MRC & Asthma UK Centre in Allergic Mechanisms of Asthma

Interim Study Report

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

Document Date: 27 July 2015

Chief Investigator: Dr Stephen Till

Sponsor: KCL/GSTT

ISRCTN: 78413121

EudraCT number: 2012-002193-31

REC number: 12/LO/0941

Stephen Till		12/8/15
Chief Investigator	Signature	Date

Protocol Synopsis

Title of clinical trial	A Randomised, Double-blind, Single-centre, Controlled Trial of Low Dose Intradermal Allergen Immunotherapy in Adults with Seasonal Allergic Rhinitis
Protocol Short Title	Pollen Low dose Intradermal Therapy Evaluation in Allergic Rhinitis (PollenLITE study)
Development Phase	II
Condition under investigation	Allergic rhinitis ('hay fever')
Purpose of clinical trial	To evaluate low dose intradermal allergen vaccination in the treatment of allergic rhinitis, and in doing so, to establish a new clinical and scientific principle.
Primary objective	To determine if low dose intradermal allergen vaccination is clinically effective.
Secondary objective (s)	1) To determine if this intervention is associated with improvement in quality of life compared to the control intervention; 2) To evaluate if this is a safe and well-tolerated form of treatment. 3) To investigate immunological mechanisms associated with this form of treatment, by examining humoral and cellular responses; 4) To explore if the intradermal desensitisation effect is long-lived.
Trial Design	Single centre randomised double-blind controlled trial
Primary Endpoint	A combined symptom and medication score during the grass pollen season period of mid May-August 2013.
Secondary endpoints	1) Symptom score during 2013 grass pollen season. 2) Medication score during 2013 grass pollen season. 3) Rhinoconjunctivitis Quality of Life Questionnaire scores during 2013 grass pollen season. 4) Visual Analogue Score during 2013 grass pollen season. 5) A global evaluation of symptoms at the end of 2013 grass pollen season. 6) Frequency of adverse events 7) Health Related Quality of Life Questionnaire (EQ-5D-5L) scores during 2013 grass pollen season. 8) Number of GP visits for hay fever during summer 2013 9) Combined symptom and medication score during the peak of the 2013 grass pollen season. 10) 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. 11) 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. 12) Individual symptoms scores (AUC) for each organ: nose, mouth, eyes and lungs. 13) Total number of days during which prednisolone used between 13th May-end August 2013
Sample Size	93
Main Inclusion Criteria	• Adults aged 18 to 65 years. • A clinical history of grass pollen-induced allergic rhinoconjunctivitis for at least 2 years with peak symptoms in May, June, or July. • A clinical history of moderate-severe persistent rhinoconjunctivitis symptoms interfering with usual daily activities or with sleep. • A clinical history of rhinoconjunctivitis that remains troublesome despite treatment with either antihistamines or nasal corticosteroids during the grass pollen season. • Positive skin prick test response, defined as wheal diameter greater than or equal to 3 mm, to Phleum pratense. • Positive specific IgE, defined as greater than or equal to IgE class 2, against Phleum pratense. • For women of childbearing age, a willingness to use an effective form of contraception for the duration of intradermal injections. • The ability to give informed consent and comply with study procedures.
Main Exclusion Criteria	• Pre-bronchodilator FEV1 less than 70% of predicted value at screening visit. • A history of uncontrolled seasonal grass pollen-induced asthma (Mild seasonal asthma may be included). • A clinical history of symptomatic seasonal allergic rhinitis and/or asthma due to tree pollen or weed pollen (mild symptoms requiring occasional antihistamines may be included). • A clinical history of symptomatic allergic rhinitis and/or asthma caused by a perennial allergen to which the participant is regularly exposed. • Emergency department visit or hospital admission for asthma in the previous 12 months. • History of chronic obstructive pulmonary disease. • History of significant recurrent acute sinusitis.

	• History of chronic sinusitis. • 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. • Current smokers or history of greater than or equal to 5 pack years. • Previous grass pollen immunotherapy within the previous 5 years. • History of life-threatening anaphylaxis or angioedema. • Ongoing systemic immunosuppressive treatment. • History of intolerance of grass pollen immunotherapy or rescue medications. • Positive pregnancy test within 72 hours of first administration of study therapy. • Lactating females. • Use of any investigational drug within 30 days of screening visit. • Ongoing treatment with leukotriene receptor antagonists, beta-blockers, calcium channel blockers, tricyclic antidepressants, monoamine oxidase inhibitors or anti-IgE monoclonal antibody. • Medical condition the investigator deems incompatible with participation in the trial. • Individuals with insufficient understanding of the trial.
IMP, dosage and administration	Grass pollen allergen; 10 biological units (BU) given in 20 mcl volume by intradermal injection
Comparator Product	20 mcl intradermal injections containing histamine (dose reducing from 100 mcg/ml to 30 mcg/ml, then to 10 mcg/ml).
Maximum duration of treatment	Intervention consists of maximum of 8 injections, given at approximately 2-weekly intervals over 3 months.
Trial duration	8th October 2012 (first visit) - 27th August 2014 (last visit)
Rescue medications	Rescue medications were provided to all participants throughout the entire pollen season with instructions for use only on an as required basis.
Follow-up	Patients were followed-up at 4 months and randomised for follow-up at either 7, 10 or 13 months following final vaccine for open label intradermal skin tests with grass pollen (10 BU)
Analysis	Unblinding and analysis took place following the final patient visit on 27th August 2014.

Table of Contents

Protocol Synopsis 2
Abbreviations 6
Trial design 7
Statistical Methods 7
Sample Size 7
Randomisation 8
Analysis 8
Study Conduct 11
Study Participants 12
Primary Outcome 12
Secondary Outcomes 14
Safety 20
Discussion 22
Conclusion 24

Abbreviations

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
DMEC	Data Monitoring & Ethics Committee
DSUR	Development Safety Update Reports
eCRF	Electronic Case Record Form
EME	Efficacy and Mechanism Evaluation
eSMS	Emergency Scientific & Medical Services
FEV1	Forced Expiratory Volume
GCP	Good Clinical Practice
IDIT	Intradermal immunotherapy
IgE	Immunoglobulin E
IMP	Investigational Medicinal Product
ISRCTN	International Standardised Randomised Controlled Trial Number
KCTU	King's Clinical Trials Unit, King's College London (UKCRC CTU)
KHPCTO	Kings Health Partners Clinical Trials Office (function of the sponsor)
MHRA	Medicines & Healthcare products Regulatory Agency
NICE	National Institute for Clinical Excellence
NIHR	National Institute for Health Research
NRES	National Research Ethics Service
PEF	Peak Expiratory Flow
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
SDW	Source Data Worksheet
TMG	Trial Management Group
TSC	Trial Steering Committee

Trial design

See protocol paper (Appendix 1, Slovick et al. Clinical and Translational Allergy 2013, 3:27).

