

STATISTICAL ANALYSIS PLAN

04 May 2015

Randomised placebo-controlled study of grass pollen allergen immunotherapy tablet (AIT) for seasonal rhinitis: time course of nasal, cutaneous and immunological outcomes

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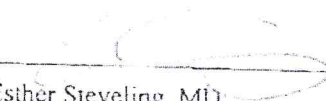
APPROVALS

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LIST OF ABBREVIATIONS

AE	Adverse event
ANCOVA	Analysis of covariance
ARIA	Allergic Rhinitis and its Impact on Asthma
AUC	Area under the curve
CRF	Case report form
DSMB	Data and Safety Monitoring Board
EPR	early phase response
ITN	Immune Tolerance Network
ITT	Intent-to-treat
LPR	late phase response
MiniRQLQ	Mini Rhinoconjunctivitis Quality of Life Questionnaire
NIAID	National institute of allergy and infectious disease
PNIF	peak nasal inspiratory flow
PP	Per protocol
SAE	serious adverse event
SAP	statistical analysis plan
SAR	seasonal allergic rhinitis
SD	Standard deviation
SCIT	Subcutaneous Immunotherapy
SLIT	Sublingual Immunotherapy
SOC	System organ class
SQ	Standardized Quality
TNSS	Total Nasal Symptom Score (0-12)
WAO	World Allergy Organization

1. PROTOCOL SYNOPSIS

Title	Randomised placebo-controlled study of grass pollen allergen immunotherapy tablet (AIT) for seasonal rhinitis: time course of nasal, cutaneous and immunological outcomes
Short title	Grass Pollen Allergen Immunotherapy Tablet (AIT) Time Course Study
Conducted by	Royal Brompton Hospital and Imperial College
Protocol Chair	Stephen Durham, MD, MA, FRCP
Accrual Objective	50 participants with moderate-severe grass pollen allergic rhinitis; 20 healthy, non-atopic participants
Study Design	<p>A randomized, double-blind, single-centre, placebo-controlled, two-arm time course study comparing grass pollen AIT with placebo.</p> <p>The study will involve a 4 month recruitment phase, a 12 month randomized, blinded treatment phase (Phase I), and finally a further 12-24 months open treatment phase and a 12 month follow up after withdrawal of treatment (Phase II). Up to 50 grass pollen allergic (atopic) participants will be enrolled to ensure randomisation of at least 44. Further we shall recruit 20 healthy, non-atopic volunteers.</p> <p>Individuals with severe grass pollen hay fever, with or without associated seasonal asthma, will be recruited after the 2013 grass pollen season, between December 2013 and April 2014. Screening will be completed before eligible atopic participants are randomized to one of the following two treatment arms in a 1:1 ratio:</p> <ul style="list-style-type: none">• Grass pollen AIT• Placebo AIT

2. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following analyses and reporting conventions will be used:

- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form “n (%).” Percentages will be rounded to one decimal place.
- Numeric variables will be summarized using n, mean, standard deviation (SD), median, minimum (min), maximum (max). Descriptive statistics will be displayed in the order: n, mean, SD, median, min, max.
- The median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics such as *t* test statistics will be reported to two decimal places.
- *P*-values will be reported to three decimal places if greater than or equal to 0.001. If less than 0.001, the value will be reported as “<0.001.”

If departures from these general conventions are present in the specific evaluations section of this SAP, then those conventions will take precedence over these general conventions.

3. ANALYSIS SAMPLES

- **Intent-to-treat (ITT) sample** will be defined as all randomized participants. ITT participants will be analyzed with the group to which they were randomized, regardless of the medication actually received. If participants drop out post-randomization, they will be invited to complete study assessments throughout the duration of the trial.
- **Per-protocol (PP) sample** will be defined as ITT sample participants who remain in the study until the primary endpoint was assessed. Participants in the PP sample must be compliant with study medication, defined as taking 75% or more of their study medication for the duration of the study. Compliance with study medication will be assessed by pill count for SLIT and placebo. Participants in the PP sample will be analyzed with the group to which they were randomized.
- **Safety sample (SS)** will be defined as all enrolled participants. Participants in the safety sample will be analyzed in regards to adverse events.

