatic response 0-2 hr (EAR_{0-2hr})

Maximum percent decrease in FEV_1 in $EAR_{0-2\ hr}$ for each allergen challenge will be calculated similarly to maximum percent decrease in FEV_1 for LAR, as defined in Section 3.1.2, using FEV_1 measurements at 10, 20, 30, 45, 60, 90 and 120 minutes post-allergen challenge.

3.4.4 AUC of time adjusted percent decrease in FEV_1 curve in early asthmatic response ($EAR_{0-2\ hr}$)

The AUC of time-adjusted percent decrease in FEV_1 in $EAR_{0-2\ hr}$ will be estimated similarly to the AUC of time-adjusted percent decrease in FEV_1 in LAR, as described in Section 3.3.1, using all available FEV_1 data between and inclusive of the 0 to 2 hour timepoints (0, 10, 20, 30, 45, 60, 90 and 120 minutes post-challenge).

3.4.5 Number of eosinophils and basophils in lung tissue biopsies for both allergen challenge and baseline inflammation

Biopsy samples are collected at Visit 5 (baseline), Visit 8 (pre-allergen challenge), and Visit 15 (24-hr post-allergen challenge). Effect on baseline inflammation will be evaluated by change from baseline (Visit 5) to Visit 8. Effect on allergen-induced response will be evaluated by change from pre-challenge (Visit 8) to post-challenge (Visit 15).

3.4.6 Eosinophil, eosinophil progenitor cells and basophils in bone marrow aspirates for both allergen challenge and baseline inflammation

Eosinophils, eosinophil progenitor cells, and basophils in bone marrow aspirates are collected in the bone marrow aspirate sub-group of subjects at Visit 4 (baseline), Visit 10 (pre-allergen challenge) and Visit 12 (post-allergen challenge). Effect on baseline inflammation will be evaluated by change from baseline (Visit 4) to Visit 10. Effect on allergen-induced response will be evaluated by change from pre-challenge (Visit 10) to post-challenge (Visit 12).

3.4.7 Eosinophil and basophil counts in blood for both allergen challenge and baseline inflammation

Similar to percentage of eosinophils in induced sputum, there are 2 periods of measurements for eosinophils in blood:

1. allergen-induced change at 7-hr and 24-hr post-allergen challenge to evaluate allergen response at Allergen Challenge 1 and Allergen Challenge 2, and

2. measurements for effect on baseline inflammation at Visits 4 (baseline), 7, 9 and 10.

For basophil counts in blood, only Allergen Challenge 1 is assessed for allergen-induced change and only Visit 4 and Visit 10 are assessed for effects of benralizumab on baseline inflammation.

Table 9 shows the timepoints for allergen-induced change in eosinophil counts in blood. Table 10 shows the timepoints for allergen-induced change in basophil counts in blood.

Table 9 Timepoints for eosinophil counts in blood during allergen challenge

Triad	Pre-challenge	Post-challenge
Allergen Challenge 1 (post-IP treatment)	Visit 10	Visit 11 and Visit 12
Allergen Challenge 2 (post-IP treatment)	Visit 13	Visit 14 and Visit 15

IP = Investigational Product

Table 10 Timepoints for basophil counts in blood during allergen challenge

Triad	Pre-challenge	Post-challenge
Allergen Challenge 1 (post-IP treatment)	Visit 10	Visit 12

IP = Investigational Product

3.4.8 MCh PC20

Methacholine (MCh) PC20 is defined as the concentration of inhaled MCh that produces a 20% fall in FEV_1. Similar to percentage of eosinophils in induced sputum, there are 2 periods of measurements for MCh PC20:

1. allergen-induced change at 24-hr post-allergen challenge to evaluate allergen response at Screening and Allergen Challenge 1, and

2. measurements for effect on baseline inflammation at Visits 4 (baseline), 7 and 10.

For allergen-induced change, see Table 11 for corresponding visits.

Table 11 Timepoints for MCh PC20 during allergen challenge

Triad	Pre-challenge	Post-challenge
Screening (pre-IP treatment)	Visit 1b	Visit 3

Table 11 Timepoints for MCh PC20 during allergen challenge

Triad	Pre-challenge	Post-challenge
Allergen Challenge 1 (post-IP treatment)	Visit 10	Visit 12

IP = Investigational Product, MCh = Methacholine

3.5 Exploratory efficacy outcome variables

3.5.1 [redacted]

[redacted]

[redacted]

3.6 Safety outcome variables

3.6.1 Adverse Events

Adverse events experienced by the subjects will be collected throughout the entire study and will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) per the Data Management Plan.

Adverse event data will be categorized according to their onset date into the following study periods:

- AEs in the pre-treatment period are defined as those with onset date after informed consent and prior to day of first dose of IP
- AEs in the on-study period are defined as those with onset between day of first dose of IP and the day of scheduled follow-up, inclusive.

If an AE has a missing onset date, then unless the stop date of the AE indicates otherwise, this will be considered an on-study event. Similarly, if an AE has a partial onset date, then unless the partial onset date or the stop date indicates otherwise, this will be considered an on-study AE. Partial AE start and end dates will be imputed as per the algorithms described in Appendix 8.1.

3.6.2 Laboratory Variables

Safety laboratory tests (list provided in Table 14) will be performed in a central laboratory. Chemistry samples will be collected at Visits 1a, 7, 9 and 16. Hematology samples will be collected at Visits 1a, 7, 16 and 17. Urinalysis samples will be collected at Visits 1a, 7 and 16.

The Investigator should make an assessment of the available results with regard to clinically relevant abnormalities. Clinically relevant abnormalities will be recorded as adverse events.

Table 14 List of Safety Laboratory Tests

Serum Chemistry		Hematology	Urinalysis
Alkaline phosphatase	G Gamma-glutamyl transpeptidase (GGT)	Hematocrit	Appearance
ALT (alanine aminotransferase)	Glucose	Hemoglobin	Blood
AST (aspartate aminotransferase)	Phosphorus	Mean corpuscular volume (MCV)	Colour
BUN (blood urea nitrogen)	Potassium	Platelet count	Glucose
C-reactive protein	Sodium	Red blood cell (RBC) count	Ketones
Calcium	Total bilirubin	RBC morphology	

Table 14 List of Safety Laboratory Tests

Serum Chemistry		Hematology	Urinalysis
Chloride	Total cholesterol	White blood cell (WBC) count with differentials[a]	
CO2 (carbon dioxide)	Uric acid		
Creatinine			

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, BUN = Blood urea nitrogen, CO2 = Carbon dioxide, GGT = gamma-glutamyl transpeptidase, MCV = Mean corpuscular volume, RBC = Red blood cell, WBC = white blood cell

[a] After Visit 1a, the total white cell count as well as neutrophil and lymphocyte counts will be reported to the investigate site. Note the eosinophil, basophil and monocyte counts will be redacted from the report.

