

STATISTICAL ANALYSIS PLAN  
21 February 2015

**A Randomized, Double-blind, Single-center,  
Placebo-controlled Study of Sublingual Immunotherapy  
and Subcutaneous Immunotherapy in Adults with  
Seasonal Allergic Rhinitis**

PROTOCOL NUMBER ITN043AD

SPONSOR

This clinical study is supported and conducted by the Immune Tolerance Network, which is sponsored by the National Institute of Allergy and Infectious Diseases.

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## **LIST OF ABBREVIATIONS**

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

## 1. PROTOCOL SYNOPSIS

<b>Title</b>	A Randomized, Double-blind, Single-center, Placebo-controlled Study of Sublingual Immunotherapy and Subcutaneous Immunotherapy in Adults with Seasonal Allergic Rhinitis
<b>Short Title</b>	Long-Term Effects of Sublingual Grass Therapy
<b>Sponsor</b>	University of California, San Francisco (UCSF)
<b>Conducted by</b>	Immune Tolerance Network
<b>Protocol Chair</b>	Stephen Durham, MD
<b>Accrual Objective</b>	Up to 114 participants
<b>Study Design</b>	<p>This is a randomized, double-blind, single-center, placebo-controlled, three-arm study comparing SLIT with placebo and SCIT with placebo. The main comparison will be between SLIT and placebo. Individuals with severe grass pollen hay fever, with or without associated seasonal asthma, will be recruited during the pollen season of March through September 2011. Eligible participants will be randomized to one of the following three treatment arms administered in a double-blind, double-dummy fashion in a 1:1:1 ratio:</p> <ul style="list-style-type: none"><li>• SLIT + SCIT placebo</li><li>• SCIT + SLIT placebo</li><li>• SLIT placebo + SCIT placebo.</li></ul> <p>Participants will receive treatment over a 2-year period followed by a 1-year blinded withdrawal phase. They will be provided with anti-allergic rescue medications (antihistamine, topical intranasal corticosteroids, and short-acting beta agonists) throughout the study. Clinical endpoint assessments will be performed at baseline, after 1 and 2 years of treatment, and after the 1-year withdrawal period at 3 years.</p>
<b>Study Duration</b>	6 months recruitment; up to 48 months study participation.
<b>Primary Endpoint</b>	The nasal response to allergen challenge at 3 years measured by Total Nasal Symptom Score (TNSS).
<b>Primary Analysis of the Primary Endpoint</b>	The AUC of the primary endpoint is calculated by taking the per-hour average of the TNSS AUC measured at 0 to 1 hours (EPR) plus the per-hour average of the AUC measured between 1 to 10 hours (LPR). The primary analysis, performed on the ITT sample, will compare the mean AUC at 3 years, adjusted for the baseline AUC, using an ANCOVA model at the 0.05 level of significance. The primary analysis consists of the comparison of SLIT + SCIT placebo versus SLIT placebo + SCIT placebo.
<b>Inclusion Criteria</b>	1. Adults age 18 to 65 years.

2. A clinical history of grass pollen-induced allergic rhinoconjunctivitis for at least 2 years with peak symptoms in May, June, or July.
3. A clinical history of moderate - severe rhinoconjunctivitis symptoms interfering with usual daily activities or with sleep as defined according to the ARIA classification of rhinitis.<sup>1</sup>
4. A clinical history of rhinoconjunctivitis for at least 2 years requiring treatment with either antihistamines or nasal corticosteroids during the grass pollen season.
5. Positive skin prick test response, defined as wheal diameter greater than or equal to 3 mm, to *Phleum pratense*.
6. Positive specific IgE, defined as greater than or equal to IgE class 2 (0.7 kU/L), against *Phleum pratense*.
7. A positive response to nasal allergen challenge with *Phleum pratense*, defined as an increase in TNSS greater than or equal to 7 points.
8. For women of childbearing age, a willingness to use an effective form of contraception for the duration of the trial.
9. The ability to give informed consent and comply with study procedures.

**Exclusion Criteria**

1. Prebronchodilator FEV1 less than 70% of predicted value at either screening or baseline visit.
2. A clinical history of moderate - severe allergic rhinitis, according to the ARIA classification, due to tree pollen near or overlapping the grass pollen season.<sup>1</sup>
3. A clinical history of persistent asthma requiring regular inhaled corticosteroids for > 4 weeks per year outside of the grass pollen season.
4. A clinical history of moderate- severe allergic rhinitis, according to the ARIA classification, caused by an allergen to which the participant is regularly exposed.<sup>1</sup>
5. History of emergency visit or hospital admission for asthma in the previous 12 months.
6. History of chronic obstructive pulmonary disease.
7. History of significant recurrent acute sinusitis, defined as 2 episodes per year for the last 2 years, all of which required antibiotic treatment.
8. History of chronic sinusitis, defined as a sinus symptoms lasting greater than 12 weeks that includes 2 or more major factors or 1 major factor and 2 minor factors. Major factors are defined as facial pain or pressure, nasal obstruction or blockage, nasal discharge or purulence or discolored postnasal discharge, purulence in nasal cavity, or impaired or loss of smell. Minor



factors are defined as headache, fever, halitosis, fatigue, dental pain, cough, and ear pain, pressure, or fullness.

