Allergy Therapeutics (UK) Ltd.: PQGrass306 Statistical Analysis Plan

Interim and Final Analysis

Status: **final**

Clinical Trial Title:	A randomised, double-blind, placebo-controlled clinical trial to evaluate the efficacy and safety of PQ Grass in subjects with seasonal allergic rhinitis and/or rhinoconjunctivitis induced by grass pollen exposure
NCT05540717	
Investigational Product:	PQ Grass
Clinical Phase:	Phase III
Sponsor:	Allergy Therapeutics (UK) Ltd. Dominion Way BN14 8SA Worthing United Kingdom
Date:	October 31, 2023

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2 LIST OF ABBREVIATIONS

Abbreviation	Text
ACT	Asthma control test
ADR	Adverse drug reaction
AE	Adverse event
AESI	Adverse event of special interest
AIT	Allergen immunotherapy
ALT	Alanine aminotransferase
AR	Allergic rhinitis
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical classification
BAT	Basophil activation test
BDRM	Blind data review meeting
BDRMIA	Blind Data Review Meeting for data relevant for the interim analysis
BDRMY1	Blind Data Review Meeting for Year 1
BDRMY2	Blind Data Review Meeting for Year 2
BMI	Body mass index
BPS	Birch pollen season
Breg	regulatory B cells
CI	Confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CRP	C-reactive protein
CSMS	Combined symptom and medication score
CSR	Clinical study report
DC	Dendritic cell
dMS	daily medication score
dSS	daily symptom score
EAACI	European Academy of Allergy and Clinical Immunology
eCRF	electronic case report form
eDiary	electronic diary
EoS	End of study
FAS	Full analysis set
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GEE	Generalised estimating equation
GGT	Gamma-glutamyl transferase
GINA	Global Initiative for Asthma
GPS	Grass pollen season
ICE	Intercurrent event
ICF	Informed consent form
ICH	International Council for Harmonisation
Ig	Immunoglobulin
IgE-FAB	IgE-facilitated allergen binding
LDH	Lactate dehydrogenase

LS mean	Least squares mean

Abbreviation	**Text**
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities
mm	millimetre
mg	milligram
mL	millilitre
MNAR	Missing not at random
MSE	Mean squared error
PD	Protocol deviation
PEF	Peak expiratory flow
PEFR	Peak expiratory flow rate
PEI	Paul-Ehrlich-Institute
PT	Preferred term
PTAE	Pre-treatment adverse event
RBC	Red blood cells
PBMC	Peripheral blood mononuclear cells
RCI	Repeated confidence interval
RQLQ	Rhinoconjunctivitis Quality of Life Questionnaire
RQLQ(S)	Rhinoconjunctivitis quality of life questionnaire with standardised activities
SAE	Serious adverse event
SAF	Safety set
SAP	Statistical analysis plan
SAR	Seasonal allergic rhinitis
SD	Standard deviation
SE	Standard error
SOC	System organ class
SOP	Standard operating procedure
SPT	Skin Prick Test
SU	Standardised unit
TEAE	Treatment emergent adverse event
TOC	Trial oversight committee
US	United States of America
WBC	White blood cells
WHO	World Health Organisation

3 GENERAL

This statistical analysis plan
follows the principles of the Guidelines ICH Topic E3, ICH Topic E9, and ICH Topic E9(R1). It gives all details for the interim and final statistical analysis of this clinical trial.

3.1 Analyses planned and already performed

One interim analysis will be conducted after the grass pollen season (GPS) of the first year (Year 1) is terminated and all subjects treated in Year 1 have completed Period 3 (PreGPS, Onset of GPS, During GPS and End of GPS assessments, including Visit 11).

Based on the results of the interim analysis one of the following 3 scenarios will occur:

- The trial stops for success.
- The trial progresses to Year 2.
- The trial stops for futility.

In case the trial progresses to Year 2 based on the interim result, a recalculation of the sample size will be performed, and the sample size may be adapted.

In case the trial is terminated based on interim analysis results and this decision is documented in writing, the results of the primary analysis will be shared with the Sponsor. Furthermore, group unblinded topline results of efficacy will be prepared and distributed to the Sponsor. As the subjects will continue with the safety follow-up, all personnel involved in the conduct of the trial will stay blinded until the database including safety data has been locked.

In case the trial will continue into Year 2, no unblinding of personnel involved in the conduct of the trial will occur after the interim analysis. After all subjects enrolled in Year 2 have completed Period 3 (including Visit 11), all data relevant for the efficacy analysis will be locked and group unblinded topline results of efficacy will be generated and distributed to the Sponsor. All personnel involved in the conduct of the trial will stay blinded until the last safety follow-up is performed and all data.

3.2 SOPs to be followed

The analysis will be carried out according to Standard Operating Procedures (SOPs).

4 OVERVIEW OF THE CLINICAL TRIAL PROTOCOL

4.1 Objectives and endpoints of the clinical trial

The objectives and endpoints of the clinical trial are shown in Table 1.

