
Revised Clinical Study Protocol

Drug Substance	Tralokinumab (CAT-354)
Study Code	D2210C00014
Edition Number	2.0
Date	15 January 2016

A Multicentre, Randomized, Double-blind, Parallel Group, Placebo Controlled, 12-Week, Phase 2 Study to Evaluate the Effect of Tralokinumab on Airway Inflammation in Adults with Asthma Inadequately Controlled on Inhaled Corticosteroid (MESOS)

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The following Amendment(s) and Administrative Changes are included in this revised protocol:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment
1	16 July 2015		
2	15 January 2016		
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Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change
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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 Multicentre, Randomized, Double-blind, Parallel Group, Placebo Controlled, 12-Week, Phase 2 Study to Evaluate the Effect of Tralokinumab on Airway Inflammation in Adults with Asthma Inadequately Controlled on Inhaled Corticosteroid (MESOS)

International Co-ordinating Investigator: Prof. Chris Brightling M.D.

The study will be conducted in collaboration with the United Kingdom Translational Research Partnership (TRP).

Study site(s) and number of subjects planned

Approximately 80 subjects will be randomized at approximately 20-25 study sites.

Study period		Phase of development
Estimated date of first subject enrolled	Q2 2015	2
Estimated date of last subject completed	Q4 2017	2

Study design

This multicentre, randomized, double-blind, parallel group, placebo-controlled, 12-week, phase 2 study will evaluate the effect of a 300 mg dose of tralokinumab administered subcutaneously every 2 weeks on airway inflammation in adults with asthma inadequately controlled on inhaled corticosteroids (ICS) with or without other controllers.

The subjects will be randomized to 12-week treatment with tralokinumab or placebo (1:1). After the end of treatment all subjects will be followed up for a period of 14 weeks.

Objectives

Primary Objective:	Outcome Measure:
To evaluate the effect of tralokinumab on eosinophilic airway infiltration in adult subjects with asthma inadequately controlled with ICS	Primary Outcome Variable: The change, expressed as a ratio, in number of airway submucosal eosinophils per mm ² determined by microscopic evaluation of bronchoscopic biopsies from baseline up to week 12

	Primary Outcome Measure: Ratio of tralokinumab to placebo at week 12
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Secondary Objective:	Outcome Measure :
To evaluate the effect of tralokinumab on blood eosinophil levels in adult subjects with asthma inadequately controlled with ICS	Outcome Variable: The change, expressed as a ratio, in number of blood eosinophils from baseline up to week 12 Outcome Measure: Ratio of tralokinumab to placebo at week 12
To evaluate the effect of tralokinumab on sputum eosinophil levels in adult subjects with asthma inadequately controlled with ICS	Outcome Variable: The change, expressed as a ratio, in number of differential sputum eosinophils from baseline up to week 12 Outcome Measure: Ratio of tralokinumab to placebo at week 12
To evaluate the effect of tralokinumab on activation of eosinophils in adult subjects with asthma inadequately controlled with ICS	Outcome Variables: The change, expressed as a ratio, in blood and sputum free eosinophil cationic protein (ECP) concentrations from baseline up to week 12 Outcome Measure: Ratio of tralokinumab to placebo at week 12

Safety Objective:	Outcome Variables:
To evaluate the safety and tolerability of tralokinumab in adult subjects with asthma inadequately controlled with ICS	Adverse Events/Serious Adverse Events Vital signs Clinical chemistry/haematology/urinalysis parameters Electro cardiograms Physical examinations

Exploratory Objectives:	Outcome Variables:
<p>To evaluate the effect of tralokinumab on other biomarkers of airway inflammation in adult subjects with asthma inadequately controlled with ICS</p>	<p>The change, from baseline up to week 12 in number of eosinophils per mm² of epithelium and airway smooth muscle bundle determined by light microscopy of bronchoscopic biopsies from baseline up to week 12</p> <p>The change, from baseline up to week 12 in number of inflammatory cells per mm² of epithelium, submucosa and airway smooth muscle bundle determined by light microscopy of bronchoscopic biopsies from baseline up to week 12</p> <ul style="list-style-type: none"> – CD3+, CD4+ and CD8+ lymphocytes, – Neutrophils, – Macrophages, – Mast cells <p>Change from baseline up to week 12 in induced sputum biomarkers:</p> <ul style="list-style-type: none"> – Number of inflammatory cells (neutrophils, lymphocytes and macrophages) and epithelial cells per mL,

- Number of inflammatory cells (neutrophils, lymphocytes and macrophages) expressed as a percentage of total inflammatory cells,
- Biomarkers which may include (but are not limited to) histamine, leukotrienes, Interleukin (IL) IL-13, IL-5, IL-12, IL-31

Change from baseline up to week 12 in serum biomarkers:

- Which may include (but are not limited to) periostin, DPP4, eotaxin, MCP1, TARC, IL-5, IL-33, STAT6, IL-13R α 2, MCP4/CCL13, TARC/CCL17, CLCA1, SERPINB2, biomarkers of tissue destruction, vascular adhesion molecules

Change from baseline up to week 12 in biomarkers obtained from nasosorption samples (Optional)

Change from baseline up to week 12 in blood total IgE

Change from baseline up to week 12 in exhaled nitric oxide

To evaluate the effect of tralokinumab on large airways remodelling in adult subjects with asthma inadequately controlled with ICS

Change from baseline up to week 12 in bronchial biopsy specimens

- Airway epithelial cell integrity (measured by light microscopy),
- Lamina reticularis and reticular basement membrane (RBM) thickening (measured by light microscopy),
- Deposition of periostin in basement membrane,
- Mucus glands and MUC5A,
- Other biomarkers of tissue remodelling and/or destruction which may include but are not limited to α -SMA and collagen type IV, fibronectin, laminin tenascin and TGF β and epithelial damage markers which may include caspase 3 and CC16/KL6,
- Airway epithelial gene expression

Change from baseline up to week 12 in large airway dimensions and estimated airway resistance determined from computed tomography (CT)

- Morphometry parameters (lumen area, wall area and wall area %) for airway generations 3, 4 and 5 (segmental, sub-segmental, sub-sub-segmental),
- Airway resistance for airway generations 3, 4 and 5 estimated based on lumen area,
- Airway volume and resistance for entire airway tree (lobar or lung level) based on Functional Respiratory Imaging (FRI) (Optional)

<p>To evaluate the effect of tralokinumab on small airways obstruction in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in</p> <ul style="list-style-type: none"> – Including (but not limited to) R5-R20 and AX as evaluated by airwave oscillometry (AO), – Including (but not limited to) S_{acin} by using multiple breath washout (MBW), – Air trapping expressed as percentage of the lung with expiratory density less than -856 HU, and as expiratory-to-inspiratory ratio of mean lung density on CT, – Regional matching of the inspiratory/expiratory CT scans to assess air trapping/small airway obstruction, – Extent of gas trapping determined from physiologic testing by total lung capacity (TLC)/residual volume (RV), vital capacity (VC)/inspiratory capacity (IC), functional residual capacity (FRC)
<p>To evaluate the effect of tralokinumab on asthma symptoms and other asthma control metrics in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in</p> <ul style="list-style-type: none"> – Daily asthma symptom scores (combined daytime and night-time score), – Rescue medication use, – Home peak expiratory flow (PEF) (morning and evening), – Number of night-time awakening due to asthma, – Asthma Control Questionnaire 6 (ACQ6)

<p>To evaluate the effect of tralokinumab on lung function and bronchial hyper-responsiveness in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in pre BD and post BD spirometry</p> <ul style="list-style-type: none"> – Forced expiratory volume in 1 second (FEV₁), – FVC, – Forced expiratory flow between 25% and 75% of the forced vital capacity (FEF_{25-75%}), <p>Change from baseline up to week 12 in</p> <ul style="list-style-type: none"> – Airway Hyper-responsiveness (AHR)
<p>To evaluate the effect of tralokinumab on symptom metrics of rhinosinusitis in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in Sino-Nasal Outcome Test -20 (SNOT-20) total score</p>
<p>To evaluate the pharmacokinetics and immunogenicity of tralokinumab</p>	<p>Pharmacokinetic parameters: Trough Concentration (C_{trough})</p> <p>Immunogenicity outcome variables: incidence rate of positive anti-drug antibodies and characterization of their neutralizing potential</p>
<p>To evaluate the effect of tralokinumab on Ribonucleic Acid (RNA) in samples obtained from adults subjects with asthma inadequately controlled with ICS.</p>	

Target subject population

This study will enrol males and females, 18 to 75 years of age inclusive, with uncontrolled asthma requiring continuous treatment with ICS (≥ 250 mcg fluticasone dry powder formulation equivalents total daily dose) with or without other asthma controllers and willing to provide informed consent.

Duration of treatment

After initial enrolment and confirmation of entry criteria (Visit 1) subjects will proceed to a run-in period of 4 weeks during which their suitability for randomization will be confirmed. The subjects' currently prescribed ICS and additional asthma controller medication will remain unchanged during the run-in and treatment period.

Subjects who meet the eligibility criteria at visit 3c will be randomized to a 12-week treatment period and receive either tralokinumab or placebo as an investigational product (IP) in addition to their existing asthma controller medications. IP will be administered at weeks 0, 2, 4, 6, 8 and 10. End of treatment (EOT) visit will be at week 12. Follow-up period for all subjects will be for 14 weeks with a telephonic follow-up visit at week 16. There will be an on-site follow-up visit, only for women of child bearing potential at week 26 to also ascertain their pregnancy status. For all other subjects this will be a telephonic FU 2 visit.

Investigational product, dosage and mode of administration

Tralokinumab 300 mg (150 mg/mL), or placebo, will be administered to subjects via subcutaneous injection using 2 accessorized 1 mL pre-filled syringes at the study site.

Statistical methods

The primary analysis of the efficacy endpoints will include all data captured during the double blind, treatment period (intent-to-treat approach). However, only subjects with complete data will be included in the primary analysis.

The primary efficacy objective will be evaluated through statistical testing of the within subject change from baseline to Week 12 in airway submucosal eosinophils. The null hypothesis test H_0 will be: The ratio tralokinumab/placebo equals 1 and will be tested vs. H_1 : The ratio is not equal to 1. The test will be based on an analysis of covariance model. Covariates and factors included in the model will include at least treatment and baseline number of airway submucosal eosinophils.

The secondary variable of within subject change from baseline up to Week 12 in the activation of eosinophils in bronchial submucosa will be analysed using a similar model as for the primary variable. The secondary variables of within subject change from baseline up to Week 12 in blood eosinophils and activation of eosinophils in blood and induced sputum will be analysed using a mixed model for repeated measures including at least treatment as a covariate.

The analyses will be performed by using log-transformed data and estimated geometric means and the ratio of geometric means with 95% confidence intervals will be presented.

The sample size is based on the primary endpoint; change from baseline to Week 12 in airway submucosal eosinophils. Given the assumed standard deviation of the log values in the two treatment groups are 1.62 and 1.82, it is estimated that 31 subjects in each treatment arm will be sufficient to achieve at least 80% power to detect a 3.5-fold difference versus placebo using a two-sided test at 5% significance level. With these assumptions the smallest difference that will yield a significant result is a 2.4-fold difference. The assumptions are based on the change in number of eosinophils per mm^2 subepithelial tissue in bronchial biopsies in studies D5890C00003 (Budesonide/ formoterol) (Pavord ID et al 2009) and MI-CP166 (Laviolette M et al 2013).

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It is assumed that a non-neglectable proportion of the subjects will not have an evaluable primary endpoint value due to failed biopsies. To account for this, 40 subjects will be randomized in each treatment arm.

The results for the exploratory variables will be summarized using descriptive statistics and graphical displays by treatment group. All safety parameters will be analysed descriptively. The safety analyses will be based on the safety analysis data set, defined as all subjects who received at least one dose of investigational product.

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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
AAER	Annualized Asthma Exacerbation Rate
ACQ-6	Asthma Control Questionnaire 6
ADA	Anti-drug antibody
AE	Adverse Event
AHR	Airway Hyper-Responsiveness
ALARA	As Low As Reasonably Achievable
ALP	Alkaline Phosphatase
α -SMA	Alpha smooth muscle actin
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
AO	Airwave Oscillometry
APFS	Accessorized Pre-filled Syringe
AST	Aspartate Aminotransferase
ATS/ERS	American Thoracic Society/European Respiratory Society
BAL	Broncho Alveolar Lavage
BD	Bronchodilator
β -HCG	Beta-Human Chorionic Gonadotropin
BMI	Body Mass Index
BSP-1	Basophil specific protein -1
BUN	Blood Urea Nitrogen
CC16/KL6	Clara-cell 16
CCL	Chemokine (C-C motif) ligand
CD	Cluster of Differentiation
CLCA1	Calcium-activated chloride channel regulator 1
CO ₂	Carbon Dioxide
COPD	Chronic Obstructive Pulmonary Disease

Abbreviation or special term	Explanation
CRP	C-Reactive Protein
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CT	Computed Tomography
CTDIvol	Volume CT dose index
DAE	Discontinuation of Investigational Product due to Adverse Event
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl Peptidase-4
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
ECP	Eosinophil Cationic Protein
eCRF	Electronic Case Report Form
ED	Emergency Department
EG	Eosinophil granule
ePEF	Electronic Peak Expiratory Flow
ER	Emergency Room
EOT	End of Treatment
ePRO	Electronic Patient Reported Outcome device
EU	European Union
EXACA	Module name of case report form to capture asthma exacerbations
FE _{NO}	Fractional Exhaled Nitric Oxide
FEF _{25-75%}	Forced Expiratory Flow between 25% and 75% of the Forced Vital Capacity
FEV ₁	Forced Expiratory Volume in 1 second
FRC	Functional Residual Capacity
FRI	Functional Respiratory Imaging
FSH	Follicle-Stimulating Hormone
FU	Follow-up

Abbreviation or special term	Explanation
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GINA	Global Initiative for Asthma
GLI	The Global Lung Function Initiative
GMP	Good Manufacturing Practice
GRand	Randomization Code Generator - Computerized System
HCP	Healthcare professional
HIV	Human Immunodeficiency Virus
HU	Hounsfield Unit
Hz	Hertz
IATA	International Air Transport Association
IB	Investigator's Brochure
IC	Inspiratory Capacity
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Inhaled Corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
IL-13R α 1	Interleukin 13 receptor subunit alpha-1
Il-13R α 2	Interleukin 13 receptor subunit alpha-2
IP	Investigational Product
IPD	Investigational product discontinuation
IRB	Institutional Review Board
ICRP	International Commission on Radiological Protection
ISF	Investigator Study File
ITT	Intent-to-Treat
IUD/IUS	Intra Uterine Device / Intra Uterine System
IVRS	Interactive Voice Response System

Abbreviation or special term	Explanation
IWRS	Interactive Web Response System
Kg	Kilogram
LABA	Long-Acting β_2 -Agonist
LCI	Lung Clearance Index
LTRA	Leukotriene Receptor Antagonists
MAb	Monoclonal Antibody
MBP	Major Basic Protein
MBW	Multiple Breath Washout
Mcg	Microgram
MCP-4/CCL13	Monocyte chemoattractant protein – 4 / Chemokine (C-C motif) ligand 13
μ M	Micromolar
MCP	Monocyte Chemoattractant Protein
MCT	Methacholine Challenge Test
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
mGy	Milli Gray
ML	Millilitre
MMRM	Mixed-Model for Repeated Measures
MSv	Millisievert
MUC5A	Mucin 5A
nAB	Neutralizing Antibodies
NHLB	National Heart Lung and Blood Institute
OAE	Other significant adverse events
OCS	Oral Corticosteroids
PEF	Peak Expiratory Flow
PGx	Pharmacogenetic research
PI	Principal Investigator
PK	Pharmacokinetics
PNT	Pneumotachometer

Abbreviation or special term	Explanation
PNV	Predicted Normal Value
PRO	Patient Reported Outcome
PT	Preferred Term
Q2W	Every 2 Weeks
Q4W	Every 4 Weeks
RBM	Reticular Basement Membrane
RNA	Ribonucleic acid
RV	Residual Volume
SABA	Short-Acting β 2-Agonist
S _{acin}	Index of acinar ventilation heterogeneity
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard Deviation
SDV	Source Data Verification
SERPINB2	Serpin peptidase inhibitor, clade B (ovalbumin), member 2
SI	Sputum Induction
SOC	System Organ Class
STAT6	Signal transducer and activator of transcription 6
TARC	Thymus and Activation Regulated Chemokine
TGF β	Transforming growth factor beta
Th2	T Helper 2 Cells
TLC	Total Lung Capacity
TO	Lung Turnover
TRP	Translational Research Partnership
ULN	Upper Limit of Normal
UNS	Unscheduled
VCAM	Vascular Cell Adhesion Molecule
WBC	White blood cell

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Abbreviation or special term	Explanation
WBDC	Web Based Data Capture
WOCBP	Women of Childbearing Potential

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Asthma is a syndrome characterized by airway inflammation, reversible airway obstruction and airway hyper-responsiveness, with a global prevalence of approximately 300 million patients (GINA 2014).

The observed variability in clinical response to currently available asthma therapies may be related, in part, to distinctive inflammatory phenotypes (Wenzel SE 2012). IL-13 is a central mediator implicated in the key pathophysiological features of asthma and its expression is up-regulated in a sub-group of patients with severe asthma, despite the use of inhaled corticosteroid (ICS) therapy. IL-13 promotes airway hyper-responsiveness (AHR), smooth-muscle proliferation and mucus production in animal models (Grunig G et al 1998, Lentsch AB et al 1999, Wills-Karp M et al 1998, Blanchard C et al 2005, May RD et al 2012). In cell culture, IL-13 up-regulates the production of eotaxin from airway epithelial cells (Li L et al 1999) and decreases trans-epithelial resistance, an indicator of epithelial barrier function (Saatian B et al 2013). IL-13 is thought to play a role in activating eosinophils and may affect eosinophil movement from blood to tissue by inducing release of vascular adhesion molecules (Bochner BS et al 1995, Wardlaw AJ 1999, Woltmann G et al 2000). More recently, studies suggested a potential role for IL-13 produced from innate natural T cells in asthma exacerbations (Cormier SA, Kolls JK 2011, Holtzman MJ 2012, Chang YJ et al 2013).