9. History of systemic disease affecting the immune system such as autoimmune diseases, immune complex disease or immunodeficiency.
10. At randomization, current symptoms of, or treatment for, upper respiratory tract infection, acute sinusitis, acute otitis media, or other relevant infectious process; serous otitis media is not an exclusion criterion. Participants may be re-evaluated for eligibility after symptoms resolve.
11. At randomization, inflammatory conditions in the oral cavity with severe symptoms such as oral lichen planus with ulcerations or severe oral mycosis.
12. Active malignancy at randomization.
13. Any tobacco smoking within the last 6 months or a history of greater than or equal to 10 pack years.
14. Previous treatment by immunotherapy with grass pollen allergen within the previous 5 years.
15. Any history of grade 4 anaphylaxis due to any cause as defined by the WAO grading criteria for immunotherapy (see appendix 7).
16. A history of allergy to vertebrate/finned fish with, if positive, an associated elevated positive skin test (or elevated specific IgE) to vertebrate/finned fish.
17. History of bleeding disorders or treatment with anticoagulation therapy
18. History of anti-IgE monoclonal antibody treatment.
19. Ongoing systemic immunosuppressive treatment.
20. History of intolerance to the study therapy, rescue medications, or their excipients.
21. For women of childbearing age a positive serum or urine pregnancy test with sensitivity of less than 50 mIU/mL within 72 hours before the start of study therapy.
22. The use of any investigational drug within 30 days of the screening visit.
23. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

### **Treatment Description**

Eligible participants will be randomized to one of the following three treatment arms in a 1:1:1 ratio:

- SLIT (Grazax<sup>®</sup>, *Phleum pratense* freeze-dried oral lyophilisate/orally disintegrating tablet) + SCIT placebo

- SCIT (Alutard SQ Grass Pollen<sup>®</sup> *Phleum pratense* ) + SLIT placebo
- SLIT placebo + SCIT placebo.

## **2. INTRODUCTION**

Unless stated otherwise, this statistical analysis plan (SAP) only includes analyses related to the clinical endpoints. Mechanistic assays and analyses will be performed and managed at the Immune Tolerance Network Biomarker Discovery Research Group (BDR) and the ITN Bioinformatics Group (BiG). A separate analysis plan will be created to detail the planned mechanistic analyses by both BDR and BiG. Relevant clinical data from the study will be submitted to ITN BiG to augment the mechanistic analyses.

### 3. 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). The min/max will be reported at the same level of significance as original data. The mean and median will be reported at one more significant digit than the precision of the data, and SD will be reported at two more significant digits than the precision of the data. 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 including *t* and *z* 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.

#### 4. 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 for at least 3 years and in whom the primary endpoint was assessed. Participants in the PP sample must be compliant with study medication, defined as taking 50% or more of their study medication for the duration of the study. Compliance with study medication will be as assessed by pill count for SLIT/SLIT placebo and by observation for SCIT/SCIT placebo. Participants in the PP sample will be analyzed with the group to which they were randomized, regardless of the medication they actually receive.
- **Safety sample (SS)** will be defined as all randomized participants who received at least one dose of study medication. Participants in the safety sample will be analyzed with the group according to the medication they actually received, regardless of their randomized assignment.

## 5. STUDY SUBJECTS

### 5.1 Disposition of Subjects

The disposition of all enrolled subjects will be summarized in tables and listed.

The numbers and percentages of subjects randomized and in each analysis sample will be displayed by randomized treatment group and overall. Reasons for early termination from the study and visit completion statistics will be presented. For subjects discontinuing study treatment early, the reasons for discontinuing study treatment early will also be presented. The number and percentage of subjects randomized will be tabulated using the interactive voice response system (IVRS) data files.

The listing of disposition data will also include dates of the first dose, randomization, last visit, treatment discontinuation, and termination from protocol. The listing will be sorted by treatment group and subject ID.

### 5.2 Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for the randomized and intent-to-treat (ITT) samples. Demographic data will include age, ethnicity, body weight and height, and sex. Baseline variables will include general, clinical, rhinitis, and local laboratory assessments listed in Appendix 1 of the protocol. 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 treatment group and subject.

## **6. STUDY OPERATIONS**

### **6.1 Protocol Deviations**

Protocol deviations will be listed separately for randomized and non-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 IRB reportable. 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 study therapy (24 months). In each of the treatment arms compliance rate will be calculated by dividing the observed doses taken by the per protocol expected doses administered. SCIT (Alutard SQ<sup>®</sup>) and SCIT placebo were administered during scheduled clinic visits under observation of a medical professional. Participants were asked to return used SLIT (Grazax<sup>®</sup>) and SLIT placebo blister packs and unused tablets at each study visit. Treatment compliance will be analyzed and presented across treatment groups and over time.

## 7. ENDPOINT EVALUATION

### 7.1 Overview of Efficacy Analysis Methods

#### 7.1.1 Assessment Time Windows

Allowable visit windows for all scheduled visits are provided in the Appendix.

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

- Visit –4 must occur from March 1 to September 30, 2011.
- Visit –3 must occur from June 1 to September 30, 2011.
- Visit -3a must occur from August 1 to September 30, 2011
- Visit –2 must occur from September 1 to December 31, 2011.
- Visit –1 must occur from October 1 to December 31, 2011.
- Visit 1 must occur from October 1, 2011 to February 28, 2012.
- Visits 2 through 15:  $\pm 3$  days of visit 1 target day.
- Visits 16 through 41:  $\pm 14$  days of visit 1 target day.

