

Depigmented–polymerized mixed grass/birch pollen extract immunotherapy is effective in polysensitized patients*

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Abstract

Background: Although many patients with allergic rhinoconjunctivitis have symptoms due to sensitization with more than one pollen allergen, and mixed pollen extracts are widely used for allergen immunotherapy in practice, there are few published trials.

Methods: We performed a 2-year multicentre, double-blind, placebo-controlled trial of subcutaneous immunotherapy with mixed depigmented–polymerized birch and grass pollen extract in 285 patients with allergic rhinoconjunctivitis symptomatic during both birch and grass pollen seasons. Primary outcome was combined symptom and medication score (SMS) assessed by daily visual analogue scales (VAS). Analysis included a placebo-based analysis examining the effect of treatment only on days when placebo patients were symptomatic.

Results: There was a significant reduction in median SMS for actively treated patients (median 5.70 (interquartile range 2.62–10.02) compared with 7.07 (3.47–10.71) for placebo, $P = 0.0385$). Rhinitis quality-of-life scores were significantly better for active compared with placebo, and other secondary endpoints were not significantly different. Placebo-based analysis showed a 33.7% reduced SMS at year 2 for active treatment compared with placebo on days when placebo patients were symptomatic. Both birch pollen- and grass pollen-specific IgG₄ increased with active treatment.

Conclusions: This study shows efficacy of mixed pollen extracts for immunotherapy for patients symptomatic to both birch and grass pollen allergens. The relatively modest effect may reflect 50% dose reduction for each allergen in the mixture. It supports VAS for symptom assessment and placebo-based analysis as useful for the analysis of immunotherapy trials. The safety of modified extracts may allow study of mixed extracts without dose reduction to improve efficacy.

Abbreviations

AR, allergic rhinitis; DBPC, double-blind, placebo-controlled; DPP, depigmented–polymerized units (equivalent to HEP units prior polymerization); Ig, immunoglobulin; ITT, intention-to-treat; MS, medication score; PP, per-protocol; RQLQ, rhinitis quality-of-life questionnaire; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SMS, symptom and medication score (combined); SS, symptom score; VAS, visual analogue scale.

Allergic rhinoconjunctivitis (AR) affects 20% of Western populations with considerable impact on quality of life (1). Common triggers are grass pollen and, mainly in Northern Europe, birch pollen (1). The only disease modifying treatment for AR is allergen immunotherapy (either by subcutaneous injection [SCIT] or by sublingual administration [SLIT]), and clinical trials have documented clinical efficacy for grass or birch pollen extracts in AR driven by either pollen (2). However, many individuals are sensitized to multiple allergens from an early age (3). There are no published data on the frequency of symptomatic dual sensitization to birch and grass pollen, but many patients have persistent AR from early spring to late summer because of sensitization to both birch and grass pollen. Although guidelines suggest that mixed pollen extracts may be effective, there are very little data, and no placebo-controlled trials we are aware of, to support this (4–6).

One limitation of using mixed allergen extracts is that conventionally the dose of each allergen is reduced proportionately to the number of allergens in the mixture to reduce the risk of allergic side effects. For this reason, guidelines recommend restricting the number of allergens mixed (4, 5).

One approach to reducing the risk of anaphylaxis with SCIT is to modify allergen extracts to reduce IgE binding. Such allergoids include extracts treated with acid ('depigmented'), prior to polymerization with glutaraldehyde (7). These extracts showed efficacy for both birch pollen- and grass pollen-related AR, with minimal side effects (8–11).

In clinical trials of SIT, the relevant days for analysing the 'symptom load' of patients are usually defined by the pollen counts (12). Recently, another approach has been suggested, restricting analysis to days when patients in the placebo group report high symptom and/or medication scores (13).

Here, we report a 2-year, double-blind, placebo-controlled (DBPC), multicentre study, including placebo-based analysis, of mixed birch and grass pollen depigmented and polymerized extracts (mixed at a 50 : 50 ratio) for AR in polysensitized patients symptomatic to both allergens.

Methods

Study design

This was a multicentre ($n = 44$), randomized, double-blind, placebo-controlled study performed over 2 years (August 2008 to October 2010), including 285 patients (aged from 12 to 70) with moderate to severe persistent allergic rhinoconjunctivitis (1) with or without allergic asthma due to sensitization to both birch pollen and grass pollen.

