

Original article

Efficacy of grass pollen sublingual immunotherapy for three consecutive seasons and after cessation of treatment: the ECRIT study

Background: Data supporting a carry-over effect with sublingual immunotherapy (SLIT) are scarce. This randomized, double-blind, placebo-controlled study evaluated the efficacy, carry-over effect and safety of grass pollen SLIT using co-seasonal treatment.

Methods: Patients (7.9–64.7 years) with grass pollen allergy received ultra-rush titration with increasing doses (30, 90, 150 and 300 IR) of a 5-grass pollen mixture every 20 min at the start of the pollen seasons, followed by 300 IR daily until the end of the pollen seasons. A baseline season (no SLIT) was followed by three consecutive treatment seasons and one follow-up season. Symptoms, medication and adverse events were documented and specific immunoglobulin (Ig)E and IgG₄ measured.

Results: Data were analysed for 183 of the 213 randomized patients. Mean treatment duration varied between seasons (81.8–92.7 days). Combined scores (symptoms and medication) improved progressively across treatment seasons (up to 44.7% improvement for SLIT compared with baseline) and fluctuated between –11.3% and –14.8% for placebo ($P < 0.05$). Similar changes were observed for symptom scores, with a successive decrease of 39.7% (SLIT) and fluctuations between +13.6% and –1.51% for placebo ($P < 0.05$). Combined score ($P = 0.0508$) and symptom score improvements ($P = 0.0144$) with SLIT continued during follow up. Increases in specific IgG₄ observed in the first season were sustained for SLIT vs placebo throughout treatment ($P = 0.0001$). Titration and daily SLIT were well tolerated. No serious systemic or anaphylactic reactions were reported.

Conclusions: Seasonal SLIT with ultra-rush titration is well tolerated and effective from the first treatment season onwards. These data indicate a carry-over effect of seasonal SLIT.

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Key words: allergic rhinitis; carry-over effect; grass pollen extract; sublingual immunotherapy; ultra-rush titration.

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Accepted for publication 18 July 2008

There is strong evidence that sublingual immunotherapy (SLIT) represents an effective treatment for allergic rhinitis in adults (1). This has been corroborated by a meta-analysis of 22 controlled clinical trials, including 979 adult patients with allergic rhinitis, which demonstrated that SLIT was significantly more effective than placebo (2). Moreover, SLIT has been developed as a promising alternative to subcutaneous immunotherapy (SCIT) and studies have shown that it possesses a superior safety profile, inducing fewer adverse events (AEs) (3) while preserving clinically relevant immunological effects (4).

In general, specific immunotherapy (SIT) has additional properties not shared by pharmacological treatments, including efficacy during and long-lasting effect after SIT (5), possible prevention of new sensitizations (6) and a risk reduction with regard to the occurrence of

asthma in children with allergic rhinitis (7, 8). However, it has recently been pointed out that further studies are warranted to demonstrate the long-term effects of SLIT, particularly in the paediatric population (5, 9).

To date, SLIT has been well tolerated by the vast majority of patients in double-blind, placebo-controlled (DBPC) trials (2). The most commonly observed side-effects were local to the site of administration or gastrointestinal and were generally considered to be mild because they proved to be self-limiting or manageable by temporary dose reduction.

Sublingual immunotherapy is currently initiated during a comparatively long and complex build-up (titration) phase and it is recommended that treatment is continued for at least 3 years (10). These recommendations entail certain inconveniences for patients who only have symptoms for 3–4 months per year during the pollen season.

Furthermore, affected individuals often start treatment for allergic rhinitis shortly before the start of the pollen season so that the time available for initiation of SIT may be not sufficient to accommodate a long titration phase. Therefore, the need for modifying SLIT titration is currently being addressed, by simplifying the schedule of administration through shortening the titration phase (11) or compressing it into less than 2 h (12, 13).

Thus, it was the aim of this study to establish maintenance of efficacy over the treatment period in the Efficacy, safety and tolerability of Coseasonal ultra-Rush sublingual ImmunoTherapy study in subjects with grass pollen-induced allergic rhinoconjunctivitis over three seasons of treatment and carry-over effect during a follow-up period over another pollen season.

Materials and methods

Study design

This was a randomized, DBPC, parallel-group, multi-centre study in subjects with grass pollen allergic rhinitis.