Statistical Methods

Sample Size

Power calculations for the primary outcome (combined symptom and medication score) were performed based on a previous clinical trial of subcutaneous grass pollen immunotherapy (Varney et al., BMJ. 1991;302(27):265-9.). The power calculation was 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 was performed after readjustment to medication scores such that the combined symptom and medication score endpoint gave equal weighting to both parameters. Using this method, group sample sizes of 35 and 35 achieve 90% power to detect a difference in combined symptom and medication scores between the null hypothesis that both arms means were 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 was 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 (Lehmann EL. Nonparametrics: Statistical methods based on ranks. 1975. San Francisco: Holden-Day. 457 pp.), we 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) was required. Screening visits commenced in October 2012, 4 months prior to visit 1. At visit 1 randomisation was performed and the first injection administered. To ensure that a minimum of 90 participants were randomised, up to 100 screened participants were booked for visit 1, allowing for a 10% drop-out rate between

screening and randomisation. 93 eligible participants attended for visit 1 and all were included in the study and randomised.

Randomisation

Randomisation to active or control intervention

The King's Clinical Trial Unit (KCTU) at King's College London hosted a 24 hour web based randomisation system. Participants were randomised 1:1 to active and comparator medications by the method of block randomisation with randomly varying block sizes, stratified by the size of skin test response to grass pollen at screening visit (the cut-off skin prick test size was the median value of all subjects randomised) and presence/absence of rhinitis symptoms outside the grass pollen season. Study medication was blinded. To minimise bias through accidental unblinding as a result of anticipated injection site reactions in the active trial arm, the control intervention was a reducing dose of histamine, which produced similar clinical effects as the active medication.

Randomisation for skin biopsy

In August 2013, the CTU randomly selected participants to be approached in rotation to undergo skin biopsies. The first 40 participants who agreed then underwent biopsy.

Randomisation for follow up intradermal skin test

In August 2013, the CTU randomised all participants for a second time to one of three groups (7 months, 10 months or 13 months post-final vaccine). These 3 groups then underwent repeat intradermal allergen injections at 7, 10 or 13 months, respectively, to assess if the low dose allergen immunotherapy was associated with prolonged suppression of skin responses.

Analysis

The Statistical Analysis Plan was finalised by the trial statistician and approved by the TSC and the DMEC prior to database lock. The study was unblinded after the final intradermal injection in September 2014. No interim analysis was

performed. Descriptive statistics were produced for DMEC reports and in the primary analysis. For each of the variables analysed, univariate descriptive statistics were summarised by randomised group to provide an overview of the data. Summary measures for the baseline characteristics of each group were 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. The Area under the Curves (AUC) of the combined symptom and medication scores for the period corresponding to the grass pollen season (mid May-Aug) were plotted against time as a summary measure of the primary outcome. This provided each patient's longitudinal outcome as a single quantity. The planned primary efficacy analysis i.e. the difference between the two arms in AUC of the combined symptom and medication scores, was analysed on randomized patients using (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 outside the grass pollen season). Sample size estimation assumed 10% of patients would not provide evaluable end of study information. Intention to treat analysis included all patients. Per protocol analysis excluded those that deviated significantly from the protocol, such as those failing to complete the treatment schedule, deviation from the injection schedule, missing greater than 50% of diary card data and failure to use rescue medications according to the protocol. Post hoc analysis was performed where data were missing by imputing mean scores. As a further sensitivity analysis, individual symptoms for each organ were analysed. Similar analyses were conducted for secondary (symptom scores, medication scores and visual analogue scores) and mechanistic outcomes. Regression models were 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 7, 10 and 13-month time points, the size of late response in the group that originally received active therapy was compared with the group that originally received the control intervention. Differences between the groups were estimated with

95% confidence intervals. The principal software package was STATA and GraphPad Prism 6.

Peak Pollen Season

The start of the peak of the grass pollen season was defined as the first of 3 consecutive days between 13 May and 31 August 2013 when grass pollen counts in London were >30 grains/cm^3, whilst the end of the peak season was defined as the first of 3 consecutive days when grass pollen counts were <30 grains/cm^3. The peak was from June 12 – July 26 2013.

Study Conduct

Protocol Amendments

Version	Amendments post-MHRA and REC approval
4	• Justification for skin biopsies rather than nasal biopsies or nasal samples for mechanistic investigations • Added: 'Patients were stratified according to rhinitis symptoms outside the grass pollen season' • Justification of follow-up period. Patients were randomised for follow-up skin tests at either 7, 10 or 13 months post final vaccine to monitor persistence of late phase response suppression. • Discontinuation criteria: A systemic reaction of Grade 2 or above • Exclusion criterion added: History of intolerance of grass pollen immunotherapy, rescue medications or their excipients. • Secondary outcome measure amended: Frequency of adverse events (AE), including the occurrence of systemic allergic reactions (see classification under Discontinuation criteria, Section 4, page 4). These data will be tabulated by organ system class, grade/severity and preferred term, as well as by treatment relatedness. • Sample size: Increased from 90 to 100 to allow for dropouts between screening and visit 1. • Statistical analysis: Primary outcome area under the curve described • Intradermal injections: All participants in this trial will be observed after the first intradermal injection for one hour, and for 30 mins after subsequent injections • Trial Steering committee members details amended • Added: Ms Joanna Kelly from the King's CTU has also joined the study team at the suggestion of the EME Board and will provide significant ongoing methodological input. • Service users involvement in recruitment and dissemination was added
5	• Recruitment sample size increased from 90 to 100
6	• Secondary outcomes added: • Number of medication free days and number of symptom free days and number of days during which prednisolone used between 13th May-end August 2013 • Individual symptoms scores (AUC) for each organ: nose, mouth, eyes and lungs.