Unscheduled visits may also occur throughout the study.

### 7.2 Primary Endpoint

The primary endpoint is the nasal response to allergen challenge at 3 years measured by Total Nasal Symptom Score (TNSS).

#### 7.2.1 Computation of the Primary Endpoint

The computation of the primary endpoint will be performed using two methods. First, the trapezoidal rule will be used to estimate the area under the curve (AUC) of the TNSS measurements during the Nasal Allergen Challenge (NAC). In addition, the mean TNSS measurements will be computed at each NAC time point using a repeated measures linear model. All computations of the primary endpoint will compare the following groups:

- SLIT + SCIT placebo versus SLIT placebo + SCIT placebo
- SCIT + SLIT placebo versus SLIT placebo + SCIT placebo
- SLIT + SCIT placebo versus SCIT + SLIT placebo.

#### 7.2.2 Primary Analysis of the Primary Endpoint

The AUC of the primary endpoint is calculated by taking the per-hour average of the TNSS AUC measured at 0 to 1 hours (EPR) plus the per-hour average of the AUC measured between 1 to 10 hours (LPR). The primary analysis, performed on the ITT sample, will compare the mean AUC at 3 years, adjusted for the baseline AUC, using an ANCOVA model at the 0.05 level of significance. The primary analysis consists of the comparison of SLIT + SCIT placebo versus SLIT placebo + SCIT placebo.

#### 7.2.3 Secondary Analyses of the Primary Endpoint

Secondary analyses of the primary endpoint will consist of the comparison of the per-hour average of the AUC as described in Section 7.2.2 for SCIT + SLIT Placebo versus SLIT Placebo



+ SCIT Placebo and SLIT + SCIT Placebo versus SCIT + SLIT Placebo using ANCOVA models at the 0.05 level of significance.

#### **7.2.4 Sensitivity Analyses of the Primary Endpoint**

Sensitivity analyses will be performed to address the effect of missing data on the primary analysis. Optimistic, pessimistic, and data-driven imputation methods will be used to provide bounds of the potential biases that could result from the missing data. These sensitivity analyses will provide a measure of robustness of the treatment effects as it relates to the consequences of missing data. The following statistical methodologies may be used to create sensitivity analyses of the missing data: Multiple Imputation, multivariate linear and logistic regression.

### **7.3 Secondary Statistical Methodology for Primary and Secondary Endpoints**

The effect of treatment on the primary and secondary endpoints will also be analyzed by a longitudinal repeated measures mixed effects model, adjusted for baseline TNSS and time of post-nasal allergen challenge (except where indicated). For example, plots of the mean TNSS values during the NAC will be used to illustrate how the treatment effect changes over time. Multiple sources of variation (fixed effects, random effects, serial correlation, and measurement error) will be investigated to determine the best covariance structure. The best fitting and most parsimonious model will be determined using Information Criteria where the model with the minimum AIC and BIC measures will be selected as described by [Kincaid, C.](#)

Models will be fit by maximum likelihood methods using all available data in the ITT population. Likelihood ratio tests between models with time as categorical and continuous variables will be used to determine if a linear trend is consistent with the data. Treatment by time interactions will be used to investigate the NAC time intervals that exhibit the greatest treatment effect (for example, 0 to 3 hours). Lastly, all analyses described for the ITT analysis population will be repeated for the PP analysis population.

#### **7.3.1 Justification for Secondary Statistical Methodology for Primary and Secondary Endpoints**

At protocol writing the dilemma was whether to focus on either the early nasal response (0-1 hr) or the late response (1-10hr) since both are clinically and biologically relevant. The early response best represents the patient's immediate response to pollen exposure and is a marker of mast cell activation whereas the late response is more modest, reflected mainly as nasal congestion and more representative of T cell activation and inflammatory cell infiltration into the nose. Our compromise was to take the mean of the equally weighted AUC nasal response/hr during the early (0-60 min) and late (1-10 hr) responses. The statistical test chosen to analyze this primary outcome was ANCOVA which was considered at the time (2009) to be the best statistical test to compare between groups whilst taking between patient variability of baseline nasal challenge data into account.

The above approaches were based on limited data – from a previous immunotherapy trial from the early 90s that employed different methodologies of challenge and analysis and a recent study of n=12 nasal challenges from which to determine our power calculations. 4 years later we have the benefit of observing raw coded data from some 400 nasal provocation tests from the participants in the ITN043 study and an independent cross-sectional pilot study of immunotherapy (Scadding et al in press Allergy 2015).

What has become absolutely clear is that the early response is striking and reproducible whereas the late response is minimal and quite variable, with little ‘window’ to detect changes if changes exist either with time or before/after immunotherapy or between treated groups. From Scadding et al the impact of immunotherapy is seen at the 0-60 min time point for the TNSS and 0-3hr for the more objective measurement of PNIF, and certainly not obvious for either measurement at 1-10 hr during the LPR.

Based on this data we recommend a particular focus in the analysis plan on the early response in terms of both TNSS and PNIF measurements rather than an equally weighted early and late nasal response. The mixed model accomplishes these objectives and is also a more powerful statistical methodology for analyzing these longitudinal data. Prior to finalizing the statistical analysis plan and prior to data lock these would now be our preferred options for our definition of the primary endpoint for the nasal response and our preferred methodology for analysis to discriminate between treatment groups.

