

Short-term subcutaneous grass pollen immunotherapy under the umbrella of anti-IL-4: A randomized controlled trial

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Background: Allergen immunotherapy is currently the only disease-modifying treatment available for allergic rhinitis and allergic asthma.

Objectives: We sought to evaluate the induction of sustained tolerance to allergen when anti-IL-4 was combined with a suboptimal course of grass pollen subcutaneous immunotherapy (SCIT) using the allergen-induced skin late-phase response (LPR) and exploratory immune monitoring as surrogate markers of therapeutic response.

Methods: In this randomized, double-blind, 3-group parallel design trial, 37 participants with seasonal allergic rhinitis received suboptimal SCIT (30,000 standardized quality units) in combination with anti-IL-4 (VAK694) and suboptimal SCIT

(30,000 standardized quality units) plus placebo antibody or double placebo (placebo SCIT and placebo antibody) restricted to 13 weeks before the grass pollen season. The primary end point was the size of the LPR at 12 months. Exploratory end points included measures of the immunomodulatory activity of treatment by using IL-4 and IL-10 FluoroSpot assays, flow cytometry of T cells, and measurement of IgE, IgG₄, and facilitated antigen binding.

Results: Both active treatment arms led to a substantial and sustained reduction of the LPR with no additional suppression with addition of anti-IL-4. Treatment with anti-IL-4 and SCIT compared with SCIT alone led to a sustained reduction in allergen-specific IL-4-producing cell counts ($P < .01$). Both active treatment arms led to induction of dual IL-4/IL-10-producing cells during the pollen season.

Conclusion: The combination of anti-IL-4 with SCIT provided no additional benefit over SCIT alone in suppressing the allergen-induced skin LPR. A larger trial is needed to assess whether the observed *ex vivo* downregulation of T_H2 responses might translate into clinical benefit. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: Allergic rhinitis, subcutaneous allergen immunotherapy, anti-IL-4, FluoroSpot, intradermal late-phase response

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Allergic rhinitis is the most prevalent worldwide increasing atopic disease, with an estimated prevalence of 500 million persons. In sensitized and allergic subjects exposure to allergen gives rise to symptoms in the upper airways, such as rhinorrhea, itching, sneezing, and nasal blockage.¹ Allergic rhinitis substantially impairs quality of life, sleep, education, productivity, and recreational activity and is a burden to the affected subjects, as well as to national economies through loss of productivity.

Allergen immunotherapy (AIT) is indicated in patients with severe allergic rhinitis that is not adequately controlled by antiallergic drugs and represents the only available disease-modifying treatment. AIT has potential to prevent onset of new allergen sensitizations and reduce disease progression from rhinitis to asthma.²⁻⁵ Allergic sensitization and allergic inflammation are characterized by a persisting T_H2 memory in which T cells produce IL-4, IL-5, and IL-13, as well as IL-31. In addition, in a relevant T_H2 subset the prostaglandin D₂ receptor chemoattractant receptor-homologous molecule expressed on T_H2 lymphocytes (CRTH2)⁶ is expressed. These are responsible for tissue inflammation in patients with ongoing allergic disease.

Consequently, targeting this T_H2 immune memory is considered a potential key target for disease modification and a curative

Abbreviations used

AE:	Adverse event
AIT:	Allergen immunotherapy
C _{max} :	Maximum drug plasma concentration
CRTH2:	Chemoattractant receptor-homologous molecule expressed on T _H 2 lymphocytes
FAB:	Facilitated antigen binding
FOXP3:	Forkhead box P3
FSC:	Forward scatter
LPR:	Late-phase response
mRQLQ:	Mini-Rhinitis Quality of Life Questionnaire
PVDF:	Polyvinylidene difluoride
SCIT:	Subcutaneous immunotherapy
SQ:	Standardized quality units
SSC:	Side scatter
Treg:	Regulatory T
VAS:	Visual analog scale

treatment strategy. Allergen-specific immunotherapy usually reduces allergen-specific T_H2 memory cell counts after 1 year of treatment, most likely through *de novo* induction of non-T_H2 memory cells (regulatory T [Treg]/T_H1 cells),⁷⁻¹¹ which compete with or even inhibit proallergic T_H2 cells. Monitoring of *de novo* induction of Treg cells during specific immunotherapy is a difficult task. It has been demonstrated by multiple groups that IL-10 expression is increased in *ex vivo* T-cell cultures after AIT,¹²⁻¹⁴ and forkhead box P3 (FOXP3)⁺IL-10⁺ T cells occur in the nasal mucosa of postimmunotherapy patients,¹⁵ whereas they are not detectable in peripheral blood of patients 3 years after AIT.¹⁶ The change in immune memory is also mediated by B cells and detectable by a shift from allergen-specific IgE to IgG₄ with blunting of the seasonal increase in IgE levels, reduction of facilitated antigen binding (FAB),^{9,17,18} and increased IL-10 secretion of PBMCs on *ex vivo* stimulation.^{19,20} Similarly, IgG₄ production also regresses years after immunotherapy, suggesting that IL-10 along with IgG₄ might represent an important but transient phenomenon in the reinduction of allergen tolerance.

More effective control of T_H2 memory T cells is likely to further improve immunotherapy or facilitate the treatment regimen. Although multiple adjuvant drugs were used to either control side effects or make allergen vaccination more effective, thus far, modulating the memory phenotype of T cells has not been attempted. Among Treg cells, the tissue-homing receptor CCR4⁺ subset has been reported to be of particular interest in the context of allergic reactions^{21,22} because this chemokine receptor is perceived to recruit allergen-specific T cells to the airways. The balance of T-cell subsets on initial antigen stimulation is dependent on induction of decisive transcription factors that determine the transcriptional program of the T-cell subsets, such as T-box transcription factor, GATA3, and FOXP3 for T_H1, T_H2, and Treg cells, respectively.²³⁻²⁸ It is anticipated that these transcription factors compete during the differentiation process, and rational vaccine or adjuvant design aims to control this process for optimal clinical outcomes.

Recent studies by our group and others revealed IL-4-dependent inhibition of FOXP3⁺ Treg cell induction.²⁵ Therefore we hypothesized that neutralization of IL-4 during a course of AIT would enhance the *de novo* induction of Treg cells over T_H2 cells. IL-4 is known to actively drive and sustain T_H2 responses to induce IgE isotype switching and FcεRI and MHC

class II upregulation and is considered a master T_H2 switch that also drives other proallergic cytokines, such as IL-5, IL-9, and IL-31,²⁹⁻³³ in allergic disease. Rational and effective disease modification based on mechanistic insight might pave the way for new curative treatment schemes in allergy that are applicable to all allergen sources. We aimed to investigate the therapeutic induction and maintenance of allergen-specific tolerance through the addition of a fully humanized anti-IL-4 antibody (VAK694) to a suboptimal grass pollen allergen subcutaneous immunotherapy (SCIT) regimen in a double-blind, placebo-controlled clinical study.

METHODS

Patients

Having been prescreened in telephone interviews, 66 participants were screened. Enrollment was according to the following inclusion criteria: age of 18 and 60 years and good health (as determined based on past medical history, physical examination, vital signs, electrocardiography, and laboratory tests); a history of seasonal allergic rhinitis for at least 2 years during the grass pollen season and a positive skin prick test response to *Phleum pratense* with a wheal difference of 3 mm or greater than that elicited by the negative control; weight of a minimum of 50 kg with a body mass index within the range of 18 and 32 kg/m²; use of 2 highly effective methods of contraception by male participants and use by the female partner of a further method of contraception, with male participants refraining from fathering a child for 3 months after the last study drug administration; and ability to communicate well with the investigator and comply with the requirements of the study and provide written informed consent before study participation. Key exclusion criteria were as follows: treatment with intranasal corticosteroids within 28 days before the first dose of anti-IL-4; symptoms of moderate-to-severe perennial allergic rhinitis; history of asthma, with treatment with inhaled or systemic corticosteroids within 6 months before first dose; FEV₁ of less than 70% of predicted value at screening; active smoker or smoking history of greater than 10 pack years; any exposure to human monoclonal or polyclonal antibodies; AIT within 3 years before screening and any prior grass pollen immunotherapy; and history of autoimmune or immunodeficiency disease, cancer, or chronic infection. Women of childbearing potential were excluded (a detailed list of inclusion and exclusion criteria is available in the [Methods](#) section in this article's Online Repository at www.jacionline.org).

Thirty-seven subjects fulfilled all criteria and were randomized. All patients provided written and informed consent. The trial was registered (<http://www.clinicaltrials.org>, NCT01018693) and approved by the NHS National Research Ethics Service (Trent REC 09/H0405/46) and institutional review of the Royal Brompton Hospital, London, United Kingdom.

