AstraZeneca

A 52-Week, Multicentre, Randomized, Double-blind, Parallel Group, Placebo Controlled, Phase 3 Study to Evaluate the Efficacy and Safety of Tralokinumab in Adults and Adolescents with Asthma Inadequately Controlled on Inhaled Corticosteroid Plus Long-acting β₂- Agonist (STRATOS 1)

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A & R Statistician

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Global Product Statistician

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LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
ACQ-6	Asthma Control Questionnaire 6
ADA	Anti-Drug Antibodies
AE	Adverse Event
AAER	Annual Asthma Exacerbation Rate
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AQLQ(S)+12	Standardised Asthma Quality of Life Questionnaire for 12 Years and Older
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
ATS/ERS	American Thoracic Society/European Respiratory Society
BD	Bronchodilator
CGIC	Clinical Global Impression of Change
CSR	Clinical Study Report
CTD	Common Technical Document
DAE	Discontinuation of Investigational Product due to Adverse Event
dECG	Digital Electrocardiogram
DL	Direct Likelihood
DNA	Deoxyribonucleic Acid
DPP-4	Dipeptidyl Peptidase-4
DRMI	Dropout Reason-Based Multiple Imputation
eCRF	Electronic Case Report Form
ER	Emergency Room
EOS	End of Study
EOT	End of Treatment
ePRO	Electronic Patient Reported Outcome device
EQ-5D-5L	European Quality of Life - 5 Dimensions - 5 Levels
$FEF_{25-75\%}$	Forced Expiratory Flow at 25-75% of the forced vital capacity
FE_{NO}	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second

Abbreviation or special term	Explanation
FVC	Forced Vital Capacity
FWER	Familywise Error Rate
GGT	S-Gamma-glutamyl transpeptidase
ICS	Inhaled Corticosteroids
IL-13	Interleukin-13
IP	Investigational Product
IPD	Investigational Product Discontinuation
ITT	Intent-to-Treat
IVRS	Interactive Voice Response System
LABA	Long-Acting β2-Agonist
LLOQ	Lower Limit of Quantification
MACE	Major adverse cardiac events
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities
nAB	Neutralizing Antibodies
NB model	Negative Binomial Model
NC	Not Calculable
NQ	Non-quantifiable
OAE	Other Significant Adverse Event
PEF	Peak Expiratory Flow
PK	Pharmacokinetic(s)
PRO	Patient Reported Outcome
PT	Preferred Term
Q2W	Every 2 Weeks
Q4W	Every 4 Weeks
SAE	Serious Adverse Event
SD	Standard Deviation
SIDES	Subgroup Identification based on Differential Effect Search
SMQ	Standardised MedDRA Query
TBL	Total Bilirubin
UC	Urgent Care

Abbreviation or special term	Explanation
ULN	Upper Limit of Normal
VAS	Visual Analogue Scale
WPAI+CIQ	Work Productivity and Activity Impairment Questionnaire and Classroom Impairment Questionnaire

AMENDMENT HISTORY

Date	Brief description of change
	Updated following CSP amendment dated and to clarify further sections to assist with the development of Mock TLFs
	Updated with Appendix B: Accounting for missing data and Appendix C: Analyses to identify predictive properties of two selected biomarkers and support the definition of a biomarker positive population
	Update to include tipping point analysis, eosinophil subgroup, and additional adverse event summaries. Approach to follow-up data clarified in the study design section. Added clarification on the handling of subjects receiving incorrect dosing of IP in the safety analysis set.

1. STUDY DETAILS

This is the statistical analysis plan (SAP) for study D2210C0007. The SAP describes the statistical analyses specified in the clinical study protocol (CSP) in more detail; any changes with regards to what is already specified in the CSP will be described in Section 6.

1.1 Study objectives

1.1.1 Primary objectives

Objective:	Outcome Measures:
To evaluate the effect of tralokinumab 300 mg administered every 2 weeks compared with placebo on the annualised asthma exacerbation rate (AAER) in adult and adolescent subjects with asthma that is inadequately controlled with inhaled corticosteroid (ICS) plus long-acting β_2-agonist (LABA)	**Primary outcome variable:** The AAER up to Week 52. **Primary outcome measure:** Asthma exacerbation rate reduction. An asthma exacerbation is defined by a worsening of asthma requiring: • Use of systemic corticosteroids for at least 3 days; a single depo-injectable dose of corticosteroids will be considered equivalent to a 3-day course of systemic corticosteroids. • An emergency room (ER) or urgent care (UC) visit (defined as evaluation and treatment for <24 hours in an ER or UC center) due to asthma that required systemic corticosteroids (as per the above). • An inpatient hospitalization due to asthma (defined as an admission to an inpatient facility and/or evaluation and treatment in a healthcare facility for ≥24 hours).

1.1.2   Key secondary objectives

Key Secondary Objectives:	Outcome Measures:
To evaluate the effect of tralokinumab 300 mg administered every 4 weeks compared with placebo on the AAER in adult and adolescent subjects with asthma that is inadequately controlled with ICS plus LABA	**Primary outcome variable:** The AAER up to Week 52 **Primary outcome measure:** Asthma exacerbation rate reduction An asthma exacerbation is defined by a worsening of asthma requiring: • Use of systemic corticosteroids for at least 3 days; a single depo-injectable dose of corticosteroids will be considered equivalent to a 3-day course of systemic corticosteroids. • An ER or UC visit (defined as evaluation and treatment for <24 hours in an ER or UC center) due to asthma that required systemic corticosteroids (as per the above). • An inpatient hospitalization due to asthma (defined as admission to an inpatient facility and/or evaluation and treatment in a healthcare facility for $\geq$24 hours).
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to lung function	**Key outcome variable:** Percent change from baseline in pre-dose/pre-bronchodilator (BD) forced expiratory volume in 1 second (FEV_1). **Key outcome measure:** Percent difference vs. placebo at Week 52.
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to asthma symptoms	**Key outcome variable:** Change from baseline in bi-weekly mean daily asthma symptom score (combined daytime and night-time score as captured in the Asthma Daily Diary). **Key outcome measure:** Mean difference vs. placebo at Week 52.

Key Secondary Objectives:	Outcome Measures:
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to asthma specific health-related quality of life	**Key outcome variable:** Change from baseline in Standardised Asthma Quality of Life Questionnaire for 12 Years and Older total score (AQLQ(S)+12). **Key outcome measure:** Mean difference vs. placebo at Week 52.
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to Asthma Control Questionnaire-6 (ACQ-6) defined asthma control	**Key outcome variable:** Change from baseline in ACQ-6. **Key outcome measure:** Mean difference vs. placebo at Week 52.

1.1.3   Other secondary objectives

Other Secondary Objectives:	Outcome Measures:
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to other endpoints associated with asthma exacerbations	• Time to first asthma exacerbation. • Proportion of subjects with ≥1 asthma exacerbation.
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to ER and UC visits and hospitalizations due to asthma	• AAER that are associated with an ER, UC visit or a hospitalization.
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards pre-dose and post BD FEV1	• Pre-dose/post-BD FEV_1.
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to health related quality of life.	• European Quality of Life - 5 Dimensions 5 Levels Questionnaire (EQ-5D-5L).

Other Secondary Objectives:	Outcome Measures:
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to health care resource utilization and productivity loss due to asthma	- Work Productivity and Activity Impairment Questionnaire and Classroom Impairment Questionnaire (WPAI+CIQ). - Asthma specific resource utilization (e.g., unscheduled physician visits, unscheduled phone calls to physicians, use of other asthma medications).
To assess the effect of 2 dosing regimens of tralokinumab compared with placebo with regards to other measurements of asthma symptoms and asthma control	- Rescue medication use. - Home peak expiratory flow (PEF) (morning and evening). - Night-time awakening due to asthma.
To evaluate the pharmacokinetics and immunogenicity of 2 dosing regimens of tralokinumab	**Pharmacokinetic (PK) parameters**: C_{trough} **Immunogenicity outcome variables:** incidence rate of positive anti-drug antibodies (ADA) and characterization of their neutralizing potential.
To identify a biomarker positive population based on periostin or dipeptidyl peptidase-4 (DPP-4) baseline values that may be associated with up-regulation of interleukin-13 (IL-13)	**Key outcome variable:** - The AAER up to Week 52 (key variable) **Other outcome variables:** - Percent change from baseline in pre-dose/pre-BD FEV_1. - Change from baseline in bi-weekly mean daily asthma symptom score (combined daytime and night-time score as captured in the Asthma Daily Diary). - Change from baseline in AQLQ(S)+12 - Change from baseline in ACQ-6.

1.1.4 Safety objectives

Safety Objective:	Outcome Measures:
To evaluate the safety and tolerability of 2 dosing regimens of tralokinumab	- Adverse Events (AE) /Serious Adverse Events (SAE) - Vital signs - Digital electrocardiograms (dECG) - Clinical chemistry/haematology/urinalysis - Physical examinations

1.1.5 Exploratory objectives

Exploratory Objectives:	Outcome Measures:
To explore periostin, DPP-4, and other biomarkers that may be associated with up-regulation of interleukin-13, as predictive biomarker for treatment of 2 dosing regimens of tralokinumab	**Key outcome variable:** • The AAER up to Week 52 (key variable) **Other outcome variables:** • Percent change from baseline in pre-dose/pre-BD FEV_1. • Change from baseline in bi-weekly mean daily asthma symptom score (combined daytime and night-time score as captured in the Asthma Daily Diary). • Change from baseline in AQLQ(S) +12. • Change from baseline in ACQ-6.
To explore change from baseline of biomarkers that may be associated with up-regulation of IL-13, and possible correlation with clinical efficacy of tralokinumab	Biomarkers will include: • Blood eosinophils • DPP-4 • FE_{NO} • Periostin • IgE Other biomarkers may be considered.
To evaluate the onset treatment effect in lung function, symptoms, and quality of life of 2 dosing regimens of tralokinumab	• Percent and absolute change from baseline in pre-dose/pre-BD FEV_1. • Change from baseline in mean daily asthma symptom score (combined daytime and night-time score as captured in the Asthma Daily Diary). • Change from baseline in AQLQ(S)+12. • Percent change from baseline in Forced Vital Capacity (FVC) and Forced Expiratory Flow 25-75% ($FEF_{25-75\%}$). • Clinical Global Impression of Change (CGIC) from baseline

Exploratory Objectives:	Outcome Measures:
To collect and store deoxyribonucleic acid (DNA) for future exploratory research into genes/genetic variation that may influence clinical response to tralokinumab and provide information on phenotypes of severe asthma (optional)	• DNA sample

Results from the exploratory analyses, if performed, may be reported separately from the Clinical Study Report (CSR).

1.2   Study design

This is a randomised, double-blind, parallel group, placebo-controlled study designed to evaluate efficacy and safety of a fixed 300 mg dose of tralokinumab administered subcutaneously in subjects with uncontrolled asthma on inhaled corticosteroid (ICS) plus long-acting β_2-agonist (LABA) and having a history of asthma exacerbations.

Approximately 1140 subjects will be randomised globally. Subjects will be stratified at randomisation by serum periostin (<16.44 or ≥16.44 ng/mL, sampled during run-in), geographical region (Asia Pacific, North America, South America, Central/Eastern Europe, Western Europe/Rest of the World), and age group [adults (18-75 years inclusive) versus adolescents (12-17 years inclusive), defined by age at Visit 1].

Subjects will receive either of the following regimens of tralokinumab, or placebo, administered via subcutaneous injection to subjects at the study site, over a 52-week treatment period:

- Tralokinumab 300 mg, or placebo, every 2 weeks (Q2W) or,

- Tralokinumab 300 mg, or placebo, every 4 weeks (Q4W)

After initial enrolment and confirmation of entry criteria, subjects will proceed to a run-in period of 6 weeks to allow adequate time for all of the eligibility criteria to be evaluated. Subjects who meet the eligibility criteria will be randomised to a 52-week treatment period. Subjects will be maintained on their currently prescribed ICS plus LABA and any additional asthma controller medication, without change, from enrolment throughout the run-in and treatment period.

Should the subject need to discontinue investigational product (IP) for any reason, every effort should be taken for the subject to be followed-up according to one of three options:

1. Ideally the subject should return for all regular clinic visits and perform all scheduled assessments until he/she completes a total of 52 weeks in the study, or

2. The subject will be offered to be followed up on a monthly basis via telephone calls while continuing eDiary completion, until the subject completes 52 weeks in the study (no further procedures will be performed) or,

3. If the subject cannot comply or does not wish to comply with the options above, the Investigator will only contact the subject at 52 weeks post randomisation. No study assessments will be performed prior to this contact

The key elements to be collected at these follow up visits or telephone contacts for options 2 and 3 are AEs/SAEs, changes in concomitant medications, and asthma exacerbation information.

Follow-up visits will be conducted at Weeks 56 and 72, the extended follow-up period is to ensure that determination of immunogenicity can be adequately determined. A graphical view of the study is shown in Figure 1.

There will be 2 database locks for the study; a primary lock when the last remaining subjects in the study have completed their Week 52 assessment. At this time, the study will be unblinded, and results will be produced based on the data up to Week 52 for inclusion in the CSR. All biomarker data will be included in the CSR addendum only (see below). However, baseline periostin data will be available and included as a covariate in the statistical analysis models as described in Section 4.2.

A second lock will occur when the last subjects remaining in the study have completed their Week 72 assessment. A CSR addendum will be produced following this secondary lock. Included within this addendum will be the following:

- Adverse event and concomitant medication summaries will be updated and included in this addendum.

- A summary of the spirometry data at Week 72 will also be produced.

- All ADA data, including neutralizing antibodies (nAB), will be summarised.

- Other laboratory data collected at Week 56 and Week 72 will be listed and included in the CSR addendum.

- Efficacy analyses including baseline biomarkers will be reported in the CSR addendum. This addendum will include analyses based on the investigational use only (IUO) assay for periostin and the standardised research only use (ROU) assay for DPP-4 assay as well as the commercial V&V assay for both biomarkers.

- Subgroups analyses in the biomarker positive population, defined for D2210C00008 (STRATOS 2), will also be presented for D2210C00007 (STRATOS 1)

The efficacy analyses reported in the CSR addendum will be performed by AZ Biostats and Informatics.

Study D2210C00007 (STRATOS 1) will be used to evaluate the value of the biomarkers (periostin or DPP-4) for predicting Tralokinumab efficacy. Based on analyses from D2210C00007 (STRATOS 1), the intent is to identify a biomarker positive population, which demonstrates an enhanced efficacy profile with tralokinumab treatment, in study D2210C00007. This biomarker positive subpopulation, identified in study D2210C00007 (STRATOS 1), will be validated prospectively in study D2210C00008 (STRATOS 2).

Figure 1   Study flow chart

Visit 1	Visit 2	Visit 3	Treatment period Visits 3-28	EOT Visit 29	Visits 30-31 FU
Week -6 to -4	Week -2	Week 0	Weeks 0-52	Week 52	Weeks 56-72

Enrolment/Run-in

Randomization 2:1:2:1

Tralokinumab 300mg, SC, every 2 weeks (n=380)

Placebo, SC, every 2 weeks (n=190)

End of treatment

Follow up

Tralokinumab 300mg, SC, every 4 weeks (n=380)

Placebo, SC, every 4 weeks (n=190)

End of treatment

Follow up

20
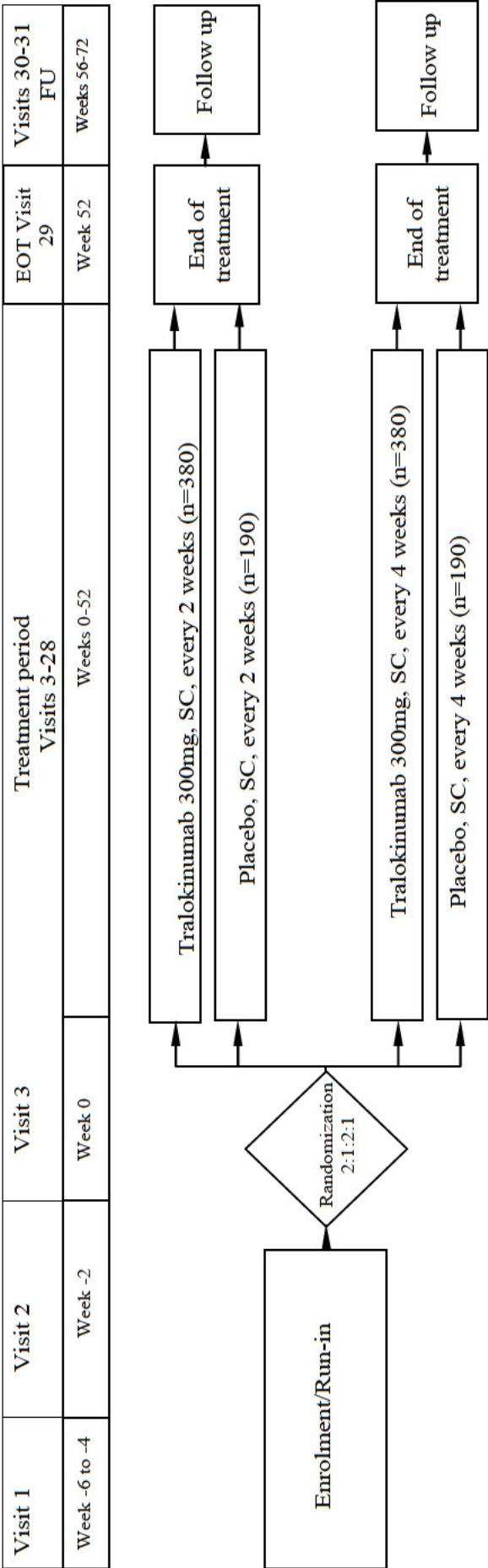
1.3 Number of subjects

A total study sample of 1,140 subjects is considered sufficient to show a reduction in AAER for the 2 dosing regimens of tralokinumab compared to the pooled placebo cohorts (380 subjects on each active dosing regimen, 190 subjects into each of the Q2W and Q4W placebo regimens).