#### 7.4 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 secondary endpoints will be analyzed using the ITT sample. The endpoints listed below will compare the following groups using the same statistical methodology as described for the primary endpoint.

- SLIT + SLIT Placebo versus SLIT Placebo + SCIT Placebo
  - SCIT + SLIT Placebo versus SLIT Placebo + SCIT Placebo
  - SLIT + SCIT Placebo versus SCIT + SLIT Placebo
1. Skin late phase response (LPR) to intradermal testing at 0, 1, 2, and 3 years.
  2. Skin early phase response (EPR) to intradermal testing at 0, 1, 2 and 3 years.
  3. Nasal LPR at 0, 1, 2, and 3 years.
  4. Nasal EPR at 0, 1, 2 and 3 years.
  5. PNIF LPR AUC at 0, 1, 2 and 3 years.
  6. PNIF EPR AUC at 0, 1, 2 and 3 years.

Depending on the distribution of the resulting data the following list of endpoints may require the application of nonparametric statistical methodology. If the data are considered to be normally distributed then the same statistical methodology used for the primary and secondary endpoints mentioned above will be used. Specifically, the data will first be tested to determine normality. If the data are not normal then, for example, a Wilcoxon test will be used across each measured time point for between group comparisons. If the data are considered normal then tests will be run to determine if the variances are equal between the two groups. If the variances are

equal, a two sample t-test assuming equal variances will be used; otherwise, a two sample t-test assuming unequal variances will be used. Longitudinal modeling of the endpoints listed below will also be performed as appropriate using the methodology specified for the primary endpoint.

1. Peak TNSS EPR at 0, 1, 2, and 3 years.
2. Skin prick test endpoint titration at 0, 1, 2 and 3 years.
3. Use of rescue medications during the pollen season at 1, 2 and 3 years.
4. Quality-of-life questionnaire score measured pre-, peak-, and post- pollen season at 1, 2, and 3 years.
5. Hay fever severity score measured at the end of each pollen season at 1, 2, and 3 years.
6. Weekly visual analog symptom scores measured pre-, peak-, and post-pollen season.

#### **7.4.1 Skin LPR and Skin EPR**

Skin LPR and skin EPR to intradermal testing will be recorded as the mean diameter of the swelling measured at the specified time points after allergen challenge at 1, 2, and 3 years. The analysis of these two endpoints will compare the mean diameter of the swelling at 1, 2, and 3 years separately, adjusting for baseline diameter using ANCOVA at the 0.05 level of significance.

#### **7.4.2 Nasal LPR and Nasal EPR**

Nasal LPR and nasal EPR will be defined as the TNSS AUC over the specified time periods after allergen challenge at 1, 2, and 3 years. The analysis of these two endpoints will compare the mean TNSS AUC at 1, 2, and 3 years separately, adjusting for baseline LPR and EPR using ANCOVA at the 0.05 level of significance.

#### **7.4.3 PNIF, PNIF LPR, and PNIF EPR**

PNIF, PNIF LPR, and PNIF EPR will be defined as PNIF AUC over the specified time periods after allergen challenge at 1, 2, and 3 years. The analyses for these three endpoints will compare the mean PNIF AUC at 1, 2, and 3 years separately, adjusting for baseline PNIF using ANCOVA and the 0.05 level of significance.

#### **7.4.4 Skin Prick Test Titration**

Skin prick endpoint titration analysis will be assessed as the mean wheal diameters (mm) in response to skin prick test in duplicate with 1000 SQ, 10,000 SQ and 100,000 SQ units of grass pollen allergen. The result will also be expressed as the concentration of allergen that caused a 5 mm skin wheal, as determined by interpolation of the dose-response curve.

#### **7.4.5 Use of Rescue medications**

Use of the following rescue medications will be assessed by asking participants to bring their medication containers to each visit, at which time any remaining pills will be counted and any remaining liquid in bottles will be weighed:

- Nasal corticosteroid (fluticasone propionate aqueous NS)

- Antihistamine (desloratidine)
- Ophthalmic antihistamine (olophtadine)
- Short-acting beta- agonists (albuterol)
- Oral corticosteroids (prednisolone)
- Inhaled corticosteroids (fluticasone)
- Combination long acting beta-agonists or steroids (salumeterol/fluticasone)

A composite rescue medication score will be derived using the following algorithm:

<b>Medication</b>	<b>Medication score</b>	<b>Daily score (recommended intake)</b>
Desloratadine 5mg	6 points / tablet	6
Loratidine 10mg	6 points / tablet	6
Cetirizine 10mg	6 points / tablet	6
Levo-cetirizine 5mg	6 points / tablet	6
Fexofenadine 120mg	6 points / tablet	6
Fexofenadine 180mg	9 points / tablet	9
Olopatadine eye drops	1.5 points / drop	6
Fluticasone Propionate nasal spray	2 points / spray	8
Fluticasone furoate nasal spray	2 points / spray	8
Mometasone furoate nasal spray	2 points / spray	8
Salbutamol inhaler	2 points / puff	8
Seretide 50 Evohaler	2 points / puff	8
Seretide 250 Accuhaler	4 points / puff	16
Seretide 500 Accuhaler	4 points / puff	16
Becotide inhaler	2 points / puff	8
Prednisolone 5mg tablet	1.6 points / tablet	16 ( 50mg, max)

The mean composite score in each treatment group at years 1, 2, and 3 will be computed and compared using ANCOVA at the 0.05 level of significance. As previously specified, non parametric methods may be implemented depending on the distribution of the data.

