

BRIEF COMMUNICATION

Efficacy and safety of *D. pteronyssinus* immunotherapy in local allergic rhinitis: a double-blind placebo-controlled clinical trial

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Abstract

The effects of allergen immunotherapy (AIT) on local allergic rhinitis (LAR) are largely unknown. We conducted the first randomized, double-blind, placebo-controlled (DBPC), phase II trial of *D. pteronyssinus* (DP) subcutaneous AIT (DP-AIT) on LAR (clinicaltrials.gov identifier: NCT02123316). Thirty-six LAR patients received Pangramin PLUS DP or placebo for 24 months. The primary endpoints were symptoms, medication scores, and medication-free days. The secondary included skin test, serum specific IgE and IgG4, nasal allergen provocation test (NAPT), and adverse events. AIT-DP produced significant improvements in both primary and secondary endpoints vs placebo. After 12 months of AIT-DP, we detected a significant and marked increase in allergen tolerance with negative NAPT in 50% of patients, and significant increases of serum sIgG4. Immunotherapy was well tolerated; no systemic reactions were reported. This study demonstrated that AIT-DP is a safe and clinically effective treatment for LAR, confirming that LAR is a new indication for AIT.

Local allergic rhinitis (LAR) is a phenotype of rhinitis characterized by localized immune response in the nasal mucosa in the absence of atopy (1–5).

The immunological characteristics of LAR include a Th2 pattern of mucosal inflammatory response and a positive

response to nasal allergen provocation test (NAPT), with release of inflammatory mediators and local production of specific IgE (sIgE) antibodies (4, 6–10).

Allergen immunotherapy (AIT) is the etiological treatment of allergic rhinoconjunctivitis and asthma with the ability to modify their natural course, increasing immunological tolerance and reducing clinical symptoms and the use of medication (11, 12).

To evaluate the potential role of AIT on LAR, we conducted an observational pilot study (13) in a group of LAR patients to grass pollen. This study showed that subcutaneous immunotherapy (SCIT) with grass pollen was a safe and effective treatment in LAR, with significant improvement in nasal tolerance to NAPTs, clinical symptoms, and reduced use of medication (13). Therefore, we decided to conduct a phase II randomized double-blind placebo-controlled (DBPC) clinical trial to investigate the efficacy and safety of a cluster schedule of SCIT with *D. pteronyssinus* (DP) in patients with LAR. The publication is performed according to the CONSORT guidelines.

Abbreviations

AIT, allergen immunotherapy; AIT-DP, allergen immunotherapy with *D. pteronyssinus*; CdSMS, combined daily symptoms–medication score; DBPC, double-blind placebo-controlled; DP, *D. pteronyssinus*; EAACI, European Academy of Allergy and Clinical Immunology; IDT, intradermal test; LAR, local allergic rhinitis; MedDRA, Medical Dictionary for Regulatory Activities; MFD, medication-free days; NAPT, nasal allergen provocation test; SAR, systemic allergic rhinitis; SCIT, subcutaneous immunotherapy; sIgE, specific IgE; sIgG4, specific IgG4; SPT, skin prick test; TdMS, total daily medication score; TdSS, total daily symptoms score; VAS, visual analog scale; VOL 2–6 cm, volume of the nasal cavity between 2 and 6 cm.

Methods

Detailed methods are described in the Online Supporting Information (Appendix S1).

We performed a 2-year, phase II, prospective, randomized, DBPC, parallel-group investigator-initiated trial in LAR patients sensitized to DP (clinicaltrials.gov identifier: NCT02123316). This first manuscript includes the methodological aspects of the study and the results of the clinical effect, immunological allergen tolerance and safety of AIT. In a second manuscript more detailed results of nasal and systemic immunological changes will be reported.

Thirty-six patients were randomized (1 : 1) to receive subcutaneous AIT with Pangramin Plus (ALK-Abelló, S.A., Spain) *D. pteronyssinus* (AIT-DP) or placebo for 24 months (Table S1). All participants provided informed written consent.

