Double-blind, randomized, placebo-controlled trial of allergen-specific immunotherapy with the major allergen Alt a 1

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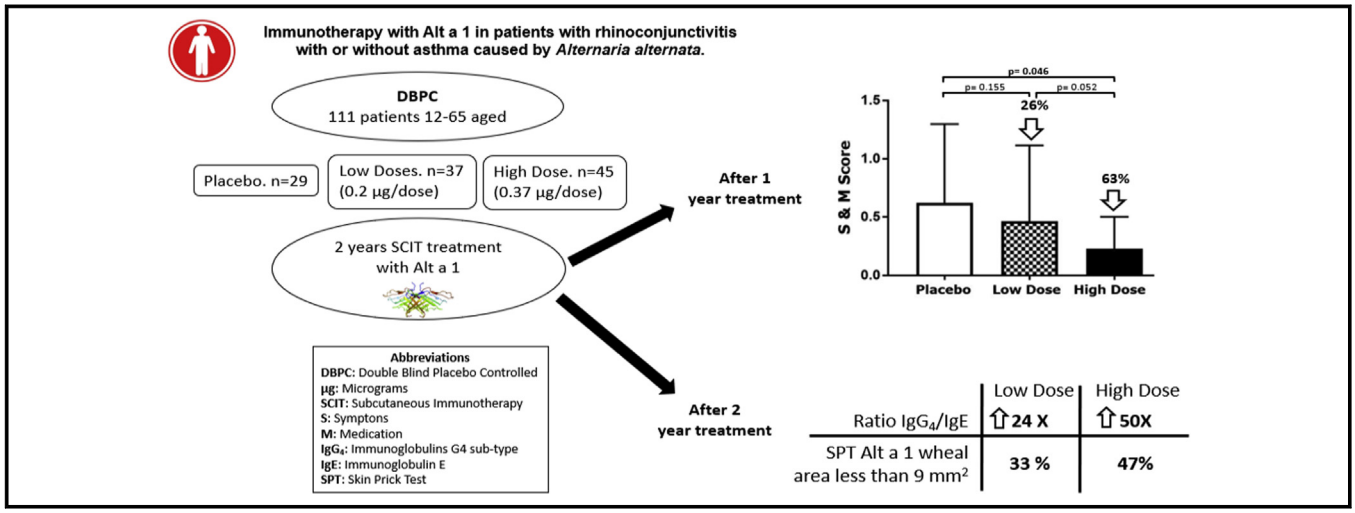
GRAPHICAL ABSTRACT



Background: There have been few studies conducted on the efficacy and safety of specific immunotherapy with allergen extracts of fungi compared with other allergen extracts, and there are no data on the major allergen Alt a 1 of the fungus *Alternaria alternata*.

Objectives: We sought to evaluate the efficacy and safety of subcutaneous immunotherapy with 2 different doses of Alt a 1 in patients with rhinoconjunctivitis caused by sensitization to *A alternata*.

Method: We performed a multicenter, randomized, double-blind, placebo-controlled trial with Alt a 1 administered subcutaneously in patients with allergic rhinoconjunctivitis with or without controlled asthma aged 12 to 65 years. Three groups were included: the placebo group and active groups receiving 0.2 or 0.37 µg of Alt a 1 per dose. The main end point was the combined symptom and medication score. Secondary end points were cutaneous reactivity and serum IgE and IgG_4 levels to Alt a 1. Recorded adverse reactions were graded according to World Allergy Organization criteria.

Results: There were significant reductions in the combined symptom and medication score for the 0.37-µg dose of Alt a 1 compared with placebo at 12 months of treatment. Reduced cutaneous reactivity and IgE levels, together with increased IgG_4 levels, were demonstrated for the 2 active groups versus the placebo group. A similar safety profile was found for both active groups compared with the placebo group. No serious adverse drug reactions were reported.

Conclusion: Immunotherapy with Alt a 1 was efficacious and safe, reducing the symptoms and medication consumption associated with rhinoconjunctivitis after only 1 year of treatment. The clinical benefits were associated with reduced skin reactivity and specific IgE levels and increased IgG_4 levels. (J Allergy Clin Immunol 2019;144:216-23.)

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Alternaria species represent probably the most important saprophytic genus from the fungal allergy point of view.[1] *Alternaria alternata* is the most abundant and best studied species, given its importance as an aeroallergen with a worldwide distribution and a high rate of involvement in patients with allergic diseases, such as rhinitis and asthma.[2] *A alternata* predominates in outdoor environments, and its spores are principally involved in sensitization,[3] although its categorization and importance as an indoor allergen have also been established.[4]

Alt a 1 is the predominant allergen of the 17 reactive IgE proteins identified in *A alternata*,[5] 12 of which are described in the International Union of Immunological Societies (www.allergen.org). It is mainly present in the spore wall[6] and responds molecularly to a 30-kDa dimer specific to the Fungi kingdom, with a β-barrel structure and an unknown biological function.[7] In reducing conditions it can be separated into subunits of 16.4 and 15.3 kDa, respectively. Sensitization to Alt a 1 is identified in more than 90% of patients with allergy to *A alternata*.[2,5]

The availability of Alt a 1 isolated in its natural form for use in *in vivo* diagnoses and immunotherapy provides an alternative to the variability of whole allergenic extracts from molds.[8,9] Alt a 1 is not present only in *A alternata* but is an orthologous allergen present in other species of the order Pleosporales, such as *Stemphylium botryosum* and *Ulocladium botrytis*. This would make it possible to apply the diagnosis and treatment with Alt a 1 to patients sensitized to these fungi as well.[10-12]

After a first clinical trial, classified as phase IV by the Agencia Española de Medicamentos y Productos Sanitarios, which demonstrated the safety of treatment with Alt a 1 in patients with symptoms of rhinitis and asthma caused by sensitization to *A alternata*,[13] the aim of this trial was to evaluate the efficacy and safety of a higher dose of Alt a 1 and compare it with the previously tested dose.

