



A Phase I clinical trial with subcutaneous immunotherapy vaccine of Timothy grass pollen extract according to EMA guidelines

Aim: A double-blind placebo-controlled study was conducted according to EMA guidelines, to evaluate safety, tolerability and short-term treatment effects of three up-dosing regimens of *Phleum pratense* subcutaneous immunotherapy. **Materials & methods:** Forty-two patients were randomized to groups: A (6 weekly doses), B (8 weekly doses) or C (eight doses, two clustered increasing doses over 3 weeks). **Results:** The most frequent adverse events were local reactions. No serious adverse events were found. Higher number and more severe systemic reactions were reported in group C. A decrease in cutaneous responses and an increase of specific antibodies was shown in all active groups even at very short-term. **Conclusion:** *Phleum pratense* subcutaneous immunotherapy in depot presentation exhibited good safety and tolerability. Group A seemed to show the best profile.

Keywords: grass pollen • Phase I • safety • SCIT • tolerability

Allergic rhinitis is a global health problem that affect patients from all ethnic groups, socioeconomic conditions and age [1].

A cross-sectional study [2] showed that the prevalence of symptomatic sensitization, that is, clinical diagnosis of allergic rhinitis, was present in 30.9% of cases of the tested sample.

Among the allergens which cause this pathology, grass pollens make up more than a third of the cases. According to the study described in *Alergológica* 2005 [3], the number of patients with rhinoconjunctivitis sensitized to grasses is greater than 61.9% in the Madrid Community. Likewise, due to increased mobility in Europe, sensitization to inhaled allergens can differ in each European region [4,5].

Allergic illnesses in Europe affect 35% of the active population and thus treatment costs as well as the socio-economic impact are noteworthy. Allergic rhinoconjunctivitis, like the common cold, produces 30% cognitive dysfunction with a corresponding deterioration in learning and work productivity [6]. Recently, the GA2LEN estimated for the EU, avoidable indirect costs per patient insufficiently treated for allergy range between €55

and €151 billion per annum due to absenteeism and presenteeism, that is, €2405 per untreated patient per year [7]. Therefore, early treatment of this illness in order to avoid its progression is also very important from the economic point of view [6].

The most effective treatments for rhinoconjunctivitis and for extrinsic asthma are based on three pillars: avoidance of the allergen (when possible), treatment of the symptoms with medication and treatment of the causes using specific immunotherapy (SIT). In particular, the group of patients with moderate or severe allergy is the one which responds poorly to symptomatic treatment, and is where the choice of using SIT should be made [8]. Along these lines, Calderón's meta-analysis recommends the use of immunotherapy in patients with rhinitis whose symptoms are not under control in spite of receiving medical treatment [9]. In fact, SIT is currently the only available medical intervention that can alter the natural course of the disease [10]. SIT can improve allergic individuals' quality of life, reduce the long-term costs and burden of allergies, and change the course of the dis-

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ease [10]. SIT not only effectively alleviates allergy symptoms, but it also has a long-term effect after the conclusion of the treatment and can prevent the progression of allergic diseases [10].

Before July 2009, clinical development of the treatment of allergic diseases was carried out in adherence to two primary guidelines [11,12]. Both documents focused exclusively on the development of medicinal products to treat allergic symptoms and did not include SIT. In consequence, clinical trials using SIT lacked a standardized approach, which made it difficult to compare vaccines and outcomes and also created uncertainty around the eventual approval for marketing. In 2009, the EMA issued new guidelines on the clinical development of products for SIT in the treatment of allergic diseases, intended to address issues of study design, efficacy and safety for this allergen products [13].

According to these guidelines, dose tolerability studies should be completed before dose-finding efficacy studies. Accordingly, products should be tested at different doses to provide preliminary data on safety and tolerability with regard to the maximum tolerated dose and a suitable dose escalation scheme. A double-blind placebo-controlled study was conducted to evaluate the safety, tolerability and short-term treatment effects of three up-dosing regimens of *Phleum pratense* subcutaneous immunotherapy (SCIT).

