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ORIGINAL RESEARCH ARTICLE

Negative Clinical Results from a Randomised, Double-Blind, Placebo-Controlled Trial Evaluating the Efficacy of Two Doses of Immunologically Enhanced, Grass Subcutaneous Immunotherapy Despite Dose-Dependent Immunological Response

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Abstract

Objective Specific immunotherapy is the only treatment for the underlying allergic disease in patients with respiratory allergies. The primary objective of this trial was to evaluate the efficacy and safety of two maintenance doses of immunologically enhanced, standardised quality (SQ+) grass subcutaneous immunotherapy (SCIT) [4,000 SQ+ and 15,000 SQ+; AVANZ® *Phleum pratense* (ALK)] compared with placebo.

Methods This was a randomised, double-blind, placebo-controlled, phase II/III trial. The primary evaluation was based on the combined rhinoconjunctivitis score during the entire grass pollen season. Adult subjects with grass pollen-induced allergic rhinoconjunctivitis interfering with usual activities or sleep despite symptomatic medication use, were enrolled.

Results Four hundred and fifty subjects were randomised to receive either 4,000 SQ+ ($n = 150$), 15,000 SQ+ ($n = 152$) or placebo ($n = 148$). The average grass pollen exposure was 27 grains/m^3/day. No statistically significant differences between the active groups and the placebo group were found for clinical endpoints ($p > 0.05$). Highly statistically significant ($p < 0.001$) increases in IgG_4 and IgE-blocking factor were found for both active groups versus placebo. The most frequently reported adverse events were mild-to-moderate local injection-site reactions; events were generally more frequent with 15,000 SQ+ than with 4,000 SQ+ and placebo. The most common adverse events leading to premature discontinuation from the trial were anaphylactic reactions (one subject from the placebo group and five subjects from the 15,000 SQ+ group).

Conclusions The inconclusive results were most probably influenced by a very low grass pollen season. Other factors such as the extent of the pre-seasonal treatment could potentially have contributed. The tolerability profile was acceptable for further development.

Key Points

This trial showed insignificant clinical results of grass subcutaneous immunotherapy despite highly statistically significant immunological data

In-field trials are mandatory for claiming efficacy of seasonal immunotherapy; however, the risk of low natural pollen exposure during the season can decrease the therapeutic window and lead to inconclusive or negative trial results

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1 Background

Allergic rhinoconjunctivitis represents a global health problem affecting 10–25 % of the population [1]. Allergy to grass pollen is one of the most common inhalant allergies in the Western world. Allergic rhinoconjunctivitis has been identified as one of the main reasons for visits to primary care clinics, and although usually not regarded as a severe disease, it significantly limits a patient's social life and affects school learning performance and work productivity [1]. A large number of patients are still uncontrolled on symptomatic medications [2].

Allergy immunotherapy (AIT) is the only causal treatment in allergic patients who are uncontrolled with symptomatic medications. Subcutaneous immunotherapy (SCIT) with intact *Phleum pratense* allergens has been widely used for decades throughout Europe and documented to be efficacious and well tolerated [3]. Aluminium is widely used as an adjuvant in SCIT for adsorption of allergens to induce slow release (depot effect), and has been shown to reduce adverse effects and increase effectiveness of the treatment [4].

The immunologically enhanced, standardised quality (SQ+) grass SCIT (AVANZ® *Phleum pratense*; ALK, Denmark) investigated in this phase II/III trial, has been formulated with an optimised allergen/aluminium ratio. This allows for a shorter updosing schedule and, based on data in mice, a lower maintenance dose of allergen while maintaining immunogenicity and reducing allergenicity [5]. While a conventional updosing schedule consists of approximately 15 injections to reach the maintenance dose, the maintenance dose in this trial was reached after five injections. Previously, two trials were conducted in humans to investigate the shorter updosing schedule (EudraCT 2008-006921-14 [6] and EudraCT 2011-000057-23 [Sastre et al., publication submitted]).

The objectives of the trial were to generate information on the efficacy and safety of the SQ+ grass SCIT with two maintenance doses (4,000 SQ+ and 15,000 SQ+) compared with placebo in subjects with grass pollen-induced allergic rhinoconjunctivitis. All subjects were offered, in addition to the investigational product, symptomatic medications as needed for the treatment of allergic rhinoconjunctivitis and asthma symptoms.

2 Methods

The trial was a randomised, double-blind, placebo-controlled, multinational trial with 53 sites in Austria, Germany and Spain (EudraCT No. 2011-000120-15).The trial was designed and conducted in accordance with the principles of the Declaration of Helsinki (1964, and its amendments and subsequent clarifications) [7] and in compliance with the principles of Good Clinical Practice [8]. Written consent was obtained prior to the start of any trial-related activities. The subjects were informed that they were free to withdraw from the trial at any time and for any reason without prejudice. No substantial trial amendments were made after treatment initiation.

The primary evaluation of efficacy was based on the combined rhinoconjunctivitis score [CRCS; sum of rhinoconjunctivitis daily symptom score (DSS) and daily medication score (DMS), obtained from daily diary recordings during the entire grass pollen season (GPS)] (see below). Other endpoints included DSS, DMS, immunological parameters, pharmacoeconomic parameters, quality of life (QoL), asthma symptoms and asthma medication used during the GPS. Safety assessment was primarily based on adverse event (AE) reporting.