4. UNBLINDING THE TRIAL FOR ANALYSIS PURPOSES

We will unblind the trial for analysis purposes after the 12 months blinded phase of this trial. The trial will be unblinded by the statistician, Dr. Fabiana Gordon. Dr. Fabiana Gordon will request and receive the unblinding list from the Inform Database team. Documentation will be performed when the randomization code was requested and provided, when the list was opened and when the randomization data was applied to the analysis dataset. This will include the time as well as the date. The documentation will be filed in the trial master file on file notes accordingly. Please also refer to SOP AIT009.

5. STUDY SUBJECTS

5.1 Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for the randomized atopic and non- atopic sample.

Demographic data will include and data will be adjusted for age, gender, Skin Prick test (screening), specific IgE (screening) and compliance.

Furthermore the following characteristics will be used for descriptive statistics only: baseline visual analogue scale (How has your Hayfever been overall in the previous season? (Appendix 2 from Protocol), Polisensitisation (this will be defined as a positive skin prick test during screening apart from skin prick test to timothy grass and the positive control (Yes/No), seasonal asthma, this information will be taken from the medical and allergy history from the participant (Yes/No), total IgE, BMI, ethnicity.

These data will be presented in the following manner:

- Continuous data (e.g., age) will be summarized descriptively by mean, SD, median, min, and max.
- Categorical data (e.g., sex and ethnicity) will be presented as frequencies and percentages.

Demographic and baseline characteristic data will also be presented in data listings by study group (atopic (placebo and active group) and non-atopic).

6. STUDY OPERATIONS

6.1 Protocol Deviations

Protocol deviations will be listed separately for randomized subjects with information such as type of deviation, severity of the deviation (major or non-major), date of occurrence, the reason for the deviation, steps taken to address the deviation, and whether or not the deviation was reportable to the regulatory authorities. The number of major protocol deviations, the number of each type of violation, the number of consented subjects with at least one deviation, the number of deviations leading to termination of a subject from study and the number resulting in an AE will be summarized in a tabular format.

6.2 Treatment Compliance

Treatment compliance will be summarized by treatment arm through the total duration of blinded study therapy (12 months). In each of the treatment arms compliance rate will be calculated as follows: first we calculated the amount of days the participant should have taken the tablet from the start of the IMP until the last day of the IMP taken before switching to the open-label follow up treatment with Grazax. Then we calculated how many days the participant took the tablet according to the returned tablets counted by Pharmacy. We calculated the resulting difference between the amount of tablets that the participant should have taken to the actual amount of tablets taken (e.g. if the participant should have taken 100 tablets and he took 80 tablets it will be calculated as follows: $100:100 \times 80 = 80$ (this would be a compliance of 80 %).

Treatment compliance will be presented across treatment groups.

7. ENDPOINT EVALUATION

7.1 Overview of Efficacy Analysis Methods

7.1.1 Assessment Time Windows

All study visits must occur within the time limits specified below:

Atopic

Phase I (all):

Screening Visit – Visit -1, from December 2013 to April 2014

Visit – 0a, after screening and before Visit 0b

Visit - 0b, from January 2014 to April 2014

Visit – 1, after Visit – 0b

Visit – 2, from February to May 2014

Visit – 3, from March to May 2014

Visit – 4, from April to June 2014

Visit – 5, from May to August 2014

Visit – 6, from July to October, 2014

Visit – 7, from January to April 2015

Phase II (active treatment group only)

Visit – 8, 24 months after start of treatment

Visit – 9, 36 months after start of treatment

Non-Atopic

Screening Visit – September 1st to November 30th, 2014

Visit – 1 coinciding with Visit 7 at 12 months of treatment of the atopic participants

Unscheduled visits may also occur throughout the study.