Table 1 Objectives and endpoints

Objectives	Endpoints
Primary Efficacy	
To evaluate the efficacy of PQ Grass 27600 SU in subjects with grass pollen induced seasonal allergic rhinitis (SAR) and/or rhinoconjunctivitis based on symptoms and medications.	• Combined Symptom and Medication Score (CSMS) averaged over the peak grass pollen season (GPS).
Secondary Efficacy	
To evaluate the potential of PQ Grass 27600 SU to reduce symptom burden and the need for medication use.	• The number of well days during the peak GPS.
To evaluate the quality of life.	• Rhinoconjunctivitis quality of life questionnaire with standardised activities (RQLQ(S)) measured within the peak GPS.
To evaluate the effect of PQ Grass 27600 SU on IgG4.	• Serum grass-specific IgG4 at Visit 7 compared to baseline.
To evaluate the treatment effect of PQ Grass on the CSMS over the entire GPS.	• CSMS averaged over the entire (or truncated) GPS.
To evaluate the treatment effect of PQ Grass on the daily Symptom Score (dSS) and daily Medication Score (dMS) components of the CSMS over the GPS.	• dSS component of the CSMS averaged over the peak GPS and entire (or truncated) GPS. • dMS component of the CSMS averaged over the peak GPS and entire (or truncated) GPS.
Safety	
To evaluate the safety and tolerability of PQ Grass in subjects with grass pollen induced SAR and/or rhinoconjunctivitis.	• Frequency, severity and relationship of adverse events (AEs) to treatment. • Frequency of AEs leading to premature discontinuation from treatment or clinical trial. • Frequency of adverse events of special interest (AESIs). • Changes in clinical laboratory values (serum chemistry, haematology and urinalysis) between screening and Visit 11. • Changes in vital signs at all treatment visits.

4.2 Clinical trial design

This is a multi-centre, randomised, parallel group, double-blind, placebo-controlled clinical trial to confirm the efficacy and safety of the optimal effective dose of PQ Grass (27600 SU). The clinical trial will be conducted in the United States of America (US) and in 5 countries in Europe at approximately 91 clinical trial sites.

The trial will have an adaptive group sequential design with one planned interim analysis that will be performed on the subjects randomised in the first year (Year 1).

The clinical trial for each subject (regardless of whether the subject is recruited in Year 1 or in Year 2) will consist of 4 periods, encompassing 11 mandatory visits, 2 safety follow-up calls per telephone.

- Period 1: Screening (Visit 1 and Visit 1a, if applicable).
- Period 2: Randomisation and treatment (Visit 2 to Visit 7).
- Period 3: Pre-GPS, Onset of GPS, During GPS and End of GPS assessments (Visit 8 to Visit 11).
- Period 4: Telephone safety follow-up at 12 and 24 weeks after the last injection.

4.2.1 Dosing schedule and allocation to treatment

Subjects will be randomly assigned to 1 of 2 treatment groups in a ratio of 1:1 to receive 1.0

5 GENERAL ASPECTS OF THE STATISTICAL ANALYSIS

5.1 Definition of analysis sets

The analysis sets that will be used for the statistical analyses are specified in Table 3.

Table 3 Definition of analysis sets

Analysis set	Description
Full Analysis Set (FAS)	All subjects who received at least 1 injection of the investigational drug/placebo. Subjects will be analysed according to the treatment they were randomised to.
Safety Set (SAF)	All subjects who received at least 1 injection of the investigational drug/placebo. Subjects will be analysed according to the treatment that they actually received.

The FAS will be the primary analysis set and will be used to evaluate efficacy. The SAF will be used for all evaluations of safety and tolerability. The criteria used for inclusion of each subject into the respective analysis sets are detailed in the BDRM plan and the final decisions are documented in the BDRM minutes.

5.2 Protocol deviations and intercurrent events

Protocol deviations (PDs) and non-compliances reported by the trial team and/or automatically identified via pre-programmed checks are reviewed on a regular basis and classified.

Summary tables will be generated for the FAS displaying the number and frequency of subjects with at least one critical or major PD, overall and by category.

Additionally, the PDs will be classified into those that have a potential effect on the primary efficacy endpoint and those for which it is not considered likely that they have an effect.

Possible intercurrent events and PDs will be reviewed during the BDRM(s) and a final decision will be made how these events will be treated for the different estimands.

All decisions and the reasons for these decisions will be documented in the BDRM minutes, which will be finalised and signed prior to the interim analysis (BDRMIA), prior to the Year 1 efficacy analysis.

6 DEFINITIONS FOR STATISTICAL ANALYSIS

The statistical analysis (interim analysis and final analysis) will be performed after completion of the following working steps:

- All relevant electronic case report form (eCRF) data is available and has been checked for errors and/or inconsistencies.
- All external subject data relevant for the analysis as described below, e.g., eDiary data, laboratory data, is available and reconciled.
- All external non-subject data, e.g., grass pollen counts, is available.
- All queries relevant for the analysis have been answered and necessary editing of the database has been performed.
- Relevant data has been locked. The evaluation and classification of all intercurrent events and potential PDs have been completed and captured in the final BDRM minutes approved by the responsible persons.
- A detailed final SAP is available and approved by responsible personnel.

6.1 Screen failures

For the purpose of summary tables and subject data listings, screen failures are defined as subjects who consent to participate in the clinical trial but are not subsequently randomised.

The number of screen failed subjects and the reason for screen failure will be tabulated.

6.2 Handling of withdrawals (drop-outs), missing values and outliers

Discontinued or withdrawn subjects will not be replaced if they withdrew due to AE. Subjects withdrawn early from the clinical trial due to other reasons may be replaced. Data collected for subjects whose participation is terminated is evaluated with all data as collected until withdrawal and during a possible follow-up for safety.

