ORIGINAL ARTICLE

In Vivo and In Vitro Immunological Changes Induced by a Short Course of Grass Allergy Immunotherapy Tablets

C Panizo,[1] M Cimarra,[2] E González-Mancebo,[3] A Vega,[4] C Senent,[5] S Martín[6]

[1]Hospital Nuestra Señora del Prado, Talavera de la Reina, Spain
[2]Hospital Clínico San Carlos, Madrid, Spain
[3]Hospital Fuenlabrada, Madrid, Spain
[4]Hospital Universitario de Guadalajara, Guadalajara, Spain
[5]Hospital Virgen del Valle, Toledo, Spain
[6]ALK-Abelló S. A., Madrid, Spain

Abstract

Background: The fast-dissolving grass allergy immunotherapy tablet (grass AIT), Grazax, has proven effective in grass pollen–induced rhinoconjunctivitis.
Objective: To investigate the immunological and cutaneous changes induced after a short course with grass AIT.
Methods: We performed a randomized, double-blind placebo-controlled trial with 78 patients randomly assigned to receive either grass AIT or placebo in a 2:1 ratio. Treatment lasted at least 8 weeks before the grass pollen season (GPS), and continued until the season finished. Specific immunoglobulin (Ig) G4, IgE, and IgE-blocking factor to *Phleum pratense* were measured at the beginning of the trial and at different intervals during treatment. Immediate and delayed skin tests with *P pratense* were also performed. Safety endpoints were defined in terms of adverse events reported.
Results: A total of 75 patients completed the trial (50 active and 25 placebo). *P pratense* IgG4, IgE, and IgE-blocking factor in actively treated patients increased significantly from baseline to the start of the GPS compared to placebo (P>.001, P=.017, and P=.005, respectively). The immediate cutaneous response was reduced during therapy in actively treated subjects, whereas placebo-treated subjects showed a decrease only after the start of the GPS. The delayed response to the intradermal test in grass AIT–treated subjects diminished, although not in a significantly different way from the placebo-treated subjects.
Conclusion: Treatment with grass AIT for grass pollen allergic rhinoconjunctivitis induces immunological changes after only 1 month of treatment.

Key words: Grass pollen allergy. Grass allergy immunotherapy tablet. Immunological changes. Sublingual immunotherapy.

Resumen

Antecedentes: La inmunoterapia con liofilizados orales de rápida disolución de gramíneas (Grazax) ha mostrado su eficacia en el tratamiento de la rinitis inducida por polen de gramíneas.
Objetivo: Investigar los cambios inmunológicos y cutáneos inducidos tras la inmunoterapia de corta duración con Grazax.
Métodos: Estudio clínico doble ciego controlado con placebo en el que se incluyeron 78 sujetos aleatorizados 2:1 para recibir Grazax o placebo. Los sujetos fueron tratados durante al menos 8 semanas antes de la época de polinización de gramíneas (EPG) continuando posteriormente hasta su finalización. Se midieron los niveles de IgG4, IgE y factor bloqueante de la IgE específicos de *P. pratense* antes y durante el tratamiento. Asimismo, se realizaron pruebas cutáneas inmediatas y tardías. La evaluación de la seguridad se basó en la recogida de acontecimientos adversos.
Resultados: 75 sujetos completaron el ensayo (50 en tratamiento activo y 25 placebo). Los niveles de IgG_4, IgE y del factor bloqueante de IgE frente a *P. pratense* en el grupo activo aumentaron significativamente respecto al placebo desde el tiempo basal hasta el comienzo de la EPG (P>0.001, P=0.017 y P=0.005, respectivamente). La respuesta cutánea inmediata se redujo durante el tratamiento en los sujetos con tratamiento activo mientras que en el grupo placebo disminuyó sólo después de comenzar la EPG. La respuesta cutánea tardía disminuyó en el grupo activo aunque no de forma significativa respecto al grupo placebo.
Conclusión: El tratamiento con Grazax de la rinoconjuntivitis alérgica al polen de gramíneas induce cambios inmunológicos ya desde el primer mes de tratamiento.

Palabras clave: Alergia al polen de gramíneas. Liofilizados orales de gramíneas. Cambios inmunológicos. Inmunoterapia sublingual.

Introduction

Treatment of allergic rhinitis is based on patient education, allergen avoidance, use of symptomatic medication, and allergen-specific immunotherapy. While most of these alternatives help to control symptoms, immunotherapy is the only treatment that has the potential to alter the natural course of the disease [1]. Immunotherapy is usually administered as injections of small doses of allergen extract in an attempt to prevent allergic reaction by desensitizing the immune system. Although high specific immunoglobulin (Ig) G titers in serum may not directly correlate with the development of the disease, induction of blocking antibodies such as IgG is important for successful allergen immunotherapy [2]. Sublingual immunotherapy for grass pollen–induced seasonal rhinoconjunctivitis has made treatment available to a broader group of individuals. However, its efficacy for inducing changes in specific serum antibodies remains controversial.

Grazax (ALK-Abelló, Hørsholm, Denmark) is a newly developed rapidly dissolving SQ-standardized grass allergy immunotherapy tablet (AIT) that can be administered at home for the treatment of allergic rhinoconjunctivitis in patients with proven grass pollen allergy. It is an efficacious and well-tolerated treatment option that reduces symptom and medication scores and increases quality of life, as demonstrated in several randomized, double-blind, placebo-controlled studies in adults and children [3-9]. Recent pharmacoeconomic analyses have also found it to be a cost-effective intervention for the treatment of grass pollen–induced rhinoconjunctivitis in Northern [10] and Southern [11] European countries.