Patients

Two-hundred and sixty-six patients (aged 7.9–64.7 years) with grass pollen allergic rhinoconjunctivitis were screened, of whom 11 were children. The diagnosis was based on clinical history (≥ 2 years of grass pollen allergy), confirmed by a positive skin prick test (wheal diameter ≥ 3 mm) (Stallergenes SA, France) and the presence of grass pollen-specific serum immunoglobulin (Ig) E reaching a level of at least 0.70 kU/l corresponding to class 2 in the utilized detection system (CAP[®] System; Phadia, Uppsala, Sweden). The skin prick test included 5-grass pollens (cocksfoot or orchard grass, *Dactylis glomerata*; meadow grass, *Poa pratensis*; perennial rye grass, *Lolium perenne*; sweet vernal grass, *Anthoxanthum odoratum*; and timothy grass, *Phleum pratense*). Patients had to suffer from persistent allergic rhinitis (> 4 weeks/year) with a severity requiring treatment by drugs recommended for patients with mild persistent rhinitis where SIT is also indicated (10). All subjects had required symptomatic treatment during the course of the preceding pollen season and had planned treatment for the first study season with either oral cetirizine, ocular or nasal azelastine, oral or ocular prednisolone, nasal beclomethasone or inhaled salbutamol.

Exclusion criteria were: perennial allergic rhinitis and/or perennial allergic asthma, a total serum IgE level > 2000 kU/l, chronic non-allergic rhinitis or sinusitis, atopic dermatitis, severe asthma (mild asthma grade 1–2 was accepted) (14), any immunotherapy in the previous 5 years and the usual contraindications to immunotherapy (15). House dust mite sensitivity was not an exclusion criterion.

All patients or their guardians gave their written informed consent to participate in the study, which was conducted in accordance with the International Conference on Harmonisation guidelines on Good Clinical Practice and approved by the local Ethics Committees and competent authorities.

Sublingual immunotherapy extracts and treatment

Of the screened patients, 213 were randomized (2 : 1) to receive either SLIT for the treatment period and placebo in the follow-up

period, or placebo during both the treatment and follow-up periods. All patients visited their study centre at the beginning and end of the first pollen season (baseline visit) to assess the use of symptomatic medication in the absence of SLIT. Starting date for treatment was the first day of the pollen season estimated by local pollen calendars. The evaluation period during the pollen seasons was defined as the period from the first day with relevant pollen exposure (≥ 100 pollen grains/m³) until the last day with at least 50 pollen grains/m³ plus 7 days.

During the next three pollen seasons, patients received study medication or placebo, as randomized. A mixture of pollen extracts of five grasses (cocksfoot or orchard, meadow, perennial rye, sweet vernal and timothy grasses; Stallergenes SA, France) was used at a concentration of 300 IR/ml (equivalent to 21 µg/ml of Phl p 5; *Phleum pratense* major allergen) and administered as sublingual drops. Rush titration was performed under close supervision. On day 1, patients took four increasing doses of SLIT, corresponding to 30, 90, 150 and 300 IR, at 20-min intervals (0, 20, 40 and 60 min), followed by a daily intake of 300 IR for the duration of the pollen season. In the event of a severe drug reaction (a reaction unacceptable to the patient), titration was interrupted and repeated the next day. If the reaction continued to be severe, the patient was considered to be a study drop out. These side-effects were considered as adverse drug reactions and were not documented separately as AEs. Rescue medications were offered to all patients.

Compliance was checked by counting the returned vials of SLIT.

The final pollen season, during which patients were not treated, was the follow-up period. All patients visited the study centre before and at the end of each respective treatment and follow-up pollen season.

Efficacy measurements

The primary efficacy measure was the baseline-adjusted sum score of combined symptom and rescue medication score respectively, which were calculated and time-adjusted for the three consecutive pollen seasons and the follow-up period.

During the pollen seasons, patients used daily diary cards to record symptoms, rescue medication use and side-effects. Symptoms of rhinitis (sneezing, rhinorrhoea and nasal blockage), conjunctivitis (itching, redness and tearing) and asthma (cough, dyspnoea and wheezing) were assessed by the patients using a visual analogue scale (VAS) and graded from 0 to 3 (0 = absent, 1 = mild, 2 = moderate and 3 = severe). The maximum score for symptoms alone was therefore 27. VAS scales were used for the combined and the total symptom score. The single symptoms (sneezing, nasal itching etc.) were measured by ordinal scores. Outside the pollen seasons, symptoms were assessed every 2 months by telephone.