Changes in hematology and clinical chemistry variables between baseline and each post-baseline assessments will be calculated. There will be no imputation for missing values. For values recorded with a leading greater than or less than ('>', '<') symbol, the reported numeric value will be used for analysis and the value with the symbol will be included in the listings, unless otherwise specified. For example, a value of <0.01 will be analyzed as 0.01 and listed as <0.01.

Absolute values will be compared to the relevant reference range and classified as low (below range), normal (within range or on limits) or high (above range). The central laboratory reference ranges will be used for laboratory variables. All absolute values falling outside the reference ranges will be flagged.

Urinalysis data will be categorized as negative (0), positive (+), or strongly positive (++, +++, or >++++) at each timepoint.

For the purposes of hematology, clinical chemistry and urinalysis shift tables, maximum or minimum value post-baseline will be calculated over the entire post-baseline period, including the post-treatment period and unscheduled visits.

For the liver function tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transpeptidase (GGT) and total bilirubin (TBL), the multiple of the central laboratory upper limit of the normal (ULN) range will be calculated for each data point.

Subjects who meet any of the following criteria at any point during the study will be flagged:

- AST≥3xULN
- AST≥3xULN
- TBL≥2xULN

3.6.3 Vital Signs

Pre-IP dose vital signs (pulse, blood pressure, respiration rate and body temperature) will be obtained at Visits 1a, 6, 7, 9 and 16. Height and weight will be collected at Visit 1a.

Changes in vital signs variables between baseline and each subsequent scheduled assessment will be calculated. There will be no imputation for missing values.

Absolute values will be compared to the reference ranges in Table 15 and classified as low (below range), normal (within range or on limits) or high (above range). All values (absolute and change) falling outside the reference ranges will be flagged.

Table 15 Vital Signs Reference Ranges

Parameter	Standard units	Lower limit	Upper limit
Diastolic Blood Pressure (DBP)	mmHg	60	120
Systolic Blood Pressure (SBP)	mmHg	100	160
Pulse Rate	Beats/min	40	120
Respiratory Rate	Breaths/min	8	28
Body Temperature	Celsius	36.5	38

DBP = Diastolic Blood Pressure, SBP = Systolic Blood Pressure

Body mass index (BMI) will be calculated from the height and weight as follows:

BMI (kg/m^2) = weight (kg) / $(\text{height (m)})^2$

3.6.4 ECGs

ECG will be performed at Visits 1a and 16.

The investigator or authorized delegate will be responsible for the overall interpretation and determination of clinical significance of any potential ECG findings.

3.6.5 Physical Examination

Complete physical examination will be performed at Visit 1a and 16. Any new finding(s) or aggravated existing finding(s), judged as clinically significant by the investigator, will be reported as an AE.

4. ANALYSIS METHODS

4.1 General Principles

The analysis of the primary, secondary and exploratory endpoints will include all data captured during the study, unless the subject withdraws consent (or assent when applicable) to study participation, regardless of whether IP was prematurely discontinued, or delayed, and/or irrespective of protocol adherence, unless otherwise specified.

The data analysis will be conducted using the SAS® System (SAS Institute Inc., Cary, NC). All SAS® programs used to generate analytical results will be developed and validated according to Rho, Inc. SAS® programming standards and validation procedures.

Descriptive statistics will also be provided for safety and efficacy data. Unless otherwise stated, the data analysis will include subjects in the full analysis set. Descriptive statistics on continuous variables will be summarized by treatment group using mean, standard deviation, minimum, median and maximum, while categorical data will be summarized using frequency counts and percentages. Data that are log-transformed will also present the geometric mean and geometric coefficient of variation (CV). When data are summarized by time, the values recorded against the scheduled time points listed in the protocol will be used. When assessing minimum/maximum increases or decreases during the study, all assessments, including unscheduled assessments will be used. For analysis assessing change and percent change from baseline, only subjects with both baseline and at least 1 evaluable post-baseline measure will be included. Nominal visit will be used for all summary and analysis, and no analysis windows will be applied.

All hypothesis testing will be reported using two-sided tests. There are two primary endpoints for this study and adjustments for multiple comparisons will be made. All other p-values will be nominal (i.e., not multiplicity adjusted). P-values will be rounded to 4 decimal places.

Since the availability of certain data (i.e. bone marrow and bronchoscopy measures) are predicated on the type of study site, and the planned sample sizes from each stratum will be small, all analyses will use the available data and there will be no stratification of data summaries based on site.

When assessing the allergen challenge triads (Screening/Allergen Challenge 1/Allergen Challenge 2) and MCh PC20 timepoints, if unscheduled or repeated visits exist then the data from the latest record associated with the nominal visit will be used for analysis. These repeated/unscheduled visits are performed only when there are procedural issues with the original allergen and methacholine challenges.

Per the protocol, the same doses of allergen as used at the Screening Allergen Challenge are administered at post-screening allergen challenges unless it is unsafe to do so. A change in the administered allergen challenge dose may influence allergen-induced change endpoints. Patients will be included in the full analysis set regardless of their allergen challenge dose; this analysis set is used for evaluation of the effect on baseline inflammation. For the occurrence

where a subject's given post-screening allergen challenge dose differs from the allergen challenge dose given at the screening challenge, efficacy outcome data will be flagged at the analysis level. Subjects where the allergen challenge dose is higher post-screening (important protocol deviation) will be excluded from both the primary efficacy analysis set and the sensitivity analyses described in Section 3.2. Subjects where the allergen challenge dose is reduced post-screening (allowed for safety reasons) will be included in the primary efficacy analysis set, but excluded from sensitivity analyses. Additionally, subjects with an IPD of use of a systemic corticosteroid within 1 week prior to allergen challenge study visit or other exclusionary IPDs identified prior to database lock will be excluded from sensitivity analyses.

During blinded data reviews, it was noted that some subjects (approximately 5) received a lower allergen challenge dose post-screening. Although this was allowed per protocol for subject safety reasons, the lower challenge doses may potentially lead to a bias in the allergen-induced change efficacy analysis results. Particularly if there is a significant imbalance between the benralizumab and placebo groups in the number of subjects with lower dose of allergen challenge at post-screening allergen challenge. If the results of the sensitivity analyses confirm that these subjects are influential to the primary endpoint point estimates and treatment effect estimates, the primary efficacy analysis set may be amended to exclude these patients and all allergen-induced change endpoints will be evaluated in this revised analysis set for conclusion purposes. The two primary efficacy endpoints will be evaluated in the full analysis set, the primary efficacy analysis set, and the sensitivity analysis to allow a full assessment of the robustness of study conclusions to deviations in allergen-challenge dosing.