Tralokinumab specifically binds to IL-13 and blocks its interaction with both interleukin 13 receptor subunit alpha-1 (IL-13R α 1) and subunit alpha-2 (IL-13R α 2), preventing IL-13 signal transduction. IL-13R α 2 may function solely as a decoy receptor, but a recently reported study from cell culture model systems suggest that it may have a role in IL-13 mediated signal transduction (Fichtner-Fiegl S et al 2006). Tralokinumab has been shown to have a wide array of effects which might prove beneficial in asthma management. *In vitro*, tralokinumab prevented activation, signalling activity and production of mediators in and from a number of cells implicated in the development of asthma, including airway smooth muscle and epithelial cells, fibroblasts, monocytes and B cells (May RD et al 2012). Tralokinumab reduced IL-13 mediated eotaxin production, CD23 up-regulation and IgE production (May RD et al 2012). In a murine model, tralokinumab attenuated IL-13-induced leukocytic inflammation, airway hyper-responsiveness (AHR) and bronchoalveolar lavage (BAL) eosinophilia (Yang G et al 2005, May RD et al 2012). In a cynomolgus monkey model tralokinumab prevented AHR and BAL eosinophilia after antigen challenge (May RD et al 2012). Periostin and dipeptidyl peptidase-4 (DPP4) may be biomarkers of an activated IL-13 pathway and may predict response to tralokinumab (Jia G et al 2012, Parulekar AD et al 2014, Brightling CE et al 2014a).

Studies in patients with severe, uncontrolled asthma suggest that tralokinumab might provide a beneficial response. In a phase 2a study (MI-CP199), tralokinumab at a dose of 300 mg administered subcutaneously (SC) every 2 weeks (the dose proposed for the phase 3 program) improved forced expiratory volume in one second (FEV₁) when added to standard asthma controller medications. A subsequent phase 2b study (CD-RI-CAT-354-1049), in a patient

population whose asthma was poorly controlled by high dose ICS/ long-acting β_2 – agonist (LABA) confirmed that 300 mg of tralokinumab administered every two weeks significantly improved FEV₁ over 52 weeks of treatment. Although the primary endpoint for this study, a reduction in the annualised asthma exacerbation rate (AAER) over 52 weeks was not met, there were trends for AAER reductions in several subgroups. A subpopulation reversible to bronchodilators on study entry had an observed AAER reduction. A further reduction of AAER was observed in a subgroup of reversible subjects with evidence of increased IL-13 activity i.e. elevated serum periostin or DPP4. Post-hoc analysis of a small (n=26) sub-study of CD-RI-CAT-354-1049 utilising quantitative computed tomography (CT), showed significant improvements in sub-segmental airway lumen parameters with tralokinumab compared with placebo, added to ICS/LABA. In tralokinumab-treated subjects, higher baseline wall area % seems to be associated with better lung function improvement (Brightling CE et al 2014b).

An important unanswered question is whether tralokinumab improves asthma outcomes by reducing airway inflammation. An interesting observation in previous clinical trials has been that patients treated with tralokinumab had an increase in blood eosinophil levels. Based on *in vitro* work which showed that tralokinumab decreased epithelial cell production of eotaxin and vascular cell adhesion molecule-1 (VCAM-1) (May RD et al 2012), this observation in humans suggested that tralokinumab might reduce airway inflammation in asthma by reducing eosinophil trafficking from the blood into the lung. This study will test this hypothesis and will ask whether tralokinumab administered to subjects with uncontrolled asthma despite use of ICS, increases blood eosinophils but decreases airway submucosal eosinophil infiltration and reduces activated blood and airway submucosal eosinophils.

In addition, this study will address several exploratory questions. First, assuming that airway inflammation is reduced by tralokinumab, radiographic and physiologic tests will be performed to determine if reduced airway inflammation results in measurable changes in both large airway and small airway function and size. Second, this study will also address whether gene expression changes occur in the airways and the nose using tralokinumab and whether these changes might correspond to changes in airway inflammation. Third, the relationship between improvement in airway inflammation and both asthma symptoms and airway hyper-responsiveness will be assessed.

1.2 Rationale for study design, doses and control groups

This multicentre, randomized, double-blind, parallel group, placebo-controlled, 12-week, phase 2 study will evaluate the effect of a 300 mg dose of tralokinumab administered subcutaneously (SC) every 2 weeks on airway inflammation in adults with asthma inadequately controlled on ICS (≥ 250 mcg fluticasone dry powder formulation equivalents total daily dose) with or without other asthma controller medications.

The rationale for this study is the need to understand the mechanism of effect of tralokinumab in improving asthma outcomes. Pre-clinical work suggests that tralokinumab might reduce

airway inflammation, but information supporting this hypothesis in humans is currently lacking. One of the hallmarks of airway inflammation in asthma is an influx of eosinophils into the bronchial submucosal layer (Djukanovic R 2001, Sietta M and Durato G 2001). Pre-clinical studies suggest that tralokinumab might reduce eosinophil influx into the bronchial submucosal layer in asthma by affecting eosinophil trafficking and activation. Thus, the primary outcome measure for this study will be the change, expressed as a ratio, in number of airway submucosal eosinophils per mm² determined by microscopic evaluation of bronchoscopic biopsies from baseline up to week 12. Secondary outcomes will include blood eosinophil numbers and evidence of activation of eosinophils in blood, sputum and airway submucosa. In addition to these analyses, exploratory efforts will be made to understand whether the tralokinumab anti-inflammatory effect results in improvement in large airways remodelling and small airways obstruction.

In order to avoid bias, the study will be randomized and double-blinded. A placebo group will be included to ensure a control for comparison of effects. The dose and dosing frequency for tralokinumab, 300 mg administered SC every two weeks, was determined by an analysis of data from both the phase 2a study (MI-CP199) and the phase 2b study (CD-RI-CAT-354-1049). These studies demonstrated that tralokinumab 300 mg Q2W significantly increased FEV₁. In contrast, only limited, if any, improvement was observed with 150 mg Q2W (in study MI-CP199) or 300 mg Q2/4W (in study CD-RI-CAT-354-1049). Furthermore, an effect on AAER was observed with the 300 mg Q2W dosing regimen, but not with the Q2/4W dosing regimen in study CD-RI-CAT-354-1049. The treatment phase will last 12 weeks because previous studies with biologics such as benralizumab (Laviolette M et al 2013) have confirmed a reduction in airway submucosal eosinophils with a similar treatment duration. Included patients will have uncontrolled asthma to increase the likelihood that there will be increased airway submucosal eosinophils at study start.

1.3 Benefit/risk and ethical assessment

There are few treatment options for subjects whose asthma remains uncontrolled on ICS/LABA (GINA 2014). The evidence base for oral add-on therapies (i.e. oral corticosteroids (OCS), leukotriene inhibitors (LTRAs), and xanthenes) is limited. Anti-immunoglobulin E (IgE) therapy (i.e., omalizumab) improves control in a subset of subjects with severe asthma associated with IgE-mediated allergy to a perennial allergen. Hence, new therapies are needed for asthma management in subjects who remain uncontrolled on standard of care.

IL-13 is targeted as it plays a role in the allergic/Th2 type response which is a signature of asthma. An anti-IL-13 treatment may therefore be useful in treatment of asthma. Data from phase 2 studies support this notion (for further details see the Investigator's brochure). Approximately 520 subjects with asthma have so far been exposed to tralokinumab at various doses and for different periods of time. The 1 year long phase 2b study has contributed 301 of these subjects with 140 of them receiving 300 mg every other week. In all studies conducted so far, tralokinumab has been well tolerated, and no safety concerns have been identified.

Tralokinumab might reduce airway inflammation in asthma by reducing eosinophil trafficking from the blood into the lung. The purpose of this study is to test the hypothesis that tralokinumab reduces airway submucosal eosinophil infiltration in subjects with uncontrolled asthma despite use of ICS, and increases blood eosinophils but is associated with a decrease in activated eosinophils.

Because it is believed that the Th2 response may be of importance in the defence against helminthic parasitic infections, a theoretical risk for such infestations exists in patients treated with tralokinumab. IL-13 may also play a role in regulating tumours ([Hallett MA et al 2012](#)), and although evidence for this is scarce and inconclusive, this theoretical risk needs to be considered. In conjunction with the performance of routine pharmacovigilance activities risk minimization measures therefore include the exclusion of subjects with untreated parasitic infection and active or recent malignancy.

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.

CT is incorporated into this study design with reference to the EU guidance ([Directorate-General – Environment, nuclear safety and civil protection 1998](#)). The potential benefits of the study are expected to be Category IIB as described in the guidance document aimed directly at the diagnosis, cure, or prevention of disease. The use of CT involves ionizing radiation that increases the risk of radiogenic tumours in subjects. Subjects will be informed of the risks associated with CT before entering the study. Because CT scans in this study may not offer direct individual benefit to the subject, a dose constraint has been applied based on the as low as reasonably achievable (ALARA) principle. The CT scan parameters in the study will be optimized to give a standardized x-ray exposure across the different scanners used in the trial. A target CTDIvol (volume CT dose index) of approximately 6 milli Gray (mGy) for the inspiratory scan and 3 mGy for the expiratory scan is required to achieve the noise index needed for the subsequent image analysis. With two visits and a scanned length of 30 cm this corresponds to an estimated radiation dose of 7.6 mSv based on ICRP60 (International Commission on Radiological Protection Publication 60) weighting factors ([AAPM, 2008](#), [ICRP, 1991](#)). Allowing for some additional variation between scanners, patient geometries and calculation models, the total effective radiation dose for the study is estimated to be equal or less than approximately 10 milli Sievert (mSv). This dose is within the accepted radiation dose range for biological research (1-10 mSv; [Directorate-General – Environment, nuclear safety and civil protection 1998](#)).

1.4 Study Design

This multicentre, randomized, double-blind, parallel group, placebo-controlled, 12-week, phase 2 study will evaluate the effect of a 300 mg dose of tralokinumab administered SC

every 2 weeks on airway inflammation in adults with asthma inadequately controlled on ICS with or without other controllers.

The subjects will be randomized to 12-week treatment with tralokinumab or placebo (1:1). After the end of treatment all subjects will be followed up for a period of 14 weeks.

Target subject population

This study will enrol males and females, 18 to 75 years of age inclusive, with uncontrolled asthma requiring continuous treatment with ICS (≥ 250 mcg fluticasone dry powder formulation equivalents total daily dose) with or without other asthma controllers and willing to provide informed consent.

Duration of treatment

After initial enrolment and confirmation of entry criteria (Visit 1) subjects will proceed to a run-in period of 4 weeks during which their suitability for randomization will be confirmed. The subjects' currently prescribed ICS and additional asthma controller medication will remain unchanged during the run-in and treatment period.

Subjects who meet all the eligibility criteria at Visit 3c will be randomized to a 12-week treatment period and receive either tralokinumab or placebo as an investigational product (IP) in addition to their existing asthma controller medications. IP will be administered at weeks 0, 2, 4, 6, 8 and 10 (Visit 3c to Visit 8). End of treatment (EOT) visit will be at week 12 (Visit 9).

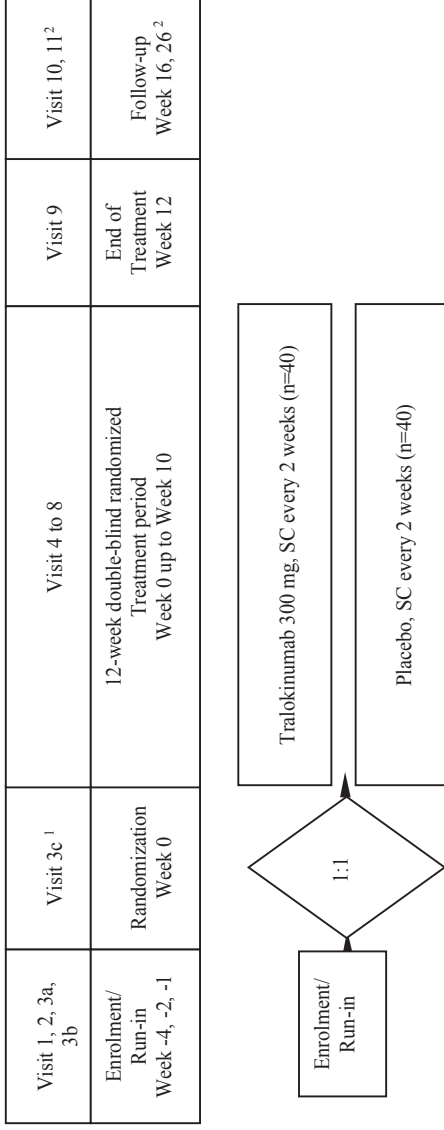
Post-treatment follow-up period for all subjects will be for 14 weeks after the EOT visit with a telephonic follow-up (FU) 1 visit (Visit 10, Week 16). There will be an on-site FU 2 visit (Visit 11, Week 26) only for WOCBP to also ascertain their pregnancy status. For all other subjects this will be a telephonic FU 2 visit

Investigational product, dosage and mode of administration

Tralokinumab 300 mg (150 mg/mL), or placebo, will be administered to subjects SC using 2 accessorized 1 mL pre-filled syringes at the study site.

Subjects will be maintained on their currently prescribed ICS therapy and any additional asthma controller medications, without changes, from enrolment until the end of treatment period.

Figure 1 Study flow chart



1. Due to multiplicity of assessments, Visit 3 is spread over 3 visits, Visit 3a, 3b and 3c. Visit 3c being the randomization visit when IP is administered for the first time.
2. WOCBP will have an on-site follow-up visit at Week 26 to also ascertain their pregnancy status.

2. STUDY OBJECTIVES

2.1 Primary objective

Primary Objective:	Outcome Measure:
To evaluate the effect of tralokinumab on eosinophilic airway infiltration in adult subjects with asthma inadequately controlled with ICS	<p>Primary Outcome Variable: The change, expressed as a ratio, in number of airway submucosal eosinophils per mm² determined by microscopic evaluation of bronchoscopic biopsies from baseline up to week 12</p> <p>Primary Outcome Measure: Ratio of tralokinumab to placebo at week 12 to baseline</p>

2.2 Secondary objectives

Secondary Objective:	Outcome Measure :
To evaluate the effect of tralokinumab on blood eosinophil levels in adult subjects with asthma inadequately controlled with ICS	<p>Outcome Variable: The change, expressed as a ratio, in number of blood eosinophils from baseline up to week 12</p> <p>Outcome Measure: Ratio of tralokinumab to placebo at week 12 to baseline</p>
To evaluate the effect of tralokinumab on sputum eosinophil levels in adult subjects with asthma inadequately controlled with ICS	<p>Outcome Variable: The change, expressed as a ratio, in number of differential sputum eosinophils from baseline up to week 12</p> <p>Outcome Measure: Ratio of tralokinumab to placebo at week 12</p>

<p>To evaluate the effect of tralokinumab on activation of eosinophils in adult subjects with asthma inadequately controlled with ICS</p>	<p>Outcome Variables: The change, expressed as a ratio, in blood and sputum free eosinophil cationic protein (ECP) concentrations from baseline up to week 12</p> <p>Outcome Measure: Ratio of tralokinumab to placebo at week 12 to baseline</p>
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2.3 Safety objectives

Safety Objective:	Outcome Measures:
<p>To evaluate the safety and tolerability of tralokinumab in adult subjects with asthma inadequately controlled with ICS</p>	<p>Adverse events (AEs)/Serious adverse events (SAEs)</p> <p>Vital signs</p> <p>Electrocardiogram (ECG)</p> <p>Clinical chemistry/haematology/urinalysis parameters</p> <p>Physical examination</p>

2.4 Exploratory objectives

Exploratory Objectives:	Outcome Variables:
To evaluate the effect of tralokinumab on other biomarkers of airway inflammation in adult subjects with asthma inadequately controlled with ICS	<p>The change, from baseline up to week 12 in number of eosinophils per mm² of epithelium and airway smooth muscle bundle determined by light microscopy of bronchoscopic biopsies from baseline up to week 12</p> <p>The change, from baseline up to week 12 in number of inflammatory cells per mm² of epithelium, submucosa and airway smooth muscle bundle determined by light microscopy of bronchoscopic biopsies from baseline up to week 12</p> <ul style="list-style-type: none">- CD3+, CD4+ and CD8+ lymphocytes,- Neutrophils,- Macrophages,- Mast cells

Change from baseline up to week 12 in induced sputum biomarkers:

- Number of inflammatory cells (neutrophils, lymphocytes and macrophages) and epithelial cells per mL,
- Number of inflammatory cells (neutrophils, lymphocytes and macrophages) expressed as a percentage of total inflammatory cells,
- Biomarkers which may include (but are not limited to) histamine, leukotrienes, Interleukin (IL) IL-13, IL-5, IL-12, IL-31

Change from baseline up to week 12 in serum biomarkers:

- Which may include (but are not limited to) periostin, DPP4, eotaxin, MCP1, TARC, IL-5, IL-33, STAT6, IL-13R α 2, MCP4/CCL13, TARC/CCL17, CLCA1, SERPINB2, biomarkers of tissue destruction, vascular adhesion molecules

Change from baseline up to week 12 in biomarkers obtained from nasosorption samples (Optional)

Change from baseline up to week 12 in blood total IgE

Change from baseline up to week 12 in exhaled nitric oxide

To evaluate the effect of tralokinumab on large airways remodelling in adult subjects with asthma inadequately controlled with ICS

Change from baseline up to week 12 in bronchial biopsy specimens

- Airway epithelial cell integrity (measured by light microscopy),
- Lamina reticularis and reticular basement membrane (RBM) thickening (measured by light microscopy),
- Deposition of periostin in basement membrane,
- Mucus glands and MUC5A,
- Other biomarkers of tissue remodelling and/or destruction which may include but are not limited to α -SMA and collagen type IV, fibronectin, laminin tenascin and TGF β and epithelial damage markers which may include caspase 3 and CC16/KL6,
- Airway epithelial gene expression

Change from baseline up to week 12 in large airway dimensions and estimated airway resistance determined from computed tomography (CT)

- Morphometry parameters (lumen area, wall area and wall area %) for airway generations 3, 4 and 5 (segmental, sub-segmental, sub-sub-segmental),
- Airway resistance for airway generations 3, 4 and 5 estimated based on lumen area,
- Airway volume and resistance for entire airway tree (lobar or lung level) based on Functional Respiratory Imaging (FRI) (Optional)

<p>To evaluate the effect of tralokinumab on small airways obstruction in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in</p> <ul style="list-style-type: none"> – Including (but not limited to) R5-R20 and AX as evaluated by airwave oscillometry (AO), – Including (but not limited to) S_{acin} by using multiple breath washout (MBW), – Air trapping expressed as percentage of the lung with expiratory density less than -856 HU, and as expiratory-to-inspiratory ratio of mean lung density on CT, – Regional matching of the inspiratory/expiratory CT scans to assess air trapping/small airway obstruction, – Extent of gas trapping determined from physiologic testing by total lung capacity (TLC)/residual volume (RV), vital capacity (VC)/inspiratory capacity (IC), functional residual capacity (FRC)
<p>To evaluate the effect of tralokinumab on asthma symptoms and other asthma control metrics in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in</p> <ul style="list-style-type: none"> – Daily asthma symptom scores (combined daytime and night-time score), – Rescue medication use, – Home peak expiratory flow (PEF) (morning and evening), – Number of night-time awakening due to asthma, – Asthma Control Questionnaire 6 (ACQ6)

