

Immunotherapy with depigmented-polymerized mixed tree pollen extract: a clinical trial and responder analysis

O. Pfaar¹, D. S. Robinson², A. Sager³ & R. Emuzyte⁴

¹Center for Rhinology and Allergology Wiesbaden, Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Mannheim, Germany; ²Laboratorios LETI SL, Tres Cantos, Spain; ³LETI Pharma, Witten, Germany; ⁴Vilnius University Faculty of Medicine, Clinic of Children Diseases Vilnius, Lithuania

To cite this article: Pfaar O, Robinson DS, Sager A, Emuzyte R. Immunotherapy with depigmented-polymerized mixed tree pollen extract: a clinical trial and responder analysis. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2010.02413.x.

Keywords

allergic rhinitis; allergoid; depigmented-polymerized allergen extract; specific subcutaneous immunotherapy (SCIT); tree pollen.

Correspondence

Oliver Pfaar, MD, Center for Rhinology and Allergology Wiesbaden, Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Mannheim, Germany.

Tel.: +49 611 8904381

Fax: +49 611 8904381

E-mail: oliver.pfaar@allergiezentrum.org

Accepted for publication 16 April 2010

DOI:10.1111/j.1398-9995.2010.02413.x

Edited by: Wytske Fokkens

The results of this study were presented in part as poster presentations at the XXVIIIth Congress of the European Academy of Allergy and Clinical Immunology (EAACI) in Warsaw, Poland, June 2009, and at the XXIXth Congress of the EAACI in London, UK, June 2010.

Abstract

Background: Rhinoconjunctivitis because of tree pollen sensitization is common in Northern Europe. Specific subcutaneous immunotherapy (SCIT) is the only disease-modifying treatment, but unmodified allergen extracts carry a risk of allergic side-effects. Our objective was to examine efficacy and safety of a depigmented-polymerized mixed tree pollen extract.

Methods: A double-blind, placebo-controlled trial of 184 tree pollen allergic adults was performed. SCIT consisted of four increasing doses at 7-day intervals, then maintenance injections every 6 weeks for 18 months. Primary outcome was combined symptom and medication score during the 2008 season. Secondary outcomes included analysis at different levels of pollen exposure and a responder analysis. Adverse events were classified using the EAACI scale. Birch pollen-specific IgE and IgG₄ were measured before and after treatment.

Results: The combined symptom and medication score of actively treated patients was significantly lower than those on placebo ($P < 0.04$). Increased efficacy was seen at high pollen exposure (median score 2.1 for active [IQR 0.7–3.4] vs 4.2 [IQR 2.4–5.3] for placebo for days with 500 or more pollen grains per m³, a 50% reduction, $P < 0.01$). A modified responder analysis revealed 64% responders in the active and 32% in the placebo group ($P < 0.01$). There were 17 systemic reactions. All were mild (grade 1 or 2) and required no treatment. Serum birch-specific IgG₄ increased in the SCIT group ($P < 0.01$).

Conclusions: SCIT with depigmented- polymerized tree pollen extract was clinically effective and well tolerated. Responder analysis suggested that one-third of patients treated with immunotherapy may not respond.

Allergic rhinoconjunctivitis represents a global health problem affecting 25% of western populations (1). Subcutaneous immunotherapy (SCIT) using inhalant allergens is clinically effective in the treatment of allergic rhinitis, induces clinical and immunologic tolerance, has long-term efficacy, may

prevent the progression of allergic disease, and also improves the quality of life of allergic patients (2–7). In tree pollen allergic patients, clinical efficacy and long-term effects in preventing progression to asthma have also been confirmed for SCIT with birch pollen extracts (8–10). For patients sensitized to several tree pollens, some studies suggest that extracts containing birch pollen alone are equally effective as those containing birch, alder and hazel (11, 12), whereas others suggest better outcomes if mixed pollen extracts are used (13). This presumably depends on geographical variation in forestation and thus tree pollen exposure. It is

Abbreviations

SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; EAACI, european academy of allergy and clinical immunology; Ig, immunoglobulin; ITT, intention to treat; PP, per protocol; SMS, combined symptom and medication score.

common practice in Northern and Eastern Europe to administer mixed tree pollen extracts to patients sensitized against hazel, alder and birch, especially when their symptoms start early in the year, when hazel pollination occurs.