The aim of this trial was to investigate the ability of AITs to induce cutaneous and immunological changes in patients with grass allergy. The kinetics of these changes and patient tolerance were also assessed.

Methods

We performed a multicenter, randomized, double-blind, placebo-controlled trial between January and August 2007 with the participation of 5 allergy departments at hospitals in Central Spain. The trial was reviewed and approved by the competent authorities and ethics committees and was conducted according to the Declaration of Helsinki.

The inclusion criteria were as follows: man or woman aged 18 to 65 years; clinical history of rhinitis (with or without concurrent asthma) caused by grass pollen for at least 1 year prior to trial entry; positive skin prick test (SPT, ALK-Abelló, S.A., Madrid, Spain) response to *Phleum pratense* 20 µg/mL; positive IgE against grass pollen (CAP class ≥2); negative pregnancy test for women of childbearing potential; and written informed consent and willingness to follow the trial protocol. The main exclusion criteria were as follows: having received immunotherapy with grass pollen in the last 5 years; presence of clinically relevant features in the physical examination; severe or uncontrolled asthma (forced expiratory volume in 1 second [FEV_1] <70% of predicted); and previous anti-IgE therapy.

Seventy-eight patients were randomly assigned to receive either grass AIT (Grazax 75 000 SQ-T) or placebo in a 2:1 ratio (Figure 1). Patients received treatment for at least 8 weeks before the grass pollen season and continued throughout the season. Patients visited the clinic at selection (visit 1, January to February), before the season (visit 3, April to May), during the season (visit 4, 1 month later), and at the end of the study (visit 5, after the season). Extra appointments were required for blood extractions to guarantee a monthly serum sample (visits 2a, 2b). Blood samples were obtained at all visits. Skin tests for immediate and delayed cutaneous response were performed at visits 1, 3, and 5 with a *Phleum pratense* allergen extract at 100, 20, 4, and 0.8 µg/mL of Phl p 5 for immediate SPT tests and by intradermal injection of 0.02 mL of a 0.1 µg/mL Phl p 5 solution. During visit 1, participants were informed about the trial and gave their informed consent. They received the first dose of treatment in the hospital and stayed in the clinic for at least 30 minutes. Afterwards, the grass AIT was self-administered at home. Patients were instructed to return all unused medication to the investigators, and adherence was assessed by counting the number of tablets returned. The reference therapy was placebo, which was also presented as an oral lyophilisate that was similar in taste, smell, and physical appearance to grass AIT, but without the active agent. Tablets had to be taken in the morning and left under the tongue without swallowing for at least 1 minute. Patients were advised not to drink or eat during the following 5 minutes. Therapies with antihistamines, corticosteroids, xanthines, or monoamine oxidase inhibitors had to be avoided before SPT, according to usual practice.

The primary efficacy endpoint was the difference in serum levels of specific IgG4 between the active and placebo groups with regard to the change in *P pratense* levels from the beginning of the trial to the start of the grass pollen season. Sample size estimation was based on a previous trial in which a mean (SD) difference of 0.0003 (0.0004) in IgG4 levels was

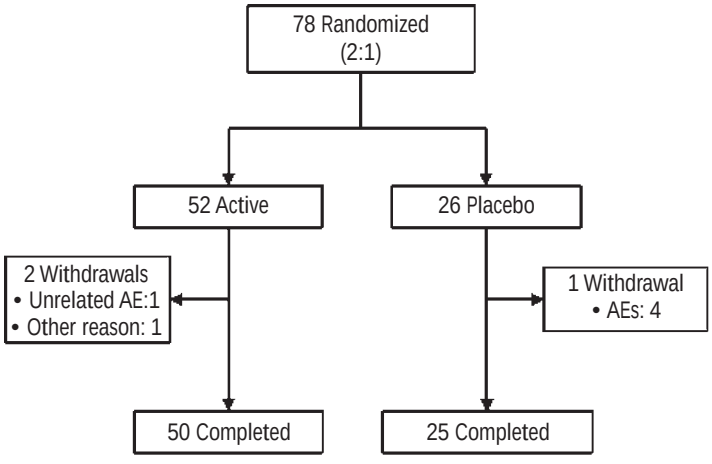

Figure 1. Patient flow chart. AE indicates adverse event.

observed in the actively treated patients and 0 (0.0002) in the placebo. With a significance level of 5%, a power of 90%, and a dropout rate of 35%, the estimated sample size was 50 patients in the active group and 25 in the placebo group. Secondary efficacy endpoints were as follows: progress of and differences between treatment groups in *P pratense*–specific IgG4, IgE, and IgE-blocking factor and in IgG4 to Phl p 1, Phl p 5, and Phl p 12 major allergens; and differences between groups in the immediate and delayed skin reactivity to *P pratense*. Safety endpoints were defined in terms of adverse events reported.

Serum sample analysis was as follows: IgG4 to *P pratense* samples was analyzed at Universidad del País Vasco (Spain) using UniCap 100 (Phadia, Uppsala, Sweden); IgE and IgE-blocking factor to *P pratense* and IgG4 to *P pratense* major allergens were analyzed at the research and development department of ALK-Abelló, S.A. using an ADVIA Centaur (IgE and IgE-blocking factor [5,12]) and monoclonal antibody–based enzyme-linked immunosorbent assay (IgG4) [13].