<p>To evaluate the effect of tralokinumab on lung function and bronchial hyper-responsiveness in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in pre BD and post BD spirometry</p> <ul style="list-style-type: none"> – Forced expiratory volume in 1 second (FEV₁), – FVC, – Forced expiratory flow between 25% and 75% of the forced vital capacity (FEF_{25-75%}), <p>Change from baseline up to week 12 in</p> <ul style="list-style-type: none"> – Airway Hyper-responsiveness (AHR)
<p>To evaluate the effect of tralokinumab on symptom metrics of rhinosinusitis in adult subjects with asthma inadequately controlled with ICS</p>	<p>Change from baseline up to week 12 in Sino-Nasal Outcome Test -20 (SNOT-20) total score</p>
<p>To evaluate the pharmacokinetics and immunogenicity of tralokinumab</p>	<p>Pharmacokinetic parameters: Trough concentration (C_{trough})</p> <p>Immunogenicity outcome variables: incidence rate of positive anti-drug antibodies and characterization of their neutralizing potential</p>
<p>To evaluate the effect of tralokinumab on Ribonucleic Acid (RNA) in samples obtained from adult subjects with asthma inadequately controlled with ICS.</p>	

3. SUBJECT SELECTION, ENROLMENT, RANDOMIZATION, 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. Provision of informed consent prior to any study specific procedures (including withholding of any asthma medications required for procedures) for subjects who are at, or over the age of majority (as per local law).
2. Female or male, ages 18 to 75 years, inclusively at time of enrolment (Visit 1).
3. Women of childbearing potential (WOCBP) (after menarche) must use a highly effective form of birth control (confirmed by the Investigator). Highly effective forms of birth control includes: true sexual abstinence ('true sexual abstinence' is defined as abstinence that is in line with the preferred and usual lifestyle of the subject. Periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not considered highly effective methods of contraception), a vasectomised sexual partner, female sterilization by tubal occlusion, Implanon[®], any effective intrauterine device/system (IUD/IUS), Depo-Provera[™] injections, oral contraceptive, or Nuvaring[™] (if approved for use as per local regulations). WOCBP must agree to use highly effective method of birth control, as defined above, from enrolment (Visit 1), throughout the study duration and within 16 weeks after last dose of investigational product (IP), and have a negative serum pregnancy test result at 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 postmenopausal if they have been amenorrhic 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 amenorrhic for 12 months or more following cessation of exogenous hormonal treatment and follicle stimulating hormone (FSH) levels in the postmenopausal range.
 - Women \geq 50 years old would be considered postmenopausal if they have been amenorrhic for 12 months or more following cessation of all exogenous hormonal treatment.
4. Weight of \geq 40 and <150 kg at enrolment (Visit 1).

5. Documented physician-diagnosed asthma for at least 12 months prior to enrolment (Visit 1) with the subject having received an asthma controller regimen requiring treatment with ICS (minimum dose of $\geq 250\mu\text{g}$ fluticasone propionate via dry powder inhaler equivalents total daily dose) alone or in combination ≥ 6 months and that has been taken at a stable dose for at least 1 month prior to enrolment (Visit 1). In order to aid the assessment, ICS equivalents for fluticasone propionate dry powder, are presented in [Appendix E](#). If the subject is on two or more different types of ICS, these can form parts of an addition, and the sum, however approximate, will be assessed.
6. Additional maintenance asthma controller medications are allowed according to standard practice of care. These medications must be given at a stable dose for at least 1 month prior to Visit 1. Furthermore, after randomization, the subject's background maintenance medication for asthma shall remain unchanged throughout the study.
7. At enrolment (Visit 1) the subject must have a predicted normal value (PNV) for the pre-bronchodilator (BD) $\text{FEV}_1 > 50\%$ and more than 1L. If this criterion is not met at Visit 1, the criterion must be met at Visit 2. Prior to the lung function measurement, the subject should withhold their BD for the effect duration specific to the BD (see [7.7.1.2](#) and [Appendix G](#)).
8. A post-BD reversibility in FEV_1 of $\geq 12\%$ and ≥ 200 mL at enrolment (Visit 1). If this criterion is not met at Visit 1, the criterion must be met at Visit 2. Prior to the lung function measurement, the subject should withhold their BD for the effect duration specific to the BD (see [7.7.1.2](#) and [Appendix G](#)).

Prior to randomization at Visit 3c, subjects should fulfil the following inclusion criteria:

9. For WOCBP only: have a negative urine pregnancy test prior to administration of the IP.
10. No requirement for a change in the subject's ICS, other asthma controller medications and/or the requirement to add asthma controller medications during the run-in period.
11. Ability to perform acceptable inhaler and spirometry techniques and use peak flow meter as judged by the investigator.
12. Minimum 70% compliance with usual asthma controller (ICS and any other) medications and eDiary completion between Visit 1 and Visit 2. Compliance is defined as completing all elements of eDiary for any 10 mornings and evenings of the last 14 days between visits as reported by the subject in the eDiary.
13. Asthma Control Questionnaire-6 (ACQ-6) ≥ 1.5 at Visit 1 or Visit 2.

14. Successful bronchial biopsy procedure as judged by the investigator.

3.2 Exclusion criteria

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

1. History of interstitial lung disease, chronic obstructive pulmonary disease (COPD), or other clinically significant lung disease other than asthma that in the opinion of the investigator may compromise the well-being of the subject or study end point assessments at the start of the study.
2. Any disorder, including but not limited to, cardiovascular, gastrointestinal, hepatic, renal, neurological, musculoskeletal, infectious, endocrine, metabolic, haematological, psychiatric, or major physical impairment that is either not stable or could in the opinion of the Investigator:
 - Affect the safety of the subject throughout the study
 - Influence the findings of the studies or their interpretations
 - Impede the subject's ability to complete the entire duration of study
3. Known history of allergy or reaction to any component of the IP formulation (see [Table 4](#)).
4. History of anaphylaxis following any biologic therapy.
5. A helminth parasitic infection diagnosed within 6 months prior to enrolment (Visit 1) that has not been treated with, or has failed to respond to, standard of care therapy.
6. History of clinically significant infection, including acute upper or lower respiratory infections, requiring antibiotics or antiviral medication within 30 days prior to enrolment (Visit 1) or during the run-in period.
7. Tuberculosis requiring treatment within the 12 months prior to enrolment (Visit 1).
8. Any clinically significant findings in physical examination, vital signs, electrocardiogram (ECG), CT scan, haematology, clinical chemistry, or urinalysis during the enrolment or run-in period, which in the opinion of the Investigator, may put the subject at risk because of his/her participation in the study, or may influence the results of the study, or the subject's ability to complete entire duration of the study.
9. History of chronic alcohol or drug abuse within 12 months of the enrolment visit (Visit 1), or a condition associated with poor compliance as judged by the Investigator.

10. Positive hepatitis B surface antigen or hepatitis C virus antibody serology. Subjects with a history of hepatitis B vaccination without a history of hepatitis B are allowed to be enrolled.
11. History of any known primary immunodeficiency disorder including a positive human immunodeficiency virus (HIV) test at enrolment (Visit 1), or the subject taking antiretroviral medications as determined by medical history and/or subject's verbal report.
12. Current tobacco smoking (smoking must have stopped for ≥ 3 months prior to enrolment (Visit 1)) or a history of tobacco smoking for >10 pack-years (one pack year = 20 cigarettes smoked per day for 1 year).
13. History of cancer with the exception of:
 - Subjects who have had basal cell carcinoma, localized squamous cell carcinoma of the skin or in situ carcinoma of the cervix provided that the subject is in remission and curative therapy was completed at least 12 months prior to enrolment (Visit 1).
 - Subjects who have had other malignancies provided that the subject is in remission and curative therapy was completed at least 5 years prior to enrolment (Visit 1).
14. Use of immunosuppressive medication (including but not limited to: methotrexate, troleandomycin, cyclosporine, azathioprine, and intramuscular long-acting depo corticosteroids) within 3 months prior to enrolment (Visit 1).
15. Chronic use of oral corticosteroids (OCS).
16. Subjects unable to safely undergo elective, flexible, fiberoptic bronchoscopy.
17. Subjects who have been hospitalised or required OCS within 6 weeks of the enrolment visit or had a history of more than three exacerbations requiring corticosteroid treatment in the previous year or history of intubation/ICU in the previous year.
18. Receipt of immunoglobulin or blood products within 30 days prior to enrolment (Visit 1).
19. Receipt of any marketed (e.g., omalizumab) or investigational biologic agent within 4 months or 5 half-lives prior to the enrolment (Visit 1), whichever is longer.
20. Receipt of live attenuated vaccines within 30 days prior to the enrolment (Visit 1) and the subject agrees not to have live attenuated vaccines during the study including the follow-up period.

- Receipt of inactive/killed vaccinations (e.g., inactive influenza) are allowed, provided they are not administered within 5 days before/after any dosing visit.
21. Receipt of any investigational non-biologic agent within 30 days or 5 half-lives prior to enrolment (Visit 1), whichever is longer.
 22. Previous receipt of tralokinumab (CAT-354).
 23. Initiation of new allergen immunotherapy or change in existing immunotherapy is not allowed within 30 days prior to enrolment (Visit 1). However, allergen immunotherapy initiated prior to this period may be continued provided there is a span of at least 5 days between the immunotherapy and IP administration.
 24. Current use of any oral or ophthalmic non-selective β -adrenergic antagonist (e.g., propranolol).
 25. Current use of five-lipoxygenase inhibitors (e.g., Zileuton) or roflumilast.
 26. Subjects who have undergone bronchial thermoplasty.
 27. Major surgery within 8 weeks prior to the enrolment (Visit 1), or planned in-patient surgery or hospitalization during the study period.
 28. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level ≥ 2.5 times the upper limit of normal (ULN) at enrolment (Visit 1).
 29. Pregnant, currently breast-feeding, or lactating women.
 30. Previous randomization in the present study.
 31. Concurrent enrolment in another clinical study where the subject is receiving an IP.
 32. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study site).
 33. Employees of the clinical study site or any other individuals directly involved with the planning or 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, the subject screening log, of subjects who entered pre-study screening. The pre-screening/screening log will be evaluated periodically by AstraZeneca and/or its delegates during routine monitoring visits.

The Investigator(s) will:

1. Obtain signed informed consent from the potential subject or their legal representative before any study specific procedures are performed.
2. Assign potential subject a unique enrolment number, beginning with 'E' via the Interactive Web Response System/ Interactive Voice Response System (IWRS/IVRS) at Visit 1.
3. Determine subject eligibility. See Section 0.
4. Assign the eligible subject unique randomization code via IWRS/IVRS at Visit 3c.

Subjects will be allocated to receive tralokinumab or placebo in a 1:1 ratio (i.e. 40 subjects on tralokinumab versus 40 subjects on placebo). Randomization numbers will be grouped in blocks. Randomized subjects who discontinue will not be replaced. If a subject withdraws from participation in the study, then his/her enrolment/randomization code cannot be reused.

Specific information concerning the use of the IWRS/IVRS will be provided in a separate instruction manual.

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.

When 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 a discussion should occur between the AstraZeneca study physician and the investigator regarding whether to continue or discontinue the subject from treatment. The AstraZeneca study physician must ensure all decisions are appropriately documented.

3.5 Methods for assigning treatment groups

Randomization codes will be assigned strictly sequentially as subjects become eligible for randomization. The randomization code will be assigned from a randomization list prepared by an internal AstraZeneca computerized system (GRand).

3.6 Methods for ensuring blinding

This is a double-blind study in which tralokinumab and placebo are visually distinct from each other. Neither the subject nor any of the Investigator or sponsor staff who are involved in the treatment or clinical evaluation and monitoring of the subjects will be aware of the treatment

received. Since tralokinumab and placebo are visually distinct, IP will be handled by an unblinded IP manager at the site and will be administered by an unblinded investigational site study team member who will not be involved in the management of study subjects (this could be the same person).

Should an issue arise with the IP (e.g. damaged kit or syringe) that has been assigned to a subject prior to administration, or any other unexpected event with the kit or syringe (e.g. a malfunction during IP administration) the unblinded IP manager at the site will contact a predetermined unblinded AstraZeneca site monitor (who is not otherwise involved in the project) to determine whether any specific actions are required.

A blinded AstraZeneca site monitor will perform IP accountability. In the event that the treatment allocation for a subject becomes known to the Investigator or other study staff involved in the management of study subjects, or needs to be known to treat an individual subject for an AE, the sponsor must be notified immediately by the Investigator and if possible, before unblinding.

All packaging and labelling of IP will be done in such way as to ensure blinding for all sponsor and investigational site staff (other than the unblinded IP manager who will directly handle the pre-filled syringes).

The following personnel will have access to the randomization list:

- IVRS/IWRS Vendor
- those generating the randomization list

The information in the randomization list will be kept from other personnel involved in the conduct of the study, and in a secure location until the end of the study.

No member of the extended study team at AstraZeneca, or any contract research organization (CRO) handling data, will have access to unblinded information during the conduct of the study, with the exception of the Supply Chain Study Management department and the Patient Safety department at AstraZeneca.

3.7 Methods for unblinding

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

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

Fertile and sexually active female subjects should use highly effective contraceptive methods throughout the study and for at least 16 weeks (5 half-lives) after last administration of the IP.

Subjects must abstain from donating blood or plasma from the time of informed consent and up to 16 weeks (5 half-lives) after last dose of IP.

3.9 Criteria for withdrawal from study

3.9.1 Screen failures

Screen 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 as 'Incorrect Enrolment' (i.e., subject does not meet the required inclusion/exclusion criteria). This reason for study withdrawal is only valid for screen failures, and not randomized subjects.

3.9.2 Post-randomization

After randomization and receiving IP, subjects are free to discontinue IP and withdraw from the study at any time without prejudice to further treatment. A subject that decides to discontinue the IP and withdraw from the study will always be asked about the reason(s) for their decision to withdraw and the presence of any AEs. If possible, they will be seen and assessed by an Investigator(s). Adverse events will be followed up (as described in Section 6.3).

Subjects will be withdrawn from the study in the following situations:

1. Subject decision. The subject is at any time free to discontinue treatment, without prejudice to further treatment
2. The subject experiences an AE that, in the opinion of the Investigator, contraindicates further dosing
3. The development of any risk to the subject as judged by the Investigator or AstraZeneca
4. Severe non-compliance with the study protocol as judged by the Investigator or AstraZeneca
5. Pregnancy

6. Lost to follow-up¹
7. Development of any study specific criteria for discontinuation, including:
 - a) An anaphylactic reaction to the IP requiring administration of epinephrine
 - b) A helminth parasitic infestation requiring hospitalization
 - c) An asthma-related event requiring intubation
 - d) Any malignancy
8. Development of one or more of the following:
 - a) Confirmed ALT or AST increase of ≥ 8 x ULN
 - b) Confirmed ALT or AST increase of ≥ 5 x ULN for more than 2 weeks
 - c) Confirmed ALT or AST increase of ≥ 3 x ULN and total bilirubin of ≥ 2 x ULN
 - d) ALT or AST of ≥ 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia ($\geq 5\%$)

The Principal Investigator will perform all the possible observation(s), test(s) and evaluation(s) including completion of questionnaires if any, as per End of Treatment (EOT) assessments mentioned in [Table 1](#) as soon as possible. Investigator will give appropriate medication and take all possible measures for the safety of the subject. They will also immediately inform AstraZeneca of the withdrawal. Subject will then be followed up for additional 16 weeks from the last dose of IP, unless the subject withdraws consent for any further follow-ups. During follow-up, the Investigator will perform all possible assessments in accordance with schedule provided in [Table 1](#).

The reason for premature study withdrawal will be documented in the source documentation and recorded in the electronic case report form (eCRF). All discontinued subjects must return the electronic patient reported outcome (ePRO) device (eDiary) and electronic ePEF devices. Early termination of IP treatment, will be registered via the IWRS/IVRS for each subject.

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

If the subject only withdraws consent for the retention of blood samples for future exploratory use (e.g., study of markers of asthma, identifying potential new drug targets for asthma, or for

¹ A subject is considered lost to follow up when any of the following attempts of contact are failed: at least 3 attempts of either phone calls, faxes or emails; having sent 1 registered letter/certified mail; or one unsuccessful effort to check the status of the subject using publicly available sources, if allowed by local regulations.

assay development purposes), the subject will not be withdrawn from the study. For details please see Section 5.7.4.

3.10 Discontinuation of the study

The entire study may be stopped if, in the judgment of AstraZeneca, trial subjects are placed at undue risk because of clinically significant findings that:

- are assessed as causally related to study drug,
- are not considered to be consistent with continuation of the study

Regardless of the reason for termination, all data available for the subject at the time of discontinuation of follow-up must be recorded in the eCRF. All reasons for discontinuation of treatment must be documented.

In terminating the study, the Sponsor will ensure that adequate consideration is given to the protection of the subjects' interests.