The use of SCIT is limited because of the risk of systemic side-effects (14, 15). In an attempt to overcome these potential hazards, allergoids were developed by polymerizing allergen extracts with glutaraldehyde (16). The aim of such chemical modification is to reduce specific IgE binding (which is responsible for immediate systemic reactions), whilst maintaining T cell reactivity (which is thought to be responsible for induction of tolerance) (17). These extracts have been further developed by introducing a depigmentation step, before the polymerization process. The depigmentation removes protein-bound pigments, enhances the solubility of the allergoids and inactivates enzymatic activity (18). The clinical safety and efficacy of depigmented allergoids has been shown in several clinical studies in patients with pollen- and house dust mite-induced allergic symptoms (19–23).

The objective of this study was to evaluate clinical efficacy and safety of 18 months SCIT with standardized, depigmented-polymerized tree pollen extract consisting of hazel, alder and birch pollen. Additionally, the effect of treatment on immunological parameters was assessed.

Methods

Patients and study design

This was a randomized, double-blind, placebo-controlled clinical study, including patients with a medical history of allergic rhinoconjunctivitis with or without allergic asthma because of tree pollen sensitization. A total of 184 patients

were recruited at 28 study centres in Lithuania, Poland and Germany in autumn 2006 and randomized to receive either SCIT or placebo (Fig. 1). Randomization was 3 : 1 verum to placebo, and the study sites included 5 academic sites, 5 general hospitals, the rest specialized allergy centres (allergological practice). Positive skin prick tests to birch pollen resulting in a wheal diameter ≥ 3 mm as well as serum level of specific IgE against birch pollen $\geq$ CAP-Class 2 = 0.7 kU/l were required.

A retrospective visual analogue score (VAS) was used in patient selection. Patients had to score the previous season's symptoms with at least 30 mm on a VAS of disease activity (on a 100-mm line from no symptoms to intolerable). Patients were excluded if they had perennial symptoms or allergic sensitization, other than to tree pollen, causing symptoms during the hazel, alder and birch pollen season, or asthma graded at 2 or more according to GINA guidelines. Written informed consent was obtained from patients. The study was approved by the relevant Ethics Committees and conducted in accordance with the latest version of the Declaration of Helsinki (24).

Immunotherapy

SCIT was performed using Depigoid® (Laboratorios LETI SL, Tres Cantos, Spain), a standardized depigmented and glutaraldehyde-polymerized tree pollen extract (33% *Corylus avellana*, 33% *Alnus glutinosa*, 34% *Betula alba*) adsorbed onto aluminium hydroxide. Depigoid® was provided at two different strengths: 100 DPP units (Depigmented and Polymerized)/ml (Vial 1) and 1000 DPP/ml (Vial 2). The biological activity unit of 1 DPP was the result of depigmenting and polymerizing 1 histamine equivalent prick test unit of allergen extract. Before polymerization, 100 DPP corresponded to