Materials & methods
Patient population

Patients were recruited from the outpatient clinic of the Allergy Division of the Ramón y Cajal Univer-

sity Hospital in Madrid (Spain). Forty-two (93.3%) patients with rhinoconjunctivitis with/without asthma out of 45-screened patients, ranging in age from 18 to 60 years, were included in the study (Table 1). Selection criteria were defined according to Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 [14] and EMA guidelines [13]. All patients had a clinical history of seasonal grass pollen-induced allergic rhinitis that was inadequately controlled for at least the previous 2 years; a positive skin prick test response (wheal diameter of ≥3 mm) to standardized *P. pratense* extract (Prick Test Diagnóstico, Bial-Arístegui, Bilbao, Spain), and a *P. pratense*-specific IgE class ≥2, (Pharmacia CAP, Uppsala, Sweden). Although monosensitized patients to gramineae pollens were preferred, patients polysensitized to other allergens were not excluded if their symptoms were not clinically relevant during data collection. The exclusion criteria were those outlined by the EAACI Subcommittee [15]: patients with serious immunological diseases, major cardiovascular disease, cancer, chronic infections were excluded, as well as patients with severe asthma with persistently reduced lung functions (FEV1) below 70% and patients on treatment with β-blockers. Patients with stable and continued use of allergy medication during the 2 weeks prior to their inclusion in the study were excluded. Even if polysensitized patient were allowed, patients with clinically relevant sensitizations to other seasonal allergens and/or sensitizations to perennial allergens if relevant in the collection data period were excluded. Lack of compliance and severe psychological disorders, pregnancy, severe atopic eczema were also listed as exclusion criteria. In addition, patients

Table 1. Demographic and baseline characteristics.						
Variable	Group A		Group B		Group C	
	Active (n = 10)	Placebo (n = 4)	Active (n = 10)	Placebo (n = 4)	Active (n = 10)	Placebo (n = 4)
Age (years) [†]	35.0 (9.7)	38.0 (14.6)	34.5 (9.3)	28.3 (7.1)	34.3 (7.0)	40.0 (6.2)
Sex (male) [‡]	4 (40.0%)	2 (50.0%)	4 (40.0%)	1 (25.0%)	5 (50.0%)	1 (25.0%)
Race (Caucasian) [‡]	3 (30.0%)	2 (50.0%)	6 (60.0%)	1 (25.0%)	6 (60.0%)	1 (25.0%)
Weight (kg) [†]	69.2 (11.3)	67.0 (17.4)	72.0 (21.5)	59.5 (7.5)	71.0 (7.6)	63.0 (7.4)
Height (cm) [†]	163.6 (12.0)	166.5 (8.2)	168.6 (8.9)	162.5 (7.4)	167.5 (11.7)	161.0 (8.6)
Time since diagnosis (years) [†]	4.6 (2.8)	2.8 (1.5)	5.3 (3.5)	5 (2.2)	7.8 (7.1)	12.0 (10.9)
CAP/PHADIA [†]	13.9 (12.8)	19.3 (20.5)	6.2 (4.4)	9.2 (8.5)	13.0 (10.8)	15.9 (12.4)
Class (n) [‡]						
2	2 (20.0%)	2 (50.0%)	3 (30.0%)	1 (25.0%)	2 (20.0%)	0 (0.0%)
3	4 (40.0%)	0 (0.0%)	7 (70.0%)	2 (50.0%)	5 (50.0%)	3 (75.0%)
4	4 (40.0%)	2 (50.0%)	0 (0.0%)	1 (25.0%)	3 (30.0%)	1 (25.0%)
[†] Mean (standard deviation). [‡] Number (%).						

with previous (5 years prior to the study) SIT for the tested allergen or a cross-reacting or that were receiving immunotherapy for any allergen were excluded.

The study was conducted in accordance with ICH6 the Declaration of Helsinki [16] and the protocol was approved by the Hospital Ramón y Cajal Ethics Committee and the Spanish Regulatory Authority (AEMPS) (EudraCT No: 2009-015000-26). Written informed consent was obtained from all patients.

Study design

The study was a randomized double blind placebo-controlled trial in three parallel groups, with different dose escalation schemes in each group (Figure 1) and it was conducted on 2010 outside the pollen season. The patients were allocated to either arm A, B or C of the treatment in entrance order, in that moment the patients were randomized to active treatment or placebo. In the three arms the proportion of active treatment was 5:2. Thus, in each group there were ten patients allocated to receive active treatment and four patients received placebo.