Revised Clinical Study Protocol
Drug Substance Tralokinumab (CAT-354)
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4. STUDY PLAN AND TIMING OF PROCEDURES

Table 1 Study plan - Treatment and follow-up period

Assessment/activity	Enrolment Run-in			Treatment							EOT ^{iv}	FU 1 ^v	FU 2 ^{vi}	UNS ^{vii}			
	V1 ⁱ	V2	V3a ⁱⁱⁱ	V3b ⁱⁱⁱ	V3c	V4	V5	V6	V7	V8					V9	V10	V11
	-4w	-2w	-7d	-5d	0	2w	4w	6w	8w	10w					12w	16w	26w
	N/A	+3	±2	±2	0	±3	±3	±3	±3	±3	±7	±7	±7	N/A			
Informed consent	X																
Inclusion/exclusion criteria	X	X	X	X	X												
Demographics	X																
Medical/Surgical and asthma history	X																
Weight, Height	X																
Asthma Daily Diary and PEF adherence check		X	X	X	X	X	X	X	X	X	X						
ACQ-6 at the site	X	X			X		X				X						
SNOT-20 at the site					X						X						
Urine pregnancy test (dipstick) ^{viii}		X			X	X	X	X	X	X	X		X				
Urinalysis (dipstick)	X										X						
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Asthma exacerbation		X	X	X	X	X	X	X	X	X	X	X	X	X			
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X			X			
Randomization					X												
Vital Signs ^{ix}	X	X			X	X	X	X	X	X	X						
Local ECG	X										X						
Complete physical examination	X				X						X						

Assessment/activity	Enrolment Run-in						Treatment							EOT ^{iv}	FU 1 ^v	FU 2 ^{vi}	UNS ^{vii}
	V1 ⁱ	V2	V3a ⁱⁱⁱ	V3b ⁱⁱⁱ	V3c	V4	V5	V6	V7	V8	V9	V10	V11				
	-4w	-2w	-7d	-5d	0	2w	4w	6w	8w	10w	12w	16w	26w				
	N/A	+3	±2	±2	0	±3	±3	±3	±3	±3	±7	±7	±7	N/A			
Brief physical exam						X	X	X	X	X				X			
Clinical chemistry	X										X			X			
Haematology	X				X		X				X			X			
Serology (hepatitis B,C; HIV-1,2)	X																
Serum pregnancy test	X																
FSH ^x	X																
Serum biomarkers					X		X				X						
Total IgE					X		X				X						
Phadiatop					X												
RNA Transcriptomics ^{xi}				X	X		X				X						
Tralokinumab PK ^{xiii}					X		X				X						
Tralokinumab ADA/nAb ^{xii}					X						X						
FE _{NO}			X				X				X						
Nasosorption and nasal epithelial cells sampling				X							X						
Pre-BD, spirometry	X	X ^{xiii}	X				X				X						
Post-BD, spirometry	X	X ^{xiii}	X ^{xiv}								X ^{xiv}						
Post-BD, whole body plethysmography (WBP)			X								X						

Assessment/activity	Enrolment Run-in				Treatment							EOT ^{iv}	FU 1 ^v	FU 2 ^{vi}	UNS ^{vii}		
	V1 ⁱ	V2	V3a ⁱⁱⁱ	V3b ⁱⁱⁱ	V3c	V4	V5	V6	V7	V8	V9					V10	V11
	-4w	-2w	-7d	-5d	0	2w	4w	6w	8w	10w	12w					16w	26w
	Visit window ⁱⁱ (days)																
	N/A	+3	±2	±2	0	±3	±3	±3	±3	±3	±7	±7	±7	±7	N/A		
Airwave oscillometry (AO)		X ^{xv}		X							X						
Multiple breath washout (MBW)		X ^{xv}		X							X						
AHR				X							X						
Computed tomography (CT)			X								X ^{xix}						
Sputum induction ^{xvi}		X ^{xv}	X				X				X						
Bronchoscopy/biopsy/brushing					X ^{xvii}												
Administration of IP ^{xviii}					X	X	X	X	X	X							

For restrictions for withholding asthma medications before visits see Section 7.7.1.2. Asthma medications should not be withheld on visits when bronchoscopy is scheduled.

- i. Visit 1 can be performed over more than one day, within a 3-working day window, with the exception of documentation of informed consent which can be completed up to 30 days prior to Visit 1.
- ii. All visits are to be scheduled from the date of randomization, not from the date of the previous visit.
- iii. All pulmonary assessments performed during V3 (a, b & c) may be performed over a 7-working day window, with a suggested order of FENO, AO, MBW and Pre-BD spirometry if performed on the same day. CT Scan assessment should precede sputum induction if performed on same day.
- iv. Based on investigator's judgement and site resources, EOT assessments will be performed over more than one day, within a 7-working day window. The sequence of pulmonary assessments during the EOT visit should be performed in the same order as completed at Visits 3a, 3b and 3c. (Refer to footnote iii).
- v. This will be a telephonic follow-up visit. Depending on investigator's judgement, subject may be called for an on-site visit for further evaluation.
- vi. On-site FU 2 visit will be performed only for WOCBP to also ascertain their pregnancy status. For all other subjects this will be a telephonic FU 2 visit.
- vii. At unscheduled visits for assessing an asthma exacerbation, the assessment/activity listed above is only the minimum needed to be performed. Other unscheduled visits may be initiated as needed, and assessments performed as per investigator's judgement.
- viii. For WOCBP urine pregnancy test (dipstick) is to be done at the centre and confirmed 'negative' prior to IP administration when scheduled.
- ix. Vital signs will be recorded pre-dose on the days of IP administration. Subjects will be observed 2 hours post IP administration for Visits 3c through Visit 6. For all later visits where IP is administered, subjects will be observed for a minimum of 1 hour. Additional assessments may be performed as per investigator's judgement.
- x. FSH test is performed only for female subjects to confirm postmenopausal status in women <50 years who have been amenorrhoeic for ≥12 month.

- xi. RNA samples will be obtained from the peripheral blood collected for haematology assessments, nasal epithelial cells and from bronchial brushing samples.
- xii. Blood samples for PK and ADA/nAb evaluations will be collected before the administration of IP. In case of any acute reaction following IP administration, additional blood samples will be drawn. For details see Section 6.7.
- xiii. Pre and post-BD spirometry to assess FEV₁ and its reversibility will be performed at Visit 2 only if eligibility criteria is not met at Visit 1.
- xiv. V3a and Visit 9 (EOT) Post-BD spirometry assessments will be performed as outlined in Appendix H. But it will stop at the end of Step 1 (i.e. using up to 4 inhalations of salbutamol).
- xv. AO, MBW and Sputum induction completed at Visit 2 will be training assessments to ensure the subjects are familiar with the procedures. They can be completed over more than one day, within a 3-working day window, with a suggested order of AO, MBW, Spirometry and Sputum induction if performed on the same day.
- xvi. V6 and EOT sputum assessment should only be performed if either V2 or V3 sputum sample is available for assessment.
- xvii. Depending on investigator's judgement, bronchoscopy along with biopsy and bronchial brushing can be performed up to 3 days before or on the day of randomization Visit 3c.
- xviii. IP will be administered after performing all the visit specific assessments.
- xix. EOT Bronchoscopy and CT scans will be performed only if baseline data were of good quality as confirmed by central analysing facility.

4.1 Enrolment and run-in period

4.1.1 Enrolment

Each potential subject who is at, or over the age of majority (as per local law) will provide informed consent prior to the start of any study specific procedures and undergo assessments applicable for this visit (see [Table 1](#)).

With the exception of documentation of informed consent which can be completed up to 30 days prior to Visit 1, all other Visit 1 procedures should be completed within a 1 to 3-working day window. The 3-day window is to enable subjects to return if necessary for the spirometry assessments on a morning when they have had their bronchodilator medications withheld in accordance with the instruction manual provided for the study. The registration of the subject's enrolment via IWRS/IVRS should occur on the day when the subject's Visit 1 procedures are performed.

Visit 1 assessments are primarily concerned with assessing the subject's eligibility (inclusion/exclusion) criteria, including their asthma disease state, the requisite level of severity based on maintenance medications and exacerbation history.

Spirometry will also be performed at Visit 1. Subjects must have a pre-BD FEV₁ value > 50% of their PNV and >1 L. Should this criterion not be met or cannot be performed at Visit 1, subjects must fulfil it at Visit 2 in order to proceed in the study. If not, the subject will be screen failed.

Subjects must have a post-BD reversibility in FEV₁ of $\geq 12\%$ and ≥ 200 mL at enrolment (Visit 1). If this criterion is not met at Visit 1, the criterion must be met at Visit 2, failing which the subject will be screen failed.

Other study assessments and procedures to be performed at enrolment Visit 1 are mentioned in [Table 1](#).

A record of physician-diagnosed asthma, ICS and other asthma controllers use is required source documentation. Current, regular use of an ICS at a stable dose for at least 1 month prior to enrolment (Visit 1) must be documented in the source data. This documentation may be in the form of a recent, active medication list as per a healthcare professional (HCP) note, or filled prescription.

After confirmation of initial entry criteria the subject will be supplied with an electronic handheld peak expiratory flow (ePEF) meter to monitor home lung function, and an electronic diary (eDiary) to record asthma symptoms and complete relevant questionnaires (see [Section 5.3.2](#) for further details).

4.1.2 Run-in period

All subjects meeting eligibility criteria at Visit 1 will enter into a 4-week run-in period. After 2 weeks subject will come back to the site for Visit 2. Subjects will continue on their current

ICS and other maintenance asthma controller medications (if applicable) with no changes during the run-in period.

Subjects who do not comply with the eDiary completion will be given a one-time option to reschedule a study visit within 72 hours. Compliance is defined as completing all required elements of eDiary for any 10 mornings and 10 evenings of the last 14 days before the visit. Subject must comply with eDiary compliance between Visit 1 and 2, before being randomized into the treatment period otherwise this will result in screen failure.

Assessments and procedures to be performed during the run-in period at Visit 2, 3a and 3b are as per [Table 1](#). Post-BD spirometry evaluation will be performed at Visit 2, only if required inclusion criteria were not met at Visit 1. Subject will be screen failed if the inclusion criteria are not met. Subject will also perform multiple breath washout (MBW), airwave oscillometry (AO) and sputum induction (SI) as training manoeuvres at Visit 2.

4.1.3 Re-screening

Subjects who experience an asthma exacerbation, have respiratory infections requiring antibiotics or antiviral medication during the run-in period should be screen failed. They may be re-screened no sooner than 30 days after their last dose of systemic agents (including steroids) administered to control the episode.

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), subjects may potentially be re-screened. These cases should be discussed with the AstraZeneca Study Physician and documented approval for re-screening should be filed in the investigator study file (ISF).

Any re-screened subject will be re-enrolled and reassigned their originally assigned enrolment number after signing a new informed consent form (ICF) and after all Visit 1 assessments have been performed as listed in [Table 1](#).

Re-enrolment is only allowed once for any subject, regardless of whether this is due to an exacerbation or infection. Subjects may be re-screened no later than 3 months after failing the initial enrolment. But the subject may not be re-screened if any other eligibility criteria have not been met.

4.2 Treatment period

The randomized treatment period begins at Visit 3c (Week 0) till Visit 8 (Week 10) with End of treatment (EOT) visit at Visit 9 (Week 12). Subject's eligibility to continue in the study will be once again confirmed before randomization during Visit 3c (see [Section 3.1](#) and [3.2](#)). Assessments and procedures to be performed during the treatment period are as per [Table 1](#).

Subjects confirmed to be eligible will be randomized in a 1:1 ratio to receive either tralokinumab 300 mg or placebo every two weeks throughout the treatment period. The first

dose of the IP will be administered at Visit 3c after the subject's randomization via IVRS/IWRS.

Following randomization the subject will receive the double-blind treatment for 12 weeks, with the last dose of tralokinumab /placebo administered at Visit 8 (Week 10).

Subjects will continue on their current ICS and other maintenance asthma controller medications (if applicable) with no changes during the entire treatment period.

4.3 Follow-up period

Post-treatment follow-up period to assess any ongoing safety issues and any potential prospective AEs for all subjects will be for 14 weeks after the EOT visit with a telephonic FU 1 visit (Visit 10, Week 16). There will be an on-site FU 2 visit (Visit 11, Week 26) only for WOCBP to also determine their pregnancy status. For all other subjects this will be a telephonic FU 2 visit. Assessments and procedures to be performed during the follow-up period are as per [Table 1](#).

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 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 eCRFs. A copy of the completed eCRFs will be archived at the study site upon study completion.

5.1 Efficacy assessments

5.1.1 Bronchoscopy biopsy and bronchial brushing

Bronchoscopy along with bronchial biopsy and brushing, will be performed in accordance with the schedule provided in [Table 1](#). Details for conducting bronchoscopy, bronchial biopsy and bronchial brushing will be described in a separate manual of procedures.

5.1.1.1 Bronchial biopsy

Bronchial biopsy will be performed in accordance with the schedule provided in [Table 1](#). Details for conducting bronchial biopsy will be described in a separate manual.

Samples will be analysed at a central facility designated by AstraZeneca. Details of assessment and analysis parameters will be described in a separate manual

5.1.1.2 Bronchial brushing

During bronchoscopy, bronchial brushing will be obtained. Details for sample collection, handling, processing, storage and transportation will be described in a separate manual.

Samples will be analysed at a central facility designated by AstraZeneca. Details of assessment and analysis of parameters including airway epithelium gene expression and transcriptomics will be described in a separate manual.

5.1.2 Sputum induction

The aim of sputum induction is to obtain satisfactory samples of sputum originating from the airways. Sputum induction will be performed in accordance with the schedule provided in [Table 1](#). Visit 2 assessment will be a training manoeuvre for the subjects. A detailed procedure for inducing sputum will be described in a separate manual. Attempts will be made to perform sputum induction as close to the baseline assessment time at each of the scheduled visits.

Detailed procedures for sample processing, storage and transportation will be described in a separate manual.

Details of assessment and analysis of parameters including inflammatory and epithelial cell counts, biomarkers which may include (but are not limited to) histamine, leukotrienes, IL-13, IL-5, IL-12, IL-31 and ECP will be described in a separate manual.

5.1.3 Nasosorption and nasal epithelial cells samples

The nasosorption procedure is a quick and simple technique for sampling nasal secretions. Samples of nasal epithelial cells will also be obtained for gene expression profiling and transcriptomics.

Nasosorption and nasal epithelial cells samples will be obtained in accordance with the schedule provided in [Table 1](#). Detailed procedures for obtaining, processing, storing, transporting and analysing the samples will be described in a separate manual.

5.1.4 Computed tomography

Chest CT will be performed in accordance with the schedule provided in [Table 1](#). Detailed procedures for the acquisition, storage, anonymization and transfer of the CT image data will be described in a separate manual.

Details for the analysis of the CT image data to obtain parameters for large airway morphometry, air trapping and functional imaging will be described in a separate imaging biomarker document.

The CT scans will be assessed clinically at each site, and the Principal Investigator is responsible for the clinical reporting of findings and its subsequent management. Clinically significant findings not related to asthma, will be reported as medical history or AEs by the investigator (also refer [Section 6.3.6](#)).

5.1.5 Multiple breath washout

Multiple breath washout (MBW) assesses ventilation distribution inhomogeneity during tidal breathing from functional residual capacity (FRC), by examining inert gas clearance over a series of breaths. MBW is included in this study as a specific small airway marker to evaluate treatment effect on small airway physiology.

Detailed procedure for performing MBW will be described in a separate manual. Subjects will be encouraged to breathe normally and comfortably through a pneumotachometer (PNT) via a mouthpiece. Each test will consist of two phases (wash-in and wash-out). Concentrations of gas in expired breath will be measured continuously using the gas analyser connected to the mouth piece. An acceptable trial should show a regular breathing pattern, without leaks from the mouthpiece, with complete wash-in and washout phases.

MBW procedures will be performed in accordance with the schedule provided in [Table 1](#). Visit 2 assessment will be a training manoeuvre for the subjects.

Number of parameters including S_{acin} (index of acinar ventilation heterogeneity) will be derived from the raw MBW data using custom software.

5.1.6 Airwave oscillometry

Airwave oscillometry (AO) is a non-invasive lung function test included in this study to evaluate treatment effect on small airway physiology.

A calibrated system will be used for measurements. Detailed procedures for performing, recording and analysing AO data will be described in a separate manual.

AO evaluation will be performed in accordance with the schedule provided in [Table 1](#). Assessment at Visit 2 will be a training manoeuvre for the subjects.

By measuring subject's airflow and response to sound waves, frequency-dependent resistance and reactance will be calculated by the AO system software. A signed and dated copy of the results printout from the equipment must be kept at the study site for SDV. The printout must be marked with the study code, subject enrolment code, date and time of measurement, and visit number. Number of parameters including (but not limited to) R5, R20 and AX for each trial will be recorded and analysed.

5.1.7 Post-BD Whole body plethysmography

Whole body plethysmography (WBP) to assess lung volumes will be performed post-BD in accordance with the schedule provided in [Table 1](#). Prior to assessment, subject will be asked to inhale 100 micrograms of salbutamol using a metered dose inhaler and a valved spacer device. This procedure will be repeated 4 times. WBP assessment should be performed within 15-60 minutes post bronchodilation.

Lung volume subdivisions which include total lung capacity (TLC), residual volume (RV), vital capacity (VC), inspiratory capacity (IC) and FRC will be performed at study sites by the investigator or qualified designee according to American Thoracic Society /European Respiratory Society (ATS/ERS) guidelines ([Wanger J et al 2005](#)) and in accordance with applicable SOP and clinical standards of care at investigational sites. The test will be performed by trained pulmonary function technicians with experience performing this assessment.

Each site will be allowed to use their own body boxes for this assessment. A signed and dated copy of the results printout from the equipment must be kept at the study site for SDV. The printout must be marked with the study code, subject enrolment code, date and time of measurement, and visit number.

5.1.8 Spirometry

5.1.8.1 General requirements

Pre and post BD pulmonary function will be measured by spirometry at the study site using equipment provided by a central vendor. For all the remaining assessments (such as sputum induction, airway hyper-responsiveness) involving spirometry, sites will use their own local equipment. Spirometry will be performed by the Investigator or authorized delegate according to ATS/ERS guidelines ([Miller MR et al 2005](#)).

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

Pre-BD spirometry testing must be performed according to the schedule provided in [Table 1](#). All post-randomization spirometry assessments should be performed within ± 1.5 hours of the time that the baseline Pre-BD spirometry (Visit 3a) was performed.

5.1.8.2 Spirometry technique

Detailed procedure for performing spirometry will be described in a separate instruction manual. A signed and dated copy of the pre- and post- BD spirogram printout must be kept at the study site for source data verification. The printout must be marked with the study code, subject enrolment code, date and time of measurement, and visit number.

Spirometry references

The Global Lung Function Initiative (GLI) equations will be used to determine the subjects PNV values and are pre-programmed into the spirometer ([Quanjer PH et al 2012](#)).

FEV₁, expressed as percent of the PNV, will be calculated as follows:

$$\text{FEV}_1\% \text{ of PNV} = (\text{FEV}_1 \text{ measured} / \text{FEV}_1 \text{ PNV}) \times 100$$

5.1.8.3 Assessments and analysis

FVC, FEV₁, forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75%}) is taken from the forced expiration with the largest sum of FEV₁ and FVC. The absolute measurement (for FEV₁), and the percentage of PNV ([Quanjer PH et al 2012](#)) will be recorded.

5.1.8.4 Post-BD spirometry and FEV₁ reversibility assessment

Post-BD spirometry procedures will be performed according to the schedule provided in [Table 1](#) and will be described in separate instruction manuals. Up to a maximum of 8 inhalations of salbutamol (100 µg metered dose) will be used as bronchodilator ([Sorkness RL et al 2008](#)). It is highly recommended to use a spacer device for this procedure. Nebulizer should not be used. The algorithm for reversibility assessment is outlined in [Appendix H](#). A lower total dose (e.g., 2 inhalations instead of 4 in the first round of puffs, and/or a total of less than 8 puffs), can be used if there is a concern about any effect on the subject's heart rate, tremor or safety; the reason should be noted in the subject's medical record. Post-BD spirometry to assess FEV₁ reversibility will be performed at Visit 2 only if eligibility criteria are not met at Visit 1. It is acceptable to stop the reversibility assessment procedure once the criteria for reversibility are met.