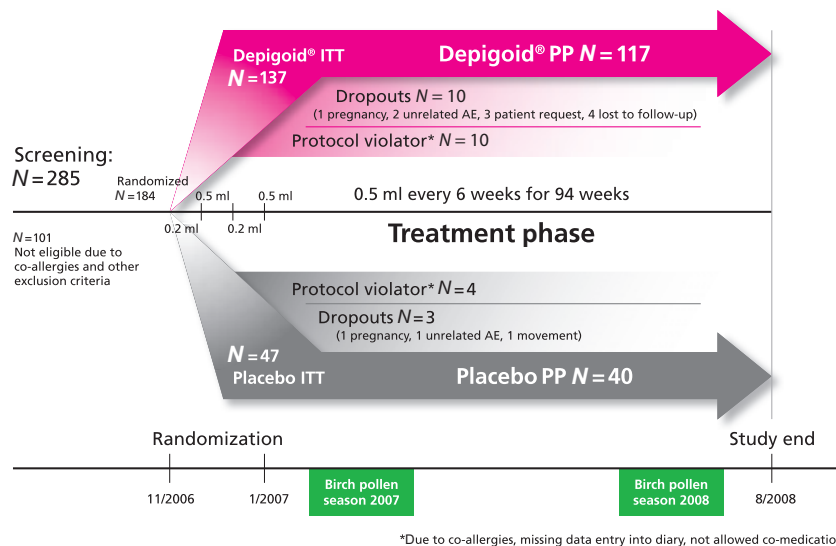


Figure 1 Study flow chart. Patients recruited, treatment schedule and timelines of study with drop outs in each group. Of the 10 protocol violations in the Depigoid group, four were because of missing diary data, four because of taking prohibited medication

and two because of other allergies. For the placebo group, two of the protocol violations were for co-allergy, and one each for non-permitted medication and missing diary data.

2.20 µg/ml Bet v1 major allergen equivalent (measured using a Bet v 1 ELISA from Indoor Biotechnologies Inc., Charlottesville, VA, USA). Placebo medication was identical in appearance and contained the excipients only. Patients initially received doses of 0.2 and 0.5 ml of Vial 1 followed by 0.2 and 0.5 ml of Vial 2 at 7-day intervals, followed by the maintenance dose of 0.5 ml of Vial 2 at 6-week intervals for 18 months. The maintenance dose corresponded to 11.0 µg Bet v1 before polymerization. All patients were observed for 30 min in the immunotherapy unit after each injection, following the recommendations of the EAACI (25).

Pollen counts

The start of the pollen season for each centre was defined as when the pollen count was ≥ 250 pollen grains/m³ per day for more than 3 consecutive days. The following period was evaluated for efficacy until the pollen count fell to ≤ 250 pollen grains/m³ per day for 3 consecutive days, indicating the end of the relevant pollen exposure. Within one pollen season, several periods of relevant pollen exposure were evaluated for the combined symptom and medication score. The local hazel, alder and birch pollen counts were determined for each study centre using a volumetric pollen trap (Burkard Scientific Ltd, Uxbridge, UK). Days with a pollen exposure of > 500 pollen grains/m³ per day were used to define the peak pollen season.

Assessment of efficacy

Using diaries patients recorded their individual rhinitis, conjunctivitis and (for safety evaluation) asthma symptoms on a daily basis: sneezing, nasal itch, rhinorrhea, nasal congestion, ocular itch, increased lacrimation and eye redness were included into the symptom score. Individual symptoms were scored on a scale from 0–3, with no symptoms = 0, mild = 1, moderate = 2 and severe = 3, respectively (4, 26).

Patients were supplied with rescue medication. Each drug was given according to the recommendation of the manufacturer. No other medication was allowed. Each dose of rescue medication was scored as follows: 0 = no medication; 1 = topical or systemic anti-histamines (desloratadine one 5 mg tablet, and azelastine eye or nose drops, 2 drops), or salbutamol (2 puffs) for asthma; 2 = nasal or inhaled corticosteroids (mometasone nasal spray 2 puffs, budesonide inhaler 100 µg); 3 = systemic corticosteroids for uncontrolled rhinitis or asthma (methylprednisolone 2 mg/kg body weight). For each patient, the daily rescue medication score was defined as sum of points for each medication used (26). The combined symptom and medication score (SMS) was defined as the time-weighted area under curve (AUC) of the sum of the daily rescue medication score and the daily symptom score for all days of the predefined pollen season.