The pollen season will be defined as in the protocol. Pollen counts will be quantitated retrospectively for the purposes of analysis. *Pollen season* is defined as follows:

Start of season	First 3 consecutive days at pollen count >10 grains/cm ³
End of season	First 3 consecutive days at pollen count < 10 grains/cm ³
Start of peak season	First 3 consecutive days at pollen count >30 grains/cm ³
End of peak season	First 3 consecutive days at pollen count < 30 grains/cm ³

A variable for the pollen season and peak pollen season (0 = no pollen season, 1 = pollen season, 2 = peak pollen season) will be created by the AIT team indicating when the season started and finished.

7.2 Primary Endpoint

7.2.1 Analysis of the primary endpoint

The primary endpoint is the total nasal symptoms score (TNSS) measured at 0, 5, 15, 30 and 60 minutes (Early phase response (EPR)) following grass pollen nasal allergen challenge at 12 months (Appendix 6).

The endpoints will compare the following groups:

- SLIT versus SLIT Placebo
- SLIT versus SLIT Placebo versus non-atopic controls

This study requires the analysis of longitudinal data since the outcome of interest is measured over a period of time. Longitudinal analysis allows estimating the marginal effect of the predictors of interest such as Age, Gender, Skin Prick test, specific IgE, baseline visual analogue scale and compliance as well as the comparison between the intervention and control group. Longitudinal analysis also allows the assessment of changes over time. One of the comparisons of interest is the difference between the intervention and control groups at baseline, 6 months and 12 months. The other relevant comparison is the average EPR at 12 months with baseline for both groups. Longitudinal data require special statistical methods because observations made on each subject are correlated. This correlation must be taken into account to draw valid statistical inferences. Some of the approaches that model the correlation structure in longitudinal data are Models for the Mean response and Random coefficient models.

Also, before the main analysis exploratory analysis will be carried out: box plots, scatter plots, cross-tabs, frequency tables and summary statistics.

This will be analysed using the ITT sample and for the PP analysis population.

7.3 Secondary Endpoints

This study is not designed or powered to perform hypothesis testing on secondary endpoints. All secondary analyses will be treated as supportive. P-values will be presented for the secondary endpoints but will not be adjusted for multiplicity. The endpoints listed below will compare the following groups using the same statistical methodology as described for the primary endpoint.

- SLIT versus SLIT Placebo
- SLIT versus SLIT Placebo versus non-atopic controls

1. Peak nasal inspiratory flow (PNIF) measured at 0, 5, 15, 30 and 60 minutes (EPR) following grass pollen nasal allergen challenge at baseline and at 12 months.

2. Mean LPR to intradermal testing recorded as the mean diameter of the swelling (longest diameter plus perpendicular diameter, exclude pseudopods) measured at the specified time points - after 8 hours of allergen challenge respectively - at baseline and after 12 months of treatment.
3. Mean EPR to intradermal testing recorded as the mean diameter of the swelling measured at the specified time points - after 15 minutes of allergen challenge respectively - at baseline and after 12 months of treatment.
4. Global seasonal visual analogue score 1 (Appendix 1 of the protocol) after the pollen season 2014 (single measurement and delta (comparing baseline with after pollen season time point))
5. Global Evaluation score 2 (retrospective individual global evaluation) (Appendix 2 of the protocol) after the pollen season 2014 (single measurement only).

7.3.1 Exploratory Endpoints

The endpoints listed below will compare the following groups using the same statistical methodology as described for the primary endpoint.