The sample size calculations are made in terms of subject years at risk and are based on an assumed exacerbation rate in the placebo group of 0.8, and shape parameter of 0.95 (over-dispersion). The methodology used is described in Keene et al 2007 and the sample size is calculated for two-sided superiority tests at a significance level of 5% to achieve a power of 90%.

Assuming a uniform loss to follow-up of 15% during the study 380 randomised subjects per group is expected to provide approximately 350 subject-years at risk per treatment group. This is expected to provide at least 90% power for treatment effects down to 32% AERR.

2. ANALYSIS SETS

2.1 Definition of analysis sets

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

2.1.1 Efficacy analysis set

Full analysis set (FAS): All subjects randomised and receiving any investigational product (IP) will be included in the FAS, irrespective of their protocol adherence and continued participation in the study. Subjects will be analysed according to their randomised treatment, irrespective of whether or not they have prematurely discontinued, according to the ITT principle. For subjects who withdraw consent or assent to participate in the study all data will be included up to the date of their study termination.

2.1.2 Safety analysis set

Safety analysis set (Safety): All subjects who received any IP 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. Subjects will be classified according to the following:

- If a subject receives at least one dose of tralokinumab, they will be included in the active treatment group based on the dosing frequency they were randomised to*.

- If a subject receives placebo only, they will be included in the placebo treatment group based on the dosing frequency they were randomised to*.

All safety summaries and ADA analysis and summaries will be based on this analysis set.

*if not randomised, classified as Q4W

Any deviations from the randomised treatment assignment will be listed and considered when interpreting the safety data.

2.1.3 PK analysis set

PK analysis set: All subjects in the FAS who received tralokinumab and who had blood samples obtained for PK, including PK blood samples that are assumed not to be affected by factors such as protocol deviations (eg, disallowed medication, or incorrect study medication received) will be included in the analysis set. All PK summaries will be based on this analysis set.

2.1.4 Patient reported outcome (PRO) analysis set

PRO outcome variables will be evaluated based on the FAS.

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

2.2 Violations and deviations

Only important protocol deviations will be listed and tabulated in the CSR for all randomised subjects. These are protocol deviations that may greatly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being include:

- Subjects who do not meet the inclusion criteria

- Subjects who do not meet the randomisation criteria

- Subjects who meet any of the exclusion criteria

- Subjects who use one or more disallowed medication (listed in Table 1, for any reason unless otherwise specified) during the randomised treatment period

Table 1   Disallowed medications considered to be important protocol deviations

Medication Details	Anatomical Therapeutic Chemical (ATC) code(s)	Preferred term[a] (if applicable)
Live Attenuated Vaccines[b]	J07BD , J07BF, J07BJ, J07BK, V04CF, J07AP, J06BB, J07BB J07BH J07BL	
Any immunomodulators or immunosuppressives	L04AX, L04AD, L01BA , L04AX	

Medication Details	Anatomical Therapeutic Chemical (ATC) code(s)	Preferred term[a] (if applicable)
Any marketed or investigational biologic treatment	R03DX, L04AC	OMALIZUMAB MEPOLIZUMAB RESLIZUMAB
Roflumilast (Daxas/ Daliresp)	R03DX	ROFLUMILAST
Oral or ophthalmic β- adrenergic antagonist[b]	S01ED, C07AA, C07AG	
Systemic corticosteroids[c]	H02AB	

[a] Preferred term will be used in combination with the ATC codes to identify medications
[b] Additional physicians review is required to identify these medications correctly. They will be programmatically isolated for review using the ATC codes.
[c] Additional physicians review is required to identify these medications correctly. They will be programmatically isolated for review using the ATC codes and duration. Only medications with a duration of >= 30 days will be flagged for review. Temporary use (< 30 days) of systemic corticosteroids for treatment of asthma exacerbations or other acute conditions is allowed.

- Subjects who received the incorrect IP or study dose at any time during the 52-week double-blind treatment period

- Subjects who developed withdrawal/discontinuation of IP criteria during the study but were not withdrawn from IP/discontinued from IP.

All important protocol deviations will be identified and documented by the AZ study physician and statisticians prior to unblinding of the data.

3. PRIMARY AND SECONDARY VARIABLES

3.1 General Definitions

3.1.1 Definition of baseline

In general, the last measurement on or prior to the date of randomisation will serve as the baseline measurement for efficacy endpoints, while the last measurement prior to first dose of study treatment will serve as the baseline measurement for safety endpoints.

For spirometry variables (FEV_1, FVC, and $FEF_{25-75\%}$) the measurement recorded at the baseline visit (Visit 3) will be used as baseline. If the Visit 3 measurement is missing, the last non-missing value before Visit 3 will be used as baseline instead. For post-BD measurements, where it is possible to have multiple spirometry records per time point, the first measurement will be used (i.e. the measurement after the first BD administration); for reversibility, this will be the first measurement when the reversibility assessment was considered complete (see Section 3.1.3).

The baseline for Electronic Patient Reported Outcome (ePRO) variables (ACQ-6, AQLQ(S)+12, WPAI-CIQ, and EQ-5D-5L) will be captured or derived from what is captured on the ePRO device at Visit 3. Baseline for Asthma Daily Diary variables will be the bi-weekly mean for data collected between the evening of day -14 and the morning of day 1, where day 1 is the day of randomisation. If more than 7 daily scores (>50%) within that period is missing, then the baseline will be set to missing.

For analysis of bi-weekly means for Asthma Daily Diary variables where 'at Week 52' is referred to, this should be interpreted as 'at Period 26', as defined in section 3.3.

For laboratory data, vital signs and physical examination, baseline will be defined as the latest non-missing assessment prior to first dose. If no time is recorded for an assessment, and the assessment takes place at Visit 3, this will be assumed to be a pre-dose assessment.

For dECG the measurement recorded at Visit 1 will be used as baseline.

3.1.2   Absolute and percent change from baseline

Absolute change from baseline outcome variables is computed as

(post-randomisation value – baseline value).

Percent change from baseline is computed as

((post-randomisation value – baseline value) / baseline value) × 100%.

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

3.1.3   Reversibility

Reversibility percentage will be computed as

% Reversibility = (post-BD FEV_1- pre-BD FEV_1) × 100/pre-BD FEV_1

The FEV_1 post-BD measurement in the reversibility derivation will be the latest measurement and can be the post-BD measurement after 4, 6 or 8 SABA inhalations, depending on when the reversibility assessment was considered complete.

3.1.4   Visit and period windows

For the exacerbation-related analyses no windows will be applied.

For local laboratory data, vital signs, physical examination, and ADA, the visit recorded in the Web Based Data Capture system will be used.

For the central laboratory results, spirometry, AQLQ(S) +12, ACQ-6, and WPAI+CIQ, the variables will be summarized based on the scheduled days with adjusted analysis-defined visit

windows as defined in Table 2. EQ-5D-5L will be summarized using the windows as defined in Appendix A, Table 6.

Any data collected at unscheduled visits will be listed, included within baseline data in shift plots, and will be included in the definition of maximum /minimum within-period value, but will not be included in summaries by visit. In case of a missing assessment at a scheduled visit followed by an unscheduled visit, the unscheduled assessment will not replace the missing result in the summary outputs by period and visit.

If appropriate, i.e. if a substantial percentage of observations for a variable falls outside the adjusted window, sensitivity analysis will be performed where observations are assigned according to the extended windows in Table 2.

Table 2   **Visit windows**

Visit	Target Day	Adjusted windows for analyses:	Extended windows for sensitivity analyses:			
			Haematology, AQLQ(S)+12	Pre BD Spirometry	Post BD Spirometry	Clinical chemistry, Urinalysis
Baseline (Week 0)[a]	1	1	1	1	1	1
Week 2	15	2-21	2-21b	2-21	-	-
Week 4	29	22-35	22-42[b]	22-35	-	2-42
Week 6	43	36-49	-	36-49	-	-
Week 8	57	50-63	43-70	50-70	2-210	43-119
Week 10	71	64-77	-	-	-	-
Week 12	85	78-91	71-98	71-91	-	-
Week 14	99	92-105	-	92-105	-	-
Week 16	113	106-119	99-126	106-147	-	-
Week 18	127	120-133	-	-	-	-
Week 20	141	134-147	127-154	-	-	-
Week 22	155	148-161	-	-	-	-
Week 24	169	162-175	155-182	-	-	-
Week 26	183	176-189	-	148-203	-	120-231
Week 28	197	190-203	183-210	-	-	-
Week 30	211	204-217	-	-	-	-
Week 32	225	218-231	211-238	204-252	-	-
Week 34	239	232-245	-	-	-	-

| | | | **Extended windows for sensitivity analyses:** | | | |
Visit	**Target Day**	**Adjusted windows for analyses:**	**Haematology, AQLQ(S)+12**	**Pre BD Spirometry**	**Post BD Spirometry**	**Clinical chemistry, Urinalysis**
Week 36	253	246-259	239-266	-	-	-
Week 38	267	260-273	-	-	-	-
Week 40	281	274-287	267-294	253-322	-	232-322
Week 42	295	288-301	-	-	-	-
Week 44	309	302-315	295-322	-	-	-
Week 46	323	316-329	-	-	-	-
Week 48	337	330-343	323-350	-	-	-
Week 50	351	344-357	-	-	-	-
Week 52	365	358-378	351-378	323-378	211-378	323-378
Week 56 (FU)	393	379-448	379-448 [c]	-	-	-
Week 72 (FU)	505	449-560	449-560 [c]	379-560	-	-

[a]   If the Day 1 assessment is missing, see section 3.1.1 on how baseline value is defined.
[b]   Week 2 is not applicable for AQLQ-12. Week 4 visit window for AQLQ-12 will be 2-42.
[c]   Not applicable for AQLQ(S)+12.

For assignment of data to time points using the visit windows, study day will be defined as follows for efficacy data:

(Date of assessment - Date of randomisation) + 1.

And as follows for safety endpoints:

(Date of assessment - Date of first dose of IP) + 1.

In case of multiple observations within a single visit window, the following rules apply:

- If there are two or more observations within the same visit window, the non-missing observation closest to the target day will be used in the analysis

- If two observations are the same distance from the target day, the non-missing observation with the earlier collection date will be used in the analysis

- If two observations are collected on the same day and have a collection time associated with them, the non-missing observation with the earlier collection time will be used in the analysis

- If two or more observations are collected on the same day, all non-missing but with no collection time associated with at least one of them, the average of the observations will be used in the analysis.

If a visit window does not contain any observation, then the data will be missing for that visit.

3.2   Calculation or derivation of efficacy variables

3.2.1   Exacerbation rate

The primary endpoint is the AAER up to Week 52. For the primary analysis the response variable is the number of exacerbations the subject experiences up to Week 52, with the time at risk included as offset in the model.

An asthma exacerbation is defined as a worsening of asthma that leads to any of the following:

- Use of systemic corticosteroids for at least 3 days; a single depo-injectable dose of corticosteroids will be considered equivalent to a 3-day course of systemic corticosteroids

- An ER or UC visit (defined as evaluation and treatment for <24 hours in an ER or UC centre) due to asthma that required systemic corticosteroids (as per above)

- An inpatient hospitalization (defined as admission to an inpatient facility and/or evaluation and treatment in a healthcare facility for ≥24 hours) due to asthma

In order to calculate the number of exacerbations experienced by a subject during the 52-week treatment period the following rule will be applied:

- The start of an exacerbation is defined as the start date of systemic corticosteroids, ER or UC visits requiring systemic corticosteroids, or hospital admissions due to asthma, whichever occurs earlier,

- The end date is defined as the last day of systemic corticosteroids or ER/UC/hospital discharge, whichever occurs later.

Two or more exacerbations with the same start date and end date will be counted as one exacerbation for the purposes of calculating the number and duration of exacerbations for a subject. In the case that one or more exacerbations are recorded as starting or ending during another exacerbation, these will be counted as one exacerbation, using the earliest exacerbation start date and the latest exacerbation stop date to calculate duration.

Additional systemic corticosteroid treatments, ER or UC visits requiring use of systemic corticosteroids, or inpatient hospitalization due to asthma occurring during an exacerbation will not be regarded as a new exacerbation. In order to be counted as a new exacerbation it must be preceded by at least 7 days in which neither criterion is fulfilled. If two or more

exacerbations are recorded less than 7 days apart, these will be counted as one exacerbation, but the duration period of each exacerbation will be considered separately when calculating exacerbation duration for subject.

Maximum follow-up time for a subject is approximately 52 weeks; defined as the time from randomisation to the date of Visit 29. For a subject lost to follow-up, this will be defined as the time from randomisation to the time point after which an exacerbation could not be assessed, (i.e., the last contact date). Any exacerbations after this time point will not be included in analyses.

For the primary analysis, exacerbations that occur after a subject has discontinued IP but before maximum follow-up time will still be accounted when deriving the total number of exacerbations. Likewise, the follow-up time will reflect the follow-up time regardless of whether or not the subject is still on IP.

For the production of summary statistics, the annual exacerbation rate per subject is calculated and standardized per a 52-week period according to the formula described below.

Annual Exacerbation Rate = No. of Exacerbations*365.25 / (Follow-up date – Date of randomisation + 1).

3.2.2 Proportion of subjects with ≥ 1 asthma exacerbation during 52 weeks of treatment

The proportion of subjects with ≥ 1 asthma exacerbation during the 52 weeks of treatment will be a supportive measurement to the primary objective. The outcome variable will categorize each subject as having at least one asthma exacerbation or not (yes=1/no=0).

The proportion of such subjects will be calculated for each treatment group as:

Number of subjects with ≥ 1 asthma exacerbation during the 52 week treatment period/ number of subjects in treatment group

3.2.3 Time to first exacerbation

Time from randomisation to the first asthma exacerbation will also be used as a supportive variable to the primary objective, and is calculated as follows:

Start Date of first asthma exacerbation − Date of Randomisation + 1.

The time to first asthma exacerbation for subjects who do not experience an asthma exacerbation during the treatment period will be censored at the date of their last visit for the 52-week double-blind treatment period, or at the time point after which an exacerbation could not be assessed (for lost-to-follow-up subjects).

3.2.4   Annual rate of asthma exacerbations that are associated with an ER or UC visit or a hospitalization

The AAER associated with an ER or UC visit or a hospitalization (as a subset of the primary variable defined in section 3.2.1), will be a secondary efficacy variable.

The number of asthma exacerbations that are associated with an ER or UC visit or a hospitalization experienced by a subject during the 52-week treatment period will be derived using the same rule for start and end as described for the primary variable in section 3.2.1

Maximum follow-up time is approximately 52 weeks, and the follow-up time is derived as described in Section 3.2.1.

Additionally, for the production of descriptive statistics, the annual rate of asthma-related ER or UC visits and hospitalizations will be calculated using the same methodology as the annual rate of exacerbations described in Section 3.2.1.

3.2.5   Forced expiratory volume in 1 second

The key secondary variable is the pre-dose/pre BD FEV_1 which will be determined by spirometry. To ensure quality control all spirometries are reviewed to ensure that they meet ATS/ERS criteria for acceptability. Only those spirometry tracings determined to be acceptable or borderline will be used to determine FEV_1 (and FVC and $FEF_{25-75\%}$), based on the best measurement selected by ERT per spirogram. Section 5.1.2 of the CSP contains further details of the spirometry recordings.

The percent change from baseline to each of the post-randomisation visits, up to and including the end of 52-week double-blind treatment visit (Visit 29), will be used as secondary efficacy outcome variable.

The absolute change from baseline to each of the post-randomisation visits up to and including the end of 52-week double-blind treatment visit (Visit 29) will be used as a supportive outcome variable.

