PollenLite Trial

A Randomised, Double-blind, Single-centre, Controlled Trial of Low Dose Intradermal Allergen Immunotherapy in Adults with Seasonal Allergic Rhinitis

Statistical Analysis Plan

Version 2.

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A) QUANTITATIVE ANALYSIS PLAN

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Abbreviations

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
DMC	Data Monitoring Committee
DSUR	Development Safety Update Reports
eCRF	Electronic Case Record Form
eSMS	Emergency Scientific & Medical Services
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FEV1	Forced Expiatory Volume
GCP	Good Clinical Practice
IgE	Immunoglobulin E
IMP	Investigational Medicinal Product
ISRCTN	International Standardised Randomised Controlled Trial Number
KCL	King's College London
KCTU	King's Clinical Trials Unit, King's College London (UKCRC registered KCTU)
KHP-CTO	Kings Health Partners Clinical Trials Office (function of the sponsor)
MHRA	Medicines & Healthcare products Regulatory Agency
NIHR	National Institute for Health Research
NRES	National Research Ethics Service
PEF	Peak Expiratory Flow
PollenLITE	Pollen Low dose Intradermal Therapy Evaluation
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SPC / SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee
UKCRN	UK Clinical Research Network

1. Description of the trial

Subcutaneous immunotherapy with high dose grass pollen was first described over 100 years ago. This treatment suppresses allergen-induced cutaneous late responses, with lesser effects on early responses. In contrast, low dose subcutaneous immunotherapy has failed to show clinical benefit. Uncontrolled reports from the early 20th century describe low dose allergen inoculation directly into the dermis, an immunologically active area containing abundant dendritic cells and lymphatics. We previously reported that repeated 2-weekly intradermal injections of grass pollen - each containing approximately 7 ng of major allergen Phl p 5 – led to a progressive suppression of the allergen-induced cutaneous response, and that by the sixth injection, this was inhibited by over 90%.

The purpose of this trial is to investigate the clinical efficacy of intradermal desensitisation with low doses of grass pollen allergen for seasonal allergic rhinitis.

1.1 Principal research objectives to be addressed

We hypothesise that low dose intradermal grass pollen allergen immunotherapy is an effective treatment for seasonal allergic rhinitis ('hay fever'), reducing symptoms and rescue medication requirements, and improving quality of life for hay fever sufferers.

Primary objectives

The primary objective is to determine if pre-seasonal low dose intradermal grass pollen allergen immunotherapy (either 7 or 8 two-weekly injections of 10 Biological Units (33.333 SQ-U)) reduces symptoms and requirements for anti-allergic drugs in seasonal allergic rhinitis during the 2013 grass pollen season compared to the control intervention (histamine only).