Study design and intervention

The study was a randomized, double-blind, placebo-controlled, 3-group, parallel-design phase IIa study at the Royal Brompton Hospital in London from November 2009 to October 2011. The intervention consisted of a fully human therapeutic antibody to human IL-4 (VAK694) in combination with simultaneous suboptimal-dose subcutaneous grass pollen-specific AIT (SCIT) compared with conventional suboptimal-dose SCIT and double-placebo groups. Patients were randomly assigned to one of the 3 treatment groups. Anti-IL-4 or placebo was administered intravenously every 4 weeks at a dose of 3 mg/kg, with overall 4 doses covering the entire 13-week period of preseasonal specific immunotherapy. Commercially available SCIT (Alutard Avanz *Phleum*; ALK-Abelló, Hørsholm, Denmark) or saline placebo injections were administered after a conventional weekly up dosing schedule to a suboptimal dose of 30,000 standardized quality units (SQ), including 2 maintenance doses with an overall administration of approximately 13 injections (equal to a total dose of 136,000 SQ cumulative dose), with minor dose adjustments for SCIT according to any intense local or mild systemic

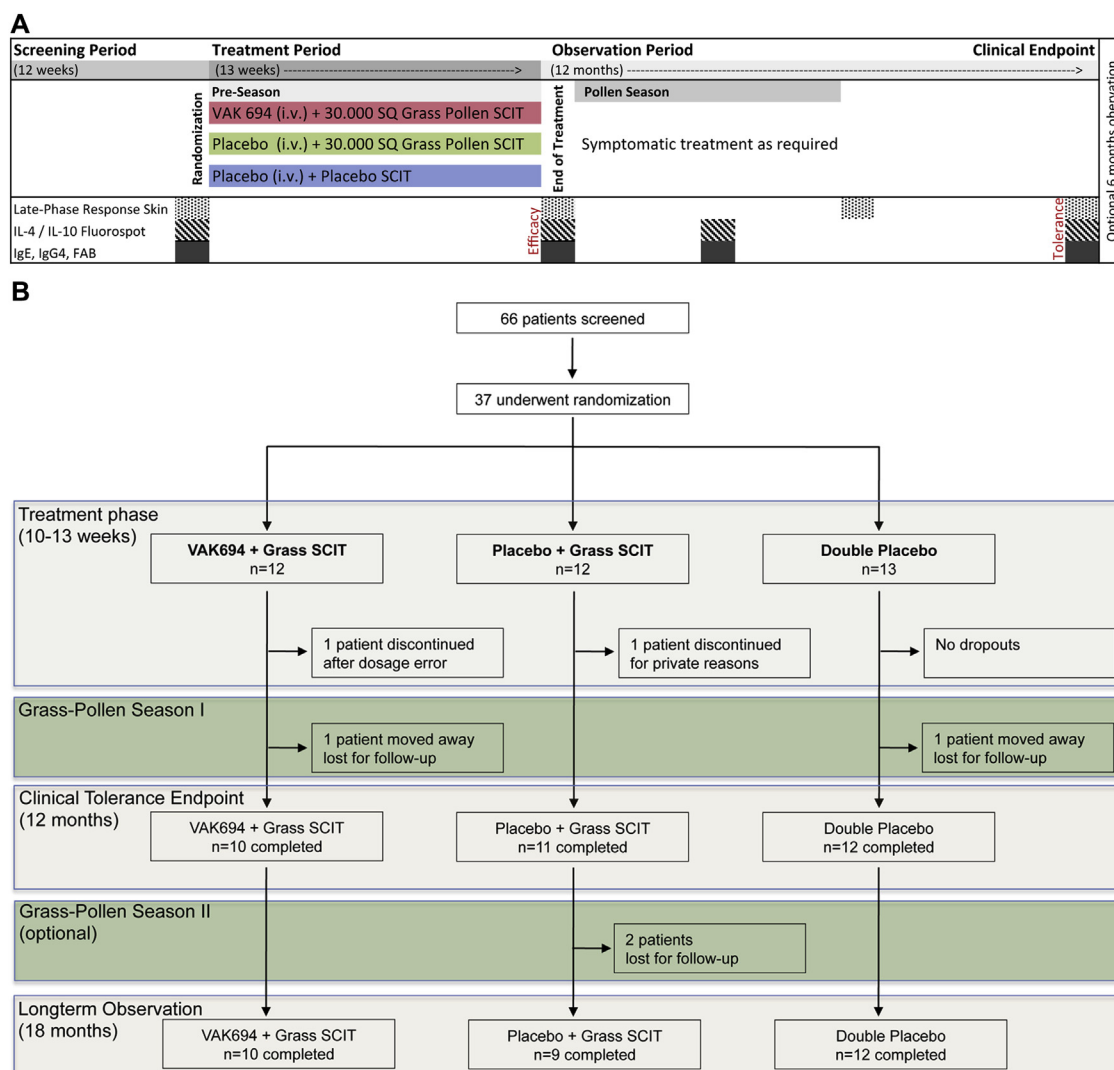


FIG 1. Study assessment schedule (A) and disposition of subjects (B).

reactions (grades 1 and 2) according to European Academy of Allergy and Clinical Immunology guidelines.³⁴

The study was exploratory in nature. The primary objectives were induction of sustained tolerance to allergen 12 months after the end of treatment, as assessed based on the cutaneous late-phase response (LPR) to allergen, when anti-IL-4 is combined with SCIT for the treatment of grass pollen allergy and assessment of the safety and tolerability of anti-IL-4 when administered in the context of grass pollen SCIT.

A 3-group parallel design was chosen to benchmark the intervention with active suboptimal SCIT and to adequately compare this treatment with placebo within this specific patient sample. We dismissed a fourth treatment arm with anti-IL-4 alone because other recent studies evaluated the sole pharmacologic intervention of anti-IL-4 in allergic disease and focused on the concept of sustained antigen-specific tolerance with the combination treatment. The antigen dose was targeted to be high enough to induce sufficient suppression to allergen but low enough to allow further reduction in the magnitude and duration of the suppressive response in the skin. A suboptimal antigen dose of 30,000 SQ instead of the recommended 100,000-SQ dose was chosen based on skin reactivity to antigen in the LPR after SCIT, as recently published.²⁰ Furthermore, the course of treatment was limited to 13 weeks (as opposed to the recommended 3 years, with a clear definition of “efficacy”

evaluation at 3 months and “tolerance” evaluation at 12 months after the end of treatment). The dose of 3 mg/kg every 4 weeks for anti-IL-4 was based on a pharmacokinetic/pharmacodynamic model and former phase 1 (NCT00620230 and NCT00929968) applications of 1 mg/kg that predicted at least 90% suppression of free tissue IL-4. Anti-IL-4 was administered in a double-blind, placebo-controlled manner as a 1-hour infusion every 4 weeks. Active and placebo SCIT injections were also administered in a double-blind manner by the investigators to the participants. Allergen and placebo injections were prepared on a daily basis by a clinician (M.M.) who was otherwise strictly independent of the clinical protocol. The sponsor had access to individual randomization data for an interim analysis at 10 months after the end of treatment. For details, also see the clinical trial database at www.novartisclinicaltrials.com.

Surrogate clinical, clinical, and laboratory assessments were performed before treatment at baseline, after the 13-week preseasonal treatment period (the “efficacy” time point), during and after the first peak pollination season, and 12 months after the end of treatment (the primary “tolerance” end point; Fig 1, A). Pharmacokinetic and pharmacodynamic analyses of anti-IL-4 were performed before the dose, 1 hour and 1 week after dosing, and at 1 and approximately 12 months after the last dose. Adverse events (AEs) were recorded from enrollment to study completion.

Skin LPR (primary end point)

The primary end point (ie, tolerance to allergen) of the study was the area of the intradermal LPR after 33 SQ of *Phleum pratense* (equivalent to 10 biological units of allergen) in 0.02 mL of diluent administered into the extensor surface of the forearm at 12 months after stopping treatment.²⁰ The LPR was performed in duplicates on the extensor aspect of the forearm and recorded as the mean swelling area at 24 hours. The LPR was assessed before the start of treatment at baseline, after the preseasonal treatment phase (efficacy time point), at the end of the first pollen season (6 months after the end of treatment), and at the clinical tolerance primary end point at 12 months after the end of treatment. In an amendment we also added an optional 18-month readout.

Symptom assessments and grass pollen counts

A visual analog scale (VAS) assessment of rhinitis symptoms was administered before the study, during the pollen season, and at the clinical end point. Total sum VAS scores were recorded based on assessments for rhinorrhea, nasal congestion, nasal itching, and sneezing. A retrospective allergy assessment score on a scale from −3 (much worse) to +3 (much better) was recorded in response to the following question: “How was your hay fever this year compared to the years before you started the treatment?”³⁵ Mini-Rhinitis Quality of Life Questionnaire (mRQLQs) were scored at baseline and in the peak season and then compared on a difference to baseline approach.

Grass pollen counts (grains per cubic meter) were obtained from the UK National Pollen and Aerobiology Research Unit in 2010 for the greater London area, and averages were displayed.

Immunologic and biomarker assessments

Frequencies of *P pratense*-specific IL-4⁺, IL-10⁺, and double-positive cells were enumerated in PBMCs from all patients at baseline, during the peak grass pollen season, and 12 months after the end of treatment by using the FluoroSpot assay, which was performed according to the recommendations of the manufacturer (Gen-Probe Diaclone SAS, Besançon, France). Briefly, isolated PBMCs were stimulated with 10 µg/mL *P pratense* extract (ALK-Abelló) or 10 µg/mL PHA (positive control) or RPMI (Gibco, Paisley, United Kingdom) with 5% human AB serum (Sigma, UK) and 0.01% penicillin/streptomycin (Sigma) for 42 hours at 37°C. PHA was used as a positive control, and medium alone was used as a negative control. Fluorescent spots were visualized and quantified under a UV light by using the iSpot reader (CADAMA; Oxford Biosystems, Oxford, United Kingdom; see the [Methods](#) section in this article's Online Repository).

Total serum IgE and grass pollen-specific IgE and IgG₄ levels were quantified by using the CAP-FEIA system, according to the manufacturer's recommendations (Phadia, Uppsala, Sweden). IgE and IgG₄ levels were assessed at baseline, after the preseasonal treatment phase, during the peak season, and at 12 months after the end of treatment. IgE-facilitated allergen binding to B cells was performed, as previously described.^{9,12} Briefly, serum from a grass pollen-sensitized donor (*P pratense*-specific IgE >100 IU/mL) was incubated with 1 µg/mL *P pratense* in the presence or absence of an equal volume of sera from study participants for 1 hour at 37°C. EBV-transformed B cells (100,000 cells per test) were added for 1 hour at 4°C and washed. Allergen-IgE complexes binding to B cells were detected by using a phycoerythrin-labeled polyclonal anti-IgE antibody (Miltenyi Biotech, Bergisch Gladbach, Germany) and analyzed by means of flow cytometry with the BD FACSCanto II flow cytometer (BD Biosciences, San Jose, Calif). IgE-FAB was assessed at baseline, after the preseasonal treatment phase, during the peak season, and 12 months after the end of treatment.