Values for serum specific IgG4, IgE, and SPT were transformed logarithmically for normalization. The change in serum *P pratense*–specific IgG4 values at selection and preseasonal visits (visit 1 and visit 3) was the primary efficacy endpoint. The *t* test was used to estimate differences between groups. Changes in immediate skin response were analyzed using the parallel line assay (AIASA CRS PLA, ALK-Abelló, S.A., Madrid, Spain) [14] and expressed as the cutaneous tolerance index (CTI), which is the ratio of the allergen extract concentrations provoking the same skin response. The CTI was calculated with its confidence interval for each visit, as well as for the difference between groups at each visit. Intradermal tests (delayed skin response) and specific immunoglobulins (IgE, IgE-blocking factor, and IgG4 to *P pratense* allergens) were analyzed using a repeated-measures ANOVA. The effect of a within-subjects factor (time), between-patients factor (treatment group), and their interaction were analyzed. All tests were 2-sided and *P* values lower than .05 were considered significant. A per protocol analysis (all patients who were randomized, exposed to trial medication, and who completed the trial) was conducted. Data obtained from visit 2b were not included in the final analysis due to the small number of patients who attended this extra visit.

Results

Seventy-eight patients were randomized 2:1 to receive either active treatment (52 participants) or placebo (26 participants) (Figure 1); 75 patients completed the trial (50 in the active group and 25 in the placebo group). Demographic baseline characteristics and measurements were similar between the 2 groups (Table 1). Three patients withdrew from the trial: 2 in the active group (1 due to an unrelated adverse event and 1 for a reason not related to the trial) and 1 in the placebo group, due to 4 simultaneous adverse events.

Patients treated with the SQ-standardized grass AIT experienced a significant increase in their IgG4 levels to the *P pratense* extract (*P*<.001, paired *t* test) from visit 1 (baseline) to visit 3 (preseason). In contrast, the placebo group did not show significant changes. The comparison of changes between the active treatment group and the placebo group was highly significant (*P*<.001, Table 2). Analysis of the IgG4 levels during treatment showed that the active treatment group behaved differently from the placebo group during the study (Figure 2A). While actively treated patients experienced a constant and significant increase from visit 1 to visit 5, the placebo group had similar IgG4 levels until visit 3, after which they increased significantly until visits 4 and 5.

Table 1. Demographic Data and Baseline Characteristics

		Placebo		Grass AIT	
		N	%	N	%
Number of participants		26	33.3	52	66.7
Sex	Male	13	50.0	19	36.5
	Female	13	50.0	33	63.5
Ethnic origin	Caucasian	21	80.8	49	94.2
	Hispanic	4	15.4	3	5.8
	Other	1	3.8	0	0.0
Smoker habit	Nonsmoker	13	50.0	37	71.2
	Smoker	6	23.1	7	13.5
	Exsmoker	7	26.9	8	15.4
Age, y, mean (SD)		32.0 (7.3)		30.8 (8.8)	
Height, cm, mean (SD)		169.5 (8.9)		167.0 (11.1)	
Weight, kg, mean (SD)		73.6 (15.5)		68.7 (13.7)	
IgE *Phleum pratense*, kU_A/L, GM (95% CI)		16.9 (8.8-32.7)		23.0 (15.0-35.5)	
IgG4 *Phleum pratense*, mg_A/L, GM (95% CI)		0.14 (0.09-0.22)		0.20 (0.15-0.28)	
SPT, mm^2, GM (95% CI)[a]		54.7 (44.4-67.4)		49.3 (42.1-57.8)	

Abbreviations: CI, confidence interval; GM, geometric mean; Ig, immunoglobulin.
[a] At the dose of 20 µg/mL of Phl p 5