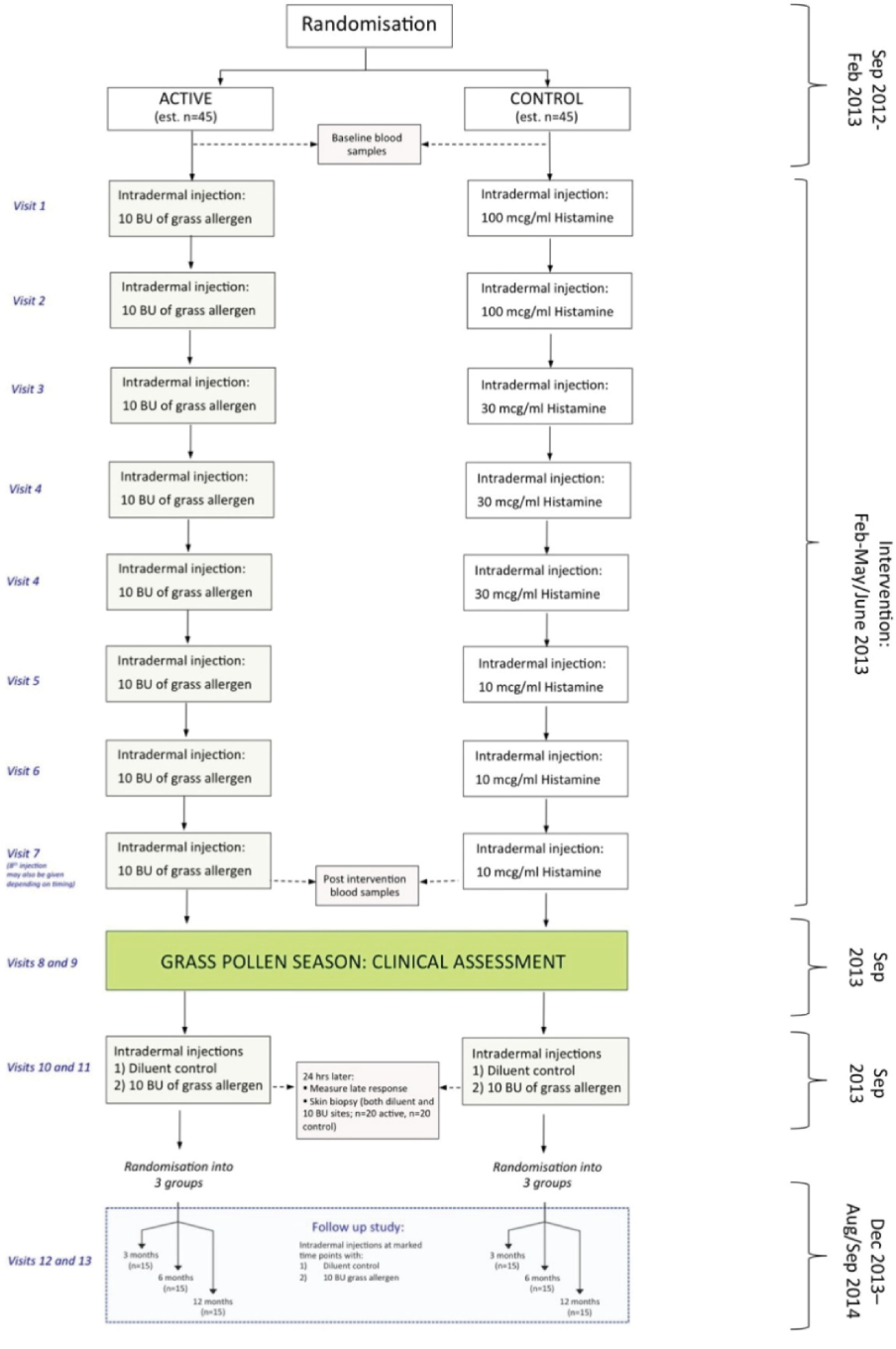
Secondary objectives

1) Determine if this intervention is associated with improvement in quality of life compared to the control intervention, as assessed during the 2013 grass pollen season.
2) Evaluate if this is a safe and well-tolerated form of treatment.
3) Investigate immunological mechanisms associated with this form of treatment, by examining humoral and cellular responses, both in peripheral blood and in tissue.
4) Explore if the intradermal desensitisation effect is long-lived i.e. persists following cessation of intradermal injections.

1.2 Trial design and flowchart

Single centre double-blind randomised parallel group controlled trial

Figure 1. Trial flowchart



1.3 Populations and Study Sample

Target Population

The target population, to which inferences from the end of the PollenLite trial are intended to generalise, is the population of patients with history of allergic rhinoconjunctivitis.

Trial Population

The trial population, from which the study sample is drawn, is further defined to be patients aged 18-65 years at commencement of pollen low dose intradermal therapy, who are screened at Guy's Hospital, King's College London, and who have history of moderate-severe persistent rhinoconjunctivitis.

Trial Sample

The achieved trial sample comprises those patients who consent to participate and are actually randomised into the PollenLite trial. These patients are the study subjects. This randomised trial sample is also the trial Intention To Treat (ITT) population. Subjects will be analysed according to the treatment group to which they are randomised. The trial ITT population comprises all randomised participants, regardless of eligibility (inclusion/exclusion) error, post-randomisation withdrawal, and whether the correct study treatments were received, or other interventions received.

Inclusion criteria

1) Adults aged 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 persistent rhinoconjunctivitis symptoms interfering with usual daily activities or with sleep.
4) A clinical history of rhinoconjunctivitis that remains troublesome despite 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, against *Phleum pratense*.
7) For women of childbearing age, a willingness to use an effective form of contraception for the duration of intradermal injections.
8) The ability to give informed consent and comply with study procedures.

Exclusion criteria

1) Pre-bronchodilator FEV1 less than 70% of predicted value at screening visit.
2) A history of seasonal grass pollen-induced asthma requiring regular treatment with salbutamol or inhaled corticosteroids. Patients with mild seasonal grass pollen-induced asthma may be included, provided symptoms are satisfactorily controlled with occasional salbutamol only.