Positive skin prick tests to both birch and grass pollen (Laboratorios LETI, S.L., Tres Cantos, Spain) and serum-specific IgE against birch and grass pollen of $\geq$ class 2 (ImmunoCAP, Phadia, Uppsala, Sweden) were required. In addition, patients had to report symptoms compatible with sensitization to both birch and grass pollen, scoring at least 3 cm on a 10-cm visual analogue scale (VAS).

Patients were excluded if they had symptomatic sensitization to other allergens or asthma graded at GINA 2 or more. The study was approved by Ethics Committees, and all patients gave written informed consent.

Immunotherapy

The randomization list was produced by the quality assurance of Pierrel Research Europe GmbH, formerly IFE, using a special validated SAS program. In this study, randomization was done for active vs placebo in a ratio 2 : 1 using blocks of six randomization numbers, each block containing 4× active and 2× placebo.

Subcutaneous immunotherapy was performed using a standardized depigmented and glutaraldehyde-polymerized birch pollen and grass pollen mix adsorbed onto aluminium hydroxide (Laboratorios LETI SL). This contained 50% *Betula verrucosa* pollen and 50% mixed grass pollen (*Dactylis glomerata*, *Festuca glauca*, *Lolium perenne*, *Phleum pratense* and *Poa pratense*). The mixed extract was provided at 1000 DPP/ml (500 DPP for each allergen). Subcutaneous immunotherapy was started before the birch pollen season. Patients initially received doses of 0.2 ml and 0.3 ml with an interval of 30 min at initial up-dosing visit, followed by injections of 0.5 ml every 4–6 weeks for 2 years (Fig. 1). Patients were observed for 30 min after each injection (4). The maintenance dose corresponded to 6.75 μ g *B. verrucosa* 1 and 15.75 μ g *P. pratense* 5 before polymerization (ELISA, Indoor Biotechnologies, Charlottesville, VA, USA). Placebo injections included all incipients present in active treatment except for allergen extracts. They were not spiked with histamine because depigmented–polymerized extracts have very low IgE binding so very few immediate local reactions occur (7).

Assessment of efficacy

The primary outcome measure was the area under the curve (AUC) for combined symptom and medication score (SMS) over the birch and grass pollen seasons for 2010. Patients recorded symptom severity in a daily diary during the pollen season, scoring nasal itching, running, sneezing, blocking and ocular itch on separate VAS with a continuous scale from 0 cm (no symptoms) to 10 cm (very severe symptoms). The SS was derived by adding the VAS for each of these five. Use of VAS to reflect symptoms of rhinoconjunctivitis has previously been validated in a large primary care population (14). In addition, VAS were used for asthma symptoms: chest tightness, breathlessness, cough and wheeze, and summed to give a separate asthma score.

The rescue medication provided and scoring was one point per spray or per drop for levocabastine nasal spray or eye drops, or per levocetirizine 5-mg tablet, or per puff of salbutamol inhaler, two points per puff for mometasone-17-(2)-furoate nasal spray or budesonide 200 μ g inhaler and three points per prednisolone 5-mg tablet (15).

Secondary endpoints were SMS for 2009 seasons, individual symptom and medication scores for both 2009 and 2010 seasons, asthma scores (in asthmatic patients) for 2009 and

