Statistical Analysis Plan

Protocol identification No.:	AQ-PRO-005
Protocol version:	3.0
Protocol date:	15-JUN-2021
EudraCT number:	2019-002849-38
CRO study ID:	BOREAS
Study title:	A prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial to assess the efficacy, safety and pharmacokinetics of a budesonide inhalation solution (AQ001S) compared to a budesonide inhalation suspension (comparator) in adults with mild asthma.
Investigational product:	AQ001S 0.125 mg/ml (budesonide 0.125 mg/ml inhalation solution)
Development phase:	1/2a
Sponsor:	Aquilon Pharmaceuticals S.A. (Aquilon) Quai de la Boverie, 59 4020 Liège BELGIUM
CRO:	
Principal investigator	Dr.
	Trial site name:
Author of SAP:	
	Senior Biostatistician
SAP version / status:	Final v2.0

Date of SAP:	29-SEP-2022

1 Version Control

Version	Date	Section	Description
Final v1.0	06-APR-2022	ALL	Initial release
Final v2.0	29-SEP-2022	8	Added log-transformation of the primary endpoint

2 Statistical Analysis Plan Approval

Author approval:

Senior Biostatistician		
Date (dd/mmm/yyyy)	Name	Signature

Statistics approval:

Head of Biostatistics		
Date (dd/mmm/yyyy)	Name	Signature

CRO approval:

Clinical Research Professional		
Date (dd/mmm/yyyy)	Name	Signature

Sponsor approval:

Clinical and Project Leader Aquilon Pharmaceuticals S.A. Quai de la Boverie, 59 4020 Liège Belgium		
Date (dd/mmm/yyyy)	Name	Signature

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4 List of Abbreviations

AE	Adverse event
av	Airway volume
ANCOVA	Analysis of Covariance
BMI	Body mass index
CRO	Contract Research Organization
CT	Computed Tomography
D	Day
ECG	Electrocardiogram
FeNO	Fractional exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second
FIH	First-In-Human
FIVC	Forced Inspiratory Vital Capacity
FVC	Forced Vital Capacity
gCV	Geometric Coefficient of Variation
gMean	Geometric Mean
HPA	Hypothalamic Pituitary Adrenocortical
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	Inhaled Corticosteroid
ID	Identifier
IMP	Investigational medicinal product

lv	Lobe volume
MCh	Methacholine (chloride)
PC20	Concentration of methacholine provoking an FEV1 fall of 20%
PD	Pharmacodynamics
PK	Pharmacokinetics
POC	Proof-Of-Concept
RMSE	Root Mean Squared Error
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TP	Treatment Period
V	Visit

5 Introduction

The BOREAS study (Protocol AQ-PRO-005 version 3.0 from 15 June 2021, hereinafter referred to as “the protocol”) was designed to compare the efficacy, the safety and the pharmacokinetics of the budesonide inhalation solution AQ001S with those of the comparator Budesonide (budesonide inhalation suspension) in adults with mild asthma. It is a phase 1/2a First-In-Human (FIH) Proof-Of-Concept (POC) prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial.

The purpose of this statistical analysis plan (SAP) is to provide details of the statistical analyses that have been outlined in the protocol of the BOREAS study AQ-PRO-005 and to ensure that the statistical methodologies that will be used, and the resulting data listings, summary tables, figures and report which will be produced are complete and appropriate to support valid conclusions regarding the study objectives of the clinical study.

The SAP follows the principles of the Guidelines ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) Topic E3 [1] and ICH Topic E9 [2].

The statistical analysis of the data will be the responsibility of and will be performed by . Of note, the statistical analysis of the PK data will be the responsibility of and will be performed by an external party ( ).

6 Study Objectives and Endpoints

6.1 Study Objectives

6.1.1 Primary objectives

The primary objectives of the clinical trial are:

- To compare the efficacy, i.e. the bronchoprotection, of AQ001S 0.125 mg/ml with the comparator.
- To assess the safety of AQ001S 0.125 mg/ml.