Assessment of safety

Adverse events were documented throughout the study. Investigators' terms of adverse events were coded using the

Medical Dictionary for Regulatory Activities (MedDRA). Systemic reactions were graded according to the EAACI classification (25).

Immunological parameters

The effects of treatment on specific IgE and IgG₄ were studied. Serum concentrations of tree pollen-specific IgE were measured before the start of the treatment and outside the season and after 18 months of treatment using IMMULITE 2000 3g Allergy, a two-step, chemiluminescent immunoassay (Siemens AG, Eschborn, Germany) (27) and birch pollen-specific IgG₄ using ELISA (REF: 10104PG, Dr. Focke Laboratorien GmbH, Neuss, Germany).

Statistics

The primary efficacy parameter of this study was the time-weighted AUC of the combined symptom and rescue medication scores (SMS) during the 2008 birch pollen season (≥ 250 pollen grains/m³ per 24 h) after 18 months of treatment. A power calculation suggested 86% power to detect a difference in means of SMS of 0.5 assuming a standard deviation of 1.0 with a sample size of 150 active and 50 placebo subjects. Because symptom scores are often relatively low during days of low pollen exposure, efficacy was also assessed using different pollen exposure cut-offs to compare median symptom, medication and combined scores for days with pollen exposure above 300, 400 and 500 grains per mm³, respectively. In addition, a modified responder analysis was performed. Because we did not have a baseline year for comparison, the median AUC for the placebo group was defined as the comparator, and responders defined as those subjects with AUC less than this value using different cut-offs (20%, 25%, 30% etc.). The optimal value for distinguishing actively treated from placebo groups was selected using receiver–operator curves, and numbers thus defined as responders and non-responders in each group were compared by chi-squared analysis. Clinical and laboratory characteristics of these groups were also compared. For further description of the responder analysis, see Data S1.

The two-tailed Wilcoxon rank-sum test was used for the treatment comparison, and the Hodges-Lehmann estimation was used to calculate median differences with confidence intervals, with a significance level of $P = 0.05$ for primary and secondary outcome parameters.

Results

A total of 184 patients aged 18–65 (mean: 38 years) were randomized to receive either SCIT with Depigoid® ($n = 137$) or placebo ($n = 47$). Baseline characteristics were balanced between the two treatment groups (Table 1). In total, 13 patients discontinued the study prematurely (Fig. 1). A further 14 patients had no diary data or concomitant allergies, thus the total per protocol population was 157 (117 Depigoid and 40 placebo).

Table 1 Demographic baseline characteristics

	Depigoid treated <i>N</i> = 137	Placebo <i>N</i> = 47
Age (years, mean and standard deviation)	38.5 (11.6)	39.0 (11.2)
Age (median and range)	38.0 (18–65)	38.0 (19–63)
Gender (male/female)	64M/73F	24M/23F
History of allergic asthma (number)	61	23
FEV1 (%predicted, mean and standard deviation)	102 (13)	103 (14)
FEV1 (%predicted, median and range)	100 (78–135)	106 (78–141)

During the 2008 pollen season, pollen exposure was within the expected range (mean: 116 pollen grains/m³ per day). In 2008, there were 43 days with exposure >250, 39 days >300 and 26 days >500 pollen grains/m³ per day.

Efficacy

At 18 months, the median AUC for the combined symptom and medication score (SMS) of the 2008 season was 2.3 (inter

quartile range 0.9–3.2) for actively treated patients and 2.6 (IQR 2.2–4.4) for placebo-treated patients (median difference −0.4 [−1.22; −0.03], *P* < 0.04), for the ITT population, and 2.2 (0.8–3.8) and 3.6 (2.3–4.5) for those patients completing the study (median difference −1.3 [−1.89; −0.34], *P* < 0.01, Fig. 2A). Median symptom scores were 0.54 (0.27–0.77) for Depigoid-treated patients completing the study and 0.61 (0.48–0.75) for placebo (median difference −0.1 [−0.20; −0.02], *P* < 0.01), whilst medication scores were 1.36 (0.4–2.9) and 2.95 (1.7–3.9), respectively (median difference −1.3 [−1.87; −0.34], *P* = 0.09).