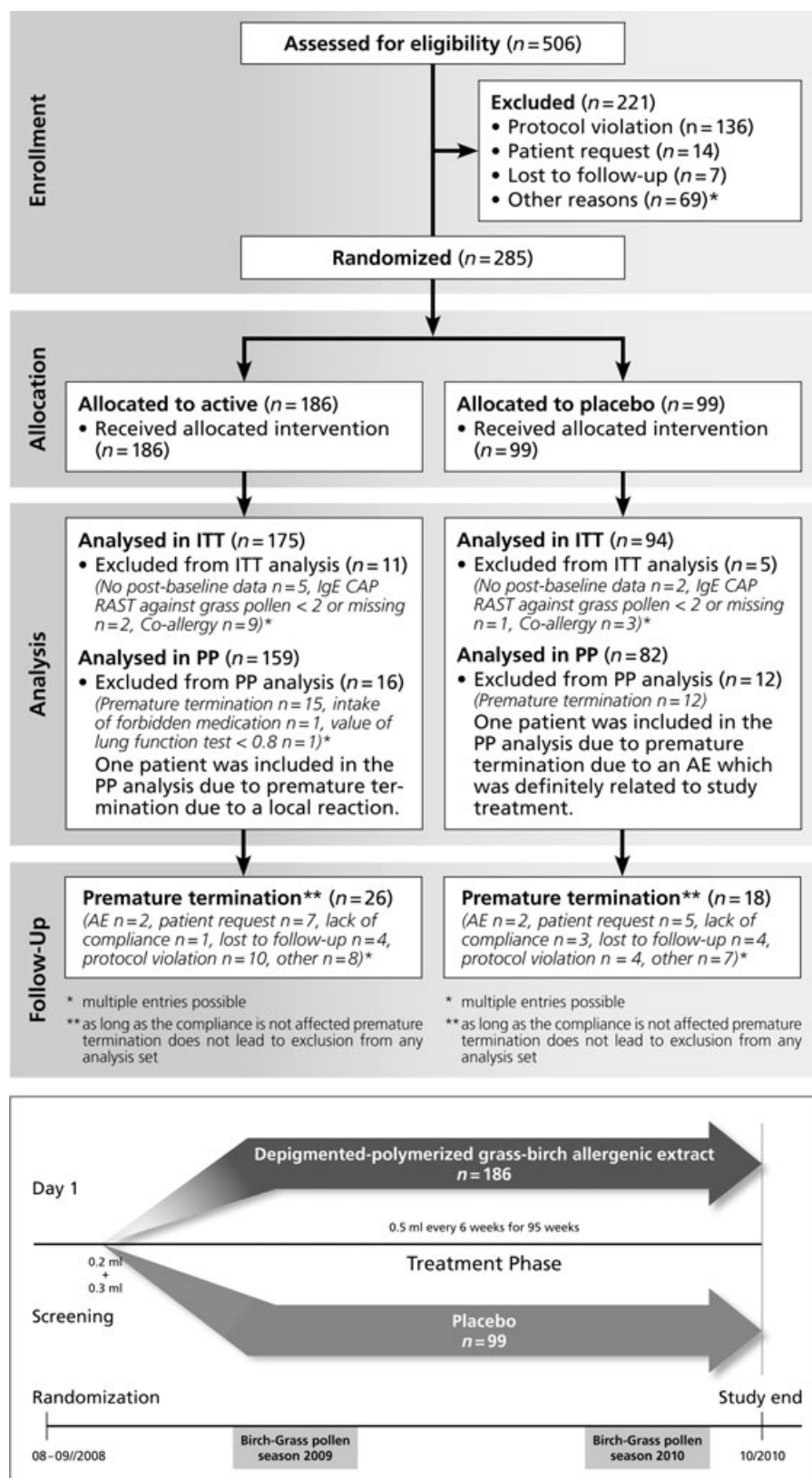


Figure 1 Patient disposition and study flow chart.

2010, and SMS for individual birch and grass pollen seasons (separately rather than combined) for both 2009 and 2010 as well as rhinitis quality-of-life questionnaire (RQLQ) (16) in 2009 and 2010.

Analysis of combined SMS was also performed for days when the SS for placebo patients was two or more (on the VAS described above); this placebo-based analysis (13) was performed for both seasons combined and for separate birch and grass pollen seasons for 2009 and 2010.

A responder analysis was performed as described (8), defining response as a 30% reduction in SMS relative to the median value for the placebo group.

Quality of life was assessed using the RQLQ score for adults and an adaptation for adolescents for patients aged 12–17 years (16, 17).

Pollen counts

The local birch and grass pollen counts were determined by volumetric pollen trap (Burkard Scientific Ltd, Uxbridge, United Kingdom). The peak pollen count was defined as the day with highest birch or grass pollen count for each season, the birch season as 1 week before and 2 weeks after the peak birch pollen count, and the grass pollen season as 1 week before and 3 weeks after the peak grass pollen count. The combined season ran from 1 week before the peak birch pollen count to 3 weeks after the peak grass pollen count.