Missing data for the primary variable on a by-day level will be handled using multiple imputation methods. Similarly, missing data in covariates included in the analysis model of CSMS will be imputed to allow the inclusion of all subjects from the FAS into the primary analysis.

ncomplete dates will be imputed temporarily for the categorisation of AEs or concomitant medications. The corresponding strategies are described in the corresponding sections.

6.3 Visit definition for analysis

All scheduled visits will be analysed as defined in the clinical trial protocol and the numbering is taken from the eCRF.

6.4 Baseline and screening

A baseline measurement will be defined as the latest measurement obtained prior to the first administered dose of investigational drug/placebo.

Screening is defined as the period before Visit 2. In case several measurements are taken during the screening period, only the later one is used for analysis.

6.5 Reference day for the statistical analysis

Day 1 is defined as the date of first administration of investigational drug/placebo. Relative days for statistical analysis will be calculated relative to Day 1. There is no Day 0 (the day before the first administration of investigational drug/placebo is day -1).

6.7 Definition of Grass Pollen Season

For the purpose of statistical analysis, the start and end of the entire grass pollen seasons (GPS) and the peak pollen seasons of each year (if applicable) will be determined based on the daily pollen counts from pollen traps local to the trial sites.

The final decisions regarding the site-specific (peak) pollen periods to be applied for the eDiary related endpoints will be determined prior to unblinding of the database and documented in the BDRM minutes for each year.

7 STATISTICAL ANALYSIS SPECIFICATION

7.1 Specifications related to whole analysis

7.1.1 Tables

Data will be summarised using suitable descriptive statistics depending on the structure of the data. Summary tables will be grouped by treatment group and by visit.

If not specified otherwise, the following treatment groups will be included:

- PQ Grass, and
- Placebo.

For continuous data, the basic statistics include the number of subjects with data available, arithmetic mean, SD, median, first and third quartile, minimum, maximum. Two-sided 95% CIs will be presented for the arithmetic mean based on the normal approximation, whenever appropriate.

In general, the minimum and maximum will have the same number of decimals as collected. Mean, median the quartiles and the CI for the arithmetic mean will be

displayed one additional decimal and the SD will be displayed with two additional decimals. For summary statistics, calculated averages will be displayed as if they were collected with one digit.

Categorical data will be displayed in frequency tables showing the number and percentage of subjects for each outcome category. Percentages will be displayed with one decimal.

7.1.2 Data listings

Individual data will be listed. All relevant generated and transformed variables will be listed next to the original data items as well. Calculated averages will be listed with 3 digits.

If not mentioned differently, data listings will be based on randomised subjects only, only a reduced set of listings will be generated for screen failures.

In general, listings will be sorted by subject number. Each listing will contain the subject number and randomised treatment group. The clinical trial period will be additionally displayed whenever applicable and reasonable.

Subject data listings for event-based data (e.g., AEs or concomitant medications) will be sorted by subject number, by event start date and then alphabetically by reported term and coded term.

7.2 Disposition of subjects

Clinical trial duration

The clinical trial duration until Visit 11 in days is defined as last subject last visit (until Visit 11) – first subject first visit + 1.

The clinical trial duration including safety follow-up in days is defined as last subject last

follow-up call – first subject first visit + 1.

The clinical trial duration until Visit 11 and the clinical trial duration including safety followup will be displayed overall, including all subjects (i.e., treated subjects as well as screen failures). Separate summaries will be generated for Year 1, Year 2 and combined, if applicable.

Subjects screened, randomised, treated, completed and discontinued prematurely

Subjects are defined as completing the clinical trial, including the safety follow-up, if the item “Premature study termination” is ticked as “No” on the “End of study evaluation” page.

Subjects are defined as prematurely discontinuing the clinical trial if this item is marked as “Yes”.

Subjects are defined as completing treatment, if the item “Premature treatment termination” is ticked as “No” on the “End of treatment” page. Subjects are defined as prematurely discontinuing treatment if this item is marked as “Yes”.

A summary table will be created including:

- the number of screenings (including re-screenings),
- the number of subjects who were screened (i.e., signed the informed consent),
- the number of screen failures,
- the number of subjects who were randomised,
- the number of subjects who were treated,
- the number and percentage of subjects who completed treatment,
- the number and percentage of subjects who prematurely discontinued treatment,
- the number and percentage of subjects who completed the clinical trial including safety follow-up, and
- the number and percentage of subjects who prematurely discontinued the clinical trial.

This analysis will be repeated by for Europe and United States separately, by pooled geographical region (if applicable), and geographical region used for randomisation.

Reason for discontinuation of treatment or clinical trial

The number and percentage of subjects discontinuing treatment including the primary reason for end of treatment and the number and percentage of subjects discontinuing the clinical trial including the primary reason for end of trial will be summarised by treatment group and overall for the FAS.

Analysis Sets

A summary table will be created showing the number of subjects randomised and the number and percentage of subjects included in each of the defined analysis sets (FAS and SAF), according to randomised treatment group and overall. Percentages will be calculated based on the randomised subjects.

Subjects per visit

The number and percentage of subjects participating at each (scheduled) visit will be provided by treatment group and overall for the FAS.

In addition, the number and percentage of subjects having the 12- and 24-weeks safety follow-up documented will be provided by treatment group and overall for the FAS.