Abbreviations used

ADR:	Adverse drug reaction
AE:	Adverse event
CRF:	Case report form
CSMS:	Combined symptom and medication score
ITT:	Intention to treat
PP:	Per-protocol
WAO:	World Allergy Organization

METHODS

Trial design

A multicenter, randomized, double-blind, parallel-group, placebo-controlled clinical trial of differing concentrations of Alt a 1 was designed. Randomization was carried out by the sponsor in blocks of 6 for each participating hospital. The treatments were assigned on a 1:1:1 basis so that each block of 6 contained 2 high-dose treatments, 2 low-dose treatments, and 2 placebos. The trial was authorized by the Clinical Research Ethics Committee, Hospital Complex of Navarra, Spain, and the Spanish Agency of Medicines and Medical Devices (EudraCT 2010-024440-15) and included 113 patients from 17 Spanish hospitals. The trial began in 2012 and finished in 2016. The treatment schedule is described in Fig 1 (see Table E1 in this article's Online Repository at www.jacionline.org). At 12 months, the placebo group was incorporated into the group receiving the highest dose (placebo-high dose). A 30-day follow-up period with 1 visit was included at the end of the study (Fig 1).

The trial was designed in accordance with European Medicines Agency guidelines on the production and control of allergens,[14] clinical development in immunotherapy with allergens,[15] and good clinical practice.[16]

Patients

Patients more than 12 years old with a clinical history consistent with allergic rhinitis/rhinoconjunctivitis associated with fungal exposure and with or without mild or moderate asthma were recruited.[17] The patients' sensitization was demonstrated, and skin test follow-up was performed throughout the trial by using skin prick tests (wheal diameter ≥3 mm) with whole extract of *A alternata* ($2HEP_D$) and the major allergen, purified natural Alt a 1, at a concentration of 10 μg/mL (Diater, Madrid, Spain). Levels of specific IgE to Alt a 1 were determined by using a commercially available system (Thermo Fisher Scientific, Waltham, Mass), with a cutoff value of 0.7 kU/L or greater. Patients polysensitized to epithelia and mites were not selected for this study nor were those with uncontrolled asthma and those who had received fungal immunotherapy in the 3 years before initiation of the trial.

We recruited 113 patients, 111 of whom received at least 1 administration (intention to treat [ITT]), who were assigned as follows: 29 to placebo, 37 to low-dose Alt a 1 (0.2 μg of Alt a 1/dose), and 45 to high-dose Alt a 1 (0.37 μg of Alt a 1/dose, Fig 2): 66 were male (59.5%) and 45 were female (40.5%) subjects, and 63 (56.8%) were aged 18 years or more and 48 (43.2%) were aged 17 years or less. The mean age was 21 years (SD, 8 years; range, 12–44 years).

Immunotherapy with Alt a 1

The major allergen Alt a 1 isoform 1.0101 (UniProt P79085) was purified from an extract of *A alternata* by using ion exchange and size exclusion chromatography, with a purity of greater than 95%.[13]

Alt a 1 was stabilized by means of lyophilization in single-dose vials with mannitol as a cryoprotectant at concentrations of 0.025 μg per vial and 0.046 μg per vial for the induction dose and 0.25 μg per vial and 0.46 μg per vial for the maintenance dose. Physiologic saline was used as the diluent, with aluminum hydroxide (0.83 mg per vial) as an adjuvant. The maximum volume of subcutaneous administration was 0.8 mL.

Trial end points

The main study end point was the combined symptom and medication score (CSMS). Nasal symptoms recorded were nasal congestion, pruritus, mucus production, and sneezing. A score based on symptom severity was used: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. Ocular symptoms recorded were hyperemia, pruritus, tearing, and exudate; an identical severity score was used. The score of each symptom was added up, resulting in a score ranging from 0 to 24. The result was then divided by the number of symptoms evaluated, resulting in a final symptom score ranging from 0 to 3. All patients had access to identical rescue medication when necessary. Medication use was graded as follows: 0, no rescue medication; 1, antihistamines (topical: levocabastine and/or systemic: loratadine); 2, nasal corticosteroids (budesonide); and 3, oral corticosteroids (deflazacort). The main end point was calculated at each of the visits during the 2 years of the trial for each of the groups by calculating mean symptoms recorded and mean medication consumed, both on a scale of 0 to 3, and dividing the sum of these by 2. This variable was recorded by patients during each month of the trial.