To obtain a relationship between pollen exposure and clinical symptoms, the SMS were evaluated at different birch pollen cut-off points: 250, 300, 400 and 500 grains/m³ per 24 h. Data are presented in Table 2 and Fig. 2B. During peak pollen season, the median AUC for the SMS was 2.1 (0.7–3.4) for actively treated patients and 4.2 (2.4–5.3) for placebo-treated patients (median difference −1.8 [−2.34; −0.81], *P* < 0.01), a 50% reduction comparing active with placebo.

Responder analysis

Receiver–operator curves suggested the optimal cut-off to define responders was a 30% reduction in AUC of SMS at

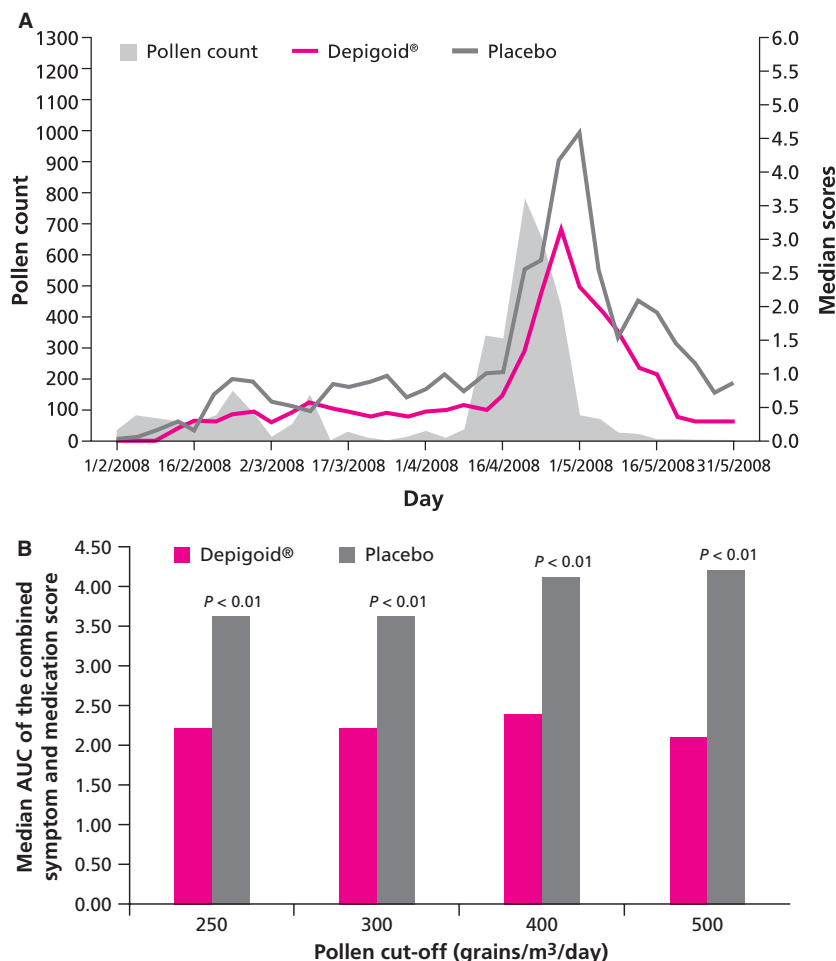


Figure 2 (A) Combined symptom and medication scores for patients completing treatment (medians) for Depigoid (pink line) and placebo (dark grey line), and time-adjusted pollen count (grains per day) for 2008. (B) Combined symptom and medication scores for patients completing treatment at different pollen exposures (median and interquartile ranges or total number of days at or above each cut-off value, with number of days shown). Comparison was by Wilcoxon rank-sum test).