Laboratory analysis

Allergen-specific IgE and IgG4 were measured at baseline and after 2 years using ImmunoCAP (Phadia, Uppsala, Sweden).

Safety data

Local reactions were assessed at 30 min after injection and measured in cm. Systemic reactions were graded according to EAACI criteria (4).

Statistical analysis

The primary efficacy parameter in this study was the combined SMS (mean daily SS plus the mean daily MS) for rhinitis and rhinoconjunctivitis calculated as the time-weighted AUC. The combined SMS within the timeframe of the predefined pollen season was taken into account. Within a hierarchical test procedure, the primary parameter was investigated after 2 years (1st step) and after 1 year (2nd step).

For comparison of depigmented–polymerized grass/birch mix and placebo, the *F*-test within an analysis of covariance (ANCOVA) model was used. Additionally, the Hodges–Lehmann estimator and the 2-sided 95% confidence interval according to Moses for the difference in the combined SMS between the two treatment groups were calculated. Analysis was performed on a (modified) intention-to-treat (ITT) population. Proportions of patients (responder analysis, safety) were compared by Fischer's exact test. Statistical analyses

were carried out using SAS[®], release 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

Patients

Two hundred sixty nine patients received at least one dose of immunotherapy and were included in the ITT (175 to active birch/grass pollen depigmented–polymerized extract, and 94 to placebo). Of these, 28 in the active group and 13 in the placebo were adolescents, and 57 and 24, respectively, had asthma. Participant flow is shown in Fig. 1, and patient baseline characteristics in Table 1.

Efficacy

Primary outcome

The median AUC for combined symptom and medication score (SMS) for both birch and grass pollen seasons for 2010 for the ITT set was 5.70 (interquartile range 2.62–10.02) for actively treated subjects and 7.07 (3.47–10.71) for patients receiving placebo, $P = 0.0385$, a 19.4% reduction comparing active and placebo (Fig. 2). Data for adolescents showed similar trends although numbers were too small for separate analysis.

Secondary outcomes

For the 2009 season, the median SMS over both birch and grass pollen seasons for the ITT set was 6.92 (3.67–13.76) for active and 8.74 (3.77–13.85) for placebo ($P = 0.508$). For 2009 and 2010, differences in total AUC for SMS between placebo and actively treated groups were not significant when grass or birch pollen seasons were analysed separately (data not shown). Individual symptom scores (SS) for 2010 were 4.37 (1.46–8.68) for active and 5.51 (2.45–9.26) for placebo-treated patients ($P = 0.185$), and medication scores (MS) were 0.77 (0.20–1.90) vs. 0.85 (0.25–2.79), respectively ($P = 0.397$). The SS and MS were also not significantly different between actively and placebo-treated patients for 2009

Table 1 Baseline patient characteristics

	Active (birch/grass mix), <i>n</i> = 175	Placebo, <i>n</i> = 94
Gender		
Female	75	48
Male	100	46
Age (mean SD)	31.3 (12.4)	31.3 (11.8)
Adolescent (aged 12–17)	28	13
Age	15.3 (1.9)	15.3 (1.8)
Asthma	57	24
Specific IgE (kU/L) median (IQR)		
Birch pollen	46.3 (11.9–94.3)	45.4 (8.89–100.0)
Grass pollen	44.3 (11.0–93.4)	40.9 (11.1–89.7)

SD, standard deviation; IQR, interquartile range.