Protocol Deviations

	IDIT	Control
Did not complete injection schedule	0	1
Deviated from injection schedule	1	1
Failure to use rescue medications according to protocol	0	5
Missing >50% of data	0	1
Patients who failed to attend follow-up skin test (but completed primary outcome diary cards)	6	6

Study Participants

Ninety-three participants allergic to grass pollen were enrolled and randomised to receive the first intradermal injection of grass pollen or histamine control between February 18 and March 1 of 2013 (Fig. 1). Study arms were well matched, with no significant differences in baseline characteristics including age, sex, race, presence of allergic rhinitis symptoms outside the grass pollen season or sensitivity to grass pollen (Table 1). All 46 participants assigned to the IDIT completed the treatment course, although one participant deviated from the administration schedule by one day for a single injection due to a scheduling conflict. Of the 47 participants assigned to the control histamine injections, one did not complete the treatment course, withdrawing after the second injection due to work commitments, and another participant deviated from the administration schedule by 4 days due to an unrelated upper respiratory tract infection. Ninety-two participants completed >50% of daily diary card data for the primary outcome; 1 completed only 48% of data. Five participants, all in the histamine control group, deviated from use of rescue medications specified in the trial protocol.

Primary Outcome

Intention to Treat Analysis

All 93 randomised participants could be evaluated for the primary outcome and were included in the intention-to-treat analysis. IDIT did not affect the primary endpoint of this trial, namely the AUC of daily combined symptom-medication score over the grass pollen season (P=0.80), although there was a clear temporal relationship with daily pollen counts in London, which peaked at levels in the above-average range (Fig. 2).

Per Protocol Analysis

The per-protocol analysis included 45 participants who received IDIT and 39 who received the histamine control treatment. The results in the per-protocol population were similar to those observed in the intention-to-treat population (Table Sx. 1, supplementary appendix), Furthermore, using mean imputation of missing data values in the intention-to-treat population gave results consistent with main intention-to-treat analysis (Table Sx. 2, supplementary appendix).