Table 2 Combined symptom and medication score in relation to pollen exposure for patients completing the study

Pollen exposure	Combined symptom and medication score (median and interquartile range)			
	Depigoid (<i>n</i> = 117)	Placebo (<i>n</i> = 40)	Median difference CI	<i>P</i> value
250 grains/m ³ per day	2.2 (0.8–3.8)	3.6 (2.3–4.5)	–1.3 [–1.9; –0.3]	<0.01
300 grains/m ³ per day	2.2 (0.8–3.9)	3.6 (2.6–5.2)	–1.3 [–2.1; –0.4]	<0.01
400 grains/m ³ per day	2.4 (0.8–3.7)	4.1 (2.6–4.8)	–1.5 [–2.0; –0.5]	<0.01
500 grains/m ³ per day	2.1 (0.7–3.4)	4.2 (2.4–5.3)	–1.8 [–2.4; –0.8]	<0.01

pollen exposure of 250 grains per m³ or more (as defined by ROC curves, Fig. S1), compared to the median placebo value. Eighty-seven of the ITT population treated with Depigoid were defined as responders (64%) and 50 as non-responders (36%). This compared with 15 apparent responders in the placebo group (32%) and 32 non-responders (68%, $P < 0.01$ by chi-squared test, chi-squared statistic 14.1). Values for the per protocol population were similar as were those for different levels of pollen exposure (data not shown). Comparison of “responders” and “non-responders” in the actively treated group did not identify any significant differences at baseline for age (mean 37.9 ± 11.0 SD for responders and 38.1 ± 12.8 for non-responders), gender (48% female for responders, 62% non-responders), diagnosis of asthma (38% of responders vs 56% of non-responders), total IgE (mean 107 IU/ml vs 149), tree-specific IgE (47.4 ± 33.2 IU/ml vs 53.4 ± 34.9) nor the ratio of specific to total IgE (0.61 ± 0.48 vs 0.61 ± 0.44). There was no difference between responders and non-responders for tree-specific IgG₄ after treatment ($437 \text{ kU} \pm 546 \text{ kU/ml}$ vs 463 ± 656 , $P = 0.2$) nor for the increase induced by treatment (233 ± 440 vs 109 ± 333 , $P = 0.6$).

Safety

Table 3 displays the patients with local and systemic reactions (all grade 1 or grade 2) according to the EAACI classification (25). In the SCIT group, the total number of systemic reactions was 17 (0.7% of all injections), which occurred in 9 patients. Of these, 12 were classified as grade 1 reactions and 5 as grade 2. There were no reactions of grade 3 and 4. None of the events in the SCIT group were considered serious and none required specific treatment.

Table 3 Side-effects by treatment group (numbers and percentage). No reaction required treatment

Symptom	Depigoid (<i>n</i> = 137)	Placebo (<i>n</i> = 47)
Local reactions	56 (40.9%)	11 (23.4%)
Systemic reactions*	17/9 (6.6%)†	1/1 (2.1%)
Conjunctival	2 (1.5%)	0
Nasal	9 (6.6%)	0
Asthma	2 (1.5%)	0
Skin (urticaria/edema)	4 (2.9%)	1 (2.1%)

*All grade 1 or 2.

†17 systemic reactions occurred in 9 patients.

Immunological effects

Mean serum concentrations of birch pollen-specific IgE remained unchanged throughout the course of the study (data not shown). There were no significant differences between baseline and study end for individual treatment groups and no difference between groups. At the end of the study, the median serum IgG₄ level significantly increased from 253 to 447 KU/l in the Depigoid® group, whereas it decreased from 302 to 255 KU/l in the placebo group (median difference 108 [69;159], $P < 0.01$).