Effector T cells and Treg cell subsets

Freshly isolated PBMCs were immunostained with anti-human CD4 (clone: OKT4), CD25 (BC96), CRTH2 (HIL-7R-M21; BioLegend, UK), CD127 (1G1), and CCR4 (BM16; BD Biosciences, UK), respectively, and

stained intracellularly with anti-human FOXP3 (clone, 259D; BioLegend) antibodies or relevant isotype controls. Cells were acquired on the BD FACSCanto II flow cytometer (BD Biosciences). Analyses were performed with BD FACSDiva V6.1.1 software (BD Biosciences; see the [Methods](#) section in this article's Online Repository). Cells were analyzed in a *post hoc* approach during the peak season and at the clinical tolerance end point (12 months after the end of treatment).

Safety assessments and pharmacokinetic/pharmacodynamic measurements

Safety parameter included vital signs, laboratory assessments (hematology, clinical chemistry, urinalysis, and immunogenicity), and repeated electrocardiograms. Medical history/current medical conditions and AEs were coded by using the Medical Dictionary for Regulatory Activities terminology. Pharmacokinetic parameters were determined as maximum drug plasma concentration (C_{max}) and time of maximum drug concentration after administration of the first and fourth doses of anti-IL-4. Pharmacodynamic parameter total IL-4 levels were assessed in plasma before and after administration of anti-IL-4. For details, see the final clinical trial report in this article's Online Repository.

Statistical analysis

The sample size was calculated on the basis of prior available data on the LPR in the skin after grass pollen SCIT.²⁰ For the LPR, a coefficient of variation of 50% was assumed. Then 11 subjects per group provided 90% power to detect a 50% reduction in the intradermal LPR in the 3 mg/kg anti-IL-4 plus SCIT group versus the placebo plus SCIT group by using a 2-sided α test at the 5% level. The primary end point (intention-to-treat analysis) was change from baseline in intradermal LPR (mean swelling area). The primary end point was examined by using a linear mixed effects model with fixed effects for treatment (anti-IL-4 plus SCIT, placebo plus SCIT, and double placebo), time of measurement, and a treatment-by-time interaction. The baseline value was included as a covariate in the model to correct for potential differences between groups in the LPR. An unstructured covariance matrix was used to allow for correlation of measurements obtained on the same subjects over time. Hypothesis testing was performed at the 2-sided α value at the 5% level. The primary comparison of interest was anti-IL-4 plus SCIT versus SCIT at the tolerance end point 12 months after the end of treatment. There were no adjustments made for multiplicity for this exploratory study. Retrospective symptom assessment scores were compared by using 2-sided *t* tests in between-group analyses. Quality of life (mRQLQ) and symptom scores (VAS) were analyzed in the same way as the primary end point. We analyzed log-transformed cell counts of IL-4, IL-10, and dual IL-4/IL-10-producing grass pollen-specific cells by using the abovementioned linear mixed effects model. Total and allergen-specific IgE and IgG₄, IgE-FAB, and *post hoc* flow cytometric results were analyzed by using nonrepeated measurement ANOVA without adjustment for multiple testing (Fisher least significant difference test). Exact *P* values were calculated by using the Welch-corrected Student *t* test when results of ANOVA were significant.

RESULTS

Baseline characteristics of participants

Baseline characteristics were similar in all groups ([Table 1](#)). The disposition of subjects is shown in [Fig 1, B](#). Of the 37 randomized participants, 33 completed the prespecified clinical tolerance end point at 12 months after the end of treatment (10, 11, and 12 subjects in the anti-IL-4 plus SCIT, placebo plus SCIT, and double-placebo treatment groups, respectively). One patient in the anti-IL-4 plus SCIT group discontinued treatment after a dosage error but remained in the intention-to-treat analysis set.

TABLE I. Patients' demographic summary

	Anti-IL-4 (VAK694) plus SCIT (n = 12)	Placebo plus SCIT (n = 12)	Double placebo (n = 13)	Total (n = 37)
Age (y)				
Mean	31.8	34.1	37.5	34.6
SD	10.84	11.74	11.67	11.36
Height (cm)				
Mean	179.33	176.42	181.15	179.03
SD	7.101	7.971	8.980	8.102
Weight (kg)				
Mean	84.65	76.60	85.88	82.41
SD	14.390	13.851	15.295	14.741
BMI (kg/m ²)				
Mean	26.24	24.49	26.13	25.62
SD	3.566	3.291	4.156	3.690
Sex				
Male	12 (100%)	11 (91.7%)	12 (92.3%)	35 (94.6%)
Female		1 (8.3%)	1 (7.7%)	2 (5.4%)
Predominant race				
White	9 (75.0%)	7 (58.3%)	12 (92.3%)	28 (75.7%)
Black	1 (8.3%)	2 (16.7%)		3 (8.1%)
Asian	1 (8.3%)	3 (25.0%)	1 (7.7%)	5 (13.5%)
Other	1 (8.3%)			1 (2.7%)
Ethnicity				
Other	10 (83.3%)	8 (66.7%)	12 (92.3%)	30 (81.1%)
Indian (Subcontinent)	1 (8.3%)	2 (16.7%)	1 (7.7%)	4 (10.8%)
Mixed	1 (8.3%)	1 (8.3%)		2 (5.4%)

Safety and AEs

All subjects who received the study drug were included in the safety analysis set. There were no clinically relevant changes over time observed in clinical laboratory test results, vital signs, or electrocardiographic results for any of the subjects. No significant immunogenicity against anti-IL-4 was observed. No serious AEs and no anaphylaxis occurred, and no adrenaline was given. None of the subjects were discontinued because of an AE. Most of the AEs were mild to moderate and 1 (injection site pruritus after SCIT) was severe in intensity. Overall, the most commonly reported individual AEs were injection-site pruritus (56.8%), injection-site mass (54.1%), injection-site erythema (32.4%), injection-site swelling (32.4%), and nasopharyngitis (54.1%). Two AEs (headache) were suspected to be related to study medication, both in the placebo group. [Table II](#) displays an overview of AEs according to a systematic organ classification (a detailed overview of AEs, see [Table E1](#) in this article's Online Repository at www.jacionline.org). Although limited by small numbers, combined treatment of anti-IL-4 with SCIT and use of SCIT alone were well tolerated in this study.

Skin LPR (primary end point)

There was no significant added suppression of the skin LPR in the anti-IL-4 plus SCIT group compared with the SCIT-only group ([Fig 2](#) and see [Table E2](#) in this article's Online Repository at www.jacionline.org). Combined treatment with anti-IL-4 and SCIT led to marked suppression of the LPR immediately after preseasonal therapy at the time point 3 months after treatment (ie, efficacy; $79.7\% \pm 7.9\%$ vs $21.6\% \pm 7.14\%$ in the double-placebo group, $P < .001$). This suppression decreased

slowly to $69.5\% \pm 10.97\%$ versus $35.2\% \pm 10.39\%$ ($P = .031$ vs the double-placebo group) after the pollen season and $67.0\% \pm 9.84\%$ versus $27.9\% \pm 9.30\%$ ($P = .007$ vs the double-placebo group) at the primary (ie, tolerance) clinical end point 12 months after the end of treatment. We observed a similar pattern for the active control group treated with SCIT alone, which reached a suppression of $76\% \pm 7.76\%$ ($P < .001$ vs the double-placebo group) after preseasonal therapy, $68.10\% \pm 10.90\%$ ($P = .036$ vs the double-placebo group) after the pollen season, and $59.2\% \pm 9.72\%$ ($P = .026$ vs the double-placebo group) 12 months after the end of treatment.

Although combined treatment with anti-IL-4 trended toward more pronounced suppression, the primary objective of a 50% difference between combined treatment and active controller was not reached. Suppression of the LPR was discernable until 18 months (optional readout for long-term suppression) after the end of treatment but lost statistical significance over placebo in both active treatment groups. A moderate decrease of the LPR was expected in the placebo group, as described in earlier studies,²⁰ and detectable as a result of repeated intradermal assessments with allergen.

Exploratory biomarkers

Frequency of allergen-stimulated IL-4- and IL-10-producing cells. Cytokine expression for IL-4 and IL-10 was measured by using FluoroSpot assays of freshly isolated PBMCs. Combined treatment with anti-IL-4 plus SCIT resulted in a substantial and sustained reduction of *P pratense*-specific IL-4-producing cell counts. IL-4-producing cell counts decreased more than 50% after treatment during the peak pollen season (least squares means from 164.76 to 81.48). In contrast, after SCIT alone, the numbers of *P pratense*-specific IL-4-producing T cells in season almost doubled (least square means from 145.97 to 269.86, $P < .01$; [Fig 3](#) and see [Table E3](#) in this article's Online Repository at www.jacionline.org). The observed suppression of allergen-driven IL-4 production in the anti-IL-4 plus SCIT group was maintained until the clinical tolerance end point at 12 months after the end of treatment. IL-4-producing cell counts in the double placebo-treated group showed a slight and nonsignificant reduction.

Although the number of IL-10⁺ T cells decreased over time in all 3 groups, they were 3-fold increased in the SCIT group compared with the combination anti-IL-4 plus SCIT group ($P = .04$) at the clinical tolerance end point at 12 months. Dual IL-4⁺IL-10⁺ cells were significantly increased in the SCIT-only group compared with placebo-treated participants. In the combined treatment group no significant increase in comparison with placebo was shown; however, dual IL-4⁺IL-10⁺ cell counts in the anti-IL-4 plus SCIT-treated group increased moderately during the peak season from baseline ([Fig 3, D](#), and see [Table E3](#)).