The same outcome variables will be derived for the secondary variable pre-dose/post- BD FEV_1.

3.2.6   Forced Vital Capacity and Forced Expiratory Flow at 25-75%

The percent change from baseline to each of the post-randomisation visits, up to and including the end of 52-week double-blind treatment visit (Visit 29) will be calculated for the exploratory variables FVC and $FEF_{25-75\%}$.

3.3   Calculation or derivation of patient reported outcome variables

Patient-reported outcomes data will be captured via an ePRO device. The definition of key secondary and secondary outcome variables based on the ePRO are provided in the following

sections. For all outcomes based on the ePRO devices, analyses will be based on data up to and including week 52.

For asthma symptom score, rescue medication use and home peak expiratory flow, 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'. Note that the first bi-weekly mean in the treatment period will be based on the evening recording on day 1 up to and including the morning recording on day 15. The daytime score is recorded in the evening and the night-time score is recorded the following morning.

Bi-weekly periods are defined as follows (where Day 1 is the day of randomisation)

Bi-weekly Period	Label
Baseline: as defined in section 3.1.1	(Day -14 to 1) Baseline
Period 1: Evening of Day 1 – Morning of Day 15	Day 1 – 15 (Week 2)
Period 2: Evening of Day 15 – Morning of Day 29	Day 15 – 29 (Week 4)
Period 3: Evening of Day 29 – Morning of Day 43	Day 29 – 43 (Week 6)
Period 4: Evening of Day 43 – Morning of Day 57	Day 43 – 57 (Week 8)
Period 5: Evening of Day 57 – Morning of Day 71	Day 57 – 71 (Week 10)
Period 6: Evening of Day 71 – Morning of Day 85	Day 71 – 85 (Week 12)
Period 7: Evening of Day 85 – Morning of Day 99	Day 85 – 99 (Week 14)
Period 8: Evening of Day 99 – Morning of Day 113	Day 99 – 113 (Week 16)
Period 9: Evening of Day 113 – Morning of Day 127	Day 113 – 127 (Week 18)
Period 10: Evening of Day 127 – Morning of Day 141	Day 127 – 141 (Week 20)
Period 11: Evening of Day 141 – Morning of Day 155	Day 141 – 155 (Week 22)
Period 12: Evening of Day 155 – Morning of Day 169	Day 155 – 169 (Week 24)
Period 13: Evening of Day 169 – Morning of Day 183	Day 169 – 183 (Week 26)
Period 14: Evening of Day 183 – Morning of Day 197	Day 183 – 197 (Week 28)
Period 15: Evening of Day 197 – Morning of Day 211	Day 197 – 211 (Week 30)
Period 16: Evening of Day 211 – Morning of Day 225	Day 211 – 225 (Week 32)
Period 17: Evening of Day 225 – Morning of Day 239	Day 225 – 239 (Week 34)
Period 18: Evening of Day 239 – Morning of Day 253	Day 239 – 253 (Week 36)
Period 19: Evening of Day 253 – Morning of Day 267	Day 253 – 267 (Week 38)
Period 20: Evening of Day 267 – Morning of Day 281	Day 267 – 281 (Week 40)
Period 21: Evening of Day 281 – Morning of Day 295	Day 281 – 295 (Week 42)

Bi-weekly Period	Label
Period 22: Evening of Day 295 – Morning of Day 309	Day 295 – 309 (Week 44)
Period 23: Evening of Day 309 – Morning of Day 323	Day 309 – 323 (Week 46)
Period 24: Evening of Day 323 – Morning of Day 337	Day 323 – 337 (Week 48)
Period 25: Evening of Day 337 – Morning of Day 351	Day 337 – 351 (Week 50)
Period 26: Evening of Day 351 – Morning of Day 365	Day 351 – 365 (Week 52)

Where a total score is calculated within a day (e.g. Asthma symptom score), this calculation will spans two calendar days - the daytime value recorded in evening of day X, and the night-time value recorded on morning of day x+1. E.g. the Asthma Symptom score on Day 1 will be the day time score recorded on the evening of Day 1 + the night-time score recorded on the morning of Day 2.

Where only night-time scores/results are of interest, the morning entries on the second day of a period up to and including the morning entry on the last day of the period (or morning of the last day of study for the last period/last IP intake) will be considered.
Where only daytime scores/results are of interest, the evening entries on the first day of the period up to and including the evening entry on the second last day of the period (or evening before the last day of study/last IP intake) will be considered.

3.3.1   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 (recorded in the evening), night-time score (recorded in the morning), and total score will be calculated and presented separately.

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

The key secondary outcome variable is the change from baseline in bi-weekly mean daily asthma symptom total score. Biweekly means and change from baseline for daytime and night-time scores will also be calculated.

The number of asthma symptom-free days will be calculated for each patient as the total number of days in the 52 week treatment period where the total asthma symptom score is 0. The proportion of asthma symptom-free days will be calculated using the total number of days with completed asthma symptom score diary during the 52 week treatment period as the denominator.

3.3.2 Rescue medication use

The number of rescue medication inhalations and nebulizer treatments taken will be recorded by the subject in the Asthma Daily Diary twice daily. Daytime use is recorded in the evening and night-time use is recorded in the morning. Inhaler usage will be reported as the number of puffs in a given period whereas nebulizer use will be reported as the number of times.

The number of inhalations of rescue medication and nebulizer treatments captured in the eDiary each day will be calculated per subject. If a subject is missing a value for either night-time or daytime rescue medication on a given day, then the total rescue medication use for that day will be set to missing.

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 change from baseline in the number of inhalations (puffs) per day will be calculated as the outcome variable.

3.3.3 Nights with awakening due to asthma

Bi-weekly mean change from baseline in the number (percentage) of nights with awakening due to asthma that required rescue medication will be calculated as the outcome variable.

3.3.4 Home peak expiratory flow (morning and evening)

Bi-weekly mean absolute changes from baseline in morning and evening PEF will be calculated.

3.3.5 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 use of short acting β-agonists. Subjects will be asked to complete ACQ-6 once every 2 weeks. The questionnaire include 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 ACQ-6 score is computed as the un-weighted mean of the responses to the 6 questions. If response to any of the questions is missing, the ACQ-6 score will be missing.

The key outcome variable for the ACQ-6 will be the change in mean score from baseline to each of the post-randomisation assessments. The change from baseline for each question will also be calculated.

Other variables based on ACQ-6 to report include:

- ACQ-6-responder (Yes=1/No=0)

    o Responder: Change from baseline ACQ-6 score $\leq$ -0.5

    o Non-responder : Change from baseline ACQ-6 score > -0.5

- ACQ-6-response (Improved/No Change / Deterioration)

    o Improvement: Change from baseline ACQ-6 score $\leq$ -0.5

    o No change: -0.5 < Change from baseline ACQ-6 score < 0.5

    o Deterioration: Change from baseline ACQ-6 score $\geq$ 0.5

- Subjects asthma control as measured by ACQ-6 score:

    o Well controlled : ACQ-6 score $\leq$ 0.75

    o Partly controlled : 0.75 < ACQ-6 score < 1.5

    o Not well controlled : ACQ-6 score $\geq$ 1.5

Subjects with missing or non-evaluable ACQ-6 score at week 52 will be considered as a non-responder for ACQ-6 responder (Yes=1/No=0).

3.3.6 Asthma quality of life questionnaire for 12 years and older (AQLQ(S) +12)

In the AQLQ(S) +12 the subjects are asked to recall their experiences during the previous 2 weeks and to score each of the 32 questions on a 7-point scale ranging from 7 (no impairment) to 1 (severe impairment). Subjects will be asked to complete AQLQ(S) +12 once every 4 weeks.

The overall score is calculated as the mean response to all questions. The 4 individual domain scores (4 domains assessing 1) symptoms, 2) activity limitations, 3) emotional function, and 4) environmental stimuli) are the means of the responses to the questions in each of the

domains. The following are the question numbers on the AQLQ(S) +12 questionnaire relating to each domain:

Table 3   AQLQ(S) +12 Domains

Domain	AQLQ(S)+12 question numbers
Symptoms	6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 29, 30
Activity Limitations	1, 2, 3, 4, 5, 11, 19, 25, 28, 31, 32
Emotional Function	7, 13, 15, 21, 27
Environmental Stimuli	9, 17, 23, 26

If response to any of the questions is missing the overall score will be missing, if response to a question within a domain is missing, the score for that domain will be missing.

The key outcome variable for the AQLQ(S) +12 will be the change in overall score from baseline to each of the post-randomisation assessments. Change from baseline in each domain will also be calculated.

Other variables based on AQLQ(S) +12 to be reported include:

- AQLQ(S) +12 -responder (Yes=1/No=0)

  o Responder: Change from baseline AQLQ(S) +12 score $\geq$ 0.5

  o Non-responder : Change from baseline AQLQ(S) +12 score < 0.5

- AQLQ(S) +12 -response (Improved/No Change / Deterioration)

  o Improvement: Change from baseline AQLQ(S) +12 score $\geq$ 0.5

  o No change: -0.5 < Change from baseline AQLQ(S) +12 score < 0.5

  o Deterioration: Change from baseline AQLQ(S) +12 score $\leq$ -0.5

Subjects with missing or non-evaluable AQLQ(S) +12 score at week 52 will be considered as a non-responder for AQLQ(S) +12 -responder (Yes=1/No=0).

3.3.7   European quality of life-5 dimensions-5 levels (EQ-5D-5L)

The questionnaire assesses 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 response options (no problems, slight problems, moderate problems, severe problems, and extreme problems) that reflect increasing levels of difficulty.

The subject will be asked to indicate his/her current health state weekly by selecting the most appropriate level in each of the 5 dimensions. The questionnaire also includes a visual analogue scale (VAS), where the subject will be asked to rate current health status on a scale of 0-100, with 0 being the worst imaginable health state.

The change from baseline in VAS will be calculated by assessment.

3.3.8 Health care resource utilization

Health care resource utilization due to asthma will be collected by the Investigator/authorized delegate at each visit as specified in the protocol and recorded in the HEVENT module in the electronic case report form (eCRF).

Study period number of days/times will be calculated for each subject for the following variables:

- Ambulance transport
- Hospitalization (number of visits and days in hospital)
  - Intensive care (days in intensive care)
  - General care (days in general care)
- ER visit
- Visit to specialist
- Visit to primary health care physician
- Other health care visit
- Home visit, physician
- Home visit, other health care
- Telephone call, physician
- Telephone call, nurse
- Spirometry
- Advanced pulmonary function test

The study period number per subject will be determined as:

Study period number = Sum of 'total No. of times/days' as entered in HEVENT up to Week 52.

3.3.9 The Work Productivity and Activity Impairment Questionnaire plus Classroom Impairment Questions (WPAI+CIQ)

The WPAI+CIQ questionnaire is a 10-item questionnaire that assesses productivity and activity impairment over the previous week. Subjects will be asked to complete WPAI+CIQ once every 2 weeks.

There are a maximum of 10 questions and a minimum of 3 questions that will be completed by subjects as follows:

1. Currently employed (yes/no)

2. Hours missed work due to health problems

3. Hours missed work due to other reasons

4. Hours actually worked

5. Degree health affected productivity while working (0-10 scale, with 0 meaning no effect)

6. Attends class in an academic setting (yes/no)

7. Hours missed class due to health problems

8. Hours actually attended class

9. Degree health affected productivity while attending class (0-10 scale, with 0 meaning no effect)

10. Degree health affected regular activities (other than work or class) (0-10 scale, with 0 meaning no effect)

If the answer to question 1 is 'No, not currently employed', then the subject should skip to question 6. If the answer to question 6 is 'No, not currently attending class', then the subject should skip to question 10.

The WPAI+CIQ provide 4 scores:

• Absenteeism (work or class time missed),

• Presenteeism (impairment at work or class/reduced on-the-job effectiveness),

• Work productivity loss (overall work or class impairment/absenteeism plus presenteeism)

• Activity impairment.

WPAI+CIQ outcomes are expressed as impairment percentages, with higher numbers indicating greater impairment and less productivity.

For each time point at which the WPAI-CIQ is administered, the following descriptive statistics (if applicable) (n, total number of hours, mean per subject, standard deviation (SD), median, minimum and maximum) will be reported for those who are employed:

• # employed

• % of all subjects employed

- # of work hours missed due to asthma

- Absenteeism due to asthma

- Presenteeism due to asthma

- Work Productivity Loss

- Activity impairment

The following formulas will be used to calculate each of the outcome measures listed above:

- # currently employed – Yes in response to Question 1

- # of hours missed due to asthma – as responded in Question 2

- Absenteeism = Q2/(Q2+Q4)

- Presenteeism = Q5/10

- Work Productivity Loss = Q2/(Q2+Q4)+[(1-Q2/(Q2+Q4))x(Q5/10)]

- Activity Impairment = Q10/10

Similarly, the following will be reported for those subjects who are in school:

- # in school

- % of all subjects in school

- # of class hours missed

- Absenteeism due to asthma

- Presenteeism due to asthma

- Class Productivity Loss

- Activity impairment

The following formulas will be used to calculate each of the outcomes measures listed above:

- # in school - Yes to Question 6

- # of class hours missed due to asthma – as responded on Question 7

- Absenteeism due to asthma - Q7/(Q7+Q8)

- Presenteeism due to asthma – Q9/10

- Class Productivity Loss – Q7/(Q7+Q8) + [(1-Q7/(Q7+Q8))x(Q9/10)]

- Activity Impairment = Q10/10

In addition, activity impairment will be presented for those who are not employed, not in school, and all subjects.

3.3.10   Clinical global impression of change CGIC)

CGIC is used for an overall evaluation of response to treatment. The Investigator (clinician) uses a 7-point scale: 1 = Very Much Improved; 2 = Much Improved; 3 = Minimally Improved; 4 = No Change; 5 = Minimally Worse; 6 = Much Worse; and 7 = Very Much Worse.

The Investigator will be asked to rate the degree of change in the overall asthma status compared to the baseline visit. The CGIC assessment was added through a protocol amendment, therefore not all subjects in the FAS will have these assessments. Calculation of percentages will be based on the number of subjects in the FAS set with a completed assessment. There will be no imputation for missing values.

Subjects will also be categorized as Improved, Much Improved and Very Much Improved according to the following responses post-baseline:

- Improved: subjects in this category will include those with responses of 'Very much improved', 'much improved 'and 'minimally improved'.

- Much Improved: subjects in this category will include those with responses of 'Very much improved', 'much improved'.

- Very Much Improved: subjects in this category will include those with responses of 'Very much improved'.

Subjects can be counted in more than one category at a given time point.

3.4   Calculation or derivation of safety variable(s)

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

Change from baseline (Visit 3) to each post-baseline time point where scheduled assessments were made will be calculated for relevant measurements.

3.4.1   Adverse events

AEs 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).

AE data will be categorized according to their onset date into the following periods:

- AEs occurring during run-in (onset date ≥ Visit 1 and before the first dose of IP)
- AEs occurring during study (onset date ≥ the first day of IP and ≤ Visit 31 (Week 72))
- AEs occurring during treatment (onset date ≥ the first day of IP and ≤ the last day of IP + dosing frequency)
- AEs occurring post-treatment (onset date > the last day of IP + dosing frequency and ≤ Visit 31 (Week 72))

Note: The 'dosing frequency' equals 2 weeks for subjects randomised to the Q2W dosing regimen, and 4 weeks for subjects randomised to the Q4W dosing regimen.

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. The same during treatment definition will be used for laboratory and physical examination data, where applicable.

3.4.2 Safety topics of special attention

Although the CSP did not describe AEs of special interest, AstraZeneca Patient Safety and study physicians review all AEs and identify those that merit special attention. These AEs fall into three categories, AEs possibly related to administration of biologics (eg, anaphylaxis/hypersensitivity reactions and injection site reactions), AEs possibly related to the mechanism of action of tralokinumab as an IL-13 blocking agent (eg, infections such as severe, viral, invasive fungal, and parasitic, malignancy, cardiovascular/cerebrovascular events, pregnancy/spontaneous abortion and increased eosinophils) and AEs reported for other biologics in this class (eg, musculoskeletal). AEs falling into the category of safety topics of special attention will be tabulated.

3.4.3 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. Blood samples for determination of haematology/haemostasis and clinical chemistry will be performed at a central laboratory. Urine samples will be analysed locally and sent for analysis at the central lab only when a positive dipstick result for any parameter is observed. The parameters outlined in Table 2, Table 3, Table 4 and Table 5 in Section 5.1.6.1 of the CSP will be collected. Laboratory data will be reported in SI units.

Changes in haematology and clinical chemistry variables between baseline and each subsequent scheduled assessment will be calculated.

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.

Urinalysis data will be categorised as negative (0), trace, or positive (+) 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, using the 'during treatment' definition as defined in Section 3.4.1.