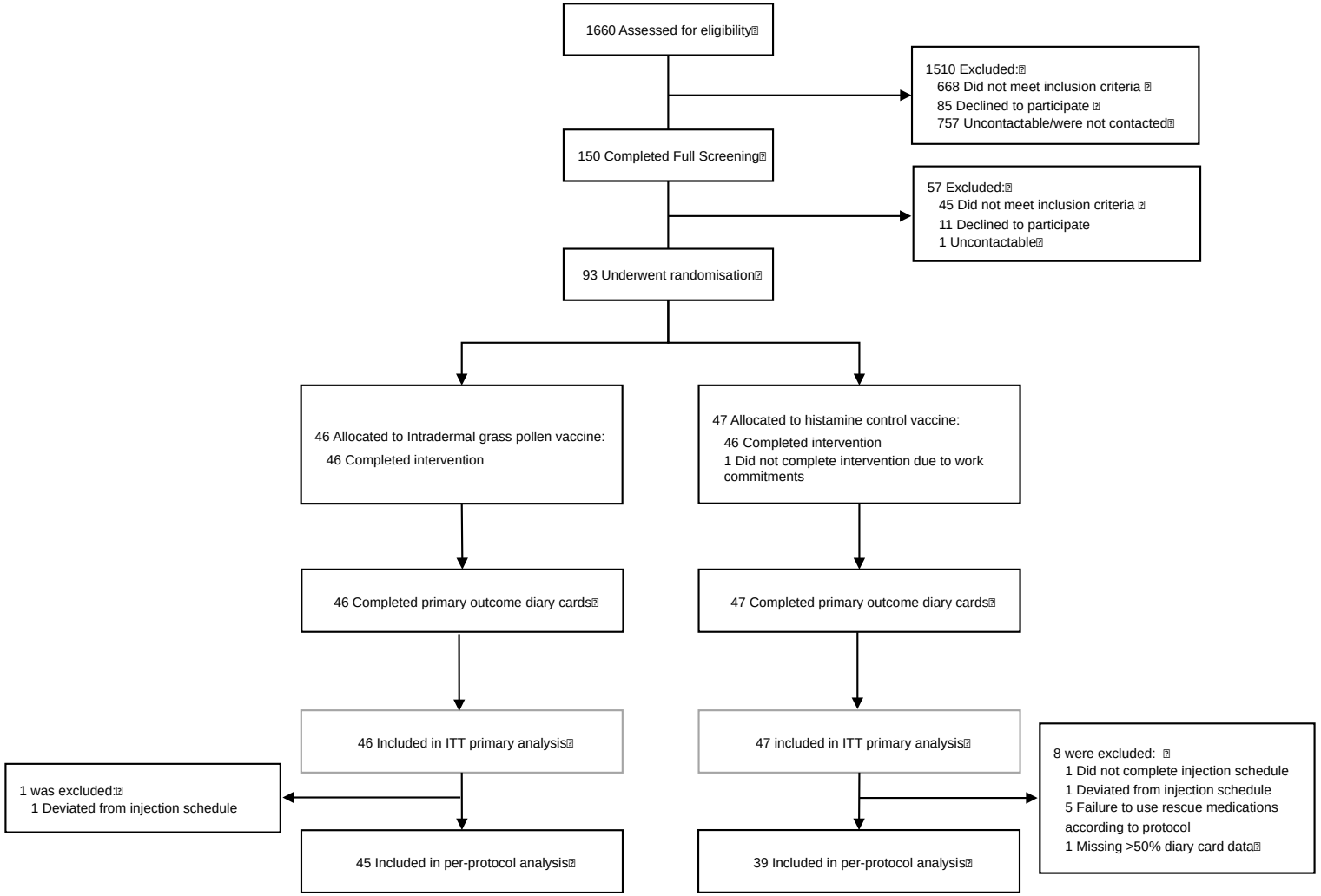

Figure 1. Disposition of Study Participants

Table 1. Baseline characteristics of study participants

	Active (IDIT) (n=46)	Control (n=47)
Age at screening (years), mean (SD)	32 (9.9)	35 (10.8)
Female sex, no. (%)	19 (41)	12 (26)
Race, no. (%)		
White	37 (80)	37 (79)
Mixed	3 (7)	2 (4)
Asian	4 (9)	3 (6)
Black	0 (0)	3 (6)
Other	2 (4)	2 (4)
Allergy symptoms outside grass pollen season, no. (%)	16 (35)	18 (38)
Total IgE (kU$_c$/L), median (IQR)	160 (80-263)	121 (64-255)
Phleum Pratense-specific IgE (kU$_A$/L), median (IQR)	22 (9-49)	27 (10-54)
Phleum pretense SPT wheal diameter (mm), mean (SD)	11 (5.0)	12 (4.2)
SPT-positive, no. (%)		
Timothy grass	46 (100%)	47 (100%)
Mixed grass	46 (100%)	47 (100%)
Silver birch	24 (52%)	19 (40%)
Mugwort	9 (20%)	11 (23%)
House dust mite	24 (52%)	28 (60%)
Cat	18 (39%)	24 (51%)
Dog	36 (78%)	41 (87%)
Horse	6 (13%)	4 (9%)
Aspergillus	2 (4%)	1 (2%)
Alternaria	7 (15%)	6 (13%)
Cladosporium	2 (4%)	2 (4%)
Vital signs		
Pulse rate (bpm), mean (SD)	72 (10.9)	69 (9.6)
Blood Pressure – systolic (mmHg), mean (SD)	133 (15.5)	137 (12.5)
Blood Pressure – diastolic (mmHg), mean (SD)	80 (9.6)	81 (9.4)
Spirometry		
FEV$_1$ (L), mean (SD)	4 (0.9)	4 (0.7)
FVC (L), mean (SD)	5 (1.2)	5 (1.0)
FEV$_1$ % Predicted spirometry, mean (SD)	101 (10.8)	101 (11.2)
Allergy History		
Asthma (controlled with salbutamol), no. (%)	15 (33)	17 (36)
Urticaria, no. (%)	13 (28)	16 (34)
Eczema, no. (%)	14 (30)	7 (15)
Food allergy, no. (%)	6 (13)	5 (11)
Drug allergy, no. (%)	5 (11)	5 (11)
Insect allergy, no. (%)	2 (4)	3 (6)
Medical History		
Respiratory, no. (%)	10 (22)	10 (21)
Dermatology, no. (%)	9 (20)	11 (23)
Musculo-skeletal, no. (%)	3 (7)	9 (19)
Gastro-intestinal, no. (%)	6 (13)	3 (6)
Genito-urinary, no. (%)	5 (11)	4 (9)
Neurological, no. (%)	1 (2)	6 (13)
ENT, no. (%)	4 (9)	3 (6)
Psychiatric, no. (%)	3 (7)	2 (4)
Haematological, no. (%)	1 (2)	3 (6)
Cardiovascular, no. (%)	2 (4)	1 (2)
Hepatic, no. (%)	1 (2)	1 (2)
Endocrine, no. (%)	1 (2)	1 (2)
Neoplasia, no. (%)	2 (4)	0 (0)
Immunological, no. (%)	1 (2)	0 (0)
Infection, no. (%)	1 (2)	0 (0)
Other, no. (%)	3 (7)	2 (4)

FEV1: Forced Expiratory Volume in 1 Second; FVC: Forced Vital Capacity; ENT: Ear, Nose and Throat; SPT: Skin prick test

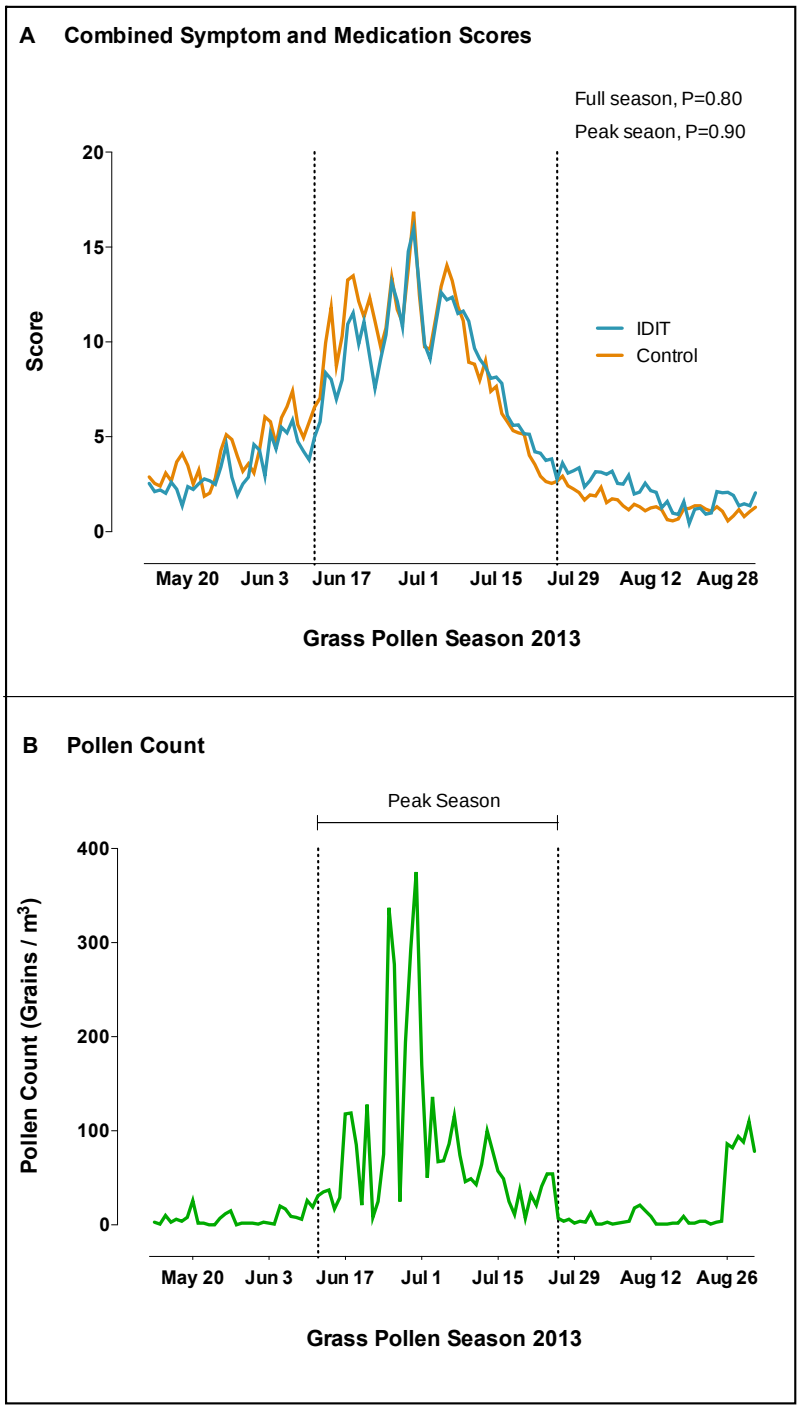

Figure 2. Primary Outcome

Panel A shows the median daily combined symptom and medication scores according to treatment group over the 2013 grass pollen season. Panel B shows the grass pollen counts during the 2013 grass pollen season (supplied by the UK Met Office). P values are based on Mann-Whitney U tests. Dates refer to the start of each week. Broken vertical lines indicate the beginning and end of the peak pollen season. (June 12 – July 26 2013).