Rescue medication use was scored as follows:

1 tablet of corticosteroid	= 6 points
1 tablet of antihistamine	= 4 points
1 puff of inhalant β -agonist	= 4 points
2 drops of corticosteroid eye drops	= 2 points
2 puffs of corticosteroid nasal spray	= 2 points
2 drops of antihistamine eye drops	= 1 point
2 puffs of antihistamine nasal spray	= 1 point

Medication score was calculated as the area under the curve of the daily medication scores for the duration of the pollen season. The combined score was calculated by adding the daily sum scores for medication intake and VAS symptoms.

Safety measurements

All AEs were reported, with a focus on the date of onset, occurrence, duration, intensity, action taken, outcome and relationship to the study drug. Gastrointestinal and buccal symptoms were graded as 1 = mild, 2 = moderate and 3 = severe. Systemic reactions were graded as 0 = no symptoms, 1 = unspecific symptoms, 2 = mild systemic symptoms, 3 = nonlife-threatening symptoms, 4 = anaphylactic shock. Side-effect symptoms, weekly frequency and intensity were documented by patients in their diaries.

Immunological measurements

Blood samples for determination of specific IgE and IgG₄ were taken before the start of treatment, after each of the treatment seasons and the follow-up season. Timothy-grass-specific IgE and IgG₄ antibodies were determined by CAP[®] (Pharmacia, Uppsala, Sweden). The 5-grass pollen mixture was used as the antigen. Sera were incubated as specified by the manufacturer and analysed on a Pharmacia ImmunoCAP[®] 250 measuring unit.

Statistical analysis

Clinical efficacy was analysed using the Wilcoxon rank-sum test, with a global α -level of 5%. Data are presented as mean $\pm$ SD and/or median values.

For secondary variables, descriptive statistics were calculated for all parameters at all time points. Both primary and secondary endpoint analyses were performed for the full analysis set (FAS), the intention-to-treat (ITT) and per protocol populations. All patients who received treatment, including those who did not complete the study, were included in the analysis.

Analysis of variance (ANOVA) was performed on the sum score. The ANOVA model included treatment, presence of concomitant allergic asthma, duration of rhinitis, duration of conjunctivitis, specific IgE, IgG₄, other immunological markers, wheal diameter in the prick test (100 IR) and data for the pollen count in the different regions as fixed effects.

The determination of sample size needed for this study was calculated, given that a difference of 10% between the two treatment

arms for the sum score was considered to be clinically relevant. A two group *t*-test with a 0.05 two-sided significance level had 80% power to detect an effect size of 0.500 when the sample sizes in the two groups were 96 and 48 (for a 2 : 1 randomization), respectively (a total sample size of 144).

Results

Study population

Two-hundred and thirteen patients were included in the overall analysis of efficacy for the three pollen seasons. Pollen seasons were of 2.5–3 months duration, depending on location of the study centre. Diaries for 145 patients were available at the end of the study and these patients were considered as the ITT population. The demographic characteristics of the ITT population included through follow up are shown in Table 1.

Because of missing diary data for some patients, the last available diary entry was used in a last observation carried forward procedure ('analysed as randomized') resulting in evaluable data for 183 patients (FAS population). Patient disposition is shown in Fig. 1.

Overall, patients had a low mean severity of allergic rhinoconjunctivitis. During the baseline season, the combined mean symptom and medication scores were 8.1 (± 6.6) for the treatment group compared with 5.8 (± 6.8) for the placebo group ($P = 0.012$), which suggests an imbalance in favour of placebo. Data entry errors, mistakes in patient allocation in the database and centre differences were excluded as accounting for the differences in baseline scores. In addition, audits failed to uncover evidence of accidental or deliberate unblinding during the study.

Patients began their treatment 13–28 days before the first day of the evaluation period of the treatment pollen seasons. Mean treatment duration varied by

Table 1. Demographic characteristics of the intention-to-treat population monitored through the follow-up period