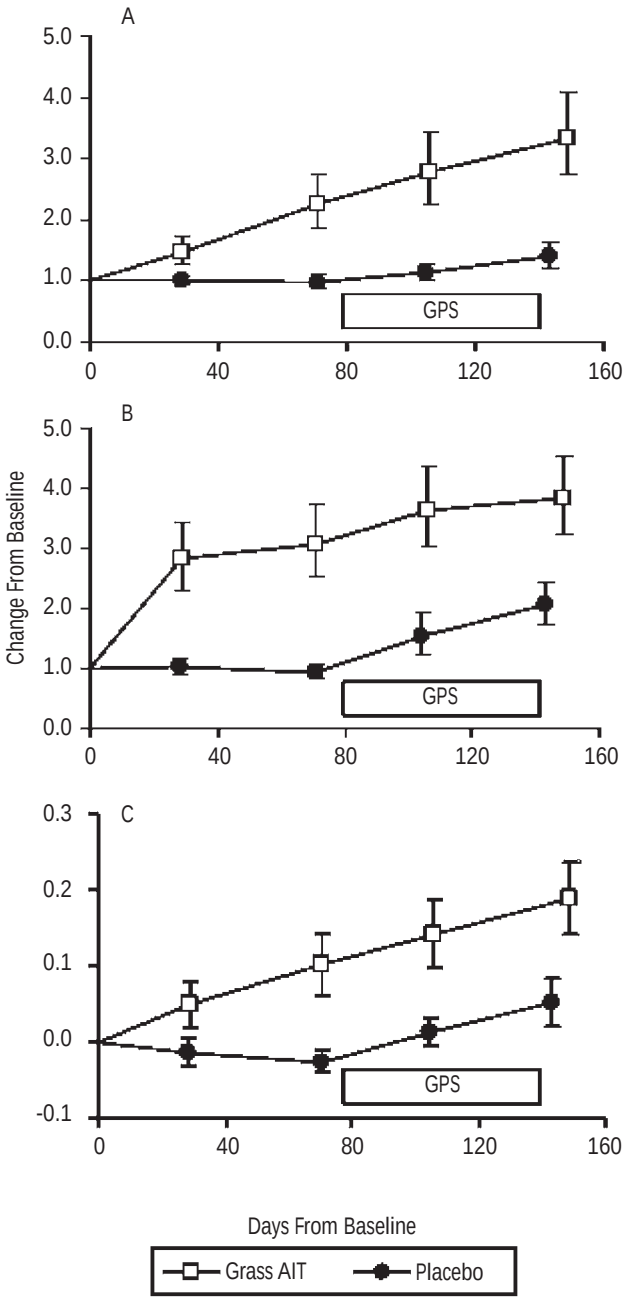

Figure 2. Change from baseline in *Phleum pratense*–specific Ig. A, IgG4; B, IgE; C, IgE-blocking factor. Data correspond to the ratio (A and B) or difference (C) of the values at each visit compared with baseline, with the respective 95% confidence intervals. GPS, approximate location of grass pollen season. AIT, indicates allergy immunotherapy tablet; Ig, immunoglobulin.

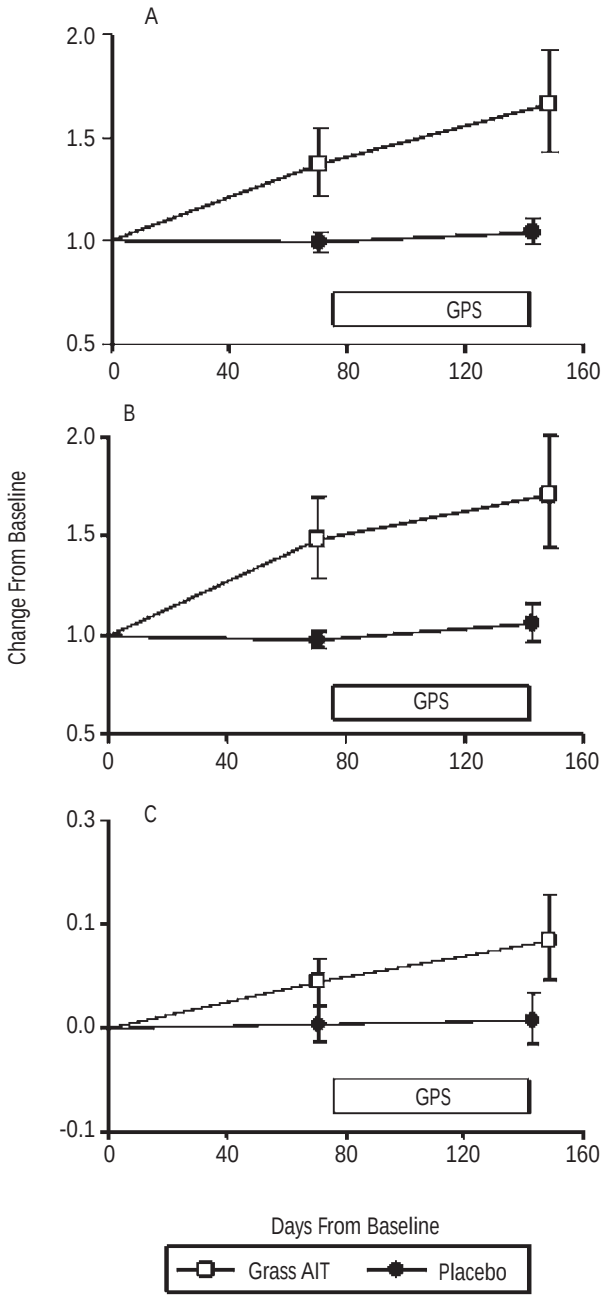

Figure 3. Change from baseline in specific IgG4 to *Phleum pratense* major allergens: A, Phl p 1; B, Phl p 5; C, Phl p 12. Data correspond to the ratio of the values at each visit compared with baseline, with the respective 95% confidence intervals. GPS, approximate location of grass pollen season.