3) A clinical history of symptomatic seasonal allergic rhinitis and/or asthma due to tree pollen or weed pollen near or overlapping the grass pollen season, although patients with mild intermittent symptoms requiring only occasional antihistamines may be included.
4) A clinical history of symptomatic allergic rhinitis and/or asthma caused by a perennial allergen to which the participant is regularly exposed, although patients with mild intermittent symptoms requiring only occasional antihistamines may be included.
5) Emergency department 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 outside the grass pollen season, which 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 discoloured 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) At randomisation, 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.
10) Current smokers or a history of greater than or equal to 5 pack years.
11) Previous treatment by immunotherapy with grass pollen allergen within the previous 5 years.
12) History of life-threatening anaphylaxis or angioedema.
13) Ongoing systemic immunosuppressive treatment.
14) History of intolerance of grass pollen immunotherapy, rescue medications or their excipients.
15) For females of childbearing age a positive serum or urine pregnancy test with sensitivity of less than 50 mIU/mL within 72 hours of first administration of study therapy.
16) Lactating females.
17) The use of any investigational drug within 30 days of the screening visit.
18) Ongoing treatment with leukotriene receptor antagonists, beta-blockers, calcium channel blockers, tricyclic antidepressants, monoamine oxidase inhibitors or anti-IgE monoclonal antibody.
19) The presence of any medical condition that the investigator deems incompatible with participation in the trial.
20) Individuals with insufficient understanding of the trial.

Safety analysis population

The safety analysis population is comprised of those randomised subjects who receive at least one treatment with pre-seasonal intradermal injections of *Phleum pratense* grass pollen extract and/or Histamine.

1.4 Method of allocation of groups

Once baseline assessments are complete (Screening visit), the individuals will be randomised to one of the treatment arms.
Randomisation will be done in a 1:1 ratio. Participants will be stratified into 2 equal groups according to i) size of skin test response to grass pollen at screening visit, and ii) presence or absence of rhinitis symptoms outside the grass pollen season and block randomised.
Females of childbearing age will be required to undergo a urine pregnancy test with sensitivity of less than 50 mIU/mL within 72 hours of randomisation and first administration of study therapy at Visit 1.
Pre-randomisation allocation concealment will be achieved through the blinding of the study medication. In addition, to minimise unconscious bias through unintentional unblinding, the control intervention will consist of a reducing dose of histamine.
24hr Emergency Code Break and Medical Information will be provided by Guy's & St Thomas' NHS Foundation Trust Emergency Scientific Medical Services (eSMS). Each randomised subject will be provided with a card detailing code break telephone numbers and emergency contact details. Subjects will be requested to carry this card with them at all times whilst participating in the trial.

1.5 Description of interventions

Intradermal grass pollen injections plus rescue medications (intervention) group will be compared to a histamine injections plus rescue medications (control) group in adults with moderate-severe grass pollen-induced allergic rhinitis ('summer hay fever')

Rescue medications

Rescue medications will be provided to all participants in both trial arms before and throughout the pollen season. These will include: desloratadine (5 mg, up to 1 tablet daily), (olopatadine eye drops, 1.0 mg/mL, up to 1 drop per eye twice daily), fluticasone propionate nasal spray 50 mcg per spray, up to 2 sprays per nostril once daily), and prednisone (for use at 30 mg per day for up to 5 days). Participants will be asked to use only these medications to treat their hay fever symptoms on an as required basis. However, participants who are not getting hay fever symptoms will be encouraged to try not to use these medications. Participants will be asked to use only these medications. A short course of prednisolone will be available if symptoms are particularly severe. Participants will be instructed to contact a trial physician prior to taking any prednisolone. The doctor will then provide instructions on dose and duration of treatment. Concurrent treatment with beta-blockers, calcium channel blockers, tricyclic antidepressants, monoamine oxidase inhibitors or anti-IgE monoclonal antibody will not be permitted.

Control group

Intradermal injection of histamine, administered at a concentration of 100 mcg/ml (histamine dose validated by Sherer et al., Clin Exp Allergy. 2007;37:39-46).

Intervention group

Intradermal injections of *Phleum pratense* grass pollen extract, each containing estimated 7 ng of major allergen Phl p 5.

1.6 Duration of the treatment period

Intervention consists of maximum of 8 injections, given at approximately 2-weekly intervals over 3 months. Two further open label injections of grass pollen (10 BO) will be given over a 3 to 12 month follow up period for mechanistic assays.

1.7 Frequency of follow-up and duration of the trial

Frequency of follow-ups is summarised in trial diagram (Figure 1), including screening and 13 visits. The duration of the trial is 2 years. The trial will end when the last subject makes the last visit to determine the late response following the final open label follow up intradermal injection at the Aug 2014 time point.