Secondary end points included serum Alt a 1–specific IgE and IgG_4 levels (ImmunoCAP; Thermo Fisher Scientific) determined at baseline and at 3, 12, 18, and 24 months. Changes in cutaneous reactivity during immunotherapy with skin prick tests to *A alternata* and Alt a 1 (Diater) were observed at baseline and 12 and 24 months.

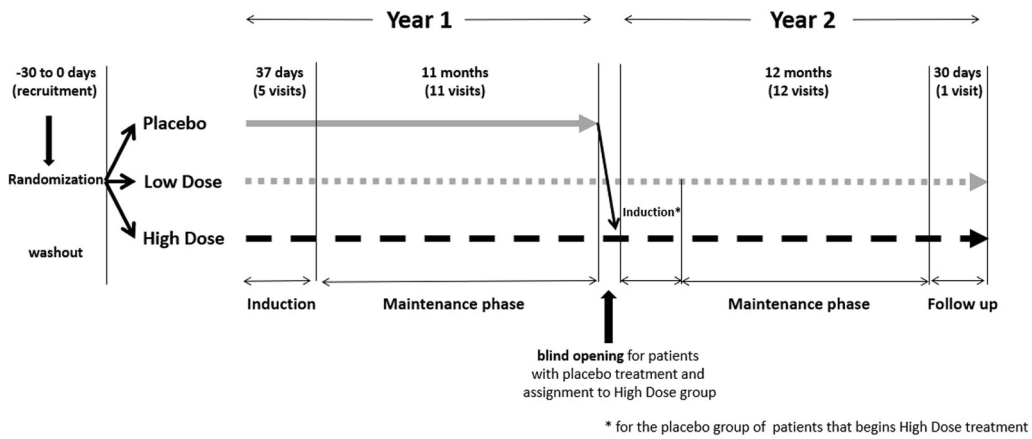

FIG 1. Study design. Low dose, 0.2 μg of Alt a 1 per dose; high dose, 0.37 μg of Alt a 1 per dose.

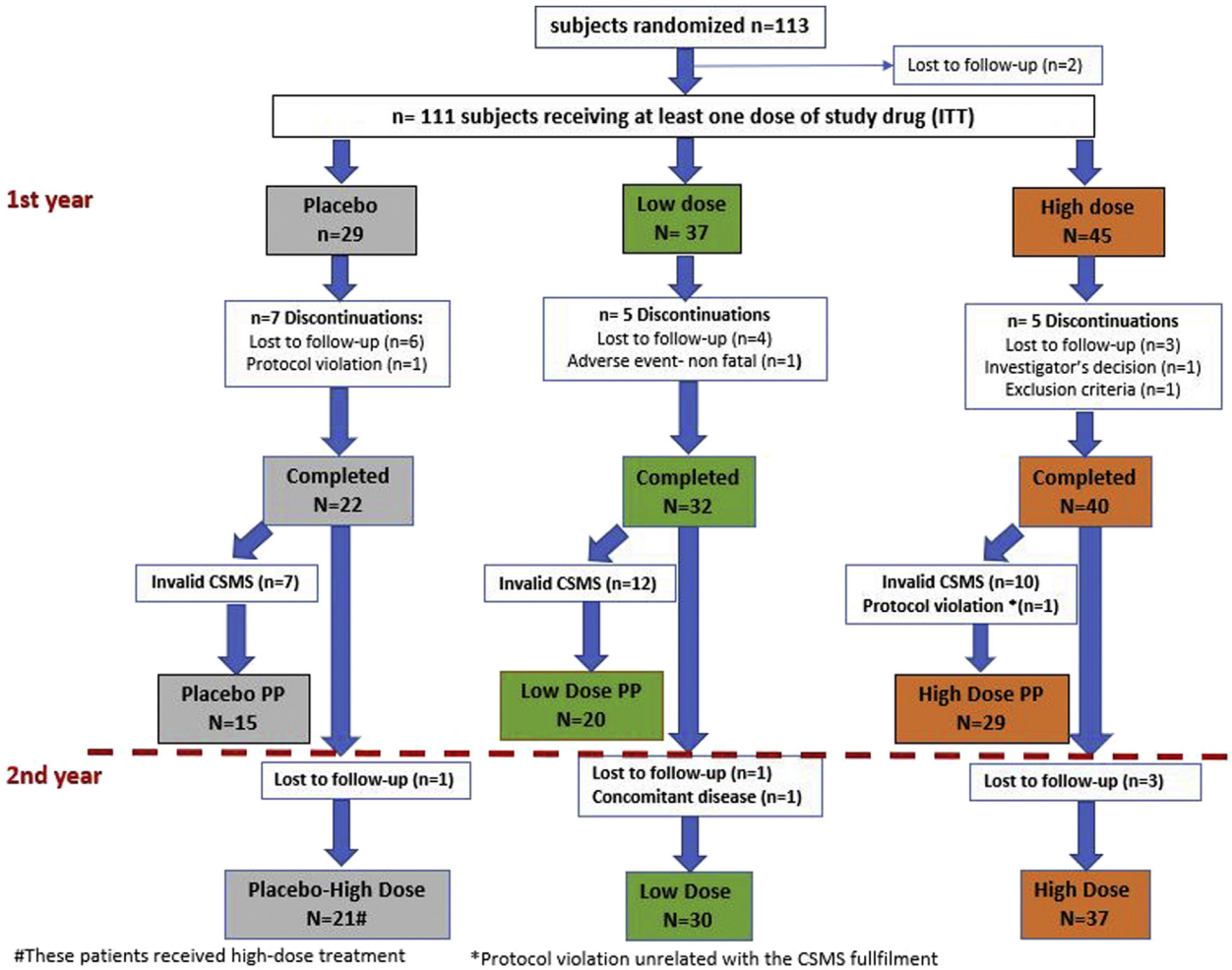

FIG 2. Flow diagram of subject disposition.