The primary endpoints were total daily symptoms (TdSS), total daily medication scores (TdMS), combined daily symptoms–medication score (CdSMS), and medication-free days (MFD). Secondary endpoints included the following: allergen tolerance measured by NAPT threshold, skin testing, serum levels of DP-sIgE and DP-sIgG4, and adverse events. The differences regarding clinical response, immunological changes, and safety between AIT-DP and placebo were analyzed at baseline, and 1, 3, 6, 12, 18, and 24 months after starting the treatment.

Results

Forty patients were included and 36 were randomized and analyzed (intention-to-treat analysis). Eight patients dropped out (22.2%), five in the active and three in the placebo group. The flowchart of patients throughout the study is shown in Fig. S1. No significant differences were observed in baseline characteristics of the included patients (Table S2).

Clinical response

AIT-DP produced a clinical improvement with an overall significant reduction in mean TdSS (42%, $P = 0.002$), TdMS (45%, $P = 0.013$), and CdSMS (42%, $P = 0.001$) and a significant increase in mean MFD (89.74%, $P = 0.031$) after 24 months compared to placebo (Fig. 1). The significant improvement in TdSS, CdSMS, and MFD started after 6 months of AIT and progressed throughout the study compared to placebo and with baseline. Patients treated with placebo showed a significant worsening in all clinical scores after 24 months of treatment (Fig. 1).

Immunological response

Patients treated with immunotherapy developed a significant increase in nasal tolerance to NAPT from the 6th to the 24th month compared to placebo and with baseline (Fig. 2A). At the end of the study, 67% of AIT-treated patients tolerated a concentration of Der p1 ≥ 10 times higher than that at baseline, and NAPT was negative in 9/18 (50%), in four of them after 3 months. Placebo group presented a progressive and

significant reduction in nasal tolerance to NAPT after 6 months compared with baseline (Fig. 2A).

In the active group, serum DP-sIgG4 antibodies progressively increased, becoming significant after 12 months compared to placebo and with baseline (Fig. 2B). AIT induced a significantly lower sIgE/sIgG4 ratio compared with placebo after 24 months ($P = 0.023$). No significant changes were observed for the placebo group.

No significant differences were found between groups for skin testing with DP. Four AIT-treated patients and two placebo-treated patients had positive SPT and IDT with detectable serum levels of sIgE. AIT-DP induced an increase in serum levels of DP-sIgE at 6 months, followed by a decrease. In contrast, patients treated with placebo and positive skin tests exhibited a progressive increase in serum levels of sIgE to DP from the 3rd month till the end of the study (Fig. 2C). The limited number of cases with detectable serum levels of allergen-sIgE did not allow us to perform statistical analysis.

Comparisons between AIT-DP patients with negative skin tests and those who developed positive response to skin tests during the study yielded a similar response, with reduction of the CdSMS, increases of MFD, negativization of nasal challenge in 3/4 of these patients, and increased levels of DP-sIgG4 (data not shown).

Adverse events

AIT-DP was well tolerated; one patient reported three moderate local reactions with the highest dose. The patient recovered fully without treatment and did not require modification of the treatment schedule. No systemic reactions occurred.

Discussion

To our knowledge, this study represents the first reported phase II, randomized, DBPC clinical trial of AIT conducted in LAR patients. The aim was to investigate the efficacy and safety of a cluster schedule of SCIT with *D. pteronyssinus* in LAR patients using standardized extracts.

The clinical efficacy of SCIT in systemic allergic rhinitis (SAR) has been established in recent meta-analyses of randomized DBPC trials (14), and in LAR in a preliminary pilot observational study of grass pollen SCIT (13).

The results of the present study confirmed the clinical effectiveness of AIT-DP in LAR, with significant reduction of symptoms score (42%), medication score (45%), and combined symptoms–medication score (42%) and a significant increase in medication-free days (89%). This study has also demonstrated the short-term clinical effect of AIT in LAR as previously reported in SAR (15). The clinical effects of SCIT started very early, after only 1 month of immunotherapy, and progressed throughout the study becoming significant from the 6th month to the end of the study. In contrast, the placebo group of patients had a worse evolution during the study, with a significant increase in the combined symptoms–medication score from the 12th month onward. The good results obtained in the active group where the dropout rate was higher than in