6.1.2 Secondary objectives

The secondary objectives are:

- To compare the PK of AQ001S 0.125 mg/ml with the comparator
- To compare the secondary efficacy/PD of AQ001S 0.125 mg/ml with the comparator.

6.2 Study overview

6.2.1 Study design

The present trial is a prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial in mild asthmatic adults (18 to 65 years old), who are “inhaled corticosteroid (ICS)”-naïve for minimum 60 days at Screening Visit, comparing two IMPs:

IMP under investigation:

- AQ001S 0.125 mg/ml is a budesonide inhalation solution administered by nebulization once daily.

IMP used as a Comparator:

- Budesonide 0.125 mg/ml is a budesonide inhalation suspension administered by nebulization once daily.

For each subject, the clinical trial will last 77 to 85 days from Screening Visit, including two treatment periods of 29 (+2) days and one washout period of 14 (+2) days, between the treatment periods (Figure 1). In case the subject is taking or has taken ICS within the last 60 days at the time of the Pre-Trial Visit (the visit before or combined to the Screening Visit), the total duration might be a maximum of 147 days from Pre-Trial Visit (Table 1.)

Figure 1. Course of the BOREAS study

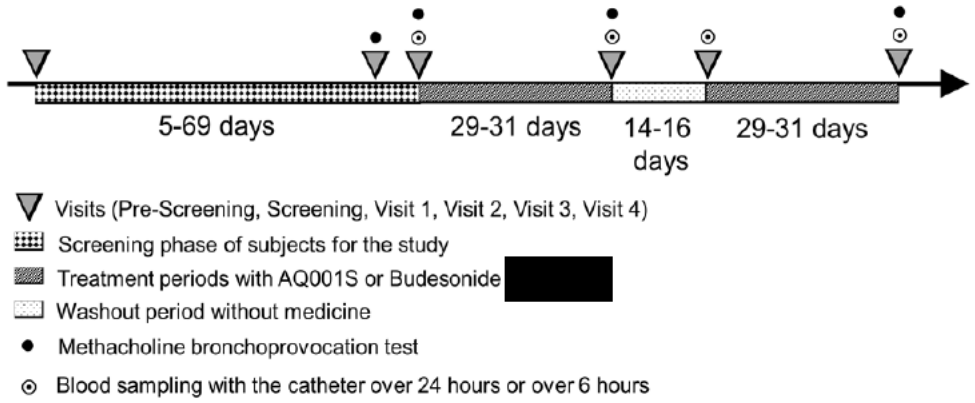

Table 1: Planned visits in BOREAS clinical trial

Periods	Visits	Timing of visits (Treatment day)
Screening	Pre-Trial Visit	Between D67 (-2) and D-5
	Screening Visit	Between D-7 and D-5
Treatment period 1 (TP1)	Visit 1 (Randomization and Baseline)	TP1 D0 and D1
	Visit 2	TP1 D28 (+2)
Washout	14 (+2) days	
Treatment period 2 (TP2)	Visit 3	TP2 D0 and D1
	Visit 4 (End-of-trial)	TP2 D28 (+2)

Regarding the main assessments performed during the visits (Figure 1 and Table 1):

- Efficacy: The concentration of the irritant methacholine (MCh) provoking an FEV1 fall of 20% (PC20) will be measured on Visits 1, 2 and 4.
- Safety (both systemic and respiratory) will be assessed during all visits
- PK of budesonide will be assessed during all visits over 24 hours (Visits 1 and 3) and over 6 hours (Visits 2 and 4).

Subject administration will be taken at trial site on D0, D1 and D28 (+2) of each treatment period. From D2 up to the penultimate day of each treatment period, subject administration will be taken at the subject's home by a study nurse.

At the first day (D0) of each treatment period, the subjects will receive one single dose of 2 ml of their assigned IMP (AQ001S 0.125 mg/ml or Budesonide 0.125 mg/ml), i.e. total dose of 0.250 mg budesonide by nebulization. Then from D1 to the end of each treatment period, the subjects will receive one dose of 1ml of their assigned IMP (once daily) for 28 (+2) days by nebulization.