- SLIT versus SLIT Placebo

1. Symptoms and medication scores (AUC) scores (Appendix 5 of the protocol) will be self-assessed weekly during the pollen season from May 2014 until end of July 2014 by the atopic participants. The symptom score will encompass nose and eye symptoms recorded on a scale from 0 to 3 (with a score of 0 indicating no symptoms, 1, 2 and 3 indicating mild, moderate and severe symptoms respectively). The maximum score is therefore 18. All possible rescue medication will be provided to each participant before the Hayfever season in May. They will be asked to use the treatment on an as required basis only, starting with the antihistamines (tablets and/or eye drops) and if this does not suffice adding treatment with nasal spray. In case this still does not relieve their symptoms they will be advised to contact the trial team to start Prednisolone 30 mg 3-5 days. Medication use will be recorded in weekly questionnaires by participants and a medication score will be calculated: desloratadine, 5 mg, up to 1 tablet daily and/or olopatadine eye drops, 1.0 mg/mL, up to 1 drop per eye twice daily (1 point per day); fluticasone nasal spray, 50 mcg per spray, up to 2 sprays per nostril once daily (2 points per day); and prednisone, 5 mg per tablet, up to 6 tablets per day (3 points per day). The maximum daily medication score will therefore be 3. The maximum weekly score will be 21. 21 will be divided by 7 and multiplied by 6, which gives us a maximum score of 18, to allow a comparison between symptom and medication scores, since maximum scores for symptoms and medications are different in magnitude, as recommended by World Allergy Organization (WAO) guidelines. A combined symptom and medication score in accordance with WAO guidance on immunotherapy trials will then be calculated by dividing both scores by 6, summarize of the results and divide by 2. This will be performed by the AIT trial team and send on excel files to the Inform database. The mean composite score in each treatment group during the pollen season (beginning of May to end of July) and during the peak pollen season (approximately

mid-June, defined as the max 14 day rolling average pollen count during the season) will be compared.

2. Mini Rhinoconjunctivitis Quality-of-Life Questionnaire (miniRQLQ) scores will be collected weekly during the pollen season (Appendix 3 of protocol). The mean composite score in each treatment group during the peak pollen season (beginning of May to end of July) will be compared. Furthermore the maximum RQLQ will be compared.
Mini RQLQ will also be recorded pre- and post-season. Adding the scores of the 14 questions to form a single score per measurement will form the score of the mini RQLQ. The Inform database team will perform this.
3. Symptoms and severity of symptoms after intake of the daily tablets will be recorded on a daily questionnaire (Appendix 9 of protocol), which the participants will fill out at home. The type of symptom (itchy mouth, swelling of the mouth or other) and the WAO Grade of the local symptoms will be calculated daily and active will be compared. The duration of the symptoms in days and the duration of symptoms in minutes will be specified, the AIT trial team according to the records assessed on the paper CRF on every visit will give this information.
4. Global Evaluation Score 3 (Visual Analogue Scale of overall Hayfever symptoms in the last week) (Appendix 4 of protocol) will be collected weekly during the pollen season. The mean composite score in each treatment group during the peak pollen season (beginning of May to end of July) will be compared. Furthermore the maximum Global Evaluation Score 3 will be compared between the placebo and the active treatment group.
5. The total nasal symptoms score (TNSS) measured at 0, 5, 15, 30 and 60 minutes (Early phase response (EPR)) following grass pollen nasal allergen challenge at baseline, at 6 and at 12 months (Appendix 6) will be assessed similar to the primary endpoint.

7.3.2 Mechanistic Assessments

The endpoints listed below will compare the following groups using the same statistical methodology as described for the primary endpoint.

- SLIT versus SLIT Placebo
- SLIT versus SLIT Placebo versus non-atopic controls

Grass pollen specific immunological markers in serum, nasal fluid and nasal brushings will be evaluated at baseline, after 1, 2, 3 months, during the peak pollen season (at 4 months) and after 12 months of treatment. Comparisons will be made between atopic active and placebo group and at the 12 months' time point also with the non-atopic participants. Biopsies will be performed at baseline, after 12 months, 24 months of treatment and after 12 months of withdrawal.

Blood samples:

Analysis will be performed on baseline, 1, 2 and 3 months, peak pollen season and 12 months of treatment samples.