Patients were assigned to their respective groups using a random assignation technique per block, so the number of treatments per group was balanced. Assignation to one of the three treatment groups was open, so both the patient and investigator knew which group they had been assigned to. However, under no circumstance did they know whether a given patient received treatment or placebo.

Treatment protocol

The active group received a standardized *P. pratense* extract adsorbed in aluminium hydroxide [0.33%] (Allergovac Depot®, Bial-Industrial Farmacéutica S.A.; Zamudio, Spain). Composition and appearance of placebo was identical to that of the active treatment but without the *P. pratense* extract. Maximum concentration (vial 3) of depot *P. pratense* extract was 1000 treatment standardized unit (TSU)/ml [17] with a Phl p 5 content of 1.5 µg/ml. Vial 2 is ten-times less concentrated, thus, the concentration of vial 2 was 100 TSU/ml with a Phl p 5 content of 0.15 µg/ml. Treatment was administered following one of the three different up-dosing regimens detailed below; the accumulated dose is described in

Figure 2:

Group A: treatment consisted of six administrations over a 5-week duration.

- Vial 2: 0.1 ml (10 TSU), 0.2 ml (20 TSU) and 0.5 ml (50 TSU) at 1-week intervals.
- Vial 3: 0.1 ml (100 TSU), 0.2 ml (200 TSU) and 0.5 ml (500 TSU) at 1-week intervals.

Group B: treatment consisted of eight administrations over a 7-week duration.

- Vial 1: 0.2 ml (2 TSU), at 1-week intervals.
- Vial 2: 0.1 ml (10 TSU), 0.2 ml (20 TSU) and 0.4 ml (40 TSU) at 1-week intervals.
- Vial 3: 0.1 ml (100 TSU), 0.2 ml (200 TSU), 0.4 ml (400 TSU) and 0.5 ml (500 TSU) at 1-week intervals.

Group C: treatment consisted of eight administrations, two administrations of clustered increasing doses over 3 weeks.

- Week 1: vial 2 – two administrations of 0.1 ml (two administrations of ten TSU), with a 30-min interval.
- Week 2: vial 2 – 0.2 ml (20 TSU) and 0.3 ml (30 TSU) with a 30-min interval.
- Week 3: vial 3 – two doses of 0.1 ml (two administrations of 100 TSU) with a 30-min interval.
- Week 4: vial 3 – 0.2 ml (200 TSU) and 0.3 ml (300 TSU) with a 30-min interval.

SCIT was administered at weekly intervals until the maintenance dose was reached (500 TSU, with Phl p 5 content of 0.75 µg) or when no higher dose could be tolerated. In the event of an adverse reaction (AR), the following dose was to be modified according to the EAACI position paper [15].

Safety & tolerability

All adverse events (AEs) and ARs were recorded throughout the study. Safety was assessed through AEs that were reported (spontaneously or not) by patients or observed by the investigators during each site visit. Tolerability of SCIT was evaluated by early and late local reactions (LLR) (i.e., local swelling and redness) and systemic reactions after each injection which are any symptoms from organs distant from the location of the injection. Reactions were classified depending on the severity and onset of the reaction according to the EAACI classification [15]:

- 0: No symptoms or nonspecific symptoms.
- I: Mild systemic reactions, which may include symptoms like localized urticaria, rhinitis or mild asthma (PF <20% decrease from baseline).
- II: Moderate systemic reactions, which may start >15 min after the injection and may comprise symptoms like generalized urticaria,

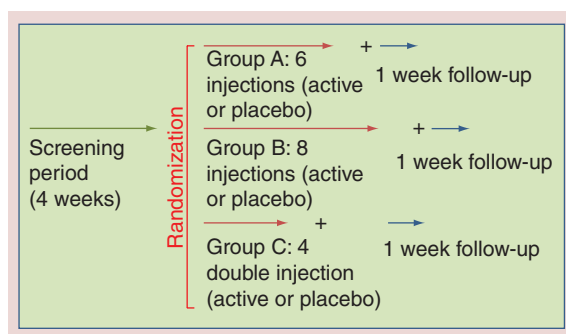


Figure 1. Study design. Patients per group: active ten, placebo four.

and/or moderate asthma (PF <40% decrease from baseline).

