

Letter to the Editor

Is intralymphatic immunotherapy ready for clinical use in patients with grass pollen allergy?

To the Editor:

Allergen-specific subcutaneous immunotherapy confers significant clinical benefits in patients with grass pollen-induced allergic rhinoconjunctivitis. Nevertheless, treatment is costly; it lasts up to 5 years and can occasionally lead to systemic adverse reactions.¹ Improving immunotherapy by means of direct administration of the allergen extract into a lymph node with 3 intralymphatic injections has been attempted in 3 previous studies. Two of the studies have shown efficacy in nasal provocations for grass pollen² and cat dander.³ One small study combining patients with grass and birch pollen allergy has demonstrated an improved global evaluation of seasonal symptoms.⁴ However, no double-blind, placebo-controlled studies have yet been published looking at efficacy based on seasonal symptom and medication scores, as proposed by the US Food and Drug Administration and the European Medical Agency (EMA).^{5,6} To study the efficacy based on patients' seasonal diaries, we performed a randomized, double-blind, placebo-controlled study testing 3 and 6 preseasonal injections of intralymphatic immunotherapy (ILIT) with alum-adsorbed *Phleum pratense*.

A detailed methodology is given in the [Methods](#) section (in this article's Online Repository at www.jacionline.org), but briefly, 45 adult subjects with a clinical history of grass pollen-induced rhinoconjunctivitis were recruited. Grass pollen allergy was confirmed by means of skin prick tests (SPTs) and specific IgE measurements. Patients with contraindications to immunotherapy were excluded.¹ Additional sensitizations were permitted as long as the patient did not have symptoms overlapping the grass pollen season. By using the minimization technique,⁷ patients were randomized into 3 groups receiving 6 injections of 1000 standard quality units (SQ-U; x6 group), 3 injections of 1000 SQ-U followed by 3 injections of placebo (x3 group), or 6 injections of placebo. The primary efficacy measure was the combined symptom and medication score (SMS) during the grass pollen season. After the treatment, as proposed by the EMA.^{5,6} Additionally, 2 assessments were made: a global seasonal assessment and assessment with the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ).

Forty-five patients were allocated to treatment, 43 started treatment, and 38 were available for evaluation of the primary efficacy parameter (see [Fig E1](#) in this article's Online Repository at www.jacionline.org). The groups were well balanced (see [Table E1](#) in this article's Online Repository at www.jacionline.org). On the basis of the primary outcome, the combined mean SMS for the entire grass pollen season, no significant clinical efficacy of ILIT could be demonstrated ([Fig 1](#)). On the contrary, the x3 group displayed a slightly negative outcome, with a 12% higher total SMS than the placebo group ($P = .612$). During the peak season, both treatment groups had a higher SMS than the placebo group. For the x3 group, the SMS was significantly higher than in the placebo group (76%, $P = .009$). Week-per-week comparison of the SMS showed a significant difference between the x3 and placebo groups for

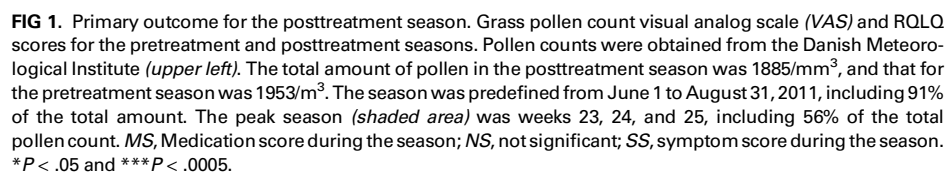
weeks 22 and 23 in favor of placebo treatment. No other weeks showed a significant difference. Comparing all treated patients (ie, combining the 2 treatment groups; [Fig 1](#) and see [Table E2](#) in this article's Online Repository at www.jacionline.org) with the placebo-treated patients did not reveal any significant difference in the mean SMS ($P = .856$, t test). The 2 treatment groups experienced no significant change in global assessment or RQLQ scores as opposed to the placebo group, who improved significantly from pretreatment to posttreatment ($P = .0014$); however, no significant differences between groups were found ([Fig 1](#) and [Table E2](#)).

In most cases, treatment was painless. Only 1 patient withdrew because of discomfort during the examination and treatment of side effects. All adverse events were mild; no moderate, severe, or life-threatening symptoms (grade 2-4¹) occurred (see [Table E1](#)). Local reactions at the injection site were seen in 83% of the actively treated patients ($P < .0001$).