2.1 Trial Population

The trial population comprised adults (18–64 years old) with documented clinically relevant symptoms of grass pollen-induced allergic rhinoconjunctivitis with or without asthma, despite having received symptomatic medications during the previous 2 years. Further major selection criteria included: clinical history of severe rhinoconjunctivitis interfering with usual activities or sleep, and an appropriate minimum level of symptoms (defined as a rhinoconjunctivitis DSS score of $\geq$10 on the worst day of the previous GPS) prior to randomisation; a positive skin prick test with wheal diameter $\geq$3 mm; a specific IgE level $\geq$0.7 kU_A/L to *Phleum pratense*; FEV_1 $\geq$70 % of predicted after adequate pharmacological treatment; no history of clinically relevant allergies other than grass overlapping the GPS; no irreversible airway damage; no previous AIT with grass pollen within 5 years; no history of anaphylaxis with cardiorespiratory symptoms; no clinically relevant chronic diseases; no immunosuppressive treatment within 3 months prior to the screening visit (except corticosteroids for allergy and asthma); and no concomitant treatment with ACE inhibitors, β-blockers, tricyclic antidepressants, catechol-*O*-methyl transferase inhibitors and monoamine oxidase inhibitors.

During the screening phase, all subjects underwent thorough examinations to ensure that only eligible subjects were randomised.

2.2 Intervention and Treatment Schedule

The active investigational medicinal product was SQ+ grass SCIT; an aluminium hydroxide adsorbed *Phleum pratense* extract administered by a subcutaneous injection.

The placebo formulation was identical to the active products, except for the *Phleum pratense* extract (i.e. placebo contains the same amount of aluminium hydroxide as the active product). The trial products were supplied in vials containing a 2.5-mL suspension for injection. The vials were to be stored in the original package in a refrigerator (2–8 °C).

The randomisation sequence was computer generated by a trial independent statistician and codes remained strictly confidential and accessible only to authorised people until the time of unblinding. Randomisation codes in sealed envelopes were retained with the sponsor and at the trial sites. Subjects were assigned the lowest available randomisation number at the site and were randomised to placebo, 4,000 SQ+, or 15,000 SQ+.

The treatment schedule consisted of five updosing injections and then maintenance treatment for approximately 1 year, ending after the GPS 2012. The dose scheduling for each treatment group is displayed in Fig. 1. After the 5-weekly updosing injections, the first maintenance dose was given 2 weeks later, the next another 4 weeks later and thereafter every 6 weeks. Schedules for dose adjustments were provided in the case of extended time between two visits or in the case of local or systemic adverse reactions.

Subjects were supplied with open-labelled symptomatic medications for rhinoconjunctivitis symptoms (desloratadine tablets, olopatadine eye drops and fluticasone nasal spray) and asthma (on-demand salbutamol and if necessary, fluticasone for inhalation).

Intake of additional concomitant medications was kept to a minimum during the trial. However, if considered necessary for the subject's well-being, concomitant medications were given at the discretion of the investigator according to local standards of care.

2.3 Grass Pollen Exposure

Pollen count data were collected for the region of trial sites by SciCon Pharma Science-Consulting GmbH (Vienna, Austria; http://www.scicon.at) to map the length and intensity of the GPS, and thereby to quantify the allergen exposure. The start and the end of the entire GPS were defined as the first and the last of 3 consecutive days with pollen counts above and below 10 grains/m^3. The peak GPS was defined as the 15 consecutive days with the highest cumulative average pollen count in the entire GPS.

2.4 Endpoints

All subjects included in the trial were issued an e-diary before the GPS 2012. Information on six rhinoconjunctivitis symptoms (four nose symptoms, two eye symptoms), four asthma symptoms and use of symptomatic medications was entered into the e-diary on a daily basis. The maximum daily scores were 18 for rhinoconjunctivitis DSS, 20 for rhinoconjunctivitis DMS, 12 for the asthma symptom score, and 16 for the asthma medication score.

Blood samples were drawn at the screening visit, after reaching the first maintenance dose (after approximately 10 weeks) and at the end of the trial (after approximately 49 weeks) for determination of specific IgE, IgE-blocking factor and IgG_4 for *Phleum pratense*.

AEs were collected from the time the subject signed the informed consent to the end-of-trial visit. AEs and serious AEs were defined according to the ICH Harmonised Tripartite Guideline E2A, Step 5 [9]. SCIT-related AEs were defined as events assessed as 'possibly' related to the treatment or with 'unknown' aetiology.

Clinical safety laboratory tests (haematology, blood chemistry, urinalysis and blood, and urine samples for aluminium content) were analysed centrally. The aluminium analysis was performed using a VARIAN AA280Z analyser (electrothermal atomic absorption spectrometry). The aluminium measurements from serum were in accordance with the German Research Community Commission for Toxicological Analysis (note 15, 1990) and from urine were based on the N.W. Tietz Clinical Guide to Laboratory Tests (2nd edition). The safety assessment also included physical examination, vital signs and lung function.