Safety was assessed by describing all reported adverse events (AEs) classified according to the MedDRA dictionary. Adverse drug reactions (ADRs) were graded according to World Allergy Organization (WAO) criteria.[18]

Statistical analysis

It was hypothesized that patients assigned to the active medication groups should show a reduction of 30% or greater in CSMSs compared with those in the placebo group to calculate the number of patients required.[19] For the

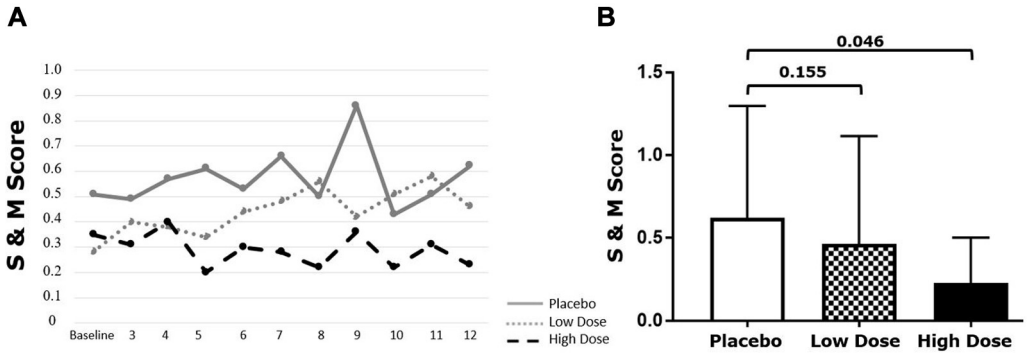

FIG 3. A, Line plot of mean scores of the main end point after 1 year of immunotherapy. Low dose, 0.25 μg/mL Alt a 1; high dose, 0.46 μg/mL Alt a 1; and placebo, treatment without Alt a 1. **B,** *t* Test comparing the 3 groups after the first year of treatment. *S & M*, Symptom and medication score.

main and secondary end points, groups were compared by using a 2-tailed *t* test to evaluate differences between the active groups and the placebo group. The paired *t* test was used to make within-group comparisons throughout the trial. In the safety section the prevalence of AEs was tested by using a contingency table and a 2-tailed χ^2 test. The CI was 95% for all tests. Data management and graphical representations were made by using the GraphPad Prism statistical package (version 7.02 for Windows; GraphPad Software, La Jolla, Calif).

RESULTS

Of the 111 patients (ITT population), 17 did not complete the first year of treatment, 13 were lost to follow-up, 1 withdrew because of an AE, there was 1 protocol violation, the investigator decided to withdraw 1 patient, and 1 met the exclusion criteria. Of the 94 ITT patients who completed the trial, 30 were not included for analysis of the main end point, 29 because they did not have a CSMS or because it was incomplete or illegible. One protocol violation was also recorded. For this reason, it was not possible to analyze the main end point in the ITT population. Therefore the main end point was evaluated in the per-protocol (PP) population of 64 patients (37 male) distributed as follows: placebo group, 15 patients; low-dose group, 20 patients; and high-dose group, 29 patients (Fig 2).

Baseline values for the main end point were similar in all groups (Fig 3, *A*), and then a trend toward reduction in CSMS in the high-dose group compared with the other 2 groups was observed (see Fig E1 in this article's Online Repository at www.jacionline.org). At 12 months, the CSMSs in the high-dose group, in which patients had received a cumulative dose of Alt a 1 of 4.99 μg, was significantly lower than those in the placebo group ($P = .046$; Fig 3, *B*). No significant differences were found between the low-dose and placebo groups after 1 year of treatment. The low-dose group showed a 26% improvement over placebo at 12 months, with a cumulative dose of Alt a 1 of 2.70 μg compared with the high-dose group, which showed a 63% improvement over placebo. Between-group analysis (low dose vs high dose) showed a reduction in the high-dose group of 50% at the end of both the first and second years, with a cumulative dose of 9.43 μg of Alt a 1 with respect to the low-dose group; the *P* value was .14 and .27 for the first and second years, respectively. In the placebo/high-dose group, which received high-dose treatment during the second year, the CSMS was reduced by 42% compared with the first year.

The ITT was included in the analysis of secondary end points. Wheal areas (n = 111) and serum samples (n = 110) were analyzed. Cutaneous skin prick test responses to Alt a 1 (10 μg/mL) and *A alternata* (2 HEP_D) during the trial showed a significant reduction in wheal area with respect to placebo in both cases ($P < .05$, Fig 4). The same analysis was performed in the 64 patients who fulfilled the main end point, and the same significant between-group comparisons were found (see Fig E2 in this article's Online Repository at www.jacionline.org).