Table 2. Effect of Immunotherapy With Grass AIT on In Vitro Parameters

		Visit 1 (Baseline)	Visit 2a (After 1 month)	Visit 3 (Preseason)	Visit 4 (Intraseason)	Visit 5 (End of Season)	Overall *P* Value[a]
IgE	Grass AIT						
	GM (95% CI)	18.3 (11.1-30.2)	51.1 (31.8-82.3)	56.5 (35.6-89.8)	66.8 (43.1-103.7)	70.2 (45.5-108.3)	*P*<.001
	Within groups *P* value	–	*P*<.001	*P*<.001	*P*<.001	*P*<.001	
	Placebo						
	GM (95% CI)	15.6 (7.1-34.2)	15.7 (7.1-34.6)	14.3 (6.7-30.6)	23.9 (10.9-52.7)	32.8 (15.3-70.3)	
	Within groups *P* value	–	*P*=.915	*P*=.210	*P*=.001	*P*<.001	*P*<.001
	Between groups *P* value	–	*P*<.001	*P*<.001	*P*<.001	*P*<.001	*P*<.001
IgG4b	Grass AIT						
	GM (95% CI)	0.21 (0.16-0.29)	0.31 (0.23-0.43)	0.48 (0.35-0.68)	0.61 (0.44-0.86)	0.71 (0.50-1.00)	*P*<.001
	Within groups *P* value	–	*P*<.001	*P*<.001	*P*<.001	*P*<.001	
	Placebo						
	GM (95% CI)	0.16 (0.10-0.24)	0.16 (0.11-0.24)	0.15 (0.10-0.23)	0.18 (0.12-0.27)	0.21 (0.14-0.32)	
	Within groups P value	–	*P*=.666	*P*=.663	*P*=.028	*P*<.001	*P*<.001
	Between groups P value	–	*P*=.003	*P*<.001	*P*<.001	*P*<.001	*P*<.001
IgG4 Phl p 1	Grass AIT						
	GM (95% CI)	0.11 (0.08-0.15)		0.15 (0.11-0.21)		0.19 (0.13-0.28)	
	Within groups *P* value	–		*P*<.001		*P*<.001	*P*<.001
	Placebo						
	GM (95% CI)	0.12 (0.08-0.16)		0.12 (0.08-0.16)		0.12 (0.09-0.17)	
	Within groups *P* value	–		*P*=.797		*P*=.159	*P*=.180
	Between groups P value	–		*P*=.001		*P*<.001	*P*<.001
IgG4 Phl p 5	Grass AIT						
	GM (95% CI)	0.32 (0.26-0.40)		0.46 (0.35-0.61)		0.55 (0.41-0.75)	
	Within groups *P* value	–		*P*<.001		*P*<.001	*P*<.001
	Placebo						
	GM (95% CI)	0.34 (0.27-0.42)		0.33 (0.26-0.41)		0.35 (0.28-0.46)	
	Within groups *P* value	–		*P*=.256		*P*=.233	*P*=.128
	Between groups *P* value	–		*P*<.001		*P*<.001	*P*<.001
IgG4 Phl p 12	Grass AIT						
	GM (95% CI)	0.09 (0.07-0.12)		0.11 (0.08-0.15)		0.13 (0.09-0.18)	
	Within groups P value	–		*P*<.001		*P*<.001	*P*<.001
	Placebo						
	GM (95% CI)	0.11 (0.07-0.17)		0.11 (0.07-0.17)		0.12 (0.07-0.18)	
	Within groups *P* value	–		*P*=.766		*P*=.539	*P*=.610
	Between groups *P* value	–		*P*=.018		*P*=.006	*P*=.006
IgE blocking factor	Grass AIT						
	Mean (95% CI)	–0.03 (–0.08 to 0.02)	0.02 (–0.03 to 0.06)	0.07 (0.02-0.12)	0.11 (0.05-0.16)	0.16 0.16 (0.10-0.21)	
	Within groups *P* value	–	*P*=.004	*P*<.001	*P*<.001	*P*<.001	*P*<.001
	Placebo						
	Mean (95% C.I.)	–0.12 (–0.23 to 0.02)	–0.12 (–0.23 to 0.02)	–0.13 (–0.24 to 0.03)	–0.10 (–0.21 to 0.00)	–0.06 (–0.16 to 0.02)	
	Within groups *P* value	–	*P*=.286	*P*=.005	*P*=.181	*P*=.007	*P*=.001
	Between groups *P* value	–	*P*=.018	*P*<.001	*P*<.001	*P*<.001	*P*<.001

Abbreviations: CI, confidence interval; GM, geometric mean; Ig, immunoglobulin.

[a]*P* values correspond to an analysis of variance for repeated measures. At each visit, the significance of the change compared with visit 1 is shown. The between-group *P* value corresponds to interaction of time (visits) and treatment group.

[b]IgG4 for major allergens were performed only at visit 1, visit 3, and visit 5.

Table 3. Summary of Treatment-Emergent Adverse Events

	Grass AIT			Placebo		
	N	(%)	Events	N	(%)	Events
Number of patients	52			26		
All adverse events	45	(86.5)	235	19	(73.1)	70
Causality						
Probably related	30	(57.7)	26	2	(7.7)	12
Possibly related	13	(25.0)	109	7	(26.9)	4
Unlikely to be related	37	(71.2)	100	18	(69.2)	54
Severity						
Mild	45	(86.5)	220	17	(65.4)	63
Moderate	6	(11.5)	15	5	(19.2)	7
Severe	0	(0.0)	0	0	(0.0)	0

Abbreviations: AIT, allergy immunotherapy tablet.

The changes observed at these later visits in the placebo group were similar to those in the active treatment group. There was a significant differential response in *P pratense*–specific IgE levels between the groups: in the active group, an increase in IgE levels was observed until visit 4. In the placebo group, IgE levels remained unchanged until visit 3, only to increase after the start of the grass pollen season (Figure 2B and Table 2). A sustained increase in IgE-blocking factor was observed in the active group from the first to the last visit, and this was already significant at the first month of treatment. In the placebo group, IgE-blocking factor remained constant until the start of the grass pollen season. After this, a significant increase was observed (Figure 2C).

IgG4 specific for *P pratense* major allergens (Phl p 1, Phl p 5, and Phl p 12) was also analyzed. The active group showed a statistically significant increase from visit 1 to visit 3 and visit 5 in IgG4 for the 3 allergens ($P<.001$), while in the placebo group the levels remained unchanged (Figure 3). Changes in patients on active treatment were significantly different from those observed in the placebo group. Table 2 shows the average values of in vitro parameters at each visit and the statistical significance of the changes as measured by repeated-measures ANOVA.

A statistically significant reduction was observed for the active group in their immediate cutaneous response, as shown by a CTI of 1.81 (95% CI, 1.4-2.4; $P<.05$) from baseline to visit 3 (preseason) and 3.0 (95% CI, 2.1-4.2; $P<.05$) to visit 5. Placebo-treated patients showed a decrease in their immediate cutaneous response only after the start of the grass pollen season (CTI=2.06; 95% CI, 1.4-3.0; $P<.05$ baseline to postseason). The delayed cutaneous response was significantly reduced from visit 3 to visit 5 (from 38.9 to 32.9 mm in mean diameter, $P=.004$, within-subjects factor) in the active group although this reduction was not significantly different from that of the placebo group.