For the liver function tests: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP), S-Gamma-glutamyl transpeptidase (GGT) and total bilirubin (TBL), the multiple of the AstraZeneca upper limit of the normal (ULN) (not extended) range will be calculated for each data point.

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

ie, 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:

- AST $\geq$ 3x ULN

- ALT $\geq$ 3x ULN

- TBL $\geq$ 2xULN

3.4.4   dECGs

Twelve-lead dECG measurements will be recorded in accordance with the protocol.

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.

3.4.5   Physical examination

Complete and brief physical examinations will be performed at time points specified in Table 1 in the CSP. What is included in the assessment will be dependent on whether the examination is complete or brief, as described in Section 5.1.7 of the CSP. For the brief physical examination, only information on whether the assessment was performed or not will be recorded.

Each component of the Visit 1 complete physical examination will be recorded as normal or abnormal. Each component of the complete physical examinations (from Visit 3 onwards) will be recorded as normal, same as Visit 1, 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.

3.4.6 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 of the CSP.

Changes in vital signs variables between baseline and each subsequent scheduled assessment will be calculated.

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.

Table 4   Vital signs reference ranges

Parameter	Standard Units	Lower Limit	Upper Limit	Change Criteria
Diastolic Blood Pressure (sitting)	mmHg	<60	>100	±15
Systolic Blood Pressure (sitting)	mmHg	<90	>160	±30
Pulse (sitting)	Beats/min	<50	>100	±20
Respiratory Rate	Breaths/Min	<8	>20	
Body Temperature	Celsius	<36	>37.5	
Weight	kg	<40	>150	

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

$$BMI = kg/m^2$$

3.4.7 Medical History

The principal for imputing incomplete diagnosis dates when calculating the number of years since diagnosis (earliest possible date) is shown in Table 5 below:

Table 5   Approach to incomplete diagnosis dates for medical history

Date of Birth (Year-Month-Day)	Diagnosis Date (Year-Month-Day)	Date for use in calculations (Year-Month-Day)
1951-09-16	1951-10-10	1951-10-10
1951-09-16	1951-10-UK	1951-10-01
1951-09-16	1951-UK-UK	1951-10-16

Date of Birth	Diagnosis Date	Date for use in calculations
(Year-Month-Day)	(Year-Month-Day)	(Year-Month-Day)
1951-09-16	1952-UK-UK	1952-01-01

UK=Unknown

3.5 Calculations or derivation of Pharmacokinetic and Immunogenicity variables

Blood samples (processed to serum) for PK and immunogenicity assessments will be collected from all subjects at baseline prior to first IP administration at Visit 3, at multiple time points before IP administrations during the treatment period, and at selected timepoints in the follow-up period of the study. ADA assessments will be conducted utilizing a tiered approach (screen, confirm, titre). 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. Titre values are reported as the reciprocal of the highest dilution that yields a value above the cut point. Samples from pre-defined study time points that confirm positive for ADA will also be tested for neutralizing ADA (nAb) activity. Both ADA and nAb will be summarized using descriptive statistics as described in Section 4.2.7.7.

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.

If possible and, if relevant, the impact of ADA occurrence on the PK and PD and safety will be summarized in the CSR addendum. Summaries of tralokinumab serum concentrations by time will be summarised by ADA status (positive vs negative) and treatment group.

4. ANALYSIS METHODS

4.1 General principles

The analysis of the study endpoints will include all data captured during the 52-week double-blind treatment period. This includes data regardless of whether IP was prematurely discontinued or delayed, and/or irrespective of protocol adherence, unless the subject withdraws consent or assent to study participation.

Only exacerbations confirmed to meet the protocol definition (CSP section 5.1.1) will be included in efficacy analysis and summaries. This includes only exacerbations that started on or after the date of randomisation, and those that are defined as a worsening of asthma that leads in at least one of the three criteria detailed in section 3.2.1 being fulfilled. From the EXACA form on the eCRF, for a given exacerbation this will include exacerbations where

EXACDRY= 'Yes' and at least one of the following a) EXSCORT = 'Yes' b) EXSCORT ='Yes' and EXERTRT ='Yes' c) HOSPIT ='Yes'. Supportive analysis of exacerbations based on adjudicated data will be based on all hospitalisations and ER/UC visits determined to be related to asthma by the independent adjudication committee. Any events with an undetermined adjudication outcome will be regarded as related to asthma if the investigator has recorded the event as a protocol defined exacerbation in the eCRF, otherwise it will be assumed to be not related to asthma.

A summary table which counts subjects who have been incorrectly randomised and who received incorrect treatment will be presented.

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, SD, median, and range. All data will be listed. Data listings will be sorted by treatment and subject number.

Minimum and maximum values will be reported to the same degree of precision as the raw data unless otherwise stated. Mean, median, SD and confidence intervals (CIs) will be reported to one further degree of precision.

All hypothesis testing will be reported using 2-sided tests. P-values will be rounded to 4 decimal places.

Efficacy and safety summaries and comparisons of tralokinumab versus placebo will be done vs. the pooled placebo cohorts using statistical models that, where suitable, includes the placebo cohorts as separate levels. This will also allow for comparisons within each dosing regimen. Although consistency across placebo cohorts is expected signs of inconsistency across placebo sensitivity analyses will be further explored. Comparisons within dosing regimen will be presented for the primary and key secondary endpoints, and for selected baseline characteristics, demography and safety tables as sensitivity analyses.

4.1.1 Testing strategy for primary and key secondary objectives

The following hierarchical testing strategy (presented in Figure 2 and further described in the text below) will be used for primary and key secondary outcome in the two dosing regimens. This testing strategy will globally strongly control the familywise error rate (FWER).

Figure 2   **Testing strategy**
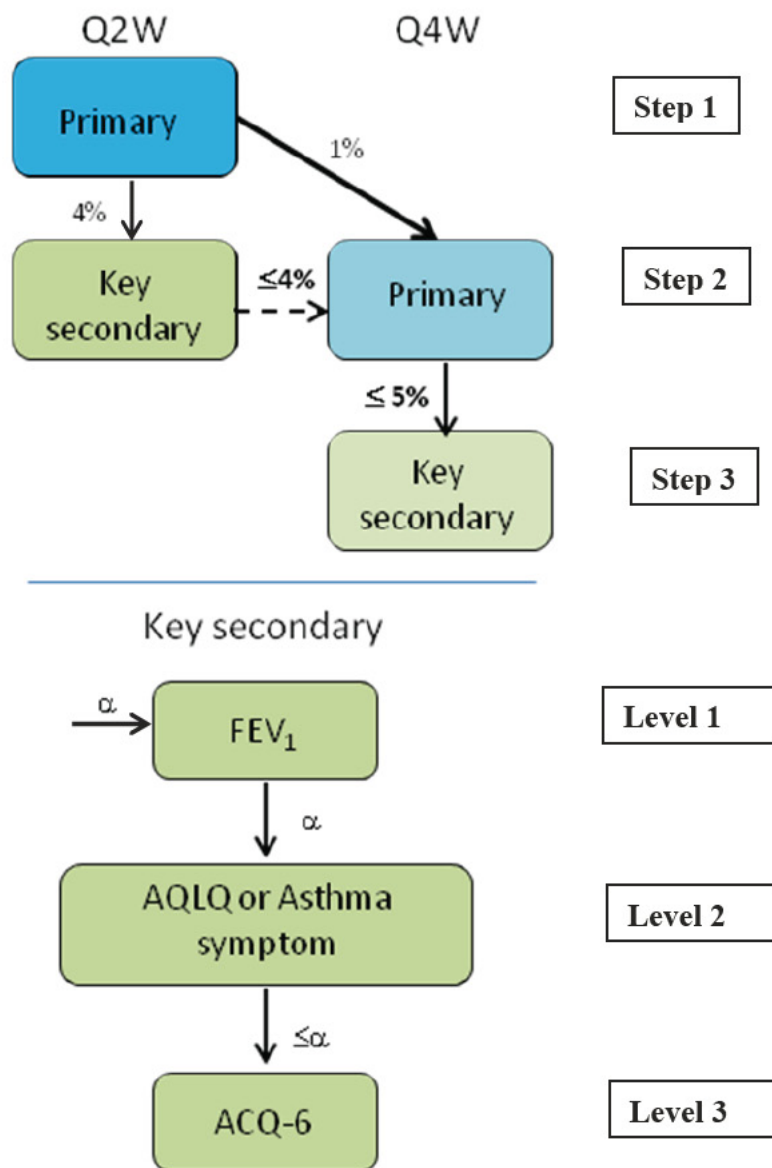
Step 1:

> The higher dosing regimen (300 mg tralokinumab Q2W) will be tested versus placebo at a two-sided 5% significance level with regards to the primary endpoint.

Step 2:

> If a treatment effect is shown with regards to the primary endpoint for Q2W in step 1, then

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a. First, the family of key secondary endpoints will be tested for Q2W at a two-sided 4% significance level using the hierarchical testing approach described below

b. Second, the primary endpoint will be tested for Q4W using a two-sided test at (at least) 1% significance level. Any significance retained from the key secondary testing family for the Q2W (bullet a) above) will be recycled to this test. Hence, the significance level in this test depends on the results from testing the family of key secondary endpoints for Q2W, as follows:

  - Case 1: All key secondary endpoints exhibits significance for Q2W. In this situation, the primary endpoint will be tested for Q4W at level $\alpha=0.05$

  - Case 2: All key secondary endpoints within the family, except one endpoint in Level 2 (see below) exhibit significance for Q2W. In this situation, the primary endpoint will be tested for Q4W at level $\alpha=0.03$

  - Case 3: In any other situation, the primary endpoint will be tested for Q4W at level $\alpha=0.01$

Step 3:.

If treatment effect is shown for the primary endpoint for Q4W in step 2, then the family of key secondary endpoints will be tested for Q4W at significance level α retained from Step 2, using the hierarchical testing approach described below

Hierarchical testing approach for key secondary endpoints

In step 2 and 3 the family of key secondary endpoints will be tested using the following hierarchical testing structure with strong local FWER control:

- Level 1: Percent change from baseline in pre-dose/pre-BD FEV_1 at Week 52 vs. placebo will be tested two-sided at significance level α retained from the previous step. If a significant treatment effect is shown, testing will proceed to the next level. Otherwise, testing will stop and all subsequent results in the family will be declared non-significant and interpreted in a descriptive, exploratory way.

- Level 2: Mean change from baseline in bi-weekly mean daily asthma symptom score at Week 52 vs. placebo and mean change from baseline in AQLQ(S) +12 Total score at Week 52 vs. placebo will be tested using a Bonferroni approach at significance α. Hence, each of the two endpoints will be tested at significance level $\alpha/2$ (=0.02 in the Q2W comparison, = 0.005, 0.015, or 0.025 in the Q4W comparison).

- If at least one significant treatment effect is shown in this level, testing will proceed to Level 3. Otherwise, testing will stop and the subsequent result within the family will be declared non-significant and interpreted in a descriptive, exploratory way.

- Level 3: Change from baseline ACQ-6 vs placebo will only be tested if at least one of the endpoints in Level 2 exhibits significance. There are two difference outcomes in Level 2 that could result in testing at Level 3:

    - Case 1: Both comparisons in Level 2 exhibit significance. In this situation change from baseline ACQ-6 vs placebo is testable and will be tested at level α (=0.04 in the Q2W comparison, = 0.01, 0.03, or 0.05 in the Q4W comparison)

    - Case 2: Only one of the comparisons in Level 2 exhibit significance. In this situation, change from baseline ACQ-6 vs placebo is testable and will be tested at level $\alpha/2$ (=0.02 in the Q2W comparison, = 0.005, 0.015, or 0.025 in the Q4W comparison).

4.1.2   Sensitivity analyses

Sensitivity analyses for the primary endpoint and the key secondary continuous endpoints based on different missing data mechanism assumptions will be performed. These analyses are detailed in Appendix B.

In addition to the methods described in Appendix B, the following analyses will also be performed;

- The interpretation of exacerbation data post-discontinuation of treatment is likely to be confounded by reduced quality of objective confirmation of deterioration, and by the use of subsequent therapies. Sensitivity analyses for the primary endpoint will be carried out to explore the impact of this. The primary analysis will be repeated, excluding data collected after discontinuation of IP.

- A Poisson regression model taking over dispersion into account will be included as a sensitivity analysis for the primary analysis. The correction for potential over dispersion will be made by Pearson chi-square. The response variable, covariates and offset variable will be the same as described for the primary analysis (section 4.2.4).

- The primary analysis will be repeated where the time at risk (which is included in the model as an offset variable) excludes any time during which a subject is having an exacerbation (see section 4.2.4).

- The main comparisons between tralokinumab and placebo will be the comparison with the pooled placebo cohort. However, the differences between tralokinumab

and placebo within each dosing regimen will also be estimated for the primary and key secondary objectives (not objectives that are supportive to the key secondary objectives), to explore the effect of pooling the placebo groups.

- If there is a relevant imbalance in either the baseline periostin or baseline DPP-4 values across the treatment groups, then an additional analysis of the primary endpoint will be performed including the baseline value of the corresponding biomarker as a continuous covariate in the analysis model. Where continuous baseline periostin is included in the model, periostin group at baseline will not be included.

For variables with adjusted analysis-defined visit windows, sensitivity analyses may be performed where observations are classified according to the extended windows described in Table 2.

4.2   Analysis methods

4.2.1   Subject disposition, demography data and subjects characteristics

Subject disposition will be summarised using the All subjects analysis set.

The number of enrolled subjects will be summarized. The number and percentage of subjects within each treatment group will be presented by the following categories; randomised, not randomised (and reason), received IP, didn't receive IP (and reason), completed treatment, discontinued treatment (and reason), completed study (subjects who completed IP and study, and subjects who discontinued IP but completed study assessments), and discontinued study (including reason).

The number and percentage of subjects, who discontinued IP, but remained in the study will be presented by treatment group and option of follow up (section 4.1) and will also be listed.

Kaplan-Meier plots will be produced summarizing the time (in days) to discontinuation of IP and withdrawal from the study.

Demographic data such as age, gender, and race will be summarized by treatment group for the FAS.

Various baseline characteristics will also be summarized by treatment for the FAS. These include medical, surgical and respiratory disease histories, weight, height and BMI, smoking status, history of allergy, FEV_1 (pre and post-BD) and FEV_1 reversibility at baseline, asthma duration, age at onset of asthma, asthma medications, the number of asthma exacerbations in the previous 12 months, number of asthma exacerbations requiring hospitalizations in the previous 12 months, phadiatop allergy test results, AQLQ(S)-12 at baseline, and ACQ-6 at baseline. Data collected at the latest pre-randomisation assessment will be summarised.

Medical and surgical histories will be summarized by MedDRA Preferred Term (PT) within the System Organ Class (SOC) level of MedDRA.

4.2.2 Prior and Concomitant Medications

The number and percentage of subjects receiving each medication (by ATC classification system codes and generic name) will be presented by treatment for the FAS. Separate tables will be presented for all medications received during the following periods

- Prior: Medications with a stop date ≤ the first day of IP.

- Concomitant – during treatment period: Medications that are still ongoing on the first day of IP and also medications with start date ≥ the first day of IP and ≤ the last day of IP + dosing frequency.

- Post–treatment period: Medications that are still ongoing one day after (the last day of IP + dosing frequency) and also medications with start date > the last day of IP + dosing frequency and ≤ Visit 31 (Week 72).

Note: The 'dosing frequency' equals 2 weeks for subjects randomised to the Q2W dosing regimen, and 4 weeks for subjects randomised to the Q4W dosing regimen.

Tables for maintenance medications (started prior to and ongoing after the first day of IP) will be produced displaying the baseline total daily dose of ICS. The number of subjects using other maintenance asthma medications at baseline will also be summarised. In addition, the total number of days of systemic corticosteroid treatment associated with asthma exacerbations per patient from the first day of IP up to Week 52 will also be summarised.

A separate table will be presented for subjects who take disallowed concomitant medications. Disallowed medications will include medications defined as prohibited according to Appendix H of the CSP). They will be defined following a physician review (prior to database lock) of the unique combinations of ATC code classifications and generic terms captured. Medications will be classified according to the AstraZeneca Drug Dictionary (AZDD). Percentages will be calculated relative to the number of subjects in the FAS.

All medications will also be listed by subject for the FAS.

Data from subjects who discontinued IP, regardless of level of follow up chosen will, where possible and relevant, be included in the appropriate medication summaries.

4.2.3 Exposure and Compliance

Extent of exposure to IP is defined as the number of days between the start and the end dates of study therapy plus the dosing frequency time:

Q2W: Extent of exposure (days) = (Last dosing date + 2 weeks) – First dosing date + 1.

Q4W: Extent of exposure (days) = (Last dosing date + 4 weeks) – First dosing date + 1.