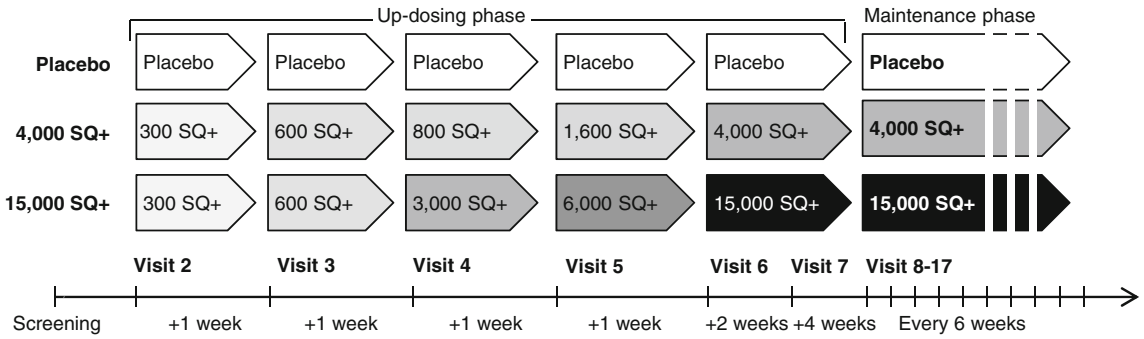


Fig. 1 Dose scheduling for each up-dosing step and for the maintenance treatment in each treatment group

2.5 Statistics

The statistical analysis plan was finalised prior to unblinding. The sample size calculation was based on (a) mean CRCS and pooled standard deviation (CRCS 7.1; SD 4.2) from a grass sublingual immunotherapy tablet trial [10]; (b) the formula for power of a two-sided, unpaired *t* test assuming equal variance; and (c) assuming a dropout rate of 10 %. Based on these numbers, 150 subjects in each arm were required to detect a difference in CRCS of 20 % with a power of 80 %.

The primary endpoint (CRCS; sum of DSS and DMS) for the entire GPS was calculated for each subject during the entire GPS divided by the number of days with diary records. The endpoint was analysed using a common linear mixed effect (LME) model for all treatment groups. The square root of the average CRCS was the response variable, treatment was a fixed class effect and pollen region was a random class variable. Estimates of adjusted means and difference in adjusted means were transformed back into the original scale. The two active treatment groups were compared with placebo using a *t* test in the LME model. The null hypothesis in the *t* test was that the mean was equal for the active dose group and placebo. The difference in (the back transformed) adjusted means for each active group compared with placebo was calculated together with the associated *p* value and 95 % confidence limit (CL).

The key secondary efficacy endpoints (average rhinoconjunctivitis DSS, average rhinoconjunctivitis DMS) were calculated for each subject as the sum of the score during the entire GPS divided by the number of days with diary records in the entire GPS as above.

The changes from baseline in IgE-blocking factor, $\log_{10}$(IgE) and $\log_{10}$(IgG_4) were analysed using a LME model. For IgE and IgG_4, change from baseline of the log-transformed immunological parameter was the response variable, treatment, visit and their two-factor interaction were fixed class variables, the log-transformed immunological parameter at baseline was a regression variable and subject was a random variable. The two active groups were compared with placebo at each visit using a *t* test in the LME model. The corresponding difference in adjusted means for each active group compared with placebo was calculated together with the associated *p* value and 95 % CL. IgE-blocking factor was analysed using the similar LME model but without any log-transformation.

The issue of multiple testing for the primary and key secondary efficacy endpoints was adjusted for using hierarchical testing; i.e. the first hypothesis was to be rejected at the 5 % level before continuing to the next hypothesis. Missing data due to premature discontinuations or diary non-compliance were not replaced by imputed data.

Safety data were analysed by summary statistics. AEs were coded by MedDRA (version 15.0) at the lower level term and summarised by system organ class and preferred term. All AEs and treatment-related AEs were broken down by severity (mild, moderate, severe), seriousness, action taken and outcome.

No changes to the statistical analyses were introduced after unblinding. The principal statistical software used was SAS®, version 9.3.

3 Results

3.1 Baseline Characteristics

Of the 517 patients screened, 450 met the inclusion criteria and were randomised to placebo ($n = 148$), 4,000 SQ+ ($n = 150$) or 15,000 SQ+ ($n = 152$). All randomised subjects were included in the analysis.

The subject disposition and the data sets used for the analyses are summarised in Fig. 2. Three hundred and ninety-four subjects completed the trial. The baseline demographics (overall 49% were male, mean age was 33 years, body mass index was 25, and 96 % were Caucasian) were similar across the treatment groups. Likewise, no major differences between groups were observed for 'worst day' rhinoconjunctivitis DSS during the previous GPS [overall mean DSS (SD): 13.4 (2.2)]. Nearly all subjects (>99 %) reported that they had taken symptomatic medications to relieve their rhinoconjunctivitis symptoms during both GPS 2010 and GPS 2011.

All subjects had a history of grass pollen-induced allergic rhinoconjunctivitis (overall mean duration 15 years) and 147 (33 %) had a history of grass pollen-induced asthma; similarly distributed among all treatment groups.

All subjects had a positive skin prick test for *Phleum pratense*. Seventy-four percent of the subjects were polysensitised with at least one additional sensitisation (the basic test set included *Phleum pratense*, *Betula verrucosa*, *Artemisia vulgaris*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, *Cladosporium herbarum*, horse, cat, and dog hair and dander; in Spain, additionally six tree and weed pollens were tested).

3.2 Exposure to Grass Pollen

The mean daily grass pollen count was 27.0 grains/m^3 and the mean length of the GPS was 47 days. This covered large regional variations in both mean daily counts (7.9–53.0 grains/m^3) and length of the GPS (11–89 days).

