Clinical Study Protocol	
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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

Sponsor: AstraZeneca AB, 151 85 Södertälje, Sweden

VERSION HISTORY

Version 4.0, 26 March 2018 Changes to the protocol are summarized below.
Protocol Synopsis (Objectives – Secondary Objectives): Section was updated to clarify that objectives related to bone marrow and bronchoscopy are done in only a sub-set of subjects. Protocol Synopsis (Statistical methods): Stratification text was clarified to show that stratification is done based on types of measurements. Section 2.2 (Secondary Objectives): Section was updated to clarify that objectives related to bone marrow and bronchoscopy are done in only a sub-set of subjects. Section 3.5 (Methods for assigning treatment groups): Stratification text was clarified to show that stratification is done based on types of measurements. Section 4 (Table 2 Study Plan – Treatment Period): Footnote (e) was updated to clarify that only a sub-set of subjects in a designated study centre will provide bone marrow. Section 4 (Table 2 Study Plan - Treatment Period): Footnote (t) was added to clarify which preceding Visit dictates the time to the FU Visit (in line with Section 4.3). Section 4.1 (Enrolment/screening period): Text updated to clarify that only a sub-set of up to 22 subjects recruited at one of the study centres will be required to provide consent to supply a sample of their bone marrow. Section 5.1.7 (Bone marrow aspiration, processing and culture): Text updated to clarify that bone marrow aspirates will only be collected from a sub-set of up to 22 subjects enrolled at one study site. Section 8.2 (Sample size estimate): The section was modified to add clarity to the number of subjects that are part of the sub-set participating in the bone marrow aspirate collection.
Version 3.0, 14 February 2017 Changes to the protocol are summarized below.
Protocol Synopsis (Study site(s) and number of subjects planned): The section was modified to clarify that study subjects will be recruited at 6 centres [redacted] Protocol Synopsis (Secondary Objectives): Objective #1 was added to clarify that the percent of eosinophils in sputum will be measured 24-hr post allergen challenge and evaluated as described in section 8.4.1.1.

Protocol Synopsis (Secondary Objectives – Objective #7 (Outcome Measure): The section was modified to correct a typo in the word "cytospins".

Section 2.2 (Secondary Objectives): Objective #1 was added to clarify that the percent of eosinophils in sputum will be measured 24-hr post allergen challenge and evaluated as described in section 8.4.1.1.

Section 2.2 (Secondary Objectives – Objective #7 (Outcome Measure): The section was modified to correct a typo in the word "cytospins".

Section 3.2 (Exclusion Criteria): For consistency, the restricted use of inhaled or intranasal corticosteroids *throughout the study* (previously mentioned in exclusion criterion #11) was moved to section 3.8 (restriction #6). Exclusion criterion #12 was revised to clarify that use of orally or systemically administered corticosteroids is prohibited within the 12 weeks prior to *enrolment* into this study. Exclusion criterion #30 was revised to clarify that allergen immunotherapy is prohibited within 4 months prior to the date informed consent is obtained. For consistency, the prohibited use of allergen immunotherapy throughout the study was moved to section 3.8 (restriction #18). Exclusion criterion #32 was modified to correct a typo in the word "non-biologic".

Section 3.8 (Restrictions): Restriction #2 was modified to be in line with Table 5. The section was also modified to clarify the prohibited use of the following medication/therapy should also be throughout the study: a) chronic use of antiplatelet agents (restriction #15), b) immunosuppressive medication (restriction #16), c) immunoglobulin and blood products (restriction #17), any marketed (eg omalizumab) or investigational biologic (restriction #18), d) live attenuated vaccines (restriction #20) and any investigational non-biologic (restriction #21). Last, the section was modified to clarify use of allergen immunotherapy in line with section 3.2 (restriction #19).

Section 3.9 (Discontinuation of Investigational Product): The section was modified to clarify that IP unblinding will be a reason for discontinuation.

Section 4 (Table 1 Study Plan – Enrollment, screening/run-in period): The table was revised to correct the section numbers referring to "Demographics" and "Medical and asthma history" and to add a footnote "d".

Section 4 (Table 2 Study Plan - Treatment Period): Visit windows were modified for Visit 6, Visit 9, Visit 10 and Visit 13. Footnote (i) was revised to clarify that basophil counts will be redacted from the lab report sent to the investigative sites for these sample collections. Footnote (i) was also revised to clarify that at visit 7 where Full Hematology and Hematology for leukocyte PD markers will be performed, only one blood sample will be required. Footnotes (j) to (u) were added to clarify the time between visits, the windows around visits and also which preceding Visit dictates the time to the next Visit.

Section 4.2.2.2 (Effect of Benralizumab on allergen-induced inflammation – Allergen Challenge Period 2): This section was revised to clarify the timing for the two allergen challenge triads.

Section 5.1.6 (Spirometry): The section was revised to clarify that Visit 4 is performed at Day-3.

Section 5.1.9 (Blood for cell assessments and serum protein markers): The section was revised to clarify that basophil counts will be recorded from each collection and redacted from the lab report sent to the investigative sites.

Section 5.3.2 (Blood Coagulation): The section was modified to remove sample collection specifications for the coagulation testing.

Section 5.4.1(Storage, re-use and destruction of biological samples): The section was modified to clarify sample storage conditions and sample use.

Section 5.4.3 (Chain of Custody of Biological Samples): The section was modified to the principal investigator will keep documentation of all shipments.

Section 7.8 (Table 5 and Table 6): Tables were modified to align the prohibited use of the medication/therapy with the revisions outlined in section 3.8 (restrictions).

Section 8.4.1 (Calculation or Deviation of Efficacy Variables): The section was modified to remove the confusing statement that the two primary endpoints will be collected in the second analysis part of the study.

Section 8.5.3 (Analyses methods for safety variables): The section was revised to clarify that ECG data will be summarized by presenting incidence of clinically notable ECG abnormalities.

Appendix D (Anaphylaxis: signs and symptoms, management – Clinical Criteria for Defining Anaphylaxis and Immune Disease Anaphylaxis): The section was modified to remove "Immune Disease Anaphylaxis" from the heading and the description of immune complex disease.

Version 2.0, 14 June 2016

Changes to the protocol are summarized below.

Table of Contents: headings 2. Clinical Criteria for Defining Anaphylaxis and Immune Disease, 3. Signs and Symptoms and Management of Acute Anaphylaxis and 4. Management

of Acute Anaphylaxis below the List of References were removed (formatting error)

List of abbreviations and definition of terms: The list was modified to add the abbreviation "PD" and its definition.

Section 1.1 (Background and Rationale for Conducting this Study): Hyperlink inserted for reference Blanchard and Rothenberg 2009.

Section 1.4 (Study Design): The section was revised to clarify the *approximate* number of subjects that will be recruited to complete the study.

Section 2.2 (Secondary Objectives): [redacted]

Section 3.1 (Inclusion Criteria): Inclusion criterion #4 was modified to revise "B2" to beta symbol (i.e. β2).

Section 3.1 (Inclusion Criteria): The section was modified to separate inclusion criterion 13 from inclusion criterion 12 (formatting error).

Section 3.6 (Methods for Ensuring Blinding): The section was modified to clarify the hematology findings (for full hematology and leukocyte PD markers) that will be reported back to the investigative site as well as those to be redacted from the lab reports. Additionally, the section was revised to clarify that procedures to mitigate unblinding should be followed from visit 4 on.

Section 3.8 (Restrictions): restriction #11 was reworded to align with the opening statement.

Section 3.9 (Discontinuation of Investigational Product): The section was revised to clarify the time when subjects who prematurely discontinue IP should return to the study centre and complete the procedures described for the IPD visit.

Section 3.10.2 (Withdrawal of the informed consent): The section was modified to clarify the visits that subjects who withdraw from the study will be asked to complete.

Section 4 (Table 1 Study Plan – Enrollment, screening/run-in period): Table 1 was modified to revise section numbering for cotinine testing and serology. Table 1 was modified to clarify that spirometry (FEV_1) is being assessed at V1b as part of the already specified MCh PC20 test.

Section 4 (Table 2 Study Plan - Treatment Period): Table 2 was modified to revise visit window for V7 and V8, to remove the ADA/nAb testing (and the parallel footnote) and the height, weight and BMI assessments from EOT and IPD visits, to revise physical examination from "brief" to "complete" at IPD visit, to add full hematology, serum chemistry and urinalysis to V7 and full hematology to UNS. In addition, Table 2 was revised to change wording of "hematology for blood eosinophil count" to "hematology for leukocyte PD

markers" will be performed at V4, V7 and V9 to V15 to clarify the type of analysis to be performed. Furthermore, footnotes were either revised or created to clarify the hematology (full hematology or for hematology for leukocyte PD markers) findings that will be reported back (and redacted) to the investigate site for these samples collections. Furthermore, footnotes were revised to clarify that subjects will be contacted prior to EOT visit (V16). Lastly, the footnote nomenclature was adjusted to reflect the modifications mentioned above.

Section 4.1 (Enrolment/screening Period (Visit 1 – Visit 3)): The number of study centres from which subjects will be required to provide consent for endobronchial biopsies was revised.

Section 4.1 (Enrolment/screening Period (Visit 1 – Visit 3)/*Repeat of screening assessments versus Rescreening/Repeat assessments*): The text "**new section"** was removed from "Methacholine PC20 (new section)". An unnecessary added "period" was also removed from the section on assessments performed at visit 4 (typo). Spirometry assessment was added to Visit 1b in line with the addition in Table 2.

Section 4.1 (Enrolment/screening Period (Visit 1 – Visit 3)/*Repeat of screening assessments versus Rescreening/Rescreen assessments*): The text was modified to clarify that if a subject requires rescreening, all procedures from screening/run-in period should be repeated with the exception of the skin prick testing.

Section 4.2.1 (Baseline Inflammation Assessments): ADA/nAb testing was removed from the assessments to be conducted at visit 4. The section was revised to change wording of "hematology (for blood eosinophils)" to "for leukocyte PD markers" to clarify the blood sample analysis to be performed at V4.

Section 4.2.2 (Randomised Treatment Period): Timing for vital signs collection was clarified.

Section 4.2.2.1 (Effect of Benralizumab on Baseline Inflammation): Timing for vital signs collection was clarified. The section was revised to change wording of "hematology (for blood eosinophils)" to "for leukocyte PD markers" to clarify the blood sample analysis to be performed at V7. Lastly, the section was modified to clarify that a predose blood sample will be collected for full hematology at V7.

Section 4.2.2.2 (Effect of Benralizumab on Allergen-induced Inflammation/*Allergen challenge period 1*): The section was revised to change wording of "hematology (for blood eosinophils)" to "for leukocyte PD markers" to clarify the blood sample analysis to be performed at V10, V11 (7 hours post allergen challenge) and V12 (24 hours (±2 hours) post allergen challenge).

Section 4.2.2.2 (Effect of Benralizumab on Allergen-induced Inflammation/*Allergen challenge period 2*): The section was revised to change wording of "hematology (for blood eosinophils)" to "for leukocyte PD markers" to clarify the blood sample analysis to be performed at V13, V14 (7 hours post allergen challenge) and V15. In addition, the section was revised to clarify that subjects will be contacted prior to EOT visit (V16). Lastly, the text was

revised to clarify the time when subjects who prematurely discontinue IP should return to the study centre and complete the procedures described for the IPD visit.

Section 5.1.8 (Bronchoscopy and processing of samples): The section was modified to remove the assessment of eosinophil progenitor cells in endobronchial biopsies.

Section 5.1.9 (Blood for cell assessments and serum protein markers): The section was reworded to clarify the different leukocytes recorded from each collection and to clarify which data should be reported back to the sites.

Section 5.2.1 (Laboratory Safety Assessments): Table 3 was modified to include assessment of RBC morphology. In addition, footnote to table was reworded to clarify data that is reported back to sites.

Section 5.3.3 (Immunogenicity): The section was removed. Immunogenicity (ADA/nAb) of benralizumab will not be assessed in this trial.

Section 7.1 (Identity of Investigational Product(s)): Table 4 was modified to revise IP manufacturer.

Section 7.3 (Management of IP related reactions): ADA testing was removed and lung function testing prior to IP administration was removed.

This Clinical Study Protocol has been subject to a peer review according to AstraZeneca Standard procedures. The clinical study protocol is publicly registered and the results are disclosed and/or published according to the AstraZeneca Global Policy on Bioethics and in compliance with prevailing laws and regulations.

PROTOCOL SYNOPSIS

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

National Co-ordinating Investigator

[redacted]

Study site(s) and number of subjects planned

The study will include approximately 38 randomised patients, recruited at 6 centres [redacted] in Canada.

Study period		Phase of development
Estimated date of first subject enrolled	Q3 2016	3
Estimated date of last subject completed	Q4 2018	3

Study design

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

Subjects who meet the eligibility criteria will be randomized to receive 3 subcutaneous (SC) doses of benralizumab 30 mg or placebo (1:1).

Following the third dose of study treatment subjects will return to the clinic for the two allergen challenge periods. The first allergen challenge will assess effects of benralizumab on allergen–induced eosinophils in induced sputum and the late asthmatic response (LAR). The second allergen challenge will assess effects of benralizumab on allergen–induced eosinophils and basophils in lung tissue. Only clinical sites participating in bronchial biopsy collections

will participate in the second allergen challenge. 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.

Objectives

(a) Primary Objectives

Objective	Outcome Measure
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: Maximal percentage decrease in Forced Expiratory Volume in 1 Second (FEV_1) 3-7 hours post allergen challenge ($LAR_{3\text{-}7\ hr}$) Supportive measure: AUC of time adjusted percent decrease in FEV1 curve in late asthmatic response ($LAR_{3\text{-}7\ hr}$)

(b) Secondary Objectives

Objective	Outcome Measure
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
To assess the effect of benralizumab on allergen-induced early (within 2 hrs post allergen challenge) asthmatic response (EAR)	Maximal 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 prechallenge 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 prechallenge 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 prechallenge 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 PC20 (concentration of inhaled methacholine that produces a 20% fall in FEV_1)

(c) Safety Objective

Objective:	**Outcome Measure :**
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/haemotology/urinalysis Physical examination

(d) Exploratory Objectives

Exploratory Objective:	Outcome Measure :
[redacted]	[redacted]
[redacted]	[redacted]

Target subject population

Men and women, 18 to 65 years of age, with mild allergic asthma, not using regular asthma controller medication and with confirmed dual (early and late) asthmatic response to inhaled allergen challenge.

Duration of treatment

Total length of the study is up to 37 weeks

- Up to 16 weeks screening/run-in period
- 13 weeks treatment period
- Follow-up period:
    - 11 weeks for subjects doing allergen challenge 1
    - 8 weeks for subjects doing allergen challenges 1 and 2

Investigational product, dosage and mode of administration

Benralizumab 30 mg/mL, or placebo, solution for injection in an accessorized pre-filled syringe (PFS) will be administered at the study centre SC every 4 weeks for 3 doses.

Statistical methods

Approximately 38 subjects in total and 19 subjects in each treatment group are needed for this study based on the choice of two primary endpoints; the change in percentage of eosinophils in induced sputum at 7-hr post allergen challenge and the maximum percent decrease in FEV_1 in late asthmatic response ($LAR_{3\text{-}7\ hr}$) both measured after the first allergen challenge. These endpoints will be tested using a hierarchical fixed-sequence approach, the first assessment being the effect on percentage of eosinophils in sputum.

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.

The primary analysis method for the allergen induced change in percentage of eosinophils in induced sputum at 7-hr and 24-hr post challenge 1 will be compared between Benralizumab and Placebo using a repeated measures analysis. Treatment group will be fitted as an explanatory variable, pre-dose allergen induced change at Screening will be fitted as a covariate and visit will be fitted as a categorical variable.

The maximum percent decrease in FEV_1 at $LAR_{3\text{-}7\ hr}$ will be compared between Benralizumab and Placebo using an ANCOVA. Treatment group will be fitted as an explanatory variable and the corresponding pre-dose FEV_1 value at Screening will be fitted as a covariate.

Efficacy analysis will be performed using the full analysis set, which consists of all randomized subjects who received at least one dose of investigational product.

All safety variables will be summarised descriptively. Safety analysis will be performed in safety analysis set which is the same as full analysis set.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
ADA	Anti-drug Antibodies
AE	Adverse Event
AHR	Airway Hyperresponsiveness
[redacted]	[redacted]
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
EAR	Early Allergic Response
FEV_1	Forced Expiratory Volume in 1 Second
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
ILC2	Type 2 Innate Lymphoid Cell
IP	Investigational Product
IPD	Investigational Product Discontinuation
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LAR	Late Allergic Response
LSLV	Last Subject Last Visit
LIMS	Laboratory information management system
MCH	Methacholine
OAE	Other Significant Adverse Event
PI	Principal Investigator
PD	Pharmacodynamics
SAE	Serious adverse event

Abbreviation or special term	Explanation
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Asthma is recognised by the presence of reversible bronchoconstriction, airway hyper-responsiveness (AHR) and airway inflammation. Environmental allergens are an important cause of asthma and can be studied in the laboratory by allergen inhalation challenge studies.

Allergen inhalation in sensitised subjects, induces an immediate bronchoconstriction (the early response), which is usually maximal within 30 minutes after allergen challenge and resolves between 1 to 3 hours. A proportion of these subjects will also proceed to develop a second, delayed bronchoconstrictor response (the late asthmatic response) which is associated with prolonged AHR and a pronounced airway eosinophilia (Weersink et al 1994, Gauvreau et al 1999). These subjects who show both an early and late response are referred to as dual responders.