For tests requiring log-transformation, on a per test basis the level of precision reported will dictate the imputation of records with a value of zero prior to log-transformation. For instance, a test reporting to zero decimals would replace records having a value 0 with ½ = 0.5. Tests reporting with 1 decimal will replace records having a value 0 with 0.1/2 = 0.05.

4.1.1 Testing strategy to account for multiplicity considerations

There are two primary endpoints, the allergen-induced change in percentage of eosinophils in sputum 7-hr post-allergen challenge and maximum percent decrease in FEV_1 in the late asthmatic response on Visit 11. These endpoints will be tested using the following hierarchical fixed-sequence approach in order to control the overall type I error rate at the 0.05 level:

Step 1: First perform the test on allergen-induced change in percentage of eosinophils in sputum 7-hr post-allergen challenge. If the two-sided p-value is ≤ 0.05, then proceed with Step 2. Otherwise, neither of the two null hypotheses will be rejected.

Step 2: Perform the test on maximum percent decrease in FEV_1 in the late asthmatic response on Visit 11, at alpha level of 0.05.

4.2 Analysis Methods

4.2.1 Subject disposition

Subject disposition will be summarized using the all subjects analysis set. The total number of subjects will be summarized for the following groups: those who enrolled, those who entered run-in, and those who were not randomized (and reason). The number and percentage of subjects within each treatment group will be presented by the following categories: randomized, received treatment with IP, did not receive treatment with IP (and reason), completed Visit 11 (Allergen Challenge 1), completed treatment with IP, discontinued treatment with IP (and reason), and withdrawn from study (and reason).

The number of subjects randomized by center will be summarized by treatment group in the full analysis set.

4.2.2 Demography data and subject characteristics

Demographics and subject characteristics will be summarized by treatment group using frequency and percentages (for categorical variables) and descriptive statistics of mean, standard deviation, minimum, median and maximum (for continuous variables) using the full analysis set. Age will be derived from the date of informed consent-date of birth, rounded down to the nearest integer.

Various baseline characteristics will also be summarized by treatment for the full analysis set. These include smoking status, number of pack years for former smokers, asthma duration, history of positive allergy test, allergy duration, results of skin prick testing, allergen selected for inhaled allergen challenge test, FEV_1 (measurements at pre-challenge, post-challenge, and change and percent change from pre-challenge), percent decrease in FEV_1 time-adjusted AUC (EAR and LAR), and sputum eosinophils, basophils, and MCh PC20 at screening (measurements at pre-challenge, post-challenge and allergen-induced change).

4.2.3 Prior and Concomitant Medications

Prior medications, categorized according to the World Health Organization (WHO) Drug Reference List dictionary which employs the Anatomical Therapeutic Chemical (ATC) classification system, will be summarized by treatment group as frequency and percentage of subjects reporting usage for the full analysis set.

Concomitant medications will be categorized according to the WHO Drug Reference List dictionary which employs the ATC classification system. Concomitant medications include pre-treatment and on-study medications per the definition in section 2.4.4. The frequency and percentage of subjects taking concomitant medications and non-pharmacological therapies during the study will be summarized by drug class and drug name using ATC code for the full analysis set. A summary will be provided for allowed and disallowed medications.

4.2.4 Study Treatments

4.2.4.1 Exposure

Exposure to investigational product will be calculated in days as:

Last dose date of IP-first dose date of IP+1

and will be summarized by treatment group for the safety analysis set.

4.2.4.2 Compliance

Subjects will receive doses of IP every 4 weeks for a total of 3 doses. The number and percentage of subjects receiving each of the three doses will be summarized by treatment group for the safety analysis set.

IP compliance will be summarized by treatment group for the full analysis set and calculated as:

IP compliance=(total doses administered/total doses expected)x100.

Subjects who received no IP will have zero compliance. Total number of doses expected includes all visits with protocol scheduled IP administration on or before a subject's IP discontinuation or completion date.

4.2.5 Primary Outcome Variable

The primary efficacy analyses will be performed using both the primary efficacy analysis set and the full analysis set. All supportive and secondary allergen-induced change efficacy analyses will be performed using the primary efficacy analysis set only. All other efficacy analyses will be performed using the full analysis set.

4.2.5.1 Primary Analysis

Allergen-induced change in percentage of eosinophils in induced sputum post-allergen challenge during Allergen Challenge 1

The null hypothesis is that the allergen-induced change in percentage of eosinophils in induced sputum 7-hr post-allergen challenge during Allergen Challenge 1 in the Benralizumab group is equal to the allergen-induced change in percentage of eosinophils in induced sputum 7-hr post-allergen challenge during Allergen Challenge 1 in the placebo group. The alternative hypothesis is that the allergen-induced change in percentage of eosinophils in induced sputum 7-hr post-allergen challenge during Allergen Challenge 1 in the Benralizumab group is not equal to that in placebo group, i.e.,:

H0: Allergen-induced Change $_{\text{7-hr post-challenge at Allergen Challenge 1}}$ (Benralizumab) = Allergen-induced Change $_{\text{7-hr post-challenge at Allergen Challenge 1}}$ (placebo)

Ha: Allergen-induced Change $_{\text{7-hr post-challenge at Allergen Challenge 1}}$ (Benralizumab) ≠ Allergen-induced Change $_{\text{7-hr post-challenge at Allergen Challenge 1}}$ (placebo)

The endpoints of the allergen-induced change in percentage of eosinophils in induced sputum at Visits 11 (7-hr post-challenge, primary endpoint) and 12 (24-hr post-challenge, secondary endpoint) will be compared between benralizumab and placebo using a repeated measures analysis. The dependent variable will be the allergen-induced change in percentage of eosinophils in induced sputum. Treatment group will be fitted as an explanatory variable. Time post-allergen challenge (7-hr and 24-hr, fitted as a categorical variable) and interaction between time post-allergen challenge and treatment will be fitted as fixed effects, and allergen-induced change at Screening will be fitted as a covariate. The model is: Allergen-induced change = treatment group + time post-allergen challenge + treatment group*time post-allergen challenge + allergen-induced change at screening.

The allergen-induced change at Screening was selected as the covariate instead of the triad baseline because the allergen-induced change at Screening was obtained prior to receiving IP and does not reflect any treatment effects. The pre-challenge value at Allergen Challenge 1 would be potentially confounding in the model, since it occurs after the IP dosing period (Visits 7-9).

The variance-covariance matrix will be assumed to be unstructured. If the procedure does not converge the compound symmetric variance-covariance matrix will be used instead. The least squares mean for the difference in treatment groups using the interaction between time post-allergen challenge and treatment group, its 95% CI, and the two-sided p-values will be reported for each visit.