In the low-dose and high-dose groups, the wheal area was reduced by 49% and 56% with the *A alternata* extract, respectively, and by 55% and 63% with the Alt a 1 skin prick test compared with placebo at 12 months. At 24 months of immunotherapy, an additional reduction of 22% in the wheal area with the extract of *A alternata* and 26% with Alt a 1 were observed in the low-dose group. In the high-dose group the reduction in wheal area was 14% for *A alternata* and 29% for Alt a 1. Between-group analysis (low dose vs high dose) showed a greater reduction in the high-dose group of 13% and 18% in the first year with skin prick tests to *A alternata* and Alt a 1, respectively, and 5% and 21%, respectively, in the second year. In some patients from both groups, there was a reduction in wheal area of less than 9 mm^2, which is not considered a positive wheal response.[20] In the low-dose group 30% of *A alternata* and 33% of Alt a 1 skin prick tests resulted in a wheal area of less than 9 mm^2 at 24 months. In active group 2 the results were 33% for *A alternata* and 47% for Alt a 1.

Levels of specific IgE to Alt a 1 increased at 3 months of immunotherapy in the 2 active groups compared with the placebo group ($P < .05$). The greatest reduction was 29% at 24 months with respect to baseline for the high-dose group ($P = .0018$; Fig 5, *A*). Specific IgG_4 levels to Alt a 1 increased continuously in the 2 active groups, whereas levels in the placebo group remained unchanged from baseline to 12 months (Fig 5, *B*).

The design of the clinical trial included blind opening of the placebo group at 12 months and assignment of patients to the high-dose group during the second year. Fig 6 shows this group behaved analogously to the high-dose group during the first year of treatment, with a significant increase in IgE levels during the first 3 months and a subsequent reduction together with a significant increase in IgG_4 levels as soon as high-dose treatment began. Skin prick test responses showed a reduction of almost 50% for both *A alternata* and Alt a 1, resulting in negative skin test results in 38% of patients.

The IgG_4/IgE (in micrograms per liter) ratio increased as doses were administered in the active groups as a consequence of the

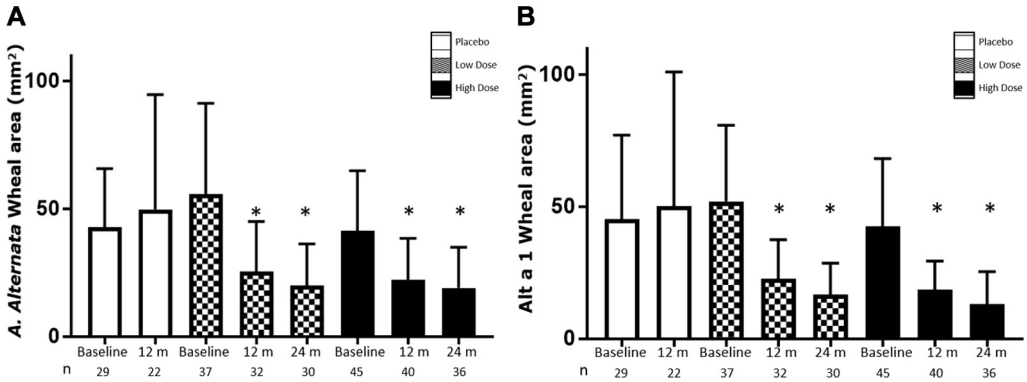

FIG 4. Mean wheal areas of skin prick tests with *A alternata* **(A)** and Alt a 1 **(B)** by group after 1 and 2 years of immunotherapy. $*P < .05$ compared with baseline. *n*, Number of patients analyzed at each visit.

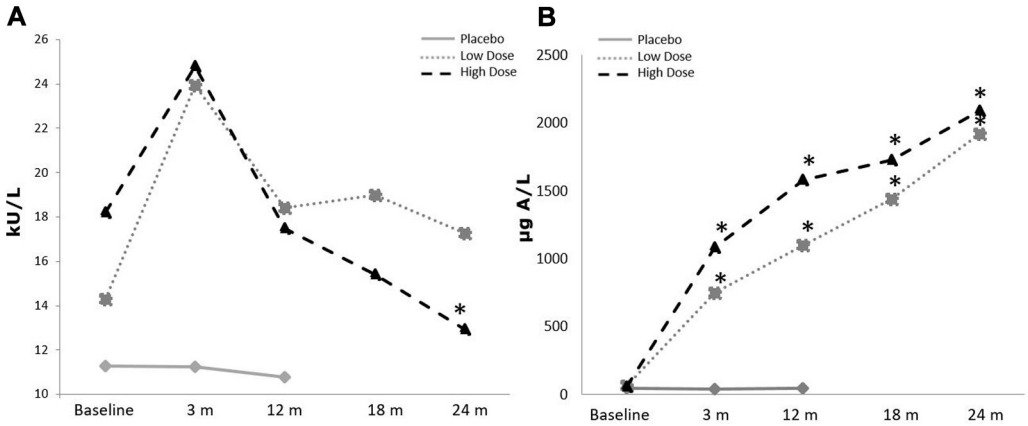

FIG 5. Specific IgE and IgG_4 Alt a 1 levels during immunotherapy for the 3 treatment groups. **A,** Specific IgE Alt a 1 level (means). **B,** Specific IgG_4 Alt a 1 level (means). $*P < .05$.

increase in IgG_4 levels and the parallel decrease in IgE levels. In the placebo group no increase was observed during the first year, but after the second year, when the high-dose concentration was administered, the ratio increased 19-fold with respect to the value of the placebo group at 12 months. The increase in the IgG_4/IgE ratio after 12 months of treatment compared with baseline was 13-fold for the low-dose group and 28-fold for the high-dose group. At 24 months, the increase was 24-fold for the low-dose group and 50-fold for the high-dose group (Table I).