	Total (<i>n</i> = 145)	Treatment (<i>n</i> = 99)	Placebo (<i>n</i> = 46)
Age (years), mean $\pm$ SD	33.3 $\pm$ 10.4	33.2 $\pm$ 11.0	33.7 $\pm$ 9.1
Height (cm), mean $\pm$ SD	173.7 $\pm$ 10.8	173.8 $\pm$ 11.9	173.5 $\pm$ 8.3
Weight (kg), mean $\pm$ SD	71.4 $\pm$ 15.4	71.8 $\pm$ 16.3	70.5 $\pm$ 13.4
BMI (kg/m ²), mean $\pm$ SD	23.4 $\pm$ 3.5	23.5 $\pm$ 3.8	23.3 $\pm$ 3.0
Gender (<i>n</i>)			
Male (%)	71 (49.0)	46 (46.5)	25 (54.4)
Female (%)	74 (51.0)	53 (53.5)	21 (45.7)
Smoking (<i>n</i>)			
Nonsmoker (%)	107 (73.8)	77 (77.8)	30 (65.2)
Occasional smoker (%)	23 (15.9)	18 (18.2)	5 (10.9)
Smoker (%)	15 (10.3)	4 (4.0)	11 (23.9)
Duration of rhinitis (mean; years)	12.8	12.9	12.6
Severity of rhinitis (symptoms scores $\pm$ SD)		4.85 $\pm$ 3.95	3.28 $\pm$ 3.39
Monosensitized patients (missing data = 16) (%)	66 (33.5)	45 (33.6)	21 (33.3)
Patients with concomitant asthma (%)	19 (13.1)	14 (14.1)	5 (10.9)
IgE (mean $\pm$ SD; kU/l)		13.35 $\pm$ 14.88	7.78 $\pm$ 8.89
IgG ₄ (mean $\pm$ SD; kU/l)		0.23 $\pm$ 0.17	0.17 $\pm$ 0.06

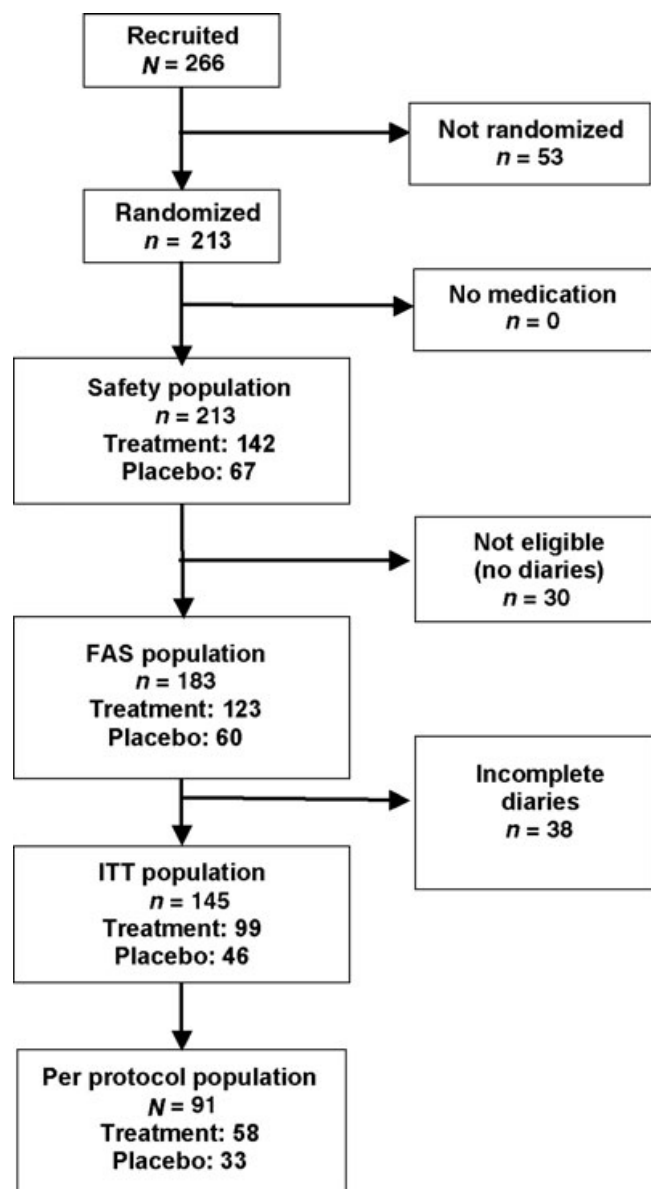


Figure 1. Study disposition.

season from 81.8 to 92.7 days. The mean cumulative dose of SLIT was approximately 22 000 IR per season (corresponding to 1500 µg of the group 5 major allergen).

Efficacy

Mean duration of SLIT was 87.7 days during the first treatment season. Compared with baseline values, the combined score (symptom and medication) decreased significantly with SLIT compared with placebo ($P = 0.043$) during the first season. The magnitude of efficacy was 33.9% for symptom score in favour of SLIT ($P = 0.0366$). The difference in intake of rescue medica-