Maximum percent decrease in FEV_1 in late asthmatic response ($LAR_{3\text{-}7\ hr}$) during Allergen Challenge 1

The null hypothesis is that the maximum percent decrease in FEV_1 in late asthmatic response ($LAR_{3\text{-}7\ hr}$) at Visit 11 in the benralizumab group is equal to the maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ at Visit 11 in the placebo group. The alternative hypothesis is that the maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ at Visit 11 in the benralizumab group is not equal to that in the placebo group, i.e.,:

H0: Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr\ Visit\ 11}$(benralizumab) = Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr\ Visit\ 11}$ (placebo)

Ha: Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr\ Visit\ 11}$ (benralizumab) ≠ Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr\ Visit\ 11}$ (placebo)

Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ at Visit 11 will be compared between benralizumab and placebo using an analysis of covariance (ANCOVA). The dependent variable will be the maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$. Treatment group will be fitted as an explanatory variable and the maximum percent decrease in FEV_1 in $LAR_{3\text{-}7hr}$ at Screening will be fitted as a covariate. The least squares mean for the difference in treatment groups and its 95% CI, and the two-sided p-value will be provided. The model will be: maximum percent decrease in FEV_1 in $LAR_{3\text{-}7hr}$ = treatment group + maximum percent decrease in FEV_1 $LAR_{3\text{-}7hr}$ at Screening.

4.2.5.2 Sensitivity Analysis

The analysis of the primary efficacy endpoints will be repeated as a sensitivity analysis using the primary efficacy analysis set, with the exclusion of subjects as described in Section 3.2.

4.2.5.3 Subgroup Analysis

Due to the small sample size in this study, no subgroup analyses are planned to be performed.

4.2.5.4 Supportive Analysis

As a supportive analysis to the primary analysis the time adjusted AUC of percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ will be analyzed and summarized descriptively in a similar manner as the maximum percent decrease in FEV_1.

The primary analysis for maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ will be repeated at Allergen Challenge 2. A line plot will be provided for the percent decrease in FEV_1 by allergen challenge and treatment group.

FEV_1 at both pre- and post-challenge time points and minimum FEV_1 post-challenge for $LAR_{3\text{-}7\ hr}$ will also be summarized descriptively by visit (Visit 2, 11 and 14).

The percentage of eosinophils in induced sputum at both pre- and post-challenge time points and the allergen-induced change in percentage of eosinophils in induced sputum at post-challenge time points will be summarized descriptively at Screening and Allergen Challenge 1. A line plot will be provided for the percentage of eosinophils throughout the study, which includes both the period of IP treatment and the period of allergen challenges.

4.2.6 Secondary Efficacy Outcome Variables

4.2.6.1 Secondary endpoints to evaluate the response to allergen challenge

Percentage of eosinophils in induced sputum 24-hr post-allergen challenge

The analysis for the allergen-induced change in percentage of eosinophil in induced sputum 24 hours post-challenge is described in Section 4.2.5.1.

Maximum percent decrease in FEV_1 in early asthmatic response ($EAR_{0\text{-}2\ hr}$)

The maximum percent decrease in FEV_1 in $EAR_{0\text{-}2\ hr}$ will be analyzed in a similar manner as the maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$, as described in Section 4.2.5.1.

Time-adjusted AUC of percent decrease in FEV_1 curve in $EAR_{0\text{-}2\ hr}$

The time-adjusted AUC of percent decrease in FEV_1 in $EAR_{0\text{-}2hr}$ will be analyzed in a similar manner as the time-adjusted AUC in FEV_1 in $LAR_{3\text{-}7\ hr}$, as described in Section 4.2.5.4.

Eosinophil, eosinophil progenitor cells and basophils in bone marrow aspirates

The allergen-induced change in absolute count and percentage of eosinophils (percentage of total cells only), eosinophil progenitor cells and basophils in bone marrow aspirates will be

analyzed using an ANCOVA. The dependent variables will be the allergen-induced change in eosinophils, eosinophil progenitor cells and basophils in bone marrow aspirates for Allergen Challenge 1. Treatment group will be fitted as an explanatory variable and baseline value (Visit 4) will be fitted as a covariate. Summary statistics for allergen-induced change in eosinophils, eosinophil progenitor cells and basophils will also be provided.

Eosinophil and basophil counts in blood

The allergen-induced change in basophil absolute counts in blood will be analyzed using an ANCOVA at Allergen Challenge 1. The dependent variable will be the allergen-induced change in absolute basophil counts in blood at Visit 12. Treatment group will be fitted as an explanatory variable and baseline (Visit 4) will be fitted as a covariate.

The allergen-induced change in absolute counts of eosinophils in blood will be analyzed using a repeated measures approach separately for the Allergen Challenge 1 and 2 triads. The dependent variable will be the allergen-induced change in absolute eosinophil counts. Treatment group will be fitted as an explanatory variable and baseline value (Visit 4) will be fitted as a covariate. Visit (7-hr and 24-hr post-challenge) and visit by treatment group interaction will be fitted as categorical fixed effects, and the variance-covariance matrix will be assumed to be unstructured. If the procedure does not converge the compound symmetric variance-covariance matrix will be used instead. The least squares mean for the difference in treatment groups using the interaction between visit and treatment group, its 95% CI, and the two-sided p-values will be reported for each visit.

Summary statistics for allergen-induced change in eosinophils (absolute count and percent) and basophils (absolute count and percent) in blood will be presented by treatment group and visit.

Number of eosinophils and basophils in lung tissue biopsies

The allergen-induced change in absolute counts of eosinophils (MBP stain) and basophils (2D7 stain) in lung tissue biopsies will be analyzed using an ANCOVA. Each analysis will be performed on epithelium assessments, submucosa assessments and combined epithelium and submucosa. The dependent variable will be the allergen-induced change in eosinophil and basophil absolute counts in lung tissue biopsies. Treatment group will be fitted as an explanatory variable and baseline (Visit 5) will be fitted as a covariate. Summary statistics for eosinophil and basophil absolute counts in lung tissue biopsies by treatment group and visit will also be presented.

Basophil counts in induced sputum from cytospins with toluidine blue staining

The allergen-induced change in basophil percentages in induced sputum from cytospins with toluidine blue staining will be analyzed in a similar manner as the allergen-induced change in percentage of eosinophils in induced sputum, as described in Section 4.2.5.1.

MCh PC20 at Allergen Challenge 1

Allergen-induced change in MCh PC20 at Allergen Challenge 1 will be analyzed after log-transformation using an ANCOVA. The dependent variable will be the change from baseline in log MCh PC20 at Visit 12. Treatment group will be fitted as an explanatory variable and allergen-induced change in log of MCh PC20 at Screening will be fitted as a covariate. Estimates from the ANCOVA will be presented after back-transformation. Summary statistics by treatment group and visit will also be presented.