Intradermal test readings after 24 hours ([Fig 2](#)) and grass pollen SPTs showed significantly reduced reactions after treatment in all 3 groups compared with those before treatment but without differences between the groups. The allergen-specific IgE level has a natural decrease out of season and an increase during the season.⁸ The IgE level for the placebo group followed this pattern. For the treated patients, an increase in IgE levels after treatment was apparent, and the IgE ratio (post/pre) was significantly 1.5- and 1.4-fold increased for the x6 and x3 groups. Actively treated patients had a significant increase in IgG₄ levels after treatment (x6: $P = .0099$, x3: $P = .0128$). In addition, a significant 2.8-fold increase ($P = .0005$) was seen in the x6 group and a 2.9-fold increase was seen in the x3 group for IgG₄ levels, indicating a recognition/modulation of the immune system in the treated patients. There was no apparent change in results of the grass pollen-induced histamine release test for any group. T-cell cytokines showed a significantly lower IFN- γ level in the x3 group in comparison with the placebo group and a tendency toward higher IL-10 levels. No group differences were seen in IL-4 and forkhead box protein 3 (FoxP3) expression ([Fig 2](#)).

Concluding on our study, the ILIT for grass pollen allergy revealed no improvement in patients' registered symptoms and use of rescue medication or in quality-of-life or intradermal test scores in spite of weak signs of immune modulation of IgE and IgG₄. The reason that ILIT appears to be effective in some studies²⁻⁴ but did not show efficacy in ours might be related to the different outcome parameters. The appropriate efficacy measures for specific immunotherapy can be discussed; nevertheless, it would seem logical that the combined symptom and medication registration should be a key factor in the primary end points, as recommended by the World Allergy Organization.⁶

Considerations as to which parameters could enhance efficacy are not straightforward. The amount of allergen could be increased because in immunotherapy the allergen dose is linked to efficacy generally.¹ However, on the basis of our study, it is not recommendable to increase the amount of allergen without an up dosing regimen because systemic side effects were seen in 9% of the patients. Perhaps the ILIT treatment could be effective in combination with hypoallergenic recombinant allergens because higher amounts of allergen can be used. Consequently, on the basis of guidelines from regulatory agencies with respect