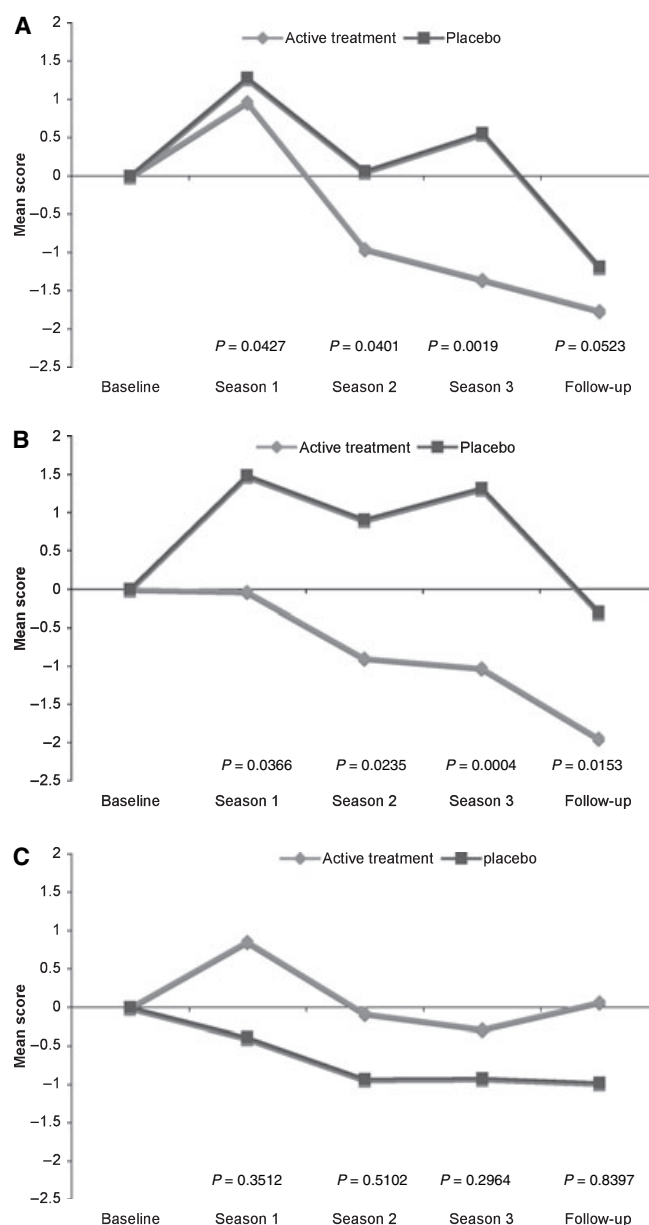


Figure 2. Changes in symptom and medication scores with sublingual immunotherapy and placebo over the course of the study (intention-to-treat population). (A) Symptom and medication scores. (B) Symptom score alone. (C) Medication score alone.

tion (−18% for SLIT) was not statistically significant ($P = 0.3512$).

Patients in the SLIT group had sequentially lower combined symptom and medication scores compared with placebo over the following two seasons and, importantly, in the follow-up period. By the third season, the median of the combined symptom and medication scores had decreased by −44.7% in the SLIT group and −14.7% in the placebo group compared with baseline values (Fig. 2A). The proportional decrease in combined

Table 2. Combined scores, and symptom and medication scores over the study period*

	SLIT				Placebo				P value
	n	Mean	SD	Median	n	Mean	SD	Median	
Combined score									
Season 1	116	0.97	13.84	-1.28	56	1.28	6.32	0.13	0.0427
Season 2	123	-0.95	14.21	-1.92	59	0.06	5.67	-0.74	0.0401
Season 3	123	-1.35	14.29	-2.32	60	0.56	6.05	0	0.0019
Follow up	124	-1.76	14.63	-2.70	60	-1.19	5.81	-1.15	0.0523
Symptom score									
Season 1	116	-0.03	4.19	-0.37	56	1.49	4.57	0.25	0.0366
Season 2	123	-0.89	4.37	-0.72	59	0.91	4.29	0	0.0235
Season 3	123	-1.02	4.54	-1.22	60	1.32	4.54	0.19	0.0004
Follow up	124	-1.94	5.05	-2.20	60	-0.30	4.40	-0.32	0.0153
Medication score									
Season 1	116	0.86	11.72	-0.54	56	-0.39	3.04	-0.12	0.3512
Season 2	123	-0.08	11.69	-0.62	59	-0.93	2.69	-0.24	0.5102
Season 3	123	-0.28	11.55	-0.88	60	-0.92	2.47	-0.22	0.2964
Follow up	124	0.07	11.69	-0.71	60	-0.98	2.61	-0.34	0.8397

*Lower scores represent fewer, less intense symptoms.

scores was calculated as follows: [(median at the given season - median at baseline)/baseline] × 100. Absolute values and standard deviations are shown in Table 2. The difference between the two treatment arms was statistically significant for each pollen season (season 1, $P = 0.043$; season 2, $P = 0.040$; season 3, $P = 0.0019$), and showed a trend towards a difference after treatment cessation ($P = 0.052$) (Table 2).