The allocation of the patients to the treatment sequences A and B is done by 1 to 1 block randomization at Visit 1 (crossover design). The treatment sequences are shown in Table 2.

Table 2: Treatment sequences

Treatment sequence A	**Treatment sequence B**
Treatment period 1: AQ001S 0.125 mg/ml for 29 (+2) days	Treatment period 1: comparator 0.125 mg/ml for 29 (+2) days
Washout for 14 (+2) days	Washout for 14 (+2) days
Treatment period 2: comparator 0.125 mg/ml for 29 (+2) days	Treatment period 2: AQ001S 0.125 mg/ml for 29 (+2) days

The randomization list will be generated using a pseudo random number generator and a seed number that ensures that the allocation is non-predictable and reproducible. The randomization list will contain a mirror list to allow for subject replacements.

This is an open-label clinical trial so there is no blinding.

This is a single-center study. Hence no pooling of different centers is necessary.

6.2.2 Sample size

A total of 24 subjects will be recruited into the trial. Considering potential dropouts, a total of 20 subjects (10 per treatment sequence) are anticipated to complete the clinical trial. As discussed above, randomisation will be in a 1:1 ratio.

The sample size was calculated considering PC20 as the primary efficacy endpoint, based on the internal animal efficacy data of the sponsor and on the work of Lipworth et al. (2005) [5] and of Kraan et al. (1988) [6]. Both Lipworth and Kraan studied the response of patients in terms of PC20 to various doses of inhaled budesonide, as detailed in the BOREAS protocol.

The sample sizes obtained based on the dose-response between PC20 and inhaled budesonide dose observed by Lipworth et al. and Kraan et al. and using a power (1-beta probability) of 80% (0.8) are given in Table 3.

Table 3 – Sample size calculation based on different assumptions of dose-response between PC20 and inhaled budesonide dose

(power=0.8)	**Required sample size - number of subjects completing the trial to show superiority of AQ001S over comparator in terms of budesonide dose reduction of:**	
	-fold	**-fold**
Lipworth et al.		

Kraan et al.		

The sample size calculations based on the PC20 as a primary endpoint in BOREAS indicate that a sample size of 20 subjects completing the clinical trial is enough to demonstrate an effect of AQ001S 0.125 mg/ml over the comparator with a -fold factor ( ) as well as with a -fold factor between AQ001S 0.125 mg/ml and the comparator with a power of 80%.

6.3 Study Endpoints

6.3.1 Primary Efficacy Endpoint

The primary efficacy endpoint will be the change from baseline in PC20 after each treatment period (baseline is defined at Visit 1).

For PC20 evaluation, the subjects will perform a methacholine (MCh) challenge test. They will inhale sequentially increasing MCh concentrations (4) with a spirometry test (measurement of % fall in Forced Expiratory Volume in 1 second, i.e. FEV1) between each concentration. The test ends when MCh induces an FEV1 drop of more than 20% from baseline. The PC20 is then calculated by linear interpolation based on the antepenultimate MCh dilution and on the last MCh dilution that induced a FEV1 that fell more than 20% from baseline.

6.3.2 Safety Endpoints

The safety will be evaluated collecting the following information:

- AEs, SAEs, including asthma exacerbations,
- General tolerability: vital signs, ECG, physical examination,
- Laboratory parameters: hematology, biochemistry and urinalysis,
- Local tolerability:
    - Increased bronchial irritability,
    - Paradoxical bronchospasm,
    - Oropharyngeal examination (e.g. vocal cord myopathy, fungal infection),
- FEV_1 and FVC, measured by spirometry,
- HPA axis function: assessment of urinary cortisol/creatinine ratio in first waking urine.

6.3.3 Pharmacokinetic Endpoints

The PK endpoint which is the PK profile of budesonide in plasma will be evaluated through the PK parameters, calculated for each treatment period both at the start (24-hour PK analysis at Visit 1 and Visit 3) and at the end (abridged PK analysis over 6 hours at Visit 2 and Visit 4) of each TP.