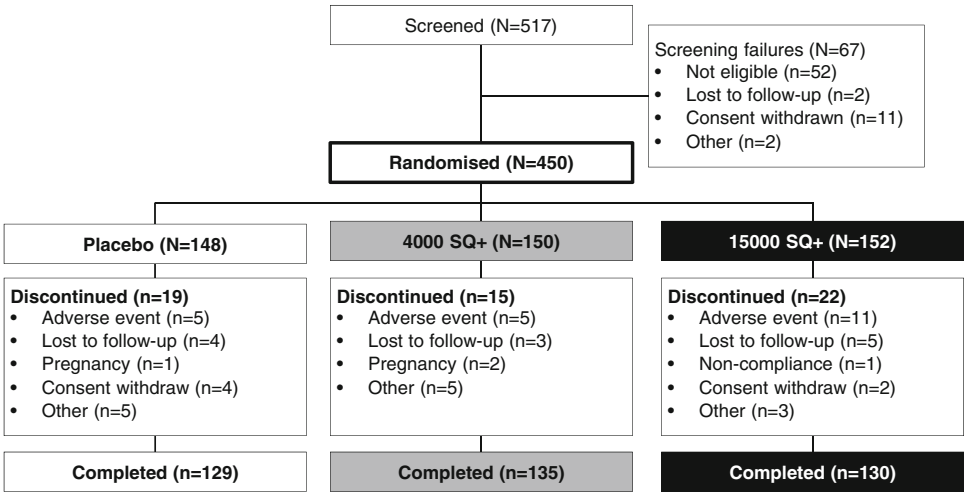


Fig. 2 Subject disposition. The passage of subjects through the trial; for each intervention group the number of subjects randomised, and thus included in the primary analysis, is shown, and the number of discontinuations and reasons for those

3.3 Exposure to Treatment

Ninety-five percent of the subjects received at least two maintenance doses (95 % in placebo, 97 % in 4,000 SQ+, and 92 % in 15,000 SQ+).

Fifty-six subjects (19 in placebo, 15 in 4,000 SQ+ and 22 in 15,000 SQ+) discontinued the trial and therefore did not receive all scheduled dosages.

Prior to the start of the GPS, the subjects had been in the trial for an overall average of 165 days and had received an average of nine injections (range 1–16); both numbers were similarly distributed among all treatment groups.

3.4 Clinical Efficacy

The CRCS over the entire GPS illustrated is in Fig. 3. The differences in adjusted means between the two active groups and placebo were just below 5 % on the relative scale (0.35 on the absolute scale for both groups) in favour of active treatment. However, the differences to placebo were not statistically significant ($p > 0.05$). For the key secondary endpoints (rhinoconjunctivitis DSS and DMS) and the additional secondary endpoints, there were no statistically significant differences between the active groups and placebo, despite numerical differences in favour of active treatment (data not shown).

3.5 Immunological Effects

A statistically significant difference in the immunomodulatory effect of active treatment was demonstrated by a dose-dependent increase in IgE-, IgG_4- and IgE-blocking factors from baseline for the active groups compared with the placebo group.

IgE was significantly increased 10 weeks after the start of treatment in both active groups, whereafter levels were decreased at the end of trial, yet significantly different from placebo ($p \leq 0.001$).

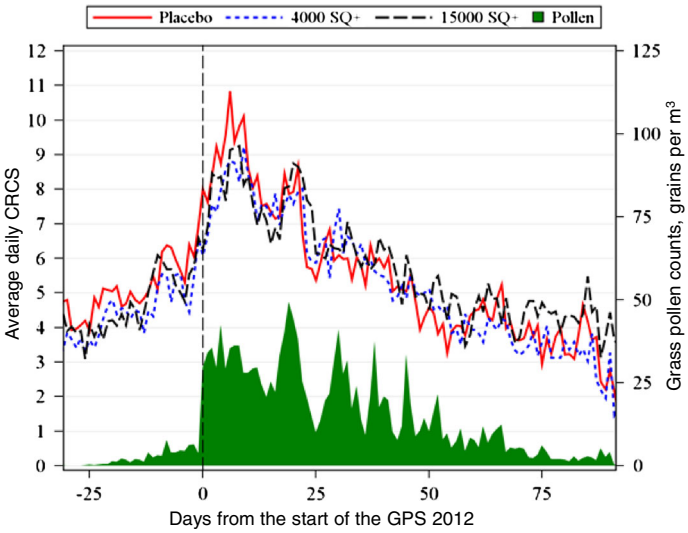
IgG_4 was increased from baseline to 10 weeks after the start of treatment and further to the end of the trial, in a dose-dependent manner (Fig. 4). The increases in both active groups were significantly different from placebo ($p < 0.001$).

IgE-blocking factor was increased from baseline to 10 weeks after the start of treatment and was on the same level at the end of the trial. The increase was larger with 15,000 SQ+ than with 4,000 SQ+ (Fig. 4). The increases in both active groups were significantly different from placebo ($p < 0.001$).