1.8 Trial efficacy end point

Pollen counts

The peak of grass pollen season will be defined as starting on the first 3 consecutive days between 13 May and 31 August 2013 when grass pollen counts in central London are >30 grains/cm3, using counts supplied by the UK Met Office. The end of the peak season will be defined as the first of 3 consecutive days when grass pollen counts are <30 grains/cm3. In the event of 2 or more peaks during the 2013 season, these individual peak periods will be analysed separately.

Efficacy assessments

Using diaries patients recorded their individual symptoms scores (reflecting the preceding 24 hours) on a daily basis from mid-May through to the end of August. The symptom scoring systems have been adapted from previous trials of grass pollen immunotherapy. The symptom score will be based on individual symptoms in the nose (sneezing, blockage, and running), eyes (itching, redness, tears, and swelling), mouth and throat (itching and dryness), and chest (breathlessness, cough, wheezing, and tightness), recorded on a scale of 0 to 3 (with a score of 0 indicating no symptoms and 1, 2, and 3 indicating mild, moderate, and severe symptoms, respectively). The maximum daily symptoms score will therefore be 39.

All possible rescue medications will be provided to each participant approximately 2 weeks before and throughout the pollen season. Each drug was given according to the recommendation of the manufacturer. No other medication was allowed. Daily medication use will also be recorded in diary cards by participants and a medication score calculated based on use according to need of the following medications: desloratadine, 5 mg, up to 1 tablet daily (6 points per day); olopatadine eye drops, 1.0 mg/mL, up to 1 drop per eye twice daily (1.5 points per drop, up to 6 points per day); fluticasone nasal spray, 50 mcg per spray, up to 2 sprays per nostril once daily (2 point per spray, up to 8 points per day); and prednisone, 5 mg per tablet, up to 6 tablets per day (2 points per tablet, up to 12 points per day). The maximum daily medication score will therefore be 32.
Since scores for symptoms and medications are different in magnitude these parameters will be normalised in accordance with World Allergy Organization guidance on immunotherapy trials. In order to make the range of the outcome measure invariant over the number of symptoms scored, we divide by the number of individual symptoms evaluated, so that the score has a range from 0 to 3. Medication scores will be then normalised to the symptoms scores so that it is given equal range 0 to 3.

Primary efficacy end point

The primary outcome measure will be combined symptom and medication score (SMS) defined as the area under curve (AUC) of the sum of the normalised daily rescue medication score and the daily symptom score for all days of the pollen season.
Efficacy will then be assessed by comparison of this combined score in active and control groups and estimate of the treatment effect will be expressed in means of median differences with confidence intervals, with a significance level of $p = 0.05$..

Secondary efficacy end points

1) Symptom scores (AUC) calculated as above.
2) Medication scores (AUC), calculated as above.
3) Rhinoconjunctivitis Quality of Life: mini Rhinitis Quality of Life Scores (RQLQ) scores (overall score and domain scores) will be recorded three times during the pollen season (June 12, June 26 and July 10) and once after the season on 4 September 2013. These values will be compared in active and control groups. The mini RQLQ covers five dimensions of health including sleep, non-nose/eye symptoms, practical problems, nasal symptoms, eye symptoms.
4) Health related quality of life: This will be evaluated using the EQ-5D-5L questionnaire three times during the pollen season (June 12, June 26 and July 10) and once after the season on 4 September 2013.
5) Visual Analogue Scores (see Additional file). These will be recorded every 2 weeks during the pollen season and AUC values calculated.
6) Global evaluation scores (see Additional file).
7) The number of primary care (i.e. general practitioner) visits for hay fever during summer 2013.

8) Combined symptom and medication scores during the peak of the 2013 grass pollen season.
9) Number of medication free days covering the grass pollen season period of 13th May-end August 2013 will be compared in active and control groups.
10) Number of symptom free days covering the grass pollen season period of 13th May-end August 2013 will be compared in active and control groups.
11) Individual symptoms scores (AUC) for each organ: nose, mouth, eyes and lungs.
12) Total number of days during which prednisolone used between 13th May-end August 2013.