The following PK parameters will be calculated based on the budesonide plasma concentrations obtained from bioanalysis, for each treatment period:

- Single dose 24-hour PK at the time of the first scheduled IMP administration of 0.250 mg budesonide (16 time points): C_{max}, t_{max}, C_{last}, t_{last}, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}6h}$, CL/F, V/F, λ_z and $t_{1/2z}$,
- Abridged PK at the time of the last scheduled IMP administration of 0.125 mg budesonide (7 time points): C_{trough}, C_{max}, t_{max}, C_{last}, t_{last}, and $AUC_{0\text{-}last}$, $AUC_{0\text{-}6h}$.

PK parameter definitions:

- C_{max}: maximum observed plasma budesonide concentration after dosing or during a dosing interval (ng/ml),
- t_{max}: time to reach the maximum observed plasma budesonide concentration (h),
- C_{last}: last observed plasma budesonide concentration after dosing (ng/ml),
- t_{last}: time to reach the last observed plasma budesonide concentration after dosing (h),
- λ_z: apparent first-order rate constant associated with the terminal slope of the semi-logarithmic budesonide concentration-time curve,
- $t_{1/2z}$: terminal elimination half-life associated with the terminal slope (λ_z) of the semi-logarithmic budesonide concentration-time curve,
- $AUC_{0\text{-}6h}$: area under the plasma concentration-time curve from time 0 to 6-hours post administration (ng.h/ml),
- $AUC_{0\text{-}last}$: area under the plasma concentration-time curve from time 0 to the last quantifiable concentration (ng.h/ml),
- $AUC_{0\text{-}inf}$: area under the plasma concentration-time curve from time 0 to infinity (ng.h/ml),
- CL/F: apparent clearance following budesonide inhalation (l/h),
- V/F: apparent volume of distribution following budesonide inhalation (l),
- C_{trough}: plasma budesonide concentration at the end of a dosing interval, immediately before next IMP administration (ng/ml).

6.3.4 Secondary Efficacy/Pharmacodynamic Endpoints

The secondary efficacy/PD endpoints will be the changes from baseline, after each treatment period, in:

- Symptom scores recorded using
  - daily day- and night-time asthma symptom scoring
  - Weekly validated Asthma Control Questionnaire™ (ACQ-5) questionnaire,
  - monthly validated Asthma Control Test (ACT) questionnaires,
- Daily use of as-needed reliever medications,
- fractional concentration of exhaled nitric oxide (FeNO) at Visit 1, 2 and 4
- Blood eosinophil count at Visit 1, 2 and 4 (also measured at Visit 3 only for safety purposes)
- FEV_1, at Visit 1, 2 and 4 (also measured at Visit 3 only for safety purposes)
- FIVC. at Visit 1, 2 and 4 (also measured at Visit 3 only for safety purposes).

7 General Considerations Pertaining to the Statistical Analysis

7.1 Definitions

Baseline

Visit 1 is defined to be the Baseline timepoint for the evaluation of the primary efficacy endpoint. Unless otherwise specified, for all other measurements the baseline refers to the last measurement collected prior to the first dose of study medication.

Baseline characteristics

Baseline characteristics concern subjects data at Visit 1. They include all patient's demographic, disease and treatment history and endpoint data recorded in the Registry prior to treatment start with AQ001S or Budesonide on Visit 1.

Change from baseline

Change from baseline refers to the endpoint values at Visit 2 (treatment period 1) or Visit 4 (treatment period 2) minus the baseline value at Visit 1 for that parameter.

Day 1

Day 1 is defined for each subject as the day of first dose of AQ001S or Budesonide for each treatment period (V1 or V3) receiving the double dose for 24h PK analysis (i.e. 0.250 mg budesonide).

Treatment sequence

Treatment sequence defines the order of treatment (A or B) which was attributed to the subject by the randomization procedure.

Treatment

Treatment refers to AQ001S or Budesonide .

Treatment period 1

Treatment period 1 refers to the first period (Day 1 to 28 from study start) the subject was under treatment (AQ001S or Budesonide).