4.2.6.2 Secondary endpoints to evaluate the effect on baseline inflammation

Percentage of eosinophils in induced sputum

The change from baseline of eosinophil absolute counts and percentages in induced sputum will be compared separately between benralizumab and placebo using mixed models for repeated measures (MMRM) analysis. The dependent variables will be the change from baseline in eosinophils (absolute count and percent, respectively) in induced sputum. Treatment group will be fitted as an explanatory variable and baseline value (Visit 4) will be fitted as a covariate. The model includes visit (as categorical) and interaction between visit and treatment group as fixed effects. The variance-covariance matrix will be assumed to be unstructured. If the procedure does not converge the compound symmetric variance-covariance matrix will be used instead. The least squares mean for the difference in treatment groups using the interaction between visit and treatment group, its 95% CI, and the two-sided p-values will be reported for each visit (Visits 7 and 10). Summary statistics of change from baseline of eosinophils (absolute count and percent) in induced sputum will be presented by treatment group and visit.

Eosinophil and basophil counts in blood

Change from baseline in the basophil absolute counts in blood will be analyzed using an ANCOVA. The dependent variable will be the change from baseline in absolute count of basophils at Visit 10. Treatment group will be fitted as an explanatory variable and baseline (Visit 4) will be fitted as a covariate. Summary statistics for change from baseline in the absolute count of basophils in blood by treatment group and visit will be provided.

Eosinophil absolute counts in blood will be analyzed using an MMRM in a similar manner as for eosinophil absolute counts in induced sputum.

Number of eosinophils and basophils in lung tissue biopsies

Change from baseline in the absolute count of eosinophils (by MBP stain) and basophils (by 2D7 stain) in lung tissue biopsies will be analyzed separately using an ANCOVA. Each analysis will be done on epithelium assessments, submucosa assessments and combined epithelium and submucosa. The dependent variables will be the change from baseline in absolute count of eosinophils and basophils, respectively, in lung tissue biopsies at Visit 8. Treatment group will be fitted as an explanatory variable and baseline (Visit 5) will be fitted as a covariate. Summary statistics for change from baseline in the absolute count of

eosinophils and basophils in lung tissue biopsies by treatment group and visit will be presented.

Basophil counts in induced sputum from cytospins with toluidine blue staining

The change from baseline in absolute and percentage basophil counts in induced sputum from cytospins with toluidine blue staining (Visits 7 and 10) will be analyzed using the same MMRM analysis as described for eosinophil counts in induced sputum.

Eosinophils, eosinophil progenitor cells and basophils in bone marrow aspirates

The change from baseline in absolute count and percentage of eosinophils (percentage of total cells only), eosinophil progenitor cells and basophils in bone marrow aspirates will be analyzed separately using an ANCOVA. The dependent variables will be the change from baseline in eosinophils (percentage), eosinophil progenitor cells (absolute count and percentage) and basophils (absolute count and percentage) at Visit 10. Treatment group will be fitted as an explanatory variable and baseline value (Visit 4) will be fitted as a covariate. Summary statistics for change from baseline by treatment group and visit will be presented.

MCh PC20

Change from baseline in MCh PC20 at Visits 7 and 10 will be analyzed after log-transformation using MMRM analysis. The dependent variable will be the change from baseline in log-transformed MCh PC20. Treatment group will be fitted as an explanatory variable and log-transformed MCh PC20 at Visit 4 will be fitted as a covariate. The model will include visit (as a categorical variable) and interaction between visit and treatment group fitted as fixed effects. The variance-covariance matrix will be assumed to be unstructured. If the procedure does not converge the compound symmetric variance-covariance matrix will be used instead. The least squares mean for the difference in treatment groups using the interaction between visit and treatment group, its 95% CI, and the two-sided p-values will be reported for each visit (Visits 7 and 10). Estimates from the MMRM will be presented after back-transformation. Summary statistics for change from baseline in MCh PC20 will be provided by treatment group and visit.

4.2.7 Exploratory Objectives

4.2.7.1 [redacted]

[redacted]

[redacted]

[redacted]

4.2.8 Safety Outcome Variables

All safety analyses will be performed using the safety analysis set, unless otherwise specified.

4.2.8.1 Adverse Events (AEs)

Adverse events will be summarized separately for the pre-treatment and on- study periods. All AEs will be listed for each subject, regardless of treatment period. All summaries will be presented by treatment groups.

An overall summary table for both the pre-treatment and on-study periods will be produced showing the number and percentage of subjects with at least 1 AE in any of the following categories: AEs, serious adverse events (SAEs), AEs with outcome of death, and AEs leading to discontinuation of investigational product (DAEs). The total number of AEs in the different AE categories in terms of AE counts will also be presented. Subjects with multiple events in the same category and study period are counted only once in that category and study period.

Adverse events, AEs with outcome of death, SAEs and DAEs will be summarised by System Organ Class (SOC) and Preferred Term (PT) assigned to the event by MedDRA. For each PT, the number and percentage of subjects reporting at least 1 occurrence will be presented, i.e., for a subject multiple occurrences of an AE will only be counted once. SAEs causing discontinuation of the IP and SAEs causing discontinuation from the study will also be summarised.

The rate of AEs per person-years at risk, calculated as (number of subjects reporting AE)/ (total period with subjects at risk of AE), will also be reported for the on-study period. The total period at risk for each subject will be defined as the period from first dose of IP to the date of the EOT or IPD visit for the on-study period. Rates will be expressed in terms of events per 100 subject-years.

A summary of the most common (frequency of >5%) AEs will be presented by PT. Adverse events, SAEs and DAEs will be summarised by preferred term and investigator's causality assessment (related vs. not related) and maximum intensity. If a subject reports multiple occurrences of the same AE within the same study period, the maximum intensity will be taken as the highest recorded maximum intensity (the order being mild, moderate, and severe).

4.2.8.2 Laboratory data

Laboratory data will be summarized by presenting shift tables using normal ranges (baseline to most extreme post-baseline value) and by presenting summary statistics of observed and change from baseline values (means, medians, quartiles, ranges). The incidence of clinically notable lab abnormalities will be summarized.

All continuous laboratory parameters will be summarised descriptively by absolute value at each visit by treatment group, together with the corresponding changes from baseline. All parameters will be summarised in SI units. Blood eosinophil and basophil counts will also be summarized in conventional units (cells/uL). Results which are reported from the central laboratory in conventional units will be converted to SI units for reporting. Shift plots showing each individual subject's laboratory value at baseline and worst post-baseline will be produced for each continuous laboratory variable. If any laboratory variables show any unusual features (high or low values or a general shift in the data points) at other time points, then shift plots of these data may be produced. Data for subjects who have treatment-emergent changes outside central laboratory reference ranges will be presented. This data presentation will include all visits for this subset of subjects.