Both the early response and the late response, after inhaled allergen challenge, are associated with inflammatory changes within the airways. These inflammatory events have been investigated in airway tissue biopsy material, bronchoalveolar lavage fluid (BALF) and induced sputum. Through the use of the allergen challenge model it is now recognised that mast cells are probably the predominant inflammatory cell involved in the early response whereas eosinophils, T lymphocytes (of the Th2 phenotype) and basophils are the major inflammatory cells contributing to the late response. Indeed recent data suggests that basophils may be key important immune function cells for the induction of the late asthmatic response observed in the inhaled allergen model in atopic asthmatic subjects (Watson et al 2015).

As well as inflammatory changes in the airways themselves, it is also recognised that there is a contribution to the inflammatory response to allergen provocation from the blood and the bone marrow, to selectively recruit and drive an eosinophilic response towards the airway (Gibson et al 1991, Sehmi et al 1997).

As such the inhaled allergen challenge model in allergic asthmatic subjects has proved to be a useful model to understand some of the mechanisms involved in the inflammatory condition of allergic asthma and also for examining the effect of anti-inflammatory and immunomodulatory agents on allergen-induced inflammatory mechanisms and inflammation that can be used to help understand the mechanism of action of these agents as reviewed by Gauvreau et al 2015.

Interleukin-5 (IL-5) is a key cytokine involved in the differentiation and maturation of eosinophils from hematopoietic stem cells in the bone marrow, their mobilization and migration from the bone marrow to the blood, and their activation and survival in tissue (Blanchard and Rothenberg 2009). The receptor for IL-5 (IL-5Rα) is exclusively expressed on eosinophils and basophils.

Benralizumab (MEDI-563) is a humanized, afucosylated monoclonal antibody (IgG1k), which binds with high affinity to IL-5Rα and induces apoptosis of eosinophils and basophils through enhanced antibody dependant cellular cytotoxicity (ADCC) (Kolbeck et al 2010). Through this mechanism a clinical study with benralizumab in mild to moderate asthmatics showed a 96% median reduction in eosinophils in the lung tissue, 90% median reduction in sputum eosinophils, 100% reduction in eosinophils in blood and 100% reduction in eosinophils and eosinophil precursors in bone marrow (Laviolette et al 2013). In this study benralizumab also reduced blood basophils by a median of 74%.

Furthermore, benralizumab has demonstrated efficacy in a Ph2b clinical trial in uncontrolled moderate to severe asthma subjects with eosinophilic inflammation reducing exacerbations, improving lung function and symptoms (Castro et al 2014) and is currently in Phase 3 trials (NCT01928771, NCT01914757).

We hypothesize that benralizumab may be effective in attenuating an allergen-induced late asthmatic response since benralizumab shows effective depletion of both eosinophils and basophils in blood and lung tissue of subjects with asthma.

1.2 Rationale for study design, doses and control groups

This will be a multi-centre, randomized, double-blind, parallel group, placebo controlled study. The primary endpoints of the study are effect of treatment on percentage of eosinophils in sputum following allergen challenge and effect on the late asthmatic response in mild allergic asthmatics.

Mild allergic asthmatics are the choice of study population in order to observe a mild respiratory physiological response and inflammatory response to inhaled allergen provocation.

Both the proportion of sputum eosinophils and the LAR are primary endpoints in the study given the eosinophil and basophil depleting properties of benralizumab.

Key secondary endpoints include the effect of treatment on the number of basophils in sputum and the number of eosinophils and basophils in the blood, bone marrow and lung tissue following allergen challenge. Sample collections for evaluating effects in the lung tissue and bone marrow have been reduced to a minimum of:

- A collection prior to treatment to establish the number of eosinophils and basophils in these tissues prior to treatment (baseline),
- At the end of the treatment period but prior to allergen challenge to evaluate the effect of treatment on baseline eosinophils and basophils
- After allergen challenge to evaluate the effect on allergen-induced increases in eosinophils and basophils

One of the aims of the study is to evaluate the effect of benralizumab on eosinophils and basophils in each of the tissue compartments where these cells are produced or migrate to

contributing to the pathology of asthma and to evaluate these effects on baseline inflammation and in response to allergen-induced inflammation.

Endobronchial biopsies are being collected to evaluate the effect of benralizumab on eosinophils and basophils in the lung tissue. It has been shown that the anti-IL-5 antibodies (e.g. mepolizumab) can significantly deplete eosinophils in sputum (median 86% reduction) but only reduced tissue eosinophils by 55% (Flood-Page et al 2003). Benralizumab has been shown to deplete eosinophils in lung tissue by median of 96% (Laviolette et al 2013) but is not known how effective benralizumab is at depleting basophils in lung tissue and whether it will reduce eosinophils and basophils in response to a provocating agent that results in enhanced eosinophilic and basophilic inflammation in the airway.

The bone marrow is the site where leukocytes are produced from progenitor cells. Mepolizumab and benralizumab (Flood-Page et al 2003; Laviolette et al 2013) have been shown to reduce eosinophils in the bone marrow but again there is little information on the effect on basophils and the effect on both eosinophils and basophils in response to allergen challenge.

In order to protect against any potential bias to the results, the study will be randomized and double-blind. The study is a parallel design, because benralizumab is a monoclonal antibody and has a long half-life and therefore precludes the possibility of a cross-over design which would prolong the washout period and have risk of carry over effects of drug between treatments.

The benralizumab dose (30 mg SC) is based on all available safety, efficacy and immunogenicity data.

The effects of treatment after 3 doses of benralizumab are being evaluated as previous data suggests that three SC doses every 4 weeks of benralizumab may be required to achieve optimal depletion of eosinophils in the lung tissue (Laviolette et al 2013). A similar dosing regimen in the current study may optimize the pharmacological effects on the late asthmatic response. The first allergen challenge is timed to be conducted when PK concentration is expected to peak in serum and lung following the third dose of benralizumab. The second allergen challenge period is timed to be conducted when benralizumab PK exposure is at steady-state trough level.

A placebo treatment group is included to evaluate the effect of treatment relative to effects in a control group.

1.3 Benefit/risk and ethical assessment

It is not expected that asthma subjects in this study will receive any clinical benefit from participation in this study. As the study population are proposed to be mild asthmatics that only use short-acting bronchodilators for treatment of symptoms, it is not expected that benralizumab will have clinical benefit in this population.

Eosinophils are a prominent feature of the inflammatory response to helminthic parasitic infections, and the presence of infiltrating eosinophils has been circumstantially associated with a positive prognosis in certain solid tumors. Therefore, there is a theoretical risk that prolonged eosinophil depletion may diminish the ability to defend against helminthic parasites, or negatively impact the natural history of certain malignant tumors. Risk minimization measures include exclusion of subjects with untreated parasitic infection and active or recent malignancy, in conjunction with the performance of routine pharmacovigilance activities.

Anti-Drug Antibodies

Development of ADA to benralizumab has been documented. Theoretical risks of developing ADA include decreased drug efficacy and hypersensitivity reactions (eg, anaphylaxis or immune complex disease).

A detailed assessment of the overall risk/benefit of benralizumab in subjects with asthma is given in the Investigator's Brochure.

Methacholine Challenge Test

Methacholine elicits airway narrowing in all individuals, but asthma subjects are hyperresponsive, in that it requires lower methacholine concentrations to achieve a response compared to non-asthmatic subjects. Severe bronchoconstriction, hyperinflation, or severe coughing are extremely rare events. In general, the methacholine challenge test is well tolerated, and respiratory symptoms in those who react to methacholine typically reverse promptly following bronchodilator administration. This test will be performed in a research laboratory with available personnel trained to treat acute bronchospasm and to use resuscitation equipment if needed.

Allergen Inhalation Challenge

Allergen inhalation challenges have been performed for over 20 years without safety concerns. Between 2003 and 2008, 965 individual research allergen inhalation challenges have been conducted across a number of centres in Canada [redacted] Allergen challenges are not without risk. When conducted by experienced laboratories however, provoked models of asthma are extremely safe (O'Byrne 2009).

Sputum Induction

The procedures for sputum induction have shown to be simple and safe, and the risks in subjects with stable asthma are acceptable (Pizzichini et al 2002). A multicenter study examining the safety of sputum induction in subjects with moderate to severe asthma concluded that sputum induction has acceptable risks, and sputum induction has a predictable risk of bronchoconstriction. Perceived risks are minimized in that our study population will be individuals with mild asthma. Additionally, sputum induction will be undertaken in carefully

monitored conditions with rigorous safeguards to identify and treat bronchoconstriction (Fahy et al 2001).

Bone Marrow Aspirates

Bone marrow aspirates will be performed using the [redacted] standard operating procedures. Aspirates are obtained from the iliac crest using a bone marrow aspiration needle. The surface of the skin is disinfected then anaesthetized with lidocaine down to the level of the bone and surrounding periosteum. Subjects may experience some local tenderness once the local anesthetic wears off. The risk of infection is very low. Subjects will be instructed to contact the study physician if they experience excessive or prolonged (>24-36 hrs) pain or bleeding at the site of insertion, or if they develop fevers, sweats, chills or any other unexpected symptoms.

Endobronchial Biopsies

Bronchoscopies will be performed using [redacted] standard operating procedures, and according to the recommendations of the U.S. National Institutes of Health. Facilities for the management of medical emergencies and cardiopulmonary resuscitation will be available. Biopsies will be performed by qualified pulmonologists experienced in research bronchoscopies, and all due precautions will be taken to minimize the risk to subjects. Subjects will receive a sedative and local anaesthetic to reduce any discomfort. Subjects will be informed of the risks associated with bronchoscopy and bronchial biopsy procedure before participating in the study.

1.4 Study Design

This randomized, double-blind, parallel group, placebo-controlled study will evaluate the effect of a fixed 30 mg dose of benralizumab administered 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 centres in [redacted] Canada. All centres will participate in the characterization of the early and late asthmatic response and induced sputum collections. A subset of 5 centres will conduct the endobronchial biopsy collections, and bone marrow aspirates will be conducted at one centre 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 participation of the study site (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.

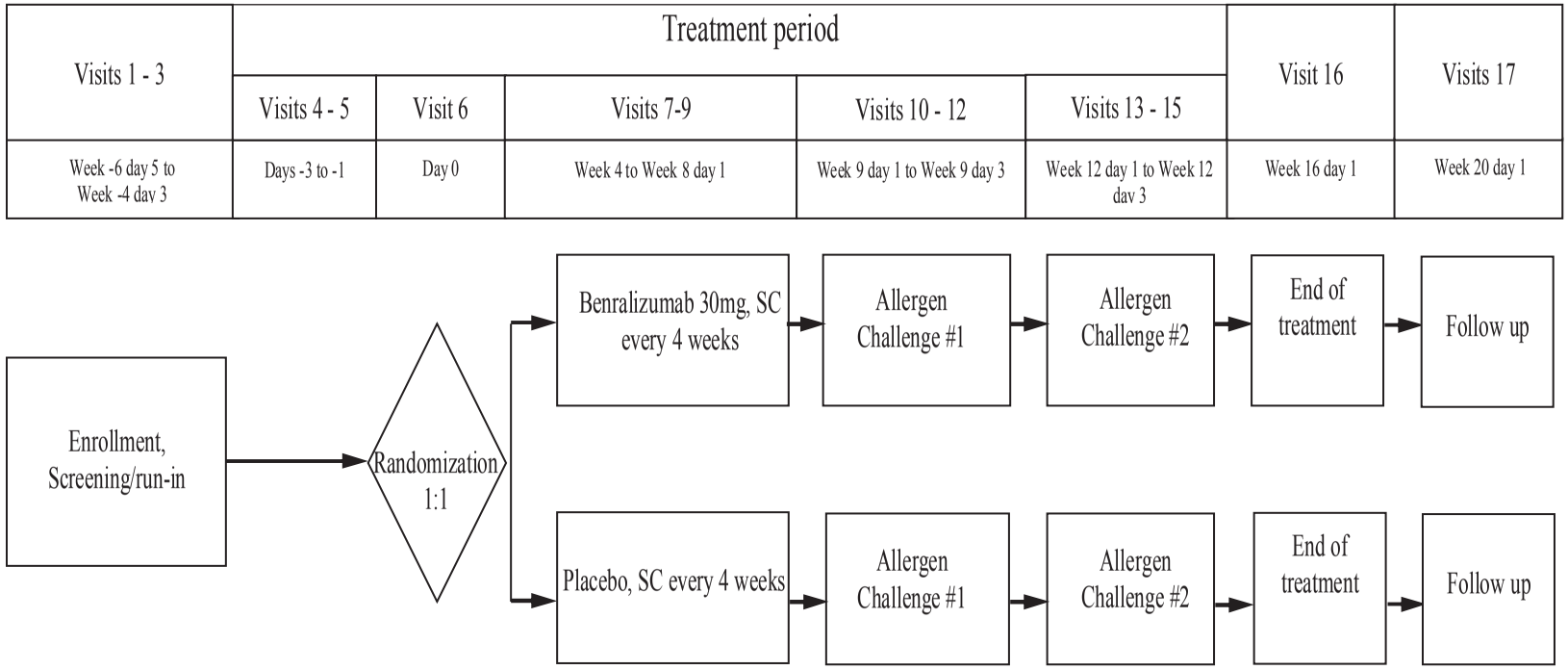
Subjects will then be randomized to receive either benralizumab 30 mg SC or matching placebo (1:1). Subjects will receive 2 additional doses of study treatment every 4 weeks for a total of 3 doses.

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 sites participating in the bronchial biopsy collections) and collection of a blood sample (all sites) for blood eosinophil measurements. Subjects will then receive a third dose of study treatment 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 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 EOT and follow-up assessments, respectively.

Figure 1 Study flow chart



2. STUDY OBJECTIVES

2.1 Primary objective

Primary Objective:	Outcome Measure:
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: Maximal percentage decrease in Forced Expiratory Volume in 1 Second (FEV_1) 3-7 hours post allergen challenge ($LAR_{3-7\ hr}$) Supportive measure: AUC of time adjusted percent decrease in FEV1 curve in late asthmatic response ($LAR_{3-7\ hr}$)

2.2 Secondary objectives

Secondary Objective:	Outcome Measure :
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
To assess the effect of benralizumab on allergen-induced early (within 2 hrs post allergen challenge) asthmatic response (EAR)	Maximal percentage decrease in FEV_1 0 – 2 hours post allergen challenge (EAR_{0-2} hr) AUC of time adjusted percent decrease in FEV_1 curve in early asthmatic response ($EAR_{0-2\ hr}$)
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 prechallenge 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 prechallenge 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 prechallenge 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 PC20 (concentration of inhaled methacholine that produces a 20% fall in FEV_1)

2.3 Safety objectives

Safety Objective:	**Outcome Measure :**

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/haemotology/urinalysis Physical examination

2.4 Exploratory objectives

Exploratory Objective:	**Outcome Measure :**
[redacted]	[redacted]
[redacted]	[redacted]

3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

Each subject should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

3.1 Inclusion criteria

For inclusion in the study subjects should fulfil the following criteria:

1. Written informed consent for study participants must be obtained prior to any study related procedures being performed and according to local guidelines.

2. Female or male aged 18 to 65 years, inclusively, at the time of enrolment.

3. General good health as declared by the investigator

4. Mild, stable, allergic asthma and asthma therapy limited to inhaled, short-acting β2 agonists (should not be used in more than 2 occasions in a week). If the subject uses the short-acting β2 agonist for prophylactic treatment this should not be included in the number of times that it is used in a 1 week-period

5. Ability to produce a sputum sample and viable cytospin for assessment of the cell differential count at screening pre, 7 and 24 hours post- challenge

6. History of episodic wheeze and shortness of breath; Pre-bronchodilator FEV_1 at screening of at least 70% of the predicted value

7. No current exposure to allergens to which a subject experiences asthmatic responses (with the exception of the house dust mite).

8. Positive methacholine challenge (PC20 ≤16 mg/mL) at screening

9. Positive skin-prick test to at least one common aeroallergen (including but not limited to cat, dust mite, grass/tree pollen) at screening

10. Positive early and late airway responses during the screening allergen challenge. The early response will be a fall in FEV1 of ≥20% during the two hours after allergen challenge. The late response will be defined as a fall in FEV1 of ≥15% during the 3-7 hours after allergen inhalation.

11. Demonstrate an increase in sputum eosinophils at 7 hrs post allergen challenge relative to the pre allergen challenge sputum sample at screening.

12. Women of childbearing potential (WOCBP) must use an effective form of birth control (confirmed by the Investigator). Effective forms of birth control include: true sexual abstinence, a vasectomized sexual partner, Implanon, female sterilization by tubal occlusion, any effective IUD intrauterine device/IUS levonorgestrel Intrauterine system, Depo-Provera™ injections, oral contraceptive, and Evra Patch™ or Nuvaring™. WOCBP must agree to use effective method of birth control, as defined above, from enrolment, throughout the study duration and within 16 weeks after last dose of IP, and have negative serum pregnancy test result on Visit 1.

    Women not of childbearing potential are defined as women who are either permanently sterilized (hysterectomy, bilateral oophorectomy, or bilateral salpingectomy), or who are postmenopausal. Women will be considered postemenopausal if they have been amenorrheic 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 postmenopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatment and follicle stimulating hormone (FSH) levels in the postmenopausal range.

- Women ≥50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatment.

13. All male subjects who are sexually active must agree to use an acceptable method of contraception (condom with or without spermicide, vasectomy) from the first dose of IP until 16 weeks after their last dose.