Treatment period 2

Treatment period 2 refers to the second period (Day 1 to 28 from end of washout period) the subject was under treatment (AQ001S or Budesonide).

Assessment time points

Expected assessment time points after initiation of AQ001S or Budesonide treatment are on Day 28 for each treatment period (Visits 2 and 4 for TP1 and TP2, respectively).

End of treatment for an individual subject

End of treatment for an individual subject is defined as the day when AQ001S or Budesonide is stopped; the day of the last dose of AQ001S or Budesonide (Day 28, 29 or 30

of each treatment period) or the day the decision is made to permanently stop treatment, whichever occurs last.

End of study for an individual subject

End of study for an individual subject is defined as the day for withdrawal from study, follow-up call or death.

End of Study

The study will conclude when all subjects will have completed the follow-up call, have withdrawn consent or died, whichever is sooner.

7.2 Analysis Sets

7.2.1 Analysis Set for Efficacy Analyses

Efficacy Analysis set (EFF)

The EFF population encompasses all subjects that completed both treatment periods and for which the primary efficacy parameter PC20 is available at baseline (Visit 1) and after both treatment periods (Visit 2 and Visit 4).
The EFF population is a per-protocol set, in the sense that it is a subset of the full analysis set, where subjects with major protocol deviations which may have an impact on the evaluation of the primary clinical trial outcome parameter will be excluded.

7.2.2 Analysis Set for Safety Analyses

Safety Analysis set (SAF)

The safety (SAF) population will include all randomized subjects who received at least one dose of IMP (AQ001S 0.125 mg/ml or comparator).

7.2.3 Analysis Set for Pharmacokinetic Analyses

Pharmacokinetics Analysis set (PK)

The PK population includes all subjects, which have two full evaluable PK profiles and did not have protocol deviations relevant to the evaluation of PK.

7.2.4 Other Analysis Sets

Screened Analysis set (SCR)

This set includes all subjects for whom the Screening Visit was performed.

Randomized Analysis set (RAND)

This set includes all randomized subjects.

7.3 DATA SCREENING AND ACCEPTANCE

7.3.1 Data Handling and Electronic Transfer of Data

All data will be captured in a web-based electronic CRF. Data will be downloaded in CSV format and transformed into SAS® data sets for the analyses.

The PK data will be captured in an electronic database in the bioanalytical laboratory (,). Data will be downloaded in CSV format by an external vendor (,). The formal statistical analysis of the PK data is covered in the Analysis Plan of and is not included in this document.

7.3.2 Handling of Missing Data

In general, missing data will not be imputed. Calculations pertaining to person-year computations will be based on observed values only. Only in case of missing body weight will the last available weight measurement be used for calculating the dose per kg bodyweight (last observation carried forward).

7.3.3 Data review meeting

In a blinded data review meeting prior to data base lock, assignment of subjects to the analysis sets will be confirmed and any necessary determinations regarding the relevance of protocol deviations will be made. These decisions will be based on study data listings (provided by data management) and other available pertinent documents such as site protocol deviation logs.

8 Statistical Analysis

This section contains the detailed description of the statistical analysis of the data from the BOREAS clinical study.

8.1 General principles

The final statistical analysis will be performed after data base lock.

In this BOREAS cross-over study, subjects will be analysed:

- according to their originally assigned treatment sequence (i.e., as randomized) in the primary efficacy analyses
- according to the treatment actually received (i.e., as treated) in all safety analyses and other analyses.

A subject will be considered randomized as soon as a subject number has been assigned.

General rules for handling missing data: In summary tables, the number of subjects with missing data will be presented when relevant but will be excluded from the calculation of percentages (numerator and denominator) unless otherwise specified.

8.2 Specifications Related to the Whole Analysis

8.2.1 Analysis Sets

The primary efficacy analysis will be evaluated on the EFF set.

The safety endpoints will be analysed for the SAF set.

The PK analysis will be performed based upon the PK set.

The secondary efficacy and PD analysis will be performed on the SAF set.