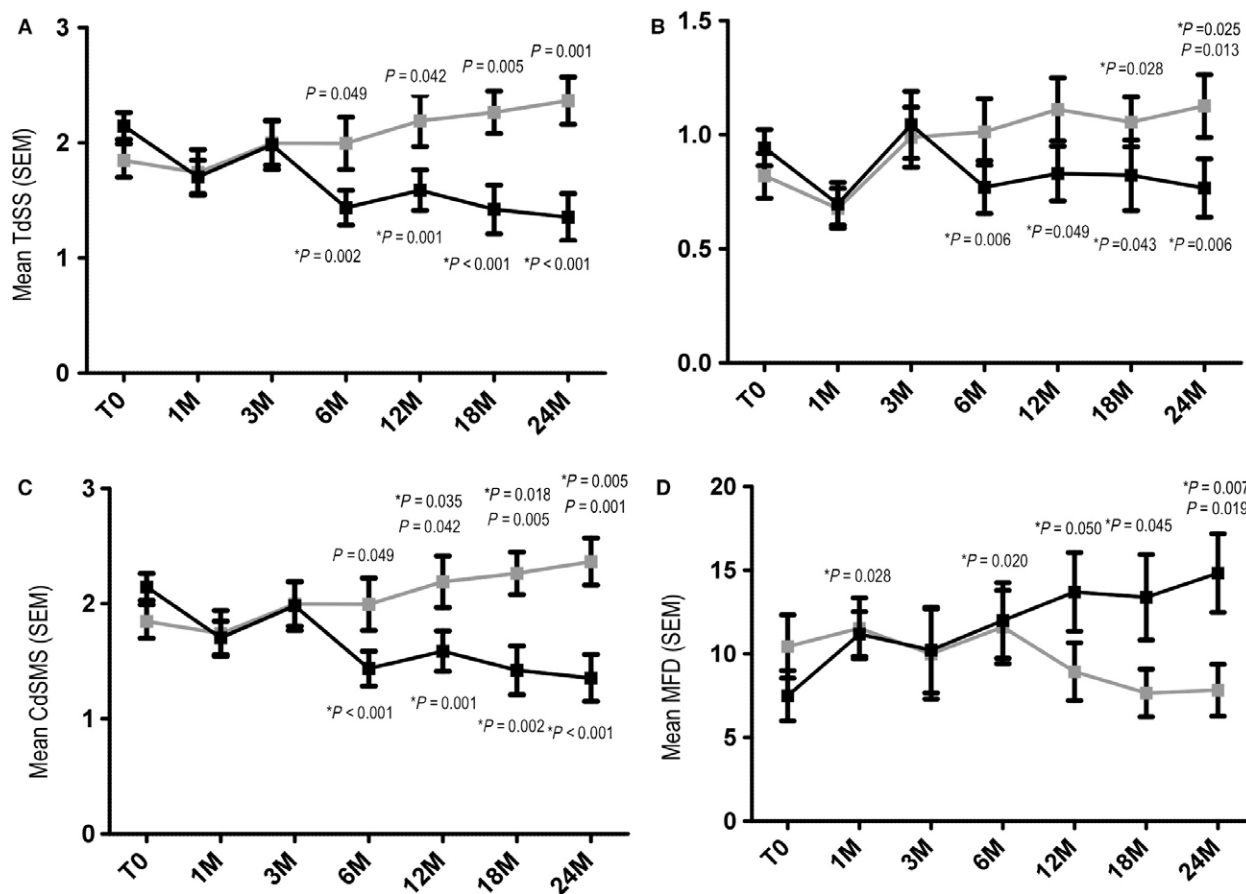


Figure 1 Clinical changes during treatment with allergen immunotherapy with *D. pteronyssinus* (AIT-DP) vs placebo. (A) Total daily symptoms score (TdSS). (B) Total daily medication score (TdMS). (C) Combined daily symptoms–medication score (CdSMS). (D) Medication-free days (MFD). Gray line: placebo group; black line: DP-AIT group.

Data are shown as mean and SEM. Comparisons between groups were made by the Mann–Whitney *U*-test (*P* values). Comparisons within groups during 24 months were made by the Wilcoxon test (**P* values).