- III: Severe but not life-threatening systemic reactions, which may comprise generalized urticaria, angioedema or severe asthma (PF >40% decrease from baseline) of rapid onset (<15 min).
- IV: Anaphylactic shock, which may include symptoms such as: immediate evoked reaction of itching, flushing, erythema, generalized urticarial, stridor (angioedema), immediate asthma, hypotension, etc.

Nevertheless, blood samples were taken at the local laboratory before and at the end of the treatment. Both hematologic and biochemical parameters were evaluated.

Efficacy end points

Dose response skin prick test

Patients were skin tested before and after treatment as previously reported [18]. The skin prick tests were conducted in duplicate, on the volar surface of the forearm using four dilutions of standardized *P. pratense* extract (5.924, 1.185, 0.5925 and 0.05925 mg/ml). Histamine HCl 10 mg/ml and glycerinated saline solution were used as positive and negative controls, respectively.

Immunoglobulins

Specific IgE and specific IgG (IgG and IgG4) to *P. pratense* were measured before and after treatment by ELISA (The enzyme-linked immunosorbent assay) technique [19] at the Protein Lab of Bial-Industrial Farmacéutica S.A. The method used was as follows: microtiter 96-well plates were coated with polyclonal rabbit antihuman IgE purified mouse, antihuman IgG and *P. pratense* extract. The coated wells were subsequently blocked with phosphate buffered saline which

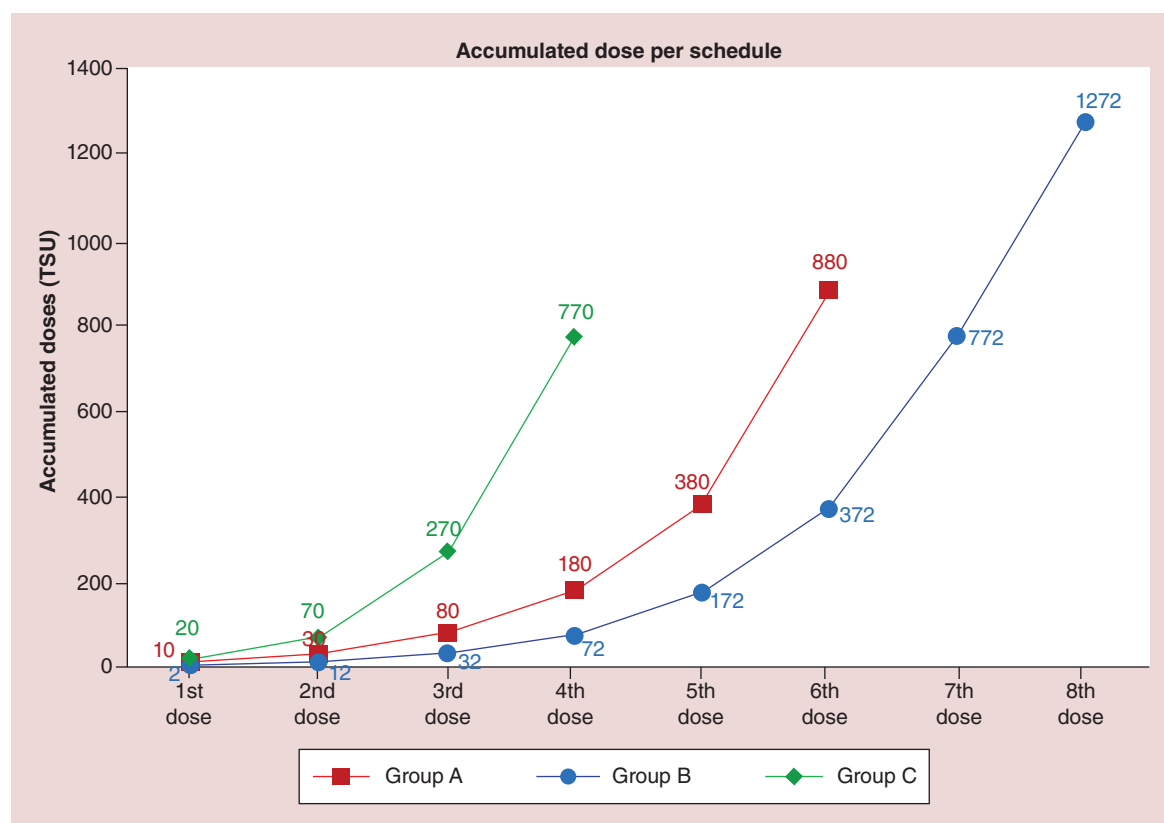


Figure 2. Accumulated dose for the three different up-dosing regimens (groups A, B and C). TSU: Treatment standardized unit.