#### 7.4.6 Quality of Life Questionnaire

Mini Rhinoconjunctivitis Quality-of-Life Questionnaire (MiniRQLQ) scores will be collected pre-, peak- and post-pollen season at 1, 2 and 3 years.

#### **7.4.7 Hay Fever Severity Scores**

Annual Global Evaluation scores for the nose and eye symptoms will be evaluated similarly to the primary outcome by comparing the mean hay fever severity scores at 1, 2 and 3 years separately, adjusting for baseline symptom score using ANCOVA at the 0.05 level of significance. Global Evaluation number 2 will be compared using a nonparametric trend test with significance of 0.05.

#### **7.4.8 Weekly Visual Analogue Scale**

Weekly visual analogue scale scores will be summarized descriptively by group and year.

#### **7.4.9 Mechanistic Assessments**

TBD

## 8. SAFETY EVALUATION

### 8.1 Overview of Safety Analysis Methods

All safety analyses will be carried out using the safety sample defined in Section 4 unless otherwise noted. Missing safety information will not be imputed. Safety will be analyzed in each of the randomized groups through the reporting of adverse events (AEs), vital signs, physical examination findings, and changes in routine laboratory values.

Listings will be prepared for all safety measurements. All listings will be sorted in order of treatment, subject identifier (ID), and time of assessment (e.g., visit, time, and/or event).

### 8.2 Adverse Events

All AEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA] version 14.0). The severity of AEs will be classified using the National Cancer Institute's (NCI's) Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0. Each AE is entered on the case report form (CRF) once at the highest severity. As such, no additional data manipulation is needed to identify events.

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

In addition, AEs classified by MedDRA SOC and preferred term will be summarized for each treatment group and overall for each of the following:

- All AEs
- AEs by maximum severity
- AEs by relationship to study drug

Summary tables will present the total number of events as well as the number and percentage of subjects experiencing the events. If a subject experiences the same AE on multiple occasions, the event will be counted once for each occurrence when reporting the number of AEs. When reporting the number of subjects experiencing the events, a subject will only be counted once if they experience an event within the particular SOC or preferred term. Percentages will be based on the number of subjects in the safety population.

### **8.3 Deaths and Serious Adverse Events**

Serious adverse events (SAEs) will be listed and summarized in the same manner described in Section 8.2. Separate displays listing and summarizing death, including time to death and cause of death, will also be created.

### **8.4 Clinical Laboratory Evaluation**

Clinical laboratory measurements include serum chemistry, urinalysis, and hematology, and IgE. Results will be converted to standardized units where possible. For numeric laboratory results, descriptive statistics of laboratory values and the change from baseline of laboratory values will be presented for each treatment group and overall. For categorical laboratory results, the number and percentage of subjects reporting each result will be presented for each treatment group and overall. Data listings sorted by treatment group, subject ID, laboratory parameter, and time of assessment will also be provided for clinical laboratory measurements. Laboratory normal ranges will be included and out-of-range flags, high (H) or low (L), will be used to denote abnormal values. The lab normal values will be those specific to the processing lab. Abnormal values will be graded per NCI-CTCAE.

Laboratory data will be plotted to show patterns over time. For each test with a numeric result, data will be plotted as a spaghetti plot where each subject's values will be plotted and connected by line segments, forming one line per subject. For each test with a numeric result, quantile plots with treatment group means (or medians) as well as 10<sup>th</sup> and 90<sup>th</sup> percentiles plotted over time will be created.

### **8.5 Vital Signs, Physical Findings, and Other Observations Related to Safety**

#### **8.5.1 Vital Signs**

Descriptive statistics of vital signs results and change from baseline of vital signs will be summarized for each treatment group and overall. Data listings sorted by treatment group, subject, vital sign parameter, and time of assessment will be provided for vital signs measurements.

#### **8.5.2 Physical Examinations**

Physical examination results of normal, abnormal, and not done will be summarized as frequencies and percentages by body system and visit. Data listings will be provided for physical examination results and sorted by treatment group, subject, body system, and time of assessment.

## **9. OTHER ANALYSES**

### **9.1 Use of Medications**

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary (version 2011.01, ITN variant). 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.

The number and percentage of subjects receiving prior, concomitant, and after medications will be presented overall and by medication class. When reporting the number of subjects receiving the medication, a subject will only be counted once if they ever received the medication within the medication class. Percentages will be based on the number of subjects in the analysis population. Separate data listings will be provided for prior, concomitant, and after medications.



## **10. INTERIM ANALYSES AND DATA MONITORING**

The progress of the study will be monitored by the Data and Safety Monitoring Board (DSMB). The Asthma and Allergy DSMB will be chartered to review safety data and to make recommendations regarding continuation, termination, or modification of the study. The DSMB will formally review the safety data at least yearly. The discontinuation of study treatment will also be periodically reported to the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the National Institute of Allergy and Infectious Diseases (NIAID) medical monitor or protocol chair to warrant review, or when an event occurs that could contribute to a predefined stopping rule specified in the protocol.