1. Immunocap (Total IgE, sIgE, sIgG4)
2. FAB-Assay
3. Basophil Activation
4. ILC2
5. IL-10 (ELISA)

Nasal Fluid:

The following analysis will be performed on baseline, 1, 2 and 3 months, peak pollen season and 12 months of treatment sample.

1. FAB-Assay
2. ISAC

The following analysis will be performed on baseline and 12 months sample.

1. Chemokines
Tryptase (baseline, 5, 15, 30 and 60 minutes and at 8 hours)
ECP and Eotaxin
 2. Cytokines (Multiplex) (baseline, 1 and 8 hours)
- Hierarchy of testing: IL-4, IL-5, IL-9, IL-13 (Th2-cytokines), possibly IFN γ , IL-10, TGF β
Eotaxin, MDC, TARC, ECP (Th2-chemokines)

8. SAFETY EVALUATION

8.1 Overview of Safety Analysis Methods

All safety analyses will be carried out using the safety sample defined in Section 3. Missing safety information will not be imputed. Safety will be analyzed through the reporting of adverse events (AEs).

8.2 Adverse Events

Side effects to the treatment will be recorded daily according to the WAO guidelines. They will be recorded as an Adverse Event (AE) only when the severity of the symptoms has a > Grade 1 according to WAO guidelines.

All AEs will be classified using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE). This will be performed by the AIT study team. Each AE is entered on the case report form (CRF) once at the highest severity and the duration of the AE will be recorded. Also information about System Organ Class – Term and CTCAE Term – Term will be included.

CTCAE classification version 4.03: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf will be used by the AIT team and send to the Inform database via excel files.

An overall summary table will be developed to report the number of events and the number and percentage of subjects having at least one event in the following categories:

- AEs
- AEs indicated as serious
- AEs that lead to study drug discontinuation
- AEs with an outcome of death
- AEs that were reported as being related to a study drug
- AEs reported by maximum severity

Exploratory analysis will be carried out for the different type of adverse events to compare the intervention with control group: frequency tables, bar graphs, summary statistics.

8.3 Deaths and Serious Adverse Events

Serious adverse events (SAEs) will be listed and summarized. Separate displays listing and summarizing death, including time to death and cause of death, will also be created.