contained bovine serum albumin. The standard curves were generated by incubating the plates with different known concentrations of IgE extended calibrator set and human IgG and IgG4. The wells coated with *P. pratense* pollen extract were incubated with different dilutions of the human sera. After several washes, bound antibodies were detected by a colorimetric reaction. Finally, the absorbance was measured at 492 nm. Levels of serum-specific antibodies were calculated by interpolating in the respective standard curve.

Statistical analysis

Following the Guideline On The Clinical Development Of Products For Specific Immunotherapy For The Treatment Of Allergic Diseases [13], the aim of the study was to obtain preliminary safety results in relation to the maximum tolerated dose and a suitable dose escalation scheme. Thus, given the nature of this clinical trial, this study was analyzed from a descriptive point of view, and no statistical tests were performed.

Numerical variables were described by mean, median, standard deviation, range (minimum and maximum) and the first and third quartiles, while categorical variables were described by frequency and percentage. The results were analyzed by treatment scheme, dose and patient.

Results

Population

Forty-two patients were randomized and allocated in one of three different groups, placebo with a 2:5 ratio in relation to active treatment was introduced in each group (Figure 1). Demographic and baseline characteristics are shown in Table 1. Overall, 30 patients received subcutaneous injections of *P. pratense* extract and 12 received a placebo. On analyzing both groups, the mean age of patients was 34.6 in the active group and 35.4 years old in the placebo group. The proportion of males in the population was 43.3% in the active and 33.3% in the placebo group. The time in years since diagnosis was 5.9 and 6.6 for the active and placebo groups, respectively. The proportion of asthmatic patients was 60% in the active group and 66.7% in the placebo groups.

Group A received 84 doses, group B 112 doses and group C 118 doses, which were higher than expected as there were dose changes according to EAACI Subcommittee: one patient received ten doses instead of eight due to a systemic reaction and another patient received 12 doses, due to mild consecutive systemic ARs.

AEs & ARs

All patients reached the maximum dose administered and none of the patients discontinued treatment throughout the study.

All AEs were mild or moderate in severity, and no serious AEs were reported in any group. The most commonly affected body system was the skin, and local reactions were the most frequent AEs.

There were 106 ARs (33.75%) after the administration of 314 doses in 34 patients (81%). The majority of ARs were local with a total of 90 local reactions out of 314 (3.49%) administered doses. Local reactions were mild and not clinically relevant, not requiring changes in up-dosing regimens. LLR (≥ 30 min after injections) were reported more often, 65 in 23 patients (64.3%), representing 23% of total administered doses. Local reactions (within 30 min), in contrast, occurred in 13 patients (31%) with a total of 18 ARs (5.73%). Most frequent local ARs were swellings at injection site and local erythema and pruritus. They resolved spontaneously or after applying antihistaminic drugs without modification of the consecutive dose of vaccine.

Eighty-two local reactions happened in the active groups: 27 in group A (8.6%), 35 in group B (11.1%) and 20 in group C (6.4%) and 8 local ARs occurred in the placebo groups. There were 5 (1.59%) immediate local reactions (ILR) versus 22 (7.00%) LLR in group A; 4 (1.27%) ILR and 31 (9.87%) LLR in group B and 8 (2.55%) ILR and 12 (3.88%) LLR in group C. Group B showed a higher rate of local reactions in 100% of patients compared with groups A and C.

Regarding the systemic reactions all were of mild to moderate intensity and did not lead to the withdrawal of any patients. There were 16 reactions reported in six patients [14.3%] among the active groups. All of them were of grade I except for four grade-II systemic reactions in group C. The number of systemic reactions in each of the active groups was four (1.28%) in group A (all in the same patient), three (0.95%) in group B (in two patients) and nine (1.59% grade I and 1.28% grade II) in immunized group C (in two patients). Exanthema and itching (SR grade I) occurred in four consecutive occasions after the administration of the last four doses in just one patient of group A and once in group B. Palpebral edema and palate pruritus (SR grade I) happened in two consecutive administrations in one patient of group B. One patient of group C had five episodes of atopic dermatitis exacerbation (SR grade I). In addition, there were registered also in group C: two generalized pruritus (SR grade II) and two events of a generalized erythema (SR grade II).