7.3 Demographics and baseline characteristics

Demographic information collected at screening (Visit 1) will include age (at date of informed consent), sex, race, ethnicity, childbearing potential (for female subjects) and methods of contraception (if applicable), as well as smoking and alcohol consumption habits.

Height at screening (latest measurement from Visit 1 and Visit 1a, if applicable) will be displayed in cm and weight at screening (latest measurement from Visit 1 and Visit 1a, if applicable) in Kilograms (kg). Body Mass Index (BMI) will be calculated as

$$BMI\ [kg/m^2] = \frac{weight\ [kg]}{}$$

$$\frac{}{height[m]_2}$$

and displayed in kg/m^2.

Demographic data will be summarised by treatment group and overall for the FAS, and listed by subject. Separate summaries will be generated for Year 1, Year 2 and combined, if applicable.

7.4 Medical History and Concurrent Diseases

7.4.1 Definitions and conventions

A medical and allergy history including details regarding all illnesses, asthma (past or current), surgeries and any kind of allergies (to assess eligibility) at screening must be recorded in the eCRF. A full history for the 5 years prior to screening and any other significant medical history is to be documented.

Concurrent diseases and medical history will be coded using the MedDRA dictionary (Medical Dictionary for Regulatory Activities) according to the version and update strategy as defined in the coding guideline for this clinical trial.

A concurrent disease is defined as any disease, for which either no stop date was documented (i.e., disease is ongoing) or the stop date is later than the date of Visit 1. All other diseases are defined as medical history. Incomplete stop dates will be estimated using the first possible date in order to define whether a disease is concurrent, i.e., it will be assumed that a disease is not ongoing at trial start whenever the end date indicated that the disease ended in the same month or year as Visit 1 occurred.

In detail, the following assumptions are made for the determination whether a disease is considered as medical history or concurrent disease:

- If the end date is incomplete, year and month are available and the day is missing, it is assumed that the disease ended at the first day of the corresponding month.
- If the end date is incomplete, year is available and month and day are missing, it is assumed that the end date is the 01-Jan of the corresponding year.
- If the end date is completely missing it will be assumed that the disease is still ongoing at Visit 1 and thus the entry is considered as concurrent disease. The only exception are cases with completely missing end date that are explicitly ticked as being “not ongoing at Visit 1” in the eCRF and the end date is unknown.

The duration of grass pollen allergy history until trial start will be calculated as:

month of first injection of investigational drug/placebo – start month of first expression of grass pollen allergy that was entered in the grass pollen history section of the eCRF.

In case the month is missing, it is assumed that the allergy started in January of the corresponding year. Entries without a year will not be considered for this calculation.

The duration of grass pollen allergy history in months will be summarised by treatment group for the FAS.

7.4.2 Medical history and concurrent diseases (non-allergy related)

Concurrent diseases and medical history not related to any allergy will be summarised by treatment group. In this summary table, the diagnoses and indications will be presented by MedDRA preferred term (PT) and grouped under the respective system organ class (SOC). The SOCs and the PTs within SOCs will be sorted by decreasing frequency in the total column.

Separate tables will be created for non-allergy related medical history and concurrent diseases for the FAS.

Non-allergy related concurrent diseases and medical history will be listed by subject.

7.4.3 Grass pollen allergy history and status at start of trial

Symptoms related to grass pollen allergy (i.e., allergic conjunctivitis, allergic rhinitis, allergic cough, allergic asthma and allergic urticaria) will be summarised separately by whether these symptoms were ongoing at trial start (i.e., occurred during at least 2 GPSs before screening) or not.

Number and percentage of subjects with the corresponding symptoms related to grass pollen allergy will be presented by treatment group, differentiating further by severity and whether allergic treatment was taken (i.e., taken any medication for allergy in the last two GPSs prior to screening).

All grass pollen allergy history related information including start and end dates of symptoms related to grass pollen allergy will be listed by subject.

Grass pollen related concurrent diseases and medical history will be summarised for the
FAS. Separate summaries will be generated for Year 1, Year 2 and combined, if applicable.

7.4.4 Other allergy history

Concurrent other allergies (ongoing at trial start) will be summarised by treatment group and reported term (including “Other” as collected in the eCRF), differentiating further by severity and whether allergic treatment was taken for the FAS.

All other allergy history (past or present) will be listed by subject.

7.4.5 Asthma status

The baseline asthma status is either “present (past and/or current)” for all subject with documented past and/or current asthma or “not present” for all subject without any documented asthma (no documented medical history also means no asthma). Frequency tables with be provided for the FAS displaying the number and percentage of subjects with present or not present asthma and the variable will be used for subgroup analysis.

7.5 Prior and concomitant medications, procedures and non-drug medical interventions

All prescription and over the counter medications for relief of allergy symptoms taken over the last 2 years and those used for other conditions in the last 1 month before screening should be recorded in the eCRF. Details of any concomitant medication used during the clinical trial including all prescription and over the counter medications taken by the subject during the clinical trial until end of study (EoS) must be documented in the eCRF at each visit (scheduled or unscheduled).

All prior and concomitant medications documented in the eCRF will be coded using the World Health Organisation (WHO) Drug dictionary according to the version and the update strategy as defined in the coding guideline for this clinical trial.