In addition the total number of dosing occasions will be calculated per subject. Compliance is defined as:

Compliance (%) = (Total number of dosing occasions/total number of dosing occasions expected) x 100

Extent of exposure to IP, compliance, and total number of dosing occasions will be summarized by treatment group, using the safety analysis set.

The date and time of all IP administrations, and all missed doses will be listed using the safety analysis set.

Compliance with the regularly scheduled ICS/LABA asthma inhaler as recorded in the daily diary will be summarized by each bi-weekly period and treatment group, together with the compliance of the use of the daily diary.

4.2.4 Analysis of the primary variable

All analyses of the primary endpoint will be based on the FAS.

The primary efficacy variable is the AAER and the primary analysis is to compare the AAER for tralokinumab with placebo. The primary comparison to support the primary objective is to compare tralokinumab Q2W with placebo. The comparison between tralokinumab Q4W vs placebo is a key secondary objective in the study.

For each dosing regimen the primary efficacy variable will be evaluated through the hypothesis test:

H_0: rate ratio (tralokinumab/placebo) equals 1 *vs.*
H_1: rate ratio does not equal 1.

The null hypothesis (H_0) is that the exacerbation rate during the 52 week double-blind treatment period on tralokinumab is equal to the corresponding exacerbation rate on placebo. The alternative hypothesis (H_1) is that the exacerbation rate during the 52 week double-blind treatment period is different on tralokinumab compared with the exacerbation rate during the 52 week double-blind treatment period on placebo.

The AAER in the tralokinumab group will be compared to that seen in the placebo group using a negative binomial model. The response variable in the model will be the number of asthma exacerbations experienced by a subject, over the 52-week double-blind treatment period. The model will include covariates using the IVRS data of treatment group (tralokinumab Q2W, tralokinumab Q4W, placebo Q2W and placebo Q4W), geographical region, age group, periostin group at baseline (with levels as defined in section 1.2), and number of exacerbations in the year before the study. The logarithm of the subject's corresponding follow-up time will be used as an offset variable in the model to adjust for subjects having different exposure times during which the events occur.

The offset variables will be considered:

1) Logarithm of the number of days at risk: Follow-up date – Date of randomisation + 1. This will be the definition included in the models used in the confirmatory analyses for primary and secondary objectives.

2) Logarithm of (Follow-up date – Date of randomisation + 1) minus the number of days the subject experiences a protocol defined exacerbation between the day of randomisation and last day of follow-up. This definition will be used in sensitivity analyses (see section 4.1.2).

Follow-up date is defined in Section 3.2.1.

The standard parameterization approach (NB2) of the Negative Binomial model will be applied (**Hilbe 2011**) using PROC GENMOD (SAS procedure).

The estimated treatment effect (i.e., the rate ratio of tralokinumab versus pooled placebo, based on a treatment contrast of tralokinumab versus placebo where the two placebo groups are given weights that are proportional to the number of patients in each group), corresponding 95% confidence interval (CI), and two-sided p-value for the rate ratio will be presented. In addition, the AAER, the asthma exacerbation rate reduction, and the corresponding 95% CI within each treatment group will be presented. Estimates (and 95% CI) for the rate ratio of tralokinumab vs. placebo within each dosing regimen, along with AAER estimates (and 95% CI) for each dosing regimen, will also be presented as supporting information. Estimates (and 95% CI) based on marginal rates may also be presented to further explore treatment effects.

The individual exacerbation criteria (ER or UC visits due to asthma that required systemic corticosteroids, hospitalization due to asthma, or use of systemic corticosteroids) will be summarized descriptively, and if appropriate (i.e. sufficient number of events) analysed using a similar model as for the primary variable, including the number of exacerbations due to ER/UC visits in the year before the study (yes/no).

4.2.4.1   Subgroup analyses

The consistency of treatment effect on the primary endpoint across different subgroups will be explored based on the FAS. For each subgroup separately, a subgroup (if not already included) and a subgroup-by-treatment term will be added to the negative binomial model used in the primary analysis. The estimates (and 95% CIs) for the interaction effects, and estimates (and 95% CI) of treatment differences within each subgroup level will be reported. Subgroups analyses for evaluating DPP-4 and eosinophils will include models excluding periostin baseline as a covariate. Any subjects with a missing value for the defined subgroup will be excluded from the analysis of that subgroup.

The subgroups to be explored will include:

- Age by category: adults (>65), adults (≥18 to ≤65) and adolescents (≥12 to <18)

- ICS dose at randomisation (high, medium) – Note: the process of categorizing ICS dose into these subgroups is detailed in a separate document (ICS Final v1.0).

- Periostin baseline group: <16.44 ng/L, ≥16.44 ng/L and by quartiles

- DPP-4 baseline group: < median, ≥median and by quartiles

- Eosinophils baseline group: <300/μL, ≥300/μL

- Geographical region (Asia Pacific *[incl. Korea and Vietnam]*, North America *[incl. US]*, South America *[incl. Argentina, Colombia, Peru]*, Central/Eastern Europe *[Bulgaria, Hungary, Poland, Slovakia, Ukraine]*, Western Europe/Rest of the World *[incl. Belgium, Germany, Spain]*)

- Country

- Race (as entered in the eCRF)

- Exacerbations in the year before study: ≤2 exacerbations, >2 exacerbations

These analyses are exploratory and the results from these analyses will not affect the choice of terms used in the model for the primary analysis.

4.2.4.2   Supportive analysis of the primary variable using adjudicated data

A supportive analysis will be performed where exacerbations associated with hospitalizations and ER/UC visits that are adjudicated not to be asthma related are removed, and hospitalizations and ER/UC visits that are adjudicated to be asthma related are added, using the same negative binomial model as described in Section 4.2.4.

4.2.5   Analysis of key secondary variable(s)

All key secondary endpoints will be analysed based on the FAS.

4.2.5.1   Lung Function

The key secondary outcome variable for lung function is: Percent change from baseline in pre-dose/pre BD FEV_1 at Week 52

The percent change from baseline in pre-dose/pre-BD FEV_1 at Week 52 will be compared between each dosing regimen of tralokinumab and placebo using a restricted maximum likelihood (REML) based repeated measures analysis (using PROC MIXED).

All subjects with a baseline pre-dose/pre-BD FEV_1 measurement in the FAS will be included in the analysis. The dependent variable will be the percent change from baseline in pre-BD FEV_1 at post-baseline protocol-specified visits (up to the EOT visit). Fixed categorical effects of treatment group, geographical region, age group, periostin group, visit and treatment-by-visit interaction will be included in the model and number of asthma exacerbations in the year

prior to the study will be a fixed covariate in the model. The variance-covariance matrix will be assumed to be unstructured. If the procedure does not converge then a compound symmetric variance-covariance matrix will be used instead. Other variance-covariance structures may be considered if required. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom. The model is:

Percent change in FEV_1 = Treatment group + geographical region + age group + periostin group + number of asthma exacerbations in the year prior + visit + treatment*visit

Results will be presented in terms of LSMEANS, treatment differences in LSMEANS, 95% confidence intervals and p-values. The treatment comparisons of primary interest for this variable will be the contrast between the dosing regimens of tralokinumab and the pooled placebo group (tralokinumab QxW vs. (placebo Q2W + placebo Q4W)) at Week 52, but estimates at all visits and overall will be presented. The contrasts between tralokinumab and placebo within each dosing regimen will also be presented at all visits and overall.

Summary statistics for the percent change from baseline at all visits in pre-dose/pre-BD FEV_1 will be produced by treatment group.

Exploratory analysis of the consistency of the treatment effect on the percent change from baseline in pre-dose/pre BD FEV_1 at Week 52 across different subgroups will be explored based on the FAS. For each subgroup separately, as described in Section 4.2.4.1, a subgroup (if not already included) and a subgroup-by-treatment term will be added to the repeated measures analysis model used for the key secondary endpoint analysis. The estimates (and 95% CIs) for the interaction effects, and estimates (and 95% CI) of treatment differences within each subgroup level will be reported. If there is a significant subgroup-by-treatment term in the model then the analysis model may be re-run fitting a 3-way subgroup-by-treatment-by-time interaction term into the model.

Supportive outcome variable: Absolute change from baseline in pre-dose/pre-BD FEV_1.

Absolute change from baseline in pre-dose/pre-BD will be analysed as described for the percent change on the FAS. Included in the model will also be the baseline pre-dose/pre-BD FEV_1.

Summary statistics for the absolute change from baseline at all visits in pre-dose/pre-BD FEV_1 will be produced by treatment group.

Other secondary outcome variable for lung function: Percent change from baseline in pre-dose/post-BD FEV_1

The percent change from baseline in pre-dose/post-BD FEV_1 will be analysed and summarised as described for the pre-dose/pre-BD FEV_1.

4.2.5.2   Asthma symptoms

The key secondary outcome variable: Change from baseline in bi-weekly mean daily asthma symptom total score (combined daytime and night-time score as captured in the Asthma Daily Diary).

The change from baseline in bi-weekly means (daily asthma symptom total score, daytime score, and night-time score) at Week 52 will each be summarized and analysed using the repeated measurement approach defined for percent change from baseline in pre-dose/pre-BD FEV_1, as described in Section 4.2.5.1. Included in the model will also be the baseline bi-weekly mean daily asthma symptom score.

The proportion of asthma symptom-free days up to Week 52 will also be summarised.

4.2.5.3   Asthma specific health-related quality of life

The key secondary outcome variable: Change from baseline in AQLQ(S) +12 total score.

The change in mean score from baseline for AQLQ(S)+12 (including the domain scores) will be summarized and analysed using the repeated measurement approach defined for percent change from baseline in pre-dose/pre-BD FEV_1, as described in Section 4.2.5.1. Included in the model will also be the baseline AQLQ(S) +12 score.

Supportive outcome variable: AQLQ(S) +12 responder (yes/no)

Responder variables AQLQ(S)+12 (yes/no) will be analysed using a logistic regression model with responder at Week 52 as the response variable and covariates of treatment, geographical region, age group, periostin group, number of asthma exacerbations in the year prior to the study, and baseline AQLQ(S)+12 total score

The number and percentage of subjects with AQLQ(S) +12 total score changes ≥ 0.5 will be summarized by treatment (identified as a meaningful change). Additionally, the number and percentage of subjects achieving an improvement, no change, or deterioration will be summarized by treatment as per Section 3.3.6.

Supportive outcome measure: change from baseline to overall post-baseline mean of AQLQ(S)+12

The change from baseline to overall post-baseline mean and the difference between treatments will be estimated from the repeated measures analysis described in section 4.2.5.1.

4.2.5.4   ACQ-6 defined asthma control

The key outcome variable: Change from baseline in ACQ-6

Change in mean score from baseline for ACQ-6 (including the individual questions) will be summarized and analysed using the repeated measurement approach defined for percent change from baseline in pre-dose/pre-BD FEV_1, as described in Section 4.2.5.1. Included in the model will also be the baseline ACQ-6 mean score.

Supportive outcome variable: ACQ-6 responder (yes/no).

Responder variable ACQ-6 (yes/no) will be analysed using a logistic regression model with responder at Week 52 as the response variable and covariates of treatment, geographical region, age group, periostin group, number of asthma exacerbations in the year prior to the study, and baseline ACQ-6 mean score.

The number and percentage of subjects achieving mean ACQ-6 ≤ 0.75, 0.75 < mean ACQ-6 <1.5 and mean ACQ-6 of ≥ 1.5 at Week 52 will be summarized by treatment. Additionally, the number and percentage of subjects achieving an improvement, no change, or deterioration as per Section 3.3.5, will also be summarized by treatment.

Supportive outcome measure: change from baseline to overall post-baseline mean of ACQ-6.

The change from baseline to overall post-baseline mean and the difference between treatments will be estimated from the repeated measures analysis described in section 4.2.5.1.

4.2.6 Analysis of other secondary variable(s)

All other secondary objectives will be analysed based on the FAS.

4.2.6.1 Biomarker positive population based on subjects baseline periostin or baseline DPP-4 values

A structured assessment of the relationship between outcome, biomarkers and treatment group will be performed to evaluate the potential prognostic and predictive value of baseline periostin and baseline DPP-4, in order to identify a biomarker positive subpopulation which will be further evaluated in study D2210C00008 to confirm the effect of tralokinumab on the primary and key secondary endpoints in this population. The relative and absolute benefit of treatment in relation to the size of the potential subpopulation will be considered in selecting the final biomarker positive subpopulation. The focus will be on the Q2W treatment regimen compared to placebo, with baseline periostin and baseline DPP-4 values as the primary biomarkers of interest. Appendix C contains further details of the process and analysis methods used to determine the biomarker positive subpopulation.

The analysis of periostin and DPP-4 data described in this SAP will be based on a IUO assay for periostin and a standardised (RUO) assay for DDP-4. Data based on a commercial assay, V&V will be available at a later stage. The differences between values for the two assays is expected to be small. However, agreement between the IUO/standardised RUO assay and the commercial V&V assay will be explored and the results included in the CSR addendum. The potential impact of differences in biomarker values between the assays on the identification of a biomarker subgroup will be assessed. Appendix C contains further details.

The other measured baseline biomarkers, eosinophils, FeNO and IgE will be presented descriptively.

4.2.6.2 Proportion of subjects with ≥ 1 asthma exacerbation

The proportion of subjects with ≥1 asthma exacerbation during the 52 weeks of treatment will be addressed as a supportive variable to the primary objective. The proportion of subjects in the active tralokinumab arms will be compared with the proportion in the placebo group using a Cochran–Mantel–Haenszel test controlled for stratifying variables.

An odds ratio will be presented together with associated 95% CI and 2-sided p-value for each active dose regimen versus placebo. The number and percentage of subjects with ≥1 asthma exacerbation will also be summarized by randomised treatment.

4.2.6.3 Time to first asthma exacerbation

Time to first asthma exacerbation will be analysed as another supportive efficacy variable to the primary objective to explore the extent to which treatment with tralokinumab delays the time to first exacerbation compared with placebo. A Cox proportional hazard model will be fitted to data including treatment, age group, geographical region, periostin group, age group and number of exacerbations in the year prior to the study as covariates. Results of the analysis will be summarized as hazard ratios, 95% confidence intervals and p-values.

Time to first asthma exacerbation will be displayed graphically using a Kaplan-Meier plot.

4.2.6.4 Emergency room or urgent care visits and hospitalizations due to asthma

AAER that are associated with an ER or UC visit or a hospitalization will be analysed using a similar Negative binomial model as outlined for the primary efficacy variable in Section 4.2.4.

The response variable in the model will be the number of asthma exacerbations that are associated with an ER or UC visit or a hospitalization experienced by a subject, over the 52-week double-blind treatment period. The model will include covariates of treatment group, geographical region, age group, periostin group at baseline, and number of exacerbations resulting in hospitalisation or ER treatment (yes/no) in the year before the study. The logarithm of the subject's corresponding follow-up time (as defined in section 3.2.1) will be used as an offset variable in the model to adjust for subjects having different exposure times during which the events occur.

Supportive outcome variable: Emergency room or urgent care visits and hospitalisations due to asthma using adjudicated data

A supporting analysis will be performed where exacerbations associated with hospitalizations and ER/UC visits that are adjudicated not to be asthma related are removed, and hospitalizations and ER/UC visits that are adjudicated to be due to an asthma exacerbation are added. The same negative binomial model as described above will be used. Any discrepancies between the secondary and this supporting analysis will be tabulated.

4.2.6.5   Health care resource utilization and productivity loss due to asthma

The total number events/days will be presented by treatment along with descriptive statistics for the treatment period mean per subject, for all variables listed in section 3.3.8.

Health care resource utilization data will be combined with economic data collected independently of the study to construct comparative health economic analyses between treatment groups. These analyses will be reported separated from the main study report.

4.2.6.6   WPAI-CIQ

For each time point at which the WPAI-CIQ is administered, descriptive statistics by treatment will be presented, as described in Section 3.3.9.

Work productivity loss data will be combined with economic data collected independently of the study to construct comparative health economic analyses between treatment groups. These analyses will be reported separated from the main study report.

4.2.6.7   Nights with awakening due to asthma

The change from baseline in the bi-weekly mean number (percentage) of nights with awakening due to asthma that required rescue medication will be analysed using the repeated measurement approach defined for percent change from baseline in pre-dose/pre-BD FEV_1, as described in Section 4.2.5.1. Included in the model will also be the baseline biweekly mean number (percentage) of nights with awakening due to asthma that required rescue medication.

4.2.6.8   Rescue medication use

The change from baseline in the bi-weekly mean rescue medication use will be summarized and analysed using the repeated measurement approach defined for percent change from baseline in pre-dose/pre-BD FEV_1, as described in Section 4.2.5.1. Included in the model will also be the baseline mean rescue medication use.