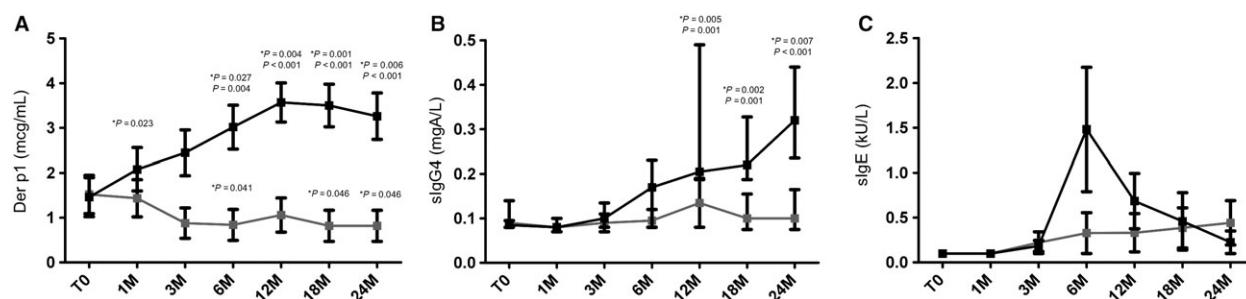


Figure 2 Immunological effect induced by allergen immunotherapy with *D. pteronyssinus*. (A) Nasal tolerance to Der p1 (mcg/mL). (B) Serum levels of specific IgG4 (sIgG4) to DP (mgA/mL). (C) Serum levels of sIgE to DP (kU/L). Gray line: placebo group; black line: DP-AIT group.

AIT group. Data are shown as mean and SEM. Comparisons between groups were made by the Mann–Whitney *U*-test (*P* values). Comparisons within groups during 24 months were made by the Wilcoxon test (**P* values).

the placebo group make the clinical effect of AIT-DP in LAR more relevant.

Clinical outcomes, although relevant to the patient, do not always adequately reflect the effectiveness of AIT, may

not detect the individual variation in allergen exposure, and do not discriminate between symptoms induced by nonallergic and allergic mechanisms because of the nasal hyperresponsiveness (16). To control variability in allergen

exposure and focus on SAR mechanisms, NAPT and, more recently, environmental exposure chambers have been used to assess responses to allergen immunotherapy in SAR (16, 17). Our study demonstrates that AIT-DP leads to an important improvement in allergen tolerance in LAR measured by NAPT, a useful and safe diagnostic test for LAR patients (1, 6–8). Two-thirds of patients tolerated an allergen concentration over 10 times higher than basal concentration, and NAPT was negative in half of the patients. The previous pilot observational study on grass SCIT conducted in LAR obtained similar results (13).

AIT induces an immune modulation of the allergen-specific IgE and IgG response in SAR, with a transient increase in serum sIgE, accompanied by an increase in the IgG4 isotype (18). Variation in the sIgE/sIgG4 ratio has been considered a biomarker for AIT evaluation in SAR (19). In our study, a similar immune response to AIT-DP was observed in LAR patients, with a progressive increase in serum sIgG4 levels, which became significant after 12 months of DP-SCIT. Also, those patients with positive skin tests presented an increase in serum sIgE during the first 6 months of DP-SCIT, followed by a decrease with a significantly lower ratio of sIgE/sIgG4 compared with placebo.

In our study, the appearance of positive skin test and serum levels of sIgE to DP in the placebo and in the active group could be related to the repetition of intradermal skin tests up to six times, as previously reported in SAR (20). However, AIT-DP-treated patients who developed positive skin tests had the same clinical and immunological improvement as the rest of treated patients with negative skin tests.

In summary, this first phase II, randomized, DBPC clinical trial of D. *Pteronyssinus* SCIT in LAR patients has clearly demonstrated that AIT-DP is a safe and clinically effective treatment for LAR, having an immunomodulatory effect comparable to that previously demonstrated in SAR.

The strong increase in nasal tolerance to the allergen and in the induction of an sIgG4 antibody response with a decrease in serum sIgE provides a proof of concept that LAR is an IgE-mediated allergic disease and confirms that LAR is a new indication for AIT. The results obtained in this study reinforce the need for an early diagnosis and etiological treatment of LAR in patients with non-atopic rhinitis.