All prior and concomitant procedures and non-drug medication interventions documented in the eCRF will be coded using the medical dictionary of regulatory activities (MedDRA) according to the version and update strategy as defined in the coding guideline for this clinical trial.

Prior medications are defined as medications that started prior to first injection of investigational drug/placebo. In case the start date is missing or incomplete with the possibility that the medication was taken prior first injection of investigational drug/placebo, it will be assumed that at least one dose was taken prior to the first injection of investigational drug/placebo and thus the entry will be considered as prior medication.

Concomitant medications are defined as any medication with at least one dose taken at or after the first injection of investigational drug/placebo, i.e., for all end dates larger or equal to first documented injection of investigational drug/placebo. In case the end date is missing or incomplete with the possibility that the medication was taken after the first injection of investigational drug/placebo, it will be assumed that at least one dose was taken after the first injection of investigational drug/placebo and thus the entry will be considered as concomitant medication.

Prior and concomitant medications will be summarised separately by display of the absolute and relative frequency by treatment group using as denominator the number of subjects in the respective treatment group and overall in the FAS. In these summary tables, medication will be classified according to WHODD ATC2 (Anatomical Therapeutic Chemical Classification) name and base substance.

Prior and concomitant procedures and non-drug medical interventions will be summarised by primary SOC and PT in the FAS by treatment group and overall.

The summary tables will be sorted by decreasing frequency in the total column within levels.

7.6 Other baseline characteristics

Other baseline characteristics are defined as the results of assessments that are assessed at screening and/or baseline only. Assessments that are performed at several visits including screening and/or baseline are presented in the corresponding sections (efficacy, safety or exploratory). Other baseline

characteristics are the results of Skin Prick Test (SPT), the results of spirometry, and immunoglobulins collected at screening for eligibility (ImmunoCAP). These data will be summarised by treatment group and overall for the FAS, and listed by subject.

7.6.1 Skin Prick Test

A SPT using various allergens will be performed at screening to assess eligibility of subjects. A standard negative control will be used and histamine control solution (1.0%) will be used for the positive control.

The reaction grade (negative, positive) will be displayed in a frequency table further differentiated by case history of symptoms.

7.6.2 Spirometry

Spirometry will be performed at screening (Visit 1) and Visit 1a (if applicable) for all subjects
to determine eligibility. At screening, the FEV_1 should be ≥70% of predicted with a FEV_1/FVC ratio >75%.

Summary statistics will be created for spirometry measurements at screening by treatment group for the FAS.

7.6.3 Immunoglobulins at screening

Blood samples will be collected at screening for evaluation of eligibility:

- Serum total IgE [U/mL]
- Serum grass-specific IgE [kUA/L]
- Serum grass-specific IgG4 [mg/L]

Results will be summarised by display of the absolute and relative frequencies per class by treatment group for the FAS.

7.7 Efficacy

7.7.1 Primary efficacy analyses

The primary efficacy variable is the daily Combined Symptom and Medication Score (CSMS) averaged over the peak grass pollen season (GPS).

7.7.1.1. Primary estimand for primary efficacy analysis

In alignment with the addendum to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E9, the estimand for the primary efficacy analysis in this clinical trial is defined by the following attributes (see Section 9.3.5.1 of the clinical trial protocol):

Table 8 Primary estimand

Treatment	“PQ Grass” (PQ Grass 27600 SU) versus “Placebo”.

Population	Subjects with seasonal allergic rhinitis and/or rhinoconjunctivitis induced by grass pollen exposure, as defined by the inclusion and exclusion criteria of the clinical trial, who received at least 1 injection of the clinical trial medication
	PQ Grass or placebo. The analysis will be based on the FAS.
Variable	CSMS averaged over the peak GPS for each subject. The daily CSMS is calculated as the sum of the dSS and the dMS data recorded in the eDiary. The average CSMS over the peak GPS will be calculated as sum of the daily CSMS within the peak GPS divided by the number of days of the peak GPS where the CSMS has been collected.
Population level summary	Difference in mean CSMS between the PQ Grass and placebo group.

7.7.1.2. Daily Combined Symptom and Medication Score

The daily CSMS is the sum of the daily Symptom Score (dSS) component of the CSMS and the daily Medication Score (dMS) component of the CSMS calculated from the data recorded in the eDiary.

The dSS is calculated as the sum of the severity of conjunctival symptoms (2 items) and nasal symptoms (4 items) divided by 6 for each subject and day (see Table 9).

The severity of symptoms is recorded on a 4-point severity scale:

- 0 = No symptoms,
- 1 = Mild symptoms
  (sign/symptom clearly present, but with minimal awareness and easily tolerated),
- 2 = Moderate symptoms
  (definite awareness of sign/symptom that is bothersome but tolerable), and
- 3 = Severe symptoms
  (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping).

Table 9: Conjunctival and nasal symptoms of the CSMS

Conjunctival Symptoms	Itchy/red eyes *
	Watery eyes
Nasal Symptoms	Blocked nose
	Runny nose

	Itchy nose
	Sneezing

* Maximum of single items “Itchy eyes” and “Red eyes” is used for analysis.

The dMS is equal to the raw score assigned to each subject and day for the medication with the highest step taken that day.

Thereby, medication is rated on a 4-point scale:

- 0 = No relief medication used,
- 1 = Oral antihistamine/Ocular antihistamine (Step 1 medication),
- 2 = Intranasal corticosteroids (Step 2 medication) with Step 1 medication(s), and • 3 = Oral corticosteroids (Step 3 medication) with Step 1 and Step 2 medications.