Discussion

In this randomized, double-blind, placebo-controlled study with tree pollen-induced allergic rhinoconjunctivitis, SCIT with depigmented-polymerized mixed tree pollen extract was clinically effective and safe. In what is to our knowledge the first reported responder analysis of a double-blind placebo-controlled immunotherapy study, there was a significantly superior proportion of responders with active treatment. Treatment with depigmented-polymerized tree pollen extract resulted in increased birch pollen-specific IgG₄, whereas birch pollen-specific IgE remained unchanged.

Because summary data over the whole pollen season include days with minimal pollen exposure and thus minimal symptoms scores, the use of SMS over the whole season may misrepresent the burden of disease for patients who often report much higher scores on days of high pollen exposure. To address this problem, we analysed symptom and medication scores on days above a series of threshold values for pollen exposure, arguing that symptoms are likely to be highest when the pollen count is highest (as was indeed the case for the placebo group). At the predefined peak pollen exposure of ≥ 500 grains/m³ per 24 h, there was a 50% reduction in the median SMS for actively treated patients who received the whole course of treatment when compared to placebo (compared to a 39% reduction over the whole season). The size of response to treatment seen in our study is comparable with that noted in previous studies of immunotherapy with unmodified birch pollen extracts (9, 10, 28, 29) and depigmented-polymerized house dust mite (22). To our knowledge, this is the first study to suggest a relationship between pollen exposure and magnitude of combined symptom and medication score caused by this exposure. These findings should be investigated in further studies.

In addition to performing efficacy analysis based on combined symptom and medication scores, current European Union regulatory bodies suggest a responder analysis should be performed (<http://www.emea.europa.eu/pdfs/human/ewp/1850406enfin.pdf>). Such analysis conventionally requires a period of baseline observation which is uncommon in large allergen immunotherapy studies for logistical reasons, and because pollen counts vary from year to year making comparison difficult. For this reason, we performed a responder analysis setting the median value for the primary outcome (AUC for SMS for the entire pollen season for the ITT population) for the placebo group as “baseline” and analysing numbers of responders and non-responders at different levels of reduction in the combined scores from this baseline. A cut-off of 30% was selected as the best discriminator between active and placebo groups using receiver–operator curves (data not shown) and seems reasonable given that Malling has suggested that immunotherapy should achieve a treatment effect of at least 30% (30). The level of placebo response seen here is not unexpected and is similar to that seen in others studies of allergic diseases such as asthma. The analysis also suggested that up to 36% of the actively treated group were “non-responders”. This will include as non-responders any patients who might otherwise have had much higher combined scores, but also will include a group of genuine non-responders, that is patients who did not respond to allergen immunotherapy. This is a very little studied area in allergen immunotherapy, and to our knowledge has never been reported before in a prospective study. One retrospective study identified 55.2% responders to treatment in 76 patients treated with SCIT based on clinical assessment (and hence 44.8% non-responders) (31). Di Lorenzo et al. suggested that the ratio of total to specific IgE might predict outcome, but we did not confirm that in this study. We did not identify any factors that were related to responder status, including the increase in allergen-specific IgG₄ induced by treatment. It would be of interest to examine functional activity of IgG₄ in blocking IgE/allergen interactions, and we plan to do so in future studies. We suggest that responder analysis should be included in all future immunotherapy studies.

The encouraging safety profile seen in our study (no grade 3 or 4 systemic reactions) is in line with some studies using unmodified allergen birch pollen extract (7, 8, 28). However, in one study, 5 grade three reactions and 1 grade four reaction occurred in 24 patients treated with unmodified extract by SCIT (29). Favourable safety profiles were demonstrated for depigmented- polymerized allergen extracts in children and adults with allergic rhinoconjunctivitis and/or asthma because of house dust mites and pollen (19, 22, 23, 28). In 3 prospective, non-interventional studies including 175, 766 and 768 patients who received a variety of different depigmented-polymerized allergen extracts, no Grade 3 or 4 systemic reactions were reported (23, 32, 33). It is of note that the reduction in IgE binding resulting from depigmentation and polymerization allows for very rapid up-dosing schedule for immunotherapy using 4 up-dosing injections over 3 weeks as in this study, or even a rush protocol achieving maintenance

dose at the first visit with two injections with a low rate of mild systemic reactions (21). This short induction-phase of therapy may encourage patient compliance.