Findings will be reported to Institutional Review Boards (IRBs) and health authorities.

## **11. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL**

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the DSMB, the study sponsor, and the health authorities. These changes will be described in the final study report as appropriate.

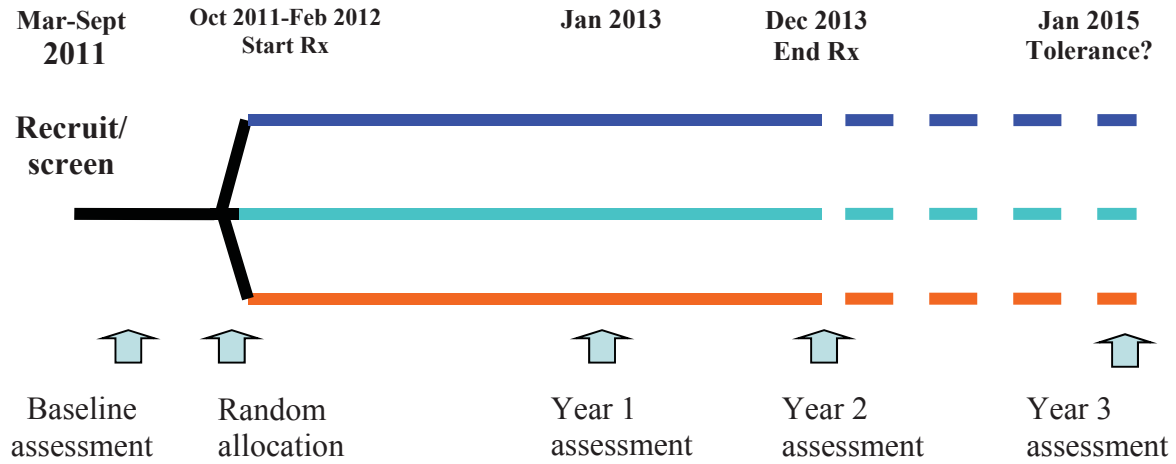
## 12. REFERENCES

1. [Kincaid, C.](http://www2.sas.com/proceedings/sugi30/198-30.pdf) Guidelines for Selecting the Covariance Structure in Mixed Model Analysis. <http://www2.sas.com/proceedings/sugi30/198-30.pdf>

### 13. APPENDICES

#### 13.1 Study Flow Chart

- █ Subcutaneous immunotherapy
- █ Sublingual immunotherapy
- █ Placebo (double dummy)



## 13.2 Schedule of Events

### 13.2.1 Screening and Baseline Assessments

Year	2011				
	In-Season		Out-of-season		
Month	Mar-Sep	Jun-Sep	Aug-Sep	Sep-Jan	Oct-Jan <sup>1</sup>
Visit	-4	-3	-3a	-2	-1
<b>General Assessments</b>					
Informed consent	X				
Medical history	X				
Allergy history	X				
Comprehensive physical exam				X	
Limited physical exam	X		X		
Vital signs	X	X	X	X	X
Pulmonary function testing (spirometry)	X		X	X	
Peak flow testing				X	
Adverse events	X	X	X	X	X
Concomitant medications	X	X	X	X	X
<b>Study Medications</b>					
SCIT + SLIT placebo <i>or</i> SCIT placebo + SLIT placebo <i>or</i> SLIT + SCIT placebo					
Rescue medications					
<b>Clinical Assessments</b>					
Screening nasal allergen challenge	X <sup>2</sup>		X <sup>2</sup>		
Nasal allergen challenge				X	
Total nasal symptom score	X		X	X	
Peak nasal inspiratory flow	X		X	X	
Intradermal skin test- Phleum pratense				X	
Skin prick test – multiple allergens	X				
Skin prick test endpoint titration - Phleum pratense					X
<b>Rhinitis Assessments</b>					
Rescue Medication Score					
Mini RQLQ		X		X	
Global Evaluation No. 1				X	
Global Evaluation No. 2					
Visual Analogue Scale	X	X	X	X	
<b>Local Laboratory Assessments</b>					
Serum pregnancy test	X			X	
Urine pregnancy test					
Hematology	X				
Comprehensive chemistry	X				
Total IgE	X				
Timothy grass RAST	X				

<sup>1</sup> Visit -2 must precede visit -1 by at least 3 weeks.

<sup>2</sup> The visit -4 screening nasal allergen challenge will be deferred for all grass-allergic patients screened during June and July as well as for all grass-allergic patients with additional tree allergy screened during April and May. These study candidates will undergo the screening nasal allergen challenge from August 1, 2011 to September 30, 2011 during visit -3a. This screening allergen challenge must precede the visit -2 baseline nasal allergen challenge by at least 3 weeks.