Change in the symptom score alone followed a similar pattern to the combined score (season 1, $P = 0.037$; season 2, $P = 0.024$; season 3, $P = 0.0004$) (Fig. 2B). Moreover, significance was also observed for this parameter in the follow-up period ($P = 0.015$) (Table 2). Standard deviation values for symptom score alone are shown in Table 2. Change in medication score alone is shown in Fig. 2C; standard deviation values are shown in Table 2.

When SLIT and placebo were compared, the differences between the third treatment period and baseline for conjunctivitis and rhinitis were statistically significant ($P = 0.0044$ and $P = 0.0060$, respectively).

Four patients (2.76%) terminated the study prematurely because of lack of efficacy: SLIT ($n = 3$), placebo ($n = 1$).

Immunological profile

An increase in specific IgG₄ compared with baseline was observed with SLIT in the first season (Table 3). In the following two treatment seasons, IgG₄ remained at a significantly higher level in SLIT-treated individuals compared with those taking placebo ($P < 0.0002$) and decreased during follow up.

Immunoglobulin E values increased markedly during the first treatment cycle with SLIT ($P < 0.0001$; Table 3). However, in contrast to IgG₄, these values

Table 3. Change in immunological parameters* over the study period

	SLIT ($n = 99$)		Placebo ($n = 46$)		P
	Mean	SD	Mean	SD	
IgE					
Season 1	15.54	22.64	0.22	2.01	<0.0001
Season 2	8.18	19.96	-0.87	4.48	<0.0001
Season 3	6.6	20.20	0.93	11.22	0.0113
Follow-up	5.74	16.88	2.02	9.78	0.2578
IgG ₄					
Season 1	0.38	1.18	0.01	0.06	0.0001
Season 2	0.38	0.98	0.01	0.06	0.0002
Season 3	0.54	1.55	0.01	0.06	<0.0001
Follow-up	0.02	0.30	-0.03	0.10	0.518

*Change in immunological parameter = (baseline value kU/l) - (treatment season value kU/l).

decreased thereafter during the following two seasons and during follow up. In the placebo group, IgE values remained almost constant throughout the study.

Mean serum concentrations of all immunological parameters returned to baseline values in the follow-up phase.

Compliance

Compliance was good. Patients took a mean value of 88% of SLIT treatment doses per day and 91% per administration.

Safety

The titration and maintenance phases of SLIT were well tolerated. No severe systemic or anaphylactic reactions were reported and no unexpected AEs were observed.

In total, 65.7% of all patients (140/213) in this study experienced a treatment-emergent AE; 98 (69.0%) of those treated with SLIT, 42 (62.7%) of those taking placebo. There was no significant difference in the number of patients reporting treatment-emergent AEs between the two treatment arms ($P = 0.50$).

Overall, 2.8% of the AEs (15 symptoms) were assessed as 'definitely' related to study medication; 10 AEs in the SLIT group (2.7%) and one in the placebo group (0.7%). Another 16 AEs (4.3%) were considered to be 'likely' related to study medication. The most common AEs, as coded by MedDRA, were nasopharyngitis, headache and bronchitis. Treatment-emergent local or systemic allergic reactions were reported in 38 patients (17.8%); 24 (16.9%) treated with SLIT and 12 (17.9%) taking placebo.

In total, 14 patients discontinued the study prematurely because of AEs. Four patients treated with SLIT withdrew from the study because of AEs assessed as 'definitely' or 'likely' related to study medication; these included upper abdominal pain, diarrhoea, application

Table 4. Adverse events and their frequency in the safety population ($n = 213$)

Adverse events	Safety population ($n = 213$)	SLIT ($n = 142$)	Placebo ($n = 67$)
Number of pat. with AE, n (%)	140 (65.7)	98 (69.0)	42 (62.7)
Number of AE episodes	511	356	147
Number of AEs according to MedDRA code	535	371	152
Causality assessment in relation to study medication, n (%)			
No relation	363 (67.9)	263 (70.9)	98 (64.5)
Unlikely	131 (24.5)	79 (21.3)	51 (33.6)
Likely	21 (3.9)	16 (4.3)	—
Definite	15 (2.8)	10 (2.7)	1 (0.7)
Missing	5 (0.9)	3 (0.8)	2 (1.3)
Causality assessment in relation to rescue medication, n (%)			
No relation	406 (75.9)	286 (77.1)	108 (71.1)
Unlikely	121 (22.6)	79 (21.3)	42 (27.6)
Likely	—	—	—
Definite	3 (0.6)	3 (0.8)	—
Missing	5 (0.9)	3 (0.8)	2 (1.3)
Nonsystemic reactions, n (%)			
Mild	214 (40.0)	146 (39.4)	66 (43.4)
Moderate	277 (51.8)	197 (53.1)	70 (46.1)
Severe	37 (6.9)	25 (6.7)	12 (7.9)
Missing	7 (1.3)	3 (0.8)	4 (2.6)