Serum total and grass pollen-specific IgE levels, specific IgG₄ levels, and inhibitory activity for IgE-FAB. In both active treatment groups, there was a moderate increase in grass pollen-specific IgE levels after specific immunotherapy with blunting of the seasonal grass pollen-specific IgE response; these changes were accompanied by similar increases in total IgE levels ([Fig 4, A and B](#), and see [Table E4](#) in this article's Online Repository at www.jacionline.org). Subjects treated with double placebo had an increase of total

TABLE II. AEs

	Anti-IL-4 (VAK694) plus SCIT (n = 12), no. (%)	Placebo plus SCIT (n = 12), no. (%)	Double placebo (n = 13), no. (%)	Total (n = 37), no. (%)
Patients with AE	12 (100.0)	11 (91.7)	13 (100.0)	36 (97.3)
System organ class				
General disorders and administration-site conditions	12 (100.0)	11 (91.7)	6 (46.2)	29 (78.4)
Infections and infestations	8 (66.7)	5 (41.7)	10 (76.9)	23 (62.2)
Respiratory, thoracic, and mediastinal disorders	7 (58.3)	6 (50.0)	7 (53.8)	20 (54.1)
Nervous system disorders	5 (41.7)	3 (25.0)	6 (46.2)	14 (37.8)
Skin and subcutaneous tissue disorders	7 (58.3)	4 (33.3)	2 (15.4)	13 (35.1)
Gastrointestinal disorders	3 (25.0)	3 (25.0)	3 (23.1)	9 (24.3)
Injury, poisoning, and procedural complications	4 (33.3)	3 (25.0)	2 (15.4)	9 (24.3)
Musculoskeletal and connective tissue disorders	5 (41.7)	2 (16.7)	2 (15.4)	9 (24.3)
Eye disorders	2 (16.7)	1 (8.3)	4 (30.8)	7 (18.9)
Immune system disorders	1 (8.3)	1 (8.3)	3 (23.1)	5 (13.5)
Ear and labyrinth disorders	1 (8.3)	2 (16.7)	1 (7.7)	4 (10.8)
Vascular disorders	0 (0.0)	0 (0.0)	3 (23.1)	3 (8.1)
Reproductive system and breast disorders	0 (0.0)	0 (0.0)	2 (15.4)	2 (5.4)
Blood and lymphatic system disorders	1 (8.3)	0 (0.0)	0 (0.0)	1 (2.7)
Investigations	1 (8.3)	0 (0.0)	0 (0.0)	1 (2.7)
Metabolism and nutrition disorders	0 (0.0)	0 (0.0)	1 (7.7)	1 (2.7)
Neoplasms: benign, malignant and, unspecified (including cysts and polyps)	0 (0.0)	0 (0.0)	1 (7.7)	1 (2.7)
Psychiatric disorders	1 (8.3)	0 (0.0)	0 (0.0)	1 (2.7)

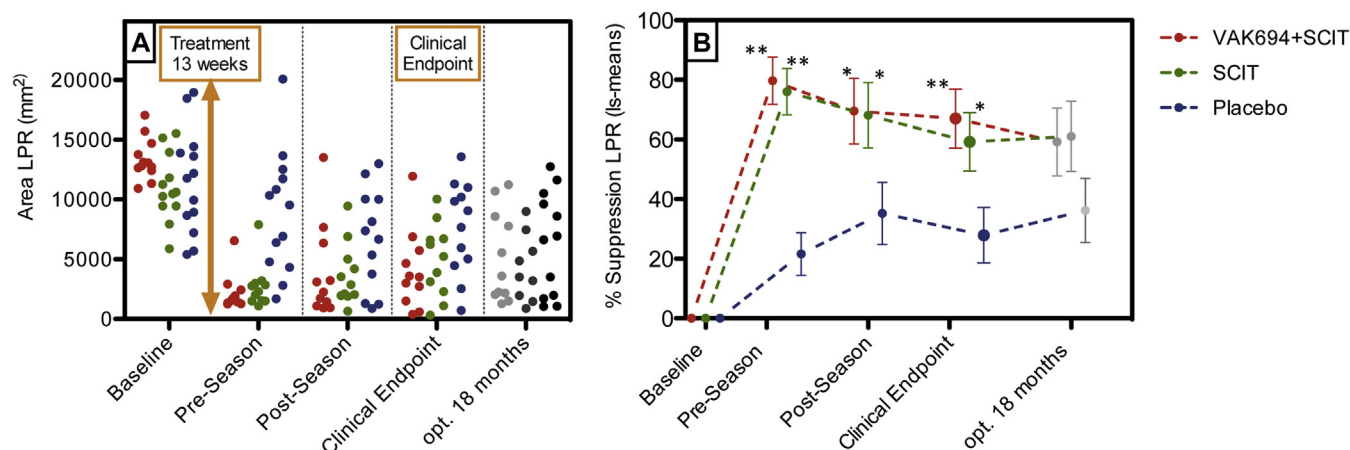


FIG 2. LPR to allergen in the skin. **A**, Size of the late allergic response in the skin to grass pollen extract after 24 hours of treatment with anti-IL-4 (VAK694) plus SCIT, SCIT alone, and double placebo (intention to treat [ITT] population) shows distribution of individual values in square millimeters in treatment groups over time. **B**, Least squares means $\pm$ SEMs of LPR suppression in percentage of baseline. * P < .05 and ** P < .01 compared with placebo.

and grass pollen-specific IgE levels only during the grass pollen season. Grass pollen-specific IgG₄ levels were markedly induced (>100-fold) after anti-IL-4 plus SCIT and SCIT alone, with a moderate decrease over time until the clinical tolerance end point (Fig 4, C, and see Table E4). These changes were accompanied by parallel increases in serum inhibitory activity for FAB in both active treatment groups, with IgE-FAB decreasing approximately 30% during specific immunotherapy (Fig 4, D, and see Table E4). Treatment with anti-IL-4 had no significant effect on the magnitude of SCIT-induced changes in grass pollen-specific IgG₄ levels or FAB.

Flow cytometric monitoring of T_H2 effector and Treg cells. We stained the tissue-homing receptor CCR4 and CRTH2 within CD4⁺ cells to examine circulating differentiated T_H2 cells. We detected a moderate increase of CCR4⁺ T-cell counts in anti-IL-4 plus SCIT-treated patients compared with those in

the SCIT-only (P = .0288, 42.06 ± 2.565 vs 34.64 ± 1.819) and placebo (P = .0488; 42.06 ± 2.565 vs 33.68 ± 3.079) groups during the peak season (see Fig E1, A, in this article's Online Repository at www.jacionline.org), whereas we did not observe relevant changes in CRTH2⁺ T cells (see Fig E1, B). However, the proportion of CCR4⁺ cells within CRTH2⁺ T cells was markedly increased during the pollen season in all groups in comparison with the clinical end point outside the pollen season (P < .001; see Fig E1, C). In a gating strategy that also enabled detection of T_R1 cells, CD4⁺CD25^{hi} cell counts were increased during the peak season in the anti-IL-4 plus SCIT-treated group (P = .036; 16.13 ± 1.747 vs 11.52 ± 1.032) in comparison with those in the SCIT-only group (see Fig E1, D). We examined the definitive population of Treg cells as FoxP3 CD127^{low} cells. These were increased *vice versa* in the SCIT-only group in comparison with the anti-IL-4 plus SCIT group (P = .0152;

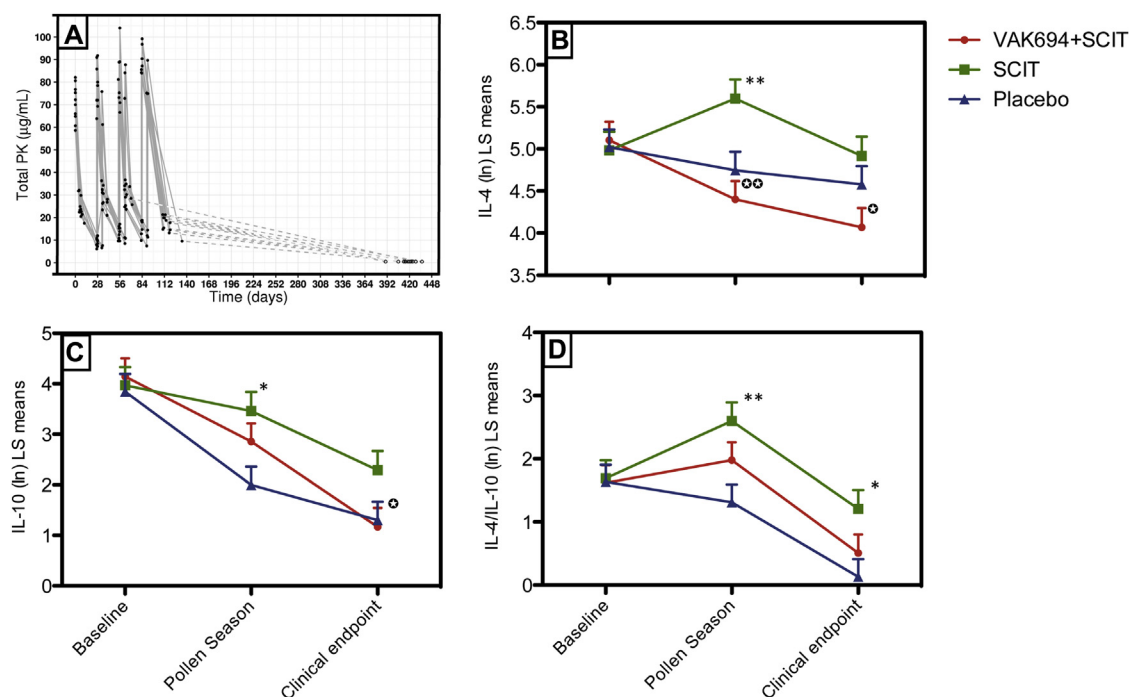


FIG 3. Exploratory end point: IL-4 and IL-10 FluoroSpots. **A**, Pharmacokinetics of anti-IL-4 (VAK694). **B-D**, Surrogate end point of grass pollen-induced IL-4 and IL-10 FluoroSpots from cultured PBMCs. All values are displayed as means and SEMs. IL-4 FluoroSpots: Fig 3, *B*, allergen-induced IL-4-producing cells; Fig 3, *C*, allergen-induced IL-10-producing cells; and Fig 3, *D*, time course of IL-4⁺/IL-10⁺ cells over time on specific immunotherapy. Asterisks indicate **P* < .05 and ***P* < .01 compared with placebo. Ⓢ *P* < .05 and ⓈⓈ *P* < .01 compared with SCIT alone.