Throughout the trial, 1168 AEs were reported, including 5 serious AEs, with no between-group differences. All serious AEs were considered unrelated to study medication; consequently, no serious ADRs occurred. Of these AEs, 240 (20.5%) were considered related to the study medication, and 13 (5.4%) were reported as local immediate reactions, 172 (71.7%) as local late reactions, 38 (15.8%) as grade 1 systemic reactions, and 17 (7.1%) as grade 2 systemic reactions. One hundred eighty-one ADRs were reported during the first year, and 59 were reported during the second year. Overall, there were no significant between-group differences in the incidence of ADRs per 100 injection visits compared with the placebo group (Table II) for each type of reaction; a significant decrease in the incidence was observed during the second year, especially in the placebo/high-dose group, in which the proportion was reduced by more than 7 points in the second year. No serious ADRs were reported throughout the study, and no systemic ADRs more severe than WAO grade 2 were reported.[18]

DISCUSSION

There are few studies of fungal immunotherapy with *A alternata*[21-25] and only 1 with the major allergen Alt a 1 purified in its natural form.[13] Alt a 1 is an allergen that is exclusive to the Fungi kingdom and has a prevalence of greater than 90% in patients sensitized to *A alternata* and species of other genera of the order Pleosporales.[12] Its use in immunotherapy provides a response to the heterogeneity of allergen extracts of *A alternata*[26-28] common to this source of sensitization.[2,29]

The results of our study show that allergen immunotherapy with Alt a 1 is associated with a significant improvement in CSMSs. The low- and high-dose groups both showed a reduction in CSMSs[15,30,31] at 12 months of treatment of 26% and 63%, respectively, compared with the placebo group, and this reduction was statistically significant in the group treated with the high dose. This suggests benefits for the patient because there are clinical improvements after a single year of treatment. The most effective dose of Alt a 1 is 0.37 µg. For pharmaceutical development reasons, the highest dose tested was 0.37 µg of Alt a 1, which was almost twice the previously tested dose (0.2 µg).

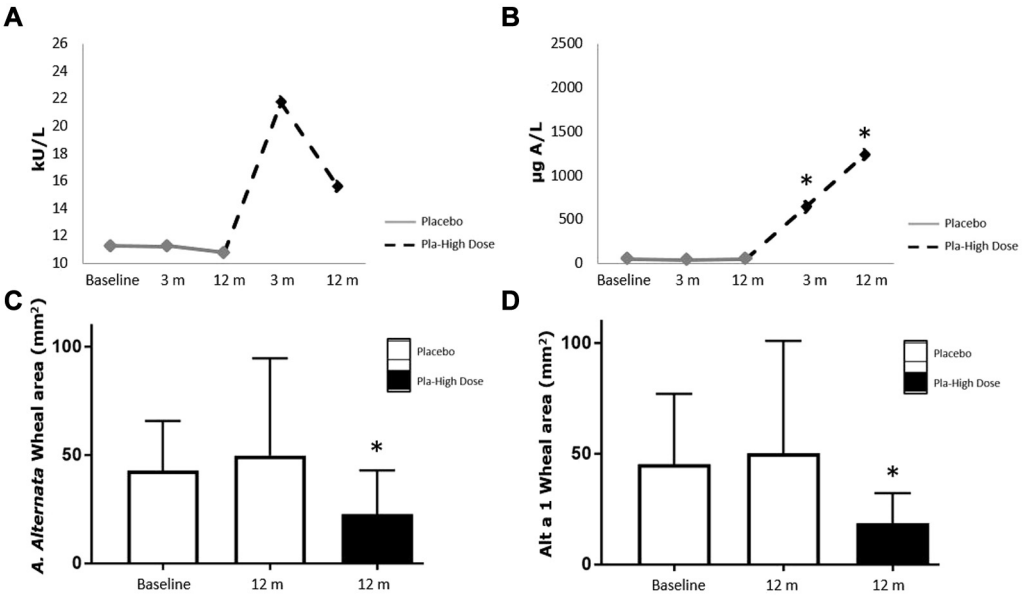

FIG 6. A and **B,** Specific IgE (Fig 6, *A*) and IgG_4 (Fig 6, *B*) Alt a 1 levels during immunotherapy for the placebo/high-dose group. **C** and **D,** Mean wheal areas of skin prick tests with *A alternata* (Fig 6, *C*) and Alt a 1 (Fig 6, *D*). $*P < .05$.