Assessment of safety

Adverse events were documented throughout the study. Systemic reactions were graded according to the EAACI classification. Details on AE are described in the protocol

1.9 Sample size estimation (including clinical significance)

Power calculations for the primary outcome (combined symptom and medication score) were performed based on a previous clinical trial of subcutaneous grass pollen immunotherapy conducted by Varney et al. The power calculation has been conservatively based on the detection of a clinical effect size 80% of that reported in the Varney trial. Since subcutaneous grass pollen immunotherapy is the gold standard treatment such an effect size would be viewed as clinically meaningful. This power calculation has been performed after readjustment to medication scores such that the combined symptom and medication score endpoint gives equal weighting to both parameters. Using this method, group sample sizes of 35 and 35 achieve 90% power to detect a difference of 80% in combined symptom and medication scores between the null hypothesis that both arms means are 638.0 with estimated group standard deviations of 271.0 and the alternative hypothesis that the mean of the intervention arm is 419.0 at a significance level of 0.05, using a two-sided Mann-Whitney test assuming that the actual distribution is normal. To adjust for the unknown distribution of the primary outcome and based on the lower bound for the asymptotic relative efficiency (ARE) of the Mann-Whitney U test. We have increased the sample size by a further 15% to 40 in each arm. Further accounting for a post-randomisation dropout rate of up to 10% consistent with previous trials of grass pollen immunotherapy, a total sample size of 90 (45 each arm) is required. Recruitment will take place several months before visit 1. At visit 1 randomisation will be performed and the first injection administered. To ensure that a minimum of 90 participants is randomised, up to 100 screened participants will be booked for visit 1, allowing for a 10% drop-out rate between screening and randomisation. In the event that more than 90 eligible participants attend for visit 1, all will be included in the study and randomised up to a maximum of 100.

1.9 Brief description of proposed analyses

Analyses will be carried out by the trial statistician. In the first instance data will be analysed under intention-to-treat assumptions (i.e. analyse all those with data in groups as randomised irrespective of treatment received).

2. Data analysis plan – Data description

2.1 Recruitment and representativeness of recruited patients

Recruitment, randomisation and follow-up for PollenLite will be summarised by arm in a CONSORT flow-diagram.
This will include the main reasons for there being missing data (withdrawal, lost to follow up) by stages of the trial, and will also include the numbers for whom this occurs per arm.
Also included will be the number randomised, who comprise the intention to treat trial population, and the numbers followed-up to be in the analyses of the primary outcome.

2.2 Baseline comparability of randomised groups

Summary measures for the baseline characteristics of each group will be presented as mean and standard deviation for continuous (approximate) normally distributed variables, medians and interquartile ranges for non-normally distributed variables, and frequencies and percentages for categorical variables. No significance testing.
The characteristics will include socio-demographic descriptors (including sex and age), randomisation stratifiers, allergy history, symptoms, rhinoconjunctivitis severity (severe/moderate), and other baseline (screening) clinical measures.
This will allow a visual assessment of whether the randomisation procedure succeeded in producing comparable arms, and will not include the improper use of p-values from statistical hypothesis testing between arms at baseline.
This will also show baseline characteristics of the trial sample for description in the main paper.

2.3 Loss to follow-up and other missing data

At least 50**%** of daily SMS scores has to be complete in order for a diary to be acceptable for evaluation. Data from subjects who do not submit valid diary data for at least 2 of the 4 peak pollen weeks will be considered as Missing data.
The proportions of participants missing each variable will be summarised in each arm and at each time point.
The baseline characteristics of those missing follow up will be compared to those with complete follow up with p-values from univariate statistical tests.
The reasons for withdrawal from the trial will be summarised.
Sample size estimation assumed 10% of patients would not provide evaluable end of study information. If this rate is observed, data for some patients will be only partially observed. Efforts were planned to reduce missing data by reminding participant to their 24 hour dairy at the beginning, midway and at

the end of pollen season. If data from one assessment point are missing, the mean value of the two adjacent ones will be used. Another alternative, the daily SMS could be determined by calculating a 3-day (or up to one week maximum) rolling average (previous, current and following days). For patients with missing data and for patients who withdrew or dropped two weeks before the peak pollen period end, multiple imputations method will be used in order to provide an overall treatment effect estimate with a standard error that is properly inflated to incorporate uncertainty associated with imputing values (i.e. between-imputation variability in the estimated treatment effect). Since this may introduce a bias if the main reason for drop-out was deterioration, sensitivity analysis will be examined to explore departures from the missing at random assumption using White et al intention to treat strategy.

2.5 Adverse event reporting

Adverse events (AE), adverse reactions (AR), serious adverse events (SAE) and serious adverse reactions (SAR) will be summarised.

2.6 Assessment of outcome measures (unblinding)

Evidence for unblinding of treatment to interviewers will be studied.

2.7 Descriptive statistics for main outcome measures

The Area under the Curves (AUC) of the individual and combined symptom and medication scores for the period corresponding to the grass pollen season (mid May-Aug) will be plotted against time as a summary measure of the primary outcome. This will provide each patient's longitudinal outcome as a single quantity, which will be calculated for Symptom and Medication scores.