3.6 Safety

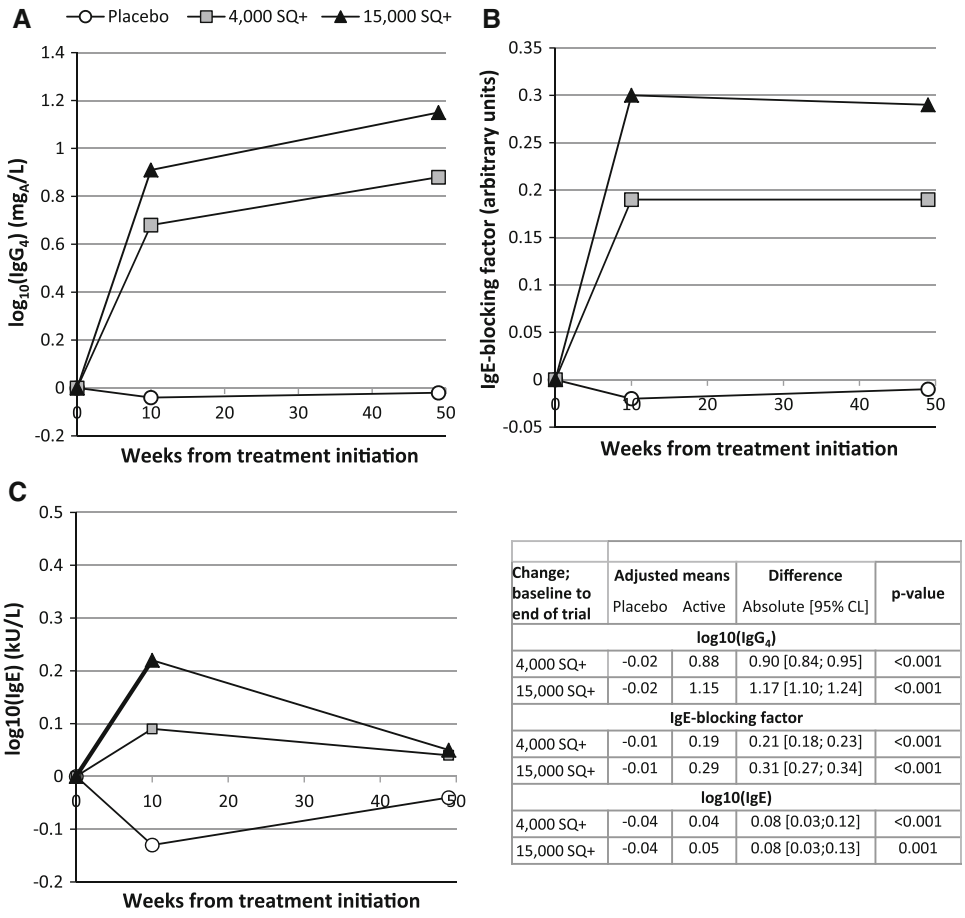
Overall, 2,101 AEs were reported, with more AEs in the active groups (721 in 4,000 SQ+, and 783 in 15,000 SQ+) than in the placebo group (597 AEs), but most AEs were mild (83 %) or moderate (15 %) in severity. The most frequently reported AE was injection-site reaction with 548 AEs in 157 subjects (26 %) (Fig. 5). Eleven anaphylactic reactions were reported by nine subjects (one subject from placebo, eight subjects from 15,000 SQ+) (Table 1). All were assessed as SCIT related. Six occurred with the same dose (0.1 mL Vial B, 3,000 SQ+). All resolved with adequate care and medication. Adrenaline was administered in the one placebo AE. Five of the reactions (in four subjects in 15,000 SQ+) were assessed as serious (because of fulfilment of the hospitalisation criterion or the event being assessed as medically important). There were no reported cases of SCIT-related anaphylactic shock. Additionally, 14 SAEs were reported by 13 subjects; none of these was assessed as SCIT related.

Few subjects discontinued the trial because of AEs [five subjects in placebo (3.4 %), five in 4,000 SQ+ (3.3%) and



FAS	Adjusted means		Difference, adjusted means		p-value
	Placebo	Active	Absolute [95% CL]	Relative/% [95% CL]	
4,000 SQ+	7.15	6.81	0.35 [-0.7; 1.4]	4.84 [-11.0; 18.5]	0.53
15,000 SQ+	7.15	6.80	0.35 [-0.7; 1.4]	4.93 [-10.7; 18.4]	0.51

Fig. 3 The CRCS over the entire GPS. In the table, the adjusted mean scores for each group and the absolute and relative differences to placebo (along with corresponding *p* values for the differences) are shown. *95 % CL* 95 % confidence limits, *CRCS* combined rhinoconjunctivitis score, *FAS* full analysis set, *GPS* grass pollen season



Change; baseline to end of trial	Adjusted means		Difference	p-value
	Placebo	Active	Absolute [95% CL]	
log10(IgG_4)				
4,000 SQ+	-0.02	0.88	0.90 [0.84; 0.95]	<0.001
15,000 SQ+	-0.02	1.15	1.17 [1.10; 1.24]	<0.001
IgE-blocking factor				
4,000 SQ+	-0.01	0.19	0.21 [0.18; 0.23]	<0.001
15,000 SQ+	-0.01	0.29	0.31 [0.27; 0.34]	<0.001
log10(IgE)				
4,000 SQ+	-0.04	0.04	0.08 [0.03;0.12]	<0.001
15,000 SQ+	-0.04	0.05	0.08 [0.03;0.13]	0.001

Fig. 4 **a** Change from baseline in log10(IgG4) against *Phleum pratense*; **b** change from baseline in IgE-blocking factor against *Phleum pratense*. In the table, the adjusted mean changes from baseline to the end of trial are shown for each group, as well as the absolute differences to placebo along with corresponding *p* values for the differences. *95 % CL* 95 % confidence limits

11 in 15,000 SQ+ (7.2 %)]. The most common AE leading to discontinuation was anaphylactic reaction (one subject from the placebo group and five subjects from the 15,000 SQ+ group; Table 1). Two hundred and ninety-one AEs in the placebo group, 394 in the 4,000 SQ+ group and 506 in the 15,000 SQ+ group were assessed as SCIT related.