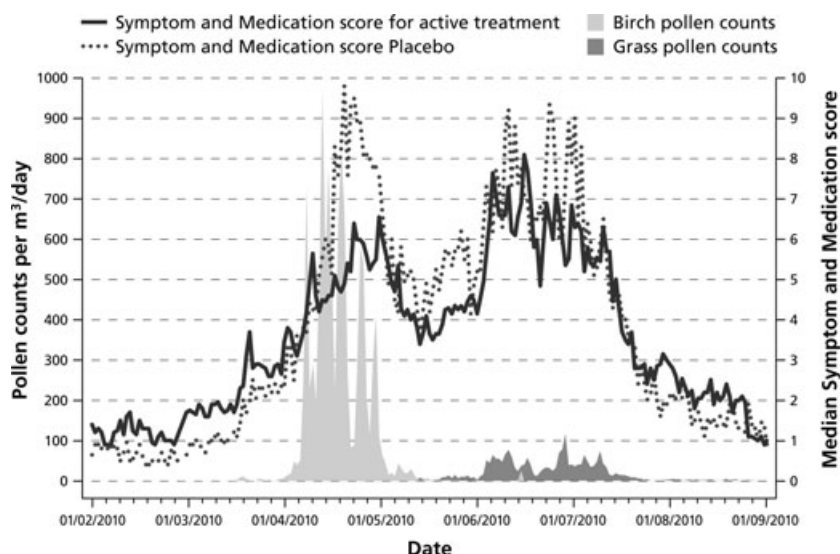


Figure 2 Combined symptom and medication score (SMS) during the 2010 birch and grass pollen season.

Table 2 Symptom scores (SS), medication scores (MS) and combined symptom and medication score (SMS) for the ITT set on days when the SS for placebo-treated patients was more than two. Median data with interquartile range

Birch/grass treatment (n = 175)	Placebo (n = 94)	P-value
2009 birch and grass pollen seasons		
SMS 5.06 (2.88–9.97)	7.97 (3.71–11.67)	0.004
SS 4.24 (1.91–8.83)	6.57 (3.23–9.05)	0.004
MS 0.77 (0.18–1.73)	0.85 (0.27–2.21)	0.793
2009 birch pollen season		
SMS 6.46 (3.02–15.04)	9.68 (4.10–15.91)	0.043
SS 5.34 (1.98–13.24)	8.99 (3.60–13.48)	0.043
MS 0.94 (0.17–2.32)	1.14 (0.36–2.50)	0.282
2009 grass pollen season		
SMS 6.35 (1.97–13.31)	7.16 (3.11–13.82)	0.374
SS 4.58 (1.12–10.93)	5.97 (2.47–10.60)	0.374
MS 0.93 (0.1–2.24)	0.97 (0.09–2.48)	0.733
2010 birch and grass pollen seasons		
SMS 4.26 (1.89–8.20)	6.43 (4.11–11.05)	0.003
SS 2.81 (1.26–6.64)	5.17 (2.62–9.36)	0.007
MS 0.59 (0.10–1.58)	0.83 (0.25–2.23)	0.306
2010 birch pollen season		
SMS 4.54 (1.71–11.41)	6.10 (3.66–11.60)	0.107
SS 3.30 (1.12–10.70)	5.72 (2.69–9.71)	0.187
MS 0.69 (0.00–1.83)	0.67 (0.21–2.36)	0.927
2010 grass pollen season		
SMS 5.65 (2.04–10.50)	8.93 (5.35–14.29)	0.015
SS 4.20 (1.19–10.02)	7.50 (3.13–12.96)	0.003
MS 0.93 (0.07–2.16)	0.83 (0.24–2.66)	0.924

(data not shown), nor were there significant differences for asthma scores for patients with asthma (not shown).

When analysis of combined SMS was performed on days with placebo SS of more than two (placebo-based analysis),

the SMS for patients treated with depigmented–polymerized birch/grass extract was 33.7% less than placebo in 2010 and 36.5% less for 2009 (Table 2). In addition, there were significant differences between actively treated and placebo for the individual birch and grass pollen seasons when analysed separately for SMS and symptom score, which were significant for the birch pollen season of 2009 and the grass pollen season of 2010 (Table 2). However, medication scores did not differ between groups (Table 2).

Responder rates were 42.6% of actively treated patients and 32.9% of placebo-treated patients for 2010 ($P = 0.16$).

Quality-of-life data

Rhinitis quality-of-life questionnaire for the 2010 birch and grass pollen seasons showed a significantly lower total score for those treated with birch/grass depigmented–polymerized extract compared with placebo (mean 1.38, SE 1.17 vs 1.72 SE 1.12, $P = 0.022$). There were also significant differences for the sleep (1.02 SE 1.43 vs 1.37 SE 1.43, $P = 0.015$) and nasal symptom (1.81 SE 1.43 vs 2.21 SE 1.36 $P = 0.024$) domains, but no significant differences for other domains. There were no significant differences for RQLQ (total or individual domains) between active and placebo-treated patients for 2009.