3. Data analysis plan – Inferential analysis

3.1 Main analysis of treatment differences

The main statistical analyses will estimate the difference in mean outcomes between patients randomised to 45 and 45 by intention to treat at the various post-treatment observation time points. Group difference estimates and associated confidence intervals will be reported.

3.1.1 Analysis of primary outcomes

The planned primary efficacy analyses, difference between the two arms in AUC of the combined symptom and medication scores, will be analyzed on randomized patients using non-parametric approach, (stratified) Mann-Whitney U test (Van Elteren test statistic),adjusted for the baseline stratification factors size of the skin test to grass pollen and presence or absence of rhinitis symptoms. And the (stratified) Hodges-Lehmann estimation to calculate median differences with confidence intervals, with a significance level of P = 0.05.

If the data distribution is normal or log-normal, analysis of covariance (ANCOVA), adjusted for the baseline stratification factors size of the skin test to grass pollen and presence or absence of rhinitis symptoms will replace the non-parametric analysis.

3.1.2 Analysis of secondary outcomes

Similar analyses as for the primary outcome measure will be conducted for secondary (symptom scores, medication scores and individual symptoms) and mechanistic outcomes. Subgroup analysis by holiday's destination will also be investigated. All patients who were on holiday in continental Europe will be included in the per protocol analysis. Those who holidayed outside of Europe are to also be in per protocol analysis but data for days where they are abroad are to be counted as missing data and >50% missing data threshold will be applied (See page 14, paragraph "Loss to follow-up and other missing data"). Extensive sensitivity analysis on all holiday destinations will be conducted.

Regression models will be also used to evaluate the change in RQLQ scores to isolate the effect of the intervention on each arm after adjusting for stratification factors.
In analysing the recovery of the cutaneous late response at each 3, 6 and 12 month time point, the size of late response in the group that originally received active therapy will be compared with the group that originally received the control intervention. As a further sensitivity analysis, all key outcomes will be re-analysed adjusting for any observed differences at baseline that are judged to be of clinical importance. Differences between the groups will be estimated with 95% confidence intervals.

3.1.3 Responder analysis

Responder analysis will be performed. Because we do not have a baseline year for comparison, the median AUC for the placebo group will be defined as the comparator, and responders defined as those subjects with AUC less than this value, using different cut-offs (20%, 25%, 30% etc.). The optimal value for distinguishing actively treated from placebo groups will be selected using receiver–operator curves, and numbers thus defined as responders and non-responders in each group were compared by chi-squared analysis. Clinical and laboratory characteristics of these groups will be also investigated.

3.1.4 Model assumption checks

If a model assume normally distributed outcomes; this will be checked when describing the data and if substantial departures from normality occur, transformations will be considered. Residuals will be plotted to check for normality and inspected for outliers.

3.2 Exploratory analyses

Any examination of subgroups, not specifically identified in the protocol, will be considered exploratory in nature and will be clearly identified.

3.4 Interim analysis

No interim analysis is planned although pre-defined stopping criteria will be discussed by the TSC and the Independent DMEC and agreed if appropriate.

4. Reporting conventions

Reporting conventions will adhere when possible to the International Conference on Harmonization (ICH) Guidance document E3, “Structure and Content of Clinical Study Reports”. Some specific conventions are outlined below:

1. All tables and listings will be in landscape format.
2. All statistical analysis software output for tables and listings will be distributed in PDF files.

5. Software

Data management: An online data collection system for clinical trials (MACRO; InferMed Ltd) will be used. This is hosted on a dedicated server at KCL and managed by the MH&N CTU. The MH&N CTU Data Manager will extract data periodically as needed and provide these in comma separated (.csv) format.

Statistical analysis: The principal software package will be STATA, with verification of results from syntax for selected analyses in SAS.

B) ECONOMIC ANALYSIS PLAN

Heath economic objectives

To assess the cost-effectiveness of low dose intradermal grass pollen allergen immunotherapy in adult patients with moderate-severe persistent rhinoconjunctivitis.

Economic measures

Economic measures will include cost of the intervention, volume of resource use for health services and related unit costs, and EQ-5D scores. Economic analyses will conform to NICE's preferred methodology. Outcomes will be reported as quality-adjusted life years (QALYs) and symptom-free days. Results will be subjected to simple and probabilistic sensitivity analysis.

Statistics

Because of the skewed nature of medication use and QoL data will be analysed using a (stratified) nonparametric test (Mann–Whitney) to compare resource use and QALYs.