The highest pre- and post-BD FEV₁ will be used to determine reversibility.

Reversibility is calculated as follows:

$$\% \text{ Reversibility} = (\text{post-BD FEV}_1 - \text{pre-BD FEV}_1) \times 100 / \text{pre-BD FEV}_1$$

V3a and Visit 9 (EOT) Post-BD spirometry assessments will be performed as outlined in [Appendix H](#). But it will stop at the end of Step 1 (i.e. using up to 4 inhalations of salbutamol).

5.1.9 Airway hyper-responsiveness

Airway hyper-responsiveness (AHR) will be assessed, according to the schedule provided in [Table 1](#), by methacholine challenge test (MCT). MCT will be performed in accordance with the ATS Guideline ([ATS Guideline 1999](#)) and applicable SOP and clinical standards of care at investigational sites.

Signed and dated worksheets from the measurements will be kept in the ISF for source data verification (SDV). If a worksheet cannot be printed, required measurements will be recorded in the subject's medical records for SDV.

5.1.10 Home PEF testing

An electronic hand-held peak expiratory flow (ePEF) meter will be provided to the subject at Visit 1. ePEF meter will be paired with an eDiary which will record, store and send the readings via a cellular network.

Home PEF testing will be performed by the subject in the morning upon awakening (prior to taking their AM asthma controller) and in the evening at bedtime (prior to taking their PM asthma controller). For details see Section [7.7.1.1](#) and [Appendix G](#). Recording of home PEF should start from the evening of Visit 1 until the morning of Visit 9 (Week 12) using an ePEF meter device.

Subjects should perform 3 successive peak flow manoeuvres while sitting or standing, but in the same position at every testing; the highest of the 3 values will be captured for the morning and for the evening manoeuvres.

The Investigator/authorized delegate will check subject's adherence to correct use of the peak flow meter at each visit as shown in [Table 1](#).

5.1.11 Fractional exhaled nitric oxide

Fractional exhaled nitric oxide (FE_{NO}) measurements will be performed in accordance with the schedule provided in [Table 1](#).

Measurements will not be performed until 2 weeks after a respiratory infection. Subjects will be asked whether they have had a respiratory infection in the 2 weeks prior to measurement, which will be recorded as "Yes or No" in the eCRF. Measurement of (FE_{NO}) will be performed prior to the spirometry measurements.

FE_{NO} will be measured using an electrochemical sensor. Information concerning the specifications and use of the analyser will be provided in a separate instruction manual. The standard single exhalation technique recommended by the ATS will be followed (Dweik RA et al 2011).

Signed and dated printouts from the measurements will be kept in the ISF for SDV. Printouts will be marked with the study code, subject enrolment/ randomization code, date and time of measurement and visit number. If a printout cannot be printed, the mean value of the measurements will be recorded in the subject's medical records for SDV.

The vendor supplying the equipment to participating sites will be responsible for ensuring that the equipment and procedures for the measurement of FE_{NO} are validated prior to the start of the study.

5.1.12 Assessment of asthma exacerbation

Assessment of asthma exacerbation will be performed in accordance with the schedule provided in Table 1.

For the purpose of the study, an asthma exacerbation will be defined as a worsening of asthma that leads to any of the following:

- A temporary bolus/burst of systemic corticosteroids for at least 3 days to treat symptoms of asthma worsening; a single depo-injectable dose of corticosteroids will be considered equivalent to a 3-day bolus/burst of systemic corticosteroids
- An emergency room or urgent care visit (defined as evaluation and treatment for <24 hours in an emergency department or urgent care centre) due to asthma that required systemic corticosteroids (as per the above)
- An in-patient hospitalization (defined as admission to an inpatient facility and/or evaluation and treatment in a healthcare facility for ≥ 24 hours) due to asthma

Worsening of asthma is defined as new or increased symptoms and/or signs (examination or lung function) that can be either concerning to the subject (subject-driven) or related to an asthma daily diary alert (diary-driven). Baseline values for diary-driven alerts will be the mean of measures collected between Visits 1 and 2.

The eDiary will be programmed to alert both the subject and study centre when certain pre-specified worsening thresholds are crossed including:

- Decrease in morning peak flow $\geq 20\%$ on at least 2 consecutive days compared with baseline, and/or
- An increase in rescue medication use of 4 or more puffs, or use of one new or additional nebulized β_2 agonist on at least 2 consecutive days compared with the average use during baseline, and/or

- An increase of 2 nocturnal awakenings due to asthma over a 7 day period compared with the average during baseline, and/or
- An increase in total asthma symptom score (the sum of day time [evening assessment] and night time [morning assessment] of at least 2 units above the baseline average or the highest possible score (daily score of 6), on at least 2 consecutive days

If an exacerbation event is not associated with deterioration in at least 1 of the pre-specified objective measurements, the Investigator will have to justify the decision for defining the event as an exacerbation and record it in the eCRF. Events that are not supported by any objective assessment will be deemed not to be a protocol-defined exacerbation.

An asthma exacerbation that occurs ≤ 7 days of the last dose of systemic steroids, prescribed for a prior exacerbation, will be counted as the same exacerbation event.

Subjects who experience an exacerbation during the run-in period should be screen failed and may be considered for re-screening. Discontinuation due to an exacerbation after randomization (Visit 3c) is not mandatory and subjects may remain on the investigative product at PI's discretion. A stable period of 6 weeks is recommended between the last dose of systemic steroids for treating an asthma exacerbation and the EOT bronchoscopy. For such subjects, the timing of EOT bronchoscopy assessments should be discussed and agreed upon with AstraZeneca study physician.

Reasonable attempts should be made by the Investigator to bring the subject into the study centre for evaluation of a diary alert or subject initiated asthma worsening, particularly when it results in additional treatment being prescribed. Study centre evaluations for asthma worsening may occur as an unscheduled (UNS) visit or as part of an ordinary centre visit if the worsening happens to be coincident with a scheduled visit window. A copy of the medical record should be obtained for exacerbations evaluated and treated at non-study centres (e.g., by the primary care HCP or at an ED/hospital) and details entered into the exacerbation eCRF (EXACA module) in a timely fashion. Changes in concomitant medication due to an exacerbation must be recorded in the appropriate module of the eCRF.

5.1.13 Serum biomarkers

Blood (serum) samples will be collected pre-dose according to the schedule in [Table 1](#) to evaluate the pharmacology of tralokinumab, including but not limited to periostin, DPP4, Eotaxin, ECP, monocyte chemoattractant protein 1 (MCP1), thymus and activation regulated chemokine (TARC), IL-5, IL-33, signal transducer and activator of transcription 6 (STAT6), IL-13R α 2, MCP4/chemokine (C-C motif) ligand 13(CCL13), TARC/CCL17, calcium-activated chloride channel regulator 1 (CLCA1), serpin peptidase inhibitor, clade B (ovalbumin), member 2 (SERPINB2), biomarkers of tissue destruction, vascular adhesion molecules. Instructions for sample collection, processing, storage, shipment and analysis will be provided in a separate laboratory manual provided to the sites.

5.1.14 Whole blood eosinophils

Samples for assessment of blood eosinophils will be collected as haematology samples according to the schedule in [Table 1](#). Blood eosinophil count will be obtained from the total and differential white blood cell (WBC) counts. Instructions for sample collection, processing, storage, shipment and analysis will be provided in a separate laboratory manual provided to the sites.

5.1.15 Total IgE

Testing for total IgE will be performed as per schedule in [Table 1](#). The analysis for this test will be managed by the central laboratory. Instructions for sample collection, processing, storage, and shipment will be provided in a separate laboratory manual provided to the sites.

5.1.16 Phadiatop (Allergy screen)

Testing for phadiatop in blood (allergy screening test) will be performed as per schedule in [Table 1](#). The analysis for this test will be managed by the central laboratory. Instructions for sample collection, processing, storage, and shipment will be provided in a separate laboratory manual provided to the sites.

5.1.17 RNA Transcriptomics

Transcriptomic analysis will be performed on the ribonucleic acid (RNA) sample obtained from peripheral blood samples, nasal epithelial cells and from bronchial brushing as per schedule in [Table 1](#). All the samples will be analysed at a central facility. Instructions for sample collection, processing, storage, shipment and analyses will be provided in a separate manual of procedures provided to the sites.

The results from the analysis related to all exploratory objectives may be reported either in the CSR itself or as an addendum, or separately in a scientific report or publication as appropriate.

5.2 Safety assessments

5.2.1 Laboratory safety assessments

Blood and urine samples for determination of clinical chemistry, haematology, and urinalysis will be taken at visits in accordance with the schedule outlined in [Table 1](#). Fasting is not required before the collection of blood and urine samples. For dosing visits, all samples will be taken prior to the administration of IP.

The laboratory variables measured are outlined in [Table 2](#).

Table 2 Laboratory safety variables

Tests	Variables
Haematology/Haemostasis (whole blood)	Haemoglobin, Red blood cell (RBC) count, Leukocyte count, Leukocyte differential count (absolute count), Platelet count, Haematocrit, Mean corpuscular volume
Clinical chemistry (Serum)	Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Bilirubin (Total), Blood urea nitrogen, Calcium (Total), Carbon dioxide (CO ₂), Chloride, Creatinine, Creatinine kinase, C-Reactive Protein (CRP), Gamma-glutamyl transpeptidase (GGT), Glucose, Phosphorus, Potassium, Sodium, Total cholesterol, Uric acid
Urine (Dipstick)	Nitrite, Bilirubin, Glucose, Blood, Protein, Ketones
Urine ¹	Microscopy, Casts, Culture (as required)

1. Urine samples will be collected and sent for analysis at the central lab only when a positive dipstick result for any parameter is observed

Blood samples for determination of haematology/haemostasis and clinical chemistry will be performed at a central laboratory. For information on methods of collection, assessment, labelling, storage and shipment of samples please refer to the separate laboratory Manual.

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

NB. In case a subject shows an AST **or** ALT $\geq 3 \times \text{ULN}$ **and** total bilirubin $\geq 2 \times \text{ULN}$ please refer to [Appendix D](#) ‘Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy’s Law’, for further instructions.

The total volume of blood that will be collected from each subject during the study is presented in [Table 3](#).

Table 3 Volume of blood to be drawn from each subject

Assessment ³		Sample volume (mL)	No. of samples	Total volume ² (mL)
Safety	Clinical chemistry	2.5	2	5
	Haematology	2	4	8
FSH, β -HCG ¹		1	1	1
Serology		7.5	1	7.5
IgE		2.5	3	7.5

Assessment ³		Sample volume (mL)	No. of samples	Total volume ² (mL)
Phadiatop		2.5	1	2.5
Serum biomarkers		16	3	48
PK		3.5	2	7
ADA/nAb		3.5	2	7
			Total	93.5

1. Female subjects only
2. The number of samples may be changed due to additional sampling at unscheduled visits, and the blood volume required may be altered to fit the assay requirements. The total volume of blood drawn from each subject over the course of the study will not exceed 450 ml.
3. Timing of assessments as per [Table 1](#)

5.2.2 Pregnancy test

The following tests are applicable to female subjects only, and will be conducted in accordance with the schedule provided in [Table 1](#).

- Serum β -human chorionic gonadotropin (β -HCG) – the test done at enrolment (Visit 1) only, for WOCBP (analysed at central laboratory)
- FSH – the test done at enrolment (Visit 1) only, for female subjects to confirm postmenopausal status in women <50 years who have been amenorrhic for ≥ 12 months
- Urine HCG – the test will be performed at the study site for WOCBP in accordance with the schedule outlined in [Table 1](#) using a dipstick. Positive urine test result must be confirmed with serum β -HCG.

5.2.3 Physical examination

Physical examinations (complete and brief) will be performed in accordance with schedule provided in [Table 1](#).

Baseline data will be collected at Visit 1. 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](#).

5.2.3.1 Complete physical examination

The complete 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.

5.2.3.2 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 will be recorded.

5.2.4 ECG

All ECG assessments must be performed using site's electrocardiogram (ECG) device according to the clinical standards of care at the site. ECGs will be performed prior to blood drawing, spirometry and IP administration, in accordance with schedule provided in [Table 1](#).

For all subjects, the printouts of the ECG will be collected and signed, dated and stored at the study site along with a signed and dated photocopy of each printout (i.e., if the printout is not on archive-quality paper). Any new finding(s) judged as clinically significant by the Investigator, will be reported as an AE as described in [Section 6.1](#).

5.2.4.1 Resting 12-lead ECG

A 12-lead ECG will be taken in supine position, after the subject has been resting for at least 5 minutes. Heart rate, P and QRS durations, PR, QT and QTc intervals will be recorded from the standard lead of the ECG.

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 Investigator's interpretation and that provided by the ECG machine (if applicable), the Investigator's interpretation takes 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 be used for all ECG assessments throughout the subject's participation in the study.

5.2.5 Vital signs

Vital signs (i.e., pulse, blood pressure, respiration rate and body temperature) will be obtained in accordance with the schedule provided in [Table 1](#). Vital signs will be taken prior to blood drawing and IP administration. At Visits 3c through 6, subjects should be observed for a minimum of 2 hours after IP administration for the appearance of any acute drug reactions. For the remaining visits involving IP administration, subjects will be observed for a minimum of 1 hour after IP administration for any such reaction. 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](#).

5.2.5.1 Pulse, blood pressure and respiration rate

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 a seated position. Pulse rate will be obtained prior to blood pressure measurement if measured manually.

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

5.2.5.2 Body temperature

Body temperature will be measured in degrees Celsius prior to IP administration, in accordance with local standards.

5.2.6 Other safety assessments

5.2.6.1 Serology

Hepatitis B surface antigen, hepatitis C antibody, HIV-1 and HIV-2 antibodies will be assessed at enrolment (Visit 1) only. All testing for these will be performed at a central laboratory.

Instructions for sample collection, processing, storage, and shipment will be provided in a separate laboratory manual provided to the sites.

5.2.6.2 Infections

Subjects experiencing serious infections, defined as life-threatening infections, infections requiring hospitalization, or infections requiring treatment with antiviral medications, intravenous antibiotics or medications for helminth parasitic infections or that lead to a permanent discontinuation of study drug should be noted in the infection module in the eCRF.

5.3 Other assessments

5.3.1 Weight and height

Weight and height will be measured in accordance with the schedule provided in [Table 1](#). The subject's weight will be recorded in kilograms, and height will be recorded in centimetres. Weight and height measurements will be performed in light clothing and with shoes off.

5.3.2 Patient reported outcomes

Patient reported outcomes (PRO) data will be captured using an ePRO device (eDiary) and also by using paper based tool. Site personnel will be trained on using the eDiary. Detailed procedures for using eDiary and training the subjects will be described in a separate instruction manual. Subjects will be trained on at-home use of the eDiary and paired ePEF meter at Visit 1. Training will include explanation of device functionality and proper use of the ePEF meter. The subject will be asked to use both devices as part of the training and will be asked to verify completion of training on the eDiary. Subjects will be provided with information on device use and issue resolution (e.g., helpdesk numbers) to conclude the training. The site staff will set assessment reminder alarms on the device. At-home PRO assessment will start the evening of Visit 1. Subjects will be asked to bring the device back at each study visit. Additional details concerning the assessments can be found in the subsequent sections (see Sections [5.3.2.1](#) to [5.3.2.3](#)).

5.3.2.1 Asthma Daily Diary

The Asthma Daily Diary will be completed each day from the evening of Visit 1 to the morning of Visit 9. The Asthma Daily Diary will include the following daily recordings: morning and evening home PEF data (obtained from the ePEF meter), asthma symptoms, inhalations of rescue medication, nights with awakenings due to asthma symptoms, maintenance medication compliance. There will be triggers in the eDiary to alert the subjects to signs of worsening of asthma and to contact their physician, please refer to Section 5.1.12.

The subject should contact the investigator for evaluation after receiving a diary alert. The Investigator/authorized delegate will check subject's adherence to the Asthma Daily Diary at each visit as shown in Table 1.

Home PEF measurement

Details regarding home PEF measurement please refer to Section 5.1.10.

Asthma symptoms

Asthma symptoms during night-time and daytime will be recorded by the subject each morning and evening in the Asthma Daily Diary, beginning the evening of Visit 1 until the morning of Visit 9.

Daytime is defined as the time period between the morning lung function assessment (upon rising in the morning) and the evening lung function assessment. Night-time is defined as the time period between the evening lung function assessment (at bedtime) and the morning lung function assessment.

Rescue medication

The number of rescue medication inhalations (puffs) and nebulizer treatments taken will be recorded by the subject in the Asthma Daily Diary twice daily (i.e., in the morning and evening) beginning the evening of Visit 1 until the morning of Visit 9. The number of inhalations taken between the morning and evening lung function assessments will be recorded in the evening. The number of inhalations taken between the evening and the morning will be recorded in the morning.

Nocturnal awakenings

Nocturnal awakenings due to asthma symptoms will be recorded by the subject in the Asthma Daily Diary each morning, beginning in the morning after Visit 1 until the morning of Visit 9, by answering question whether he/she woke up during the night due to asthma symptoms by a "yes" or "no" response.

Maintenance medication

Maintenance medication compliance will be captured daily from the evening of Visit 1 until the morning of Visit 9. Maintenance medication will be recorded in the Asthma Daily Diary.

The maintenance medication question will ask about compliance with regularly scheduled asthma medications.

5.3.2.2 Asthma Control Questionnaire (ACQ-6)

The Asthma Control Questionnaire (ACQ)-6 is a shortened version of the ACQ that assesses asthma symptoms (night-time waking, symptoms on waking, activity limitation, shortness of breath, wheezing, and short-acting β_2 -agonist use) omitting the FEV₁ measurement from the original ACQ score.

Subjects will be asked to recall how their asthma has been during the previous week by responding to 1 question regarding their BD use, and 5 questions pertaining to their asthma symptoms. Subjects will be instructed to count each use of nebulizer as 2 puffs of inhalers.

Questions are weighted equally and scored from 0 (totally controlled) to 6 (severely uncontrolled). The mean ACQ-6 score is the mean of the responses. Mean scores of ≤ 0.75 indicate well-controlled asthma, scores between 0.75 and ≤ 1.5 indicate partly controlled asthma, and a score >1.5 indicates uncontrolled asthma (Juniper EF et al 2006). Individual changes of at least 0.5 are considered to be clinically meaningful.

ACQ-6 will be completed at the study site using eDiary in accordance with the schedule provided in [Table 1](#).

5.3.2.3 Sino-Nasal Outcome Test - 20

Sino-Nasal Outcome Test – 20 (SNOT-20) is a health-related and disease specific instrument for assessing quality of life for rhinosinusitis. This questionnaire contains 20 questions divided across five subgroups (nasal symptoms, paranasal symptoms, sleep-related symptoms, and social and emotional impairment). Subjects will rate their experiences during the previous 2 weeks and score each question on a six-point scale (0-no problem, 5-most serious problem) and in addition, they will mark which of the five items they consider to be the most important.