42.44 ± 3.913 vs 28.46 ± 3.546) during the peak season and also in the subset of CCR4⁺FoxP3⁺ T cells (*P* = .04, see Fig E1, *E* and *F*). Interestingly, in anti-IL-4 and placebo-treated subjects CCR4⁺FoxP3⁺ T-cell counts were significantly increased out of the pollen season (*P* = .009 and *P* = .035, respectively).

Symptoms

Symptoms of allergic rhinitis measured by means of 2-week VAS assessments from mid-May to early August increased in line with natural grass pollen exposure (see Fig E2, *A*, and Table E5 in this article's Online Repository at www.jacionline.org). No significant differences between groups were detected. There was no obvious trend observed within or across treatment groups. Data were highly variable. During the peak season, an increase in mRQLQ scores was observed. The data were variable, with the majority of subjects within each treatment group having low scores at baseline and variable increases in scores during the peak season. There was no statistically significant difference in mRQLQ scores between treatment groups. Nevertheless, at the clinical end point, retrospective allergy assessment scores revealed that, compared with placebo, both active treatment cohorts reported better symptom control (see Fig E2, *B*).

Pharmacokinetic and pharmacodynamic outcomes of anti-IL-4 (VAK694)

The mean C_{max} concentrations achieved after the first and fourth doses of anti-IL-4 were 70.7 and 85.7 µg/mL, respectively (Fig 3, *A*). The C_{max} ratio between the first and fourth doses was 1.24, indicating that limited accumulation in systemic exposure occurred. The interindividual pharmacokinetic variability in C_{max} after the first and fourth doses was low (coefficients of

variation, 11.8% and 9.7%, respectively). Total plasma IL-4 concentrations comprised of both free IL-4 and IL-4 bound to anti-IL-4 increased after each antibody administration, reflecting efficient capture of IL-4 (see Fig E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

We have shown that a preseasonal and suboptimal-dose treatment regimen with a well-standardized and validated allergen SCIT vaccine with or without the addition of intravenous anti-IL-4 was highly effective in suppressing the skin LPR as a surrogate of clinical efficacy. This effect was apparent after only a 13-week preseasonal treatment course (demonstrating efficacy) and persisted out to 12 months (which is consistent with induction of antigen-specific tolerance), as previously shown with the known optimal dose regimen with the same SQ-standardized allergen vaccine alone.^{3,17,36,37} Furthermore, both active treatment regimens were accompanied by a significant reduction in retrospective global seasonal symptom assessment scores.

Although we could not detect additional clinical tolerance to allergen with the particular combination treatment regimen we used, we were able to show, for the first time in a double-blind placebo-controlled clinical trial, that targeted immunomodulation in grass pollen SCIT through addition of an anti-IL-4 antibody resulted in prolonged inhibition of *ex vivo* antigen-specific production of IL-4 for up to 12 months after the end of treatment.

On combined treatment with anti-IL-4 and SCIT, allergen-specific production of IL-4 detected by using the FluoroSpot assay was sustainably reduced, whereas IL-10⁺ and dual IL-4⁺IL-10⁺ cells showed a heterogeneous picture. During

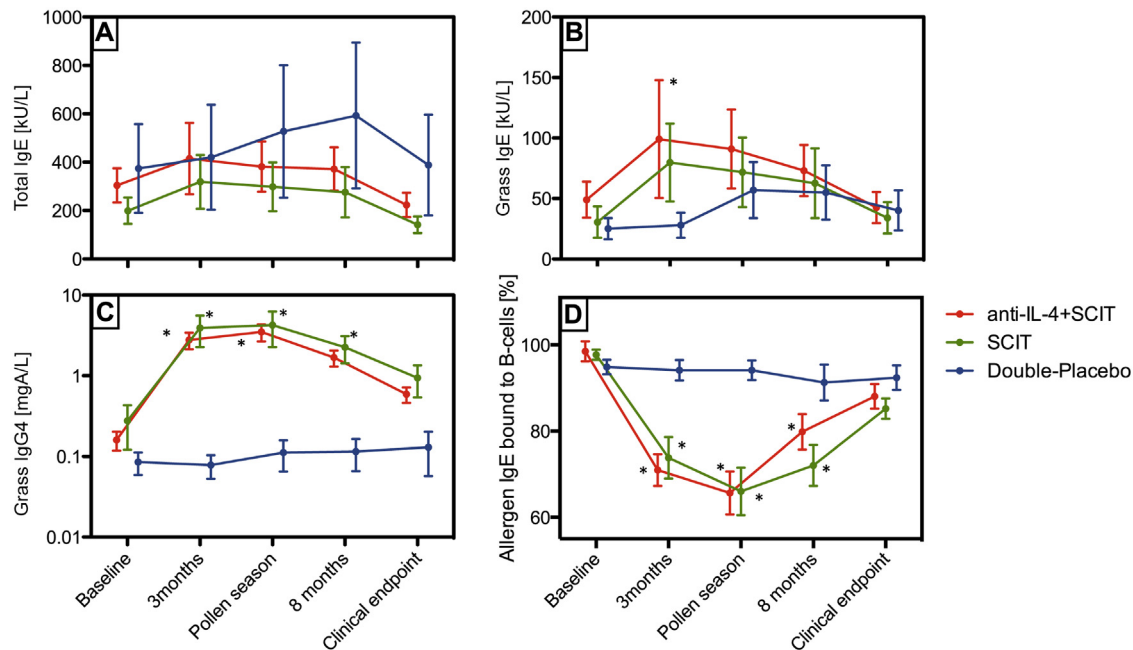


FIG 4. IgE levels, IgG₄ levels, and inhibitory activity for IgE-FAB. **A** and **B**, Total IgE levels in serum (Fig 4, A) and grass pollen-specific IgE levels (*P pratense*; Fig 4, B) display blunting of the seasonal IgE increase. **C** and **D**, Induction of IgG₄ on SCIT and anti-IL-4 plus SCIT (Fig 4, C) and inhibition of allergen-specific IgE bound to B cells (as a percentage) on SCIT (Fig 4, D) without an additional effect of VAK694. Data are means and SEMs (intention-to-treat data set). **P* < .05 compared with placebo.

SCIT alone, we detected an increase of allergen-specific IL-4-producing cells that is in line with the known initial increase in specific IgE levels during AIT. Interestingly, specific IgE and IgG₄ responses were not altered in the combined treatment cohort. Therefore we demonstrate a proof of concept in which the increase in IL-4-producing cell counts throughout specific immunotherapy is inhibited and a sustained reduction in antigen-specific IL-4-producing cells was still apparent 12 months after the last administration of the drug, a time when the anti-IL-4 antibody VAK694 was effectively cleared (Fig 3, A).

However, this immunologic proof of concept did not translate into clinical advantage in comparison with SCIT. Interestingly, model-based predictions based on plasma pharmacokinetic/pharmacodynamic data revealed that free plasma IL-4 levels returned to baseline quickly at the end of treatment with anti-IL-4 (see Fig E3, A). This might explain why the *ex vivo* proof of concept did not translate into clinical efficacy for the patients.

The blunting of seasonal IgE and induction of IgG₄ and FAB observed with SCIT alone (Fig 4) was unaffected by coadministration of anti-IL-4, thus challenging the view that IL-4 is necessary for induction of IgG₄ or maintenance of IgE responses. Additional neutralization of IL-13 could be either necessary or sufficient to block this effect on IgE and the desired induction of protective IgG₄ responses.

Flow cytometric monitoring used freshly isolated PBMCs but was not antigen specific and therefore limited. Although anti-IL-4 plus SCIT-treated subjects showed a moderate increase in expression of the tissue-homing receptor CCR4 on T cells, we did not detect differences among treatment groups for the frequency of circulating differentiated CCR4⁺CRTH2⁺ T cells. Interestingly, out of the pollen season, these T_H2 cell counts were markedly reduced in all groups. The CRTH2 gene codes for the

prostaglandin D₂ receptor, depends on IL-4 exposure and antigen stimulation,^{6,38} and has been described as a reliable marker of T_H2 cells. Anti-IL-4 plus SCIT did not globally change this T_H2 environment during the peak season but acted selectively and in an antigen-specific manner, as detected by using IL-4 FluoroSpots. As previously described, in AIT studies¹²⁻¹⁴ we observed an induction of CD4⁺CD25^{hi} T cells in the anti-IL-4 plus SCIT-treated group, whereas we found in the SCIT only-treated group an increased proportion of FoxP3⁺ definitive Treg cells. We consider FoxP3 to be the current gold standard for Treg cells. However, FoxP3 expression in T_R1 cells is not constant, which is why we chose to gate also on CD4⁺CD25^{hi} cells. The lack of antigen specificity in this approach and the IL-4-dependent regulation of CRTH2 and CD127³⁹ limit the conclusions that can be drawn from these findings.