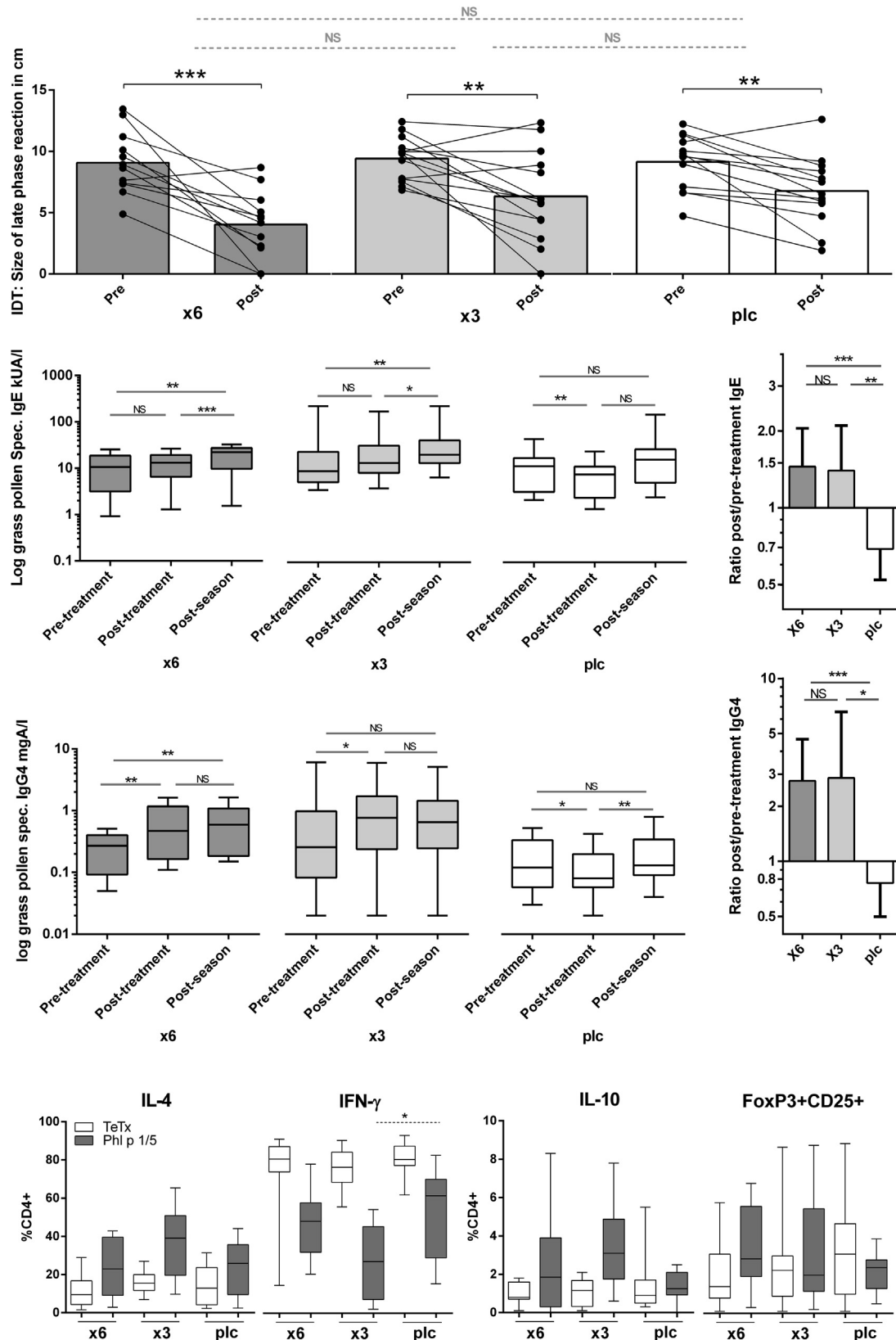


FIG 2. Paraclinical parameters: intradermal skin tests (IDT) and IgE, IgG₄, T-cell intracellular cytokine, and regulatory T-cell marker measurements. IgE and IgG₄ concentrations and posttreatment/pre-treatment ratios (logarithmic scale) are shown. The ratio increased significantly after treatment in the treated groups. Blood samples for determination of allergen-specific IgE and IgG₄ levels by using CAP were drawn before treatment, after 3 and 6 treatments, and after the posttreatment season. Intracellular T-cell cytokines after allergen or tetanus toxoid stimulation of PBMCs were identified. **P* < .05, ***P* < .005, and ****P* < .0005.

to accepted primary outcomes, efficacy still has to be documented for this treatment. Even though the number of treated patients in our study exceeds the sum of the 2 previous placebo-controlled studies, it was small, and further adequately performed large-scale, double-blind, placebo-controlled studies focusing on optimizing allergen extracts, dose regimens, or both are required before ILIT is ready for clinical use.

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METHODS

Study design and inclusion of subjects

The study was randomized, double-blind, and placebo-controlled and performed at the Allergy Clinic, Copenhagen University Hospital, Gentofte, Denmark. Male and female subjects aged 18 to 60 years with a clinical history of grass pollen-induced rhinoconjunctivitis for the last 2 years were recruited primarily by means of advertisement. Grass pollen allergy was confirmed by using SPTs (SPT wheal diameter ≥ 3 mm with Soluprick SQ 10 HEP *Phleum pratense*; ALK-Abelló, Hørsholm, Denmark) and specific IgE measurement (Timothy grass pollen-specific antibody [IgE] class ≥ 2 ; Phadia CAP-FEIA, Uppsala, Sweden). Patients with additional sensitizations were permitted as long as they did not have symptoms overlapping the grass pollen season. Patients with severe or uncontrolled seasonal asthma or grass pollen immunotherapy within the last 5 years were excluded, as were patients with conditions contraindicatory to immunotherapy.^{E1}

Randomization and masking

The group allocation by minimization^{E2} and the preparation of study medicine were performed by persons not involved in the trial. Staff administering medication and assessing outcomes were blind to the treatment. One doctor performed all treatments and assessments. Blinding was maintained until all assessments and recordings were completed. The study was conducted in accordance with good clinical practice guidelines^{E3} with written informed consent and was monitored by the Danish GCP Unit, Bispebjerg University Hospital, Copenhagen, Denmark. The protocol was approved by the Danish Medicines Agency and the local ethics committee (no, H-2-2010-020) and complies with the Consort 2010 guidelines for reporting randomized trials.^{E4}