3.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

1. A worsening of asthma or a respiratory tract infection within 6 weeks preceding enrolment

2. Current lung disease other than mild allergic asthma

3. History of clinically significant hypotensive episodes or symptoms of fainting, dizziness, or light headedness, as judged by the investigator

4. Any history or symptoms of cardiovascular disease, particularly coronary artery disease, arrhythmias, hypertension, or congestive heart failure

5. Any history or symptoms of significant neurologic disease, including transient ischemic attack (TIA), stroke, seizure disorder, or behavioral disturbances

6. Any history or symptoms of clinically significant autoimmune disease

7. Any history of clinically significant haematologic abnormality, including coagulopathy or any history of chronic treatment with anticoagulants (e.g. warfarin, etc) or antiplatelet agent (e.g, aspirin, etc)

8. Clinically significant abnormalities in laboratory test results at enrolment and during the screening period (including complete blood count, coagulation, chemistry panel and urinalysis) unless judged not significant by the investigator

9. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level ≥2.5 times the upper limit of normal (ULN) confirmed during screening period

10. Being pregnant or lactating or have positive serum pregnancy test at enrolment or positive urine pregnancy test during the study

11. Use of regular treatment with inhaled or intranasal corticosteroids within the 4 weeks prior to enrolment (Visit 1) into the study

12. Use of orally or systemically administered corticosteroids within the 12 weeks prior to enrolment into this study

13. Use of nonsteroidal anti-inflammatory drugs (NSAIDs) 72 hours before or aspirin prn within 7 days of enrolment (Visit 1), as judged by the investigator

14. Use of long-acting bronchodilators such as tiotropium and salmeterol in the 2 weeks prior to enrolment (Visit 1) into the study

15. Have chronic use of any other medication for treatment of allergic lung disease other than short- acting β2-agonists

16. Blood draws of 100 mL or more within 45 days prior to enrolment (Visit 1) into the study

17. Current smokers. Ex-smokers must not have smoked for a minimum of 6 months, and should not have a smoking history ≥10 pack years. Subjects who administer nicotine in other forms (patches, chew tobacco, e-cigarette, etc) will also be excluded from the study. Cotinine testing must be conducted at screening (Visit 1) to support confirmation of non-smoking status.

18. Concomitant disease or condition which could interfere with the conduct of the study, or for which the treatment might interfere with the conduct of the study, or which would, in the opinion of the investigator, pose an unacceptable risk to the subject in this study, including, but not limited to, cancer, alcoholism, drug dependency or abuse, or psychiatric disease

19. History of cancer:

    - Subjects who have had basal cell carcinoma, localized squamous cell carcinoma of the skin, or in situ carcinoma of the cervix are eligible provided that the subject is in remission and curative therapy was completed at least 12 months prior to the date informed consent, and assent when applicable was obtained,

    - Subjects who have had other malignancies are eligible provided that the subject is in remission and curative therapy was completed at least 5 years prior to the date informed consent, and assent when applicable, was obtained.

20. Alcohol or drug abuse (past or present) or any conditions suggesting the potential for poor study compliance

21. Subject who has a scheduled in-patient surgery or hospitalization during the study.

22. History of anaphylaxis to any biologic therapy or vaccine

23. History of Guillain-Barré syndrome

24. A helminth parasitic infection diagnosed within 24 weeks prior to the date informed consent is obtained that has not been treated with, or has failed to respond to standard of care therapy

25. Positive hepatitis B surface antigen, or hepatitis C virus antibody serology, or a positive medical history for hepatitis B or C. Subjects with a history of hepatitis B vaccination without history of hepatitis B are allowed to enrol

26. A history of known immunodeficiency disorder including a positive human immunodeficiency virus (HIV) test

27. Use of immunosuppressive medication (including but not limited to: methotrexate, troleandomycin, cyclosporine, azathioprine, intramuscular long-acting depot corticosteroid, oral corticosteroid, or any experimental anti-inflammatory therapy) within 3 months prior to the date informed consent is obtained

28. Receipt of immunoglobulin or blood products within 30 days prior to the date informed consent is obtained

29. Receipt of any marketed (eg omalizumab) or investigational biologic within 4 months or 5 half-lives prior to randomization is obtained, whichever is longer

30. Any allergen immunotherapy within 4 months prior to the date informed consent is obtained.

31. Receipt of live attenuated vaccines 30 days prior to the date of randomization

    - Receipt of inactive/killed vaccinations (eg, inactive influenza) are allowed provided they are not administered within 1 week before/after any IP administration.

32. Receipt of any investigational non-biologic within 30 days or 5 half-lives prior to the date informed consent is obtained, whichever is longer

33. Previously received benralizumab (MEDI-563)

34. AstraZeneca staff involved in the planning and/or conduct of the study

35. Employees of the study centre or any other individuals involved with the conduct of the study, or immediate family members of such individuals

Procedures for withdrawal of incorrectly enrolled subjects see Section 3.4

3.3 Subject enrolment and randomization

Investigator(s) should keep a record of subjects considered for, and included in the study. This pre-screening/screening log will be evaluated periodically by AstraZeneca or its delegates during routine monitoring visits.

The investigator or designee will:

1. Obtain signed informed consent, and assent when applicable, from the potential subject before any study specific procedures are performed.

2. Assign each potential subject a unique enrollment number, beginning with 'E1001001 via interactive web/voice response system (IWRS/IVRS).

3. Determine subject's eligibility. See sections 3.1 and 3.2

- During the screening period, subjects will be screened with medical history, physical examination, spirometry and routine laboratory tests. These assessments may be carried out over two test days in order to minimize tests while entry criteria are being established

  If subjects continue to meet entry criteria their allergic status will be documented by skin testing against a panel of common airborne allergens.

  Note: If subjects meet entry criteria to progress to the screening allergen challenge triad then the visits for the screening allergen challenge must be conducted over three consecutive days.

- An allergen challenge will be performed to confirm the presence of an early and late phase response. Only subjects with a documented early (≥20% fall in FEV_1) and late (≥15% fall in FEV_1) asthmatic response to inhaled incremental allergen challenge will be eligible for randomisation. The screening allergen challenge can be repeated at the discretion of the Investigator, as per [redacted] SOPs.

4. Assign eligible subject unique randomization code via IWRS/IVRS. Randomisation codes will be assigned strictly sequentially per site, ie, lowest available randomisation number will be assigned.

If a subject withdraws from participation in the study, then his/her enrolment/randomisation code cannot be reused.

3.4 Procedures for handling incorrectly enrolled or randomized subjects

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

are enrolled, but subsequently found not to meet all the eligibility criteria must not be randomized or initiated on treatment, and must be withdrawn from the study.

Where a subject does not meet all the eligibility criteria but is randomized in error, or incorrectly started on treatment, the Investigator should inform the AstraZeneca study physician immediately, and the subject should be discontinued from the study

3.5 Methods for assigning treatment groups

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 IWRS/IVRS 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.

3.6 Methods for ensuring blinding

The study will be conducted in double-blind fashion. AstraZeneca staff involved in the study, the subjects, and the investigators involved in the treatment of the subjects or in their clinical evaluation will not be aware of the treatment allocation.

Placebo solution will be visually matched with benralizumab solution. Both benralizumab and placebo will be provided in an accessorized pre-filled syringe (APFS).

Maintaining the blind to the subject's blood, sputum and bone marrow eosinophil and basophil counts

While not entirely specific, subjects on active benralizumab treatment are expected to have lower eosinophil and basophil counts than subjects on placebo. Procedures to mitigate unblinding on this basis include:

- From Visit 4 on, eosinophil, basophil and monocyte counts will be redacted from the full hematology and leukocyte PD markers reports
- From Visit 4 on, eosinophil and basophil assessments conducted in induced sputum, endobronchial biopsies, blood and bone marrow at McMaster University Laboratory will be conducted by separate

laboratory staff who do not have communication with the subjects and are not involved in any other data collection in the study (i.e. adverse event reporting, clinical testing procedures conducted at the study site, laboratory procedures conducted at the study site). To further maintain blinding of personnel at the McMaster clinical site the following procedure will be followed by the McMaster central laboratory staff:

- Samples at the time of collection by study site staff will be labelled with subject identification, study, test and date.

- An individual not involved with the study will prepare an additional layer of blinding so that each sample cannot be traced back to the subject identification number. This will require a separate coded number.

- The results will be collected and maintained on a password protected computer that is regularly backed up. Results will not be shared with others who collect data.

- Once all procedures have been completed for a subject, the separate code can be broken and subject data entered into the database

3.7 Methods for unblinding

Individual treatment codes, indicating the treatment randomisation for each randomised 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 centre.

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

AstraZeneca retains the right 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.

3.8 Restrictions

Subjects will be required to:

1. Withhold the use of short acting β2-agonist for at least 8 hours before each visit.

2. Withhold the intake of xanthines and caffeine-containing products or medications for 4 hours before each visit where spirometry will be conducted.

3. Limit the use of alcohol. The use of alcohol by adults will be limited to no more than 1 drink per day (1 drink being equivalent to 12 ounces of regular beer, 5 ounces of wine, or 1.5 ounces of 80 proof distilled spirits). Subjects must withhold the intake of alcohol for 48 hours prior to each visit.

4. Withhold the use of aspirin products for 7 days before each visit and nonsteroidal anti-inflammatory drugs (NSAIDs) for 3 days before each visit.

5. Abstain from vigorous physical activity (such as long distance running) on the days of laboratory visits and during the 24 hours following allergen challenges and bronchial biopsy.

6. Withhold the use of corticosteroids (oral, systemic, nasal or inhaled) in line with the exclusion criteria (Section 3.2) and throughout the study. Withhold the use of long-acting bronchodilators including anticholinergics (e.g. tiotropium) for two weeks prior to enrolment and throughout the study.

8. Withhold the use of anti-leukotriene therapies for two weeks prior to enrolment and throughout the study.

9. Withhold the use of cromoglycate or nedocromil for 4 weeks prior to enrolment and throughout the study.

10. Withhold the use of short-acting antihistamines for 3 days, intermediate anti-histamines for 7 days and long acting-antihistamines for 9 days prior to skin prick testing and inhaled allergen testing (refer to [redacted] SOPs for further guidance).

11. Abstain from the use of nicotine or tobacco containing products (including but not limited to: snuff, chewing tobacco, cigars, cigarettes, pipes, or nicotine patches).

12. Abstain from donating blood, plasma from the time of informed consent, and assent when applicable, and for 16 weeks (5 half-lives) after last dose of IP.

13. Withhold the use of theophylline for four weeks prior to enrolment and throughout the study.

14. Withhold the use of herbal remedies for the treatment of allergic, inflammatory, or respiratory diseases for 30 days prior to enrolment and throughout the study.

15. Withhold the chronic use of anticoagulant and antiplatelet agents in line with section 3.2 (exclusion criterion #7) and throughout the study. Note PRN use of aspirin is allowed in line with restriction #4.

16. Withhold the use of immunosuppressive medication in line with Section 3.2 (exclusion criterion #27), during treatment period and 3 months or 5 half-lives (whichever is

longer) after last dose of the IP. Topical administration may be allowed at the discretion of the Investigator after discussion with the AstraZeneca physician.

17. Withhold the receipt of immunoglobulin or blood products in line with Section 3.2 (exclusion criterion #28) and throughout the study.

18. Withhold the use of any marketed (eg omalizumab) or investigational biologic in line with Section 3.2 (exclusion criterion # 29), during treatment period and 4 months or 5 half-lives (whichever is longer) after the last dose of the IP.

19. Withhold the use of any allergen immunotherapy in line with Section 3.2 (exclusion criterion # 30) and throughout the study.

20. Abstain from the use of live attenuated vaccines in line with Section 3.2 (exclusion criterion #31), during treatment period and 4 months (5 half-lives) after the last dose of the IP.

21. Withhold the use of any investigational non-biologic in line with Section 3.2 (exclusion criterion # 32) and throughout the study.

3.9 Discontinuation of investigational product

Subjects will be discontinued from investigational product (IP) in the following situations:

- Subject decision. The subject is at any time free to discontinue treatment, without prejudice to further treatment (see Section 3.1)
- Adverse event (AE) that, in the opinion of the investigator, contraindicates further dosing
- Risk to subject as judged by the investigator or AstraZeneca
- Severe non-compliance to study protocol
- Eligibility requirement found not to be fulfilled (see section 3.4)
- Pregnancy
- Lost to follow-up[1]
- IP unblinding

[1] Subject is considered lost to follow up when the following two attempts of contact are failed: 3 attempts of either phone calls, faxes or emails and having sent 1 registered letter/certified mail.

[2] Oral corticosteroids may be used to rescue after allergen challenge if needed. If the subject has to use inhaled or oral corticosteroids, the remaining secondary and exploratory outcomes of the allergen challenge triad should be aborted. Subjects will report for safety assessments, including spirometry.

- Development of any study specific criteria for discontinuation:

(a) Anaphylactic reaction to the investigational product requiring administration of epinephrine.

(b) Development of helminth parasitic infestations requiring hospitalization.

(c) If one dose of IP is missed during course of the study.

(d) An asthma-related event requiring an ER visits or use of oral corticosteroids[2]

(e) Worsening of asthma, as judged by the investigator

All subjects who prematurely discontinue IP should return to the study centre and complete the procedures described for the IPD (Premature IP Discontinuation) visit and Follow-up visit within 4 weeks (+7 days) and 12 weeks (±7 days) after the last dose of IP, respectively.

Reasons for premature discontinuation of IP should be recorded in the eCRF.

3.9.1 Procedures for discontinuation of a subject from investigational product

A subject that discontinues will always be asked about the reason(s) for discontinuation and the presence of any adverse events. The Principal Investigator/Investigator will perform the best possible observation(s), test(s) and evaluation(s) as well as give appropriate medication and all possible measures for the safety of the subject. They will also immediately inform AstraZeneca of the withdrawal. Adverse events will be followed up (See Section 6).

If a subject is withdrawn from study, see Section 3.1.

3.10 Criteria for withdrawal

3.10.1 Screen failures

Screening failures are subjects who do not fulfil the eligibility criteria for the study, and therefore must not be randomized. These subjects should have the reason for study withdrawal recorded in eCRF.

3.10.2 Withdrawal of the informed consent

Subjects are free to withdraw from the study at any time (IP and assessments), without prejudice to further health treatment.

A subject who withdraws consent, and assent when applicable will always be asked about the reason(s) and the presence of any adverse events (AE). The investigator will follow up AEs outside of the clinical study.

Subjects who withdraw from the study before Visit 9 will be asked to return to the study centre and complete procedures described for the IPD (Premature IP Discontinuation) visit

and Follow-up visit within 4 weeks (+7 days) and 12 weeks (±7 days) after the last dose of IP, respectively.

Subjects who withdraw from the study after Visit 9 will be asked to return to the study centre and complete procedures described for the End-of-Treatment (EOT) visit and Follow-up visit within 8 weeks (+7 days) and 12 weeks (±7 days) after the last dose of IP, respectively.

If a subject withdraws from participation in the study, then his/her enrolment/randomisation code cannot be reused. Withdrawn subjects may be replaced.

4. STUDY PLAN AND TIMING OF PROCEDURES

Table 1 Study Plan - Enrollment, screening/run-in period

Assessment/activity	Refer to	Enrollment	Screening/run-in		
		V1a[a]	V1b	V2 [d]	V3 [d]
		W-6 d5	W-4 d5	W-4 d4	W-4 d3
Written informed consent	10.4	X			
Inclusion/exclusion criteria	3.1/3.2	X			
Demographics	4.1	X			
Complete Physical examination	5.2.2	X			
Weight, Height, BMI	5.3.1	X			
Medical and asthma history	4.1	X			
12-lead ECG	5.2.4	X			
Vital signs	5.2.5	X			
Serum chemistry	5.2.1	X			
Cotinine testing	5.3.3.2	X			
Full Hematology	5.2.1	X			
Urinalysis	5.2.1	X			
Blood coagulation	5.3.2	X			
FSH	5.2.1.1	X			
Urine pregnancy test	5.2.1.1	X			
Serum pregnancy test	5.2.1.1	X			
Skin prick test	5.1.2	X			
Skin titration test [b]	5.1.3	X			
Serology (HIV-1; HIV-2; Hep B,C)	5.3.3.1	X			
Spirometry	5.1.6	X	X	X[c]	X
MCh AHR	5.1.4		X		X
Induced sputum for cell differential count	5.1.5		X	X	X
Allergen Challenge	5.1.1			X	
Concomitant medication	7.8	X		X	X
Adverse event review (AEs and SAEs)	6.1	X		X	X

[a] The window between Visit 1a and Visit 1b would be dictated by the time taken to receive blood safety results to allow entry criteria to be met before proceeding with V2.