A total of 305 adverse events were reported: 235 in the active treatment group and 70 in the placebo group. More than 90% were mild, and none were severe or serious. In the active group, 135 adverse events (57.4%) were considered related to treatment; 16 adverse events (22.9%) were considered related to treatment in the placebo group. The number of treatment-emergent adverse events is summarized in Table 3 by treatment group and association with treatment. Respiratory, gastrointestinal, nervous system, and ocular disorders, as well as general and injection site effects were the most frequently reported treatment-related adverse events.

Discussion

The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines recommend the use of allergen-specific immunotherapy in patients with demonstrable IgE-mediated diseases and long-term symptoms, or in patients for whom pharmacotherapy has been partially or totally ineffective or has induced side effects [1]. Grazax is a rapidly dissolving grass AIT that provides grass pollen–allergic patients with a clear clinical benefit throughout the grass pollen season after a pretreatment period of only 8 weeks [9]. Efficacy increases with the duration of preseasonal treatment [15], provides relief for individual rhinoconjunctivitis symptoms [6], and is sustained after 2 [4] and 3 years [16] of treatment, and even 1 year after cessation [17], indicating a persistent positive effect on the immune system.

The association between immunologic changes and the efficacy of immunotherapy is not completely understood, although the success of immunotherapy depends to a large extent on the following factors: *a)* the increase in specific IgG serum levels and inhibitory activity of the IgE-facilitated allergen-presentation pathway [18]; *b)* the initial increase in specific IgE levels followed by a gradual decrease [19] and a reduction in the seasonal increase in specific IgE levels [20]; *c)* the reduction in the release of mediators, such as histamine, from basophils and mast cells, which is relevant in the immediate phase of allergic reactions; and *d)* the suppression of late-phase inflammatory responses in the skin and respiratory tract blocking both the immediate and late-phase allergic response [21]. Although there is prior evidence

that treatment with the SQ-standardized grass AIT induces immunological changes [22,23], this is the first trial aimed specifically at demonstrating this effect on in vivo and in vitro immunological markers and on major *P pratense* allergens after short preseason treatment and throughout the pollen season.

The level of IgG4 was selected as the primary trial endpoint, because available evidence suggests that it may constitute an initial marker in the development of tolerance to allergens [24]. Although there is evidence suggesting that increases in allergen-specific IgG antibody titers may not predict the duration of the efficacy of immunotherapy, it is hypothesized that alterations in IgG specificity with immunotherapy might be crucial to clinical efficacy [25].

Secondary trial in vitro endpoints such as IgE and IgE-blocking factor to the whole *P pratense* extract and IgG4 to *P pratense* major allergens would give a fuller picture of the activity of immunotherapy and of the development of sustained efficacy [26,27]. Immediate skin response was selected as an in vivo secondary endpoint because of its close correlation to the immunological changes occurring as a result of immunother-apy [28]. Intradermal skin reactions would provide information on the cellular response (late phase) to the allergen [20,29]. Although beyond the scope of this trial, determination of lymphoproliferative responses to different allergens, changes in helper T cytokine profiles and generation of regulatory T lymphocytes would have enriched the information gathered on successful immunotherapy and immunologic tolerance with grass AIT treatment and should be studied in further trials.

Blood extractions (baseline, after 4 weeks, preseason, intraseason, and postseason) were selected to provide information about the kinetics of the changes that can be observed shortly after treatment starts and during the grass pollen season.

The present trial shows that grass AIT in patients with grass pollen allergy induces immune responses after 1 month of treatment. To our knowledge, this is the first report to show that sublingually administered AIT provides a rapid immunomodulatory effect on allergen-specific IgG4. In comparison with placebo, immunotherapy with grass AIT resulted in highly significant immunological changes. This result is consistent with observations from previous trials with grass AIT [22,23]. Within-subject ANOVA (during the treatment period) showed that IgG4 levels to *P pratense* allergen extract in the active group changed significantly soon after the start of treatment and increased constantly and significantly until the end of the grass pollen season. The placebo group showed unchanged, and considerably lower, IgG4 levels until the start of the season, after which an increase was observed. Once the season started, and up to its end, changes in IgG4 levels were similar in the 2 treatment groups. The IgE and IgE-blocking factor results are similar to those of IgG4 and are confirmed by changes in IgG4 to *P pratense* major allergens with a clear induction of response to the major allergens Phl p 1 and Phl p 5 and, to a lesser extent, Phl p 12. IgE-blocking factor is a dimensionless number that assesses the effect of serum components that compete with IgE binding to an allergen and has been associated with clinical efficacy [30]. In this trial, it showed a significant correlation with IgG4 levels (Pearson r=0.632, N=48, $P<.001$) at the end of the trial (data not shown). Similarly, a decrease in immediate and delayed cutaneous reactivity was observed in patients treated with grass AIT, although this increase was absent in the placebo group. Other authors have demonstrated a reduction in the late-phase reactions after subcutaneous IT (SCIT), and this reduction has even been proposed as a useful parameter in the follow-up of patients treated with SCIT [31]. The short-term treatment, unbalanced randomization scheme, or both may explain the absence of significant differences between groups in the reduction of the delayed response.