This study was not powered to detect changes in symptom scores or disease-related quality of life between the groups. Nonetheless, suppression of the skin LPR was accompanied by significant improvement in the overall retrospective assessment total score by the participants at the primary end point (see Fig E2, B). Symptom reduction was apparent in both active treatment groups compared with the placebo group, whereas no differences between the anti-IL-4 plus SCIT group versus the SCIT-only group were detected.

Our study illustrates the utility of a placebo-controlled suboptimal immunotherapy regimen with the skin LPR as a clinical surrogate end point for future testing of combination regimens to improve the efficacy and safety of currently available AIT. The number of subjects required to detect a marked effect on the LPR is very few compared with the number required to detect an effect on symptoms. Further optimization of the allergen dose and duration for the immunotherapy regimen and for the late-phase skin outcome should enhance the approach.

We conclude that anti-IL-4 treatment with VAK694 is effective in modulating T_H2 memory when applied during administration of specific immunotherapy, although this did not translate into additional clinical efficacy. It remains unclear whether the deletion of peripheral antigen-specific T_H2 cells adds any benefit for treatment. Upcoming clinical studies need to clarify which concept of combined intervention with allergen, allergen dose, and biological agent is optimal for sustainable induction of clinical efficacy and sustained immune tolerance.

We thank Ms Andrea Goldstone and Mrs Benedikte Hovmand, specialist nurses in allergy; Mrs Natalia Klimowska-Nassar, study administrator; Ms Delica Cheung and Ms Mimi Poon, technicians; and members of the VAK694 team at Novartis for their excellent assistance. We also thank the study patients, who supported this study with high commitment and personal dedication. We would further like to commemorate Professor Christoph Walker, who supported us in setting up, mentoring, and sponsoring this trial and who passed away before the study began.

Clinical implications: Coinjection of allergen under the umbrella of anti-IL-4 during preseasonal AIT did not provide additional suppression of the allergen-induced skin LPR. A larger trial is needed to determine whether the observed *ex vivo* downregulation of T_H2 responses might translate into clinical benefit.

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METHODS

Preparation of PBMCs

Heparinized venous blood from each patient was diluted 1:1 with RPMI-1640 (Invitrogen, UK), layered on Histopaque-1077 (Sigma-Aldrich, UK), density gradient (density 1077) and centrifuged for 25 minutes at 1136g at room temperature. The PBMC layer was collected, washed, and resuspended in RPMI-1640. Cell viability was greater than 95%, as determined by means of trypan blue exclusion.

Flow cytometric assay

Frequencies of effector T and Treg cells were determined by using multicolor multiparameter flow cytometric analysis. Freshly isolated PBMCs were immunostained with anti-human anti-CD4 (clone: OKT4), anti-CD25 (BC96; BioLegend, UK), anti-CCR4 (1G1), anti-CRTH2 (BM1; BD Bioscience, UK), or relevant isotype controls for 40 minutes at room temperature in the dark. Samples were incubated for 10 minutes with 2 mL of 1:10 BD FACS Lysing solution (BD Biosciences) and then washed with 3 mL of PBS and resuspended in 450 μ L of BD CellFix solution (BD Biosciences). For Treg cells, the PBMCs were subjected to surface immunostaining by using anti-CD4, anti-CD25, and anti-CD127 (HIL-7R-M21). After fixation and permeabilization, cells were immunostained with anti-FOXP3 (259D) (BioLegend, Uithoorn, The Netherlands), according to the manufacturer's recommendations.

FluoroSpot assay

Frequencies of *P. pratense*-specific IL-4⁺ and IL-10⁺ cells were assessed by using the FluoroSpot assay. Briefly, polyvinylidene difluoride (PVDF)-backed plates were preincubated with 25 μ L of 70% ethanol for 30 seconds at room temperature. All wells were precoated with anti-IL-10 (10 μ g/mL) and anti-IL-4 (10 μ g/mL) antibodies overnight at 4°C. Wells were blocked with 100 μ L of 10% FCS in RPMI media and incubated for 2 hours at room temperature. PBMCs were stimulated 10 μ g/mL *P. pratense* (ALK-Abelló, Hørsholm, Denmark) in quadruplicates. PHA (5 μ g/mL; ICN Biomedicals, Aurora, Ohio) was added to control wells. After 42 hours of incubation at

37°C, PVDF plates were washed 3 times with 0.1% Tween 20 in PBS, and 100 μ L of anti-IL-4 (10 μ g/mL) and anti-IL-10 (10 μ g/mL) detection antibodies in 1% BSA/PBS were added to each well. PVDF plates were incubated for 1 hour and 30 minutes at room temperature in the dark. Plates were washed, and then 100 μ L of anti-fluorescein isothiocyanate green fluorescence conjugate, and streptavidin-phycoerythrin conjugate (1:1000 in 1% BSA/PBS) was added to each well. After 1 hour and further washing, plates were allowed to dry for 2 hours. Fluorescent spots were visualized and quantified under a UV light by using the iSpot reader.

Fluorescence-activated cell-sorting gating strategy

CD4⁺CCR4⁺ cells. CD4⁺ T cells were gated from lymphocyte populations within the forward scatter (FSC) and side scatter (SSC) dot plots. The proportion of CCR4⁺CD4⁺ cells was derived from the total CD4 T-cell population.

CD4⁺CRTH2⁺ cells. CD4⁺ T cells were gated from the lymphocyte population within the FSC and SSC dot plots. The proportion of CRTH2⁺CD4⁺ cells was derived from the total CD4⁺ T-cell population.

CCR4⁺CRTH2⁺ cells. CD4⁺ T cells were gated from the lymphocyte population within the FSC and SSC dot plots. The proportion of CRTH2⁺CD4⁺ cells was derived from the CD4⁺ T-cell population.

CD25^{hi}CD4⁺ cells. CD4⁺ T cells were gated from the lymphocyte population within the FSC and SSC dot plots. The proportion of CD25^{hi}CD4⁺ cells was derived from the total CD4 population.

CD127⁺FoxP3⁺ cells. CD4⁺ T cells were gated from the lymphocyte population within the FSC and SSC dot plots. CD25^{hi}CD4⁺ T cells were quantified, and the proportion of CD127^{lo}FoxP3⁺ cells was derived from the CD25^{hi}CD4⁺ T-cell population.

CCR4⁺CD127⁺FoxP3⁺ cells. CD4⁺ T cells were gated from the lymphocyte population within the FSC and SSC dot plots. CD25^{hi}CD4⁺ T cells were quantified, and the proportion of CD127^{lo}FoxP3⁺ cells was derived from CD25^{hi}CD4⁺ T cells. The proportion of CCR4⁺CD127⁺FoxP3⁺ cells was derived from the CD127^{lo}FoxP3⁺ T-cell population.

The instrument (FACSCanto II) was calibrated by using STS beads on a daily basis. Appropriate controls included relevant matching isotype controls.

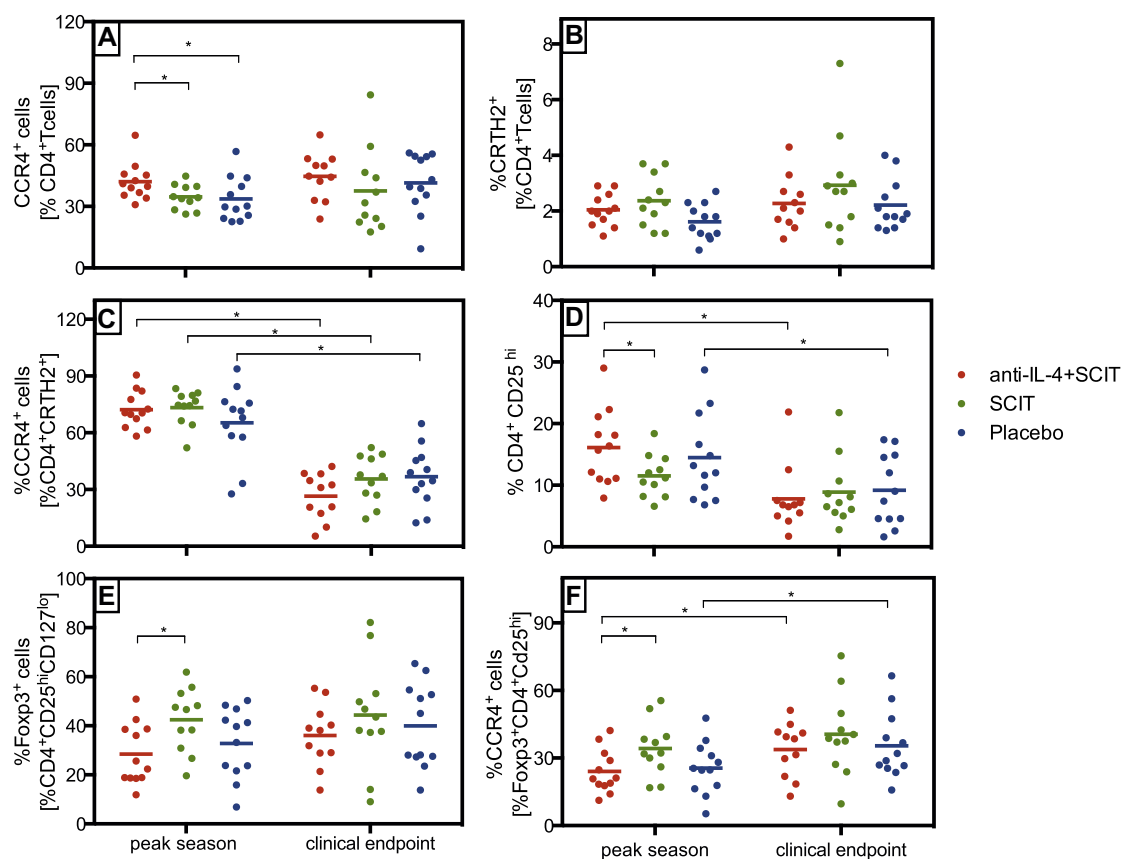


FIG E1. Fluorescence-activated cell sorting in PBMCs in season and at the clinical end point out of the pollen season. **A**, CCR4⁺ cells within CD4⁺ T cells on anti-IL-4 (VAK694) plus SCIT and SCIT. **B**, CD4⁺ CD25^{hi} T cells. **C**, FoxP3⁺ Treg cells within CD4⁺ CD25^{hi} CD127^{lo} cells. **D**, Proportion of CCR4⁺ cells within this Treg cell subset. **E**, CRTH2⁺ T cells. **F**, CCR4⁺ T cells within CD4⁺ CRTH2⁺ cells in and off season. **P* < .05.