Secondary Outcomes

Combined symptom and medication score during peak season

No difference was seen between the trials arms in the AUC of the combined symptom-medication scores during the peak of the grass pollen season (June 12 – July 26 2013) (P=0.90; Table 2).

Total symptom scores & medication scores during entire season

No difference was seen between groups in daily overall symptom scores (P=0.24) or rescue medication use (P=0.44) during the entire season (Table 2). For both groups the symptom and medication scores closely paralleled the pollen counts.

Total daily symptom scores for nose, mouth, eyes and lungs

Rhinitis symptoms, measured by total daily nasal symptom scores, were paradoxically 44% higher in the IDIT group compared to the control group with a median difference of 35 (95% confidence interval [CI], 4.0-67.5; P=0.03)(Fig. 3). No significant differences were seen between groups in total daily eye or lung symptoms (Table 2), although there was a trend for median mouth symptoms to also be higher in the IDIT treated group (median difference 10.0; 95% CI, -3.8-24; P=0.05).

Visual Analogue Scores during entire 2013 grass pollen season.

Rhinitis symptoms measured by AUC of visual-analogue scale were also 28% higher in the IDIT group, with a median difference of 53 (95% CI, -11.6-125.2; P=0.05) (Fig. 3). No differences were observed in eye symptoms measured by AUC of visual-analogue scale.

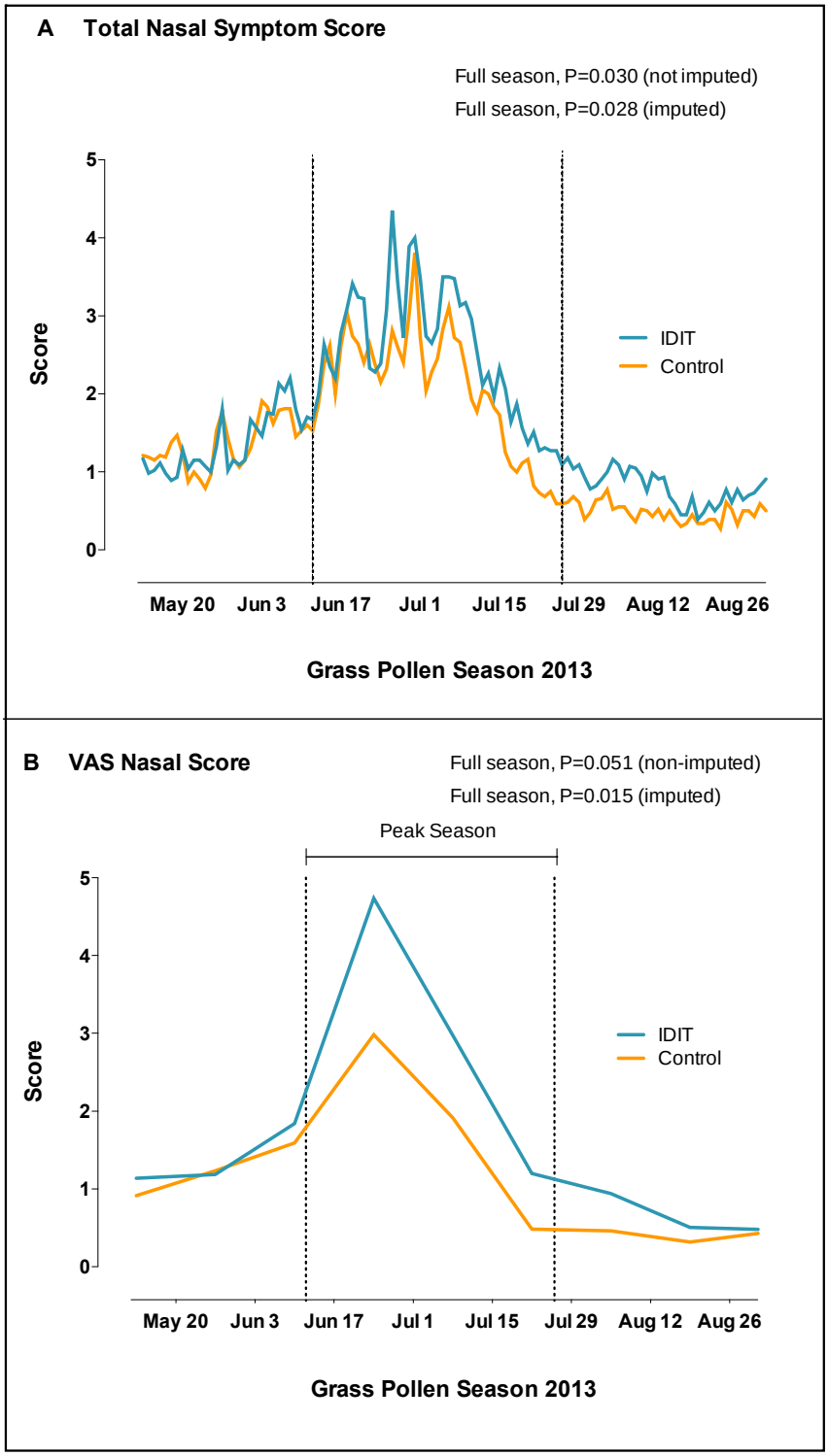

Figure 3. Nasal daily symptom scores and nasal visual analogue scores

Panel A shows the median daily nasal symptom diary scores. Panel B shows nasal visual analogue scores (VAS), according to treatment groups and week of the 2013 pollen season. P values are based on Mann-Whitney U tests. Dates refer to the start of each week. Broken vertical lines indicate the beginning and end of the peak pollen season. (June 12 – July 26 2013).