A subject will be assigned 1 score (i.e., 1, 2 or 3) per day based on at least 1 dose of the medication of the highest step taken that day, regardless of if more than 1 medication from different steps has been taken. In case, no relief medication is taken, a score of 0 is assigned. For example, a subject taking antihistamine eye drops (Step 1), an antihistamine tablet (Step 1) and intranasal corticosteroids (Step 2) on the same day would be allotted a score of 2 for the Step 2 medication taken.

7.7.1.4. Average CSMS over the peak GPS

The average CSMS over the peak GPS is calculated as the sum of the daily CSMS within the peak GPS divided by the number of days of the peak GPS where the CSMS has been collected or imputed.

For the primary analysis, all subjects in the FAS will be included. This will be done by using multiple imputation methods on a by-day level. The average CSMS over the peak GPS can then be calculated based on all days within the peak GPS per imputation.

An analysis providing summary statistics based on observed cases will also be performed

Summary statistics will be provided for the average CSMS over the peak GPS by treatment group based on observed cases.

7.7.1.5. Statistical analysis method

The primary efficacy analysis for this clinical trial will be a comparison of the mean average CSMS during the peak GPS in the PQ Grass versus the placebo group.

The following null and alternative hypotheses will be tested for the primary endpoint, based on all subjects who received at least 1 injection of the clinical trial medication, i.e., are included in the FAS (two-sided test):

$$H0\colon \mu_{Placebo} = \mu_{PQ\ Grass}$$

$$H1: \mu_{Placebo} \neq \mu_{PQ\ Grass}$$

μ: arithmetic mean of the average CSMS over the peak GPS

The primary efficacy endpoint will be evaluated using a linear mixed model.

The difference between PQ Grass and placebo will be estimated using least squares (LS) means. Two-sided 95% repeated confidence intervals (RCIs) for the difference between treatment groups will be provided at the interim and (if applicable) at the final analysis, which will ensure 95% probability despite calculation at the interim and (if applicable) final analysis.

The difference between treatment groups will also be presented in percent relative to the LS mean in the placebo group, and the CI will be calculated by approximating the SE using the delta method.

7.7.1.6. Adaptive design and interim analysis

The interim analysis will be performed based on the subjects randomised in Year 1. Objectives of this interim analysis include an evaluation whether a second recruitment season for the remaining subjects is actually required, and an evaluation whether the planned sample size is adequate or should be adapted.

7.7.2 Key secondary efficacy analyses

The key secondary efficacy endpoints will only be addressed with statistical hypothesis tests if the primary endpoint shows a statistically significant difference between treatment groups. Multiplicity in the key secondary endpoints will be addressed with the Fallback method. The full 5.0% two-sided alpha for the Fallback method will be distributed across the 8 key secondary endpoints. If the preceding hypothesis test is not significant, subsequent tests will be performed at the aforementioned alpha-level. If tests are significant, the alpha is added to the subsequent hypothesis test.

All p-values for the analyses of key secondary endpoints will be presented without adjusting for multiplicity. The evaluation whether the null hypotheses can be rejected or not will be done based on the raw p-values. CIs (for example for the LS means) will also be presented in an explorative way, i.e., two-sided 95% CIs will be presented without a formal multiplicity adjustment.

7.7.2.2. Average CSMS over the entire GPS

The average CSMS over the entire GPS is calculated in the same way as the average CSMS over the peak GPS. The following null and alternative hypotheses will be tested for this endpoint, based on all subjects who received at least 1 injection of the clinical trial medication, i.e., are included in the FAS (two-sided test):

$$H_0: \mu_{Placebo} = \mu_{PQ\ Grass}$$

$$H_1: \mu_{Placebo} \neq \mu_{PQ\ Grass}$$

μ: arithmetic mean of the average CSMS over the entire GPS.

The primary efficacy analysis will be repeated for the average CSMS over the entire GPS.

Summary statistics will be provided for the average CSMS over the entire GPS by treatment group.

7.7.2.3. Average dSS and dMS over the peak and the entire GPS

The average dSS over the peak and the entire GPS and the average dMS over the peak and the entire GPS is calculated in the same way as the average CSMS over the peak GPS.

The following null and alternative hypotheses will be tested for these endpoints, based on all subjects who received at least 1 injection of the clinical trial medication, i.e., are included in the FAS (two-sided test):

$$H_0: \mu_{Placebo} = \mu_{PQ\ Grass}$$

$$H_1: \mu_{Placebo} \neq \mu_{PQ\ Grass}$$

μ: arithmetic mean of the average dSS/dMS over the peak / entire GPS.

Summary statistics will be provided for all average scores by treatment group.

7.7.2.4.2. Key secondary efficacy analysis of well days during peak GPS using generalised estimating equation

The probability of a well day during the peak GPS will be analysed using data on a by-day level per subject using generalised estimating equation (GEE).

The following null and alternative hypotheses will be tested for this endpoint, based on all subjects who received at least 1 injection of the clinical trial medication, i.e., are included in the FAS (two-sided test):

$$H_0: p_{Placebo} = p_{PQ\ Grass}$$

$$\neq$$

$$H_1: p_{Placebo}\ \ p_{PQ\ Grass}$$

p: probability of well days during the peak GPS

Thereby, the expected probability for a well day on a specific day of the peak GPS will be modelled in terms of a GEE for a binary response. As the observations of well days for one subject during the peak GPS are correlated (it can be assumed that days close to each other have a higher correlation than more distant days), a within-subject working correlation matrix is assumed.