TABLE I. IgG_4/IgE ratios in micrograms per liter and increases at 12 and 24 months

Group	Baseline	12 mo	Increase at 12 mo	24 mo	Increase at 24 mo
Placebo	1.673	1.743	No increase	NA	NA
Low dose	1.888	24.887	13-fold	46.224	24-fold
High dose	1.349	37.614	28-fold	67.337	50-fold
Placebo–high dose	NA	NA	NA	32.853	19-fold

NA, Not applicable.

The graphic representation of the main end point shows a serrated profile that is attributable to the number of patients per group and the presence of spores in the environment. However, in the study of CSMS trends (see Fig E1), it can be clearly seen how, from the start, there is a tendency in the high-dose group toward a decrease in score and therefore an improvement in CSMS.

In addition, wheal areas of skin prick test responses with *A alternata* and Alt a 1 were significantly reduced in the 2 active groups at 12 months: 47% of patients in the high-dose group and 33% in the low-dose group had a wheal area of less than 9 mm^2 on skin prick tests with Alt a 1.

Serologic studies showed the classic increase in specific IgE levels at the beginning of immunotherapy and a subsequent reduction of 29% at 24 months in the high-dose group. The increase in the IgG_4 response, which was associated with a nonpathogenic[32] and protective[33] role induced by immunotherapy,[34,35] was evident in the low- and high-dose groups from the third month of immunotherapy onward, with significant differences in both groups compared with placebo at 3 and 12 months. At 12 and 24 months, IgG_4 levels were greater in the high-dose group than in the low-dose group. This suggests that the increase in IgG_4 levels is, at least in part, dependent on the dose of allergen administered and is consistent with the importance of the route of exposure in the response.[36] The significance of the behavior of immunoglobulins as biomarkers in hypersensitivity, as advocated by the European Academy of Allergy and Clinical Immunology Position Paper,[37] was shown by the Alt a 1–specific IgG_4/IgE (in micrograms per liter) ratio, which showed 13- and 28-fold increases in the low-dose and high-dose groups, respectively, at 12 months and 24- and 50-fold increases at 24 months.

No between-group differences were found in the ratio of patient-reported ADRs per 100 injection visits at 12 months. After inclusion of the placebo group in the high-dose group, no between-group differences were found in the ratio of patient-reported AEs and ADRs per 100 injection visits at 24 months. This suggests that the safety profile of the 2 active groups was similar to that of the placebo group. Despite the study not being able to identify ADR reporting differences, these were reported by 55% of patients, showing a predominantly lower prevalence than published results.[18] As expected for immunotherapy treatment, ADRs were more frequently reported by immunotherapy group patients. However, 77% were local delayed ADRs, for which prevalence and incidence per injection visit were significantly lower and showed no between-group differences in the second year.

Each reported symptom was coded according to the MedDRA dictionary, each event reported (other than those which were unrelated) was considered an ADR, and nonspecific systemic ADRs, probably not IgE mediated and without clinical relevance (24% of reported ADRs), were included in the analysis in line

TABLE II. Ratio of ADRs per 100 injection visits according to group and year of treatment

	Year 1			Year 2		
Type of ADR	Placebo (n = 29)	Active 1 (n = 37)	Active 2 (n = 45)	Placebo–active 2 (n = 22)	Active 1 (n = 32)	Active 2 (n = 40)
Local immediate	0.3	0.4	0.5	0.0	0.3	1.3
Local delayed	8.7	7.8	9.3	2.1	3.2	3.5
Total local	9.0	8.2	9.8	2.1	3.5	4.8
Grade 1	0.5	2.7	1.5	0.6	2.1	0.2
Grade 2	0.5	0.5	0.9	0.0	1.3	0.2
Total	10.0	11.4	12.2	2.7	6.9	5.2

with regulatory requirements.[18] No serious ADRs or systemic ADRs more severe than WAO grade 2 were reported throughout the study.

This is the first clinical trial that has shown the efficacy and safety of allergen immunotherapy with a single protein, Alt a 1. The demonstration of its efficacy is based on the results obtained for the main end point, according to the application guideline,[15] is based on the patient's CSMSs in 2 one-year treatment periods. There are several problems with this measuring tool, such as its subjectivity because it consists of self-assessment by the patient. However, we have seen that for the high dose, it was sensitive enough to demonstrate a significant clinical improvement. Although in the low-dose group the improvement was not statistically significant, contradicting the surrogate efficacy results studied as secondary end points, such as reduction in skin test results and immunoglobulin levels, which showed an improvement.

Another of the factors that could explain this loss of sensitivity is the exclusion of patients associated with noncompliance with the case report form (CRF). As many as 29 patients from the 3 treatment groups had to be excluded from the analysis of the main end point as a consequence of losing or failing to complete the CRF or providing an illegible CRF. This meant that analysis of the main end point had to be performed on the PP rather than ITT population, as recommended for this type of trial. However, in our opinion this does not invalidate the results obtained because the randomization effect has not been violated or biased in the PP population because the original proportion of patients per group has been maintained, the percentage of patients excluded from each of the groups for the PP population was similar (between 27.5% and 32%), and this patient exclusion was not related to a differential intervention in the groups.

Another of the limitations of this study is the number of patients available for the efficacy analysis. However, this number is in line with previous studies with fungi, allowing us to make conclusion on the efficacy and safety of immunotherapy with fungi.