[b] May be conducted at Visit 1a or Visit 1b

[c] Serial FEV_1 measurements will be performed

[d] Visit is timed from when V1b occurs

Table 2 Study Plan - Treatment period

Assessment/ activity	Refer to	Treatment												EOT	FU	IPD[g]	UNS
		Dosing & effect on baseline inflammation						Ag challenge #1			Ag challenge #2 [f, s]						
		V4[j]	V5[k]	V6[l]	V7[m]	V8[n]	V9[o]	V10[p]	V11	V12	V13[q]	V14	V15	V16[r]	V17[t]	N/A	N/A
		d-3	d-1	d0	W4	W8	W8 d1	W9 d1	W9 d2	W9 d3	W12 d1	W12 d2	W12 d3	W16 d1	W20 d1		
		Visit window (days)															
		+ 14 days	+ 1 day	+ 6 day	-3/+7 days	-3/+7 days	+3 days	+7 days			+7 days			+/- 3 days	+/- 3 days		
Inclusion/exclusion criteria	3.1/3.2	X	X	X													
Complete Physical examination	5.2.2													X		X	
Brief Physical examination	5.2.3														X		X
12-lead ECG	5.2.4													X		X	
Vital signs	5.2.5			X	X		X							X		X	X
Serum chemistry	5.2.1				X		X							X		X	X
Full hematology	5.2.1				X[h]									X[h]	X[h]	X[h]	X[h]
Hematology for leukocyte PD markers	5.1.9	X[i]			X[i]		X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]				
Urinalysis	5.2.1				X									X		X	X
Urine Pregnancy Test	5.2.1.1	X		X[a]	X[a]	X[b]	X[a]	X			X			X		X	X

Assessment/ activity	Refer to	Treatment												EOT	FU	IPD[g]	UNS
		Dosing & effect on baseline inflammation						Ag challenge #1			Ag challenge #2 [f, s]						
		V4[j]	V5[k]	V6[l]	V7[m]	V8[n]	V9[o]	V10[p]	V11	V12	V13[q]	V14	V15	V16[r]	V17[t]	N/A	N/A
		d-3	d-1	d0	W4	W8	W8 d1	W9 d1	W9 d2	W9 d3	W12 d1	W12 d2	W12 d3	W16 d1	W20 d1		
		Visit window (days)															
		+ 14 days	+ 1 day	+ 6 day	-3/+7 days	-3/+7 days	+3 days	+7 days			+7 days			+/- 3 days	+/- 3 days		
Spirometry	5.1.6	X	X		X	X		X	X[c]	X	X	X[c]	X	X		X	
MCh AHR	5.1.4	X			X			X		X	X						
Induced sputum:	5.1.5																
differential count		X			X			X	X	X							
flow cytometry		X						X		X							
biomarkers		X			X			X	X	X							
Blood for serum protein biomarkers	5.1.9	X			X			X	X	X							
Blood for flow cytometry assessments	5.1.9	X						X		X							
Bronchoscopy [d]	5.1.8		X			X							X				

Assessment/ activity	Refer to	Treatment												EOT	FU	IPD[g]	UNS
		Dosing & effect on baseline inflammation						Ag challenge #1			Ag challenge #2 [f, s]						
		V4[j]	V5[k]	V6[l]	V7[m]	V8[n]	V9[o]	V10[p]	V11	V12	V13[q]	V14	V15	V16[r]	V17[t]	N/A	N/A
		d-3	d-1	d0	W4	W8	W8 d1	W9 d1	W9 d2	W9 d3	W12 d1	W12 d2	W12 d3	W16 d1	W20 d1		
		Visit window (days)															
		+ 14 days	+ 1 day	+ 6 day	-3/+7 days	-3/+7 days	+3 days	+7 days			+7 days			+/- 3 days	+/- 3 days		
Bone marrow aspirate for cell count, flow cytometry, protein biomarkers and functional activity [e]	5.1.7	X						X		X							
Allergen Challenge	5.1.1								X			X					
Concomitant medication	7.8	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse event review (AEs and SAEs)	6.1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization	4.2.2			X													
Administration of investigational product	7			X	X		X										

[a] For all females except those NOT of child bearing potential as defined in inclusion criterion 12, urine HCG test to be done at centre on each treatment visit before IP administration

[b] Urine HCG test to be performed before bronchoscopy
[c] Serial FEV_1 will be performed
[d] Designated study centres only.
[e] Sub-set of subjects at designated study centre only.
[f] Subjects not participating in the bronchial biopsy collection will not participate in Allergen Challenge #2 and will continue to V16 according to the study schedule. Subjects will be contacted by telephone 4 weeks after last dose of study medication, prior to the EOT visit (V16). Please see section 4.2.2.2
[g] Subjects will be contacted by telephone 4 weeks after last dose of study medication, prior to the EOT visit (V16). Please section 3.9
[h] After visit 1a, lymphocyte, neutrophil and total white cell count will be reported to sites. Eosinophil, basophil and monocyte counts will be redacted from the lab report, please refer to 5.1.9, 5.2.1 and 3.6 for further details.
[i] Lymphocyte, neutrophil and total white cell count will be reported to investigative sites. Eosinophil, basophil, and monocyte counts will be redacted from the lab report, please refer to 5.1.9, 5.2.1 and 3.6 for further details. At Visit 7 where Full Hematology is also performed, only one blood sample collection will be required for all assessments.
[j] Must take place at least 28 days after Visit 3 without exceeding 42 days.
[k] Must take place 48 hours after Visit 4 without exceeding 3 days.
[l] Can take place between 1 and 7 days after V5.
[m] Must take place 28 days after V6 with a window of -3/+7 days.
[n] Must take place 56 days after V6 with a window of -3/+7 days.
[o] For Subjects participating in the bronchial biopsy collection, Visit 9 should take place 1 day after V8 with a window of +3 days. For Subjects not participating in the bronchial biopsy collection, Visit 9 should take place 57 days after V6 with a window of +/-3 days.
[p] Must take place 7 to 14 days after V9
[q] Must take place 20 to 27 days after V11
[r] Visit is timed from time of Visit 9 (Please refer to section 4.3)
[s] Please see section 4.2.2.2 (Allergen Challenge period 2) for details on the required timing between the two allergen challenge triads.
[t] Visit is timed from time of Visit 9 (Please refer to section 4.3)

D Days; EOT End-of-Treatment; FU Follow-up; R Randomization; V Visit; UNS Unscheduled; W Week.

4.1 Enrolment/screening period (Visit 1 – Visit 3)

Each potential subject must provide written informed consent prior to any study specific procedures and undergo assessments applicable for the visit (see Table 1).

Subjects must sign the Informed Consent Form (ICF) prior Visit 1 procedures.

A sub-set of up to 22 subjects recruited at one of the study centres will be required to provide consent to supply a sample of their bone marrow. Subjects recruited at 5 of the 6 study centres will be required to provide consent for endobronchial biopsies. These consents are included in the main subject informed consent form.

At Visit 1a the following assessments will be conducted:

- Collection of demographics and evaluation of the health and asthma status of the subject including height and weight, physical examination, vital signs, ECG, clinical chemistry and hematology, urinalysis, spirometry, medical and asthma history, pregnancy test

Other study assessments and procedures to be performed at enrolment Visit 1 are mentioned in Table 1. It is preferred that Visit 1a is conducted over two days, however, Visit 1a may be conducted over a period of up to two weeks to allow any repeat assessments that may need to be made.

Subjects who do not meet the Visit 1a assessment criteria must not be enrolled in the study.

Subjects who meet eligibility criteria from V1a will return for the allergen challenge screening assessments at Visit 1b, Visit 2 and Visit 3. Visit 1b, Visit 2 and Visit 3 must be conducted over 3 consecutive days.

At Visit 1b the following assessments will be conducted:

- Spirometry
- Methacholine PC20 test to determine that a subject has hyper-responsive airways for inclusion criteria and to calculate the concentration of allergen to be used on each subject for the inhaled allergen challenge test at Visit 2 (Cockcroft et al 1987). The calculated MCh PC20 at the site will be recorded in the CRF.
- Induced sputum procedure to determine ability of subject to provide a viable sputum sample and to determine the pre-allergen challenge eosinophil count.

Note that the skin prick test to confirm atopy and identify the specific allergen and allergen sensitivity for inhalation challenge testing may be conducted at Visit 1a or Visit 1b at the preference of the study site investigator.

At Visit 2 assessment the following assessments will be conducted:

- Spirometry (this will include serial measurements to at least 7 hours post allergen challenge)
- Inhaled allergen challenge
- Induced sputum collection 7 hours post allergen challenge

At Visit 3 subjects will return to the clinic for the following assessments 24 hours post allergen challenge:

- Spirometry
- Methacholine PC20 test to assess airway responsiveness post allergen challenge
- Induced sputum for assessment of sputum eosinophils 24 hours post allergen challenge.

Methacholine PC20 and induced sputum will be performed 24 hrs (± 2) after allergen challenge (Visit 3).

To be eligible for continuation in the study subjects must meet the following criteria:

- A positive skin prick test to at least one aeroallergen
- A MCh PC20 test result of ≤16 mg/mL at Visit 1b
- Produce a viable and evaluable sputum cytospin at Visit 1b, at 7 hours post allergen challenge at Visit 2 and at 24 hrs post allergen challenge at Visit 3
- Demonstrate a fall in FEV_1 of ≥20% from baseline during the period of early reaction (within 2hrs post challenge) and a fall in FEV_1 of ≥15% from baseline during the period of late reaction (3 – 7 hrs post challenge) to inhaled allergen challenge at Visit 2
- Demonstrate an increase in sputum eosinophils in the 7 hrs post challenge sputum sample when compared with pre-allergen challenge.

Repeat of screening assessments versus Rescreening

Repeat assessments

Some assessments conducted at Visit 1a may be repeated at the discretion of the investigator. These include:

- Pre-bonchodilator FEV_1 if <70% predicted
- Clinical chemistry and/or hematology

These assessments may be repeated on one occasion, unless they are for follow-up safety, and should be conducted within the 2 week time frame between Visit 1a and Visit 1b.

At Visit 1b the following assessments may be repeated:

- Induced sputum assessment

The induced sputum assessment may only be repeated on one occasion and should be repeated within 2 weeks of the initial assessment. If a subject returns for a repeat induced sputum the methacholine PC20 assessment must also be repeated.

Note that if a subject fails an initial induced sputum assessment and this must be repeated, the Visit 2 and Visit 3 assessments must be delayed and run on consecutive days after the induced sputum assessment.

- Methacholine PC20

The methacholine PC20 can be repeated on one occasion within 2 weeks at the discretion of the investigator.

Rescreen assessments

Re-screening is allowed only once for the subject. Subjects should keep the same Ecode. The following assessments may allow for a re-screening:

- If the reason for screen failure was transient (including but not limited to study-supplied equipment failure, unforeseen personal events that mandate missed screening visits), which would mean that the subject would fall outside of the enrolment/screening window.
- If a subject fails to achieve a late asthmatic response the allergen inhalation challenge triad may be repeated as per Investigator discretion.

The rescreen as a result of needing to repeat the allergen challenge triad should be conducted as early as 2 but not later than 6 weeks after the first allergen challenge.

If a subject requires rescreening then subjects should re-sign informed consent before the re-screening procedures. All procedures from screening/run-in period should be repeated with the exception of the skin prick testing.

Subjects may not be re-screened if:

- They fail to show an skin prick response after confirmation of antihistamine washout
- They fail to show an increase in sputum eosinophils at 7 hours post allergen challenge

4.2 Treatment period

4.2.1 Baseline Inflammation assessments

No less than 4 and no greater than 6 weeks following screening and allergen challenge (to allow inflammation to return to baseline), subjects will return to the clinic for assessment of baseline inflammation prior to dosing with study treatment. These assessments will take place during two visits over three days (Visit 4 and 5).

At Visit 4 subjects who have met screening eligibility criteria will undergo the following assessments:

- Urine pregnancy test
- Spirometry. FEV_1 should be ≥70% predicted.

  If FEV_1 is <70%, procedures may continue at the discretion of the investigator, [redacted] If it is not possible to continue with procedures then the Visit should be postponed and rescheduled.
- MCh PC20 test. The MCh concentration to induce a 20% fall in FEV_1 should not be more than one doubling concentration lower than the value at enrolment (Visit 1). If the MCh PC20 is more than one doubling dose lower, the remainder of the visit should be postponed and the visit rescheduled.
- A blood sample collection for leukocyte PD markers, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and serum protein biomarkers.
- An induced sputum sample for cell differential count, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and protein biomarkers
- A bone marrow aspirate (in a sub-group of subjects at one site only) will be collected for eosinophil count, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and protein biomarkers.

At least forty eight hours after Visit 4 subjects participating in endo-bronchial biopsy sub-group assessments will attend Visit 5. The endobronchial biopsy is collected at least 48 hours

after the induced sputum assessment to allow transient neutrophilic inflammation from the isotonic saline inhalation to resolve. At Visit 5 this sub-group of subjects will undergo:

- Spirometry. FEV_1 should be ≥70% predicted.

  If FEV_1 is <70%, procedures may continue at the discretion of the [redacted]. If it is not possible to continue with procedures then the Visit should be postponed and rescheduled according to the visit window in the schedule of assessments.

- Endobronchial biopsy procedure

4.2.2 Randomised treatment period

Subjects confirmed to be eligible will be randomized at Visit 6 in a 1:1 ratio to benralizumab or placebo.

A 30 mg dose of benralizumab or matching placebo will be administered subcutaneously (SC) every 4 weeks for 8 weeks (Day 0 (Visit 6), Week 4 (Visit 7) and Week 8 D1 (Visit 9)).

At Visit 6 subjects will undergo the following procedures:

- Urine pregnancy test (females)
- Randomization
- Dose with study medication. Vital signs will be obtained prior to dosing (see section 5.2.5).

4.2.2.1 Effect of Benralizumab on baseline inflammation

Twenty eight days after the first dose subjects will return to the clinic for Visit 7 assessments. Visit 7 will assess the effect of treatment on baseline inflammatory markers one month after dosing. At Visit 7 subjects will undergo:

- Spirometry. FEV_1 should be ≥70% predicted.

  If FEV_1 is <70%, procedures may continue at the discretion of the investigator, as [redacted]. If it is not possible to continue with procedures then the Visit should be postponed and rescheduled up to 1 week later.

- Pregnancy test (females)
- Collection of a predose blood sample for full hematology
- Blood sample for leukocyte PD markers and serum protein biomarkers

- MCh PC20 test
- An induced sputum sample for cell differential count and protein biomarkers
- Administration of the second dose of study medication. Vital signs will be obtained prior to dosing (see section 5.2.5).

Twenty eight days after the second dose the sub-group of subjects participating in the endobronchial biopsy assessments will return to the clinic for collection of an endobronchial biopsy (at those sites conducting this assessment) (Visit 8). This lung biopsy will provide data for the effect of treatment on baseline inflammation prior to allergen challenge testing. Subjects not participating in collection of endobronchial biopsies will not attend Visit 8.

The sub-group of subjects participating in the endobronchial biopsy assessments at Visit 8 will undergo the following procedures:

- Spirometry. FEV_1 must be ≥ 70% predicted.

  If FEV_1 is <70%, procedures may continue at the discretion of the investigator[redacted]. If it is not possible to continue with procedures then the Visit should be postponed and rescheduled up to 1 week later.

- Urine pregnancy test (females)
- Endobronchial biopsy procedure to evaluate the effect of treatment on eosinophilic inflammation prior to allergen challenge

All subjects will attend Visit 9 for administration of the third dose of study medication. At this visit subjects will undergo the following procedures:

- Urine pregnancy test (females)
- Blood sample for leukocyte PD markers and serum protein biomarkers
- Administration of the third dose of study medication. Vital signs will be obtained prior to dosing (see section 5.2.5).

4.2.2.2 Effect of Benralizumab on allergen-induced inflammation

Allergen challenge period 1

A minimum of 7 days after receiving the third dose of study medication all subjects will return to the clinic (Visit 10) for allergen challenge and related assessments. The first allergen challenge period will take place over 3 consecutive days (Visits 10, 11 and 12).

At Visit 10 all subjects will undergo the following procedures:

- Spirometry. FEV_1 should be ≥70% predicted.

  If FEV_1 is <70%, procedures may continue at the discretion of the investigator, [redacted]. If it is not possible to continue with procedures then the Visit may be postponed and rescheduled up to one week later. If the visit cannot be rescheduled in the 7 day window, then subjects who are participating in the endobronchial biopsy assessments will skip visits 10, 11 and 12 and return to the clinic according to the protocol schedule for Visit 13 for the second allergen challenge. Subjects who are not participating in the endobronchial biopsy assessments will be withdrawn.

- Urine pregnancy test (females)

- Blood sample collection for leukocyte PD markers, for flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and serum protein biomarkers

- MCh PC20 test to evaluate effect of treatment on airway hyperresponsiveness

- An induced sputum sample for cell differential count, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and protein biomarkers

- A bone marrow aspirate (in a sub-group of subjects at one site only) will be collected for eosinophil count, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and protein biomarkers

These assessments conducted prior to allergen challenge will provide the data for effect of study treatment on baseline inflammation in each of the tissue compartments.

Twenty four hours after Visit 10, subjects will return to the clinic for Visit 11 to undergo allergen challenge procedure. At Visit 11 the following procedures will be conducted:

- Prior to allergen exposure a pre-challenge FEV_1 will be determined. FEV_1 should be ≥70% predicted. If FEV_1 is <70%, procedures may continue at the discretion of the investigator, [redacted]. If it is not possible to continue with procedures then the visit will be postponed and it may be possible to reschedule the complete allergen triad visits again, repeating the pre-allergen challenge assessments from Visit 10. If Visit 10 has not already been extended by 1 week, the allergen challenge triad may be repeated 1 week later, repeating the Visit 10 prechallenge assessments followed by Visit 11 and Visit 12. If the Visit 10 assessment has already been extended by 1 week then the allergen challenge triad may not be repeated. Subjects who are participating in the endobronchial biopsy assessments should skip allergen challenge period 1 and return to the clinic according to the protocol schedule for Visit 13 and the start of allergen challenge triad 2. Subjects who are not

participating in the endobronchial biopsy assessments should be withdrawn from the study.