8.2.2 Tabulation

8.2.2.1 Listings

Important Case Report Form data as well as all relevant generated and transformed variables together with the original data items will be listed. Unless otherwise specified, treatment and/or treatment sequence will always be included in listings, and listings will be sorted first by treatment if applicable, then by treatment sequence, subject identifier and finally, if applicable, by time point/visit number and/or a relevant date (e.g., date of onset of AE).

The reference date for calculation of study days will be the day of first study medication (called Day 1). For subjects randomized but not treated the date of randomization will denote Day 1. All computed durations when expressed in number of days will be calculated by the difference of the two selected dates plus one day.

8.2.2.2 Tables

Descriptive summary tables will be grouped by treatment and time point/visit as appropriate. For tables, which cannot be grouped by treatment, like e.g. subject disposition or demographics and baseline characteristics, the treatment sequence will be used as grouping variable.

For quantitative data, number of subjects, number of observations, number of missing values, mean, standard deviation, minimum, 25%-percentile, median, 75%-percentile and maximum will be shown. Unless otherwise specified, confidence intervals will be calculated at the 95% level.

Categorical data will be displayed in frequency tables showing number of missing values, absolute frequencies, and relative frequencies (in %). Presentation of these summary tables is referred to in the following as “X is/will be summarised”.

8.3 Subject Disposition

Subjects in the screened set will be listed with eligibility status, visit dates, study completion status, occurrence of temporary and/or permanent investigational product discontinuation, and reasons for study or product discontinuation (if applicable). Ineligible subjects will be listed with pertinent inclusion and/or exclusion criteria.

Assignment of subjects in the randomized set to the sets EFF, SAF, and PK, and the reasons for exclusion from any of these analysis sets, if available, will be listed. Assignment of subjects in the randomized set to EFF, SAF, and PK will be summarised by treatment sequence.

Visit completion status will be summarised by treatment and time point/visit for the EFF set.

8.4 Demographics and Baseline Characteristics

The demographic and baseline characteristics include sex and age recorded at Screening Visit will be listed and summarised by treatment sequence for the EFF set and for the SAF set, if the two sets differ.

8.5 Medical/Surgical History

Medical and surgical history items will be listed with indication, date of onset, date of resolution, treatment (if available), and outcome for the EFF set and for the SAF set, if the two sets differ. If a coding is available, then coded entities will each be summarised by treatment sequence for the EFF set and for the SAF set, if the two sets differ.

Respiratory and asthma history of subjects will be listed with date of asthma diagnosis, date of appearance of first symptoms and asthma exacerbations in the last year for the EFF set and for the SAF set, if the two sets differ. Other respiratory history will also be listed additionally if available.

8.6 Prior and Concomitant Medication

Prior and concomitant medication items will be listed with the name of the drug, the pharmaceutical form, the strength, the dose regimen, the frequency, the route of administration, the date of initiation and discontinuation, and the reason for use for the EFF set and for the SAF set, if the two sets differ. The coded prior and concomitant medication entities will be summarised by treatment sequence for the EFF set and for the SAF set, if the two sets differ. For coding the most recent WHODD version available at the time of database lock is used.

8.7 Compliance

Study product intake compliance with respect to the whole treatment phase will be listed and summarised by treatment sequence for the EFF set.

8.8 Efficacy Analysis

The primary efficacy analysis will be done using the EFF set. Statistical tests and confidence intervals will be two-sided at 5% significance level. The secondary efficacy and PD analysis will be performed on the SAF set.

8.8.1 Descriptive Analyses of Primary Efficacy Endpoint

For the parameter PC20 (concentration of MCh provoking an FEV_1 fall of 20%), baseline measurements (Visit 1) and measurements at Visit 2 and Visit 4 will be listed by subject. Baseline measurements, measurements at Visit 2 and Visit 4, and changes from baseline will be summarised by treatment group. Line plots for baseline measurements and measurements at Visit 2 and Visit 4 will be produced by treatment sequence.