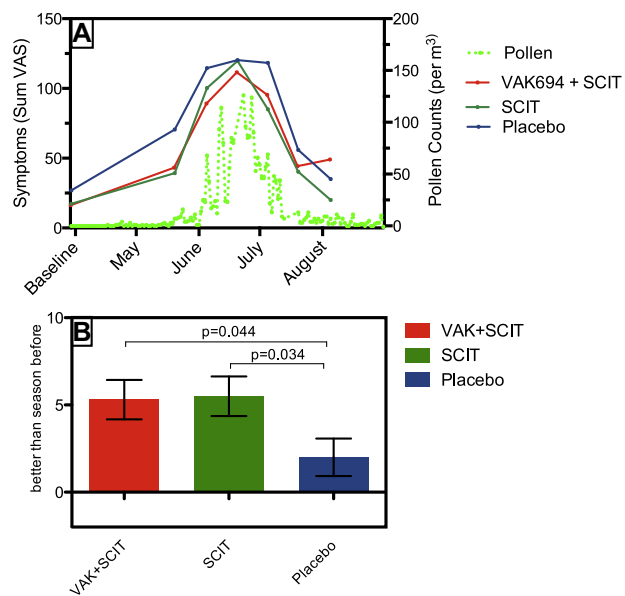


FIG E2. Symptom data. **A**, Sum of VAS symptoms and grass pollen grains per cubic meter in greater London displayed in means. **B**, Retrospective assessment total score (least square means).

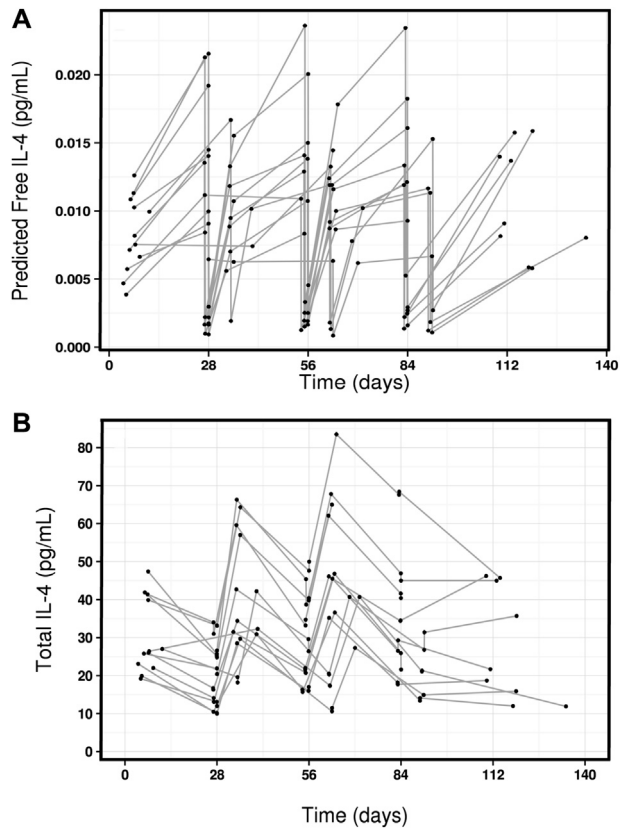


FIG E3. Pharmacodynamics of serum IL-4. **A**, Predicted free IL-4 levels in serum in VAK plus SCIT-treated patients with immediate increase of free IL-4 after discarding treatment. **B**, Concentration of total IL-4 in serum, including antibody-bound IL-4.

TABLE E1. AEs (overall by most frequent events when experienced by at least 2 subjects)

AE	Anti-IL-4 (VAK694) plus SCIT (n = 12), no. (%)	Placebo plus SCIT (n = 12), no. (%)	Double placebo (n = 13), no. (%)	Total (n = 37), no. (%)
Patients with AEs	12 (100.0)	11 (91.7)	13 (100.0)	36 (97.3)
Preferred term				
Injection-site pruritus	10 (83.3)	10 (83.3)	1 (7.7)	21 (56.8)
Injection-site mass	10 (83.3)	10 (83.3)	0 (0.0)	20 (54.1)
Nasopharyngitis	6 (50.0)	4 (33.3)	10 (76.9)	20 (54.1)
Injection-site erythema	5 (41.7)	7 (58.3)	0 (0.0)	12 (32.4)
Injection-site swelling	4 (33.3)	8 (66.7)	0 (0.0)	12 (32.4)
Headache	3 (25.0)	2 (16.7)	5 (38.5)	10 (27.0)
Cough	3 (25.0)	3 (25.0)	3 (23.1)	9 (24.3)
Rhinorrhea	1 (8.3)	2 (16.7)	3 (23.1)	6 (16.2)
Epistaxis	3 (25.0)	0 (0.0)	2 (15.4)	5 (13.5)
Injection-site urticaria	3 (25.0)	2 (16.7)	0 (0.0)	5 (13.5)
Oropharyngeal pain	1 (8.3)	2 (16.7)	2 (15.4)	5 (13.5)
Urticaria	2 (16.7)	2 (16.7)	1 (7.7)	5 (13.5)
Allergic pruritus	1 (8.3)	3 (25.0)	0 (0.0)	4 (10.8)
Rash	3 (25.0)	1 (8.3)	0 (0.0)	4 (10.8)
Sneezing	1 (8.3)	1 (8.3)	2 (15.4)	4 (10.8)
Arthralgia	2 (16.7)	1 (8.3)	0 (0.0)	3 (8.1)
Arthropod bite	2 (16.7)	0 (0.0)	1 (7.7)	3 (8.1)
Back pain	1 (8.3)	1 (8.3)	1 (7.7)	3 (8.1)
Diarrhea	1 (8.3)	0 (0.0)	2 (15.4)	3 (8.1)
Dyspnea	0 (0.0)	1 (8.3)	2 (15.4)	3 (8.1)
Eye pruritus	1 (8.3)	1 (8.3)	1 (7.7)	3 (8.1)
Mouth ulceration	0 (0.0)	2 (16.7)	1 (7.7)	3 (8.1)
Nasal congestion	0 (0.0)	1 (8.3)	2 (15.4)	3 (8.1)
Peripheral edema	2 (16.7)	0 (0.0)	1 (7.7)	3 (8.1)
Pain in extremity	2 (16.7)	1 (8.3)	0 (0.0)	3 (8.1)
Allergy to animal	1 (8.3)	1 (8.3)	0 (0.0)	2 (5.4)
Asthma	1 (8.3)	1 (8.3)	0 (0.0)	2 (5.4)
Chills	2 (16.7)	0 (0.0)	0 (0.0)	2 (5.4)
Contusion	1 (8.3)	0 (0.0)	1 (7.7)	2 (5.4)
Ear pain	1 (8.3)	0 (0.0)	1 (7.7)	2 (5.4)
Erythema	2 (16.7)	0 (0.0)	0 (0.0)	2 (5.4)
Eye pain	0 (0.0)	0 (0.0)	2 (15.4)	2 (5.4)
Fall	0 (0.0)	0 (0.0)	2 (15.4)	2 (5.4)
Herpesvirus infection	2 (16.7)	0 (0.0)	0 (0.0)	2 (5.4)
Lacrimation increased	0 (0.0)	1 (8.3)	1 (7.7)	2 (5.4)
Oral herpes	1 (8.3)	1 (8.3)	0 (0.0)	2 (5.4)
Pharyngitis	2 (16.7)	0 (0.0)	0 (0.0)	2 (5.4)
Pruritus	1 (8.3)	1 (8.3)	0 (0.0)	2 (5.4)
Pyrexia	1 (8.3)	0 (0.0)	1 (7.7)	2 (5.4)
Sinus headache	2 (16.7)	0 (0.0)	0 (0.0)	2 (5.4)
Throat irritation	1 (8.3)	1 (8.3)	0 (0.0)	2 (5.4)
Wheezing	1 (8.3)	0 (0.0)	1 (7.7)	2 (5.4)

TABLE E2. LPR to allergen in the skin

Time point	Anti-IL-4 (VAK694) plus SCIT (no. [SE])	Placebo plus SCIT, (no. [SE])	Double placebo (no. [SE])	Δ Anti-IL-4 (VAK694) plus SCIT vs double placebo (95% CI)	Δ SCIT vs double placebo (95% CI)	Δ Anti-IL-4 (VAK694) plus SCIT vs SCIT (95% CI)
LPR (mean % suppression of baseline area)*						
Preseason (after 13 wk treatment)	79.7 (11 [7.90])	76.0 (11 [7.76])	21.6 (13; 7.14)	58.2 (36.2 to 80.1), $P < .001$	54.4 (33.0 to 75.8), $P < .001$	3.7 (−19.1 to 26.6), $P = .741$
Postseason	69.5 (11 [10.97])	68.1 (11 [10.90])	35.2 (12 [10.39])	34.3 (3.3 to 65.3), $P = .031$	32.9 (2.2 to 63.6), $P = .036$	1.4 (−30.4 to 33.1), $P = .931$
Clinical end point (1 y after end of treatment)	67.0 (11 [9.84])	59.2 (11 [9.72])	27.9 (12 [9.30])	39.1 (11.3 to 66.9), $P = .007$	31.3 (3.9 to 58.7), $P = .026$	7.8 (−20.7 to 36.2), $P = .582$

*Data are presented as least squares means of percentage suppression of the recorded baseline area in the skin at before the season (ie, after a course of 13 weeks of treatment), after the pollen season in autumn, and at the clinical end point (ie, 12 months after the end of treatment and before the start of the next pollen season).