On evaluating systemic reactions by groups, 20% of the patients in the active groups reported at least one. These percentages were 10.0, 20.0 and 30.0% for group A, B and C, respectively.

With regards to systemic reactions by grade, groups A and B showed a better safety profile, since systemic reactions were grade I: all reactions of group A were

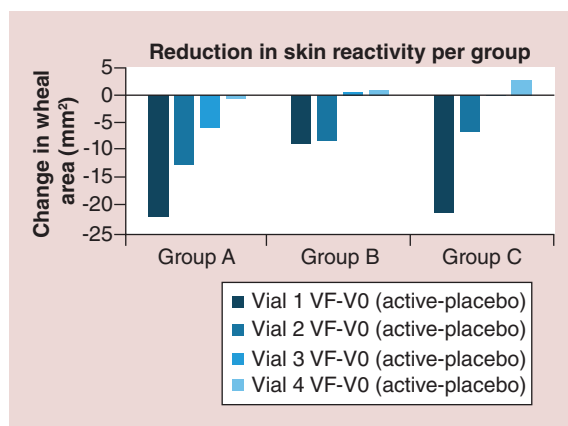


Figure 3. Changes in skin prick test response: changes in wheal area (mm²) per treatment group (groups A, B and C) between final visit and basal visit versus its placebo.

concentrated in only one patient. Group C presented higher incidences of grade I ARs and also showed grade II of generalized pruritus and exacerbation of atopic dermatitis.

Regarding AEs, the most frequently reported AEs (not related to the vaccine) were headache, which occurred in 21.7 and 18.2% of patients in the active groups and placebo groups, respectively, and influenza reported in 20.0% of patients in the active group.

Short-term treatment effects

Dose-response prick test

Dose-response skin prick tests results are shown in Figure 3. All groups showed a decrease in the skin prick test values, and this decrease was higher in the active groups than in the placebo groups. Group A showed more consistency when compared with placebo.

Specific IgG & IgG4 to *P. pratense*

Changes in specific IgG and IgG4 to *P. pratense* throughout are shown in Figures 4 & 5. Active treat-

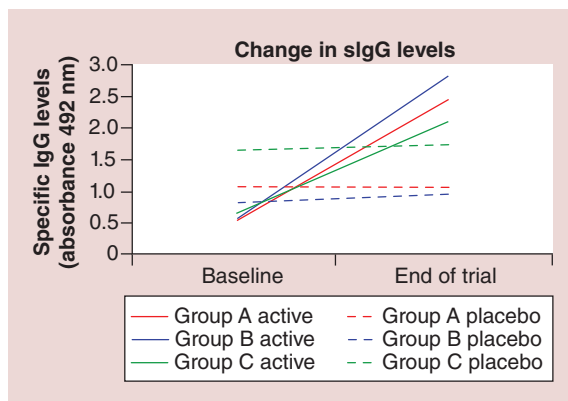


Figure 4. Changes in specific IgG levels between final visit and basal visit per treatment group (groups A, B and C), active and placebo.

ment elicited a substantial increase of specific IgG and IgG4 which was not found in the placebo group.

Specific IgE to *P. pratense*

The three active groups showed an increase of the specific IgE at the end of trial, while the three placebo groups presented a decrease of the specific IgE at the final visit. The results in IgE level changes are shown in Figure 6.

Discussion

Safety, tolerability and exploratory short-term effects of three different up-dosing regimens with *P. pratense* (Allergovac Depot) SCIT have been evaluated in grass pollen-allergic patients. Results of the study indicated that *P. pratense* SCIT in doses up to 500 TSU was safe with early signs of potential treatment effects. This study constitutes the first step toward searching the optimal dose of *P. pratense* (Allergovac Depot) SCIT in a subsequent dose-finding study following the EMA 2009 guidelines.