The duration of preseasonal treatment is crucial when attempting to achieve a meaningful clinical response. Treatment with grass AIT starting 8 weeks before the grass pollen season has demonstrated a significant reduction in symptom and medication scores [9]. Moreover, the magnitude of the reductions in rhinoconjunctivitis symptoms and medi-cation scores increased with longer duration of preseasonal treatment, thus improving clinical efficacy during the season [15]. In the present trial, samples for in vitro studies were obtained after 1 month of treatment and before, during, and after the pollen season, and the duration of treatment for the preseason visit was between 8 and 16 weeks. The results obtained confirm those obtained by Malling et al [22], who studied the dose and time response of specific Ig and IgE-blocking factor after treatment with grass AIT at different doses. The daily dose administered to patients in this trial was consistent with other doses reported in the literature [32], and the immunological changes for the 75 000–SQ-T doses were consistent with those of the previous dose-finding trial [8].

As expected when administering specific allergens to allergic individuals, there is a possibility of short-lasting, local treatment-related side effects and systemic allergic effects. In this trial, most of the adverse events recorded were mild, resolved spontaneously, or were easily treated with routine measures and in line with previous observations on grass AIT [33]. The most frequently reported related adverse events were those affecting the respiratory and gastrointestinal tracts (mouth) and ear disorders. Similar adverse reactions have been reported in the literature in children [5,34] and in adults [26,35], in whom the mouth was the most frequently affected area [36]. Treatment-emergent adverse events reported with grass AIT tablets usually consist of mild oral or ear pruritus, oral edema, and throat irritation, although they require no specific treatment and resolve spontaneously.

This trial showed that sublingual immunotherapy with grass AIT for grass pollen allergy possesses an immunomodulatory effect already seen in the first month of treatment and which develops during at least the first 3-4 months of treatment.

Acknowledgments

This trial was funded by ALK-Abelló. The authors would like to thank co-investigators Ricardo Abengózar, Juan María Beitia, Nieves Cabañes, Galicia Dávila, Montserrat Fernández Rivas, Mar Gandolfo, David González de Olano, Magdalena Lluch, Belén Mateo, Ángel Moral, Mónica Rodríguez, María Jesús Trujillo, and Sonia Vázquez, as well as all the nursing

staff involved. We are grateful to Agustín Galán and Amalia Ledesma for the in vitro assays and to María Arina for her excellent support in the monitoring of the trial.

This study was presented in part as a poster at the XXVII Congress of the European Academy of Allergology and Clinical Immunology in Barcelona, Spain, 7-11 June, 2008.

Sponsored by ALK-Abelló, S.A., Madrid, Spain.

Disclosure of potential interest: S. Martín is employed by ALK-Abelló, S.A.

References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C, Agache I, Aït-Khaled N, Bachert C, Blaiss MS, Bonini S, Boulet LP, Bousquet PJ, Camargos P, Carlsen KH, Chen Y, Custovic A, Dahl R, Demoly P, Douagui H, Durham SR, van Wijk RG, Kalayci O, Kaliner MA, Kim YY, Kowalski ML, Kuna P, Le LT, Lemiere C, Li J, Lockey RF, Mavale-Manuel S, Meltzer EO, Mohammad Y, Mullol J, Naclerio R, O'Hehir RE, Ohta K, Ouedraogo S, Palkonen S, Papadopoulos N, Passalacqua G, Pawankar R, Popov TA, Rabe KF, Rosado-Pinto J, Scadding GK, Simons FE, Toskala E, Valovirta E, van Cauwenberge P, Wang DY, Wickman M, Yawn BP, Yorgancioglu A, Yusuf OM, Zar H, Annesi-Maesano I, Bateman ED, Ben Kheder A, Boakye DA, Bouchard J, Burney P, Busse WW, Chan-Yeung M, Chavannes NH, Chuchalin A, Dolen WK, Emuzyte R, Grouse L, Humbert M, Jackson C, Johnston SL, Keith PK, Kemp JP, Klossek JM, Larenas-Linnemann D, Lipworth B, Malo JL, Marshall GD, Naspitz C, Nekam K, Niggemann B, Nizankowska-Mogilnicka E, Okamoto Y, Orru MP, Potter P, Price D, Stoloff SW, Vandenplas O, Viegi G, Williams D. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008;63 Suppl 86: 8-160.
2. Till SJ, Francis JN, Nouri-Aria K, Durham SR. Mechanisms of immunotherapy. J Allergy Clin Immunol. 2004;113:1025-34.
3. Dahl R, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, Rivas MF, Ribel M, Durham SR. Efficacy and safety of sublingual immunotherapy with grass allergen tablet for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006;118:434-40.
4. Dahl R, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, Riis B, Grønager PM, Durham SR. Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. J Allergy Clin Immunol. 2008;121:512-8.
5. Bufe A, Eberle P, Franke-Beckmann E, Funck J, Kimmig M, Klimek L, Knecht R, Stephan V, Tholstrup B, Weisshaar C, Kaiser F. Safety and efficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. J Allergy Clin Immunol. 2009;123:167-73.
6. Durham S, Riis B. Grass allergen tablet immunotherapy relieves individual seasonal eye and nasal symptoms, including nasal blockage. Allergy 2007;62:954-7.
7. Rak S, Yang WH, Pedersen MR, Durham S. Once-daily sublingual allergen-specific immunotherapy improves quality of life in patients with grass pollen-induced allergic rhinoconjunctivitis: a double-blind, randomised study. Qual Life Res. 2007;16(2):191-201.
8. Dahl R, Stender A, Rak S. Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. Allergy. 2006;61(2):185-90.
9. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol. 2006;117(4):802-9.
10. Bachert C, Vestenbaek U, Christensen J, Griffiths UK, Poulsen PB. Cost-effectiveness of grass allergen tablet (GRAZAX®) for the prevention of seasonal grass pollen induced rhinoconjunctivitis-a Northern European perspective. Clin Exp Allergy. 2007;37:772-9.
11. Canonica GW, Poulsen PB, Vestenbaek U. Cost-effectiveness of GRAZAX® for prevention of grass pollen induced rhinoconjunctivitis in Southern Europe. Respir Med. 2007;101:1885-94.
12. Petersen AB, Gudmann P, Milvang-Grønager P, Mørkeberg R, Bøgestrand S, Lin-neberg A, Johansen N. Performance evaluation of a specific IgE assay developed for the ADVIA centaur immunoassay system. Clin Biochem. 2004;37:882-92.
13. Jimeno L, Carreira J, Lombardero M. Obtention of monoclonal antibodies against human IgG4 using two different immunization strategies: development of a biotin-based ELISA for IgG4 quantitation. Int Arch Allergy Appl Immunol. 1990;92:175-82.
14. Martín S, Cuesta P, Rico P, Cortés C. A computer program based on parallel line assay for analysis of skin tests Allergy. 1997;52:97-100.
15. Calderon MA, Birk AO, Andersen JS, Durham SR. Prolonged preseasonal treatment phase with Grazax sublingual immunotherapy increases clinical efficacy. Allergy. 2007;62(8):958-61.
16. Emminger W, Dahl R, Kapp A, Colombo G, de Monchi J, Rak S, Denlow B, Durham S. Continuous clinical efficacy and progressive immunological effect of long-term specific immunotherapy with the ALK grass allergen tablet: Findings from a 3-yr randomised adult trial. Allergy. 2008;63(Suppl 88):614.
17. Durham SR, Dahl R, Kapp A, Colombo G, de Monchi J, Rak S, Riis B, Emminger W. Sustained, disease-modifying effect of grass allergen tablet immunotherapy 1 year after completion of treatment Allergy. 2009;64(Suppl 90):Abstract 333.
18. Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. J Allergy Clin Immunol. 2003;112:915-22.
19. Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, Kay AB, Durham SR. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. J Clin Invest. 1993;92:644-51.
20. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri-Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med. 1999;341(7):468-75.
21. Durham SR, Varney VA, Gaga M, Jacobson MR, Varga EM, Frew AJ, Kay AB. Grass pollen immunotherapy decreases the number of mast cells in the skin. Clin Exp Allergy. 1999;29:1490-6.
22. Malling HJ, Lund L, Ipsen H, Poulsen L. Safety and immunological changes during sublingual immunotherapy with standardized quality grass allergen tablets. J Investig Allergol Clin Immunol.2006;16:162-8.