site irritation, eye swelling, application site oedema, application site paraesthesia and respiratory problems. The other symptoms were considered to be 'unlikely' or 'not related' to SLIT. A summary of AEs is shown in Table 4.

Overall, treatment-emergent serious AEs (SAEs) occurred in 16 patients (7.5%) during the study period (11 SLIT and five placebo). No treatment-emergent SAEs were considered to be related to study medication.

Ultra-rush titration was well tolerated. In two patients, titration was repeated the next day, with good tolerability. Seven patients stopped at the titration phase and withdrew from the study because of gastrointestinal symptoms that they had self-assessed as severe. The number of patients with adverse symptoms during titration decreased from 101 in the first year to 88 in the second and 59 in the third year of treatment in the SLIT group compared with 29, 19 and nine, respectively, in the placebo group. Overall, only nine systemic reactions (0.26%) were observed with SLIT and three (0.19%) with placebo during treatment. No systemic reaction occurred with SLIT vs three (0.36%) with placebo during follow up.

Most of the side-effects reported by patients during the study were local reactions, particularly mild-to-moderate local itching and burning in the oral cavity and tongue, followed by gastrointestinal reactions, unspecified reactions and systemic reactions. The number of local reactions decreased during the study in both treatment arms. No gastrointestinal symptoms and only sporadic unspecific symptoms were reported during follow up.

Discussion

During the past decade, substantial research has investigated the short-term efficacy and safety of SLIT in both paediatric and adult patients. However, reports of its long-term benefits remain scarce. The available long-term studies are either not randomized, placebo-controlled studies (16) or contain only small numbers of patients (17). Thus, we assessed the efficacy and safety of SLIT in children and adults with allergic rhinitis who were followed up over 5 years.

In this study, efficacy of a co-seasonal treatment was demonstrated over 3 years. Therefore, although it is optimal to begin treatment in advance of the pollen season, this study suggests that treatment of patients who present late (at or after the start of the pollen season) to their practitioner can benefit from co-seasonal treatment. Nevertheless, this investigation does not assess the optimal starting point and duration of this form of SLIT treatment. Future studies for the tablet formulation of a similar 5-grass pollen SLIT will address this issue.

Interestingly, we were able to demonstrate the efficacy of grass pollen SLIT from the first season onwards. Patients taking SLIT had sequentially lower combined symptom and medication scores over the three treatment seasons and showed a trend towards this clinical amelioration during the follow-up period, compared with placebo patients. Additionally, symptom scores for SLIT patients decreased progressively during treatment, whereas scores for the placebo group showed no change. In particular, the difference between the third treatment season and baseline was statistically significant (SLIT, -1.35 ; placebo, 0.56 ; $P = 0.0019$), with corresponding median percentage changes of -39.7% for SLIT and -1.5% for placebo. In a separate analysis of single symptom scores for rhinitis, conjunctivitis and asthma revealed a similar trend to the total symptom score. According to a recent comprehensive meta-analysis of SLIT trials, these differences for all treatment seasons can be considered as clinically relevant (18). Furthermore, our data agree with other randomized SLIT trials of similar study design, which also demonstrated SLIT efficacy with a significant mean reduction of symptom scores (2).

Likewise, intergroup analysis indicated a decrease of medication scores in both groups during the study (median percentage change from the third treatment period to baseline: -63.9% for SLIT and -58.8% for placebo). However, intake of additional anti-symptomatic medication was low during the study for both groups and, consequently, no significant differences were found.

The combined score and symptom score alone decreased in both groups in the follow-up period compared with baseline. We assume that the lower pollen exposure in the respective seasons (mean pollen count 3573 pollen grains/ m^3 in the baseline season vs 2880

pollen grains/m³ in the follow-up season) contributed to this effect. However, despite the low scores, the difference between treatment and placebo combined scores showed a trend towards statistical significance ($P = 0.052$). The difference in the symptom score was significant ($P = 0.015$).