Further studies are necessary to compare the clinical efficacy and immunological response to different routes, schedules, allergens, and allergen components for LAR immunotherapy.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed Materials and Methods

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Online Supporting Information

Appendix S1

Detailed Materials and Methods

Efficacy and safety of *D. pteronyssinus* Immunotherapy in Local Allergic Rhinitis: Double-Blind Placebo-Controlled Clinical Trial

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METHODS

Study design

We performed a 2-year, phase II, prospective, randomized, DBPC, parallel-group study in LAR patients sensitized to DP. All participants provided informed written consent and the study was approved by the Ethic Committee of Malaga and the Spanish Drug Agency. This study was an investigator-initiated trial conducted in compliance with the principles established in the World Medical Association Declaration of Helsinki (1). The clinicaltrials.gov identifier was NCT02123316.

Procedures

Thirty-six patients were randomized 1:1 according to a computer-generated randomization schedule to receive subcutaneous immunotherapy with an extract of *D. pteronyssinus* (AIT-DP) or placebo for 24 months (Table S1). Randomization numbers were assigned to subjects by providing the next available number and treatment kit (ordered sequentially). Placebo and active treatments were produced and blinded by ALK-Abelló S.A, Madrid, Spain, and were identical in appearance, smell, taste, and packaging to ensure treatment blinding was maintained. The differences regarding clinical response, immunological changes, and safety between

AIT-DP and placebo were analyzed at baseline, and 1, 3, 6, 12, 18 and 24 months after starting the treatment.

Forty-three eligible patients with LAR to DP, diagnosed consecutively, were invited to participate in the study, 3 of them declined to participate (6.9%). Forty included subjects underwent 4-week run-in periods to evaluate their baseline clinical and immunological situation by means of symptom and medication scores, SPT, NAPT, and determination of serum total IgE, and sIgE and sIgG4 against DP.

The primary endpoints of the study were total daily symptoms (TdSS), total daily medication scores (TdMS) and medication free days (MFD). Following the indications of a recent EAACI (European Academy of Allergy and Clinical Immunology) position paper the average combined daily symptom-medication score (CdSMS) (2) was also included as a primary endpoint for evaluating the clinical response to treatment.

Secondary endpoints included nasal tolerance to DP tested by NAPT threshold, skin testing, serum levels of DP-sIgE and DP-sIgG4, and adverse events.

Patients

Patients had to be aged from 18 to 55 years with a history of LAR sensitized to DP confirmed by a positive response to NAPT, and/or a positive level of nasal DP-sIgE (> 0.35 kU/L), with negative results in skin testing (SPT and intradermal test (IDT) to DP, and serum determination of DP-sIgE DP (<0.35 kU/L). Determination of DP-sIgE in nasal lavage was performed as previously described (3).

Patients with immunologic diseases, severe psychological disorders, severe atopic dermatitis, FEV1 <70% after appropriate drug therapy, uncontrolled asthma, chronic rhinosinusitis and/or nasal polyp, treatment with β -blockers, previous specific immunotherapy, history of hypersensitivity or intolerance to the excipients and/or test medication or other medications in the protocol, inability to adequately perform diagnostic tests or treatment, or pregnancy were excluded.

Immunotherapy protocol

The active treatment consisted of a *D. pteronyssinus* extract titrated in Mass Units,

i.e., with the major allergens Der p 1 and 2 quantified in µg/mL (Pangramin Plus® , ALK-Abelló S.A, Madrid, Spain). During the initial phase a cluster schedule was used (Table S1). The placebo treatment contained the same ingredients as the active treatment except for the active substance. The duration of the treatment was 24 months. All doses were administrated at the Allergy Department and under the strict supervision of the investigators-involved in the trial.

Clinical response

Patients were instructed to keep a diary during the previous 4 weeks (28 days) of all visits (0, 1, 3, 6, 12, 18 and 24 months), for a daily evaluation of nasal symptoms (sneezing, rhinorrhea, nasal itch, nasal obstruction) and conjunctival symptoms (itchy/red/watery eyes), and the use of rescue medication.