- Inhaled allergen challenge using the same allergen and concentration as determined at screening
- An induced sputum sample collection 7 hours post allergen challenge for cell differential count and protein biomarkers
- A blood sample collection 7 hours post allergen challenge for leukocyte PD biomarkers and serum protein biomarkers

Subjects will then return to the clinic 24 hours following the allergen challenge procedure (Visit 12) for collection of assessments 24 hours (+/- 2 hours) post allergen challenge. At Visit 12 subjects will undergo the following procedures:

- Spirometry
- Blood sample collection for leukocyte PD markers, for flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and serum protein biomarkers.
- MCh PC20 test to evaluate effect of treatment on airway hyperresponsiveness
- An induced sputum sample for cell differential count, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and protein biomarkers
- A bone marrow aspirate (in a sub-group of subjects at one site only) will be collected for eosinophil count, flow cytometry assessments (basophils, eosinophil progenitor cells, ILC2s) and protein biomarkers

Allergen challenge period 2

The sub-group of subjects who participate in the endobronchial biopsy assessments will return to the clinic for a second allergen challenge triad. Subjects should return for the start of the second allergen triad (Visit 13) no earlier than 20 days after the first allergen challenge (Visit 11).

At Visit 13 subjects will undergo the following pre-allergen challenge procedures:

- Spirometry. FEV_1 should be ≥70% predicted.

  If FEV_1 is <70%, procedures may continue at the discretion of the investigator, [redacted]. If it is not possible to continue with procedures then the Visit should be postponed and rescheduled up to 1 week later.

- Urine pregnancy test (females)
- Blood sample collection for leukocyte PD markers
- MCh PC20 test

Twenty four hours after Visit 13, subjects will return to the clinic for Visit 14 to undergo the allergen challenge procedure. At Visit 14, subjects will undergo the following procedures:

- Prior to allergen exposure a pre-challenge FEV_1 will be determined. FEV_1 should be ≥70% predicted. If FEV_1 is <70%, procedures may continue at the discretion of the investigator [redacted]. If it is not possible to continue with procedures then the visit will be postponed and it may be possible to reschedule the complete allergen triad visits again, repeating the pre-allergen challenge assessments from Visit 13. If Visit 13 has not already been extended by 1 week, the study allergen challenge triad may be repeated 1 week later, repeating the Visit 13 prechallenge assessments followed by Visit 14 and Visit 15. If the subjects Visit 13 assessment has already been extended by 1 week then the allergen challenge triad may not be repeated and the subject should be withdrawn.
- Spirometry will be performed throughout the procedure at regular intervals up to 7 hrs post challenge.
- Inhaled allergen challenge using the same allergen and concentration as determined at screening
- A blood sample collection 7 hours post allergen challenge for leukocyte PD markers

Twenty four hours after the allergen challenge procedures subjects will return to the clinic for Visit 15. At Visit 15 the endobronchial biopsy subject sub-group will undergo the following procedures:

- Spirometry.
- Blood sample collection for leukocyte PD markers
- Endobronchial biopsy procedure to evaluate the effect of treatment on eosinophilic inflammation following allergen challenge

Subjects not participating in the bronchial biopsy collection will not participate in allergen challenge 2 and will continue to V16 according to the study schedule. Subjects will be contacted by telephone 4 weeks after last dose of study medication to collect information on AEs and concomitant medication as well as to remind them to return for the EOT visits (V16).

Subjects who prematurely discontinued IP (see Section 3.9) should return to the study centre and complete procedures described for the IPD visit (Premature IP Discontinuation) and Follow-up visit within 4 weeks (+7 days) and 12 weeks (±7 days) after the last dose of IP, respectively. Study assessments and procedures to be performed at IPD and Follow-up visits are mentioned in Table 1.

Reasons for premature discontinuation of IP should be recorded in the eCRF.

4.3 Follow-up period

Subjects will return to the clinic 8 and 12 weeks after the last dose of study medication for EOT and follow-up assessments as indicated in the table of assessments (Table 2), respectively.

5. STUDY ASSESSMENTS

The Rave Web Based Data Capture (WBDC) system will be used for data collection and query handling. The investigator will ensure that data are recorded on the eCRFs as specified in the study protocol and in accordance with the instructions provided.

The investigator ensures the accuracy, completeness, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. The investigator will sign the completed eCRFs. A copy of the completed eCRFs will be archived at the study site upon study completion. .

The Principal Investigator/Investigator will record data on the observations, tests and assessments specified in the protocol on the eCRFs provided by AstraZeneca. The CRF will be accompanied with 'Instructions for the Investigator', which should be followed. These instructions provide guidance for the recording of study data in the CRF including how to change data incorrectly recorded.

5.1 Efficacy assessments

5.1.1 Allergen Inhalation Challenge

Allergen inhalation challenge is performed as described by O'Byrne and colleagues (O'Byrne et al 1987) in accordance with [redacted] SOPs.

Inhalation with specific allergen agent (as determined from skin prick tests (see Section 5.1.2) will be performed on Day 2 (Visit 2) of Screening (to determine whether the subject demonstrates a dual response), at Visit 11 and at Visit 14 (only for subjects participating in the endobronchial biopsy procedures). Allergen inhalation periods will be separated by a minimum of 3 weeks between challenges.

Prior to beginning each inhaled allergen challenge, baseline FEV_1 will be measured with 3 technically satisfactory readings. The highest value will be entered into the CRF and be used for calculation of the percentage fall in FEV_1 (see section 5.1.6 for spirometry assessments).

The allergen challenge should be performed in a designated area, with emergency equipment and medical facilities readily available. Emergency drugs should be available, including salbutamol (and ipratopium if applicable) MDI and nebules: steroids (injectable and inhaled); injectable antihistamine (eg, Diphenhydramine) epinephrine, and atropine. A resuscitation bag and wheelchair should also be available. The test will be carried out by qualified study personnel under physician supervision defined as the investigator or delegate being aware of the procedure, and being present in the laboratory or available immediately by phone/pager.

Screening Allergen Challenge

The FEV_1 will be required to be within 10% of Visit 1a FEV_1 to proceed with the challenge and should be >70% predicted. The concentration of allergen extract for inhalation will be 2-4 doubling concentrations below predicted to result in a 20% decrease in FEV_1 as determined from a formula described by Cockcroft *et al* (Cockcroft et al 2005).

Allergen will be inhaled by 2 minutes of tidal breathing and FEV_1 will be measured at 10 minutes post-inhalation. Inhalations will therefore occur at 12 minute intervals.

- If FEV_1 falls 10-15%, the next concentration of allergen should be inhaled after waiting an additional 10 minutes and measuring another FEV_1.
- If FEV_1 falls between 15-20% the next concentration may be inhaled for 1.5 minutes or 2 minutes (as determined by the investigator or delegate). Allergen will be inhaled until the FEV_1 falls by a minimum of 20%.

Maximum decreases in FEV_1 will be chosen to quantify the early and late response magnitudes.

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 early bronchoconstrictor response (i.e., EAR) is taken to be the largest percentage fall in FEV_1 within 2 hours post allergen inhalation, and the late response (i.e., LAR) is taken to be the largest percentage fall in FEV_1 between 3 hours and 7 hours post allergen inhalation. The area under the curve (AUC) is determined during the early (0-2 h) and late (3-7 h) responses. Only subjects who achieve a fall in FEV1 of ≥20% during the period of early response and a fall in FEV1 of ≥15% during the period of late response at screening V2 will be randomised.

Post treatment Allergen Challenge Triads

The same doses of allergen as used at screening are administered during each subsequent challenge unless it is unsafe to do so. If this happens the procedure will proceed with the highest allergen dose that can be administered as outlined in [redacted] SOPs.

Note: If the subject has to use inhaled or oral corticosteroids, the remaining secondary and exploratory outcomes of the allergen challenge triad should be aborted. Subjects will report for safety assessments, including spirometry.

5.1.2 Allergen Skin Testing

An allergy skin test, also called a skin prick test, will be used to determine the allergen that is given to each subject in the inhalation allergen challenge in accordance with [redacted] SOPs. It is performed by applying extract of an allergen to the skin of the volar aspect of the forearm, scratching or pricking the skin to allow exposure, and then evaluating the local reaction in the skin. Allergen extracts will include, but are not limited to: ragweed, tree mix, grass mix, dog, cat, horse, feathers, dust mites (Dermatophagoides farinae and D. pteronyssinus), Alternaria, and Aspergillus. A positive control (1 mg/mL histamine) and a negative control (diluent) are applied to the skin. If an allergen provokes an allergic reaction, a raised itchy bump (wheal) develops. The size of the wheal (the raised area, not the redness) will be measured and recorded with a ruler in millimeters in the horizontal and vertical directions, perpendicular to each other after approximately 15 minutes. The size of the wheal for each antigen will be recorded on the Skin Test Form, along with any observed adverse reaction or event and any actions taken. A reaction greater than 2 x 2 mm will be regarded as positive, provided that the positive and negative controls are appropriately positive (histamine) and negative (diluent), respectively. The investigator is to choose an allergen for inhalation on the basis of the largest skin response. This procedure will be completed in accordance with the allergen skin testing using the epicutaneous method SOP [redacted].

5.1.3 Allergen Skin Titration Testing

The allergen skin titration test will be performed in accordance with [redacted] SOPs. An allergy skin titration test, is used to identify the lowest titration of allergen which causes a skin wheal at least 2 x 2 mm in size. It is performed by applying serial dilutions of a selected allergen extract to the volar aspect of the forearm skin (opposite to arm used for determination of the appropriate allergen), scratching or pricking the skin to allow exposure, and then measuring the skin's reaction with a ruler. The diameter of each wheal is measured in two perpendicular directions after 10 minutes. The average wheal diameter for each dilution in the horizontal and vertical directions is recorded on the worksheet (i.e 4 x 3 mm). The weakest dilution of allergen which produces an average wheal of at least 2 x 2 mm is the ``skin test endpoint``. This is used to calculate the starting allergen dose for inhalation.

5.1.4 Methacholine Inhalation Tests

Methacholine inhalation challenge will be performed as described by Cockcroft (Cockcroft DW 1985) and in accordance with the [redacted] SOPs using tidal breathing, from a Wright nebulizer (Roxon Meditech, Montreal, PQ, Canada). Subjects inhale normal saline, then doubling concentration of methacholine for 2 minutes using a nebulizer with a mouthpiece on a one-way Hans-Rudolph valve. Spirometry is measured at regular intervals after each inhalation. The test will be terminated when a fall in FEV_1 of at least 20% of the baseline value occurs, and the methacholine PC20 has been calculated. The methacholine PC20 assessment will be performed as a measure of airway hyper responsiveness during the

screening period (24 hours before and 24 hours following allergen challenge), at the start of the treatment period pre-dose, and at the end of the treatment period (24 hours before and 24 hours following allergen challenge). The methacholine PC20 measured at the start of the treatment period (Visit 4) must not be more than 1 doubling dose lower than that measured during screening pre-allergen (Visit 1) to proceed with treatment. The methacholine inhalation challenge will be performed before any induced sputum procedures.

5.1.5 Sputum Induction

Sputum will be induced and processed using modified methods adapted from the method described by Pizzichini (Pizzichini et al 1996) and in accordance with the [redacted] SOPs. In brief, subjects will inhale 3%, 5% and 7% saline for 7 minutes at each concentration [redacted] SOPs.

The sputum samples collected will be used in the following ways:

- The total cell count will be determined using a Neubauer hemocytometer chamber (Hausser Scientific, Blue Bell, PA) and expressed as the number of cells per milliliter of sputum.
- Cells will be prepared on glass slides for differential counts and stained with DiffQuik (American Scientific Products, McGaw Park, IL). Using sputum cytospins, the differential cell count and the number of eosinophils will be counted (Visits 1b, 2, 3, 4, 7, 10, 11 and 12).
- Separate cytospin slides will be prepared and stained with toluidine blue to determine basophil counts (Visits 1b, 2, 3, 4, 7, 10, 11 and 12)
- Remaining cells from the sputum processing will be used to determine the presence of basophils, eosinophil progenitor cells and ILC2s by flow cytometry (Visits 4, 10 and 12).
- The sputum supernatant will also be analyzed for cytokines and protein biomarkers (Visits 4, 7, 10, 11 and 12).

The cell differential counts will be performed at a central [redacted] sputum laboratory. The sputum processing procedure and procedures for cytospin staining, counting and flow cytometry analysis and supernatant assessments will be defined in a lab manual separate to the study protocol.

5.1.6 Spirometry

Spirometry (FVC, FEV_1, FEV_1 /FVC ratio, FVC % predicted, FEV_1 % predicted) will be measured in accordance with the [redacted] SOPs. Prior to administration of study medication and at baseline, pre allergen challenge, pre MCh PC20 or pre induced sputum FEV_1 measurements will be measured from 3 technically satisfactory readings. The highest FEV_1 value will be entered into the CRF and used for analysis.

The spirometry procedure will be conducted according to American Thoracic Society (ATS) European Respiratory Society (ERS) Guidelines 2005 (Miller et al 2005). In general:

- The centre will be responsible for calibrating and recording the calibration of the spirometer according to the recommendations of the manufacturer. Unless otherwise advised, this should be on a daily basis and where there is a significant fluctuation in temperature or barometric pressure.
- The subject should rest for 15 minutes before lung function tests are performed.
- The subject should be sitting with the head level and tight clothing should be loosened.
- The subject should perform the manoeuvre with a nose clip.

All subjects must have a Day -3 (Visit 4) FEV_1 within 10% of that measured pre-screening allergen challenge to demonstrate asthma stability.

5.1.7 Bone Marrow Aspiration, processing and culture

Bone marrow aspirates will only be collected from a sub-set of up to 22 subjects enrolled at one study site (McMaster University). Bone marrow aspirates will be collected at Visits 4, 10 and 12.

Following local freezing, a bone marrow aspirate will be obtained from the iliac crest using a bone marrow aspiration needle. 10 ml of bone marrow will be collected into a syringe pre-filled with heparin. Total cell count will be performed as per Cell Counting SOP, and slides for differential counts will be prepared.

Bone marrow aspirates will be used for the following assessments:

- Slides for differential cell counts and measurement of mature eosinophils
- Basophils, eosinophil progenitor cells and ILC2s by flow cytometry
- Plasma for measurement of cytokines and protein biomarkers
- Evaluation of functional activity of eosinophil progenitor cells by an in vitro methylcellulose method evaluating IL-5 induced growth.

The cell counts, flow cytometry assessments and in vitro cell growth assays will be performed at a central [redacted] sputum laboratory. The bone marrow aspirate processing procedure and procedures for cytospin staining, counting and flow cytometry analysis, plasma assessments and in vitro assay will be defined in a lab manual separate to the study protocol.

5.1.8 Bronchoscopy and processing of samples

Endobronchial biopsy will be performed at 5 of the 6 participating clinical study sites in accordance with the [redacted] SOPs. Subjects who participate in the endobronchial biopsy assessments will have endobronchial biopsies collected at Visits 5, 8 and 15.

Spirometry is measured on fasted subjects, and pulse oximetry is monitored throughout the procedure. Subjects are pre-medicated as per McMaster Bronchoscopy SOP (if required midazolam IV and/or Fentanyl IV is used for sedation and titrated to response by physician). Nasopharyngeal anaesthetic is administered to vocal cords and the trachea. Bronchoscopy is conducted using a fibreoptic bronchoscope, which is passed through the vocal cords and introduced into the airways and wedged into a segmental or sub segmental bronchus. Five endobronchial biopsies are taken from the carini at the take-off of the bronchi of the lower and upper lobes, using Radial Jaw single use forceps from Boston Scientific M00515181. The sampling time of the last biopsy will be recorded in the CRF. Throughout the procedure, 100% oxygen is delivered via a nasal cannula at a rate of 2 L/min whilst oxygen saturation is monitored continuously using an oximeter with the probe placed on a finger.

Subjects will be allowed to recover in hospital and will be released when a study physician deems it is safe to do so. If the patient has received sedation they will be kept under observation for 1-4h or according to the bronchoscopist. They will be allowed to eat and drink 2-4 hours after the procedure or according to the bronchoscopist. Release time will be noted in the patient's charts. Patients will be reminded of contact information of study physician should complications due to the procedure arise. Subjects should be instructed to return if they experience bleeding; if they develop fevers, sweats, chills or any other unexpected symptoms.

Endobronchial biopsies will be used for the following assessments:

- Number of eosinophils and basophils
- Eosinophil activation markers
- Other cell assessments associated with asthma and the pharmacology of benralizumab

The processing and evaluation of the endobronchial biopsy tissue will be performed at a central [redacted] sputum laboratory. The endobronchial biopsy tissue processing procedures and procedures for staining, counting and analysis will be defined in a lab manual separate to the study protocol.

5.1.9 Blood for cell assessments and serum protein markers

Blood will be collected from all participating subjects for the following pharmacodynamics assessments:

- Samples for full hematology will be collected at Visit 1a, Visit 7, Visit 16 and Visit 17 (see section 5.2.1). In regards to WBC count and

differentials the following will be recorded: total cell count, neutrophils, lymphocytes, monocytes, basophils and eosinophils as percent and absolute counts. After Visit 1a, the total white cell count as well as neutrophil and lymphocyte counts will be reported to the investigative site. Note the eosinophil, basophil and monocyte counts will be redacted from the report.

- Samples for leukocyte PD markers will be collected at Visits 4, 7 and 9 to 15. In regards to WBC count and differentials the following will be recorded: total cell count, neutrophils, lymphocytes, monocytes, basophils and eosinophils as percent and absolute counts. These hematology results will be reported back to the sites redacting eosinophil, basophil and monocyte counts.