The final composition of the groups, the number of patients in the high-dose was almost double that of the placebo group and 27% more patients in the low-dose group than in the placebo group. However, this does not affect the results because designs in which the active groups contain twice the numbers of the placebo group are valid.

In summary, our results show the efficacy and safety of subcutaneous immunotherapy with Alt a 1, the major allergen of *A alternata*, at a greater dose than usual in patients with allergic rhinitis. There was a clear association between the increased dose of Alt a 1 and greater clinical improvement, as shown by the CSMS and a reduction in IgE levels together with an increase in IgG_4 levels. The reduction in skin test reactivity was similar in both active groups. In addition, there was an association between clinical improvements in patients treated with the high dose of Alt a 1 and 2 biomarkers, skin tests against extracts of *A alternata* and Alt a 1, with a percentage of negative wheals of nearly 50% and an increase in the IgG_4/IgE ratio of up to 50-fold.

We would like to acknowledge the other principal investigators: A. Ferrer from Hospital General de Almansa (Albacete), P. Guardia from Hospital Universitario Virgen de la Macarena (Seville), A. M. Martínez-Cañavate from Hospital Universitario Virgen de las Nieves (Granada), A. Millán from Hospital de Jerez de la Frontera (Cádiz), C. Alías from Clínica Corachán (Barcelona), and F. Moreno from Clínica Dr. Lobatón (Cádiz), as well as all the participating patients. The statistical analysis was provided by BioClever 2005 S.L.

Clinical implications: Treatment of hypersensitivity with a single protein demonstrates efficacy and safety. There was improvement of 63% in CSMSs in a single year of treatment. No ADRs were more severe than WAO grade 2.

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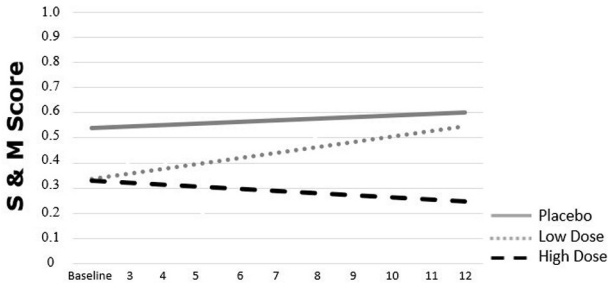

FIG E1. Trends obtained by means of linear regression of CSMSs of each of the groups during the first year of the trial. Low dose, 0.25 μg/mL Alt a 1; high dose, 0.46 μg/mL Alt a 1; and placebo, treatment without Alt a 1. *S & M*, Symptom and medication score.

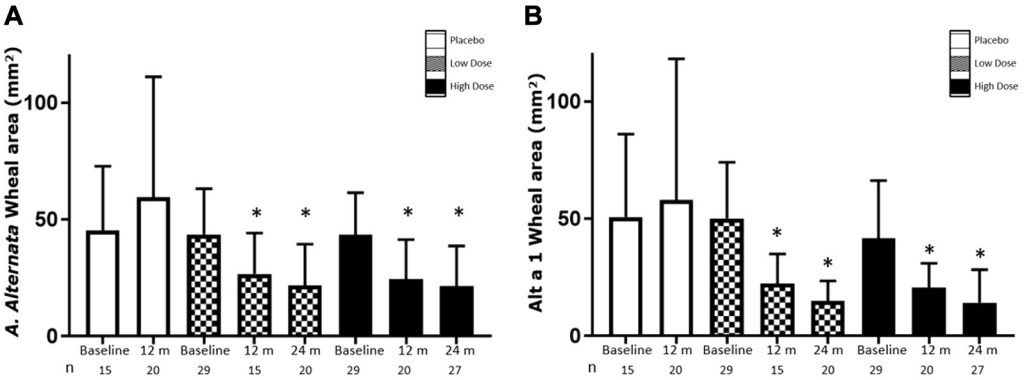

FIG E2. Mean wheal areas of skin prick tests with *A alternata* **(A)** and Alt a 1 **(B)** by group after 1 and 2 years of immunotherapy in the PP population. $*P < .05$ compared with baseline. *n*, Number of patients analyzed at each visit.

TABLE E1. Administration schedule and cumulative doses of Alt a 1 per group

				Low-dose group	High-dose group	Placebo group
Days	**Doses**	**Vial**	**Volume (mL)**	**Alt a 1 (μg) per dose**	**Alt a 1 (μg) per dose**	**Alt a 1 (μg) per dose**
1	1	2	0.1	0.0025	0.0046	
	2	2	0.2	0.005	0.0092	
8	3	2	0.4	0.01	0.018	
	4	2	0.4	0.01	0.018	
15	5	3	0.1	0.025	0.046	0
	6	3	0.2	0.05	0.092	
22	7	3	0.4	0.1	0.183	
	8	3	0.4	0.1	0.183	
37	9	3	0.8	0.2	0.37	
Cumulative dose, induction phase				0.503	0.924	
Monthly	11	3	0.8	0.2	0.37	
Cumulative dose, first year				2.70	4.99	0
Monthly	12	3	0.8	0.2	0.37	0.37
Cumulative dose, second year				5.10	9.43	9.43*

*Includes induction phase and maintenance doses in the same regimen as the high-dose group during the first year of treatment.