The number and percentage of subjects within each treatment group who received rescue medication will be summarized by each bi-weekly period.

4.2.6.9   Home PEF (morning and evening)

The change from baseline in bi-weekly mean morning and evening PEF will each be summarized and analysed using the repeated measurement approach defined for percent change from baseline in pre-dose/pre-BD FEV_1, as described in Section 4.2.5.1. Included in the model will also be the baseline morning and evening PEF.

4.2.6.10   European quality of life-5 dimensions-5 levels (EQ-5D-5L)

The EQ-5D-5L responses from each dimension and the VAS will be summarized by treatment group. The number and percentage responses to each dimension will be summarized by assessment, and shift tables for baseline to Week 52 will be presented for each dimension. The

mean and mean change from baseline to each assessment in VAS will be summarized with descriptive statistics.

Utility derived from EQ-5D will be calculated to construct comparative health economic analyses between treatment groups. These analyses will be reported separate from the main study report.

4.2.7 Safety and tolerability

All safety variables will be summarized using the safety analysis set and data presented according to treatment received.

4.2.7.1 Adverse events (AEs)

AEs will be summarized separately for the treatment and study periods defined in Section 3.4.1. AEs occurring during the run-in period, or occurring post-treatment (as per the definition in Section 3.4.1) will be listed, but not summarized separately.

An overall summary table will be produced showing the number and percentage of subjects with at least 1 AE in any of the following categories: AEs, serious adverse events (SAEs), deaths due to AE, AEs causing discontinuation of IP (DAEs), and OAEs. OAEs will be defined following medical review of system organ classes/preferred terms after unblinding of the data. The total number of AEs in the different AE categories in terms of AE counts will also be presented (ie, accounting for multiple occurrences of the same event in a subject).

AEs will be summarized by SOC and PT assigned to the event using MedDRA. For each PT, the number and percentage of subjects reporting at least one occurrence will be presented ie, for a subject multiple occurrences of an AE will only be counted once.

AEs (by SOC and PT) will be summarized by causality and maximum intensity. If a subject reports multiple occurrences of the same AE, the maximum intensity will be taken as the highest recorded maximum intensity for each SOC and PT (the order being mild, moderate, and severe).

The following will also be summarised by SOC and PT

SAEs
OAEs in the category of safety topics of special attention
DAEs
DAEs causally related to IP
SAEs leading to discontinuation of IP
Most common AE's (frequency of >3%) (by PT only)
Deaths

Injection site reactions will be reported by preferred term for the treatment period, summarised by treatment group with unpooled and pooled placebo groups.

The approach to identifying possible anaphylaxis/hypersensitivity AEs occurring within 3 days of IP administration is described in a separate charter. Those identified AEs meeting the criteria described in this charter will be summarised by preferred term and treatment group for the treatment period and study period.

Subjects experiencing a severe infection are defined as having an AE which met one of the following:

- life-threatening,
- requiring hospitalisation,
- requiring treatment with antiviral medications, intravenous antibiotics or medications for helminth parasitic infections,
- permanent discontinuation of study drug.

Severe infections will be summarised by MedDRA high level group term, high level term and preferred term by treatment group for the treatment period and study period.

The rate of AEs per person-years at risk, calculated as (number of subjects reporting AE)/(total time at risk of AE), will also be reported for the treatment period. Rates will typically be expressed in terms of events per 100 subject-years. Total time at risk will be defined as (the date of last day of IP + dosing frequency) – date of randomisation +1.

Separate listings of subjects with AEs, SAEs, death due to AE, discontinuations due to AEs, or severe infections will be presented.

Adjudicated events (MACE (major adverse cardiac events) and malignancies) will be summarised by treatment group and listed.

4.2.7.2   Laboratory data

All continuous laboratory parameters will be summarized by absolute value at each visit by treatment group, together with the corresponding changes from baseline. The summary statistics presented will be the minimum, 1st quartile, median, 3rd quartile, maximum, mean and SD. Mean changes from baseline over time will also be plotted by treatment group.

Central laboratory reference ranges will be used for the identification of individual clinically important abnormalities, and a shift table will be produced for each laboratory parameter to display low, normal, high, and missing values. The shift tables will present baseline and maximum/minimum on-treatment values, as applicable for each parameter.

Shift plots showing each individual subject's laboratory value at baseline and at maximum/minimum will be produced for each continuous laboratory variable. If any laboratory variables show any unusual features (high or low values or a general shift in the data points) at other time points then shift plots of these data may be produced. A diagonal line indicating no change, and horizontal and vertical reference lines indicating the limits of the reference ranges will also be displayed on the shift plots.

The frequency of changes with respect to normal ranges between baseline and each post-treatment time point will be tabulated. Frequencies of clinically noteworthy values (using reference ranges) occurring during the clinical study will also be given.

In order to identify potential Hy's Law cases, maximum post baseline TBL will be plotted against maximum post baseline ALT, expressed as multiples of ULN. This plot will be repeated to show maximum post baseline TBL against maximum post baseline AST, expressed as multiples of ULN. These plots will be produced on a log scale and reference lines will be included at 2xULN for TBL and at 3xULN for ALT/AST.

For all subjects who meet the biochemical criteria for Hy's law (potential Hy's Law), a Subject Safety Narrative will be produced, and the relevant laboratory parameters will be tabulated showing all visits for these subjects. Subjects with elevated ALT or AST, and elevated TBL, at any time may be explored further graphically using individual subject profile plots.

For urinalysis data, a shift table will be generated to present changes from baseline to last observation in the on-treatment period (as defined in Section 3.4.1). The number of subjects with treatment-emergent changes will also be summarized. Here, treatment-emergent changes are defined as None/Trace at baseline to Positive, at any visit after baseline.

Any data outside the central laboratory reference ranges will be explicitly noted on the listings that are produced.

4.2.7.3   dECGs

The Investigator's assessment of the 12-lead dECG (normal or abnormal) will be listed for all subjects, along with detailing whether any abnormalities were clinically significant or not.

The number and percentage of subjects with clinically significant abnormal dECGs will be summarized by treatment group and visit.

4.2.7.4   Physical Examination

Shift tables (normal, abnormal (same as Visit 1, new or aggravated)) of Visit 1 versus last observation during treatment (as defined in Section 3.4.1) will be generated, presenting the assessment for each component of the complete physical examination separately.

A similar shift table (normal, abnormal) of baseline (typically Visit 3) versus the last observation during treatment will also be generated.

Listings of results will be produced, including the date of assessments of the brief physical exam.

4.2.7.5   Vital Signs

All vital signs parameters will be summarized by absolute value at each visit by treatment group, together with the corresponding changes from baseline. The summary statistics presented will be the minimum, 1st quartile, median, 3rd quartile, maximum, mean and SD.

AstraZeneca defined reference ranges will be used for the identification of individual abnormalities, and a shift table will be produced for each vital signs parameter to display low, normal, high, and missing values. The shift tables will present baseline and maximum/minimum during treatment value, as applicable for each parameter.

Shift plots showing each individual subject's vital signs value at baseline and at maximum/minimum will be produced for each continuous vital signs parameter.

Data for subjects who have treatment-emergent changes outside the predefined criteria will be presented, using AstraZeneca clinically important change criteria. This data presentation will include all visits for each parameter with treatment-emergent changes for this subset of subjects. A change is treatment-emergent if it occurred during treatment, using the same definition as in Section 3.4.1.

All recorded vital signs data will be listed.

4.2.7.6   Weight and BMI

Weight, BMI and height (for adolescents only) will be summarized by absolute value at each visit by treatment group, together with the corresponding changes from baseline.

4.2.7.7   Analysis of Immunogenicity variables

ADA status (positive vs. negative) at each visit will be summarized by treatment group. Descriptive statistics including number of subjects, mean, SD, median, and range of the actual ADA titres by treatment group and visit, where possible, will be provided. The ADA status across the study for each subject will also be classified and summarized by treatment group. The association of ADA status across the study with AEs/SAEs and exacerbation data may be evaluated. In addition, the association of ADA titres ($\geq$ median titre in positive subjects vs. < median titre) with AE/SAEs may be evaluated for ADA-positive treated subjects only. The following ADA results will be evaluated as proportion of subjects in cohorts together with corresponding titre summaries. However, if the number of ADA positive subjects in the safety analysis set < 5% then the ADA variables may be listed only in the CSR addendum and the analysis of immunogenicity variables performed outside of the CSR addendum based on pooled data from STRATOS 1 and STRATOS 2.

- Subjects who are ADA positive at any time (including baseline).

- Subjects who are ADA positive at baseline only.

- Subjects who are ADA positive at baseline and positive in at least one post baseline measurement.

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- Subjects who are positive at baseline regardless of post-baseline result.

- Subjects who are ADA positive post-baseline.

- Subjects who are ADA positive post-baseline and ADA negative at baseline.

- Subjects who are persistently positive; persistently positive is defined as at least 2 post-baseline ADA positive measurements or an ADA positive result at the last available assessment.

- Proportion of subjects who are transiently positive; transiently positive is defined as at least one post-baseline ADA positive measurement and not fulfilling the conditions for persistently positive.

- Subjects who are ADA positive by visit.

- Subjects who are ADA positive at a post-baseline measurement for the first time by visit.

- Subjects who are ADA positive within the Placebo group at any time.

For ADA summaries at a single time point (e.g. baseline ADA or by visit) the corresponding titre summary will be based on the titre of the positive sample for that particular visit.

For proportions summarizing across visits (e.g. any ADA post-baseline) the corresponding titre summaries will be based on the maximum titre of all positive samples for each subject.

The ADA-positive subjects across the study may also be divided into persistent positive versus transient positive. A subject will be considered as persistent positive if he/she has positive ADAs for at least two consecutive visits (with ≥ 16 weeks between the first and last positive value), or positive at the last post-baseline visit. Otherwise, the subject will be considered as transient ADA positive. The associations between ADA and AE/SAEs may be summarized for both persistent positive subjects versus transient positives subjects.

The presence of neutralizing antibodies (nAb) will be tested in all post-dose ADA-positive samples using a ligand binding assay.

Neutralizing ADA evaluations will be conducted on those serum samples that test positive for ADA at end of treatment, end of study 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.The following variables will be evaluated:

- Proportion of ADA positive subjects who are nAb positive at any time.

- Proportion of ADA positive subjects (at any time) who are nAb positive for the first time by visit.

- Subjects who are persistently nAb positive; persistently positive is defined as at least 2 post-baseline nAb positive measurements.

Proportion of subjects who are transiently positive; transiently positive is defined as at least one post-baseline nAb positive measurement and not fulfilling the conditions for persistently positive sample.

For ADA, all subjects with titre information will be shown in the data listing.

Results of the ADA analyses will be included in the CSR addendum only.

4.2.8   Analysis of pharmacokinetics

All analyses of PK variables will be based on the PK analysis set. All analyses on immunogenicity variables will be based on the safety analysis set.

Due to the limited sampling schedule, the PK assessment will be primarily based on the observed serum trough (predose) concentrations, C_{trough}. If possible and if relevant, empirical evaluation of potential impact of demographic covariates and ADA on C_{trough} may be conducted.

For descriptive statistics of C_{trough}

- if, at a given time point, 50% or less of the concentrations are non-quantifiable (NQ), the geometric mean, coefficient of variation (CV), arithmetic mean and SD will be calculated by substituting the lower limit of quantification (LLOQ) divided by 2 for values which are NQ.

- if more than 50%, but not all, of the concentrations are NQ, the geometric mean, CV, arithmetic mean and SD will be reported as not calculable (NC)

- if all the concentrations are NQ, the geometric mean and arithmetic mean will be reported as NQ and the CV and SD as NC

- the median, minimum and maximum will also be reported.

The LLOQ of tralokinumab in serum will be 0.100 μg/mL.

The PK data will be merged with those from other clinical studies for a population-based meta-analysis. Results of the meta-analysis will be presented in a separate pharmacometrics report outside of the CSR.

4.2.9 Exploratory analyses

4.2.9.1 Baseline periostin and DPP-4 levels as predictive biomarkers

The utility of subject's baseline periostin level and baseline DPP-4 levels as predictive continuous biomarkers for treatment effect on asthma exacerbation rate and asthma symptom control will be explored. Appendix C contains further details of the process and analysis methods used.

4.2.9.2 Change in biomarkers associated with up-regulation of IL-13

The change from baseline in biomarkers that may be associated with up-regulation of IL-13, eosinophils, FeNO and IgE will be explored. Summary statistics and graphical presentations of change from baseline to each assessment will be presented for each biomarker. Possible correlation between biomarker and clinical efficacy will be explored graphically.

4.2.9.3 Onset of treatment effect

Onset of treatment effect will be evaluated through graphical presentations of the results from analyses presented in sections 4.2.5.1, 4.2.5.2, and 4.2.5.3.

In addition, percent change from baseline in $FEF_{25-75\%}$ and FVC will each be summarized and analysed using the repeated measures analysis approach defined for percent change from baseline in pre-dose/pre-BD FEV1, as described in section 4.2.5.1. Graphical presentations of the results will be displayed.

The number and percentage of subjects will be presented for CGIC as described in Section 3.3.10. The number and percentage of subjects defined as responders based on categorized responses for CGIC (Improved, Much Improved, and Very Much Improved) will also be presented by treatment group and visit.

4.2.9.4 Deoxyribonucleic acid collected for future exploratory research

Results relating to the exploratory objective "To collect and store DNA for future exploratory research into genes/genetic variation that may influence clinical response to tralokinumab and provide information on phenotypes of severe asthma (optional)" will be reported outside of the CSR.

5. INTERIM ANALYSES

No interim analysis is planned for this study.

The exacerbation rate and dispersion will be monitored in a blinded fashion during the execution of the study. If the blinded estimate of the exacerbation rate and/or dispersion indicates that the assumptions in the power calculation are incorrect, appropriate analyses (blinded) will be undertaken to investigate this further. The result of these analyses may lead to an increase in sample size. Any decision to increase the sample size will be taken when

sufficient data is collected but before last subject has been randomised in the study. The analyses will be performed by AstraZeneca.

An independent Adjudication Committee, blinded to the treatment of the subjects, will evaluate cases of ER or UC visits and hospitalizations, as well as all deaths, to determine whether they are due to asthma or not. The adjudication committee will also review MACE and malignancies occurring after randomisation

An independent Data and Safety Monitoring Board (DSMB) will safeguard the interest of adolescent subjects by assessing the safety of the intervention. The DSMB will review safety data on a regular basis as set out in a DSMB charter. The data for review will be outlined in a DSMB charter. The DSMB will have access to individual treatment codes and will be able to merge these with the collected study data while the study is ongoing. For reference, the DSMB will also have access to study data from adults.

An independent review of all potential anaphylaxis/hypersensitivity events will be performed by a clinical expert (external to AZ). Results from the external review will be included in the CSR addendum. Further details of the identification and review process are contained in the Hypersensitivity and Anaphylaxis Process Charter.

6. CHANGES OF ANALYSIS FROM PROTOCOL

A number of clarifications and minor corrections have been made and are listed below:

- Section 4.2.6.2 updated to detail that odds ratio, rather than weighted difference will be presented

- Section 3.3.2/4.2.6.8/3.3.3/4.2.6.7: clarification added that change from baseline will be summarised and analysed.

- Section 3.1.1: clarification of baseline for the diary variables added

- Section 2.1.2: updated definition of the safety set to clarify the handling of subjects receiving incorrect dosing of IP.

7. REFERENCES

Hilbe 2011
Hilbe J. M. (2011) "Negative Binomial Regression" Cambridge University Press ISBN: 978-0521-19815-8.

Keene et al 2007

Keene ON, Jones MRK, Lane PW, et al. Analysis of exacerbation rates in asthma and chronic obstructive pulmonary disease: example from the TRISTAN study. Pharm Stat 2007;6:89–97.

Appendix A   Analysis windows for EQ-5D

Table 6   **Analysis windows for EQ-5D**

Visit	Target Day	Adjusted windows for weekly measures
Baseline (Week 0)[a]	1	1
Week 1	8	2-11
Week 2	15	12-18
Week 3	22	19-25
Week 4	29	26-32
Week 5	36	33-39
Week 6	43	40-46
Week 7	50	47-53
Week 8	57	54-60
Week 9	64	61-67
Week 10	71	68-74
Week 11	78	75-81
Week 12	85	82-88
Week 13	92	89-95
Week 14	99	96-102
Week 15	106	103-109
Week 16	113	110-116
Week 17	120	117-123
Week 18	127	124-130
Week 19	134	131-137
Week 20	141	138-144
Week 21	148	145-151
Week 22	155	152-158
Week 23	162	159-165
Week 24	169	166-172
Week 25	176	173-179
Week 26	183	180-186
Week 27	190	187-193

Visit	Target Day	Adjusted windows for weekly measures
Week 28	197	194-200
Week 29	204	201-207
Week 30	211	208-214
Week 31	218	215-221
Week 32	225	222-228
Week 33	232	229-235
Week 34	239	236-240
Week 35	246	241-249
Week 36	253	250-256
Week 37	260	257-263
Week 38	267	264-270
Week 39	274	271-277
Week 40	281	278-284
Week 41	288	285-291
Week 42	295	292-298
Week 43	302	299-305
Week 44	309	306-312
Week 45	316	313-319
Week 46	323	320-326
Week 47	330	327-333
Week 48	337	334-340
Week 49	344	341-347
Week 50	351	348-354
Week 51	358	355-361
Week 52	365	362-368

[a]   If the Day 1 assessment is missing, see section 3.1.1 on how baseline value is defined.