8.8.2 Primary Efficacy Analysis

For primary efficacy analysis, the mean PC20 changes from baseline between AQ001S 0.125 mg/ml and the comparator will be compared by analysis of covariance (ANCOVA) for crossover design. A p-value will be calculated for the difference of the parameter between AQ001S 0.125 mg/ml and comparator. If this p-value is significant and the difference prefers AQ001S 0.125 mg/ml, superiority is shown.

Additionally, an adjusted 95%-confidence interval for the mean difference between AQ001S 0.125 mg/ml and the comparator will be obtained by the ANCOVA.

As the PC20 values are expected to follow a log-normal distribution, a $\log_{10}$-transformation is done before performing the primary efficacy analysis.

Analysis of Covariance (ANCOVA)

The ANCOVA will be used to estimate the residual error, which is used to construct the confidence intervals, moreover to evaluate the presence of period or sequence effects. The

effects considered in the ANCOVA model will be: baseline value, treatment sequence, period, and within-subject sequence.

8.8.3 Secondary Efficacy/Pharmacodynamic analysis

The secondary efficacy/PD parameters mentioned in Section 6.2.4 will also be evaluated descriptively. The individual PD parameters will be listed by subject. Frequencies/proportions for categorical (qualitative) parameters and descriptive statistics for continuous (quantitative) parameters will be calculated and summarised by treatment.

- Symptom scores recorded using
  - Daily day- and night-time asthma symptom scoring
  - Weekly validated asthma questionnaires, i.e. Asthma Control Questionnaire (ACQ)-5
  - Monthly validated Asthma Control Test (ACT) questionnaires,
- Use of as-needed reliever medications,
- fractional concentration of exhaled nitric oxide (FeNO),
- Blood eosinophil count,
- FEV_1,
- FIVC.

8.8.4 Subgroup Analyses

No subgroup analyses will be done.

8.9 Safety Analysis

All safety analyses will be done using the SAF set unless otherwise specified. Safety analyses will be descriptive in nature and no hypothesis testing is planned.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs).

8.9.1 Physical and Clinical Examination

Physical examination results (normal, abnormal – not clinically significant, abnormal – clinically significant) and description of abnormality will be listed by organ system and time point/visit. Physical examination results will be summarised as appropriate, and shift in examination results categories will be presented by treatment group and time point/visit.

Clinical examination (hight, weight, BMI) will be summarised by treatment group and visit and listed accordingly.

8.9.2 Vital Signs

For each recorded vital sign (systolic & diastolic blood pressure, body temperature, heart rate, respiratory rate, and blood oxygen saturation), all available measurements, and changes from baseline to all post-randomisation time points/visits will be listed. Vital sign measurements, and changes from baseline to post-randomisation visits, will be summarised by treatment group and time point/visit.

8.9.3 ECG

ECG assessments will be listed with date-time, ECG parameters, interpretation, and abnormalities (if any). ECG interpretation (normal, abnormal – not clinically significant, abnormal – clinically significant) will be summarised by treatment group and time point/visit.

8.9.4 Laboratory measurements

Laboratory measurement results (normal, abnormal – not clinically significant, abnormal – clinically significant) and description of the abnormality will be listed by parameter and time point/visit. Laboratory data will be compared to their reference ranges. Values outside the reference range as well as possibly clinically significant values will be highlighted in the listings.

For all recorded laboratory parameters, all available measurements will be listed. Changes from baseline to all post-randomisation time points/visits will be listed for quantitative parameters. Laboratory parameter measurements will be summarised by treatment group and time point/visit for all time points/visits. Changes from baseline to post-randomisation time points/visits will be summarised by treatment group for quantitative parameters. For laboratory parameters where normal ranges are available, shift from baseline will be tabulated by treatment group for all post-randomisation time points/visits.

8.9.5 Adverse Events

AEs will be assigned to treatments based on the recorded time of AE onset (concept of treatment emergent AEs). An overall listing of AEs and SAEs (including asthma exacerbations) by subject with information on reported term, system organ class, preferred term, date of onset, time of onset, stop date (when applicable), stop time (when applicable), actions taken, severity, seriousness, relationship to IMP and to trial procedures, and outcome will be produced.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity, and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. Absolute and relative frequencies of subjects with at least one AE will be presented and the total number of AEs will be given by treatment group. SAEs and other significant AEs will be evaluated separately.