The intensity of symptoms was recorded according to a 0-3 symptoms score: 0, no symptoms; 1, mild symptoms (symptom clearly present, but minimal awareness; easily tolerated); 2, moderate symptoms (definitive awareness of symptom that is bothersome but tolerable); and 3, severe symptoms (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping). The maximum total daily symptom score (TdSS) was 12 for patients with nasal symptoms only, and 15 for patients with nasal-conjunctival symptoms.

The use of rescue medication was scored as follows: 1 tablet of antihistamines (1 point), 1 administration of eye drops (1 point), and 1 dose of 400 mcg nasal beclomethasone or its equivalent (1.4 points) (4). The maximum total daily medication score (TdMS) was 3.4 points.

The average Combined daily Symptom-Medication score (CdSMS) was calculated as $CdSMS = (TdSS / \text{number of symptoms.}) + TdMS$.

Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 14.0. Systemic adverse drug reactions were classified according to the EAACI grading system (5).

85

86 **Immunological changes**

87 Skin testing

88 For the initial evaluation and diagnosis SPT with a panel of common prevalent
89 inhalant allergens, including pollens (*lollium*, *phleum*, *cupressus*, *olea europeae*,
90 *platanus orientalis*, *chenopodium*, *salsola*, *artemisia*, and *parietaria judaica*) , house
91 dust mites (*D. pteronyssinus*, *D. Farinae*, and *L. Destructor*), molds (*Alternaria*
92 *alternata*, *aspergillus fumigatus*, *cladosporium herbarum* and *penicillium notatum*),
93 and animals dander (dog, cat and hamster) (ALK, Madrid, Spain), was carried out.
94 IDT with freshly reconstituted freeze dried *D. Pteronyssinus* (0.4 mg/mL) (ALK,
95 Madrid, Spain) was performed. For the follow-up, SPT and ID were carried out with
96 DP (ALK, Madrid, Spain).

97

98 Inmunoglobulins

99 Concentrations of total IgE, sIgE, and sIgG4 antibodies against DP were determined
100 by ImmunoCAP-FEIA in serum samples, according to manufacturer instructions
101 (Thermo Fisher Scientific, Uppsala, Sweden). The limit of detection of the assay was
102 ≥ 0.35 kU/l for sIgE and 0.07 mgA/L for sIgG4.

103 Nasal allergen provocation test

104 Bilateral NAPT, considered the gold standard in the diagnosis of LAR (3,6,7), was
105 carried out as described previously (3,6). Briefly, patients without/mild symptoms were
106 challenged by intranasal route with 100 mL of saline in each nostril to exclude nasal
107 hyperreactivity. If negative, 15 minutes later NAPT with 100 mcL of reconstituted
108 freeze-dried allergen solutions of DP (0.04-0.4-1-2-4 mcg/mcL. ALK, Madrid, Spain) at
109 room temperature were applied in each nostril at 15 minute intervals. The response
110 was monitored by acoustic rhinometry (SRE 2000 rhinometer, Rhinometrics, Lyngø,
111 Denmark) and VAS. Acoustic rhinometry was performed following the guidelines of the
112 Standardization Committee on Acoustic Rhinometry (8), measuring the volume of the

nasal cavity that corresponds to the lower turbinate (VOL 2–6 cm) in each nostril. Symptoms of nasal obstruction, rhinorrhea, itching, sneezing and ocular symptoms were monitored at each time point by placing a vertical mark on a horizontal VAS of 100 mm. The total range of the VAS during NAPT (VAS-NAPT) was 0–500 mm. A positive NAPT response was considered when a decrease $\geq 30\%$ in the nasal cavity (VOL 2–6 cm) and an increase $\geq 30\%$ in the total VAS was observed, compared to baseline (3,6). The nasal response was monitored at baseline, 15 minutes after each aeroallergen concentration, and at 1 hour and 24 hours after the last concentration. The NAPT was stopped when a positive response occurred.