Table 2. Effect of IDIT on primary and secondary outcomes

	AUC (IQR)			
	Active (IDIT)	**Control**	**Median Difference (95% CI)***	**Adjusted P value**
Primary Outcome				
CSMS during entire season	502 (333-841)	487 (365-717)	14 (-172.5-215.1)	0.80
Secondary Outcomes				
Symptom score during entire season	335 (183-503)	264 (156-398)	59 (-1.3-110.9)	0.24
Medication Score during entire season	242 (116-405)	263 (129-482)	-19 (-153.0-100.2)	0.44
CSMS Score during peak season	356 (232-521)	365 (278-508)	-8 (-75.8-66.3)	0.90
Nasal symptom score during entire season	174 (120-207)	121 (81-200)	35 (4.0-67.5)	0.03
Mouth symptom score during entire season	34 (8-90)	14 (5-45)	10 (3.8-24)	0.05
Eye symptom score during entire season	79 (41-153)	78 (52-180)	-7 (-18.5-2.9)	0.54
Lung symptom score during entire season	17 (3-32)	12 (0-34)	4 (-1-15)	0.17
Nasal Allergic Symptoms measured by VAS	156 (104-275)	122 (54-184)	53 (-11.6-125.2)	0.05
Eye Allergic Symptoms measured by VAS	84 (32-197)	144 (41-176)	-3 (-46.0-35.8)	0.40
Mini RQLQ	N/A	N/A	-0.3 (-4.23 - 3.69)**	0.89
EQ-5D-5L	N/A	N/A	9 (-24.8 - 43.6)**	0.59
Global Evaluation of Symptom Scores	3 (2-4)	3 (1-4)	0 (0-1)	0.48
Symptom Free Days	35 (19-53)	41 (23-61)	-6 (-17-3)	0.15
No. days prednisone used during entire season	0 (0-0)	0 (0-0)	0 (0-0)	0.36
Medication Free Days	81 (65-93)	76 (65-94)	4 (-11-21)	0.22

Area under the curve (AUC) scores are expressed as median (IQR)

* The median difference was the difference between the active group relative to placebo group using stratified Hodges-Lehmann.

** The mean difference calculated from linear mixed model adjusted for stratification factors and time

Statistical comparison by means of Mann-Whitney U test adjusted for stratification factors (Van Elteren's test)

Entire season: 13th May-31st August 2013. Peak season: 12th June-26th July 2013.

CSMS: combined symptom & medication score, VAS: Visual annalogue scale, mini-RQLQ: mini-Rhinoconjunctivitis Quality-of-Life Questionnaire.
EQ-5D-5L: EuroQol instrument

Global Evaluation of Symptoms scores: assessment of symptom severity at worst during monitored season and compared with previous years

Quality-of-Life Questionnaires, Number of GP visits, Symptom or medication free days, Total number of days prednisolone used for hay fever during summer 2013

Mini RQLQ or EQ-5D-5L quality of life measures, global evaluation of symptoms scores, numbers of symptom or medication free days, or number of days during which prednisolone was used, as rescue medication were also not significantly different between the groups.

Intradermal skin test responses

Early (15 minutes) and late phase (24 hour) skin responses were measured in 86 participants 4 months after the seventh and final IDIT injection in September 2013, and then repeated later at either 7, 10 or 13 months. The size of late responses in the control group was consistent with that which we previously reported under the same conditions (Rotiroti et al., 2012)) (shown for comparison in Fig 4). In the present trial, the late response still appeared suppressed 4 and 7 months after completing IDIT (P=0.03 for both time points), but not at 10 or 13 months. In comparison with the historical data however, the degrees of suppression at these times was considerably less than that which we observed immediately after completing 6 injections (Fig. 4), suggesting that the suppressive effect on late responses was wearing off by the 4 month time-point.