Maximum post-baseline bilirubin elevations by maximum post-baseline ALT and AST will be presented, expressed as multiples of ULN. Bilirubin will be presented in multiples of the following ULN ≤1.5, >1.5 to 2, >2, and AST and ALT will be presented in multiples of the following ULN ≤1, >1 to 3, >3 to 5, >5 to 10, >10. Maximum post-baseline total bilirubin will be presented (<2 and ≥2 x ULN) and plotted against maximum post-baseline ALT (<3, ≥3 to <5, ≥5 to <10, and ≥10 x ULN), expressed as multiples of ULN. This will be repeated to show maximum post-baseline total bilirubin against maximum post-baseline AST.

For all subjects who meet the biochemical criteria for confirmed Hy's law, an SAE Narrative will be produced.

For urinalysis data, a shift table will be generated to present changes from baseline to maximum post-baseline value for each parameter and will include subjects with both baseline and post-baseline data.

4.2.8.3 Vital Signs

Vital sign data will be summarized by presenting summary statistics of observed and change from baseline values. The incidence of clinically notable vital sign abnormalities will be summarized.

4.2.8.4 ECGs

The incidence of clinically notable ECG abnormalities will be summarized.

4.2.8.5 Physical Examination

Abnormal physical examination results will be part of the AE summaries.

5. INTERIM ANALYSIS

No interim analysis is planned for this study.

6. CHANGES OF ANALYSIS FROM PROTOCOL

For the occurrence where a subject's given post-screening allergen challenge concentration differs from the concentration given at the screening challenge, a sensitivity analysis for the two primary efficacy endpoints will be performed. Records where the concentration is higher post-baseline will be excluded from all allergen-induced change endpoint analyses. Records where concentration is reduced post-screening will be included in the primary efficacy analyses but excluded from sensitivity analyses. A change in the administered allergen challenge concentration will only influence the validity of records pertinent to allergen-induced change efficacy outcomes. All other efficacy endpoints will be evaluated in the full analysis set.

Subjects who received a higher allergen concentration post-screening compared to that at Screening Allergen Challenge may impact the allergen challenge outcomes. In this scenario it is possible subjects did not respond to the same allergen concentration post-screening to that at screening due to investigational product induced reasons and increasing to a higher allergen concentration may overcome the effects of treatment affecting outcomes of the allergen-induced outcome measures (e.g allergen-induced eosinophilia and physiological responses). Therefore, we are not evaluating these subjects under the same allergen challenge conditions post-screening as were done at screening. As such, these cases are classified as important protocol deviations and will be excluded from all allergen-challenge endpoint analyses.

In the scenario where subjects received a lower allergen challenge concentration post-screening compared to that at Screening Allergen Challenge, these will not be classified as important protocol deviations. As defined in the protocol these subjects may have to stop the allergen challenge procedure early for safety reasons. Subjects in this scenario will be included in the primary efficacy analysis set for the allergen challenge endpoints (which includes the primary endpoint analyses). However, similarly to the above scenario, we will not be evaluating subjects under the same allergen challenge conditions post-screening as were done for screening. Where a lower allergen challenge concentration is used post-screening, it is possible that these subjects will have been under-challenged and so not achieve a similar level of allergen-induced inflammation and a late asthmatic response as was determined at screening with a higher allergen concentration. Therefore, a sensitivity analysis will be conducted to determine whether these subjects have any impact to the allergen challenge outcome measures. Furthermore, we will examine whether there was an imbalance in the numbers of these subjects across the treatment groups and whether the imbalance impacts the allergen challenge outcome measures. If it is determined that subjects who received a lower allergen challenge concentration post-screening compared to the Screening Allergen Challenge concentration has an impact on the allergen challenge outcome measures, then the sensitivity analysis population will become the study population for the primary analyses and conclusions from the study.

Similarly, any subject who may have been treated with systemic corticosteroids outside the permitted protocol time periods will be classified as important protocol deviations and excluded from the sensitivity analysis population. This is because systemic corticosteroids

inhibit allergen-induced inflammation and outs (such as the late asthmatic response) and can therefore impact the evaluation of investigational product on the study endpoints.

7. REFERENCES

Gauvreau et al 2014
Gauvreau GM, O'Byrne PM, Boulet L-P, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. N Engl J Med 2014; 370:2102-10.

8. APPENDIX

8.1 Partial Dates for AEs and Prior/Concomitant Medication

Dates missing the day or both the day and month of the year will adhere to the following conventions in order to classify on-study AEs and to classify prior/concomitant medications:

Adverse events

- The missing day of onset of an AE will be set to:
    - First day of the month that the event occurred, in the onset YYYY-MM is after the YYY-MM of first IP
    - The day of the first IP, in the onset YYYY-MM is the same as YYYY-MM of the first IP
    - The date of informed consent of the onset YYYY-MM is before the YYYY-MM of the first treatment.
- The missing day of resolution of an AE will be set to:
    - The last day of the month of the occurrence. If the subject died in the same month, then set the imputed date as the death date.
- If the onset date of an AE is missing both the day and month, the onset date will be set to:
    - January 1 of the year of onset if the onset year is after the year of the first IP
    - The date of the first treatment, if the onset year is the same as the year of the first IP
    - The date of informed consent, if the onset year is before the year of the first treatment
- If the resolution date of an AE or end date of a IP is missing both the day and month the date will be set to:
    - December 31 of the year of occurrence. If the subject died in the same year, then set the imputed date as the death date.

Prior/concomitant medication

- The missing day of start date of a therapy will be set to the first day of the month that the event occurred.

- The missing day of end date of a therapy will be set to the last day of the month of the occurrence.

- If the start date of a therapy is missing both the day and month, the onset date will be set to January 1 of the year of onset.

- If the end date of a therapy is missing both the day and month, the date will be set to December 31 of the year of occurrence.

- If the start date of a therapy is null and the end date is not a complete date the start date will be set to the date of the first study visit.

- If the start date of a therapy is null and the end date is a complete date

    - And the end date is after the date of the first study visit then the start date will be set to the date of the first study visit.

    - Otherwise the start date will be set to the end date of the therapy.

- If the end date of a therapy is null and the start date is not a complete date then the end date will be set to the date of the last study visit.

- If the end date of a therapy is null and the start date is a complete date

    - And the start date is prior to the date of the last study visit then the end date will be set to the date of the last study visit.

    - Otherwise, the end date will be set to the start date of the therapy.