7.7.2.5. Serum grass-specific IgG4 at Visit 7 compared to baseline

Blood samples are collected at screening, Visit 7 and Visit 10 for the evaluation of several immunoglobulin parameters, including grass-specific IgG4 [mg/L].

Summary statistics will be created by visit and treatment group for the absolute values (Baseline, Visit 7 and Visit 10) and the absolute change from baseline (to Visit 7 and Visit 10).

The change from baseline to Visit 7 in grass-specific IgG4 [mg/L] will be additionally analysed using the same linear mixed model as specified in the primary analysis with the baseline value as additional covariate.

7.7.2.6. Standardised Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ[S]) averaged over the peak GPS

7.7.2.6.1. Standardised rhinoconjunctivitis Quality of life questionnaire

Rhinoconjunctivitis quality of life will be assessed using the RQLQ(S). The RQLQ(S) is a questionnaire which consists of 28 questions in 7 domains (activity limitation, sleep problems, nose symptoms, eye symptoms, non-nose/eye symptoms, practical problems, and emotional function). Each question is scored on a scale from 0 to 6 (0 = not impaired at all to 6 = severely impaired). Subjects will be issued the RQLQ questionnaire at Visits 2, 9 and 10. The Visit 2 RQLQ will be the baseline RQLQ.

The total score defined as the mean of all 28 responses will be aggregated as an outcome variable on a subject level by taking the mean of the scores from Visits 9 and 10 that can be attributed to the peak GPS.

7.7.2.6.2. RQLQ[S] averaged over the peak GPS

The average total RQLQ score during the peak GPS will be calculated if at least one score at Visit 9 or 10 is reported during the peak GPS. It will be considered missing if the RQLQ questionnaire has not been filled at any of these visits or both visits do not fall within the peak GPS. Missing values of the RQLQ score will not be imputed.

The total RQLQ score and its change from baseline (Visit 2) will be summarised by visit and treatment group for the FAS. In addition, summary statistics will be created for the RQLQ during the peak GPS and its change from baseline.

7.8 Safety

Analysis of all safety and tolerability variables including solicited pre-specified local and systemic reactions will be based on the SAF. Summary statistics will be provided for safety variables. Alert ranges will be defined for laboratory values lying outside the normal range to a clinically relevant degree. Any measurement fulfilling these criteria will be presented in subject listings.

7.8.1 Investigational drug/placebo exposure and compliance

The number and percentage of subjects having received treatment will be summarised per treatment group and treatment visit (scheduled injections as well as unscheduled injections

for dose repeat and dose reduction) for the FAS (randomised treatment) and the SAF (actual treatment).

The actually received cumulative dose in SU is calculated based on the actually received kit and vial numbers entered in the eCRF. The planned cumulative dose is based on the randomised treatment (27600 SU for the PQ Grass treatment group).

Summary statistics will be provided for the number of injections received, the actually received cumulative dose in SU and the ratio of the cumulative dose actually received and planned [%]. This ratio can only be calculated for the PQ Grass treatment group and the corresponding planned cumulative dose is 27600 SU for every subject. Please note that ratios greater than 100% might result for subjects receiving additional unscheduled injections. This summary will be provided for the FAS (randomised treatment) and the SAF (actual treatment).

Any subject with dose modifications or unscheduled injections will be listed in a separate listing providing all details on all injections received.

For this clinical trial, any dose of investigational drug/placebo greater than the dose prescribed (for each of the dosing visits) within the clinical trial protocol represents an overdose. A separate listing will be generated including only subjects that experienced an overdose.

7.8.2 Adverse events

All Adverse events during the course of the study will be coded using the medical dictionary of regulatory activities (MedDRA) current at the start of the study and summarised by treatment group. AEs will be summarised by treatment, primary system organ class (SOC), preferred term (PT), relationship assessment and severity.

All solicited AEs identified via separate phone calls for the US will also be entered in the general AE section of the eCRF. Therefore, no separate analysis will be conducted.

The SOCs as well as the PTs within each summary table will be sorted by frequency in the total column in decreasing order.

AEs will be reported separately as pre-treatment or screening period AEs (any AE occurring prior to the first injection of study medication) and treatment emergent AEs (any AEs after first injection of study medication).

In general, all adverse events will be listed by treatment group, subject number, and period. For AE onset and end dates, the study day relative to the first injection will be listed.

7.8.2.1 Definitions related to adverse events

Pre-Treatment adverse event: Any AE with date and time of onset prior to the first injection of study medication. Pre-treatment AEs might also be termed Screening Period AEs.

Treatment emergent AE (TEAE): Any AE with date and time of onset after first injection of study medication. In case of (partially) missing date or time that could indicate a TEAE, it will be analysed as treatment emergent.

TEAEs during the treatment period: Any AE with date and time of onset after first injection of study medication and up to and including date of last injection of study medication + 28 days.

Adverse Drug Reactions (ADRs): Any AE that is possibly, probably or definitely related to study medication or the assessment of the relationship to study medication is missing as reported in the eCRF.