It has been reported that a higher dose led to greater improvement in allergic patients [20], but a general risk of SIT with higher allergen extract doses is a potential increase in ARs, particularly systemic ones [21,22], and it is very important to evaluate the safety of doses used in SIT. Although some doses of Phl p 5 have been recommended as optimal for SIT [22,23], the presence of several major allergens in the same extract, differences based on the method used for quantification, as well as differences in the content of other nonquantified major allergens or even minor allergens, presence of adjuvants, etc. made it necessary to research and document optimal doses for each allergen extract commercially available for SIT [13,24,25].

If we consider the dose injected to the patients in terms only of Phl p 5 content, we can say that it is in the range of other SCIT products based on native allergenic extracts. In addition, we must have in mind that different ELISA kits using different monoclonal antibodies and calibrators can give rise to somehow different results until they are not quantified using the same certified reagents.

Obviously, SLIT preparations or hypoallergenic allergoids for SCIT can take advantage of the higher safety profile to administer a higher amount of Phl p 5 to the patients.

It must be said also that although common allergy practice is to target the maximum tolerated dose as the therapeutic objective; on the other hand, recent reviews have suggested that maintenance dosing over a fourfold range of antigen is therapeutic. In the studies selected for this analysis, doses varying twofold to 1000-fold have been shown to have equivalent therapeutic effects [26].

In this sense, few reported Phase I studies have focused on therapeutic vaccines for allergic diseases, most of them being for sublingual immunotherapy in tablets for pollen-allergic patients [27–29], and only one of them for SCIT [30]. The last one was performed by using a polymerized mite extract [31]. To the best of our knowledge, this SIT study is the first one performed using nonpolymerized depot extract, particularly with grass-pollen extract (*P. pratense*).

P. pratense extract was tested at three up-dosing regimens, which were very well-tolerated without severe ARs. All patients reached the maximum dose of 500 TSU, and having in mind the limitations of an exploratory trial, this dose seemed to be safe as all patient tolerated it with early signs of potential treatment effects. All ARs were mild or moderate in intensity and most were local reactions. However, as is expected when administering specific allergens to allergic individuals, there was a high incidence of mild local reactions that did not require changes in dose administration.

On evaluating systemic reactions by groups, 20% of the patients in the active groups reported at least one systemic reaction. These percentages were similar to other published work, in a study with SCIT [31] in patients with allergic rhinoconjunctivitis, 48 out of 224 patients [21%] had a systemic reaction and most of the reactions were classified as mild (grade I or II according to EAACI). Likewise, other recent publications on short-time up-dosing regimen with SCIT [32] presented a 51.6% of patients with ARs and in a prospective study of a short up-dosing schedule with mites [33] was shown that the 24.1% of patients had at least one AR.

Systemic reactions were mild and all were grade I, except four grade II reactions. There were no severe reactions (grade III or IV) or life-threatening reactions. Groups A and B showed a better safety profile compared with group C. Group A received 84 doses and group B 112 doses. Apparently, the more doses are administered the risk of ARs is higher even if group B started with a lower dose (2 TSU). However, it is important to highlight that the four systemic reaction grade I of group A occurred in just one patient, whereas there were three systemic reaction grade I of group B happened in two patients.

In addition, similar results have been described by other authors [33] even with two different up-dosing schedules that differ in just one dose.

With regards to skin reactivity, the active groups showed a higher decrease than the placebo groups. Besides, the skin reactivity reduction in each of the active groups versus its placebo seemed to be more consistent in group A (Figure 3). On the other hand, specific IgG and IgG4 levels increased only with SIT and without apparent differences among the three

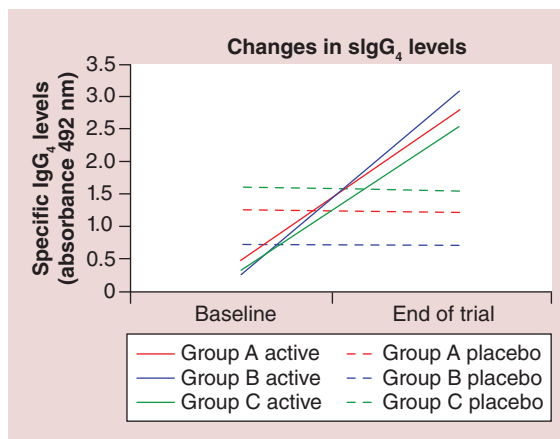


Figure 5. Changes in specific IgG₄ levels between final visit and basal visit per treatment group (groups A, B and C), active and placebo.