Intervention

The patients were treated 6 times with an aseptic ultrasound-guided intralymphatic injection of 0.1 mL of 10,000 SQ-U/mL of depot grass pollen extract (Alutard, *Phleum pratense*, ALK-Abelló), which corresponds to 0.2 μ g of Phl p 5, or saline. Placebo and active medication were identical in color and amount. The 6 treatments were conducted with a minimal interval of 14 days from the beginning of February until April 2011. All patients were observed for 1 hour after the injection.

Treatment was postponed in cases of peak flow of less than 80% of normal value or increased allergy symptoms. All injections were performed in the left groin. Lymph nodes were easily detected by using the NanoMaxx ultrasound system (transducer L38N) with color Doppler measurements. The correct placement of the fluid was documented by means of photography.

Assessments

The primary efficacy measure was the combined SMS during the grass pollen season after treatment. The patients recorded nasal and eye symptoms on a 4-point scale (symptom score [SS]) and daily medication (medication score [MS]), as proposed by the EMA and the US Food and Drug Administration guidelines.^{E5,E6} Rescue medication to be used in the grass pollen season was provided by the clinic and scored as follows:

- 1 tablet of antihistamine (8 mg of acrivastine) = 4 points (maximum $\times 3$);
- 1 drop of antihistamine eye drops (0.5 mg/mL levocabastine) = 2 points (maximum $\times 3$); and
- 1 tablet of corticosteroid (5 mg of prednisolone) = 6 points (maximum $\times 1$).

The combined daily SMS was calculated as follows:

$$0.5 \times (\text{Mean daily SS}/18) + 0.5 \times (\text{Mean daily MS}/24).$$

Additionally, 2 assessments were made: a 10-point visual analog scale and the RQLQ^{E7} with 28 items in 7 domains.

SPTs and intradermal tests were performed in duplicate before and after treatment. Soluprick *Phleum pratense* and disposable prick

lancets (ALK-Abelló) were used for SPTs; saline and histamine, 10 mg/mL, were used as negative and positive controls, respectively. Intradermal tests were performed with 0.01 mL of 5000 SQ-U/mL Aquagen *Phleum pratense*. Readings were made after 20 minutes (immediate reaction) and 24 hours (late-phase reaction).

Immunologic tests

Blood samples for determination of allergen-specific IgE and IgG₄ levels were drawn before the start of treatment, after 3 and 6 treatments, and after the posttreatment season. Timothy grass-specific IgE and IgG₄ antibody levels were determined by using CAP (Pharmacia), as specified by the manufacturer. The histamine release test was performed before and after treatment by using the histamine release glass-fiber technique with 10-fold titrations of grass pollen extract (RefLab, Copenhagen, Denmark).^{E8,E9} All patients reacted positively to grass pollen extract, but only 8 patients changed their cellular sensitivity (ie, reacting to a different threshold dose from before to after treatment), and thus we found nonsignificant changes both within and between treatment groups (results not shown). Intracellular T-cell cytokines after allergen or tetanus toxoid stimulation of PBMCs were identified. PBMCs were purified from 20 mL of whole blood by using Lymphoprep from Medinor (Oslo, Norway) and stained with carboxyfluorescein succinimidyl ester (CFSE) from Invitrogen (Carlsbad, Calif), according to the method of Parish et al.^{E10} The cells were cultured at 2×10^6 cells per mL in 6- or 12-well flat-bottom plates (Nunc, Roskilde, Denmark) in RPMI 1640 medium (Sigma, Saint Louis, Mo) supplemented with 100 U/mL penicillin and 100 μ g/mL streptomycin, 1 mmol/L L-glutamine (Invitrogen), 50 μ mol/L β -mercaptoethanol (Sigma), and 5% (vol/vol) human AB serum from (Sigma). The PBMCs were cultured for 7 days with either medium control, 10 μ g/mL tetanus toxoid from SSI (Copenhagen, Denmark), or 2 μ g/mL Phl p 1 and Phl p 5 kindly provided by ALK-Abelló.