23. W. Aberer, T. Hawranek, N. Reider, C. Schuster, G. Sturm, B. Kränke. Immunoglobulin E and G antibody profiles to grass pollen allergens during a short course of sublingual immunotherapy. J Investig Allergol Clin Immunol. 2007;17(3):131-6.
24. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol. 2004;4:313-8.
25. Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol. 2007:120:S25-S85.
26. Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. J Clin Invest. 1973;52:472-82.
27. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J Allergy Clin Immunol. 1982;70:261-71.
28. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Compari-son between parameters assessing the efficacy of immunotherapy. J Allergy Clin Immunol. 1988;82:439-46.
29. Parker WA Jr, Whisman BA, Apaliski SJ, Reid MJ. The relationships between late cutaneous responses and specific antibody responses with outcome of immunotherapy for seasonal allergic rhinitis. J Allergy Clin Immunol. 1989;84:667-77.
30. Ljorring C, Frew AJ, Durham SR, Jacobsen L, Johansen N, Jarsen JN Ipsen H. In-duction of a competitive non-IgE antibody response by specific immunotherapy is related to clinical efficacy. J Allergy Clin Immunol. 2006;117:S264.
31. Parker WA Jr, Whisman BA, Apaliski SJ, Reid MJ. The relationships between late cutaneous responses and specific antibody responses with outcome of immunotherapy for seasonal allergic rhinitis. J Allergy Clin Immunol. 1989;84:667-77.
32. Rossi RE, Monasterolo G, Coco, G Silvestro L, Operti D. Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. Vaccine. 2007; 25:957-64.
33. Kleine-Tebbe, Ribel M, Herold DA. Safety of a SQ-standardised grass allergen tab-let for sublingual immunotherapy: a randomized, placebo-controlled trial. Allergy. 2006; 61:181-84.
34. Ibáñez MD, Kaiser F, Knecht R, Armentia A, Schöpfer H, Tholstrup B, Bufe A. Safety of specific sublingual immunotherapy with SQ standardised grass allergen tablets in children. Pediatr. Allergy Immunol. 2007;18:516-22.
35. Calderón M, Essendrop M. Specific immunotherapy with high dose SQ-standardised grass allergen tablets was safe and well tolerated. J Investig Allergol Clin Immunol. 2006;16(6):338-44.
36. Larsen TH, Poulsen LK, Melac M, Combebias A, Andre C, Malling HJ. Safety and tolerability of grass pollen tablets in sublingual immunotherapy-a phase 1 study. Allergy. 2006;61:1173-6.

Manuscript received November 19, 2009; accepted for publication January 20, 2010.

Santiago Martín

Medical Department
ALK-Abelló, S.A
C/ Miguel Fleta, 19
28037 Madrid, Spain
E-mail: santiago.martin@alk-abello.com