C) SCHEDULE OF ASSESSMENTS AND MEASURES

By Visit

Visit -1 (Screening visit; Sep 2012-Jan 2013):
- Informed consent
- Medical history
- Allergy history
- Skin prick testing
- Recording of concomitant medications
- Limited Physical Examination
- Vital signs
- Spirometry
- Blood sample (5 ml) for total IgE and specific IgE (hospital lab)
- Blood sample (10 ml) for mechanistic assays (baseline sample)

Visit 1 (first intervention visit; 18th Feb-1st Mar 2013)
- Urine pregnancy test
- Recording of concomitant medications
- Intradermal injection with active or control drug
- Clinical observation for one hour
- Recording of adverse events (adverse events before randomisation at Visit 1 will not be recorded)

Visits 2-6 (Mar-May 2013)
- Recording of concomitant medications
- Intradermal injection with active or control drug
- Clinical observation for 30 minute
- Recording of adverse events

Visit 7 (May 2013)
- Recording of concomitant medications
- Blood sample (10 ml) for mechanistic assays (baseline sample)
- Intradermal injection with active or control drug (if visit 7 falls before 13 May, this injection will be repeated 12-16 days later)
- Clinical observation for 30 minutes
- Recording of adverse events

Visit 8 (early Jul 2013)
- Recording of concomitant medications
- Collection of May and June symptom/medication use diary cards
- Collection of Visual Analogue Scores for May/June
- Collection of Mini RQLQ and EQ-5D-5L forms for 12 and 26 June
- Recording of adverse events

Visit 9 (early Aug 2013)
- Recording of concomitant medications
- Collection of July symptom/medication use diary cards
- Collection of Visual Analogue Scores for July
- Collection of Mini RQLQ and EQ-5D-5L forms for 10 July

- Recording of adverse events

Visit 10 (Sep 2013)
- Recording of concomitant medications
- Collection of Aug symptom/medication use diary cards
- Collection of Visual Analogue Scores for Aug
- Collection of Mini RQLQ and EQ-5D-5L forms for 4 September
- Global assessment score (1) and (2) completion
- Record number of GP visits over summer for hay fever
- Verify blinding: All participants to guess if received active or control intervention
- Additional informed consent – skin biopsy specific form (n=40)
- Intradermal injection with diluent (negative control) and 10 BU (33.333 SQ-U) grass pollen allergen (open label)
- Measurement of skin early response size (after 15 mins)
- Clinical observation for 30 minutes
- Recording of adverse events

Visit 11 (24 hrs after Visit 10 in Sep 2013)
- Recording of concomitant medications
- Measurement of skin late response size (all participants)
- Skin biopsy of diluent and allergen intradermal injection sites (40 random participants only)
- Recording of adverse events

Visit 12 (randomised to either Dec 2013, Mar 2013 or Aug 2014)
- Recording of concomitant medications
- Intradermal injection with diluent (negative control) and 10 BU (33.333 SQ-U) grass pollen allergen (open label)
- Measurement of skin early response size (after 15 mins)
- Recording of serious adverse events

Visit 13 (24 hrs after Visit 12)
- Recording of concomitant medications
- Measurement of skin late response size
- Recording of serious adverse events

Laboratory Tests

Visit -1 (Screening visit):
A sample of venous blood (5 ml) will be collected for total IgE and specific IgE, which will be analysed routinely by the Immunology department of Guy's and St Thomas' NHS Foundation Trust. A sample of blood (10 ml baseline sample) will also be collected at the same time for mechanistic studies in academic laboratories. This sample will be centrifuged and serum aliquoted and stored at -20oC in the Chief Investigator's KCL laboratory pending analysis in the laboratory of Professor Durham (co-investigator) at Imperial College. All identifying data will be in anonymised form. Study participants will be asked to provide informed consent storage of their samples for a minimum

of ten years for future studies as novel serum-based assays of immune tolerance become available.

Visit 7:
A further sample of blood (15 ml post-intervention sample) will be collected for mechanistic studies in academic laborotories. As previously, a 10 ml sample will be centrifuged and serum aliquoted and stored at-20oC in the Chief Investigator's KCL laboratory pending analysis in the laboratory of Professor Durham (co-investigator) at Imperial College. Study participants will again be asked to provide informed consent storage of their samples for a minimum of ten years for future studies as novel serum-based assays of immune tolerance become available. The additional 5 ml will be collected into a heparinised tube for basophil activation studies in fresh whole blood.