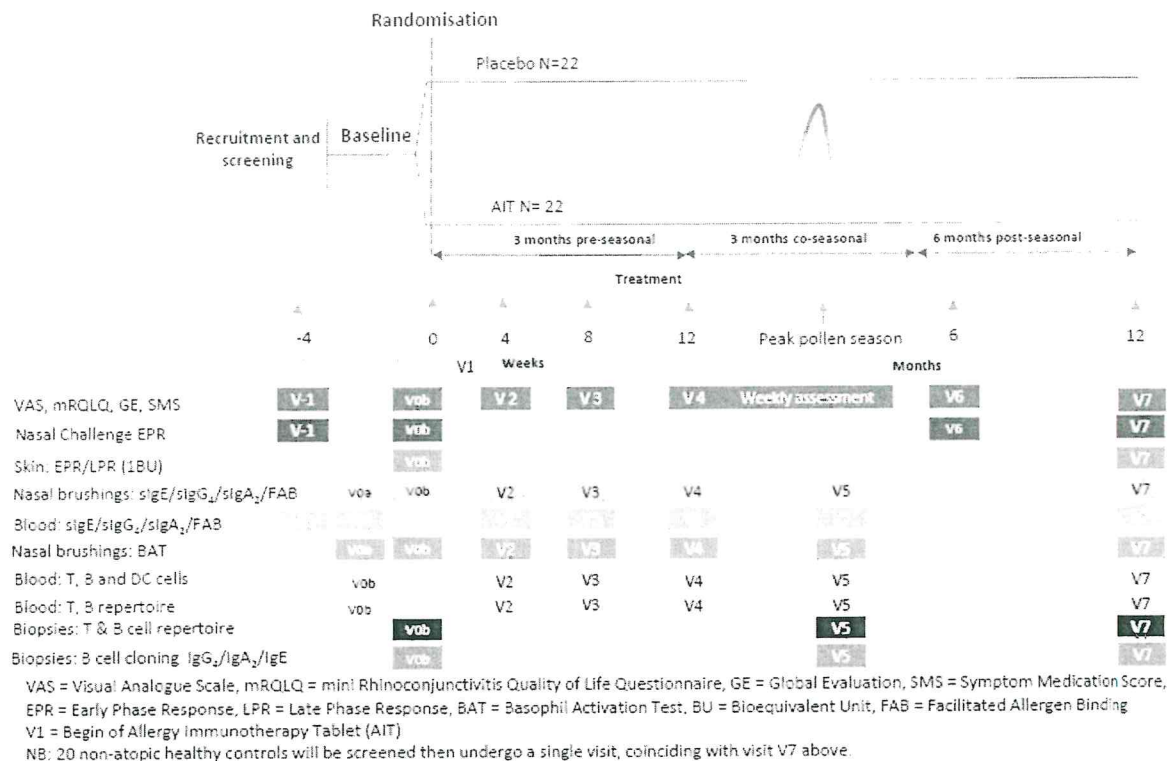
9. OTHER ANALYSES

9.1 Use of Medications

Medications will be coded according to the British National Formulary (BNF) wherever possible. Medications reported on the CRF will be categorized for analysis as prior, concomitant, or after study treatment by comparing the medication start and stop dates with the first and last dose of study medication dates. Prior medications will have both the medication start and stop dates prior to the first dose of study medication date. After medications will have both the medication start and stop dates after the last dose of study medication date. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study medication by at least one day. Descriptive statistics will be performed indicating the number and percentage of subjects receiving prior, concomitant, and after medications.

10. APPENDICES

10.1 Study Flow Chart (Phase I)



10.2 Schedule of Events

10.2.1 Assessments (Phase I and Phase II)

	Visit →	Screening Atopic	Screening Non Atopic	Visit 0a Atopic	Visit 0b Atopic	Visit 1 Atopic	Visit 2 Atopic	Visit 3 Atopic	Visit 4 Atopic	Visit 5 Atopic	Visit 6 Atopic	Visit 7 Atopic	Visit 8 Atopic active treated	Visit 9 Atopic active treated
General Assessments														
Informed Consent	Y	X	X											
Eligibility for the study		X	X											
Demographics		X	X											
Allergy History		X	X											
Medical History		X	X											
Drug History		X	X											
Limited Physical Exam		X	X											
Vital Signs		X	X			X	X	X	X	X	X	X	X	X
Haematology		X	X											
IgE, sIgE		X	X											
Spirometry		X	X											
Smoking History		X	X											
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of compliance		X	X	X	X	X	X	X	X	X	X	X	X	X
Randomisation						X								
Intervention														
Grazax vs Placebo						X	X	X	X	X	X	X	X	X
Clinical Assessments														
Intradermal Allergen Challenge (1 BU)						X						X	X	X
Skin Test		X	X											
Urine Pregnancy Test		X	X											
Clinical Outcomes														
Diary Cards														
Global Evaluation (GE) Score 1		X	X										X	X
Global Evaluation (GE) Score 2		X	X										X	X
Global Evaluation (GE) Score 3		X	X										X	X
Mini Rhinoconjunctivitis Quality of Life		X	X											
Questionnaire		X	X											
Symptom Score		X	X											
Medication Score		X	X											
Nasal Allerg Challenge		X	X									X	X	X
Mechanistic Laboratory Assessments														
Serum for antibody assays				X		X	X	X	X	X	X	X	X	X
Whole blood for basophil assays				X		X	X	X	X	X	X	X	X	X
Whole blood for LC2				X		X	X	X	X	X	X	X	X	X
Whole blood for T-regs				X		X	X	X	X	X	X	X	X	X
Biopsies					X								X	X