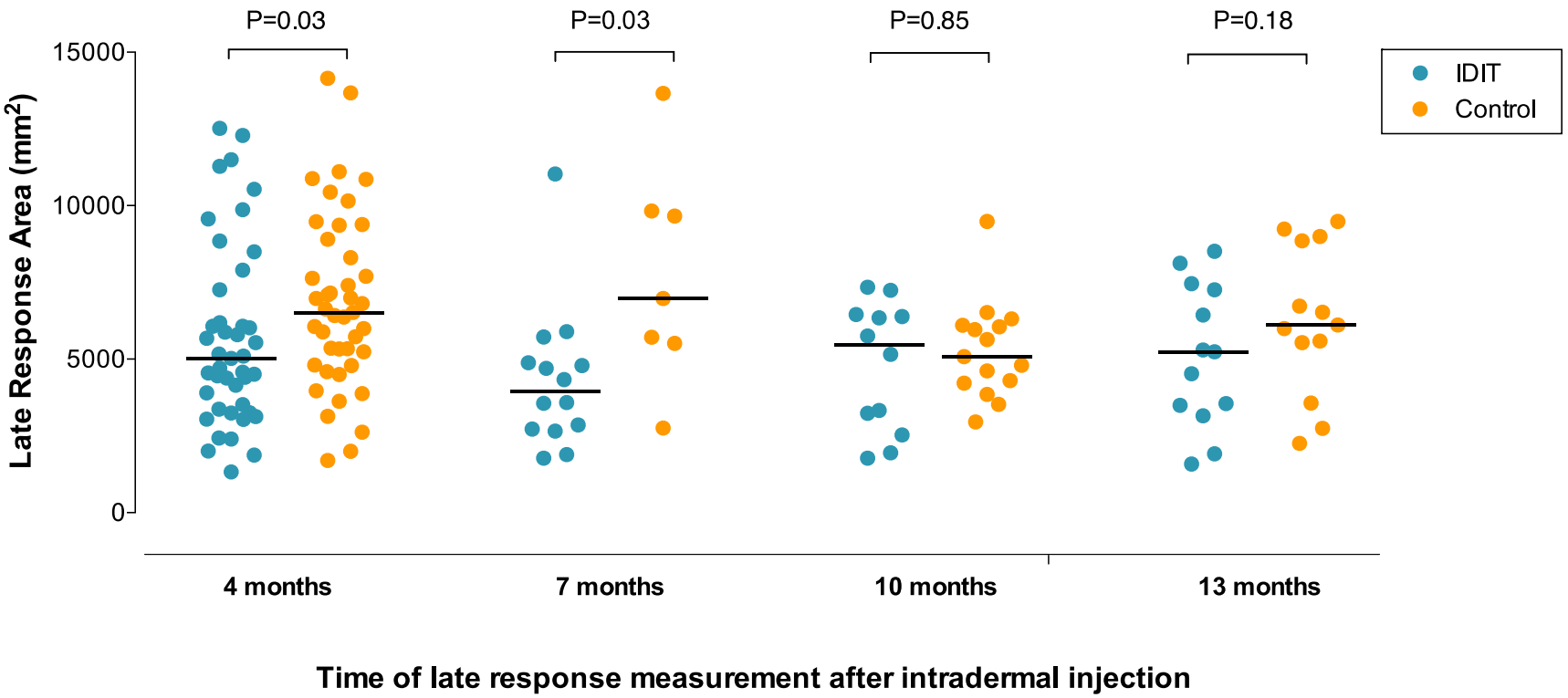

Figure 4: Cutaneous late phase responses

Late phase responses were measured 24 hours after intradermal injection of *Phleum Pratense* at 4 months and then either 7 months, 10 months and 13 months after the final treatment injection. Comparison between the IDIT and histamine control group was with Mann Whitney U test. Solid bars represent median values

Safety

Serious Adverse Events

There were 3 serious adverse events, all considered to be unrelated to treatment: one participant in the IDIT group was hospitalised for severe tonsillitis, one control participant was admitted for overnight polysomnography and another control participant required treatment to remove an infected dental plate. (Table 3).

Suspected Unexpected Serious Adverse Reactions

There were no deaths or suspected unexpected serious adverse reactions.

Adverse Reactions

Three participants in the IDIT group and 6 in the control group were recorded with treatment-related adverse events, all mild grade 1 systematic reactions. These reactions manifested as generalized pruritus without wheals, except for one IDIT participant who developed erythema that tracked from the injection site in a lymphatic distribution (‘IgE-mediated lymphangitis’) approximately 20 minutes after each administration. (Table 3)

Table 3: Frequency of Adverse Events reported from first IDIT or control injection until end of the pollen season

	Active (IDIT) (N=46)				Control (N=47)			
	No. Participants with ≥1 AE	% Participants	No. Events	Event Rate (%)	No. Participants with ≥1 AE	% Participants	No. Events	Event Rate (%)
Any AEs	40	87.0	148	100.0	42	89.4	145	100.0
Serious Adverse Events	1	2.2	1	0.7	2	4.3	2	1.4
Tonsillitis	1	2.2	1	0.7	0	0.0	0	0.0
Overnight stay for Polysomnography	0	0.0	0	0.0	1	2.1	1	0.7
Exraction of infected dental plate	0	0.0	0	0.0	1	2.1	1	0.7
Relation of AE to treatment								
Definite/Probable	3	6.5	15	10.1	6	12.8	14	9.7
Possible	0	0.0	0	0.0	0	0.0	0	0.0
Remote	30	65.2	68	45.9	34	72.3	70	48.3
None	32	69.6	65	43.9	34	72.3	61	42.1
AE withdrawals	0	0.0	0	0.0	0	0.0	0	0.0
Systemic Adverse Reactions	3	6.5	15	10.1	6	12.8	13	9.0
Generalised Pruritus	2	4.3	8	5.4	4	8.5	9	6.2
IgE-mediated lymphangitis**	1	2.2	7	4.7	0	0.0	0	0.0
Light-headedness	0	0.0	0	0.0	2	4.3	2	1.4
Facial flushing/feeling hot	0	0.0	0	0.0	2	4.3	3	2.1
Systemic Adverse Reactions, graded according to WAO*								
Grade 1	3	6.5	15	10.1	6	12.8	12	8.3
Grade 2	0	0.0	0	0.0	0	0.0	0	0.0
Grade 3	0	0.0	0	0.0	0	0.0	0	0.0
Grade 4	0	0.0	0	0.0	0	0.0	0	0.0

*World Allergy Organization Subcutaneous Systemic Reaction Grading System, Cox L et al. JACI 125:569-574, e567.

Light-headedness not classifiable per WAO

Fisher's Exact test was used when 5 or less event, Chi2 test otherwise

**P<0.05 for IgE-mediated lymphangitis

Discussion

In this study, we conclusively demonstrate that preseasonal treatment with intradermal grass pollen extract injections was not associated with clinical efficacy, as measured by the primary endpoint of a combined symptom and medication score during the 2013 summer grass pollen season. Although this trial was not specifically designed or powered to detect harm the intention-to-treat analysis of secondary endpoints this treatment showed that IDIT was associated with 44% worse allergic rhinitis symptoms as measured by both a daily scores and 28% worse symptoms as measured by a two weekly visual analogue scale.

In our study, no serious adverse events occurred that were attributable to grass pollen IDIT and 92 of the 93 participants completed the 7 injection course. One participant withdrew during the treatment period for unrelated reasons. Although a total of nine participants experienced grade one systemic reactions, six of these occurred in the control group receiving histamine injections only. One participant in the IDIT group experienced self-limiting erythema in a lymphatic distribution 20 minutes after each injection. Five participants deviated from the protocol in the use of rescue medication, mainly taking antihistamines above the prescribed dose. Two participants also used prednisolone without reference to the study physician. We are unable to account for why these five participants were all in the control arm, although their exclusion from the per-protocol analysis did not affect the findings of the study.

For this trial, we selected an allergen dose equivalent to 7 ng of the major Timothy grass pollen allergen Phl p5 for several reasons. Firstly, we previously reported in a proof of concept study conducted in a similar grass pollen allergic population that six injections at the same dose given every two weeks led to almost complete attenuation of the cutaneous late response induced by these injections. This is comparable to the effect on cutaneous late phase responses seen following high-dose subcutaneous immunotherapy and exceeds that following treatment with sublingual grass pollen vaccines. Secondly, the

average diameter of the late response induced by this dose is approximately 10 cm, which we considered to be at the upper limit of tolerability for patients for a routine treatment. Although the precise intradermal grass dosages used in the uncontrolled historic studies of Phillips are unknown, it is notable that he described the typical size of the 'delayed response' as representing 'roughly the size of the palm of the hand'. One possible limitation of our study is that the dose of grass pollen was not increased during the treatment course. We did not do this because of our previous observation that repeating the same dose was sufficient for suppression of the late phase response. Another possible weakness is that injections were not continued through the grass pollen season, although previous randomised controlled trials of pre-seasonal subcutaneous grass pollen immunotherapy have demonstrated efficacy.