Total score will be calculated for each assessment. SNOT-20 will be completed by the subject at study site using a paper based tool in accordance with the schedule provided in [Table 1](#). Completed paper questionnaires will be kept in subject's chart for SDV and data from the questionnaire transcribed into the respective fields of eCRF.

5.4 Pharmacokinetics

5.4.1 Collection of samples

Blood samples for determination of tralokinumab in serum will be collected pre-dose at the times presented in [Table 1](#). It is important that date and time of each SC injection and sample collection be recorded for each subject.

Instructions for sample collection, labelling, processing, storage, and shipment will be provided in a separate laboratory manual provided to the sites.

The volume of blood that will be collected from each subject for these assessments is presented in [Table 3](#).

5.4.2 Determination of drug concentration

Samples for determination of tralokinumab concentration in serum will be analyzed by central laboratory on behalf of AstraZeneca, using a validated bioanalytical method. Details of the analytical method used will be described in a bioanalytical report. A summary of pharmacokinetics (PK) analysis results will be reported in the CSR; details of the PK analysis will be reported separately in a bioanalytical report.

5.4.3 Storage and destruction of pharmacokinetic samples

Pharmacokinetic samples will be retained for future use at AstraZeneca, or designee, for a maximum of 15 years following the date of Last Subject's Last Visit. They may be disposed of or destroyed and anonymized by pooling. Additional analyses may be conducted on the anonymized, pooled pharmacokinetic samples to further evaluate and validate the analytical method. Any results from such analyses may be reported separately from the CSR.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. The results from the evaluation will not be reported in the CSR but separately in a bioanalytical report.

Any residual back-up PK samples may be used for future exploratory biomarker research (in this case, residual back-up PK samples will be shipped to the AstraZeneca Biobank; see details in the laboratory manual and in [Section 5.7](#)).

5.5 Immunogenicity

Instructions for immunogenicity (ADA and nAb) sample collection, processing, storage, and shipment will be provided in a separate laboratory manual provided to the sites.

Samples used for immunogenicity analyses will be retained at AstraZeneca or designee for a maximum of 15 years following the Last Subject's Last Visit. A summary of the analyses will be presented in the CSR. Details of the analytical method used will be described in a bioanalytical report.

Anti-tralokinumab antibodies

The pre-dose serum samples to measure presence of ADA will be collected according to the schedule of study procedures as per [Table 1](#).

The presence or absence of ADA will be determined in the serum samples using a validated bioanalytical method. A tiered testing scheme will be employed, with the first step being screening. Samples found positive in the screening step will be further tested in the confirmatory step. Samples confirmed positive for ADA in the confirmatory step will undergo endpoint titer determination and will be analysed for the presence of nAb.

Neutralizing antibodies

Neutralizing antibodies will be assessed as per [Table 1](#) according to the tiered testing scheme outlined above, as well as at any discontinuation, as indicated. The presence or absence of nAb will be determined using a validated bioanalytical method. A summary of nAb incidence rate will be reported in the CSR and details of the nAb assessment will be reported separately in a bioanalytical report.

5.6 Pharmacogenetics

Not applicable.

5.7 Biomarker analysis in future

The subject's consent to the use of donated biological samples for future analysis purpose is mandatory.

Biological samples (e.g., sputum, nasal secretions, blood, plasma, serum, bronchial tissue) will be collected and may be analysed in future for additional exploratory biomarkers to assess correlations with disease activity, effects of study drug, clinical outcomes and toxicity.

5.7.1 Storage, re-use and destruction of biological samples

Samples will be stored for a maximum of 15 years from the date of the Last Subject's Last Visit, after which they will be destroyed. The results of this biomarker research will be reported either in the CSR 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.

5.7.2 Labelling and shipment of biological samples

The Principal Investigator ensures that biological 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 C](#) '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.

5.7.3 Chain of custody of biological samples

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

The Principal Investigator, or delegate at each site will keep full traceability of collected biological samples from the subjects while in storage at the site until shipment and/or disposal (where appropriate) and keeps documentation of receipt of arrival.

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

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

5.7.4 Withdrawal of consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples for future analysis purpose, 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. Subjects who withdraw their consent for the use of their donated samples for future exploratory use, will be allowed to continue in the study.

The Principal Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is documented in subject's source documentation and 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)/facility(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)/facility(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 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 is an AE occurring during any study period (i.e., run-in, treatment, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient 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

Adverse Events will be collected from the time the subject signs the informed consent form, throughout the treatment period and till end of follow-up period at FU 2 Visit.

6.3.2 Follow-up of unresolved adverse events

AEs that are unresolved at the time of telephonic FU 2 Visit (or on-site FU 2 visit for WOCBP) will be followed up by the Investigator for as long as medically indicated, but without further recording in the eCRF. If deemed necessary and depending on their judgement, Principal Investigator may even call the subject for an on-site visit for further evaluation. 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.

6.3.3 Variables

The following variables will be collected for each AE;

- AE (verbatim)
- The date when the AE started and stopped
- Maximum intensity of the AE
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- Outcome.

*Intensity rating scale:

- 1 mild (awareness of sign or symptom, but easily tolerated)
- 2 moderate (discomfort sufficient to cause interference with normal activities)
- 3 severe (incapacitating, with inability to perform normal activities)

In addition, the following variables (if applicable) will be collected in eCRF 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 unless it meets the criteria shown in Section 6.2. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE when it satisfies the criteria shown in Section 6.2.

6.3.4 Causality collection

The Investigator will assess causal relationship between investigational product 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 B](#) to the Clinical Study Protocol.

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 eCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

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 CSR. Deterioration as compared to baseline in protocol-mandated laboratory values, 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 investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (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 untoward findings or events encountered during or after the conduct of study procedures that are not related to the disease under investigation will be reported as an AE(s)/SAE(s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

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.

6.3.7 Hy's Law

Cases where a subject shows elevations in liver biochemistry may require further evaluation and occurrences of AST or ALT ≥ 3 xULN together with total bilirubin ≥ 2 xULN may need to be reported as SAEs. Please refer to [Appendix D](#) for further instruction on cases of increases in liver biochemistry and evaluation of Hy's Law.

6.3.8 Disease progression

Symptoms of the disease under study

Asthma symptoms or signs, such as, wheeze, cough, chest tightness, dyspnoea, breathlessness and phlegm, will be recorded as AEs when:

- the sign or symptom is serious according to definitions, see Section [6.2](#)
- the subject discontinues the study due to the sign or symptom, and/or
- the sign or symptom is new to the subject or not consistent with the subject's pre-existing asthma history (defined as within 1 year of Visit 1) as judged by the Investigator.

Asthma exacerbations should not be recorded as AEs after randomization, unless it fulfils any of the above criteria. All asthma exacerbations should be recorded in the exacerbation eCRF as per Section [5.1.12](#).

If a subject discontinues IP due to a study specific discontinuation criterion, this should always be recorded as 'Development of study specific withdrawal' on the termination form in the eCRF. In addition, the Investigator must assess whether the asthma deterioration should also be reported as an AE leading to discontinuation of IP (DAE)/AE leading to withdrawal from study on the AE form.

6.4 Reporting of serious adverse events

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

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

The designated AstraZeneca representative works with the Investigator to ensure that all the necessary information is provided to the AstraZeneca Patient 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 adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

Once the Investigators or other site personnel indicate an AE is serious in the WBDC system, an automated email alert is sent to the designated AstraZeneca representative(s). If the WBDC system is not available, then the Investigator or other study site personnel reports a SAE to the appropriate AstraZeneca representative(s) by telephone. The AstraZeneca representative(s) will advise the Investigator/study site personnel how to proceed.

The reference document for definition of expectedness/listedness is the Investigator's Brochure for the AstraZeneca IP.

6.5 Overdose

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

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

The designated AstraZeneca representative(s) 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 investigational product should be discontinued immediately.

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product 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 informs the appropriate AstraZeneca representative(s) within 1 day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative(s) will work with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 or 5 calendar days for SAEs (see Section 6.4) and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

The pregnancy module in the eCRF will be used to report the pregnancy and the pregnancy outcome module will be used to report the outcome of the pregnancy.

6.6.2 Paternal exposure

There is no restriction on fathering children or donating sperm during the study.

6.7 Management of IP related toxicities

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 when IP is being administered. Study site personnel must be trained to recognize and treat anaphylaxis (Lieberman P et al 2010). Details on anaphylaxis management are provided in Appendix F.

Anaphylaxis will be defined as serious reaction that is rapid in onset and may cause death (Sampson HA et al 2006). Anaphylaxis typically manifest 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.

In order to help understand the potential drug-relatedness of any acute reaction, a blood sample should be drawn during the event for anti-drug antibody/neutralizing anti-body (ADA/nAb) testing. If an anaphylactic reaction occurs, a blood sample will be drawn from the subject as soon as possible after the event, at 60 minutes \pm 30 minutes after the event, and at discharge for analysis of serum tryptase. The sample will be tested at the local lab or central laboratory where applicable.

6.8 Study governance and oversight

Not applicable.

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1 Identity of investigational product(s)

The IP will be manufactured in accordance with Good Manufacturing Practice (GMP).

Tralokinumab and placebo administered in the study will be a clear to opalescent, colorless to yellow solution free from, or practically free, from visible particles.

Subjects will be randomized in a 1:1 ratio to receive 300 mg tralokinumab or placebo every 2 weeks.

Each subject will receive two SC injections of 150 mg tralokinumab at each dosing interval to receive a total dose of 300 mg, or placebo. The identity details for the IP are found in [Table 4](#).

Table 4 Identity of investigational product

Investigational product	Concentration and Formulation	Dosage form and strength	Manufacturer
Tralokinumab	Formulated at a nominal concentration of 150 mg/mL in 50mM sodium acetate/acetic acid buffer, 85mM sodium chloride, 0.01% (w/v) polysorbate-80 pH 5.5 solution.	150 mg/mL solution for injection in an accessorized pre-filled syringe, 1.0 mL fill volume.	MedImmune
Placebo	Placebo contains the same excipients, in the same concentration only lacking tralokinumab	Placebo solution for injection in an accessorized pre-filled syringe, 1.0 mL fill volume.	MedImmune

The accessorized pre-filled syringe (APFS) is a single use, disposable system that is designed to administer the labelled dose of the system to the subcutaneous space during one injection and automatically provide a safety mechanism to reduce the occurrence of accidental needle sticks during disposal of the system.

The APFS consists of a pre-filled syringe sub-assembly (PFS-SA; 1 mL fill volume, pre-filled syringe barrel with a 27 gauge thin wall, 1/2 inch long staked in needle, rigid needle shield, plunger stopper) and a safety device.

7.2 Dose and treatment regimens

The IP will be administered at the study site on treatment visits and within visit windows as specified in [Table 1](#). IP will be administered after performing all other visit specific assessments.

IP administration

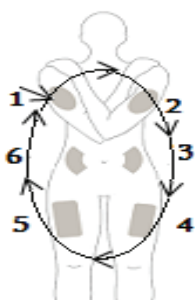
IP will be administered by a qualified, unblinded healthcare professional. The two injections should be administered within the same body location, separated by at least 3 cm. The injection site must be recorded in the source documents at each treatment visit and recorded in the eCRF.

IP **must** be equilibrated to room temperature for a minimum of 30 minutes prior to dose administration.

The person administering the dose will wipe the skin surface of the upper arm, anterior thigh or abdomen with alcohol, and allow to air dry. The skin will be pinched to isolate the SC tissue from the muscle. The needle will be inserted at a 90-degree angle approximately halfway into the SC tissue. The IP will be slowly injected (at least 5-second duration is recommended) into the SC tissue using gentle pressure. The area should not be massaged after injection.

It is advised that the site of injection of IP be rotated such that the subject receives IP at a different anatomical site at each treatment visit. The suggested injection site rotation sequence is presented below in [Figure 2](#).

Figure 2 Injection sites and rotation scheme



In cases when rotation of the injection site is not favorable for the subject and/or Investigator, the injection site, along with the reason why the site was changed, should be recorded in the source documents and eCRF for each such occurrence.

Further details on IP administration are provided in the IP Handling Instructions. IP administration must be carried out according to these instructions.

After IP administration

At Visits 3c through 6, subjects should be observed for a minimum of 2 hours after IP administration for the appearance of any acute drug reactions. For the remaining visits involving IP administration, subjects will be observed for a minimum of 1 hour after IP administration for any such reaction.

Conditions requiring IP administration rescheduling

If any of the following should occur, the Investigator should reschedule the visit and 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 (e.g., viral illnesses)
- The subject, in the opinion of the Investigator, is experiencing an acute or emerging asthma exacerbation
- The subject is febrile (defined as $\geq 38^{\circ}\text{C}$; $\geq 100.4^{\circ}\text{F}$) within 72 hours prior to IP administration

7.3 Labelling

Labelling of the IP will be carried out by AstraZeneca or designee in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines. The labels will fulfil GMP Annex 13 requirements for labelling. Label text will be translated into local language as applicable.

7.4 Storage

Tralokinumab/placebo is to be stored at the study site in a secured facility with limited access and controlled temperature. The temperature should be monitored on 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.

In the following cases:

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

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

7.5 Compliance

The date and time of all IP administrations, as well as any missed doses, should be recorded in the appropriate section of the eCRF.

7.6 Accountability

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

It is the Investigator's responsibility to ensure that a procedure is established and maintained for the operation of the unblinded study drugs, this includes but is not limited to:

- The study drugs are administered only by a qualified, unblinded health professional named in the delegation of responsibility log.
- An unblinded health professional will account for all study drugs dispensed to the subjects.

An unblinded health professional, if applicable, or the blinded AstraZeneca monitor will account for all study drugs received at the site, unused study drugs and for appropriate destruction. Certificates of delivery and destruction should be signed.

In case of malfunctioning APFS, the site should contact the unblinded AstraZeneca monitor to initiate a product complaint process according to applicable guidelines.

7.7 Concomitant and other treatments

Information about any treatment in the 3 months prior to the date of the informed consent and all the concomitant treatments given during the study, with reason for the treatment, will be collected by the Investigator/authorized delegate at each visit and recorded in the eCRF.

Maintenance of asthma controller medication

All subjects are required to be treated with a stable dose of ICS corresponding to ≥ 250 μg fluticasone propionate dry powder formulation equivalents (as outlined in [Appendix E](#)) total daily dose for at least 1 month prior to Visit 1 and during the treatment period. Subjects may also receive other physician prescribed asthma controller medications. The maintenance asthma controller therapy should be maintained at a stable dose from Visit 1 until the end of the treatment period.

Changes to the subject's maintenance asthma controller medication regimen are discouraged during the treatment period, unless judged medically necessary by the Investigator. Ideally, such changes should be discussed with the AstraZeneca Study Team Physician, prior to any change being made.

All changes in the subject's maintenance medication should be documented in source along with rationale for change and recorded in eCRF.

Maintenance medication is not regarded as an IP, but will be provided/reimbursed by AstraZeneca according to local regulations, in order to maintain appropriate oversight and access to this concomitant therapy.

As theophylline has narrow therapeutic window, please note that subjects on maintenance treatment with theophylline should have blood concentration levels within therapeutic range

documented before Visit 1. If this is not documented before signing the informed consent, it can be obtained after informed consent has been given or as part of the Visit 1 procedures. The sample can be analysed at the central or local lab as applicable. Investigator can use their clinical judgement about the therapeutic range of theophylline levels on the basis of sampling time and other factors that may impact the results.

Rescue medication

Salbutamol, albuterol, or levalbuterol will be used as rescue medication during the study in the event of a worsening of asthma symptoms. Rescue medication is not regarded as an IP, but will be provided/reimbursed by AstraZeneca according to local regulations, in order to ensure access to essential rescue therapy.

7.7.1 Restriction during and after the study

7.7.1.1 Asthma medication restrictions

Use of short-acting β_2 -agonists (SABA)

Regularly scheduled SABA use in the absence of any asthma symptoms is discouraged from enrolment (Visit 1) and throughout the study duration.

Prophylactic use of SABA (e.g., prior to planned exercise) if deemed necessary by the subject and the Investigator, may be used, but should **not** be recorded in the Asthma Daily Diary. Any such use should be documented in medical notes and recorded in the eCRF.

SABA via a metered dose device is permitted as needed for worsening asthma symptoms (i.e., rescue use) and will be recorded in the Asthma Daily Diary as number of inhalations.

Rescue use of SABA administered via jet or ultrasonic nebulization is discouraged, except as urgent treatment during an asthma exacerbation. Occasions where SABA was administered via nebulization will be recorded separately from metered dose inhaler inhalations in the Asthma Daily Diary.

Use of short acting anticholinergics

The use of short acting anticholinergics (e.g., ipratropium) as a rescue treatment for worsening asthma symptoms outside of managing an asthma exacerbation event is not allowed from enrolment and throughout the study duration.

Use of long-acting β_2 -agonists (LABA) as a reliever

The use of LABA as a reliever (e.g., Symbicort maintenance and reliever treatment) is not allowed from enrolment and throughout the study duration.

Use of theophylline and once daily bronchodilators

Use of theophylline and once daily BDs is allowed at the discretion of the Investigator. However use of these medications should be stabilized at least 1 month prior to the subject entering the study. Should the subject be taking the once daily BD or theophylline in the

evening, it is advised that the Investigator ask the subject to reschedule their BD or theophylline regimen to morning use, if there are no medical reasons to prevent this change.

Restrictions prior to home PEF testing

Subjects should avoid taking their morning asthma controller medication prior to the morning home PEF testing, and should conduct the evening home lung function testing before taking evening asthma controller medication.

7.7.1.2 Restrictions before study visits

Asthma medication restrictions on unscheduled visits

Asthma medication restrictions on unscheduled visits may not be feasible, and may be applied at the discretion of the Investigator. Timing of recent controller and reliever SABA use relative to the unscheduled pulmonary assessment should be noted in the record.

Asthma medication restrictions for scheduled visits

Subjects should be instructed not to take their usual asthma controller medication prior to scheduled study visits (Except on visits when bronchoscopy is scheduled). Subjects should be advised to bring their scheduled asthma medications along with them to study site and take them after the study visit assessments as per [Table 1](#) are complete. Following restrictions apply for scheduled visits:

- Use of SABA within 6 hours
- Use of twice daily LABA or ICS/LABA within 12 hours
- Use of once daily LABA within 24 hours
- Use of theophylline and once daily BD within 48 hours

If the above restrictions are not adhered to Investigator should reschedule the study visit. However, for Visits 4, 5, 7 and 8, Investigator can decide if the same restriction should apply.

7.7.1.3 Medication restrictions with study procedures

Details of permitted use and restrictions of medications before, during and after study procedures, if any are provided along with procedure details in a separate manual.