Visit 11:
Two 3-mm skin punch biopsies will collected 24 hours after diluent and allergen intradermal injections. The biopsy will be taken from the injection site under local anaesthesia. This will only be performed in a sub-group of 40 participants identified at random by the King's Clinical Trials Unit (who are performing randomisation for the whole trial). Biopsies will be fixed in paraformaldehyde, processed, and stored at -80oC in the Chief Investigator's KCL laboratory prior to analysis by immunochemistry. In addition, the first 20 biopsies will be divided into 2 equal pieces using a sterile scalpel: one piece will be processed as above, and the second piece will be cultured in vitro for T cell analysis in the Chief Investigator's KCL laboratory.

- **Figure 1. Trial flowchart**

	Screening	Intervention period													
Year	2012-3	2013												2013-4	
		2-weekly intervals (range: 12-16 days)*													
Month	Oct-mid Feb	18 Feb-1 Mar	4-15 Mar	18-29 Mar	1 Apr- 12 Apr	15-26 Apr	29 Apr-10 May	13-24 May	(if visit 7 falls before May 13, futher injection approx 2 wks later	Start July	Start Aug	Sep	Sep	Dec 2013 or March 2014 or Aug 2014 (randomised)	
Visit	-1	1	2	3	4	5	6	7	*Repeat 'Visit 7'*	8	9	10	11	12	13
General Assessments															
Informed consent	X														
Informed consent - skin biopsy specific form												X			
Medical history	X														
Allergy history	X														
Limited physical exam	X														
Vital signs	X														
Spirometry	X														
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	SAE only	SAE only
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X													
Re-randomisation for skin biopsy and follow-up intradermal injeciton											X				
Clinical Assessments															
Skin prick tests	X														
Urine pregnancy test		X													
Local Laboratory Assessments															
Total IgE	X														
Timothy grass RAST	X														
Intervention															
Active or control intradermal injection		X	X	X	X	X	X	X	X						
1 hour observation		X													
30 mins observation			X	X	X	X	X	X	X			X		X	
Clinical outcomes															
Symptom score								diary card completion daily mid May - end Aug							
Medication score								diary card completion daily mid May - end Aug							
Visual Analogue Score								scores completed fortnightly mid May - end Aug							
miniRQLQ									to be completed 12 Jun, 26 Jun, 10 Jul & 4 Sep						
EQ-5D-5L									to be completed 12 Jun, 26 Jun, 10 Jul & 4 Sep						
Visit for diary/score card collection										X	X	X			
Record number of GP visit for hay fever over summer												X			
Global assessment (1)												X			
Global assessment (2)												X			
Verify blinding (participants to be asked if received active or control)												X			
Mechanistic Laboratory Assessments															
Serum for antibody assays	X							X							
Whole blood for basophil assays								X							
Intradermal allergen challenge (diluent and 10 BU grass pollen)												X		X	
Measurement of skin early response (15 mins post challenge)												X		X	
Measurement of skin late response (24 hrs post challenge)													X		X
Skin biopsy (diluent and allergen sites) (n=40 only)													X		

Amendments to versions

Previous:

- Version 1.0: 02-04-2012 Developed from the EME-NIHR submission research protocol

- Version 1.2: 08-05-2013 Emails/Phones discussion with Steve Till and Janet Peacock

    - Addition of four additional secondary outcomes concurred with various immunotherapy and regulatory guidelines
    - Number of medication free days covering the grass pollen season period of 13th May-end August 2013 will be compared in active and control groups
    - Number of symptom free days (well days) covering the grass pollen season period of 13th May-end August 2013 will be compared in active and control groups
    - Individual symptoms scores (AUC) for each organ: nose, mouth, eyes and lungs
    - Total number of days during which prednisolone used between 13th May-end August 2013
    - Addition of responder analysis in planned statistics as recommended by various immunotherapy and regulatory guidelines

- Version 1.3: 08-07-2014 with Steering committee
    - Page 14: lower the percentage of permissible data to 50%, previously this was 75%.
    - Page 16: To support the primary outcome finding, a subgroup analysis by holiday's destination will also be investigated.

Current:

Principal amendments from previous version:

- Version 2.0: 05-09-2014:

    - Page 13: the sentence "Investigators' terms of adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA)" was removed from "Assessment of safety" paragraph.
    - Page 16: the sentence "All patients who were on holiday in continental Europe will be included in the per protocol analysis. Those who holidayed outside of Europe are to also be in per protocol analysis but data for days where they are abroad are to be counted as missing data and >50% missing data threshold will be applied (See page 14, paragraph "Loss to follow-up and other missing data"). Extensive sensitivity analysis on all holiday's destination will be conducted." was added in "Analysis of secondary outcomes" paragraph

Reference list

Reference List

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