- Basophils, eosinophil progenitor cells and ILC2s by flow cytometry (collected at Visits 4, 10, 12).

- Serum protein biomarkers (collected at Visits 4, 7, 10, 11, 12)

The blood eosinophil counts will be performed by the central lab. If possible collection of blood should be at same time each day (+/- 2 hrs). Flow cytometry assessments and in vitro cell growth assays will be performed at a centra[redacted] sputum laboratory. Serum protein biomarkers will be sent to a 3rd party vendor for analysis. Blood processing procedure and procedures for collection, counting and flow cytometry analysis, serum assessments and in vitro cell growth assays will be defined in a lab manual separate to the study protocol.

5.2 Safety assessments

5.2.1 Laboratory safety assessments

Safety laboratory tests (list provided in Table 3) will be performed in a central laboratory. For information on methods of collection, assessment, labelling, storage and shipment of samples please refer to the separate Laboratory Manual. Safety samples will be collected in accordance with the schedules provided in Table 1 and Table 2.

The Investigator should make an assessment of the available results with regard to clinically relevant abnormalities. The laboratory results should be signed and dated and retained at centre as source data for laboratory variables. For information on how AEs based on laboratory tests should be recorded and reported, see Section 6.3.

In case a subject shows an AST or ALT ≥3xULN or total bilirubin ≥ 2xULN please refer to Appendix C 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

Table 3 List of Safety Laboratory Tests

Serum Chemistry		Haematology	Urinalysis
Alkaline phosphatase	Gamma-GT (gamma-glutamyl transpeptidase)	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	
Chloride	Total cholesterol	WBC count with differentials [a]	
CO2 (carbon dioxide)	Uric acid		
Creatinine			

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

5.2.1.1 Pregnancy Test

The following tests are applicable to female subjects only and will be conducted in accordance with the schedules provided in Table 1.

- Serum beta-HCG : To be done in all females at screening Visit 1a except for those who are NOT of child bearing potential as defined in inclusion criterion 3. This test is to be sent to and analyzed at the central laboratory.
- FSH: To be done at screening Visit 1a only, for female subjects to confirm postmenopausal status in women <50 years who have been amenorrheic for >12 month
- Urine HCG: To be performed at the study centre for all females at each treatment visit before IP administration using a dipstick except for those females who are NOT of child bearing potential as defined in inclusion criterion 3. A positive urine test result must be confirmed with serum beta HCG.

5.2.2 Physical examination

Complete physical examination will be performed in accordance with schedule provided in Table 1 and Table 2.

The physical examination will include an assessment of the following: general appearance, skin, head and neck (including eyes, ears, nose, mouth, and throat), lymph nodes, abdomen, musculoskeletal (including spine and extremities), cardiovascular, respiratory, and neurological systems.

Baseline data will be collected at Visit 1a. Any new finding(s) or aggravated existing finding(s), judged as clinically significant by the investigator, will be reported as an AE as described in Section 6.1.

5.2.3 Brief physical examination

The brief physical examination will include an assessment of the general appearance, abdomen, cardiovascular and respiratory system. For the brief physical examination only information on whether the assessment was performed or not is to be recorded.

5.2.4 ECG

ECG will be performed in accordance with schedule provided in Table 1.

In all subjects, the printouts of the ECG will be collected and signed, dated and stored at the study centre along with a signed and dated copy (if the printouts are not on archive-quality paper).

A 12-lead ECG will be taken in supine position, after the subject has been resting for at least 5 minutes

The investigator or authorized delegate will be responsible for the overall interpretation and determination of clinical significance of any potential ECG findings. In case of discrepancy between the investigators interpretation and that provided by the ECG machine (if applicable), the investigators interpretation take precedence and should be noted on the printout and recorded in the eCRF. Two identical copies of the ECG will be produced and quality checked and kept in case of further need for re-evaluation.

It is highly recommended that the same machine is used for assessment throughout the subject's participation in the study.

ECG data and evaluation will be recorded in the eCRF.

5.2.5 Vital signs

Pre-dose vital signs (pulse, blood pressure, respiration rate and body temperature) will be obtained in accordance with schedule provided in Table 1.

The vital signs will be taken prior to IP administration, and, if possible, blood draw. If it is not logistically possible, 10 minutes should be allotted between phlebotomy and vital signs assessment. Vital signs should also be taken prior to per protocol bronchodilator administration if applicable for that visit.

Pulse rate and blood pressure should be measured after the subject has been resting for at least 5 minutes. The measurement will be taken in sitting position. Pulse rate will be obtained before blood pressure.

Respiration rate will be obtained after subject has been resting for at least 5 minutes, by counting number of breaths (how many times the chest rises) for 1 minute.

Body temperature will be measured in Celsius before IP administration in accordance with local standards.

5.3 Other assessments

5.3.1 Weight and height

Weight and height will be measured in accordance with the schedules provided in Table 1.

The subject's weight will be recorded in kilograms; height will be recorded in centimetres.

Weight and height measurements will be performed in light clothing and with shoes off.

5.3.2 Blood coagulation

The blood coagulation test will be performed at Visit 1a screening.

Samples will be tested for APTT using standard techniques.

5.3.3 Other screening/run in assessments

5.3.3.1 Serology

Hepatitis B surface antigen, hepatitis C antibody: To be done only at screening; test to be performed at central laboratory.

HIV-1 and HIV-2 antibodies: To be done only at screening; test to be performed at central laboratory.

Instructions for sample collection, processing, storage, and shipment can be found in a separate laboratory manual provided to the centres.

5.3.3.2 Cotinine testing

Cotinine testing should be done only at screening; test to be performed at central laboratory.

Instructions for sample collection, processing, storage, and shipment can be found in a separate laboratory manual provided to the centres.

5.4 Biomarker analysis

The subject's consent to the use of donated biological samples (i.e. blood, sputum, endobronchial biopsies and bone marrow aspirates) is mandatory.

5.4.1 Storage, re-use and destruction of biological samples

Samples may be stored for a maximum of 15 years from the date of the Last Subject's Last Visit, after which they will be destroyed. If a subject withdraws consent to allow samples for future use they may continue with their samples being used for the main study. The results of this biomarker research will be reported either in the Clinical Study Report itself or as an addendum, or separately in a scientific report or publication. The results of this biomarker research may be pooled with biomarker data from other studies with the study drug to generate hypotheses to be tested in future research. Any residual samples may be used for future biomarker research.

5.4.2 Labelling and shipment of biological samples

The Principal Investigator ensures that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B Regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix B 'IATA 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

5.4.3 Chain of custody of biological samples

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

The Principal Investigator keeps full traceability of all collected samples from subjects (including blood, sputum, bronchial biopsies and bone marrow aspirates) during storage at the centre until shipment or disposal (where appropriate) and keeps documentation of all shipments.

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

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca Biobank during the entire life cycle.

5.4.4 Withdrawal of Informed Consent for donated biological samples

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

The Principal Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study site
- Ensures that the subject and AstraZeneca are informed about the sample disposal.

AstraZeneca ensures the central laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed and the action documented and returned to the study site.

6. SAFETY REPORTING AND MEDICAL MANAGEMENT

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

6.1 Definition of adverse events

An adverse event (AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

6.2 Definitions of serious adverse event

A serious adverse event (SAE) is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death

- Is immediately life-threatening
- Requires in-subject hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

6.3 Recording of adverse events

6.3.1 Time period for collection of adverse events

All AEs, including SAEs, will be collected from the time the subject signs the informed consent throughout the treatment period and including the follow-up period.

6.3.2 Follow-up of unresolved adverse events

Any AEs that are unresolved at follow-up in the study will be followed up by the investigator for as long as medically indicated, but without further recording in the CRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

The requirement to follow-up AEs is not intended to delay database lock or production of the Clinical Study Report (CSR). These activities should proceed as planned with ongoing AEs if necessary.

Any follow-up information of ongoing SAEs after database lock will be reported to AstraZeneca.

6.3.3 Variables

The following variables will be collect for each AE;

- AE (verbatim)
- The date and when the AE started and stopped
- Maximum intensity of the AE
- Whether the AE is serious or not

- Investigator causality rating against the IP (yes or no)
- Action taken with regard to IP
- AE caused subject's withdrawal from study *(*yes or no*)*
- Outcome.

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

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Description of AE.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.2. 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 . 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.

6.3.4 Causality collection

The Investigator will assess causal relationship between IP and each AE, 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 investigational product?'

For SAEs causal relationship will also be assessed for other medication and study procedures. 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 A to the CSP.

6.3.5 Adverse events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: *'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 CRF. 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.

6.3.6 Adverse events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the IP.

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 will use the clinical, rather than the laboratory term (e.g., anaemia versus low haemoglobin 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. Cases in which a patient shows an AST or ALT ≥3xULN or total bilirubin ≥ 2xULN may need to be reported as SAEs (please refer to Appendix C 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions).

An adverse event will also include any untoward or unfavorable medical occurrence associated with the following study mandated procedures and/or occurring within 48 hours after:

- Allergy Skin Test
- Sputum Induction
- Bronchoscopy
- Whole Lung Allergen Challenge
- Methacholine Challenge
- Whole blood collection

- Bone Marrow Aspirate

The following clinical situations, when associated with study procedures will be considered to be outside of the normal range of findings and will be recorded as Adverse Events. These situations do not limit the principal investigator from reporting any other events, associated or not with these procedures, from being recorded and reported as AEs.

Blood Draws

- Fainting /Vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 5 minutes
- Swelling at puncture site larger than 2 cm

Allergen Skin Testing

- Prolonged (>24 hours) itching at test site
- Swelling (> 10 cm) at site of test lasting more than 24 hours
- Nasal allergic symptoms within 30 minutes from the procedure
- Fainting /Vasovagal event within 30 minutes from the procedure
- Systemic reaction

Pulmonary Function Testing

- Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure
- Coughing requiring treatment with bronchodilators within 30 minutes from the procedure

Methacholine Challenge

- FEV_1 has not returned to at least 90% of the baseline (pre-diluent) value with 4 puffs of salbutamol
- FEV_1 drops by more than 50% during the procedure

Allergen Inhalation challenge

- Systemic Reaction

- Asthma exacerbation requiring prednisone therapy
- Significant decrease in lung function

a). FEV_1 decreases by >60% from baseline (defined at the highest pre-test FEV_1 on the day of the allergen challenge Visit)

or

b) FEV_1 decreases by 40-59% from baseline AND does not begin to recover spontaneously during the first 60 minutes post immediate decline in FEV_1, OR does not respond to salbutamol therapy employed to reverse bronchoconstriction

or

c) Failure to recover to within 80% of baseline FEV_1 at discharge. Salbutamol therapy may be used to assist in recovery.

Bronchoscopy

- Asthma exacerbation requiring prednisone in response to bronchoscopy
- Significant decrease in lung function

a) FEV_1 decreases by >60% from baseline

or

b) FEV_1 decreases by 40-59% from baseline AND does not begin to recover spontaneously during the first 60 minutes post immediate decline in FEV_1, OR does not respond to salbutamol therapy employed to reverse bronchoconstriction

or

c) Failure to recover to within 80% of baseline FEV_1 at discharge. Salbutamol therapy may be used to assist in recovery.

- Significant hypoxemia during bronchoscopy
- The bronchoscopy procedure results in hypoxemia of <85% oxygen saturation for greater than 30 seconds despite supplemental O2.

6.3.7 Symptoms of the disease under study

When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. Asthma symptoms or signs, such as wheeze, cough, chest tightness, dyspnoea, breathlessness and phlegm, will be recorded as AEs only when:

The sign or symptom is serious according to definitions, see Section 6.2.

The patient discontinues the study due to the sign or symptom

The sign or symptom is new to the patient or not consistent with the patient's pre-existing asthma history (defined as within 1 year of Visit 1a) as judged by the investigator.

6.4 Reporting of serious adverse events

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

If any SAE occurs in the course of the study, then Investigators or other site personnel will inform the appropriate AstraZeneca representatives within 1 day, i.e., immediately but **no later than 24 hours** from 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 Subject Safety data entry site **within 1 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 1 calendar day**, i.e., immediately but **no later than 24 hours** from when he or she becomes aware of it.

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

If the WBDC system is not available, then the Investigator or other study site personnel is to report a SAE to the appropriate AstraZeneca representative by telephone.

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

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug.

6.5 Overdose

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

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

The designated AstraZeneca representative will work with the Investigator to ensure that all relevant information is provided to the AstraZeneca Subject Safety data entry site.

For overdoses associated with a SAE, the standard reporting timelines apply, see Section 6.4. For other overdoses, reporting must occur within 30 days.

6.6 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to AstraZeneca.

6.6.1 Maternal exposure

If a subject becomes pregnant during the course of the study IP should be discontinued immediately.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/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 abnormality) should be followed up and documented even if the subject was discontinued from the study.

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel will inform the appropriate AstraZeneca representatives within 1 day, i.e., immediately but **no later than 24 hours** from 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 Subject Safety data entry site within 1 or 5 calendar days for SAEs (see Section 6.4) and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

The pregnancy (PREGREP) module in the CRF will be used to report the pregnancy and the pregnancy outcome (PREGOUT) module will be used to report the outcome of the pregnancy.

6.6.2 Paternal exposure

Male subjects should refrain from fathering a child or donating sperm during the study and for 16 weeks (5 half-lives) following the last dose.

Pregnancy of the subject's partners will not be considered an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented for conceptions

occurring from the date of the first administration of IP until 16 weeks (5 half-lives) after the last administration of IP.

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1 Identity of investigational product(s)

All investigational products will be manufactured in accordance with Good Manufacturing Practice (GMP).

Benralizumab and placebo administered in the study will be a clear to opalescent, colourless to yellow solution.

Table 4 Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer
Benralizumab	30 mg/mL solution for injection in accessorized pre-filled syringe, 1mL fill volume	MedImmune
Placebo	Matching placebo solution for injection in accessorized pre-filled syringe, 1mL fill volume	MedImmune

7.2 Dose and treatment regimens

The IP will be administered at the study centre on treatment visits and within visit windows as specified in Table 2.

Before IP administration

Prior to each IP administration:

Investigator/authorized delegate will assess injection site as per standards of medical care

For WOCBP urine pregnancy test will be done; IP will be administered only when the result of the test is negative (see Section 5.2.1)

IP administration

The IP will be administered by the Investigator/authorized delegate. It is suggested that the site of injection of the IP is rotated such that the patient receives IP at a different anatomical site (see Figure 2) at each treatment visit. The injection site must be recorded in the source documents and the eCRF at each treatment visit.

Figure 2 Injection sites and rotation scheme

Further details on IP administration are provided in the IP Handling Instruction. IP administration must be carried out in line with the Instruction.

After IP administration

After IP administration the subject should be observed for a minimum of 2 hours for the appearance of any acute drug reactions.

Conditions requiring IP administration rescheduling

If any of the following should occur, the investigator should reschedule the visit and the IP should not be administered until the rescheduled visit:

- The subject has an intercurrent illness, that in the opinion of the investigator may compromise the safety of the subject in the study (eg, viral illnesses)
- The subject is febrile (≥38°C; ≥100.4°F) within 72 hours prior to IP administration

7.3 Management of IP related reactions

Appropriate drugs, such as epinephrine, H1 and H2 antihistamines, and corticosteroids, as well as medical equipment to treat acute anaphylactic reactions must be immediately available. Study personnel must be trained to recognize and treat anaphylaxis (Lieberman et al 2010). Details on anaphylaxis management are provided in Appendix D.

Anaphylaxis will be defined as a serious reaction that is rapid in onset and may cause death (Simpson et al 2006). Anaphylaxis typically manifests as 1 of 3 clinical scenarios:

1. The acute onset of a reaction (minutes to hours) with involvement of the skin, mucosal tissue, or both, and at least one of the following: a) respiratory compromise; or b) reduced blood pressure or symptoms of end-organ dysfunction

2. Two or more of the following that occur rapidly after exposure: involvement of the skin/mucosal tissue, respiratory compromise, reduced blood pressure or associated symptoms, and/or persistent gastrointestinal symptoms

3. Reduced blood pressure after exposure

Subjects will have had a pre-assessment (ie, vital signs) prior to IP administration and should be observed after IP administration for a minimum of 2 hours for the appearance of any acute drug reactions.

Serum tryptase or other blood or urine testing relevant to the diagnosis of anaphylaxis may be obtained at a local lab at the discretion of the investigator.

7.4 Labelling

Labelling of the IP will be carried out by AstraZeneca or designee in accordance with current Good Manufacturing Practice (GMP) and regulatory requirements of each country participating in the study. The labels will be translated into local languages where applicable.

The labels will include the following information:

- Study code
- Investigational product/study drug dosage form, route of administration, and quantity of dosage units
- Kit ID
- P Lot ID
- Expiry date
- Investigator name (to be written on the label)
- E-code (to be written on the label)
- Sponsor name
- Directions for use
- Storage conditions
- Standard statements required by Regulatory Authorities
- Sponsor name and contact details

7.5 Storage

Benralizumab/placebo is to be stored at the study centre in a secured facility with limited access and controlled temperature. The temperature should be monitored on a daily basis and documented in the temperature monitoring log.

The IP must be kept in the original outer container and under conditions specified on the label (between 2–8°C (36–46°F), protected from the light).

In the following cases:

- Temperature excursion upon receipt or during storage at the study
- Damaged kit upon receipt
- Damaged syringe/cartridge

The centre staff should not use affected IP and should immediately contact an AstraZeneca representative for further guidance. Damaged IP should be documented via IWRS/IVRS (please refer to IWRS/IVRS manual for further details).