7.7.1.4 Other medication restrictions

Use of any off-label medications, for example medications locally approved for Chronic Obstructive Pulmonary Disease but not for asthma, are also not allowed from 30 days prior to Visit 1 and throughout the study.

Use of immunosuppressive medication including oral corticosteroids is not allowed.

Receipt of live attenuated vaccines within 30 days prior to enrolment (Visit 1) and during the study, including the follow up period, is not allowed. Inactive/killed vaccines (e.g., inactive influenza vaccine) are allowed provided they are not administered within 5 days before/after any dosing visit.

Subject should not receive any new allergen immunotherapy during the study. However, allergen immunotherapy initiated at least 30 days prior to Visit 1, may be continued provided there is no dose change and there is a span of at least 5 days between the immunotherapy and IP administration.

Subjects should not take any other excluded medications including Oral or ophthalmic non-selective β -adrenergic antagonist (e.g., propranolol).

A table with medication-related restrictions is presented in [Appendix G](#).

7.7.2 Other concomitant treatment

Subjects should not undergo bronchial thermoplasty during the study. Other medication other than that described above, which is considered necessary for the subject's safety and wellbeing, may be given at the discretion of the Investigator and recorded in the appropriate sections of the eCRF.

8. STATISTICAL ANALYSES BY ASTRAZENECA

8.1 Statistical considerations

A comprehensive Statistical Analysis Plan (SAP) will be prepared prior to database lock and unblinding of the data. Analyses will be performed by AstraZeneca or its representatives.

All personnel involved with the analysis of the study will remain blinded until database lock and protocol violators identified.

8.2 Sample size estimate

The study is powered to show a reduction in airway submucosal eosinophils, from baseline to week 12 for tralokinumab, versus placebo in the overall study population.

The sample size is based on the primary endpoint; change from baseline to Week 12 in airway submucosal eosinophils. Given the assumed deviation of the log values in the two treatment groups are 1.62 and 1.82, it is estimated that 31 subjects in each treatment arm will be sufficient to achieve at least 80% power to detect a 3.5-fold difference versus placebo using a two-sided test at 5% significance level. With these assumptions the smallest difference that will yield a significant result is a 2.4-fold difference. The assumptions are based on the change in number of eosinophils per mm² subepithelial tissue in bronchial biopsies in studies D5890C00003 (Budesonide/ formoterol) (Pavord ID et al 2009) and MI-CP166 (Laviolette M et al 2013). Though there are some differences between the design of these studies and MESOS, they are considered the most relevant available studies.

It is assumed that a non-neglectable proportion of the subjects will not have an evaluable primary endpoint value due to failed biopsies. To account for this, 40 subjects will be randomized in each treatment arm.

8.3 Definitions of analysis sets

All efficacy analyses will be performed using an ITT approach based on the full analysis set. It should however be noted that only subjects with complete data will be included in the primary analysis. For consistency, demographic and baseline characteristics will be presented using the full analysis set. Safety objectives will be analysed based on the Safety population.

All subjects analysis set (All subjects): This analysis set comprises all subjects screened for the study and will be used for the reporting of disposition and screening failures.

8.3.1 Efficacy analysis set

Full analysis set: All subjects randomized and receiving any investigational product will be included in the full analysis set, irrespective of their protocol adherence and continued participation in the study. Subjects will be assigned according to their randomized treatment, irrespective of whether or not they have prematurely discontinued. Subjects who withdraw consent to participate in the study will be included up to the date of their study termination.

All efficacy analyses will be based on the full analysis set and analysed according to the ITT principle.

8.3.2 Safety analysis set

Safety analysis set (Safety): All subjects who received any investigational product will be included in the safety analysis set. Subjects will be classified according to the treatment they actually received. A subject who has on one or several occasions received active treatment will be classified as active. All safety summaries will be based on this analysis set.

8.3.3 PK analysis set

Pharmacokinetic analysis set (PK): All subjects in the full analysis set who received Tralokinumab. PK blood samples are assumed not to be affected by factors such as protocol deviations (eg, disallowed medication, or incorrect study medication received). All PK summaries will be based on this analysis set.

8.3.4 PRO analysis set

PRO outcome variables will be evaluated based on the full analysis set.

8.4 Outcome measures for analyses

8.4.1 Definition of baseline and subject baseline analyses

The baseline for outcome variables based on efficacy biomarkers is defined as value recorded at the Visit 3 (a, b or c); if a measurement is not scheduled to be measured at Visit 3 or if the Visit 3 measurement is missing, the last non-missing value before Visit 3 will be used as baseline instead.

The baseline for ACQ-6 and SNOT-20 will be captured using an eDiary and paper based tool respectively at Visit 3c. Baseline for Asthma Daily Diary variables will be the mean for data collected between Visit 1 and 2. If more than 7 daily measures/scores (>50%) within a period is missing, then the bi-weekly mean for that period is set to 'missing'.

For laboratory data and physical examination, baseline will be defined as the latest non-missing assessment prior to first dose (Visit 3c).

Absolute change from baseline outcome variables is computed as (post-randomization value – baseline value).

Percent change from baseline is computed as $100 \times 1 \left(\frac{\text{post-randomization value} - \text{baseline value}}{\text{baseline value}} \right) \%$. If either the post-randomization value or the baseline value is missing, then the absolute or percent change from baseline value will also be set to missing.

The ratio post-randomization value / baseline value will also be computed when stated below.

8.4.2 Primary outcome measure

The change, expressed as a ratio, in number of airway submucosal eosinophils per mm² determined by microscopic evaluation of bronchoscopic biopsies from baseline (Visit 3c) to week 12 (Visit 9) i.e. (Visit 9/Visit 3c) will be compared between treatments.

An absolute change variable is considered as a supportive variable to the primary variable.

8.4.3 Secondary outcome measures

- The change, expressed as ratio, in blood eosinophil counts levels from baseline up to week 12.
- The change, expressed as a ratio, in number of differential sputum eosinophils from baseline up to week 12.
- The change, expressed as a ratio, in blood and sputum free eosinophils cationic protein (ECP) from baseline up to week 2.

Absolute change variables are considered as supportive variables as well as the change in sputum eosinophils as percentage number of inflammatory cells.

8.4.4 Exploratory outcome measures

8.4.4.1 Other biomarkers of airway inflammation

- Change from baseline to week 12 in number of eosinophils per mm² of epithelium and airway smooth muscle bundle
- Change from baseline to week 12 in inflammatory cell counts/mm² of epithelium, submucosa and airway smooth muscle bundle for:
 - CD3+, CD4+, CD8+ lymphocytes,
 - Neutrophils,
 - Macrophages,
 - Mast cells,
- Change from baseline to week 12 in induced sputum for:
 - Number of neutrophils, lymphocytes and macrophages per mL
 - Number of neutrophils, lymphocytes and macrophages as percentage number of inflammatory cells
 - Soluble biomarkers including (but not limited to) histamine, leukotrienes, IL-13, IL-5, IL-12, IL-31
- Change from baseline to week 12 in serum:
 - Biomarkers which may include (but are not limited to) periostin, DPP4, eotaxin, MCP1, TARC, IL-5, IL-33, STAT6, IL-13R α 2, MCP4/CCL13, TARC/CCL17, CLCA1, SERPINB2 and other standard biomarkers of tissue destruction, vascular adhesion molecules
- Change from baseline to week 12 in biomarkers from nasosorption samples
- Change from baseline to week 12 in blood total IgE
- Change from baseline to week 12 in exhaled nitric oxide

8.4.4.2 Effect on large airways remodelling

Change from baseline to week 12 in:

- Airway epithelial cell integrity,
- Lamina reticularis and reticular basement membrane thickening,
- Deposition of periostin in basement membrane
- Mucus glands, MUC5A,
- Biomarkers of tissue remodelling and/or destruction which may include but are not limited to α -SMA and collagen type IV, fibronectin, laminin tenascin and TGF β and epithelial damage markers which may include caspase 3 and CC16/KL6
- Airway epithelial gene expression
- Large airway dimensions and estimated airway resistance determined from CT

8.4.4.3 Effect on small airways obstruction

Change from baseline to week 12 in:

- R5-R20 and AX as evaluated by AO
- S_{acin} by using MBW
- Air trapping expressed as percentage of the lung with expiratory density less than -856 HU, and as expiratory-to-inspiratory ratio of mean lung density on CT
- Regional matching of the inspiratory/expiratory CT scans to assess air trapping/small airway obstruction
- Extent of gas trapping determined from physiologic testing by TLC/RV, VC/IC, FRC

8.4.4.4 Effect on asthma symptoms and other asthma control metrics

For asthma symptom score, rescue medication use and home PEF, biweekly means will be calculated. A biweekly mean is calculated as the sum of all non-missing daily measures/scores over 14 sequential days divided by the number of non-missing daily measures/scores. For nights with awakenings due to asthma, the bi-weekly mean will be the percentage of times the subject answered “yes” to ‘did your asthma cause you to wake up’ and “yes” to ‘did you use rescue medication upon awakening’. If more than 7 daily measures/scores (>50%) within a period is missing, then the bi-weekly mean for that period is set to ‘missing’.

Percent change from baseline will be calculated as described in Section 8.4.1.

Mean daily asthma symptom score

Asthma symptoms during night-time and daytime will be recorded by the subject each morning and evening in the Asthma Daily Diary. Symptoms will be recorded using a scale 0-3, where 0 indicates no asthma symptoms. Asthma symptom daytime score, night-time score, and total score will be calculated separately.

The total daily symptom score will be calculated by taking the sum of the daytime and night-time asthma symptom scores for each day. If a subject is missing a value for either daytime or

night-time asthma symptom score on a given day then the total score for that day will be set to missing.

The outcome variable is the biweekly mean daily asthma symptom total score. The daily asthma symptom total score is defined as the sum of the daytime and night-time symptom scores as captured in the Asthma Daily Diary. The biweekly mean is calculated as the mean daily asthma symptom total over 14 sequential days. If more than 7 daily scores (>50%) within that period is missing, then the daily asthma symptom mean score is set to 'missing'.

Rescue Use

The number of rescue medication inhalations and nebulizer treatments taken will be recorded by the subject in the Asthma Daily Diary twice daily.

The number of inhalations of rescue medication captured in the eDiary each day will be calculated per subject. If a subject is missing a value for either morning or evening rescue medication on a given day, then the total rescue medication use for that day will be set to missing.

Total rescue medication use, defined as the average number of inhalations (puffs) per day will be calculated as the outcome variable.

The number of inhalations (puffs) per day will be calculated as follows:

Number of night inhaler puffs + 2 x [number of night nebulizer times] + number of day inhaler puffs + 2 x [number of day nebulizer times].

Biweekly mean number of inhalations (puffs) per day will be calculated as the outcome variable.

Nights with awakening due to asthma

The total biweekly number of nights with awakening due to asthma that required rescue medication will be calculated as the outcome variable.

Home peak expiratory flow (morning and evening)

Biweekly mean absolute changes from baseline in morning and evening PEF will be calculated.

Asthma Control Questionnaire (ACQ-6)

In the ACQ-6 questionnaire the subjects are asked to recall the status of their asthma during the previous week with regards to symptom and BD. The questionnaire includes questions on

1. Awoken at night by symptoms
2. Limitation of normal daily activities
3. Waking in the morning with symptoms
4. Dyspnoea

5. Wheeze
6. Daily rescue medication

The questions of the ACQ-6 are measured on a 7-point scale scored from 0 (totally controlled) to 6 (severely uncontrolled). The main outcome variable for the ACQ-6 will be the ACQ-6 score, computed as the un-weighted mean of the responses.

Other variables based on ACQ-6 to report include:

- ACQ-6-responder (Yes=1/No=0)
 - Responder: Change from baseline ACQ-6 score ≤ -0.5
 - Non-responder : Change from baseline ACQ-6 score > -0.5
- ACQ-6-responder (improved/No Change / Deterioration)
 - Improvement: Change from baseline ACQ-6 score ≤ -0.5
 - No change: $-0.5 < \text{Change from baseline ACQ-6 score} < 0.5$
 - Deterioration: Change from baseline ACQ-6 score ≥ 0.5
- Subjects asthma control as measured by ACQ-6 score:
 - Well controlled : ACQ-6 score ≤ 0.75
 - Partly controlled : $0.75 < \text{ACQ-6 score} < 1.5$
 - Not well controlled : ACQ-6 score ≥ 1.5

8.4.4.5 Effect on rhinosinusitis symptom metrics

Using the SNOT-20 questionnaire , subjects are asked to recall their experiences during the previous 2 weeks and to score each of the 20 questions on a 6-point scale ranging from 0 (no problem) to 5 (problem as bad as it can get).

The total score is calculated for all the questions answered.

The main outcome variable for the SNOT-20 will be the change in total score from baseline to week 12. The change from baseline will be derived as post-randomization score minus baseline score, there will be no imputation for missing values.

8.4.4.6 Effect on lung function and bronchial hyper-responsiveness

- Change from baseline to week 12 in Pre and Post BD FEV₁,FVC, FEF25-75%
 - FEV₁, FVC and FEF25-75% variables will be calculated based on the pre BD and post BD measurements.
- Change from baseline to week 12 in AHR
 - Percent change and absolute change from baseline (supportive variable) will be calculated as described in Section 8.4.1.

8.4.4.7 Calculation or derivation of pharmacokinetics and immunogenicity variables

Blood samples (processed to serum) for pharmacokinetic and immunogenicity assessments will be collected from all subjects in accordance with schedule in Table 1. ADA assessments will be conducted utilizing a tiered approach (screen, confirm, titer). These validated methods

are conducted using a bridging assay format and statistically determined floating screening assay cut point factor and confirmatory assay cut point. The minimal sample dilution is 1:13. Titer values are reported as the reciprocal of the highest dilution that yields a value above the cut point. Samples that confirm positive for ADA will also be tested for nAb activity.

Pharmacokinetics and immunogenicity of tralokinumab

Tralokinumab serum concentrations will be tabulated by time along with descriptive statistics. Population PK modelling may also be performed to better characterize the PK of tralokinumab, but will be reported separately from the CSR.

ADA status (positive vs. negative) at each visit will be summarized by treatment group. Descriptive statistics including number of subjects, mean, standard deviation, median, and range of the actual ADA titers by treatment group and visit, where possible, will be provided. The association of ADA status across the study (positive vs. negative) with AEs/SAEs may be evaluated.

Neutralizing antibody evaluations will be conducted on those serum samples that test positive for ADA at end of treatment and also during the study follow up period. The test sample is deemed positive or negative for the presence of nAb to tralokinumab relative to a pre-determined (in assay validation), statistically derived cut point. Samples positive for nAb to tralokinumab are then titered to determine relative amounts of nAb present in each test sample.

8.4.5 Calculation or derivation of safety variable(s)

The following safety data will be collected: vital signs, physical examination, 12-lead ECG, haematology, clinical chemistry, urinalysis, and reported AEs.

Change from baseline to each post-baseline time point where scheduled assessments were made will be calculated for relevant measurements.

8.4.5.1 Adverse events

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

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

- AEs occurring during **run-in** (onset date \geq Visit 1 and before the first dose of study treatment)
- AEs occurring **during treatment** (onset date \geq the first day of study treatment and \leq the last day of study treatment + 2 weeks)
- AEs occurring during **follow-up**
 - (onset date $>$ the last day of study treatment + 2 weeks and \leq the last day of study treatment + 16 weeks)

- AEs occurring **post-treatment** (onset date > the last day of study treatment + 16 weeks; *this is only applicable for subjects that prematurely discontinued treatment*)

The timing of AEs will be assigned to the period in which they first occurred. If an AE has a missing onset date, then unless the stop date of the AE indicates otherwise, this will be considered an on treatment event. Similarly, if an AE has a partial onset date, then unless the partial onset date or the stop date indicates otherwise, this will be considered an on treatment AE.

8.4.5.2 Other significant adverse events

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs or discontinuations due to AEs.

Based on the expert's judgment, significant AEs of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered other significant AEs (OAEs) and reported as such in the CSR.

Examples of these are marked haematological and other laboratory abnormalities, and certain events that led to intervention (other than those already classified as serious), dose reduction, or significant additional treatment.

8.4.5.3 Laboratory variables

Blood and urine samples for determination of clinical chemistry, haematology and urinalysis parameters will be taken at the times detailed in the CSP, and will be assessed in a central laboratory. The parameters outlined in [Table 2](#) in Section 5.2.1, will be collected. Laboratory data will be reported in SI units.

Changes in haematology and clinical chemistry variables between baseline and each subsequent on treatment assessment will be calculated as described in Section 8.4.1. There will be no imputation for missing values.

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

Urinalysis data will be categorised as negative (0), positive (+), or strongly positive (++, +++, or >++) at each time-point.

For the purposes of haematology, clinical chemistry and urinalysis shift tables, baseline will be defined as the latest non-missing assessment prior to first dose, and on-treatment will be defined as the latest non-missing assessment whilst the subject is ongoing on treatment.

For the liver function tests: AST, ALT, ALP, GGT and total bilirubin, the multiple of the AstraZeneca ULN (not extended) range will be calculated for each data point.

$$\text{Multiple} = \text{Value} / \text{ULN}$$

i.e., if the ALT value was 72 IU/L (ULN 36) then the multiple would be 2.

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

- $\text{AST} \geq 3 \times \text{ULN}$
- $\text{ALT} \geq 3 \times \text{ULN}$
- $\text{TBL} \geq 2 \times \text{ULN}$

8.4.5.4 ECGs

Twelve-lead ECG measurements will be recorded in accordance with the protocol, with the baseline visit being defined as Visit 1.

The outcome of the overall evaluation is to be recorded as normal/abnormal in the eCRF, with any abnormalities being recorded as not clinically significant or clinically significant.

8.4.5.5 Physical Examination

Complete and brief physical examinations will be performed at time points specified in [Table 1](#). What is included in the assessment will be dependent on whether the examination is complete or brief, as described in [Section 5.2.3](#). For the brief physical examination, only information on whether the assessment was performed or not will be recorded.

Each component of the baseline visit (i.e., Visit 3c) complete physical examination will be recorded as normal or abnormal. Each component of the follow-up physical examinations will be recorded as normal, same as baseline, or new/aggravated.

Any new finding(s), or aggravated existing finding(s), judged as clinically significant by the Investigator, will be reported as an AE.

8.4.5.6 Vital signs

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

Changes in vital sign variables between baseline and each subsequent scheduled assessment will be calculated as described in [8.4.1](#). There will be no imputation for missing values.

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

Body mass index (BMI) will be calculated from the height (in meters) and weight (in kilograms) as $\text{BMI} = \text{kg}/\text{m}^2$.

8.5 Methods for statistical analyses

The main focus for the statistical analyses is to compare tralokinumab to placebo with regards to primary key secondary, and safety objectives.