Statistical analysis

Based on previous data (9), a sample size of 18 participants per group was calculated for a 95% chance of detecting a 45% improvement in symptoms-medication score after immunotherapy with a statistical power of 85% and taking into account an estimated dropout rate of 25% between randomization and end of follow-up. Statistical analysis was performed using data from all participants (intention to treat analysis). Comparisons between groups were performed by Student t-test for normal distributions, and non-parametric Mann-Whitney U-test for non-normal distributions. Comparisons within groups before and at different time points after starting treatment were performed by the Wilcoxon signed-rank test. Qualitative variables were analyzed by Chi² test. Least square means estimated from the analysis of covariance model were used to determine the percentage treatment difference relative to placebo, which was calculated as follows: (Active - Placebo) / Placebo x 100%. All reported p-values represent two-tailed tests, with values $p \leq 0.05$ considered statistically significant. The Bonferroni correction was applied for multiple comparison testing.

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168 after preseasonal immunotherapy with grass pollen. J Allergy Clin Immunol
169 2011; 127:1069-71.
170

170 **ONLINE Table S1. Treatment schedule for initial phase of treatment**

Day	Vial	Volume (ml)*	Dose (mcg Der p 1 - Der p 2)
1	A	0.1 / 0.2	0.03/0.02 - 0.06/0.04
7		0.4 / 0.6	0.12/0.08 - 0.18/0.12
14	B	0.1 / 0.2	0.3/0.2 - 0.6/0.4
21		0.4 / 0.4	1.2/0.8 - 1.2/0.8

171 *The interval between doses was 30 minutes.

172 The first full dose of 0.8 mL (vial B) given 15 days after this initial phase was followed
 173 by monthly doses of 0.8 mL.

174

175

175 **ONLINE Table S2. Baseline demographic and clinical characteristics of**
176 **participants**
177

	Placebo	DP-AIT	P value
Subjects, N	18	18	
Women, N (%)	14 (77.8)	13 (72.2)	1.000
Age*, mean $\pm$ SD	42.6 $\pm$ 11.5	37.22 $\pm$ 10.8	0.203
Duration of LAR (years) *, mean $\pm$ SD	8.94 $\pm$ 6.0	7.39 $\pm$ 3.4	0.345
Rhinitis severity, N (%)			
Moderate	13 (72.2)	12 (66.7)	0.717
Severe	5 (27.8)	6 (33.3)	
Comorbidities			
Conjunctivitis, N (%)	12 (66.7)	13 (72.2)	0.717
Asthma, N (%)	4 (22.2)	6 (33.3)	0.457
TdSS *Y	5.2 $\pm$ 1.5	5.8 $\pm$ 1.2	0.133
TdMS *Y	0.8 $\pm$ 0.4	0.9 $\pm$ 0.3	0.204
CdSMS *Y	1.8 $\pm$ 0.6	2.1 $\pm$ 0.5	0.117
MFD *Y	10.4 $\pm$ 8.0	7.5 $\pm$ 6.4	0.232
Positive NAPT			
N (%)	18 (100)	18 (100)	1.000
<i>Der p1</i> (mcg/mL), median (range) **	0.4 (0.04 – 4)	0.4 (0.04 – 4)	0.584
Local poly-sensitization***			
N (%)	2 (11.1)	3 (16.7)	1.000
Grass pollen	2 (11.1)	2 (11.1)	
Olea pollen	0 (0)	1 (5.6)	
Alternaria	0 (0)	0 (0)	
Serum levels, median (range) **			
Total IgE (kU/L)	30 (4 – 134)	18 (4 – 120)	0.823
DP-sIgE (kU/L)	<0.35	<0.35	1.000
DP-sIgG4 (mgA/L)	0.09 (0.05 – 0.18)	0.09 (0.07 – 0.11)	0.416

178 *: Student's t test (data expressed as mean $\pm$ SD). **: Mann-Whitney U test (data
179 expressed as median (range)). ***: Fisher's exact test. Y: Average score during the 4
180 weeks prior to the first dose of treatment. CdSMS = (TdSS / number of symptoms) +
181 TdMS). MFD (medication free days). TdMS (total daily medication score). TdSS (total
182 daily symptom score).
183

183 **LEGENDS FOR ONLINE SUPPLEMENTARY FIGURES**

184 **ONLINE Figure S1. Flowchart of patients throughout the study**