Appendix B   Accounting for missing data

To minimize the amount of missing data in the study subjects are encouraged to remain in the study after premature discontinuation of IP and complete visits according to the protocol. However, subjects dropping out of the study will potentially lead to unobserved events and measurements.

This section summarizes how we will describe the pattern of and reasons for missing data from the study. It will also describe how we plan to account for missing data, including both the primary and sensitivity analyses to assess the robustness of the treatment effect under different underlying assumptions to account for missing data.

Accounting for missing data for recurrent events (exacerbation rate endpoint)

Missing data descriptions

Tabular summaries for the percentage of subjects by the reason for discontinuation of randomised treatment as well as for withdrawal from the study will be presented by treatment to describe why subjects discontinue from randomised treatment or withdraw from the study. The time to discontinuation of randomised treatment and withdrawal from the study by treatment will be presented using Kaplan Meier plots. Since the imputation methods presented below are based on reason for discontinuation and withdrawal, the plots will also be split by treatment related/not treatment related reason for discontinuation (as defined in Table 7 and Table 8). Dependent on these outputs additional exploratory analyses may be produced as deemed necessary to further understand the pattern of missing data.

Primary analysis under the Treatment Policy Estimand using the Missing at Random (MAR) assumption

The primary analysis is under the treatment policy estimand, which implies the inclusion of all data until subjects withdraw from the study regardless of if they discontinue from randomised treatment. This allows for differences in outcomes over the entire study treatment period to reflect the effect of initially assigned randomised treatment as well as if subsequent treatments are taken. The primary analysis uses the negative binomial regression model with (logarithm of) the observation period as an offset term and assumes that missing data is MAR, a will be applied where all of the available observed data are analysed without deletion nor imputation. This is a so called direct likelihood approach (DL).

Sensitivity analyses under the Treatment Policy Estimand using both MAR and MNAR assumptions

To examine the sensitivity of the results of the primary analysis to departures from the underlying assumptions, additional analyses will be performed using the controlled multiple imputation method introduced in [1] and further developed and assessed at AstraZeneca [2, 3]. As with the primary analysis, the sensitivity analyses includes all data until subjects withdraw from the study regardless of if they discontinue from randomised treatment.

For this method, post study withdrawal counts will be imputed conditional upon the observed number of events prior to the withdrawal, a post-withdrawal model assumption, the baseline covariates included in the primary analysis model and the time remaining after discontinuation to end-of study (52 Weeks).

The method involves first fitting the primary analysis i.e. negative binomial regression model to the observed data and drawing independent samples from the joint distribution of the model parameters, creating a number of parameter sets that consist of the linear regression terms and the log of the dispersion parameter. It is assumed that the dispersion parameter is asymptotically independent of the other model parameters. This distribution is approximately the same as the posterior distribution for the parameters of a Bayesian log-linear negative binomial model with non-informative priors.

Imputed post-withdrawal counts are then generated for each discontinued subject by, for each generated set of model parameters, drawing a random number from the probability function for post-withdrawal counts, conditioned on the observed number of events prior to withdrawal for that subject. The conditional distribution for subject i is a negative binomial distribution with probability of event p_j and dispersion parameter $\gamma + y_i$, where y_i is the number of counts before withdrawal from the study, γ is the dispersion parameter estimated from observed data. The j denotes the treatment arm. Furthermore,

$$p_j = \frac{p_{j,2} - p_{j,1} p_{j,2}}{1 - p_{j,1} p_{j,2}} \qquad (1)$$

where $p_{j,1}$ is the negative binomial distribution parameter for probability of event before withdrawal from the study, and $p_{j,2}$ is the corresponding post withdrawal parameter determined by various assumptions and the baseline covariates included in the primary analysis model (the intensity λ_j is $p_j / (1 - p_j)$)

The imputed number of exacerbations is then combined with the observed exacerbations and data is analysed using the primary analysis methodology . This analysis is repeated multiple times and the results combined using Rubin's formulae [7, 8].

The following assumptions that will be used to determine $p_{j,2}$ and impute the missing data who withdraw early from the study:

(a)   MAR: Missing counts for a subject is imputed using the observed event rate within the treatment group of that subject ($p_{j,2} = p_{j,1}$).

(b)   Partial Dropout Reason-based Multiple Imputation (Partial-DRMI): Missing counts will be imputed differently depending on the reason for dropout; counts for subjects in the Tralokinumab arms who dropped out for a treatment related reason are imputed based on the expected event rate in the placebo arm ($p_{T,2} = p_{P,1}$), whereas the remaining subjects who have dropped out are imputed assuming MAR. Treatment related reasons include (1) AEs, (2) Death and (3) development of study specified reasons to stop active treatments.

(c)   Dropout Reason-based Multiple Imputation (DRMI): As for Partial-DRMI with treatment related reasons and also including severe non-compliance of protocol.

Together with the primary analysis these sensitivity analyses are considered to cover the range from realistic to plausible worst case assumptions about missing data. The MAR multiple imputation approach is expected to correspond closely to the primary analysis, and is included to allow for comparisons with MNAR assumptions (specifically methods b and c) using the same multiple imputation methodology

The dropout reason-based multiple imputation (DRMI) approach was selected as the most conservative approach based on the fact that placebo subjects are receiving standard of care and are not expected to change to a substantially more effective treatment after withdrawing from study or study treatment. For subjects receiving Tralokinumab who withdraw from the study due to treatment related reasons it is assumed that at worst they would be on the standard of care treatment i.e. the placebo arm. For subjects receiving Tralokinumab who withdraw from the study due to non-treatment related reasons it seems reasonable to assume they would be similar to those subjects who complete treatment.

Some reasons for withdrawal are clearer to define as treatment related (*Adverse Events, Death, Development of study-specific discontinuation criteria*) or non-treatment related (*Subject lost to follow up, eligibility criteria not fulfilled*). Other reasons are less clear such as subject decision and 'Other'; a review of each subject who withdraws from the study will therefore be carried out prior to unblinding the study. The review will include assessment of the reason for discontinuation of randomised treatment for those subjects who discontinued randomised treatment and then withdrew from the study and also free text for when the reason for withdrawal or discontinuation of randomised treatment is subject decision or other. Based on this review the default assumptions for Partial-DRMI and DRMI as described in b), c) and Table 7 may be changed. A list of these subjects and the assumptions made under DRMI and Partial-DRMI will be documented prior to unblinding of the study.

A summary of reasons for subjects withdrawing from the Tralokinumab treatment arms and the corresponding treatment arm used to calculate the imputation exacerbation rate under MAR, Partial-DRMI and DRMI is given in Table 7.

Table 7   **Treatment arms used to calculate imputation rate, by reason for withdrawal**

Reason for withdrawal	MAR	Partial-DRMI	DRMI
Adverse Event	Tralokinumab	Placebo	Placebo
Development of study-specific discontinuation criteria*	Tralokinumab	Placebo	Placebo
Death	Tralokinumab	Placebo	Placebo

Reason for withdrawal	MAR	Partial-DRMI	DRMI
Severe non-compliance to protocol	Tralokinumab	Tralokinumab	Placebo
Eligibility criteria not fulfilled	Tralokinumab	Tralokinumab	Tralokinumab
Subject lost to follow up	Tralokinumab	Tralokinumab	Tralokinumab
Subject decision	Tralokinumab	Based on review prior to study unblinding	Based on review prior to study unblinding
Other	Tralokinumab	Based on review prior to study unblinding	Based on review prior to study unblinding

Note: All subjects on exacerbation rate in the placebo arm are imputed using the placebo arm rate
*Development of study-specific discontinuation criteria are based on the following: anaphylactic reaction to the IP requiring administration of epinephrine, development of helminth parasitic infestations requiring hospitalization, intensive care unit admission for asthma-related event, malignancy.

In addition, a tipping point analysis may be performed using the same model as above; Missing counts for a subject will be imputed, according to the rate of the arm that the subject belongs to multiplied by a factor delta (p_j is calculated using the MAR assumption and then the imputation rate is calculated as $\lambda_j^* = \delta_j \lambda_j$).

A series of analyses will be performed with a range of increasing deltas for the two arms (δ_P and δ_T for placebo and tralokinumab groups respectively) so that one could assess at which point the study conclusions would change from favorable to unfavourable; i.e., to identify a tipping point.

In this assessment, the placebo group is assumed to improve after withdrawal and the tralokinumab group is assumed to worsen after withdrawal. Therefore, $\log(\delta_P)$ will be varied from -1.5 to 0 in increments of 0.5 and $\log(\delta_T)$ will be varied from 0 to 1.5 in increments of 0.5. This corresponds to deltas between 0.22 and 1 for the placebo group and deltas between 1 and 4.5 for the tralokinumab group. If statistical significance ($p \leq$ the alpha level used according to the testing strategy described in section 4.1.1) is maintained among the matrix of possible δ combinations, the comparison is deemed robust to missing data. For a given comparison, if a tipping point was observed with analysis at 0.5 increments, the δ values will be further refined down to 0.25 increments for the relevant interval. For example if a tipping point is identified when increasing $\log(\delta_T)$ from 1 to 1.5, the matrix will be expanded to include also the value $\log(\delta_T) = 1.25$.

On-Treatment Analyses (Efficacy and Effectiveness estimands)

In addition primary and sensitivity analyses described previously, two alternative estimands will be estimated using only the on initial randomised treatment data:

- Efficacy estimand - what would have been the outcome if all subjects had stayed on study treatment: This will be estimated using the primary analysis method but

including only data from subjects whilst being on initial randomised treatment, and assuming MAR subsequently.

- Effectiveness estimand with assumed loss of effect post discontinuation of Tralokinumab: This will be estimated using the DRMI and Partial-DRMI controlled imputation approaches including only data from subjects whilst on treatment.

Therefore the primary analyses and sensitivity analyses will be repeated including only data from subjects whilst being on initial randomised treatment i.e. excluding data once subjects discontinue from randomised treatment.

A summary of reasons for subjects withdrawing from the Tralokinumab treatment arms and the corresponding treatment arm used to calculate the imputation exacerbation rate under MAR, Partial-DRMI and DRMI are given in Table 8. As for subjects who withdraw from the study, a review of each subject who discontinued randomised treatment will be carried out prior to unblinding the study where the default assumptions for DRMI and Partial-DRMI as described in Table 8 may be changed. Again a list of these subjects and the assumptions made under DRMI and Partial-DRMI will be documented prior to unblinding of the study.

Table 8   **Treatment arms used to calculate imputation rate, by reason for discontinuation of treatment**

Reason for discontinuation of IP	MAR	Partial-DRMI	DRMI
Adverse Event	Tralokinumab	Placebo	Placebo
Development of study-specific discontinuation criteria*	Tralokinumab	Placebo	Placebo
Severe non-compliance to protocol	Tralokinumab	Tralokinumab	Placebo
Subject lost to follow up	Tralokinumab	Tralokinumab	Tralokinumab
Subject decision	Tralokinumab	Based on review prior to study unblindng	Based on review prior to study unblindng
Other	Tralokinumab	Based on review prior to study unblindng	Based on review prior to study unblindng

Note all subjects on exacerbation rate in the placebo arm are imputed using the placebo arm rate
*Development of study-specific discontinuation criteria are based on the following: anaphylactic reaction to the IP requiring administration of epinephrine, development of helminth parasitic infestations requiring hospitalization, intensive care unit admission for asthma-related event, malignancy.

Using on treatment data is easier to interpret as it is not impacted by any subsequent pattern of alternative treatments once subjects discontinue from randomised treatment. The efficacy estimand together with the reason for and timing of why a subject might not tolerate the treatment allows for the simplest interpretation as it describes the treatment effect for subjects

who adhere to treatment together with why and when they might not adhere to treatment. Sensitivity analyses using the effectiveness estimands under the Partial-DRMI and DRMI allow for alternative assumptions to be made based on reasons for discontinuation.

Overall summary of analyses to account for missing data

A summary of the different analyses to be carried out under different estimands and assumptions are described in Table 9.

Table 9   **Summary of analyses by estimands**

	Treatment Policy Estimand				On-Treatment Analyses (Efficacy and Effectiveness estimands)			
	DL	MAR	Partial-DRMI	DRMI	DL	MAR	Partial-DRMI	DRMI
Population	On-treatment + post-discontinuation of randomised treatment				On-treatment			
Estimand	Treatment policy (MAR)		Treatment policy (MNAR)		Efficacy (MAR)		Effectiveness (MNAR)	
Exacerbation rate for imputation in Tralo arm**	No explicit imputation*	Tralo rates for all reasons for withdrawal	Placebo rate for AE, Death and development of study specified reasons to stop active treatments, otherwise Tralo or based on review prior to study unblinding	Placebo rate for AE, Death, development of study specified reasons to stop active treatments and Severe non-compliance to protocol, otherwise Tralo or based on review prior to study unblinding	No explicit imputation*	Tralo for all reasons for withdrawal	Placebo rate for AE and development of study specified reasons to stop active treatments, otherwise Tralo or based on review prior to study unblinding	Placebo rate for AE, development of study specified reasons to stop active treatments and Severe non-compliance to protocol, otherwise Tralo or based on review prior to study unblinding
Definition for $p_{j,1}$ and $p_{j,2}$ in formula (2) ****.		$p_{j,2} = p_{j,1}$ For all treatment arms j=T and P	$p_{T,2} = p_{T,1}$ for reasons above otherwise $p_{T,2} = p_{P,1}$ $p_{P,2} = p_{P,1}$	$p_{T,2} = p_{T,1}$ for reasons above otherwise $p_{T,2} = p_{P,1}$ $p_{P,2} = p_{P,1}$		$p_{j,2} = p_{j,1}$ For all treatment arms j=T and P	$p_{T,2} = p_{T,1}$ for reasons above otherwise $p_{T,2} = p_{P,1}$ $p_{P,2} = p_{P,1}$	$p_{T,2} = p_{T,1}$ for reasons above otherwise $T = p_{P,1}$ $p_{P,2} = p_{P,1}$

* Implicitly assumes unobserved rate the same as observed

** All subjects on exacerbation rate in the placebo arm are imputed using the placebo arm rate (i.e. $p_{P,2} = p_{P,1}$)

*** P denotes Placebo, T denotes Tralokinumab Q2 or Q4

**** Note can be over written by review prior to study unblinding

Forest plots will be used to show the primary analysis results along with the missing data sensitivity and alternative estimand analysis results.

It is noted that if the primary analysis is statistically significant, it is not necessarily expected that all sensitivity analyses will also give statistically significant results. If the results of the sensitivity analyses provide reasonably similar estimates of the treatment effect to the primary analysis, this will be interpreted as providing assurance that neither the lost information nor the mechanisms which cause the data to be missing have an important effect on primary analysis conclusions. Based on these outputs and the drug's mechanism of action, the plausibility of the assumptions we make about missing data in the different analyses will be considered and described in the clinical study report.

Accounting for missing data for selected continuous key secondary endpoints (Percent change from baseline in pre-dose/pre-BD FEV1 and Change from baseline in ACQ-6 at Week 52)

Sensitivity analyses of change from baseline in bi-weekly mean daily asthma symptom score and change from baseline in AQLQ(s)+12 total score may be available if requested. These will be using the same approaches as specified below.

Missing data descriptions

In addition to the tables and figures suggested above, plots of change from baseline vs time, by dropout pattern (e.g. completers vs non-completers, split by reason for dropout and/or split by last available visit) will also be produced.

Primary analysis under the Treatment Policy Estimand using the MAR assumption

As for the primary variable, the primary analysis of the continuous key secondary endpoints includes all data captured during the trial and is therefore considered to be under the treatment policy estimand. The Mixed Model Repeated Measures model (MMRM) used is a DL approach which is valid under the MAR assumption.

Sensitivity analysis under the Treatment Policy Estimand using MNAR assumptions

Sensitivity analyses of the repeated measures analyses will be performed for the continuous key secondary endpoints using controlled sequential multiple imputation methods based on pattern mixture models, as described in [8].