8.2 Important Protocol Deviation List

IPD reporting Code	IMPACT Deviation Code	Deviation	Data Management checks (programmable)	Manual ID by Site Monitor (observable)
1		**Inclusion criteria deviations**		
1.1	1.4	Absence of mild, stable, allergic asthma and asthma therapy limited to inhaled, short-acting β2 agonists (criterion 4)	X	
1.2	1.5	Not able to produce a sputum sample and viable cytospin for assessment of the cell differential count at screening pre, 7 and 24 hours post-challenge (criterion 5)	X	
1.3	1.10	Do not demonstrate a positive early and late airway responses during the Screening Allergen Challenge (criterion 10)	X	
1.4	1.11	No increase in sputum eosinophils at 7 hrs post allergen challenge relative to the pre-allergen challenge sputum sample at screening (criterion 11)	X	
2		**Exclusion criteria deviations**		
2.1	1.14	A worsening of asthma or a respiratory tract infection within 6 weeks preceding enrolment (criterion 1)	X	
2.2	1.15	Current lung disease other than mild allergic asthma (criterion 2)	X	

2.3	1.24	Use of regular treatment with inhaled or intranasal corticosteroids within the 4 weeks prior to enrolment (criterion 11)	X	
2.4	1.25	Use of orally or systemically administered corticosteroids within the 12 weeks prior to enrolment into this study (criterion 12)	X	
2.5	1.27	Use of long-acting bronchodilators in the 2 weeks prior to enrolment (criterion 14)	X	
2.6	1.28	Chronic use of Leukotriene Receptor Antagonist (LTRA) for treatment of allergic lung disease (criterion 15 - modified)	X	
2.7	N/A	Subject meets key safety-related exclusion criteria that could confound interpretation of safety as determined by the study physician	X	X
3		**Deviations from informed consent procedures**		
3.1	6.2, 6.3	Study procedures performed prior to signed informed consent or re-consent	X	X
4		**Discontinuation criteria for IP met but subject not withdrawn from IP**		
4.1	5.7	Study-specific criteria for IP discontinuation is met but subject was not discontinued • Anaphylactic reaction to IP requiring administration of epinephrine • Helminth parasitic infection	X	X

		requiring hospitalization • Missed one dose of IP • Asthma-related event requiring an emergency room (ER) visit or use of oral corticosteroids (beyond protocol allowed) prior to last dose of IP (IMPACT) • Worsening of asthma according to the PI (IMPACT)		
5		**IP management and administration**		
5.1	3.3	Administration of IP with temperature excursion confirmed not fit for use		X
5.2	3.4	Incorrect randomized treatment administered	X	X
5.3	3.2	Use of expired or damaged IP		X
5.4	5.2, 5.3	IP administered in the presence of condition(s) contraindicating dosing		X
6		**Received prohibited/restricted concomitant medication**		
6.1	2.7	Use of systemic corticosteroids within 1 weeks prior to a study visit	X	
6.2	2.1	Use of Short-acting Beta Agonist (SABA) within 8 hours of a study visit		X
6.3	2.4	Use of aspirin product within 7 days or Non-Steroidal Anti-Inflammatory Drug (NSAIDs) within 3 days of a bronchoscopy study visit		X
6.4	2.9	Use of anti-leukotriene therapies	X	

6.5	2.10	Use of cromoglycate or nedocromil	X	
6.6	2.6	Use of short-acting antihistamines within 3 days, intermediate antihistamines within 7 days and long-acting antihistamines within 9 days prior to allergen challenge		X
6.7	2.13	Use of theophylline	X	
6.8	2.14	Use of herbal remedies for the treatment of allergic, inflammatory, or respiratory diseases	X	
6.9	2.15	Chronic use of anticoagulant and antiplatelet agents	X	
6.10	2.16	Use of immunosuppressive medication	X	
6.11	2.17	Receipt of immunoglobulin or blood products	X	
6.12	2.18	Use of marketed or investigational biologic	X	
6.13	2.19	Use of allergen immunotherapy	X	
6.14	2.20	Receipt of live attenuated vaccine	X	
6.15	2.21	Use of investigational non-biologic	X	
7		**Other potential important deviations**		
7.1	N/A	Allergen challenge dose different than the dose used for the screening challenge not due to safety	X	

7.2	3.7, 5.6	Unblinding of treatment assignment for reasons unrelated to subject safety		X
7.3	N/A	Missing or incorrectly performed pre or post-challenge assessment for sputum eosinophils and spirometry at any scheduled allergen challenge	X	
7.4	4.1, 4.2, 5.4	Severe non-compliance to protocol		X

ER = Emergency Room, IP = Investigational Product, IPD = Important Protocol Deviation, LTRA = Leukotriene Receptor Antagonist, NSAID = Non-Steroidal Anti-Inflammatory Drug, SABA = Short-acting Beta Agonist

8.3 Efficacy endpoint laboratory test mapping

Endpoint	Test Name	Raw dataset	Specimen Type	Test Method	Test Name	Lab Code	Unit	Reporting Test Name
Allergen induced change in percentage of eosinophils in induced sputum post allergen challenge during allergen challenge 1	Eosinophils in induced sputum	BMRES	Induced Sputum	Cytospin	Eosinophils/ Leukocytes	L60033	%	Eosinophils
Percentage of eosinophils in induced sputum for the effect on baseline inflammation	Eosinophils in induced sputum	BMRES	Induced Sputum	Cytospin	Eosinophils/ Leukocytes	L60033	%	Eosinophils
Basophil count in induced sputum for both allergen challenge and baseline inflammation	Basophil count in induced sputum	BMRES	Induced Sputum	Cytospin	Basophils/ Leukocytes	L18034	%	Basophils
Number of eosinophils and basophils in lung tissue biopsies for both allergen challenge and baseline inflammation	Eosinophils in lung tissue biopsies	BMRES	Bronchial Biopsies	MBP	Eosinophils (MBP Antibody) Epithelium	L77S44	cells/mm2	Eosinophils in epithelium
	Eosinophils in lung tissue biopsies	BMRES	Bronchial Biopsies	MBP	Eosinophils (MBP Antibody) submucosa	L77S11	cells/mm2	Eosinophils in submucosa
	Eosinophils in lung tissue biopsies	BMRES	Bronchial Biopsies	MBP	Eosinophils (MBP Antibody) Epithelium + Eosinophils (MBP Antibody) Submucosa	L77S44 + L77S11	cells/mm2	Eosinophils, total
	Basophils in lung tissue biopsies	BMRES	Bronchial Biopsies	2D7	Basophils (2D7 Antibody) Epithelium	L77S41	cells/mm2	Basophils in epithelium
	Basophils in lung tissue biopsies	BMRES	Bronchial Biopsies	2D7	Basophils (2D7 Antibody) submucosa	L77S05	cells/mm2	Basophils in submucosa