At day 7, the cultures were harvested and fixed and permeabilized with IntraPrep from Beckman Coulter (Fullerton, Calif), according to the manufacturer's protocol, and stained for CD4-PE-cy5 or PE-cy7 and IL-10-PE-cy7 from BioLegend (San Diego, Calif); IFN- γ -PE, IL-4-PE-cy7, or FoxP3-PE from eBioscience (San Diego, Calif); and CD25-ECD from Beckman Coulter and analyzed by using a Beckman Coulter FC500 MPL flow cytometer and FlowJo from Treestar software (Ashland, Ore).^{E11,E12} Production of cytokines and expression of FoxP3 from the allergen-specific cells were determined from the CD4⁺CFSE^{low} population.

Adverse events and allergic side effects

Safety data were analyzed separately as local or systemic allergic side effects and other adverse events. Side effects occurring within 1 hour of injection were documented by the investigator. Systemic reactions were graded according to the 2006 European Academy of Allergy and Clinical Immunology Position Paper.^{E1}

Statistical methods

The determination of sample size needed for this study was calculated, given that 13 patients per group were required to detect a 25% relative effect of 3 allergen injections compared with the placebo group with a 5% risk of type 1 or 2 error. Fifteen patients were assigned to each group to account for eventual dropouts, resulting in a total of 45 patients. Safety analysis followed the intention-to-treat format and included all subjects who received at least 1 injection. Primary end point analysis followed the efficacy population, being intention to treat with missing data according to recommendations.^{E13}

SMSs were normalized, averaged over the grass pollen season, and analyzed by means of ANOVA with Tukey correction. Additionally, all treated patients were compared with the placebo group by using a *t* test. The mean visual analog scale and RQLQ (domain and overall) scores were analyzed by using ANOVA with the Tukey correction. *P* values of less than .05 were considered statistically significant. GraphPad Prism 6.00 software (GraphPad Software, San Diego, Calif) was used.

Role of the funding source

The sponsor of the study had no role in any part of the study. L.K.P., H.-J.M., and M.W. had full access to data collected for the trial and final responsibility for the decision to submit for publication.

RESULTS

Study population and blinding

Forty-five patients were allocated to treatment, 43 patients started treatment, and 38 were available for evaluation of the primary efficacy parameter. A flowchart of the study is shown in Fig E1. The 3 groups were well balanced (Table E1).

The double blinding was maintained because the patients' self-evaluation of group allocation revealed that 55% of all treated patients and 42% of placebo-treated patients guessed their allocation correctly. The investigator assessed the correct allocation in 75% of the treated and 50% of the placebo-treated patients based on adverse events.

In the x6 group 1 patient dropped out before treatment started because of lack of time and was not included in safety or efficacy analyses. Additionally, 2 patients were excluded from the efficacy analysis: 1 because of treatment with depot steroid prescribed by her general practitioner after receiving 4 injections of allergen and 1 after only 2 treatments because of the infectious disease mononucleosis. Both were included in the safety analysis. One patient diary was lost, and this patient was excluded from primary outcome analysis.

In the x3 group 1 patient withdrew consent after only 1 treatment because of treatment side effects due to an intramuscular injection of antihistamine and a laryngoscopy because of a sensation of a swollen throat. The examination revealed no swellings or other abnormal findings. The patient was included in the safety but not in the efficacy analysis.

In the placebo group 1 patient became pregnant before treatment and was excluded from both the safety and efficacy

analyses. Another patient diary was missing, resulting in exclusion from primary outcome analysis.

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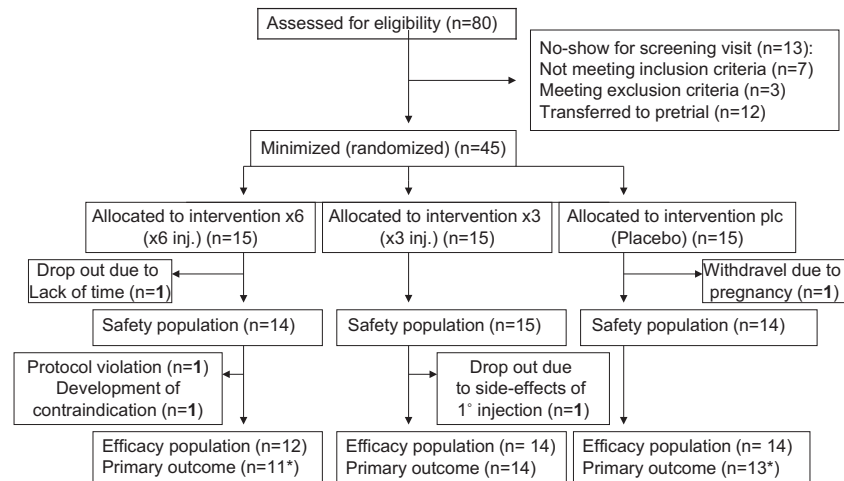


FIG E1. Participant flowchart. *Reduced number because of some patients with inadequate diary data.