Year	2011				
	In-Season		Out-of-season		
Month	Mar-Sep	Jun-Sep	Aug-Sep	Sep-Jan	Oct-Jan <sup>1</sup>
Visit	-4	-3	-3a	-2	-1
<b>Mechanistic Laboratory Assessments</b>					
Nasal biopsies					
Nasal fluid cytokines <sup>3</sup>				X <sup>3</sup>	
Nasal brushing <sup>4</sup>				X <sup>4</sup>	
Tetramers in-season – baseline		X			
Tetramers out-of-season					X
PBMC in-season		X			
PBMC out-of-season					X
DNA Methylation <sup>5</sup>		X		X <sup>5</sup>	
Serum FAB antibody assay <sup>5</sup>		X		X <sup>5</sup>	
Serum assays (pre nasal allergen challenge) <sup>5</sup>				X <sup>5</sup>	
Serum assays (post nasal allergen challenge) <sup>6</sup>				X <sup>6</sup>	
PBMC T-cell Assay (pre nasal allergen challenge) <sup>5</sup>				X <sup>5</sup>	
PBMC T-cell Assay (post nasal allergen challenge) <sup>6</sup>				X <sup>6</sup>	
Whole blood flow cytometry (pre allergen challenge) <sup>5</sup>				X <sup>5</sup>	
Whole blood flow cytometry (post nasal allergen challenge) <sup>6</sup>				X <sup>6</sup>	
Whole blood DNA–HLA genotypes		X			

<sup>3</sup> Collected before and at intervals up to 10 hours after the nasal allergen challenge.

<sup>4</sup> Collected at visit -2 baseline and annually after the nasal allergen challenge at 10 (+/-2 hours).

<sup>5</sup> Collected prior to the nasal allergen challenge.

<sup>6</sup> Collected 6 to 7 hours after the nasal allergen challenge.

### 13.2.2 Updosing Year 1

Updosing Year 1																	
Week	1 <sup>7</sup>	2	3	4	5	6	7	8	9	10	11	12	13	14	16		
Visit	1 <sup>8</sup>	2	3	4	5	6	7	8	9	10	11	12	13	14	15	UN	
<b>General Assessments</b>																	
Informed consent																	
Medical history																	
Allergy history																	
Comprehensive physical exam	X																
Limited physical exam																	X
Vital signs	X																X
Pulmonary function testing (spirometry)																	
Phone contact																	
Pre and post peak flow testing	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	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	X	X	X	X
Randomization	X																
<b>Study Medications</b>																	
SCIT + SLIT placebo <i>or</i> SCIT placebo + SLIT placebo <i>or</i> SLIT + SCIT placebo,	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
<b>Local Laboratory Assessments</b>																	
Serum pregnancy test																	
Urine pregnancy test	X																
Hematology																	
Comprehensive chemistry																	
Total IgE																	
Timothy grass RAST																	

<sup>7</sup> The dosing schedule for SCIT updosing is listed in section 5.2.2. Each visit is associated with a target dose. If the participant requires additional visits before reaching the next target dose the additional visits will be denoted as described in section 5.2.2 (e.g. 3a, 3b, etc).

<sup>8</sup> Visit 1 may occur between October 1, 2011 through February 28, 2012. All baseline assessments must be completed prior to initiating study treatment.

### 13.2.3 Maintenance and Withdrawal Visits (Years 1 through 3)

Year	Maintenance												Maintenance												Withdrawal						UN
	Year 1												Year 2												Year 3						
	Month	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	28	30	32	34	36				
Visit	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41					
Informed consent																															
Medical history																															
Allergy history																															
Comprehensive physical exam																															
Limited physical exam																											X				
Vital signs																											X				
Pulmonary function testing (spirometry)																															
Phone Contact																					X	X	X	X	X	X					
Pre and post peak flow testing	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							X				
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	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	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Randomization																															
SCIT + SLIT placebo or SCIT placebo + SLIT placebo or SLIT + SCIT placebo <sup>9 10</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											

<sup>9</sup> Additional visits at the discretion of the study physician may be required for adjustment of allergen doses for individual participants during the maintenance phase of immunotherapy (refer to section 5.2.2)

<sup>10</sup> The total duration of study therapy must be 24 months.



13.2.4 In and Out of Season Assessments (Years 1 through 3)

Year	Seasonal Assessments Year 1							Seasonal Assessments Year 2							Seasonal Assessments Year 3						
	2012					2012-2013	2013					2013-2014	2014					2014-2015			
	In-season					Out-of-season		In-season					Out-of-season		In-season					Out-of-season	
Month	Apr	May	Jun	Jul	Aug	Oct-Jan	Oct-Mar	Apr	May	Jun	Jul	Aug	Oct-Jan	Oct-Mar	Apr	May	Jun	Jul	Aug	Oct-Jan	Oct-Mar
Visit	101	102	103	104	105	106	107	201	202	203	204	205	206	207	301	302	303	304	305	306	307
<b>General Assessments</b>																					
Limited physical exam							X							X							X
Vital signs							X							X							X
Pulmonary function testing (spirometry)							X							X							X
Pre and post peak flow testing																					
Adverse events							X							X							X
Concomitant medications							X							X							X
<b>Study Medications</b>																					
Rescue medications	X	X	X	X	X			X	X	X	X	X			X	X	X	X	X		
<b>Clinical Assessments</b>																					
Screening nasal allergen challenge																					
Nasal allergen challenge							X							X							X
Total nasal score							X							X							X
Peak nasal inspiratory flow							X							X							X
Intradermal skin test - Phleum pratense							X							X							X
Skin prick test endpoint titration Phleum pratense						X							X							X	
Skin prick test																					
<b>Rhinitis Assessments</b>																					
Rescue Medication Score		X	X	X					X	X	X					X	X	X			
Rescue Medication Diary									X	X	X					X	X	X			