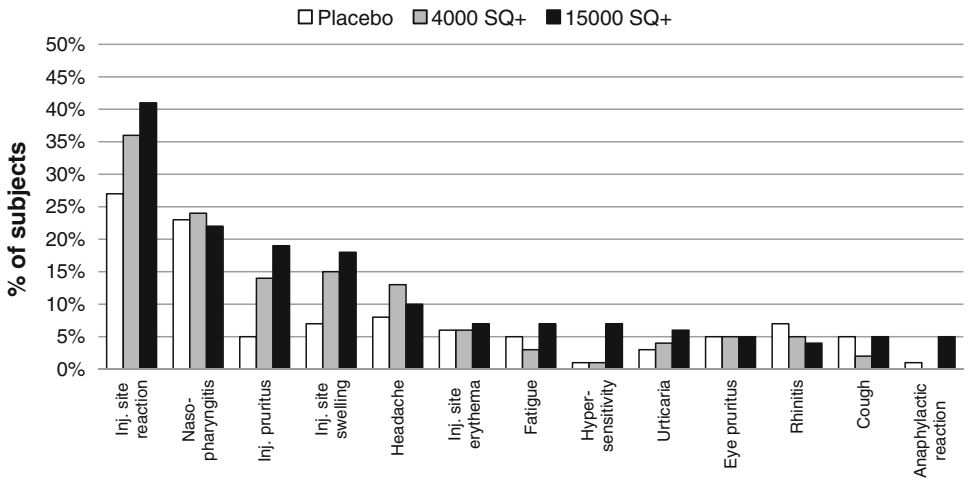


Fig. 5 Most frequently reported adverse events by MedDRA preferred terms. The figure displays all MedDRA preferred terms reported by ≥5 % in any active group. All adverse events from first subcutaneous immunotherapy administration to the end of trial were included regardless of causality and severity assessment. *inj* injection

No significant differences in clinical safety laboratory tests from baseline to follow-up were found. Overall, average aluminium concentrations at baseline were 8.2 µg/L in serum and 20.3 µg/L in urine. No accumulation of aluminium in serum or urine in any group was detected after treatment. No major findings in vital signs, physical examination or lung function were observed.

4 Discussion

Despite numerical trends, this trial did not show significant differences in primary and secondary clinical parameters between active treatment and placebo. The finding was unexpected and inconsistent with the findings from previous trials that evaluated the efficacy of other grass pollen SCIT products with similar or lower allergen content. In a double-blind, randomized, placebo-controlled study conducted at 26 UK hospital clinics, maintenance doses of 100,000 SQ-U and 10,000 SQ-U were administered [3]. The trial concluded that both doses were effective, demonstrating clinical and statistically significant differences in the symptom scores during the entire and peak GPS between the active groups and the placebo group [3]. Similar positive results were also observed in trials conducted by Zenner et al. [11] and Klimek et al. [12] where lower doses than in the present trial were administered. A trial including both traditional aluminium-adsorbed SCIT and immunologically enhanced SCIT would be valuable to directly compare clinical and immunological parameters between the two SCIT formulations.

The limited differences between active and placebo for the clinical endpoints may be explained by the low pollen exposure during this trial (27 grains/m^3/day). The pollen counts in this trial were lower than the observed pollen counts in the UK trial mentioned above (67 grains/m^3/day) and in all five grass seasons in a long-term trial with a grass SLIT tablet across Europe (36–57 grains/m^3/day across the 5 years) [3, 13]. Recently, Durham and colleagues examined the influence of pollen exposure on efficacy measurements from pooled grass SLIT-tablet trials [14]. They concluded that a treatment effect measured with grass AIT trials is 'highly dependent on pollen exposure (and that), regardless of the clinical parameter assessed, the magnitude of the treatment effect was greater with higher pollen exposure'. It is thus reasonable to assume that the treatment effect observed in the present trial could be influenced by the low pollen exposure similar to what has been reported in a previously published sublingual immunotherapy trial [15]. The World Allergy Organisation emphasises that an ideal randomised controlled trial should include 'adequate pollen counts in trials on pollen-allergic subjects' [16], which is, however, easier said than done. To be able to control the pollen exposure, a trial carried out in an environmental challenge chamber might be an appealing alternative to the field trial [17].

Another potential explanation could be related to the number of injections prior to the start of the GPS. It could be speculated that the pre-seasonal treatment of on average nine injections was insufficient in relation to measuring a clinical effect. From grass SLIT-tablet trials, it is known that sufficient pre-seasonal treatment is required for clinical effect in the first GPS [18].

In this trial, highly statistically significant increases ($p < 0.001$) in IgG_4 and IgE-blocking factor for both 4,000 SQ+ and 15,000 SQ+ versus placebo were demonstrated. This finding is similar to what has previously been observed with grass SCIT [19] and grass SLIT-tablet [20, 21].