Late phase skin responses were only measured at the end of the 2013 grass pollen season because performing such measurements before or during collection of clinical outcome data would have risked unblinding the trial. Therefore the first late phase response measurements could be obtained only four months after the final pre-seasonal injection. Late phase responses were still significantly lower in the IDIT group than the control group at this time point, and also at the 7 month time point. Nonetheless this difference was significantly less than we previously observed immediately after six intradermal injections in the proof of concept study, suggesting that late phase response suppression is transient and mostly reversed within four months.

Conclusion

This trial provides evidence that repeat intradermal injection of grass pollen is not clinically effective, despite suppressing skin late phase responses. Furthermore, the finding that this resulted in worsening of allergic rhinitis symptoms and supports the concept that dermal allergen exposure has the potential to exacerbate allergic responses.

Supplementary Appendix

Table S1. Effect of IDIT on primary and secondary outcomes (per protocol analysis, non-imputed)

	AUC (IQR)		Median Difference (95% CI)*	Adjusted P value
	Active (IDIT)	Control		
Primary Outcome				
CSMS during entire season	517 (344-841)	453 (279-685)	82 (-121.8-280.1)	0.23
Secondary Outcomes				
Symptom score during entire season	340 (189-503)	241 (150-398)	76 (25.9-133.5)	0.09
Medication Score during entire season	255 (119-405)	254 (113-358)	21 (-125.0-157.0)	0.83
CSMS Score during peak season	363 (242-546)	342 (242-476)	18 (-73.2-127.5)	0.51
Nose symptom score during entire season	173 (123-207)	119 (80-205)	40 (13.3-71.5)	0.02
Mouth symptom score during entire season	38 (8- 90)	14 (4- 43)	14 (4.9-32.0)	0.02
Eye symptom score during entire season	80 (41-153)	72 (48-145)	0 (-16.0-17.6)	0.85
Lung symptom score during entire season	17 (3- 32)	11 (0- 21)	9 (1.0-17.0)	0.05
Nose Allergic Symptoms measured by VAS	162 (105-275)	118 (50-154)	68 (8.3-134.6)	0.01
Eye Allergic Symptoms measured by VAS	90 (32-197)	114 (42-159)	1 (-52.8-62.0)	0.49
Mini RQLQ	N/A	N/A	-3(-5.46 - 0.01)**	0.31
EQ-5D-5L	N/A	N/A	3 (-28.4 - 35.2)**	0.83
Global Evaluation of Symptom Scores	3 (2-4)	3 (1-3)	1 (0.0-1.0)	0.25
Symptom Free Days	34 (19- 47)	44 (25- 67)	-12 (-22.0--2.0)	0.04
No. days prednisone used during entire season	0 (0-0)	0 (0-0)	0 (0 to 0)	0.33
Medication Free Days	80 (65- 92)	78 (66- 98)	-1 (-20.0-17.0)	0.87

Area under the curve (AUC) scores are expressed as median (IQR)

*The median difference was the difference between the active group relative to placebo group using stratified Hodges-Lehmann.

** The mean difference calculated from linear mixed model adjusted for stratification factors and time

Statistical comparison by means of Mann-Whitney U test and Van Elteren's test adjusted for stratification factors

Entire season: 13th May-31st August 2013. Peak season: 12th June-26th July 2013. ? Need to clarify how we define peak here?

CSMS: combined symptom & medication score, VAS: Visual annalogue scale, mini-RQLQ: mini-Rhinoconjunctivitis Quality-of-Life Questionnaire.

EQ-5D-5L: EuroQol instrument

Global Evaluation of Symptoms scores: assessment of symptom severity at worst during monitored season and compared with previous years

Table S2. Effect of IDIT on primary and secondary outcomes (intention to treat analysis, imputed)

	AUC (IQR)			
	Active (IDIT)	Control	Median Difference (95% CI)*	Adjusted Pvalue
Primary Outcome				
CSMS during entire season	502 (333-841)	509 (365-738)	8 (-174.7-210.9)	0.91
Secondary Outcomes				
Symptom score during entire season	335 (183-525)	264 (156-434)	61 (-7.8-123.2)	0.22
Medication Score during entire season	242 (116-405)	263 (129-482)	-24 (-173.1-107.5)	0.39
CSMS Score during peak season	363 (232-570)	370 (292-573)	-11 (-95.8-77.5)	0.80
Nose symptom score during entire season	178 (120-218)	131 (80-200)	33 (0.3-68.5)	0.03
Mouth symptom score during entire season	39 (8- 90)	14 (6- 45)	11 (3.1-26.1)	0.05
Eye symptom score during entire season	79 (41-158)	78 (52-180)	-7 (-20.0-3.0)	0.51
Lung symptom score during entire season	20 (3- 32)	12 (0- 40)	4 (-1.0-15.3)	0.17
Nose Allergic Symptoms measured by VAS	162 (107-275)	124 (66-166)	59 (-3.7-133.2)	0.02
Eye Allergic Symptoms measured by VAS	97 (37-197)	112(42-169)	2 (-45.6-49.0)	0.56
Mini RQLQ	N/A	N/A	-2 (-4.6-0.9)**	0.89
EQ-5D-5L	N/A	N/A	9 (-25.9 - 42.9)**	0.59
Global Evaluation of Symptom Scores	3 (2-4)	3 (1-3)	0 (0 to 1)	0.43
Symptom Free Days	35 (19- 53)	41 (23- 61)	-6 (-17 to 3)	0.15
No. days prednisone used during entire season	0 (0-0)	0 (0-0)	0 (0 to 0)	0.36
Medication Free Days	81 (65- 93)	76 (56- 94)	4 (-11.0-21.0)	0.22

Area under the curve (AUC) scores are expressed as median (IQR)

*The median difference was the difference between the active group relative to placebo group using stratified Hodges-Lehmann.

** The mean difference calculated from linear mixed model adjusted for stratification factors and time

Statistical comparison by means of Mann-Whitney U test and Van Elteren's test adjusted for stratification factors

Entire season: 13th May-31st August 2013. Peak season: 12th June-26th July 2013. ? Need to clarify how we define peak here?

CSMS: combined symptom & medication score, VAS: Visual annalogue scale, mini-RQLQ: mini-Rhinoconjunctivitis Quality-of-Life Questionnaire.

EQ-5D-5L: EuroQol instrument

Global Evaluation of Symptoms scores: assessment of symptom severity at worst during monitored season and compared with previous years