Year	Seasonal Assessments Year 1								Seasonal Assessments Year 2							Seasonal Assessments Year 3							
	2012						2012-2013	2013					2013-2014	2014					2014-2015				
	Month	In-season				Out-of-season			In-season				Out-of-season			In-season					Out-of-season		
Visit	Apr	May	Jun	Jul	Aug	Oct-Jan	Oct-Mar	Apr	May	Jun	Jul	Aug	Oct-Jan	Oct-Mar	Apr	May	Jun	Jul	Aug	Oct-Jan	Oct-Mar		
Mini RQLQ <sup>1</sup>	101	102	103	104	105	106	107	201	202	203	204	205	206	207	301	302	303	304	305	306	307		
Visual Analogue Scale <sup>1</sup>		X <sup>1</sup>	X <sup>1</sup>	X <sup>1</sup>			X <sup>1</sup>		X <sup>1</sup>	X <sup>1</sup>	X <sup>1</sup>			X <sup>1</sup>		X <sup>1</sup>	X <sup>1</sup>	X <sup>1</sup>				X <sup>1</sup>	
Modified Rhinitis Symptom Utility Index			X	X			X			X	X			X <sup>1</sup>									
Global Evaluation No. 1 <sup>2</sup>					X <sup>2</sup>		X <sup>2</sup>					X <sup>2</sup>		X <sup>2</sup>					X <sup>2</sup>			X <sup>2</sup>	
Global Evaluation No. 2 <sup>2</sup>					X <sup>2</sup>		X <sup>2</sup>					X <sup>2</sup>		X <sup>2</sup>					X <sup>2</sup>			X <sup>2</sup>	
<b>Mechanistic Assessments</b>																							
Nasal biopsies <sup>3</sup>																						X <sup>3</sup>	
Nasal brushing <sup>3</sup>							X <sup>3</sup>							X <sup>3</sup>									X <sup>3</sup>
Nasal fluid cytokines <sup>4</sup>							X <sup>4</sup>							X <sup>4</sup>									X <sup>4</sup>
Tetramers in-season <sup>5</sup>			X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>				X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>						X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>				
Tetramers out-of-season <sup>6</sup>						X <sup>6</sup>							X <sup>6</sup>								X <sup>6</sup>		
PBMC in-season <sup>5</sup>			X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>				X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>						X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>				
PBMC out-of-season						X <sup>6</sup>							X <sup>6</sup>								X <sup>6</sup>		
DNA Methylation <sup>5</sup>			X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>	X			X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>	X					X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>	X			
Serum FAB antibody assay <sup>5,7</sup>			X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>		X <sup>7</sup>		X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>			X <sup>7</sup>			X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>				X <sup>7</sup>

<sup>1</sup> Collected every week during the pollen season from mid-May to end of July as defined in section 3.3.3, and once out-of-season.

<sup>2</sup> Collected in August/September after the pollen season as defined in section 3.3.3, and once out-of-season.

<sup>3</sup> Collected 10 to 11 hours after the nasal allergen challenge. Nasal brushing will be performed in the contralateral nostril at the time of nasal biopsy.

<sup>4</sup> Collected before and at intervals up to 10 hours after antigen nasal challenge.

<sup>5</sup> Collected once June through August during the pollen season as defined in section 3.3.3.

<sup>6</sup> The out-of-season Tetramers assay collections should occur prior to conducting visits 107, 207, and 307 or at least three weeks after the nasal allergen challenge is performed at these visits.

Year	Seasonal Assessments Year 1								Seasonal Assessments Year 2							Seasonal Assessments Year 3									
	2012							2012-2013	2013						2013-2014	2014						2014-2015			
	Month	In-season					Out-of-season		Oct-Jan	Oct-Mar	In-season					Out-of-season		Oct-Jan	Oct-Mar	In-season					Out-of-season
Apr		May	Jun	Jul	Aug	Oct-Jan	Oct-Mar	Apr			May	Jun	Jul	Aug	Oct-Jan	Oct-Mar	Apr			May	Jun	Jul	Aug	Oct-Jan	Oct-Mar
Visit	101	102	103	104	105	106	107	201	202	203	204	205	206	207	301	302	303	304	305	306	307				
Serum assays (pre - nasal allergen challenge) <sup>7</sup>							X <sup>7</sup>							X <sup>7</sup>							X <sup>7</sup>				
Serum assays (post nasal allergen challenge) <sup>8</sup>							X <sup>8</sup>							X <sup>8</sup>							X <sup>8</sup>				
PBMC T-cell Assay (pre - nasal allergen challenge) <sup>7</sup>							X <sup>7</sup>							X <sup>7</sup>							X <sup>7</sup>				
PBMC T-cell Assay (post- nasal allergen challenge) <sup>8</sup>							X <sup>8</sup>							X <sup>8</sup>							X <sup>8</sup>				
Whole blood Flow Cytometry pre-nasal allergen challenge <sup>7</sup>							X <sup>7</sup>							X <sup>7</sup>							X <sup>7</sup>				
Whole blood Flow Cytometry post- nasal allergen challenge <sup>8</sup>							X <sup>8</sup>							X <sup>8</sup>							X <sup>8</sup>				
Whole blood DNA-HLA genotypes																									

<sup>8</sup> Collected 6 to 7 hours after the nasal allergen challenge.

## 14. ATTACHMENTS