In this study, the baseline symptom and medication scores were low and, therefore, improvement was limited. Nevertheless, an analysis that stratified all patients according to their baseline disease severity was performed as an additional sensitivity analysis to exclude a regression-to-the-mean effect for the patients receiving active treatment. Those receiving SLIT in the highest quintile for disease severity showed an improvement in the combined score and in the symptom score whereas no change was seen in the placebo group. Thus, a regression to the mean could be excluded (data not shown).

Although there is an urgent need for paediatric studies, because of the small number of children recruited to this study (11 children), this study is unable to provide further insight into the treatment of this age group beyond that provided by existing studies (19, 20).

Following ultra-rush titration, co-seasonal administration of the maintenance dose over 3 years was well tolerated by the children and adults included in our study. The most frequently observed AEs during the titration and maintenance phases were mild-to-moderate local itching and burning in the buccal cavity. Gastrointestinal symptoms were rare. No severe systemic or anaphylactic AEs and no SAEs related to study medication or unexpected suspected AEs were observed. These results are in accordance with earlier DBPC studies where the incidence of AEs varied between 17% and 60% among SLIT-treated patients, and 8% and 14% within the respective placebo group. Systemic reactions, the majority of which were respiratory symptoms, occurred in 17% and 12% of patients respectively (5). An extensive review of the literature revealed only 17 SAEs during SLIT consisting mainly of asthma exacerbations (21), while only one life-threatening anaphylactic reaction after SLIT with latex allergens has been substantiated by a physician so far (22) and no fatal events have been reported.

Immunoglobulin E and IgG₄ remained constant over the 3-year treatment in the placebo group, whereas they both increased markedly in the first season of SLIT and IgE decreased thereafter. IgE and IgG₄ returned to baseline levels during follow up in the SLIT group. These data indicate that different immune responses contribute to the carry-over effect for high-dose SLIT when compared with SCIT and further studies are needed to evaluate this effect. Similar immunological changes in the first treatment year have been observed in another SLIT study with grass pollen mixtures (23) and in trials evaluating SIT (24, 25). SLIT treatment of

house dust mite-allergic patients also resulted in a sustained IgG₄ elevation within 6 months and throughout the 2-year treatment period (26), which indicates that the allergen extract used was sufficiently immunogenic. SLIT has been shown to increase IL-10 levels in peripheral blood monocytes (27). As an early marker of a therapeutic efficacy, elevated IL-10 levels could be related to activation of regulatory T cells in the mucosa of the upper respiratory tract stimulating B cells to produce immunoglobulin A (IgA). While IgA levels were not assessed in our study, this may be considered for future studies because the presented SLIT regimen represents a mucosal immunization strategy. In addition, only antibodies to timothy grass pollen major allergen phl p 5 were measured in this investigation. However, in order to establish a more comprehensive antigen recognition pattern of included patients, it may have been useful to additionally measure the respective antibodies specific for the other four allergens employed during SLIT treatment.

In conclusion, SLIT with a standardized grass pollen extract was well tolerated, even when administered during pollen exposure. The safety profile of seasonal treatment with ultra-rush titration was comparable to that of standard SLIT, as shown in meta-analyses (2, 28). The next step may be to administer SLIT without a titration phase, bearing in mind that another study reported no difference between immediate treatment (without titration) and titrated treatment in terms of AEs (29).

This study shows that the seasonal treatment concept is efficacious from the first treatment season onwards. The follow-up results are indicative of a carry-over effect of seasonal SLIT, although significance was not achieved, which may have been affected by the low pollen count in the follow-up season. The safety profiles for seasonal high-dose SLIT with ultra-rush titration and conventional SLIT are similar. Efficacy and carry-over effects of seasonal SLIT are evident in this proof-of-concept study, but the results now need to be confirmed in a larger randomized, controlled trial. Our data are applicable to an adult population and further studies are needed to confirm the effect in children.

Acknowledgments

We thank the investigators who made this study possible: Udo Amann (Lingen), Michael Bohndorf (Kamp-Lintfort), Wolfgang Czech (Villingen-Schwenningen), Hans Engelke (Husum), Frank Friedrichs (Aachen), Markus Grewe (Düsseldorf), Heinke Hardung (Kiel), Bettina Hauswald (Dresden), Hans-Jürgen Hessler (Rendsburg), Nicolas Hunzelmann (Köln), Irene Kaufmann (Kerpen), Walter Trettel (Preetz), Irene Tausch (Preetz), Holger Weiss (Kiel).

This study was sponsored by Stallergenes GmbH, Germany.

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