7.6 Compliance

The administration of all study drugs (including investigational products) should be recorded in the appropriate sections of the eCRF.

The IP will be administered at the study centre on treatment visits and within visit windows as specified in Table 2. If 1 dose of the IP is missed during course of the study the subject should be discontinued; please refer to Section 3.9.

7.7 Accountability

The study drug provided for this study will be used only as directed in the study protocol.

The study personnel will account for all study drugs dispensed to the patient.

The monitor will account for all study drugs received at the centre, unused study drugs, and for appropriate destruction. Certificates of delivery, destruction, and/or return should be signed.

In the case of a malfunctioning accessorized prefilled syringe (APFS), the centre should contact the study monitor to initiate a product complaint process according to applicable guidelines.

7.8 Concomitant and other treatments

The Informed Consent form must be signed before conducting any study-related procedures, e.g. discontinuation of pre-study treatment.

During the study, all subjects should have access to a SABA.

Salbutamol pMDI, inhaled corticosteroid, and prednisolone will be provided in connection with study assessments, when/if applicable as per the [redacted] SOPs.

Restricted medication during the study is shown in Table 5.

Table 5 Restricted Medications

Restricted Medication prior to Visits 1a-17	Time limits prior to Visits 1a-17
Aspirin prn (all doses)	7 days
NSAIDs	72 hours
Ephedrine-containing drugs	24 hours
Inhaled/oral short-acting β2-agonists (SABA)	8 hours
Short-acting anti-histamines [a]	3 days
Intermediate anti-histamines [a]	7 days
Long-acting anti-histamines [a]	9 days
Xanthine (tea, coffee, chocolate) [b]	4 hours

[a] Prior to skin prick test and inhaled allergen testing only. Please refer to [redacted] SOPs for further guidance.
[b] Applicable only to visits where spirometry will be conducted.

Medication not allowed during the study, from Visit 1a to end of Visit 17 are shown in Table 6.

Table 6 Non-allowed medication

Non-allowed medication during the study (Visits 1a-17)	Time limit prior to Visit 1a
Inhaled [a] and nasal [b] steroids	4 weeks
Oral, rectal, parenteral (including periarticular injections) steroids [c]	3 months
Topical GCS for dermal use, group II – IV [d]	from Visit 2
Chronic use of anticoagulants and antiplatelet medication	4 weeks
Herbal remedies for the treatment of allergic, inflammatory, or respiratory diseases	30 days
Inhaled disodium cromoglycate and inhaled nedocromil sodium	4 weeks
Inhaled/oral long-acting β2-agonists (LABA)	14 days
Leukotriene antagonists and 5-Lipoxygenase Inhibitors	14 days

Non-allowed medication during the study (Visits 1a-17)	Time limit prior to Visit 1a
Inhaled short-acting anticholinergics	8 hours
Inhaled long-acting anticholinergics	14 days
Theophyline	4 weeks
Betablockers including eyedrops	from Visit 2
Macrolide antibiotics	from Visit 2
Immunosuppresives [e]	3 months
Immunoglobulins and blood products	30 days
Allergen immunotherapy	4 months
Any marketed or investigational biologic [e]	4 months or 5 half-lives (whichever is longer)
Live attenuated vaccines [e]	30 days
Any investigational non-biologic	30 days or 5 half-lives (whichever is longer)

[a] Use of inhaled steroids is prohibited during the study (please see section 3.8 for further details). Inhaled ICS may be used acutely after allergen challenge or to stabilize asthma during the study if needed, provided dosing is acute (3-5 days maximum) and washout (1 week) is applied before study visits.
[b] Use of nasal steroids is prohibited during the study (please see section 3.8 for further details). If there is a need for acute use of nasal steroids a 12 hour washout period is required prior to any study related procedures.
[c] Use of systemic steroids is prohibited during the study (please see section 3.8 for further details). Systemic steroids may be used acutely after allergen challenge or to stabilize asthma during the study if needed, provided a washout (1 week) is applied before study visits.
[d] Topical GCS group I are allowed on areas up to the size of the subject's palm.
e Please refer to section 3.8 for further details.

7.8.1 Other concomitant treatment

Medications other than those described above which are considered necessary for the safety and well-being of the subject, may be given at the discretion of the Investigator and recorded in the appropriate sections of the Case Report Form.

8. STATISTICAL ANALYSES BY ASTRAZENECA

8.1 Statistical considerations

- All personnel involved with the analysis of the study will remain blinded until database lock and protocol violators identified.
- Analyses will be performed by AstraZeneca or its representatives.
- Full details of the analyses for this study will be detailed in the Statistical Analysis Plan (SAP) which will be completed prior to unblinding of the data.

8.2 Sample size estimate

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 the allergen induced change in percentage of eosinophils in induced sputum at 7-hr post allergen challenge during Allergen Challenge 1, and 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 step down approach (first assessing the effect on percentage of eosinophils in sputum) as described in 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 it is expected to be sufficient for all endpoints using an estimation type approach which has been used for most studies of this type.

8.3 Definitions of analysis sets

All efficacy analyses will be performed using an Intent-to-Treat (ITT) approach based on the full analysis set. For consistency, demographic, baseline characteristics and safety objectives will be presented using the full analysis set.

8.3.1 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.

8.3.2 Full analysis set

The full analysis analysis set consists of all randomized subjects who received at least one dose of investigational product 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, and assent when applicable 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.

8.3.3 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.

8.4 Outcome measures for analyses

8.4.1 Calculation or derivation of efficacy variables

This study has two main analysis parts for efficacy evaluations as described below:

1. Visit 4 up to Visit 10 to evaluate the effect on baseline inflammation

2. Screening Allergen Challenge, post-dose Allergen Challenge 1 and Allergen Challenge 2 to evaluate response to allergen challenge

The study also 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

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

There are 2 triads where percentages of eosinophils post allergen challenge are collected in the study – at Screening and at Allergen Challenge 1. At each triad, a pre-challenge, 7-hr and 24-hr post challenge samples will be taken (refer to table below for visit associated with measurements). The 7-hr post allergen challenge measure during allergen challenge 1 will be considered as primary.

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

The allergen induced change at 7-hr and 24-hr post challenge will be derived by percentages of eosinophils post-challenge minus the percentages of eosinophils pre-challenge within each triad:

Allergen Induced Change $_{\text{screening}}$ = post-challenge $_{\text{screening}}$ – pre-challenge $_{\text{screening}}$

Allergen Induced Change $_{\text{Allergen Challenge 1}}$ = post-challenge $_{\text{Allergen Challenge 1}}$ – pre-challenge $_{\text{Allergen Challenge 1}}$

8.4.1.2 Maximum percent decrease in FEV_1 in late asthmatic response $(LAR)_{3\text{-}7\ hr}$ during allergen challenge

FEV_1 measurements will be taken for up to 7 hrs post-allergen challenge at the following 3 visits: Screening, Visit 11 (Allergen Challenge 1), and Visit 14 (Allergen Challenge 2).

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

Maximum percent decrease in FEV_1 in $LAR_{3\text{-}7\ hr}$ in each triad will be defined as 100* (Minimum value of FEV_1 between 3 – 7 hours post allergen challenge – pre-challenge value)/(pre-challenge value). The FEV_1 in $LAR_{3\text{-}7\ hr}$ on Visit 11 is considered as the primary endpoint.

8.4.1.3 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 for percent decrease FEV_1 in $LAR_{3\text{-}7\ hr}$ will be estimated using the linear trapezoidal rule (LxHour) using all available FEV_1 data between and inclusive of the 3 to 7 hours. The time adjusted total AUC FEV_1 in $LAR_{3\text{-}7\ hr}$ is calculated as total AUC/ (the length of time (hr) the total AUC is calculated).

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

Eosinophils in induced sputum are collected on Visit 4 (pre-dose, will be defined as baseline for the summary of effect on baseline inflammation), and on Visit 7 and Visit 10. The change from baseline (Visit 4) of percent eosinophils to each post-baseline visit will be used in the analyses.

8.4.1.5 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 types of measurements for basophil count in induced sputum: allergen induced change at 7-hr and 24-hr post allergen challenge to evaluate allergen response at Screening and Allergen Challenge 1, and measurements for effect on baseline inflammation at Visit 4, Visit 7 and Visit 10. Basophil count in sputum will be obtained by two types of readings: flow cytometry and cytospins with toluidine blue staining, refer to tables below for the scheduling of each type of reading.

Allergen Challenge related:

	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

Baseline Inflammation related:

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

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

Maximum percent decrease in FEV_1 in $EAR_{0-2\ hr}$ at each visit will be defined as 100* (Minimum value of FEV_1 between 0 – 2 hours post allergen challenge – pre-challenge value)/(pre-challenge value).

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

The total AUC for FEV_1 in $EAR_{0-2\ hr}$ will be estimated using the linear trapezoidal rule (LxHour) using all available FEV_1 data between and inclusive of the 0 to 2 hours. The time

adjusted total AUC FEV_1 in $EAR_{0\text{-}2\ hr}$ is calculated as total AUC/ (the length of time (hours) the total AUC is calculated).

8.4.1.8 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 challenge). Effect on baseline inflammation will be evaluated by change from baseline (Visit 5) to Visit 8. Effect on allergen response will be evaluated by change from pre-challenge (Visit 8) to post-challenge (Visit 15).

8.4.1.9 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 on Visit 4 (baseline), Visit 10 (pre-challenge) and Visit 12 (post-challenge).

Effect on baseline inflammation will be evaluated by change from Visit 4 to Visit 10. Effect on allergen response will be evaluated by change from pre-challenge (Visit 10) to post-challenge (Visit 12).

8.4.1.10 Eosinophils and basophils counts in the blood

Similar to percentage of eosinophils in induced sputum, there are 2 types of measurements for eosinophils: 1) allergen induced change to evaluate allergen response, and 2) measurements for effect on baseline inflammation at Visit 4, 7, 9 and 10. For basophil counts in blood only allergen challenge 1 is assessed for allergen induced change and Visit 4 and Visit 10 for baseline inflammation. For allergen induced change, see table below for corresponding visit:

For Eosinophils:

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

For Basophils:

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

8.4.1.11 Methacholine PC20 (concentration of inhaled methacholine that produces a 20% fall in FEV_1)

Similar to percentage of eosinophils in induced sputum, there are 2 types of measurements for Methacholine PC20: 1) allergen induced change to evaluate allergen response, and 2) measurements for effect on baseline inflammation at Visit 4, 7 and 10. For allergen induced change, see table below for corresponding visit:

Triad	Pre-challenge	Post-challenge
Screening (pre-dose)	Visit 1	Visit 3
Allergen Challenge 1 (post-dose)	Visit 10	Visit 12

8.4.1.12 Soluble inflammatory markers associated with pathology of asthma or the pharmacology of Benralizumab

Details of the analyses will be specified in a separate analyses plan.

8.4.2 Calculation or derivation of safety variables

8.4.2.1 Safety variables

The following safety data will be collected: vital signs, physical examination and ECG, haematology, clinical chemistry, urinalysis, reported AEs and SAEs.

8.5 Methods for statistical analyses

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.

The prior medications, categorized according to the 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.

The concomitant medication will be categorized according to the WHO Drug Reference List dictionary which employs the Anatomical Therapeutic Chemical (ATC) classification system. The frequency and percentage of subjects taking concomitant medications and non-drug therapies during the treatment period will be summarized by drug class and drug name using ATC code.

The analysis of the primary, secondary and exploratory endpoints will include all data captured during the study, unless the subject withdraws consent, and assent when applicable to

study participation, regardless of whether study treatment was prematurely discontinued, or delayed, and/or irrespective of protocol adherence.

Descriptive statistics will also be provided for safety and efficacy data. Unless otherwise stated, the data analysis 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 were summarized using frequency counts and percentages. When data are summarized by time, the values recorded against the scheduled time points listed in the protocol were used. When assessing minimum/maximum increases or decreases during the study, all assessments, including unscheduled assessments will be used. For analysis assessing 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, no analysis window will be applied.

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 p-value is <= 0.05, then proceed with Step 2. Otherwise, neither of the two hypothesis 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.

8.5.1 Analysis of the primary variable (s)

8.5.1.1 Allergen induced change in percentage of eosinophils in induced sputum 7-hr 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, ie,:

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 primary analysis method for the allergen induced change on Visit 11 (7-hr post challenge) and 12 (24-hr post challenge) 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 at 7-hr and 24-hr post allergen. Treatment group will be fitted as an explanatory variable pre-dose allergen induced change at Screening will be fitted as a covariate. Visit will be fitted as a categorical variable, 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 2-sided p-values will be reported for each visit.

8.5.1.2 Maximum percent decrease in FEV_1 in late asthmatic response ($LAR_{3\text{-}7\ hr}$) on Visit 11 during Allergen Challenge 1

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

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 at $LAR_{3\text{-}7\ hr}$ on Visit 11 will be compared between Benralizumab and Placebo using an ANCOVA. The dependent variable will be maximum percent decrease in FEV_1 at $LAR_{3\text{-}7\ hr}$. Treatment group will be fitted as an explanatory variable and the corresponding pre-dose FEV_1 value at Screening will be fitted as a covariate. The least squares mean for the difference in treatment groups and its 95% CI, and the 2-sided p-values will be provided.

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

The above analyses will be repeated at allergen challenge 2 triad.

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

8.5.2 Analysis of the secondary variable(s)

Endpoints for secondary objectives to evaluate the response to *allergen challenge* in this study are:

- Maximum percent decrease in FEV_1 in early asthmatic response 0-2 hr ($EAR_{0\text{-}2\ hr}$)
- AUC of time adjusted percent decrease in FEV_1 curve in $EAR_{0\text{-}2\ hr}$
- Eosinophil, eosinophil progenitor cells and basophils in bone marrow aspirates after allergen challenge
- Eosinophil and basophil counts in blood
- Number of eosinophils and basophils in lung tissue biopsies
- Basophil count in induced sputum

The maximum percent decrease in FEV_1 in $EAR_{0\text{-}2\ hr}$ will be analysed as for the maximum percent decrease and AUC in FEV_1 in $LAR_{3\text{-}7\ hr.}$

The change from pre-allergen challenge in basophil count in induced sputum from cytospins with toluidine blue staining will be analysed as for the allergen induced change in percentage of eosinophils in induced sputum.

The change from pre-allergen challenge eosinophil, eosinophil progenitor cells and basophils in bone marrow aspirates after allergen challenge will be analysed using an ANCOVA. The dependent variable will be the change in the number of eosinophils and absolute eosinophil progenitor cells and basophils in bone marrow aspirates all on Visit 12 from Visit 10. Treatment group will be fitted as an explanatory variable and baseline (Visit 4) will be fitted as a covariate.

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

The change from pre-allergen challenge eosinophils counts in blood after allergen challenge will be analysed 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 at 7-hr and 24-hr post challenge. Treatment group will be fitted as an explanatory variable and baseline (Visit 4) will be fitted as a covariate. Visit (7-hr, 24-hr) will be fitted as a categorical variable, 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 2-sided p-values will be reported for each visit.

The change from pre-allergen challenge in the number of eosinophils and basophils in lung tissue biopsies will be analysed using an ANCOVA. The dependent variable will be the change in the number of eosinophils and absolute basophils in lung tissue biopsies from Visit 8 to Visit 15. Treatment group will be fitted as an explanatory variable and baseline (Visit 5) will be fitted as a covariate.

Allergen induced change in methacholine PC20 at Allergen Challenge 1 will be analysed using ANCOVA with treatment as explanatory variable and allergen induced change at Screening fitted as a covariate.

Endpoints for secondary objectives to evaluate the effect on *baseline inflammation* in this study are:

- Percentage of eosinophils in induced sputum for the effect on baseline inflammation
- Eosinophils and basophils counts in blood
- Number of eosinophils and basophils in lung tissue biopsies
- Basophil count in induced sputum from cytospins with toluidine blue staining

The primary timepoint for this evaluations will be Visit 10 (except Visit 8 for bronchoscopy measures).

The change from baseline of percent eosinophils in induced sputum will be compared between Benralizumab and Placebo using a repeated measures analysis. The dependent variable will be change from baseline in percent eosinophils in induced sputum. Treatment group will be fitted as an explanatory variable and baseline (Visit 4) will be fitted as a covariate. Visit will be fitted as a categorical variable, 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 2-sided p-values will be reported for each visit (Visit 7 and 10).

Change from baseline for basophil counts in induced sputum from cytospins with toluidine blue staining (Visit 7 and 10) will be analyzed as for the percentage of eosinophils in inducted sputum. Eosinophils count (Visit 7, 9 and 10) in blood will also be analysed in this way.

Change from baseline in the number of basophils counts in the blood will be analysed using an ANCOVA. The dependent variable will be the change from baseline in number of basophils on Visit 10. Treatment group will be fitted as an explanatory variable and baseline (Visit 4) will be fitted as a covariate.

Change from baseline in the number of eosinophils and basophils in lung tissue biopsies will be analysed using an ANCOVA. The dependent variable will be change from baseline in number of eosinophils and basophils in lung tissue biopsies on Visit 8. Treatment group will be fitted as an explanatory variable and baseline (Visit 5) will be fitted as a covariate.

Change from baseline in methacholine PC20 at visit 7 and 10 will be analyzed after log transformation using repeated measures analysis with treatment as explanatory variable, and baseline (visit 4) fitted as a covariate.