Adverse events related to study procedure: Any AE that was documented with causal relationship to a study procedure of “yes” in the eCRF.

Adverse events of special interest (AESI): Any AE that was documented as “AESI confirmed by Adjudication Committee” in the eCRF.

Local AE: Any AE that is documented with the category “Local (injection site) adverse event” in the eCRF.

Systemic AE: Any AE that is documented with the category “Systemic adverse event” in the eCRF.

Other AE: Any AE that is documented with the category “Other adverse event” in the eCRF, i.e. any adverse event that is neither local nor systemic.

AEs leading to premature discontinuation of treatment: Any AE for which action taken is documented as “Permanent discontinuation of the IMP” in the eCRF.

AEs leading to temporary discontinuation of treatment: Any AE for which action taken is documented as “Temporary discontinuation of the IMP” in the eCRF.

AEs leading to premature discontinuation of study: for which action taken is documented as “Withdrawal from the Study” in the eCRF.

Duration of adverse event (expressed in [days]) is calculated as follows:

- End date/time of AE – onset date/time of AE
- In case of missing onset time or missing end time: end date of AE – onset date of AE + 1
- In case of (partially) missing onset date or (partially) missing end date no calculation will be done

In case of ongoing end date/time (outcome = recovering, not recovered or unknown), date of study termination will be used as documented at the end of the study page or the early termination page of the eCRF.

7.8.2 Summary tables for analysis of adverse events

The number and percentage of subjects with

- TEAEs
- ADRs
- treatment emergent serious adverse events (SAEs)
- serious ADRs
- treatment emergent adverse events of special interest (AESIs)
- TEAEs leading to premature discontinuation of treatment
- ADRs leading to premature discontinuation of treatment
- TEAEs leading to temporary discontinuation of treatment
- ADRs leading to temporary discontinuation of treatment
- TEAEs leading to premature discontinuation of study

- ADRs leading to premature discontinuation of study
- local AEs
- systemic AEs
- other AEs, not classified as local or systemic AEs

will be summarised by treatment group, MedDRA SOC and PT.

For the analysis of the severity and the relationship of AEs, the worst severity and the strongest relationship per subject and type of AE will be used. The number and percentage of subjects with at least one AE by MedDRA SOC, PT and severity/relationship will be displayed only for

- all TEAEs
- all ADRs

all serious adverse events (SAEs).

7.8.2.2 Overview summary tables for adverse events

An overview summary of the following AE types will be provided, presenting number and percentage of subjects experiencing at least one AE of the respective AE type in each treatment group as well as the corresponding number of events.

- Any AE
- Any ADR
- Any severe AE
- Any severe ADR
- Any AE of special interest
- Any serious AE
- Any serious ADR
- Any non-serious AE
- Any AE leading to premature discontinuation from treatment
- Any AE leading to temporary discontinuation from treatment
- Any ADR leading to premature discontinuation from treatment
- Any ADR leading to temporary discontinuation from treatment
- Any AE leading to premature discontinuation of study
- Any ADR leading to premature discontinuation of study
- Any local TEAE
- Any systemic TEAE
- Any other TEAE, not classified as local or systemic AE

7.8.3 Safety laboratory

Serum Chemistry	Glucose, Sodium, Uric acid, Urea, Potassium, Calcium, Creatinine, Chloride, Total protein, Phosphorus, Cholesterol, Albumin, Total Bilirubin, Alkaline phosphatase, LDH, AST, ALT, GGT, CRP
Haematology	Haemoglobin, haematocrit, total WBC and differentials, total RBC, RBC indices, and platelet count.
Urinalysis (using a urine dip-stick)	pH, Protein, Glucose, Ketones, Bilirubin, Blood, Nitrite, Urobilinogen, Leukocytes.

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRP = C-reactive protein; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; RBC = red blood cells; WBC = white blood cells.

Summary tables will be created for serum chemistry and haematology.

For urinalysis, a frequency table will be created summarizing the overall sample.

7.8.4 Vital signs

Vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate, and body temperature) will be measured in a semi-supine position after 5 minutes rest for the subject in a quiet setting without distractions using a completely automated device.

Vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate, and body temperature) will be measured after the subject has been in the supine position for at least 5 minutes at Visit 2 and Visit 7.

Summary statistics will be created per vital sign parameter and visit/timepoint (pre- and post-injection) for all scheduled treatment visits by treatment group including the absolute values per visit/timepoint and the absolute change from baseline.

7.8.5 Physical examination

A complete physical examination includes assessment of the following: general appearance, skin, head, neck, eyes, ears, nose, throat, lymph nodes, abdomen, musculoskeletal, cardiovascular, respiratory and neurological systems. Rectal, breast, pelvic, gynaecological, and/or urogenital examinations will not be performed. Complete physical examination will be performed at Visit 1 and Visit 11, or at the early termination visit if applicable.

An assessment of the subjects' mental status to determine their ability to participate in the clinical trial will be included at the Visit 1 physical examination assessment only.

7.8.6 Other safety data

Urine pregnancy tests will only be performed on female subjects of childbearing potential. on every visit (except safety follow-up calls). If positive, a serum pregnancy test should be done.

The corresponding results will only be listed by subject.

8 SOFTWARE AND STATISTICAL PROGRAMMING

The statistical analysis will be performed using the SAS® statistical software package in Version 9.4 or higher.

9 REFERENCES

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