TABLE E1. Demographics, baseline characteristics, and adverse events

Demographics and baseline characteristics	x6 Safety population (n = 14)/primary outcome (n = 11)	x3 Safety population (n = 15)/primary outcome (n = 14)	Placebo safety population (n = 14)/primary outcome (n = 13)
Sex			
Male (%)	7 (50)/5 (46)	7 (46.7)/7 (50)	8 (57.1)/7 (58)
Female (%)	7 (50)/6 (55)	8 (53.3)/7 (50)	6 (42.9)/5 (42)
Age (y), mean (SD [range])	35.9 (11.6 [22-58])	34.5 (11.4 [20-55])	35.9 (8.53 [24-52])
VAS (cm), preseason mean (SD [range])	36.3 (13.1 [22-58])	33.2 (10.6 [20-55])	36.9 (8.48 [26-52])
	4.6 (2.52 [0.8-7.8])	6.4 (2.76 [1-10])	5.4 (2.21 [1.5-7.8])
	4.6 (2.62 [0.8-7.8])	6.2 (2.67 [1-9.7])	5.4 (2.34 [1.5-7.8])
Patients with concomitant asthma (%)	3 (21)/3 (27)	3 (20)/3 (21)	5 (36)/3 (25)
Patients with concomitant birch pollen allergy (%)	5 (35)/3 (27)	4 (27)/4 (29)	5 (36)/4 (33)
Patients with depot steroid treatment before season (%)	1 (7)/1 (9)	2 (13)/2 (14)	2 (14)/2 (17)

Adverse events	x6 (n = 14)		x3 (n = 15)		Placebo (n = 14)	
	No. of patients (%)	No. of episodes	No. of patients (%)	No. of episodes	No. of patients (%)	No. of episodes
Local swellings	11 (78.5)*	23	13 (86.6)*	28	0	0
Swollen eye surroundings	1 (7.1)	1	1 (6.6)	1	0	0
Sneezing	2 (14.2)	2	3 (20)	5	1 (7.1)	1
Runny nose	1 (7.1)	3	3 (20)	5	1 (7.1)	1
Itching of eyes	1	1	6	10	2	2
Axillary itching	1	1	2	2	0	0
Itching, other location	6	6	6	7	5	7
Cold	6 (42.9)	6	10 (66.6)	11	4 (28.6)	4
Headache	3 (21.4)	4	4 (26.7)	6	1 (7.1)	1
Sore throat	0	0	1 (6.6)	1	1 (7.1)	1
Fatigue	2 (14.2)	2	3 (20)	3	1 (7.1)	1
Influenza	4 (28.6)	4	4 (26.7)	4	7 (50)	7
Gastrointestinal infection	4 (28.6)	4	2 (13.3)	2	4 (28.6)	4
Cough	3 (21.4)	3	3 (20)	3	3 (21.4)	3

There were no significant demographic differences before treatment among the 3 groups with respect to sex and age, a global evaluation score (the visual analog scale) of the pretreatment season, seasonal asthma, concomitant birch allergy, and steroid treatment in the 2010 season.

VAS, Visual analog scale.

* $P < .001$. A significant difference was seen in the appearance of local swelling in the 2 treated groups in comparison with the placebo group by using the t test.