TABLE E3. Cytokine-producing cells (FluoroSpots)

Cytokine/time point	VAK694 plus SCIT† (no. [CI]‡)	Placebo plus SCIT† (no. [CI]‡)	Double placebo† (no. [CI]‡)	VAK694 plus SCIT/double placebo§	SCIT/double placebo§	VAK plus SCIT/SCIT§
IL-4						
Baseline	164.76 (12 [106.60-254.65])	145.97 (12 [94.46-225.54])	151.73 (13 [99.94-230.35])			
Peak season	81.48 (12 [52.71-125.93])	269.86 (11 [171.42-424.79])	115.23 (12 [74.63-177.90])	<i>P</i> = .27	<i>P</i> < .01*	<i>P</i> < .01*
Clinical end point	58.52 (11 [37.15-92.18])	136.51 (11 [86.69-214.95])	97.33 (12 [63.02-150.31])	<i>P</i> = .11	<i>P</i> = .29	<i>P</i> = .01*
IL-10						
Baseline	62.93 (12 [30.70-128.99])	52.92 (12 [25.85-108.33])	46.75 (13 [23.45-93.21])			
Peak season	17.37 (12 [8.47-35.60])	31.89 (11 [15.09-67.40])	7.38 (12 [3.60-15.11])	<i>P</i> = .10	<i>P</i> = .01*	<i>P</i> = .24
Clinical end point	3.21 (11 [1.52-6.80])	9.88 (11 [4.68-20.89])	3.68 (12 [1.80-7.55])	<i>P</i> = .79	<i>P</i> = .06	<i>P</i> = .04*
IL-4/IL-10 dual cells						
Baseline	5.04 (12 [2.87-8.83])	5.42 (12 [3.10-9.51])	5.12 (13 [2.99-8.78])			
Peak season	7.23 (12 [4.12-12.68])	13.44 (11 [7.49-24.11])	3.71 (12 [2.12-6.49])	<i>P</i> = .10	<i>P</i> < .01*	<i>P</i> = .13
Clinical end point	1.66 (11 [0.93-2.98])	3.35 (11 [1.86-6.03])	1.14 (12 [0.65-2.00])	<i>P</i> = .36	<i>P</i> = .01*	<i>P</i> = .10

Frequencies of cytokine-expressing cells in PBMCs in response to stimulation with *P pratense* extract, as measured at baseline, during the height of the pollen season, and before the following season is shown. Data are presented as follows: †counts of cytokine-producing cells per 100,000 cells as estimated geometric means of back-transformed natural log-scaled data, ‡number of subjects at a specific time point and CIs of cytokine-producing cells, and §*P* values of 2-sided tests.

*Statistically significant results.

TABLE E4. Total and grass pollen-specific IgE levels, IgG₄ levels, and FAB (allergen-IgE complexes bound to B cells)

Analyte/time point	VAK694 plus SCIT (no. [SD])	Placebo plus SCIT (no. [SD])	Double placebo (no. [SD])	VAK694 plus SCIT/double placebo	SCIT/double placebo	VAK plus SCIT/SCIT
Total IgE in serum (kU/L)						
Baseline	303.81 (12 [245.32])	199.16 (12 [189.82])	374.31 (13 [661.76])	<i>P</i> = .7534	<i>P</i> = .4355	<i>P</i> = .6475
Preseason	414.77 (11 [490.14])	318.64 (11 [369.46])	420.26 (13 [783.74])	<i>P</i> = .9809	<i>P</i> = .6589	<i>P</i> = .6883
Peak season	381.87 (12 [359.89])	298.17 (11 [334.46])	527.48 (12 [948.98])	<i>P</i> = .5248	<i>P</i> = .3278	<i>P</i> = .7206
Postseason	371.71 (11 [297.51])	275.57 (11 [346.76])	592.92 (12 [1043.40])	<i>P</i> = .3451	<i>P</i> = .1762	<i>P</i> = .6876
Clinical end point	222.75 (11 [166.07])	141.31 (11 [114.30])	338.38 (12 [722.35])	<i>P</i> = .4794	<i>P</i> = .2918	<i>P</i> = .7333
Grass pollen-specific IgE in serum (kU/L)						
Baseline	48.96 (12 [51.74])	30.50 (12 [45.01])	25.04 (13 [31.36])	<i>P</i> = .4592	<i>P</i> = .8658	<i>P</i> = .5752
Preseason	99.15 (11 [161.52])	79.79 (11 [106.63])	27.98 (13 [37.24])	<i>P</i> = .0325	<i>P</i> = .1184	<i>P</i> = .5738
Peak season	90.89 (12 [113.22])	71.70 (11 [95.38])	56.95 (12 [80.21])	<i>P</i> = .3036	<i>P</i> = .6615	<i>P</i> = .5689
Post season	73.11 (11 [70.25])	62.57 (11 [95.55])	54.86 (12 [77.90])	<i>P</i> = .5882	<i>P</i> = .8189	<i>P</i> = .7594
Clinical end point	42.53 (11 [42.79])	33.95 (11 [42.73])	40.15 (12 [57.65])	<i>P</i> = .9437	<i>P</i> = .8541	<i>P</i> = .8032
Grass pollen-specific IgG ₄ in serum (mg/L)						
Baseline	0.16 (12 [0.15])	0.28 (12 [0.54])	0.09 (13 [0.10])	<i>P</i> = .9404	<i>P</i> = .8486	<i>P</i> = .9093
Preseason	2.77 (11 [2.11])	3.92 (11 [5.47])	0.08 (13 [0.09])	<i>P</i> = .0090	<i>P</i> = .0002	<i>P</i> = .2818
Peak season	3.50 (12 [2.90])	4.26 (11 [6.61])	0.11 (12 [0.16])	<i>P</i> = .0011	<i>P</i> < .0001	<i>P</i> = .4648
Post season	1.68 (11 [1.25])	2.27 (11 [2.78])	0.12 (12 [0.17])	<i>P</i> = .1340	<i>P</i> = .0401	<i>P</i> = .5817
Clinical end point	0.59 (11 [0.42])	0.94 (11 [1.34])	0.13 (12 [0.25])	<i>P</i> = .6590	<i>P</i> = .4360	<i>P</i> = .7406
Allergen-IgE complexes bound to B cells (%)						
Baseline	98.54 (12 [8.14])	97.70 (12 [4.08])	94.87 (13 [6.09])	<i>P</i> = .4445	<i>P</i> = .5548	<i>P</i> = .8643
Preseason	70.98 (11 [12.28])	73.81 (11 [15.98])	94.15 (13 [8.64])	<i>P</i> < .0001	<i>P</i> < .0001	<i>P</i> = .5800
Peak season	65.64 (12 [17.34])	66.05 (11 [18.24])	94.13 (12 [7.94])	<i>P</i> < .0001	<i>P</i> < .0001	<i>P</i> = .9354
Post season	79.87 (11 [13.71])	72.07 (11 [15.80])	91.30 (12 [14.41])	<i>P</i> = .0235	<i>P</i> = .0002	<i>P</i> = .1287
Clinical end point	88.05 (11 [9.47])	85.23 (11 [8.01])	92.39 (12 [9.99])	<i>P</i> = .3864	<i>P</i> = .1543	<i>P</i> = .5825

TABLE E5. Clinical symptoms at peak season and retrospective allergy symptom assessment

Assessment	Anti-IL-4 (VAK694) plus SCIT (no. [SE])	Placebo plus SCIT (no. [SE])	Double placebo (no. [SE])	Δ Anti-IL-4 (VAK694) plus SCIT vs double placebo (SE [95% CI]), <i>P</i> value	Δ SCIT vs double placebo (SE [95% CI]), <i>P</i> value	Δ Anti-IL-4 (VAK694) plus SCIT vs SCIT (SE [95% CI]), <i>P</i> value
Total VAS (change from baseline)*						
Peak season	94.6 (12 [25.57])	107.9 (10 [28.01])	99.0 (12 [25.63])	−4.4 (36.21 [−78.3to 69.4]), <i>P</i> = .904	8.9 (37.97 [−68.5to 86.3]), <i>P</i> = .816	−13.3 (37.92 [−90.7 to 64.0]), <i>P</i> = .728
mRQLQ (change from baseline)*						
Peak season	13.3 (12 [4.59])	18.4 (11 [4.80])	15.6 (11 [4.69])	−2.3 (6.56 [−15.7to 11.0]), <i>P</i> = .723	2.8 (6.71 [−10.9to 16.5]), <i>P</i> = .682	−5.1 (6.78 [−19.0 to 8.7]), <i>P</i> = .456
Total RAAS†						
Clinical end point (1 y after end of treatment)	5.3 (11 [1.13])	5.5 (11 [1.13])	2.0 (12 [1.08])	3.3 (1.56 [0.1 to 6.5]), <i>P</i> = .044	3.5 (1.56 [0.3 to 6.6]), <i>P</i> = .034	−0.2 (1.59 [−3.4 to 3.1]), <i>P</i> = .910

Data are presented as least square means as follows: *change from baseline of total VAS symptom assessment or mRQLQ at the peak of the pollination season in June (visit 22); †total RAAS compared with hay fever season year before (−3 to +3) as inquired at the clinical tolerance end point 12 months after the end of treatment.

RAAS, Retrospective allergy assessment score.