The SCIT-related AEs reported in the present trial are similar to what have been observed in a previous SQ+ grass SCIT trial [6], except for a higher incidence of anaphylactic reactions in the present trial. These occurred primarily during up-dosing, i.e. outside of the pollen

Table 1 Overview of anaphylactic reactions in the trial

ID	Dose	Onset after SCIT Administration	Symptoms	Blood Pressure	Treatment	Recovery/discontinuation
Placebo						
50495	0.5 mL placebo	20 min	Generalised urticaria, generalised erythema	NK	Corticosteroid p.o., antihistamine (nasal), adrenaline s.c.	Recovered on the same day/ discontinued the trial
15,000 SQ+						
50282*	0.1 mL vial B (3,000 SQ+)	2 h	Generalised urticaria, itching and stinging sensation of the lip	125/84 mmHg (baseline 116/76 mmHg)	Corticosteroid i.v., antihistamine i.v., volume substitution (0.9 % NaCl) i.v.	Recovered ~11 h after onset time of event/discontinued from the trial
50383	0.1 mL vial B (3,000 SQ+)	30 min	Moderate generalised symptoms of angioedema, pruritus and upper airway swelling	Suspicion of hypotension (not measured)	Antihistamine p.o.	Recovered after app. 8 h later/ discontinued from the trial
50415*	0.1 mL vial B (3,000 SQ+)	2.5 h	Asthma, generalised urticaria, generalised pruritus and re/ watery eyes	120/80 mmHg (baseline 120/80 mmHg)	Corticosteroid i.v., antihistamine i.v.	Recovered 3 h after onset of the reaction/SCIT up-dosing was restarted from step 1
50445	0.1 mL vial B (3,000 SQ+)	90 min	Generalised pruritus (neck and palms), bronchospasm and cough	100/60 mmHg (baseline 100/60 mmHg)	Corticosteroid i.v., anti-histamine i.v., β2-agonist for inhalation	Recovered 2 h after onset time/ SCIT dose was reduced at next injection
50512-1	0.1 mL vial B (3,000 SQ+)	2 h	Dyspnoea and generalised urticaria	Not available	Corticosteroid i.m., antihistamine i.m.	Recovered 1 h later/SCIT dose was reduced at next injection
50512-2	0.5 mL vial B (15,000 SQ+)	4 h	Dyspnoea and generalised urticaria	Not available	Corticosteroid i.m., antihistamine i.m.	Recovered 2 h later/ discontinued from the trial
50580*	0.5 mL vial A (300 SQ+)	5 min	Circulatory collapse, swelling in left arm and convulsions in both hands. No respiratory or skin reactions and no loss of consciousness	90/60 mmHg (baseline 140/97 mmHg)	Antihistamine i.v., corticosteroid i.v, volume substitution (0.9 % NaCl) i.v.	Hospitalised for observation; discharged 4 h later (possible vasovagal reaction)/ discontinued from the trial
50591	0.2 mL vial B (6,000 SQ+)	45 min	Generalised urticaria, itching palms, mouth, tongue and ears. Redness palms and swelling neck	120/60 mmHg (baseline 100/63 mmHg)	Antihistamine p.o.	Recovered after app. 6 h later
50637-1[a]	0.1 mL vial B (3,000 SQ+)	25 min	Redness in head, swollen and itchy ears and generalized swelling of the body	140/70 mmHg (baseline value 125/80 mmHg)	Antihistamine p.o. and i.v., corticosteroid p.o., β2-agonist inh., histamine H_2-antagonist i.v.	Hospitalised for observation; discharged next day in recovered condition
50637-2[a]	0.2 mL vial B (6,000 SQ+)	50 min	Exanthema on head, neck and upper body	130/85 mmHg (baseline value 125/80 mmHg)	Antihistamine p.o, coticosteroid p.o.	Hospitalised for observation; discharged next day/ discontinued from the trial

i.m. intramuscular, *i.v.* intravenous, *p.o.* peroral, *s.c.* subcutaneous

[a] Assessed as serious

season. All were manageable with standard care. Treatment typically included corticosteroids (intravenous, intramuscular or peroral) and antihistamines, and the one event treated with adrenaline occurred in a placebo patient. Although it is difficult to compare safety profiles between different trials because of differences in safety reporting methodology, the pattern of AEs observed in this trial seems to be in line with what has been previously observed with other SCIT products. The tolerability profile was thus considered acceptable for continuing the clinical development of SQ+ grass SCIT to determine its potential efficacy.

The reported clinically insignificant trial results carry important information. First, immunological changes are necessary, but not a sufficient condition for predicting clinical effects of SCIT with grass pollen extracts. Second, in-field trials are mandatory for demonstration of efficacy of immunotherapy with allergens of seasonal exposure; however, the risk of low pollen exposure during the season can decrease the therapeutic window and lead to inconclusive or negative trial results. Last, considering the robust design of this trial, the inconclusive results indirectly strengthen the necessity of multi-site-based, randomized, placebo-controlled trials for the proper definition of clinical efficacy in AIT.

5 Conclusions

A 1-year trial did not reveal statistically significant differences between SQ+ grass SCIT and placebo with respect to clinical efficacy parameters despite numerical differences. Dose-dependent increases in IgG_4 and IgE-blocking factor were observed, with the changes being statistically significant ($p < 0.001$) for both 4,000 SQ+ and 15,000 SQ+. The most frequently reported treatment-related AEs were mild-to-moderate local injection-site reactions. The trial results were probably influenced by the low natural pollen exposure during the GPS. Other factors such as the extent of the pre-seasonal treatment could potentially have contributed. The tolerability profile was acceptable for further development.