Endpoint	Test Name	Raw dataset	Specimen Type	Test Method	Test Name	Lab Code	Unit	Reporting Test Name
	Basophils in lung tissue biopsies	BMRES	Bronchial Biopsies	2D7	Basophils (2D7 Antibody) epithelium + Basophils (2D7 antibody) submucosa	L77S41 + L77S05	cells/mm2	Basophils, total
Eosinophil, eosinophil progenitor cells and basophils in bone marrow aspirates for both allergen challenge and baseline inflammation	Eosinophil in bone marrow aspirates	BMRES	Bone Marrow	Diff-quik	Eosinophils, Particle Conc.	L57042	10**9/L	Eosinophils
	Eosinophil progenitor cells in bone marrow aspirates	BMRES	Bone Marrow	Flow Cytometry	Eosinophil Progenitor CD45+CD34+IL5Ra+ Cells, Quant	L57R75	1/mL	Eosinophil progenitor cells
	Basophils in bone marrow aspirates	BMRES	Bone Marrow	Flow Cytometry	Basophils Lin+CD45+FCER1+CCR3+/CRTH2+/123+ Cells, Quant	L57R90	1/mL	Basophils
Eosinophils and basophils counts in the blood for both allergen challenge and baseline inflammation	Eosinophils counts in the blood	CLAB	Blood	Hematology count	Eosinophils, particle conc.	L04042	x10^3/uL	Eosinophils
	Basophils counts in the blood	BMRES	Blood	Flow Cytometry	Basophils Lin+CD45+FCER1+CCR3+/CRTH2+/123+ Cells, Quant	L04R90	1/mL	Basophils
Change in basophil count in induced sputum by flow cytometry for both allergen challenge and baseline inflammation	Basophil count in induced sputum by flow cytometry	BMRES	Induced Sputum	Flow Cytometry	Basophils Lin+CD45+FCER1+CCR3+/CRTH2+/123+ Cells, Quant (/g)	L18R73	1/g	Basophils
Change from baseline in the number of ILC2, ILC2 expressing IL-5Rα and Eosinophil Progenitor (EoP)	ILC2 in induced sputum by flow cytometry	BMRES	Induced Sputum	Flow Cytometry	ILC2 Lin-CD45+127+CrTH2+ Cells, Quant (per gram)	L18R81	1/g	ILC2

Endpoint	Test Name	Raw dataset	Specimen Type	Test Method	Test Name	Lab Code	Unit	Reporting Test Name
Cells in induced sputum by flow cytometry	ILC2 expressing IL-5Rα in induced sputum by flow cytometry	BMRES	Induced Sputum	Flow Cytometry	ILC2 Expressing IL5Ra Lin-CD45+127+CrTH2+IL5Ra+ Cells (/g)	L18R80	1/g	ILC2 expressing IL-5Rα
	Eosinophil Progenitor (EoP) Cells in induced sputum by flow cytometry	BMRES	Induced Sputum	Flow Cytometry	Eosinophil Progenitor CD45+CD34+IL5Ra+ Cells, Quant (/g)	L18R76	1/g	Eosinophil progenitor cells
ILC2, ILC2 expressing IL-5Rα cells in bone marrow aspirates for both allergen challenge and baseline inflammation	ILC2 in bone marrow aspirates	BMRES	Bone Marrow	Flow Cytometry	Innate Lymphoid Cells Grp 2 Lin-CD45+127+CrTH2+ Cells, Quant	L57R72	1/mL	ILC2
	ILC2 expressing IL-5Rα cells in bone marrow aspirates	BMRES	Bone Marrow	Flow Cytometry	ILC2 Expressing IL5Ra Lin-CD45+127+CrTH2+IL5Ra+ cells, Quant	L57R97	1/mL	ILC2 expressing IL-5Rα
ILC2, ILC2 expressing IL-5Rα and eosinophil progenitor cells in blood	ILC2 cells in blood	BMRES	Blood	Flow Cytometry	Innate Lymphoid Cells Grp 2 Lin-CD45+127+CrTH2+ Cells, Quant	L04R82	1/mL	ILC2
	ILC2 expressing IL-5Rα in blood	BMRES	Blood	Flow Cytometry	ILC2 Expressing IL5Ra Lin-CD45+127+CrTH2+IL5Ra+ Cells, Quant	L04R97	1/mL	ILC2 expressing IL-5Rα
	Eosinophil progenitor cells in blood	BMRES	Blood	Flow Cytometry	Eosinophil Progenitor CD45+CD34+IL5Ra+ Cells, Quant	L04R75	1/mL	Eosinophil progenitor cells
Neutrophils and macrophages in sputum	Neutrophils in sputum	BMRES	Induced Sputum	Cytospin	Neutrophils/ Leukocytes	L60049	%	Neutrophils
	Macrophages in sputum	BMRES	Induced Sputum	Cytospin	Macrophages	L60G20	%	Macrophages

Endpoint	Test Name	Raw dataset	Specimen Type	Test Method	Test Name	Lab Code	Unit	Reporting Test Name
Biomarkers of eosinophil activation in sputum, blood and bone marrow aspirates	EDN in sputum	BMRESSP	Induced Sputum	Elisa	Eosinophil Derived Neurotoxin, Quant	L60M48	ng/mL	Eosinophil Derived Neurotoxin
	ECP in serum	BMRESSE	SERUM	FEIA	Eosinophil Cationic Protein	L02062	ug/L	Eosinophil Cationic Protein
	Interleukin 5 in serum	BMRESSE	SERUM	Elisa	Interleukin 5	L02277	pg/mL	Interleukin 5
	Interleukin 5 in bone marrow	BMRESBM	Bone Marrow	Elisa	Interleukin 5	L57277	pg/mL	Interleukin 5
	Eotaxin in bone marrow	BMRESBM	Bone Marrow	Elisa	Eotaxin, Total	L57R39	pg/mL	Eotaxin
	EDN in serum	BMRESSE	SERUM	Elisa	Eosinophil Derived Neurotoxin, Quant	L02M48	ng/mL	Eosinophil Derived Neurotoxin
	Eotaxin in serum	BMRESSE	SERUM	Elisa	Eotaxin, Total	L02R39	pg/mL	Eotaxin
	IL5 in sputum	BMRESSP	Induced Sputum	Elisa	Interleukin 5	L60277	pg/mL	Interleukin 5
	Eotaxin in sputum	BMRESSP	Induced Sputum	Elisa	Eotaxin-1	L60E75	pg/mL	Eotaxin
	EDN in bone marrow	BMRESBM	Bone Marrow	Elisa	Eosinophil Derived Neurotoxin, Quant	L57M48	ng/mL	Eosinophil Derived Neurotoxin
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