The method is analogous to the multiple imputation of exacerbation events and the imputation process consists of a sequence of MI steps, where each step is intended to impute missing values at one time-point only. This model will assume that some pre-specified subset of subjects who withdraw from the study have correlations with future (unobserved) visits similar to subjects in the placebo arm. As for the exacerbation events, this allows us to assess various deviations from the MAR assumption.

The assumptions that will be used to impute the missing data who withdraw early are as follows:

(a)   MAR: Assumes that the trajectory for subjects who dropped out in each arm is similar to those observed in their own treatment arm

(b)   Partial-DRMI: Assumes that the trajectory for subjects in the Tralokinumab arms who dropped out for treatment related reasons (according to the same classification as for the Partial-DRMI analysis of the primary endpoint) is similar to that of the placebo subjects, whereas the remaining subjects who has dropped out are imputed assuming MAR.

(c)   DRMI: As for Partial-DRMI with treatment related reasons being the same as for the DRMI analysis of the primary endpoint

Approaches b) and c) can be considered more conservative than the approach for the primary analysis because the assumptions mean that as soon as subjects withdraw for a treatment related reason, they begin to worsen immediately.

Imputation will be done in two steps, the non-monotone (intermediate) missing values will be imputed first (Markov chain Monte Carlo (MCMC) method is used to partially impute the data using SAS PROC MI) and then the missing value at each visit will be imputed using a sequential regression method (using MONOTONE REG option of SAS PROC MI). The imputation model will include the baseline covariates used in the primary analysis model.

The MNAR imputation is achieved by only including selected data at each stage of the imputation. For example, to impute missing values at time t for subjects in the Tralokinumab arms, that dropped out for treatment related reasons, include only placebo observations up to and including time t, plus observations from subjects in the Tralokinumab arms, that dropped out for treatment related reasons, up to and including time t-1. This is done for each visit, one at a time using observed data. Placebo missing observations and Tralokinumab observations that are not missing for treatment related reasons are imputed assuming MAR and follow the pattern of observed placebo observations in each treatment arm respectively. 100 imputations will be carried out, and a seed of 784478 will be used.

The imputation models will based on absolute values (including the baseline value) and change from baseline will be calculated in imputed datasets. The analysis of each of these imputed datasets will be as described for the primary analysis in section 4.2.4 and these will be combined using SAS procedure PROC MIANALYZE.

In addition, an exploratory tipping point analysis may be performed using the same methodology as above; subjects who withdrew will have their first imputed efficacy score worsened by some amount delta. This results in a one-time shift towards a worse value in the outcomes of subjects that withdrew after a given visit. Again, a series of analyses will be performed with a range of increasing deltas for the two arms (δ_P and δ_T for placebo and tralokinumab groups respectively) to identify a tipping point.

In this assessment, the placebo group is assumed to improve after withdrawal and the tralokinumab group is assumed to worsen after withdrawal. Therefore, for FEV_1 δ_P will be varied from 0 to 300ml in increments of 100ml and δ_T will be varied from 0 to -300ml in increments of 100ml. If statistical significance (p≤ the alpha level used according to the testing strategy described in section 4.1.1) is maintained among the matrix of possible δ combinations, the comparison is deemed robust to missing data. For a given comparison, if a tipping point was observed with analysis at 100 increments, the δ values will be further refined down to 50ml increments for the relevant interval. For example if a tipping point is identified when increasing δ_T from -300ml to -200ml, the matrix will be expanded to include also the value δ_T = -250ml.

Similarly for the ACQ-6 score, δ_P will be varied from 0 to -3 in increments of -1 and δ_T will be varied from 0 to 3 in increments of 1.

On-Treatment Analyses (Efficacy and Effectiveness estimands)

Analogously to the approach for the primary endpoint, efficacy and effectiveness estimands will be estimated using on-treatment data and the methods described above.

Results for continuous endpoints will be presented as per the recurrent event sensitivity analyses

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Appendix C   Analyses to identify predictive properties of two selected biomarkers and support the definition of a biomarker positive population

This appendix to the statistical analysis plan of D2210C00007 outlines the pre-defined analyses that will assess the potential predictive properties of baseline periostin and baseline DPP-4 for the treatment effect of Tralokinumab and to support the definition of a biomarker sub-population where Tralokinumab is expected to have enhanced efficacy. Baseline periostin and baseline DPP-4 were selected based on both biological plausibility and clinical relevance. Analysis of the other biomarkers included in this study, eosinophils, FeNO and IgE, will be performed as supportive measures.

It should be noted that the analyses presented below are not intended to automatically define a biomarker and biomarker population. Instead, the intention is that the results will, as a whole, provide the basis for decision making before un-blinding D2210C00008. It should also be noted that no data or results from study D2210C00008 will be a part of these analyses.

Further details on the design and analysis of the D2210C00007 and D2210C00008 studies can be found in respective study CSP/SAP. The analysis specified here include and expand upon what is stated in Section 8.5.7.5 of the CSP for D2210C00007. This appendix will not replace the analyses stated in the SAP for D2210C00007 and D2210C00008 in any way.

The assessments will initially be based on baseline periostin and DPP-4 values derived using an investigational assay (IUO for periostin and a standardised RUO for DPP-4), when commercial V&V assay for the selected biomarker is available the agreement between assays will be assessed and selected results from this initial analysis will be confirmed using the V&V assay values. This will be done prior to unblinding study D2210C00008.

In addition, supportive exploratory analyses of baseline eosinophils will be provided to add context to the periostin and DPP4 findings.

Three possible outcomes are considered:
1. A biomarker sub-population based on baseline periostin is defined where patients are included in the sub-population if their baseline value exceeds a pre-defined cut-off level.
2. A biomarker sub-population based on baseline DPP-4 is defined where patients are included in the sub-population if their baseline value exceeds a pre-defined cut-off level.
3. No biomarker sub-population is defined; this would be an outcome if no biomarker sub-population with enhanced effect could be identified. In that case, no subgroup will be pre-defined in D2210C00008 and the confirmatory testing strategy will be adjusted by allocating all alpha to the all-comer population.

The analyses will be performed on D2210C00007 data and will support the definition of a biomarker sub-population to be pre-defined in the SAP for study D2210C00008, prior to un-

blinding of that study. The effect of tralokinumab on the primary and key secondary endpoints in the biomarker positive population is planned to be formally tested in D2210C00008 (details are given in the D2210C00008 SAP).

The primary aim of these analyses is to, based on D2210C00007 only:
1. Assess the potential predictive properties of baseline periostin and baseline DPP-4 for the treatment effect of Tralokinumab in reducing the asthma exacerbation rate and to characterize the relationship between biomarkers, exacerbation rate and treatment.
2. Identify key candidate definitions for the biomarker positive population to be confirmatory tested in D2210C00008. This assessment will be primarily based on enhanced effect of Tralokinumab in reducing asthma exacerbation rate. Analyses based on key secondary and other endpoints are considered supportive.

Prior to assessing the potential predictive properties of the biomarkers, the homogeneity in baseline biomarker distribution within the FAS population will be assessed together with potential confounding effects of non-biomarker baseline covariates. This will help establish the potential limitations that this may pose on the interpretation of predictive properties and the selection of a subgroup with enhanced effect.

General principles

The focus of the analyses will be on the Q2W treatment regimen compared to placebo and the two placebo regimens will be pooled in all analyses. The Q4W treatment regimen may be used to support findings for Q2W. The analyses supporting the definition of a biomarker positive population, including the assessment of the predictive properties of the biomarkers will be based on the full analysis set based on an ITT as randomised approach.

The key efficacy variable used to identify a biomarker positive subgroup is the number of asthma exacerbations during the randomised treatment period (up to week 52). Percent change from baseline to week 52 in pre-dose/pre-BD FEV1, and change from baseline to week 52 in asthma symptom score, ACQ-6 and AQLQ (S) +12 score will be included in supportive analyses.

As a preliminary step, the homogeneity in baseline biomarker distribution within the overall study population will be assessed:

- Descriptive statistics will be presented to assess the distribution of biomarkers and the potential confounding effect of non-biomarker baseline covariates within the study population, including
  - Histograms/density plots showing the distribution of baseline periostin and DPP-4, over all and by treatment group.
  - Scatterplots and boxplots showing the relationship between each biomarker and various non-biomarker baseline covariates
- Non-biomarker covariates that are considered likely to be correlated with either of the biomarkers may be further assessed with regards to the impact on the assessment of the biomarkers. If a non-biomarker covariate clearly confounds the predictive

properties of baseline periostin or DPP-4, it may for example lead to a biomarker being excluded from further assessments or that only a subset of the patient population is included when identifying a biomarker positive population (e.g. only adults). The non-biomarker covariates of interest currently includes: geographical region, number of exacerbations in the year before entering the study, body mass index (BMI), smoking status, ICS dose, race and age.

Assess the predictive properties of baseline periostin and DPP-4

To assess the potential predictive properties of baseline periostin and baseline DPP-4, a number of analyses will be considered:

- Graphical presentation of parametric and non-parametric models assessing and characterizing the relationship between exacerbation rate and baseline biomarker value by treatment group; including
    - Scatterplots (with smooths and a graphical representation of negative binomial regression model) and
    - forest plots based on negative binomial regression models for asthma exacerbation counts and partitions of data based on each biomarker
- A likelihood ratio test to assess whether at least one of the continuous biomarkers generally have predictive properties of the tralokinumab treatment effect. The test will compare a model with only treatment and biomarkers as covariates to a model that also includes biomarker-by-treatment interactions
- The probability of observing a significant interaction in D2210C00008, and pooled D2210C00007 + D2210C00008, will be estimated based on simulations using the estimated negative binomial model of asthma exacerbations with regression terms for treatment, biomarkers, other baseline covariates and interactions between biomarker and treatment in D2210C00007. The probability will be calculated for DPP-4 and periostin separately.

In addition, the biomarker may also be assessed for their predictive properties of Tralokinumab effect on the key secondary endpoints listed above.

Define the biomarker subgroup

If potential predictive properties for treatment effect on asthma exacerbation rate is supported by the above analyses the next step is to define candidate biomarker subgroups. The subgroup definition will consist of a biomarker and a cut-off value: a patient is to be included in the biomarker subgroup if their continuous baseline biomarker value exceeds the specified cut-off value. Subgroups based on either baseline periostin or baseline DPP-4 and cut-offs with an observed proportion of subjects of between 30% and 70% of the study population are to be considered.

The analyses supporting the identification of possible biomarker population definitions, and the selection of a single biomarker population to be tested in D2210C00008, include:

- Application of a structured search algorithm: A search algorithm, Subgroup Identification based on Differential Effect Search (SIDES, Lipkovich and Dmitrienko, 2014), will be applied to identify potential subgroups with desirable properties. The uncertainty in the determination of a subgroup will be assessed, via various robustness checks, for example by comparing the results to what is expected when there is no differential treatment effect across biomarker levels and obtaining the distribution of cutoff values identified by the search algorithm using a resampling approach. Also, the effect of rounding the cut-off value identified by SIDES (e.g to the nearest integer) will be assessed.

- Forest plots based on negative binomial regression models for asthma exacerbation counts will be presented to assess subgroups based on individual biomarkers, both for the subgroups identified by the search algorithm and subgroups based on various cut-off values (quartiles and decentiles) for each biomarker (and the corresponding complementary subgroups).

- The probability of a significant treatment effect on asthma exacerbation rate in the biomarker positive population in D2210C00008, given the observed effect for this population in D2210C00007 will be estimated based on simulations (with the prevalence of the biomarker positive population estimated to be that observed in D2210C00007). As above, the simulations will be based on the estimated negative binomial model of asthma exacerbations with regression terms for treatment, biomarkers, other baseline covariates and interactions between biomarker and treatment. The probability will be calculated for DPP-4 and periostin separately. The sensitivity to changes in placebo exacerbation rate will be assessed as well as higher or lower prevalence estimates for the biomarker positive population (+/- 10% from observed D2210C00007 prevalence).

- Forest plots will also be applied for the supportive variables; change from baseline to week 52 in pre-dose/pre-BD FEV_1, asthma symptom score, ACQ-6 and AQLQ (S) +12 score. In the forest plots, results for these endpoints will be presented for the biomarker positive subgroups that are identified by the search algorithm above. The search algorithm above may also be applied to these variables to further assess the consistency across endpoints and treatment comparisons.

The final decision of what subgroup to be confirmatory tested in study D2210C00008 will be made considering all produced outputs as well external knowledge and clinical utility. The choice of biomarker threshold will be solely based on D2210C0007 data. As previously noted, a potential outcome of the analyses is that the data does not sufficiently support the selection of any subgroup.

Safety concerns are not expected to be related to any of the biomarkers and are therefore not considered when assessing data to find a suitable biomarker subgroup. However, when the subgroup has been defined based on an assessment of efficacy, selected safety output will be produced for that subgroup and its complementary subgroup.

Sensitivity analyses

Sensitivity analyses will be performed to:
- Assess whether the subgroup identification is largely affected by restricting the subgroup size to being between 30% and 70% of the overall population. There may, for example, be a subgroup smaller than 30% with a substantially higher observed enhanced effect.
- Assess impact of missing data, both for missing biomarker values at baseline and unobserved exacerbations due to study withdrawals
- Assess potential imbalance of biomarker distribution within treatment groups: The randomisation is stratified with regards to periostin. For DPP-4 there is a small risk that the distribution differs between the treatment groups and this may potentially lead to an artificial increase or decrease of the observed predictive properties of DPP-4. To aid the interpretation of the results, histograms of the biomarkers by treatment group will be produced and the potential impact of outliers will be assessed.

Agreement of IUO/Standardised RUO and V&V assays

The assessment to identify predictive biomarkers and a biomarker positive subgroup in D2210C00007 will initially be based on values from an investigational assay (IUO for periostin and a standardised RUO for DPP-4). The definition of the biomarker subgroup in D2210C00008 will however be based on values from the final commercial (V&V) assay. The two versions of the assay are expected to give very similar results. They will however be explored in D2210C00007 with regards to the within sample agreement of the two assays.

The agreement within patient for the baseline biomarker values between the different assays will be assessed; i.e. agreement between the standardized RUO (DPP-4) or IUO (periostin) and the corresponding commercial V&V assays. Only the biomarker selected in the initial definition of the biomarker subgroup (based on the IUO/standardized RUO) will be assessed with regards to its commercial V&V assay.

The assessment of agreement will include:

- Assessment of the univariate distribution of each assay
- Assessment of the bivariate distribution of the two assays; standardized RUO vs. V&V and IUO vs. V&V respectively. These analyses will include
  - Graphical output (scatterplots of RUO/IUO data vs V&V data, Bland-Altman plot of RUO/IUO vs V&V
  - Spearman and Pearson correlation coefficients will be calculated
  - Contingency tables presenting number of subject above and below the defined cut-off (percentile) based for the RUO vs V&V.

If the assessment of agreement show a non-ignorable difference (e.g a Spearman correlation coefficient < 0.85 or if >10% are not in agreement in the contingency tables) between the 2 assays additional analyses may be performed to assess the consistency in predictive properties for the 2 assays. These analyses may include the analyses specified in Table 10.

Table 10   Analyses repeated with the V&V assay

Endpoint	Analysis	Population
Primary efficacy variable AERR	Negative Binomial model (see section 4.2.4) for the biomarker positive and biomarker negative subgroups, defined using the V&V assay.	Biomarker positive & Biomarker negative using V&V assay
Key secondary variable: Percent change from baseline in pre-dose/pre BD FEV$_1$ at Week 52	Restricted maximum likelihood (REML) based repeated measures analysis (see section 4.2.5.1)	Biomarker positive & Biomarker negative
Change from baseline in bi-weekly mean daily asthma symptom total score (combined daytime and night-time score as captured in the Asthma Daily Diary).	Restricted maximum likelihood (REML) based repeated measures analysis (see section 4.2.5.2)	Biomarker positive & Biomarker negative
Change from baseline in AQLQ(S) +12 total score.	Restricted maximum likelihood (REML) based repeated measures analysis (see selction 4.2.5.3)	Biomarker positive & Biomarker negative
Change from baseline in ACQ-6	Restricted maximum likelihood (REML) based repeated measures analysis (see section 4.2.5.4)	Biomarker positive & Biomarker negative

If an acceptable agreement between assays are found, and based on the analysis specified in Table 10 there is sufficient consistency in the potential predictive properties for the biomarker using commercial assays values, then the cut-off for the proposed biomarker subgroup defined based on the V&V assay is derived as the cut-off in ng/mL that corresponds to the same percentile as for the cut-off earlier proposed based on the IUO/standardized RUO assay.

If there is not merely a difference in location of the mean of the distribution of the selected biomarker when using the V&V assay, then an alternative subgroup threshold will be defined using the methods described in the 'Define the biomarker subgroup' section within Appendix C. Forest plots based on partitions of data and scatterplots with smooths may also be produced based on V&V assay data to re-assess the prognostic and predictive properties of the biomarker.

References

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