The immunological changes seen in this study are in line with previous reports of SCIT with unmodified allergen extracts, where increases in allergen-specific IgG₁ and IgG₄ are reported (33–35). Indeed, it has been suggested that part of the mechanism of immunotherapy may be via induction of IgG antibodies that block interaction of allergen and IgE with effector cells (34). Here, we confirm that depigmented-polymerized tree pollen extract immunotherapy also induced birch pollen-specific IgG₄ which suggests retained immunogenicity of this product. Further studies will be required to determine whether these antibodies have IgE blocking activity.

In conclusion, SCIT with standardized depigmented- polymerized tree pollen extract was clinically effective and safe and resulted in immunological changes similar to those of native birch pollen extracts.

Acknowledgments

The authors thank all participants in this study, Dr Carlos Badiola for discussions on responder analysis, Dr Jeronimo Carnes for comments on the manuscript and Birgit Schnieiders for statistical analysis.

We also thank the following investigators for participating in this study: R. Brehler, Münster, Germany; H. Brüning, Kiel, Germany; M. Hoffmann, Witten, Germany; T. Scholten, Hagen, Germany; L. Volgmann, Hannover, Germany; M. Worm, Berlin, Germany; A. Blaziene, Vilnius, Lithuania; J. Greiciuviene, Siauliai, Lithuania; P. Leisyte, Kaunas, Lithuania; B. Sitkauskiene, Kaunas, Lithuania; I. Valatkiene, Vilnius, Lithuania; A. Bodzenta-Lukaszyk, Bialystok, Poland; T. Hofman, Poznan, Poland; B. Majorek-Olechowska, Tarnów, Poland; J. Malolepszy, Wrocław, Poland; G. Mincewicz, Gdansk, Poland; D. Madra-Rogcka, Poznan, Poland; M. Roczen-Fortuna, Szczecin, Poland; B. Samolinski, Warszawa, Poland.

Disclosure of potential conflict of interests

O. Pfaar has received research grants from ALK-Albello, Denmark; Allergopharma, Germany; Stallergenes, France; HAL, Netherlands; Artu Biologicals, Netherlands; Bencard, Great Britain; Hartington, Spain; Lofarma, Italy; Novartis/Leti, Germany/Spain; Roxall, Germany and has served as advisor and on the speaker's bureau for the above mentioned pharmaceutical companies.

Funding

This study was funded by Leti Pharma GmbH, Germany.

Supporting information

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

Figure S1. Receiver-operator curves for defining optimum discriminator between responders and non-responders to Depigoid.

Data S1. Responder analysis.

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Supporting information

Responder analysis

Responder analysis was performed as described in the methods section. Values for the AUC of combined symptom and medication score for both actively treated and placebo treated intention- to-treat populations were compared to the median value for the placebo group which was defined (arbitrarily) as the “baseline value”. The percentage reduction (or increase) from this value was calculated and different cut-offs used to define responders (20,25, 30,35 etc%). True positive values were taken as responders to active treatment, whilst false positives were responders in the placebo treated group. These data were then used to calculate sensitivity and specificity which was plotted for each cut off at different pollen thresholds to generate receiver-operator curves (Figure S1). The optimal cut off was defined as that giving maximal sensitivity and specificity and was a 30% reduction in AUC combined SMS for 250, 300 and 400 pollen grains per m³ per day and 35% for 500 pollen grains per m³ per day.

Responder analysis:
Time weighted AUC of the combined symptom and medication score – ROC curves (intention-to-treat 2008)