up-dosing regimens. These findings are in agreement with the ones previously reported on SIT [34] in which grass-pollen SIT elicits an increase in specific IgG and IgG4 levels accompanying clinical improvement [35,36]. Grass-pollen SIT induces allergen-specific, IL-10-associated protective IgG4 responses in which IgG4-dependent blocking of IgE binding to B cells occurs [37]. IL-10 and Treg cells potentially suppress both total and allergen-specific IgE and simultaneously increase IgG4 production [38,39]. Thus, in addition to generating tolerance in T cells, IL-10 regulates specific antibody isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype. Our results are noteworthy due to the very short term of the SIT treatment, 4 weeks for group C, and because they confirmed previous results after 7 treatment weeks [40]. Overall, the short-term treatment effects showed the ability to induce changes in immune response following SIT. These data must clearly be confirmed in extended placebo-controlled studies, and such studies are currently ongoing. Also,

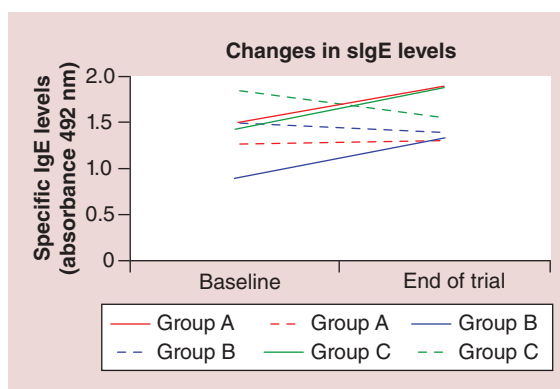


Figure 6. Changes in specific IgE levels between final visit and basal visit per treatment group (groups A, B and C), active and placebo.

clinical efficacy must be evaluated with the selected up-dosing scheme.

Regarding IgE level early changes, they are compatible with recent publications [41] where it is confirmed that when SIT is initiated, allergen-specific IgE levels usually increases and then gradually decreases during the years of treatment to pretreatment levels [42].

We should point out some of the limitations of the study, most of which are intrinsic to Phase I studies: the primary criticism of the standard Phase I studies are based on the low number of patients included. In many cases, low study populations do not allow for deep statistical analysis of the results, and, as in this study, only descriptive analyses may be carried out; larger samples are required to detect events that happen in a small percentage of patients; and since the evaluation of efficacy is not the objective of a Phase I trial, good results found on evaluating short-term treatment effects has to be confirmed by further studies.

Conclusion

In conclusion, the data supported the safety and tolerability, as well as early signs of immune response changes of the three up-dosing schemes. Considering that groups A and B showed a more favorable trend in terms of safety, and that all reactions of group A occurred in only one subject, and taking into account other issues such as the convenience for the patient, the treatment schedule A seemed to show the best profile. Thus, it was the up-dosing regimen selected to continue with the clinical development of the vaccine. These data has been used for design of the subsequent dose-finding study (Eudra CT 2011-000814-21) which results will be published shortly.

Future perspective

Although immunotherapy for allergic disease has developed over more than a century, only recently

have solid evidence-based data been available, with the achievement of substantial improvements in the quality of clinical studies. In the near future, it is anticipated that existing internationally agreed guidelines for immunotherapy and evidence-based recommendations will be used more extensively and will undoubtedly improve therapeutic outcomes in specific clinical scenarios.

At the same time, clinical trial designs based on regulatory agency recommendations will help to define the best dose, formulation, composition, release form and route of administration for SCIT products. SCIT products have become a very promising therapeutic tool in the management of allergic diseases and it is anticipated that their acceptance and use will continue to increase in the coming years.

Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- *Phleum pratense* subcutaneous immunotherapy in depot presentation have shown a good safety and tolerability profile.
- Very short-term treatment induced changes in both humoral and prick dose response.
- Group A showed a better profile than Groups B and C.
- Additional Phase II studies are under way to evaluate optimal dose in a larger group of patients.

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• of interest; •• of considerable interest.

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