8.10 Pharmacokinetic analysis

The PK analysis will be performed on the PK set. The statistical analysis of this section will describe and characterize the PK of AQ001S 0.25 mg/ml and Budesonide 0.25 mg/ml for the

24-hour PK at the start of each TP, and the PK of AQ001S and Budesonide 0.125 mg/ml for the 6-hour abridged PK at the end of each TP. There are no formal hypotheses being tested.

The individual plasma concentrations of budesonide will be listed and tabulated by treatment and time point.

Descriptive statistics for the PK parameters described in Section 6.3.3 will be calculated and summarised by treatment. This analysis is just mentioned here for completeness but will not be performed by .

9 Reporting Conventions

P-values will be reported to three (3) decimal places; p-values less than 0.001 will be reported as “<0.001”. Minimum and maximum will use the same number of decimal places as the original data. All other statistics, including percentage values, will be reported to one (1) decimal place greater than the original data.

Data in columns of a table will be formatted as follows:

- Alphanumeric values are left-justified (in mixed upper- and lower-case)
- Integer numbers (e.g., counts) are center-justified
- Numbers containing fraction portions are decimal aligned

All fraction numeric values will be printed with a zero to the left of the decimal point (e.g., 0.12, 0.3 etc.)

Dates will be printed according to the alpha-numerical definition in the database. Missing portions of dates should be represented on patient listings as dashed (--JAN99). Dates that are missing because they are not applicable for the patient are output as “NA” (= not applicable), unless otherwise specified.

In case that the definition of a date variable is numeric, dates will be printed in SAS® DATE9. Format (“DDMMMYYYY”: 01JAN2008).

If measurements are repeated, the first one (scheduled one) will be used in the summary table, except for screening and Day -1: then the latest measurement (nearest to first dosing) will be used. All measurements including the repeated one will be presented in the individual listings.

10 Technical Details

The analysis will be carried out according to standard operating procedure SOP001_PROGRAMMING [3]. The statistical analysis, including all tables, listings, figures of clinical and laboratory data will be performed using SAS® 9.4 or higher.

SAS® programming will be performed according to standards as defined in SOP001_PROGRAMMING [3] and related work instructions. Special attention will be paid to planning and performance of quality control measures as documented in the quality control plan for the analysis of this study (see also SOP002_PROGRAM_QC [4]).

11 List of Planned Contents of Tables, Figures and Listings

Based on this SAP, an excel file is developed which covers all tables, figures and listings to be included in the clinical study report. This document is appended to this SAP. In accordance with ICH E3 [1] the statistical output is organised in Tables and Figures as follows:

14 TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

- 14.1 Demographic data and baseline characteristics
- 14.2 Efficacy data
- 14.3 Safety data
- 14.4 Pharmacokinetics data

16 APPENDICES

- 16.2 PATIENT DATA LISTINGS

12 References

[1] International Conference on Harmonisation. Note for Guidance on Structure and Content of Clinical Study Reports (ICH E3). CPMP/ICH/137/95, 1996.

[2] International Conference on Harmonisation. Note for Guidance on Statistical Principles for Clinical Trials (ICH E9). CPMP/ICH/363/96, 1998.

[3] SOP001_PROGRAMMING, “Standard Operating Procedure for Statistical Programming”, current version

[4] SOP002_PROGRAM_QC, “Standard Operating Procedure for Quality Control of programs”, current version

[5] Lipworth BJ, Sims EJ, Das SK, Buck H, Paterson M. Dose-response comparison of budesonide dry powder inhalers using adenosine monophosphate bronchial challenge. Ann Allergy, Asthma Immunol 2005; 94:675–681.

[6] Kraan J, Koëter GH, van der Mark TW, et al. Dosage and Time Effects of Inhaled Budesonide on Bronchial Hyperreactivity. Am Rev Respir Dis 1988; 137:44–48.