TABLE E2. Primary and secondary efficacy parameters

Category	Group	No.	Adjusted mean (SE)	Difference vs placebo (95% CI)	Difference of x6 vs x3 (95% CI)	Difference of all treated vs placebo (95% CI)
Combined symptom and medication score during season (SMS)	x6	11	0.12 (0.02)	−0.05 (−0.16 to 0.07)	−0.07 (−0.18 to 0.04)	−0.007 (−0.09 to 0.07)
	x3	14	0.19 (0.03)	0.02 (−0.08 to 0.13)		
	Plc	13	0.17 (0.04)			
Medication score during season (MS)	x6	11	0.12 (0.03)	−0.08 (−0.22 to 0.07)	−0.11 (−0.25 to 0.03)	−0.13 (−0.12 to 0.09)
	x3	14	0.23 (0.04)	0.04 (−0.10 to 0.18)		
	Plc	13	0.19 (0.05)			
Symptom score during season (SS)	x6	11	0.13 (0.02)	−0.02 (−0.12 to 0.09)	−0.03 (−0.13 to 0.07)	0.001 (−0.07 to 0.07)
	x 3	14	0.15 (0.02)	0.01 (−0.09 to 0.11)		
	Plc	13	0.14 (0.04)			
Nose symptom score during season	x6	11	0.12 (0.03)	−0.04 (−0.16 to 0.08)	−0.05 (−0.16 to 0.07)	−0.01 (−0.09 to 0.07)
	x3	14	0.17 (0.03)	0.007 (−0.10 to 0.12)		
	Plc	13	0.16 (0.04)			
Eye symptom score during season	x6	11	0.13 (0.03)	0.03 (−0.08 to 0.13)	0.01 (−0.10 to 0.11)	0.02 (−0.05 to 0.09)
	x3	14	0.12 (0.02)	0.02 (−0.08 to 0.12)		
	Plc	13	0.10 (0.03)			
Symptom and medication score during peak season (wk 23 + 24 + 25)	x6	11	0.23 (0.05)	0.02 (−0.14 to 0.17)	−0.14 (−0.30 to 0.01)	0.10 (−0.01 to 0.21)
	x3	14	0.37 (0.03)	0.16 (0.01 to 0.30)*		
	Plc	13	0.21 (0.05)			
Medication score during peak season (wk 23 + 24 + 25)	x6	11	0.22 (0.06)	−0.01 (−0.20 to 0.18)	−0.20 (−0.39 to −0.01)*	0.10 (−0.04 to 0.24)
	x3	14	0.42 (0.05)	0.19 (0.01 to 0.37)*		
	Plc	13	0.23 (0.05)			
Symptom score during peak season (wk 23 + 24 + 25)	x6	11	0.23 (0.05)	0.04 (−0.12 to 0.20)	−0.08 (−0.24 to 0.07)	0.09 (−0.02 to 0.20)
	x3	14	0.32 (0.03)	0.13 (−0.02 to 0.27)		
	Plc	13	0.19 (0.05)			
RQLQ score, change from pretreatment season	x6	12	4.75 (6.88)	−13.75 (−39.94 to 12.44)	−17.04 (−43.23 to 9.16)	−4.58 (−22.8 to 13.6)
	x3	14	21.79 (7.37)	3.29 (−21.88 to 28.45)		
	Plc	14	18.50 (7.39)			
VAS score, change from pretreatment season	x6	12	0.13 (0.81)	−1.83 (−4.20 to 0.54)	−0.55 (−2.92 to 1.82)	−1.53 (−3.13 to 0.07)
	x3	14	0.69 (0.68)	−1.28 (−3.56 to 1.00)		
	Plc	14	1.96 (0.49)			
SPT, change from pretreatment season	x6	12	3.29 (0.59)	−0.13 (−2.97 to 2.70)	0.75 (−2.03 to 3.54)	0.54 (−1.39 to 2.47)
	x3	14	2.54 (0.78)	−0.89 (−3.62 to 1.84)		
	Plc	13	3.42 (0.92)			
ID (20 min), change from pretreatment season	x6	11	0.35 (0.17)	0.10 (−0.53 to 0.73)	−0.15 (−0.46 to 0.77)	−0.02 (−0.45 to 0.42)
	x3	14	0.19 (0.14)	−0.06 (−0.66 to 0.55)		
	Plc	13	0.25 (0.21)			
ID (24 h), change from pretreatment season	x6	12	5.04 (1.05)	2.67 (−0.43 to 5.77)	1.99 (−1.06 to 5.04)	−1.60 (−3.77 to 0.58)
	x3	14	3.05 (0.87)	0.68 (−2.31 to 3.66)		
	Plc	13	2.37 (0.61)			

Plc, Placebo; VAS, visual analog scale.

* $P < .05$.