8.5.3 Analyses methods for safety variables

Adverse events will be coded using the MedDRA dictionary. Number of subjects with events and percentages will be tabulated by preferred term and system organ class. An event that occurred once or more times during the study treatment period will contribute one observation to the numerator of the proportion. The denominator of the proportion will comprise all subjects in the Safety set. Adverse events will also be summarized by intensity/severity and separately, by causality/relatedness (as determined by the investigator). Should a subject report the same preferred term/system organ class within multiple intensity/severity or causality/relatedness categories, the subject's worst occurrence (most severe/most related) will be tabulated. Serious AEs, AEs leading to discontinuation, and commonly occurring AEs will be summarized in a generally similar manner. Adverse events, SAEs, AEs leading to death, and AEs leading to study discontinuation will be summarized for each treatment group as applicable

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.

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.

The incidence of clinically notable ECG abnormalities will be summarized.

8.5.4 Exploratory analysis

[redacted]

9. STUDY AND DATA MANAGEMENT BY ASTRAZENECA

9.1 Training of study site personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and WBDC, IWRS/IVRS and other systems to be utilised.

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

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

9.2 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in accordance with the Laboratory Manual and that study drug accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (e.g., clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

The AstraZeneca representative will be available between visits if the Investigator(s) or other staff at the centre needs information and advice about the study conduct.

9.2.1 Source data

Please refer to the Clinical Study Agreement (CSA) for location of source data.

9.2.2 Study agreements

The Principal Investigator at each centre should comply with all the terms, conditions, and obligations of the Study Agreement with the Principal Investigator, or equivalent, for this study. In the event of any inconsistency between this CSP and the Study Agreement with Principal Investigator, the terms of CSP shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the Study Agreement with Principal Investigator shall prevail.

Agreements between AstraZeneca and the Principal Investigator should be in place before any study-related procedures can take place, or any subjects are enrolled.

9.2.3 Archiving of study documents

Study files. AstraZeneca will provide the Principal Investigator with a file in which to organise and retain all study-related documents. All study documents (including letters from AstraZeneca) should be retained in this file by the Principal Investigator. The monitor will regularly check the file to ensure that all relevant documents are retained. The contents of the file may be audited/inspected by AstraZeneca's auditor, regulatory authorities, or IRB.

Period of record retention. The study site (and the Principal Investigator) will retain the essential documents specified in the ICH GCP (e.g., source document such as medical records, contract, signed consent form). Essential documents should be retained at the study site for at least 15 years following completion of the study, or per regulatory obligations if longer, and thereafter destroyed only after agreement with AstraZeneca. However this is not always applied to those that are not preservable such as blood samples. In the event of any inconsistency between the above-mentioned contents and the contract with the study site, the contract shall prevail. These documents should be retained for a longer period however if needed by AstraZeneca, and the specific period and method of retention will be separately discussed between the study site and AstraZeneca. AstraZeneca should notify the head of the study site in writing when the study related records are no longer needed. The records should be managed by a responsible person appointed by the head of the study site

9.2.4 Deviation from the clinical study protocol

The Investigator(s) must not deviate from or make any changes to the protocol without documented agreement between the Principal Investigator and AstraZeneca or the IRB approval based on its deliberations. However, this shall not apply to cases where the deviation or change is necessary to avoid an immediate hazard to the subjects or for other compelling medical reasons, or where the changes involve only logistical or administrative aspects of the

clinical study (e.g., changes to the organisation/structure of the AstraZeneca, the name/department name of the study site, the address or phone number of the study site or AstraZeneca, the job title of the Investigator, and monitors).

The Investigator(s) should document any deviation from the protocol regardless of their reasons. Only when the protocol was not followed in order to avoid an immediate hazard to the subjects or for other medically compelling reason, the Investigator should prepare and submit the records explaining the reasons thereof to AstraZeneca and the head of study site, and retain a copy of the records.

The Investigator(s) may deviate from or make a change to the protocol without documented agreement between the Principal Investigator and AstraZeneca or the IRB approval, only in the event of a medical emergency, e.g., it is only way to avoid an immediate hazard to the subjects. In such case, the Principal Investigator must notify details of the deviation or change, the reason, and a proposed revision in the protocol if required, to AstraZeneca and the head of the study site and IRB via the head of the study site as soon as possible, in order to obtain their approval. A certificate of approval by the head of the study site as well as AstraZeneca should be obtained via the head of the study site.

9.3 Study timetable and end of study

Planned duration of the study: 3 years

Study period: July 2016 – December 2018

Discontinuation or suspension of the whole study programme

If AstraZeneca decides to prematurely terminate or suspend the study, the Principal Investigator/Investigator, the head of the study site, and regulatory authorities should receive written notification of the reasons for the premature termination or suspension.

The Principal Investigator/Investigator will immediately notify the decision to the subjects, give appropriate medical treatment; take necessary measures, and record treatment or measures provided on the source documents.

Completion of the study

Upon terminating the study, the Principal Investigator/Investigator will report in writing the completion of the study as well as the summary of the results to the head of the study site in accordance with the study site's rules. The head of the study site, who is informed of the termination by the Investigator, will provide a written notification of the results to the IRB and AstraZeneca.

9.4 Data management by AstraZeneca

Data management will be performed by AstraZeneca Data Management Centre staff, according to the Data Management Plan.

AEs and medical/surgical history will be classified according to the terminology of the latest version the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the AstraZeneca Drug Dictionary. All coding will be performed by the Medical Coding Team at the AstraZeneca Data Management Centre.

The Rave Web Based Data Capture (WBDC) system will be used for data collection and query handling. The Investigator will ensure that data are recorded on the electronic Case Report Forms as specified in the study protocol and in accordance with the instructions provided.

The investigator will ensure the accuracy, completeness, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. The investigator will sign the completed electronic Case Report Forms. A copy of the completed electronic Case Report Forms will be archived at the study centre.

Data associated with biological samples will be transferred from laboratories internal or external to AstraZeneca.

Serious Adverse Event (SAE) Reconciliation

SAE reconciliation reports are produced and reconciled with the Subject Safety database and/or the investigational site.

10. ETHICAL AND REGULATORY REQUIREMENTS

10.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

10.2 Subject data protection

The Master Informed Consent Form will explain that:

- Study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation
- Subject data will be maintaining confidentiality in accordance with national data legislation
- For data verification purposes, authorised representatives of AstraZeneca, a regulatory authority, an IRB may require direct access to parts of the hospital or practice source records relevant to the study, including subjects' medical history

- All data computer processed by AstraZeneca will be identified by study code and enrolment code (E-code)

10.3 Ethics and regulatory review

An IRB should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The head of the study site will ensure the distribution of these documents to the applicable IRB, and the Principal Investigator to the Investigator and study site staff.

The opinion of the IRB should be given in writing. The head of the study site should submit a notification of direction/determination as well as a copy of the IRB written approval to AstraZeneca and the Principal Investigator before enrolment of any subject should into the study.

The IRB should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

A valid contract between the study site and AstraZeneca should be signed before the Investigator can enrol any subject into the study. The protocol should be re-approved by the IRB annually.

The head of the study site should seek the opinion of the IRB with respect to the appropriateness of continuing the study at the study site at least once a year when the duration of the study exceeds one year. The Principal Investigator should submit progress reports to the IRB via the head of the study site at the time of the protocol re-approval.

Before enrolment of any subject into the study, the final study protocol, including the final version of the ICF, should be approved by the national regulatory authority with notification provided, according to local regulations. AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide Regulatory Authorities, IRB, the head of the study site and the Principal Investigator with safety updates/reports according to local requirements.

The head of the study site should submit a written report to the IRB providing the details of all safety relative information reported by AstraZeneca.

10.4 Informed consent

The Principal Investigator(s) at each study centre will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the

study (before any study procedures are performed) as per local requirements.

- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated Informed Consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File and kept for a period that is complaint with GCP/local regulatory requirements, whichever is longer
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the Informed Consent Form that is approved by an Ethics Committee EC.

10.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal Investigator and AstraZeneca. If it is necessary for the study protocol to be amended, the amendment should be submitted to the Head of the Study Site and be approved by its IRB. If applicable, AstraZeneca should submit a notification to the regulatory authority before it is implemented. If a protocol amendment requires a change to a particular centre's Informed Consent Form, then AstraZeneca and the centre's IRB should be notified. Approval of the revised Informed Consent Form by AstraZeneca and by the IRB is required before the revised form is used. If an administrative change is required, such a change should be notified to or approved by each IRB according to local requirements.

10.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency or other body about an inspection or an audit at the study centre.

All study data may undergo a reliability review and onsite-GCP inspection by the regulatory authorities.

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Appendix A Additional Safety Information

Further Guidance on the Definition of a Serious Adverse Event (SAE)

Life threatening

'Life-threatening' means that the subject 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 subject'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).

Hospitalisation

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

Important medical event or medical intervention

Medical and scientific judgement 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, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention 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 judgement 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 anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation

Development of drug dependency or drug abuse

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 subject 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 aetiology 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 limited or insufficient information in the case, it is likely that the event(s) will be assessed as 'not related'.

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

Appendix B International Airline Transportation Association (IATA) 6.2 Guidance Document

Labelling and shipment of biohazard samples

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories. For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

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:

- are to be packed and shipped in accordance with IATA Instruction 602.

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, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B

- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations

- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging

- Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content

- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

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

Introduction

This Appendix describes the process to be followed in order to identify and appropriately report cases of Hy's Law. 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 subject meets potential Hy's Law (PHL) criteria at any point during the study.

The Investigator participates, together with AstraZeneca clinical project representatives, in review and assessment of cases meeting PHL criteria to agree whether Hy's Law (HL) criteria are met. HL criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than Drug Induced Liver Injury (DILI) caused by the Investigational Medicinal Product (IMP).

The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting Adverse Events (AE) and Serious Adverse Events (SAE) according to the outcome of the review and assessment in line with standard safety reporting processes.

Definitions

Potential Hy's Law (PHL)

Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥ 3x Upper Limit of Normal (ULN) **together with** Total Bilirubin (TBL) ≥ 2xULN at any point during the study following the start of study medication irrespective of an increase in Alkaline Phosphatase (ALP).

Hy's Law (HL)

AST or ALT ≥ 3x ULN **together with** TBL ≥ 2xULN, 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 (i.e. on the same day) the elevation in TBL, but there is no specified timeframe within which the elevations in transaminases and TBL must occur.

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 subject who meets any of the following identification criteria in isolation or in combination:

- ALT ≥ 3xULN

- AST ≥ 3xULN
- TBL ≥ 2xULN

When a subject meets any of the 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 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
- Complete the appropriate unscheduled laboratory CRF 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 subject meets PHL criteria (see Definitions within this Appendix for definition) by reviewing laboratory reports from all previous visits (including both central and local laboratory results)

The Investigator will without delay review each new laboratory report and if the identification criteria are met will:

- Notify the AstraZeneca representative
- Determine whether the subject meets PHL criteria (see Definitions within this Appendix for definition) by reviewing laboratory reports from all previous visits
- Promptly enter the laboratory data into the laboratory CRF

Follow-up

Potential Hy's Law Criteria not met

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

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

Potential Hy's Law Criteria met

If the subject does meet PHL criteria the Investigator will:

- Determine whether PHL criteria were met at any study visit prior to starting study treatment (See Actions Required When Potential Hy's Law Criteria are Met Before and After Starting Study Treatment)

- Notify the AstraZeneca representative who will then inform the central Study Team

The Study Physician contacts the Investigator, to provide guidance, discuss and agree an approach for the study subjects' follow-up and the continuous review of data. Subsequent to this contact the Investigator will:

- Monitor the subject until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated

- Investigate the etiology of the event and perform diagnostic investigations as discussed with the Study Physician

- Complete the three Liver CRF Modules as information becomes available

- If at any time (in consultation with the Study Physician) the PHL case meets serious criteria, report it as an SAE using standard reporting procedures

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.

No later than 3 weeks 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. The AstraZeneca Medical Science Director 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.

If 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 CRF

- If the alternative explanation is an AE/SAE, record the AE /SAE in the CRF accordingly and follow the AZ 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:

- Report an 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 3 weeks, 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:

- Report an SAE (report term 'Potential Hy's Law') applying serious criteria and causality assessment as per above
- 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 met. Update the SAE report according to the outcome of the review

Actions Required When Potential Hy's Law Criteria are Met Before and After Starting Study Treatment

This section is applicable to subjects who meet PHL criteria on study treatment having previously met PHL criteria at a study visit prior to starting study treatment.

At the first on study treatment occurrence of PHL criteria being met the Investigator will:

- Determine if there has been a significant change in the subjects' condition[#] compared with the last visit where PHL criteria were met[#]
  - If there is no significant change no action is required
  - If there is a significant change notify the AstraZeneca representative, who will inform the central Study Team, then follow the subsequent process described in Potential Hy's Law Criteria met of this Appendix

[#] A 'significant' change in the subject's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) 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.

Actions Required for Repeat Episodes of Potential Hy's Law

This section is applicable when a subject meets PHL criteria on study treatment and has already met PHL criteria at a previous on study treatment visit.

The requirement to conduct follow-up, review and assessment of a repeat occurrence(s) of PHL is based on the nature of the alternative cause identified for the previous occurrence.

The investigator should determine the cause for the previous occurrence of PHL criteria being met and answer the following question:

- Was the alternative cause for the previous occurrence of PHL criteria being met chronic or progressing malignant disease or did the subject meet PHL criteria prior to starting study treatment and at their first on study treatment visit as described in Actions Required When Potential Hy's Law Criteria are Met Before and After Starting Study Treatment?

If No: follow the process described in Potential Hy's Law Criteria met of this Appendix

If Yes:

Determine if there has been a significant change in the subject's condition[#] compared with when PHL criteria were previously met

- If there is no significant change no action is required
- If there is a significant change follow the process described in Potential Hy's Law Criteria met

[#] A 'significant' change in the subject's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) 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.

References

FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation':
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Appendix D Anaphylaxis: signs and symptoms, management

1. Introduction

As with any antibody, allergic reactions to dose administration are possible. The World Health Organization has categorized anaphylaxis into 2 subgroups, which are clinically indistinguishable: immunologic [IgE-mediated and non-IgE-mediated (eg, IgG and immune complex mediated) and nonimmunologic (Johansson et al, 2004). The clinical criteria for defining anaphylaxis for this study are listed in section 2. A guide to the signs and symptoms and management of acute anaphylaxis is provided in section 3. Appropriate drugs, such as epinephrine, antihistamines, corticosteroids, etc, and medical equipment to treat anaphylactic reactions must be immediately available at study sites, and study personnel should be trained to recognize and treat anaphylaxis according to local guidelines.

If an anaphylactic reaction occurs, a blood sample will be drawn from the patient as soon as possible after the event, at 60 minutes ± 30 minutes after the event, and at discharge for analysis of serum tryptase.

2. Clinical Criteria for Defining Anaphylaxis

In adults, anaphylaxis is highly likely when any one of the following 3 criteria is fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING

   (a) Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia).

   (b) Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence).

2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):

   (a) Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula).

   (b) Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia).

   (c) Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence).

   (d) Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting).

3. Reduced BP after exposure to known allergen for that patient (minutes to several hours): Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that patient's baseline.

3. Signs and Symptoms and Management of Acute Anaphylaxis

Anaphylaxis is an acute and potentially lethal multi-system allergic reaction in which some or all of the following signs and symptoms occur:

- Diffuse erythema
- Pruritus
- Urticaria and/or angioedema
- Bronchospasm
- Laryngeal edema
- Hypotension
- Cardiac arrhythmias
- Feeling of impending doom
- Unconsciousness
- Shock

Other earlier or concomitant signs and symptoms can include:

- Itchy nose, eyes, pharynx, genitalia, palms, and soles
- Rhinorrhea
- Change in voice
- Metallic taste
- Nausea, vomiting, diarrhea, abdominal cramps and bloating
- Lightheadedness
- Headache
- Uterine cramps

- Generalized warmth

4. Management of Acute Anaphylaxis

4.1 Immediate intervention

1. Assessment of airway, breathing, circulation, and adequacy of mentation

2. Administer epinephrine intramuscularly every 5-15 minutes, in appropriate doses, as necessary, depending on the presenting signs and symptoms of anaphylaxis, to control signs and symptoms and prevent progression to more severe symptoms such as respiratory distress, hypotension, shock and unconsciousness.

4.2 Possibly appropriate, subsequent measures depending on response to epinephrine

(a) Place patient in recumbent position and elevate lower extremities.

(b) Establish and maintain airway.

(c) Administer oxygen.

(d) Establish venous access.

(e) Normal saline IV for fluid replacement.

4.3 Specific measures to consider after epinephrine injections, where appropriate

(a) Consider epinephrine infusion.

(b) Consider H1 and H2 antihistamines.

(c) Consider nebulized β2 agonist [eg, albuterol (salbutamol)] for bronchospasm resistant to epinephrine.

(d) Consider systemic corticosteroids.

(e) Consider vasopressor (e.g. dopamine).

(f) Consider glucagon for patient taking b-blocker.

(g) Consider atropine for symptomatic bradycardia.

(h) Consider transportation to an emergency department or an intensive care facility.

(i) For cardiopulmonary arrest during anaphylaxis, high-dose epinephrine and prolonged resuscitation efforts are encouraged, if necessary.

Adapted from: Kemp SF, Lockey RF, Simons FE; World Allergy Organization ad hoc Committee on Epinephrine in Anaphylaxis. Epinephrine: the drug of choice for anaphylaxis. A statement of the World Allergy Organization. Allergy. 2008; 63(8):1061-70.

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