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References

1. Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group; World Health Organization. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001;108:S147–S334.
2. White P, Smith H, Baker N, Davis W, Frew A. Symptom control in patients with hay fever in UK general practice: how well are we doing and is there a need for allergen immunotherapy? Clin Exp Allergy. 1998;28:266–70.
3. Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006;117:319–25.
4. Bousquet J, Lockey RF, Malling HJ; WHO Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998;53:1–42.
5. Rask C, Lund L, Lund G, Heydenreich B, Wurtzen P, Bellinghausen I, Saloga J, Lund K. An alternative allergen: adjuvant formulation potentiates the immunogenicity and reduces allergenicity of a novel subcutaneous immunotherapy product for treatment of grass-pollen allergy. Clin Exp Allergy. 2012;42:1356–68.
6. Pfaar O, Jung K, Wolf H, Decot E, Kleine-Tebbe J, Klimek L, Wustenberg E. Immunological effects and tolerability of a new fast updosed immunologically enhanced subcutaneous immunotherapy formulation with optimized allergen/adjuvant ratio. Allergy. 2012;67:630–7.
7. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. 2008 (adopted by the WMA General Assembly in Helsinki (1964) and as amended by the WMA General Assembly).
8. ICH. ICH harmonised tripartite guideline topic E6 (R1): guideline for good clinical practice. 1996.
9. ICH. ICH harmonised tripartite guideline topic E2A. Clinical safety data management: definitions and standards for expedited reporting. 1994.
10. Dahl R, Kapp A, Colombo G, de Monchy J, Rak S, Emminger W, Fernandez-Rivas M, Ribel M, Durham S. Efficacy and safety of

sublingual immunotherapy with grass allergen tablet for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006;118: 434–40.
11. Zenner HP, Baumgarten C, Rasp G, Fuchs T, Kunkel G, Hauswald B, Ring J, Effendy I, Behrendt W, Frosch PJ, Przybilla B, Brunner FX, Merk HF, Kapp A, Schnitker J, Wolf H. Short-term immunotherapy: a prospective, randomized, double-blind, placebo-controlled multicenter study of molecular standardized grass and rye allergens in patients with grass pollen-induced allergic rhinitis. J Allergy Clin Immunol. 1997;100:23–9.
12. Klimek L, Mewes T, Wolf H, Hansen I, Schnitker J, Mann WJ. The effects of short-term immunotherapy using molecular standardized grass and rye allergens compared with symptomatic drug treatment on rhinoconjunctivtis symptoms, skin sensitivity, and specific nasal reactivity. Otolaryngol Head Neck Surg. 2005;133:538–543.
13. Durham SR, Emminger W, Kapp A, De Monchy JG, Rak S, Scadding GK, Wurtzen PA, Andersen JS, Tholstrup B, Riis B, Dahl R. SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. J Allergy Clin Immunol. 2012;129: 717–25.
14. Durham S, Andersen J, Li Z, Nolte H, Bernstein D, Creticos P, Nelson H. The influence of pollen exposure on efficacy measurements in grass allergy immunotherapy trials. EAACI-WAO World Allergy and Asthma Congress; Milan, Italy, 25 June 2013.
15. Amar SM, Harbeck RJ, Sills M, Silveira LJ, O'Brien H, Nelson HS. Response to sublingual immunotherapy with grass pollen extract: monotherapy versus combination in a multiallergen extract. J Allergy Clin Immunol. 2009;124:150–6.
16. Canonica GW, Bousquet J, Casale T, Lockey RF, Baena-Cagnani CE, Pawankar R, Potter PC, Bousquet PJ, Cox LS, Durham SR, Nelson HS, Passalacqua G, Ryan DP, Brozek JL, Compalati E, Dahl R, Delgado L, van Wijk RG, Gower RG, Ledford DK, Filho NR, Valovirta EJ, Yusuf OM, Zuberbier T, Akhanda W, Almarales RC, Ansotegui IJ, Bonifazi F, Ceuppens J, Chivato T, Dimova D, Dumitrascu D, Fontana L, Katelaris CH, Kaulsay R, Kuna P, Larenas-Linnemann D, Manoussakis M, Nekam K, Nunes C, O'Hehir R, Olaguibel JM, Onder NB, Park JW, Priftanji A, Puy R, Sarmiento L, Scadding GK, Schmid-Grendelmeier P, Seberova E, Sepiashvili R, Solé D, Togias A, Tomino C, Toskala E, Beever HV, Vieths S. Sub-lingual immunotherapy: World Allergy Organization position paper 2009. World Allergy Organ J. 2009;2:233–81.
17. Day JH, Horak F, Briscoe MP, Canonica GW, Fineman SM, Krug N, Leynadier F, Lieberman P, Quirce S, Takenaka H, Van Cauwenberge P. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. Clin Exp Allergy Rev. 2006;6:31–59.
18. Calderon MA, Birk AO, Andersen JS, Durham SR. Prolonged preseasonal treatment phase with Grazax sublingual immunotherapy increases clinical efficacy. Allergy. 2007;62:958–61.
19. Shamji MH, Ljorring C, Francis JN, Calderon A, Larche M, Kimber I, Frew AJ, Ipsen H, Lund K, Wurtzen PA, Durham SR. Functional rather than immunoreactive levels of IgG(4) correlate closely with clinical response to grass pollen immunotherapy. Allergy. 2012;67:217–26.
20. Panizo C, Cimarra M, Gonzalez-Mancebo E, Vega A, Senent C, Martin S. In vivo and in vitro immunological changes induced by a short course of grass allergy immunotherapy tablets. J Investig Allergol Clin Immunol. 2010;20:454–62.
21. Bufe A, Eberle P, Tholstrup B, Henmar H, Durham S. Clinical efficacy and immunological response in children is similar to that of adults after the first treatment season with SQ-standardized grass allergy immunotherapy tablet in two randomized trials. Allergologie. 2013;36:238–45.