The analysis of the study endpoints will include all data captured during the 12-week double-blind treatment period. This includes data regardless of whether study treatment was prematurely discontinued or delayed, and/or irrespective of protocol adherence, unless the subject withdraws consent to study participation.

The statistical analyses will compare tralokinumab to placebo. Summary data will be presented in tabular format by treatment. Categorical data will be summarized by the number and percentage of subjects in each category. Continuous variables for parametric data will be summarized by descriptive statistics including N, mean, standard deviation (SD), geometric mean, SD of log values, median, and range. All data will be listed and data listings will be sorted by treatment and subject number.

The primary and secondary variables will be analysed using geometric means. Geometric mean is often used to evaluate data covering several orders of magnitude, and for evaluating ratios, percentages, or other data sets bounded by zero. A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if an arithmetic mean was calculated. The geometric mean is also a log-transformation of data to enable meaningful statistical evaluations; it accounts for some of the expected skewness in the data, but with the possibility to use a parametric analysis with a p-value directly linked to the confidence interval for the treatment effect. Both the difference in number of cells and the %change or ratio will be reported, but the statistical analysis will only be based on the ratio. Nonparametric analyses will be applied as sensitivity analyses.

All hypothesis testing will be reported using 2-sided tests. Nominal p-values will be reported for primary and secondary variables i.e., no adjustment of multiplicity will be performed. P-values will be rounded to 3 decimal places.

8.5.1 Analysis of the primary variable (s)

The primary efficacy objective will be evaluated through statistical testing of the within subject change from baseline to week 12 in airway submucosal eosinophils. The null hypothesis test H₀ will be: The ratio tralokinumab/placebo equals 1 and will be tested vs. H₁: The ratio is not equal to 1.

The primary variable of within subject change from baseline to Week 12 in airway submucosal eosinophils will be analysed using an analysis of covariance (ANCOVA) including at least baseline value and treatment as covariates. The analysis will be performed by using log-transformed data and estimated geometric means and the ratio of geometric means with 95% confidence intervals will be presented.

If the change from baseline for a subject is zero, the value will be replaced by half the smallest observed value among the subjects with non-zero values, before doing the logarithmic transformation.

Available values at both baseline and week 12 are required for a subject to be included in the analysis and it is unlikely that a subject with a missing baseline value will undergo a second biopsy.

8.5.2 Analysis of the secondary variable(s)

The secondary variables of within subject change from baseline up to Week 12 in blood and sputum eosinophils and blood and sputum free eosinophil cationic protein (ECP) will be analysed using a mixed model for repeated measures (MMRM) including at least treatment as covariate. The analysis will be performed by using log-transformed data.

8.5.3 Subgroup analysis

Potential sub-group analyses are currently under discussion and will then be conducted for the primary, secondary as well as selected CT and lung function parameters.

8.5.4 Interim analysis

Not applicable.

8.5.5 Sensitivity analysis

For the primary and secondary variables, non-parametric analysis methods will be applied as sensitivity analyses.

It is likely that some biopsy specimens will not be evaluable. Also, due to the invasive procedures performed, it is likely that some subjects will withdraw from the study. We cannot rule out that there is a relationship between unobserved values and the likelihood of data being missing and therefore the effect of missing data will be assessed using multiple imputations and a range of assumptions for the missing data.

Full details of the sensitivity analyses will be pre-specified in SAP and documented prior to database lock of the studies.

8.5.6 Exploratory analysis

Exploratory variables will be summarized using descriptive statistics and graphical displays. How changes in biomarkers correlate with changes in airway inflammation and remodelling will be explored graphically.

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, the WBDC and IVR/IWR systems, ePRO and ePEF devices, spirometers, and any other system(s) that may be utilised in the study.

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 ensure that a record of all individuals involved in the study (medical, nursing and other staff) is maintained.

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 eCRFs, that biological samples and CT image data are handled in accordance with the instruction manuals and that study drug accountability checks are being performed
- Perform source data verification (a comparison of the data in the eCRFs 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(s) will be available between visits if the Investigator(s) or other staff at the site 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 site should comply with all the terms, conditions, and obligations of the CSA, or equivalent, for this study. In the event of any inconsistency between this Clinical Study Protocol and the CSA, the terms of Clinical Study Protocol 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 CSA shall prevail.

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

9.2.3 Archiving of study documents

The Investigator should follow the principles and terms outlined in the CSA pertaining to the archival of study documents.

9.3 Study timetable and end of study

The end of the study is defined as ‘the last visit of the last subject undergoing the study’.

The study is expected to start in Q2, 2015 and to end by Q4, 2017.

The study may be terminated at individual sites if the study procedures are not being performed according to Good Clinical Practice (GCP), or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with tralokinumab.

9.4 Data management by AstraZeneca

Data management will be performed by the AstraZeneca Data Management Centre according to the Data Management Plan (DMP). Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. The DMP will also clarify the roles and responsibilities of the various functions and personnel involved in the data management process.

Data will be entered in the WBDC system at the study site.

Site personnel will be trained on use of the WBDC system and will be responsible for entering data on the observations, tests and assessments specified in the protocol into the WBDC system. eCRF instructions will be provided to the personnel at the study site as guidance for performing data entry. Data entered in the WBDC system will be immediately saved to a central database and all changes will be tracked in the system’s audit trail. All data will be Source Data Verified (SDV) by an AstraZeneca site monitor (or representative), reviewed /queried and updated as needed.

Data queries will be raised for inconsistent, impossible, or missing data, and must be resolved in a timely manner. Clean file occurs when all data have been declared clean and signed off by

all Investigators. Data will be frozen and then locked to prevent further data entry/editing. Any treatment revealing data may thereafter be added and the final database will be locked. A copy of the eCRFs will be provided to and archived at the study site when the study has been locked.

Data collected through third party sources will be obtained and reconciled against study data.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version of 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.

Serious Adverse Event (SAE) Reconciliation

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

Data associated with human biological samples

Data associated with biological samples will be transferred from laboratory(ies) internal or external to AstraZeneca.

Management of external data

Data Management will determine the format of the data to be received from external vendors and coordinates the flow of data to an external environment or clinical database (as applicable).

Data Management will ensure that the data collection tool (e.g., eDiary, IVRS/IWRS, etc.) will be tested/validated as necessary. External data reconciliation will be done with the clinical database as applicable.

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 International Conference on Harmonisation (ICH)/GCP, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

10.2 Subject data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

10.3 Ethics and regulatory review

An Ethics Committee (EC)/Institutional Review Board (IRB) should approve the final study protocol, including the final version of the Informed Consent Form (ICF) and any other written information and/or materials to be provided to the subjects. The Investigator will ensure the distribution of these documents to the applicable EC/IRB, and to the study site staff.

The opinion of the EC/IRB should be given in writing. The Investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study.

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

AstraZeneca should approve any modifications to the ICF that are needed to meet local requirements.

If required by local regulations, the protocol should be re-approved by the EC/IRB annually.

Before enrolment of any subject into the study, the final study protocol, including the final version of the ICF, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide Regulatory Authorities, ECs/IRBs and Principal Investigators with safety updates/reports according to local requirements.

Each Principal Investigator is responsible for providing the EC/IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Principal Investigator so that he/she can meet these reporting requirements.

10.4 Informed consent

The Principal Investigator(s) at each site will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- 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 ICFs are stored in the Investigator's Study File
- Ensure a copy of the signed ICF 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 EC/IRB.

10.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the International co-ordinating Investigator, and AstraZeneca.

If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Revised Clinical Study Protocol).

The amendment is to be approved by the relevant EC/IRB and if applicable, also the national regulatory authority approval, before implementation. Local requirements are to be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to each Principal Investigator(s). For distribution to EC/IRB, see Section [10.3](#).

If a protocol amendment requires a change to a site's ICF, AstraZeneca and the site's EC/IRB are to approve the revised ICF before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by each EC/IRB.

10.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an EC/IRB may perform audits or inspections at the site, 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, GCP, guidelines of the ICH, and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the site.

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Clinical Study Protocol Appendix B

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Appendix B
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

Outpatient 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.



Clinical Study Protocol Appendix C

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Appendix C
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 (http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm). 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 (http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)
- **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.



Clinical Study Protocol Appendix D

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

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1. 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 patient 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.

2. DEFINITIONS

Potential Hy's Law (PHL)

Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) $\geq 3x$ Upper Limit of Normal (ULN) together with Total Bilirubin (TBL) $\geq 2xULN$ 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 $\geq 3x$ ULN **together with** TBL $\geq 2x$ ULN, where no other reason, other than the IMP, can be found to explain the combination of increases, e.g., 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.

3. IDENTIFICATION OF POTENTIAL HY'S LAW CASES

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

- ALT $\geq 3x$ ULN
- AST $\geq 3x$ ULN
- TBL $\geq 2x$ ULN

When a patient 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 patient meets PHL criteria (see Section 2 of 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 patient meets PHL criteria (see Section 2 of this Appendix for definition) by reviewing laboratory reports from all previous visits
- Promptly enter the laboratory data into the laboratory CRF

4. FOLLOW-UP

4.1 Potential Hy's Law Criteria not met

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

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

4.2 Potential Hy's Law Criteria met

If the patient does meet PHL criteria the Investigator will:

- 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 patients' follow-up and the continuous review of data. Subsequent to this contact the Investigator will:

- Monitor the patient 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. This includes deciding which of the tests available in the Hy's law lab kit should be used
- 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

5. REVIEW AND ASSESSMENT OF POTENTIAL HY'S LAW CASES

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

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

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6. 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>



Clinical Study Protocol Appendix E

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Appendix E
Maintenance Therapy Equivalence Table

Clinical Study Protocol Appendix E

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Appendix E
Maintenance Therapy Equivalence Table

Estimated daily doses for inhaled corticosteroids.

Inhaled Corticosteroid	Total Daily Dose (µg/day)	
	Low / Medium¹	High
Beclomethasone dipropionate	500 - 1000	>1000 - 2000
Beclomethasone HFA	240 - 480	>480
Beclomethasone dipropionate (Fostair)	200 - 400	>400 - 800
Ciclesonide	160 - 320	>320 - 1280
Triamcinolone acetonide	1000 - 2000	>2000
Flunisolide	1000 - 2000	>2000
Fluticasone furoate	100	200
Fluticasone propionate	250 - 500	>500 - 1000
Fluticasone propionate HFA	364 - 440	>440
Budesonide	400 - 800	>800 - 1600
Budesonide, if as delivered dose (e.g. Symbicort)	320 - 640	>640 - 1280
Mometasone furoate	200 - 400	>=800

¹ The acceptable ICS dose for this study is equal to or above the bolded dose.



Clinical Study Protocol Appendix F

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Appendix F
Anaphylaxis: definition criteria, signs and symptoms, and 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 (e.g., IgG and immune complex mediated) and nonimmunologic (Johansson SGO 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 \pm 30 minutes after the event, and at discharge for analysis of serum tryptase.

2. CLINICAL CRITERIA FOR DEFINING ANAPHYLAXIS AND IMMUNE COMPLEX DISEASE

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 (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

AND AT LEAST ONE OF THE FOLLOWING:
 - (a) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
 - (b) Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (e.g., 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 (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)
 - (b) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)

- (c) Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)
 - (d) Persistent gastrointestinal symptoms (e.g., 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.

Immune Complex Disease

Immune complex disease or Hypersensitivity Type III is evoked by the deposition of antigen-antibody or antigen-antibody-complement complexes on cell surfaces, with subsequent involvement of breakdown products of complement, platelets, and polymorphonuclear leukocytes, and development of vasculitis; serum sickness and nephritis is common.

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 β_2 agonist [e.g., albuterol (salbutamol)] for bronchospasm resistant to epinephrine

- (d) Consider systemic corticosteroids
- (e) Consider vasopressor (e.g. dopamine)
- (f) Consider glucagon for patient taking β -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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Clinical Study Protocol Appendix G

Drug Substance	Tralokinumab (CAT-354)
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Appendix G
Restricted and prohibited medications

PROHIBITED AND RESTRICTED MEDICATIONS

Asthma medication restrictions

Medication	Prohibited/restricted	Details
Maintenance treatment with long-acting bronchodilators (including ICS/LABA combinations)	Restricted	<p>Changes in dose and regimen should not be done from enrolment and throughout the study treatment (unless there is a medical need as judged by the Investigator)</p> <p>Usual ICS/LABA should not be taken prior to scheduled study visits defined in the protocol and home lung function assessments (to be administered once assessments are completed)</p> <p>Subjects should be instructed not to use their twice daily LABA within 12 hours of the scheduled site visit. For once daily LABA a 24 hour washout period is required</p> <p>Subjects will not need to washout of their asthma medications for unscheduled visits due to asthma worsening</p>

<p>Short acting β2-agonists (SABA)</p>	<p>Restricted</p>	<p>Regular scheduled use not allowed from enrolment through the study duration</p> <p>Rescue use of SABA administered via nebulization is discouraged, except as urgent treatment during an asthma exacerbation</p> <p>SABA should not be used within 6 hours prior to scheduled study visits defined in the protocol and home lung function assessments with the exception of any unscheduled visits due to asthma worsening and home lung function assessments</p>
<p>Additional Maintenance Controllers</p>	<p>Allowed with restriction</p>	<p>Stable dose for 1 month prior to Visit 1; stable dose during the treatment period</p> <p>Subjects on theophylline should have blood concentration levels within therapeutic range documented before Visit 1</p> <p>Subjects should be instructed not to use additional once daily bronchodilators within 48 hours of the scheduled study visits defined in the protocol with the exception of any unscheduled visits due to asthma worsening</p>
<p>Short acting anticholinergics (e.g. ipratropium)</p>	<p>Restricted</p>	<p>Not allowed from enrollment and throughout the study as a rescue treatment for worsening asthma symptoms outside of managing an asthma exacerbation event</p> <p>May be used for managing an asthma exacerbation event</p>

Long-acting β -agonists as a reliever (e.g. Symbicort Maintenance and Reliever Treatment)	Prohibited	Not allowed from enrolment and throughout the study duration
Zileuton	Prohibited	Not allowed 30 days prior to Visit 1; during treatment period

Other medication restrictions

Medication	Prohibited/restricted	Details
Live Attenuated Vaccines	Prohibited	Not allowed 30 days prior to Visit 1; during treatment period and 4 months (5 half-lives) after the last dose of the investigational product
Inactive/killed vaccinations (e.g. inactive influenza)	Restricted	Allowed provided they are not administered within 5 days before or after any study visit
Any immunomodulators or immunosuppressives (including long-acting depo corticosteroids)	Prohibited	Not allowed 3 Months or 5 Half Lives (whichever is longer) prior to Visit 1; during treatment period; 3 Months or 5 Half Lives (whichever is longer) after the last dose of the investigational product
Blood products or immunoglobulin therapy	Prohibited	Not allowed 30 days prior to Visit 1; during treatment period
Any marketed (eg omalizumab) or investigational biologic treatment	Prohibited	Not allowed 4 months or 5 half-lives (whichever is longer) prior to Visit 1; during treatment period; 4 months or 5 half-lives (whichever is longer) after the last dose of the investigational product
Other investigational products (including investigational use of an	Prohibited	Not allowed 30 Days or 5 Half Lives (whichever is longer) prior to Visit 1; during treatment period

Medication	Prohibited/restricted	Details
approved drug)		
Allergen Immunotherapy	Restricted	Allowed if on stable therapy for at least 30 days prior to Visit 1; no anticipated changed during treatment period and provided there is a span of at least 5 days between the immunotherapy and IP administration
Herbal remedies for the treatment of allergic, inflammatory, or respiratory diseases	Prohibited	Not allowed 30 days prior to Visit 1; during treatment period
Roflumilast	Prohibited	Not allowed 30 days prior to Visit 1; during treatment period
Oral or ophthalmic non-selective β -adrenergic antagonist (e.g. propranolol)	Prohibited	Patients currently using any oral or ophthalmic non-selective β -adrenergic antagonist at the time of enrolment are not eligible for the study Not allowed during treatment period
Medications not currently licensed for use in the treatment of asthma, for example medications approved for Chronic Obstructive Pulmonary Disease and not part of current standard of care	Prohibited	Not allowed 30 days prior to Visit 1; Not allowed throughout the duration of the study



Clinical Study Protocol Appendix H

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Appendix H
Post-BD spirometry and FEV₁ reversibility assessment algorithm

POST-BD SPIROMETRY AND FEV₁ REVERSIBILITY ASSESSMENT ALGORITHM

1. Verify with the subject that the medication restrictions for performing spirometry assessment have been met. For details please see sections 5.1.8 and 7.7.1.
2. Complete the initial (pre-BD) FEV₁ measurement (refer section 5.1.8).

The standard metered dose for each inhalation of bronchodilator in the reversibility assessment is salbutamol 100µg.

Step 1: FEV₁ measurement after 4 bronchodilator inhalations

For administration of salbutamol, the subject will:

- i. Perform a gentle, complete expiration
- ii. Inhale Dose 1 of bronchodilator to total lung capacity (TLC) and hold their breath for 5-10 seconds before the subject exhales
- iii. Rest for approximately 30 seconds before the next dose
- iv. Repeat this for Dose 2, Dose 3 and Dose 4, resting for a further 15 – 20 minutes after Dose 4

After resting for 15 – 20 minutes FEV₁ is measured following the technique described in section 5.1.8.

If the inclusion criterion for reversibility is met ($\geq 12\%$ and ≥ 200 mL) this is the end of the reversibility assessment. If it is not met the subject proceeds to Step 2.

Step 2: FEV₁ measurement following a further 2 (total of 6) bronchodilator inhalations

The subject will repeat the inhalation procedures for Dose 5 and Dose 6, resting for 15 – 20 minutes after Dose 6. (See Step 1 above for instructions).

After resting for the 15 – 20 minutes FEV₁ will be measured following the technique described in Section 5.1.8.

This is the end of the reversibility assessment if either of the following occur:

- the inclusion criterion for reversibility is met ($\geq 12\%$ and ≥ 200 mL) or
- the incremental change in FEV₁ between Step 1 and 2 is $\leq 5\%$ and the inclusion criterion is not met

Subjects proceed to Step 3 if the incremental change in FEV₁ between Step 1 and Step 2 is $>5\%$ but the criterion for reversibility has not yet been met.

Step 3: FEV₁ measurement following a further 2 (total of 8) bronchodilator inhalations

The subject will repeat the inhalation procedures for Dose 7 and Dose 8, resting for 15 – 20 minutes after Dose 8. (See Step 1 above for instructions).

After resting for the 15 – 20 minutes FEV₁ will be measured following the technique described in Section 5.1.8.

The reversibility assessment is now complete.

V3a and V9 (EOT) Post-BD spirometry assessment will stop at the end of Step 1.