Immunological data

There was no significant change in allergen-specific IgE for either birch or grass pollen (data not shown). Birch pollen-specific IgG4 increased over 2 years from a mean (standard deviation) of 141.6 (291.6)–230.8 (445.9) kU/l in the patients treated with birch/grass pollen mix ($P < 0.001$), with minimal reduction in the patients receiving placebo (132.9 [210.5]–104.3 [135.6]). There was also a significant increase in grass pollen-specific IgG4 with active treatment (147.9 [228.5]–718.6

Table 3 Systemic reactions*

Reaction	Birch/grass treatment (n = 175)	Placebo (n = 94)
Vertigo/anxiety	2	–
Asthma	2	2
Conjunctivitis	2	–
Rhinitis	1	–
Throat irritation	1	2
Headache	1	–
Chills	2	–
Urticaria	–	1
Pruritus	1	–
Feeling hot	1	–
Total	13 (10 patients)	5 (4 patients)

*All reactions were grade 1 except for 2 asthmatic reactions with falls in PEF of 20% (grade 2), both with active treatment. All these reactions resolved spontaneously and required no specific treatment.

[828.6] kU/l, $P < 0.001$), which was not seen with placebo (139.3 [248.1]–180.6 [265.0] kU/l). There was no significant correlation between either specific IgG4 at the end of the study or increase in specific IgG4 from baseline and SMS for 2010.

Safety

Treatment was well tolerated. All patients achieved rapid up-dosing with no dose reductions. Eighteen systemic reactions occurred in 14 patients. These reactions are summarized in Table 3. All resolved rapidly without specific treatment. There were no grade three or four reactions.

Local reactions occurred in 66 (35.5%) patients for active treatment and 29 (29.3%) patients for placebo. None required treatment or dose adjustment. There were no drug-related serious adverse events.

Discussion

In what we believe to be the first report of a double-blind, placebo-controlled trial of mixed allergen extracts for rhinoconjunctivitis due to birch and grass pollen allergy (or indeed any pollen allergens), we show a significant reduction in combined SMS. In addition, actively treated patients reported significantly better rhinitis quality-of-life scores and significant increases in pollen-specific IgG₄ levels.

In Northern Europe, many patients are sensitized to both tree and grass pollen, resulting in symptoms from early spring to late summer. The relative proportion of such patients, as opposed to monosensitized subjects to grass or tree pollen alone, varies with geographical location and is not adequately defined. Most reported trials of allergen immunotherapy report on patients treated with one allergen, although European and US guidelines recognize that in practice many patients are polysensitized, and mixed extracts are widely used (4, 5, 18). Guidelines suggest mixing is limited to two or possibly three allergens whilst recognizing that, particularly in the US, mixing of multiple allergens for immunotherapy is still

used. Evidence for efficacy of such extracts is limited. Uncontrolled studies suggest efficacy for mixed pollen extracts for both SCIT and SLIT, and sequential up-dosing using separate allergens has also been used (6, 18–20). However, in one of the few large DBPC trials of mixed allergen extracts for asthma, there was no efficacy for the outcomes measured (21).

One potential problem with using mixed allergen extracts is the need to reduce the amount of each allergen because of safety concerns. There is a widespread assumption that efficacy of allergen immunotherapy is related to maintenance dose and that the reduced effective dose of each component allergen in a mixed extract will reduce the efficacy of that allergen compared to therapy with a single allergen extract. Here, we also reduced the amount of each allergen by 50% compared with single allergen preparations of depigmented-polymerized extracts. This may explain the relatively modest 19.4% reduction in combined SMS comparing active treatment with placebo seen here. In addition, changes in secondary endpoints such as individual symptom and medication scores and the responder analysis showed nonsignificant trends only in favour of active treatment. Although one cannot directly compare with previous studies performed with single allergens for depigmented-polymerized extracts (8–10), because both patients and pollen exposure differed, these do on the whole show larger treatment effects. Given the safety of depigmented-polymerized extracts, it may be possible to mix extracts without dose reduction. Further studies will be required for the safety and efficacy of such mixes. Nonetheless, our current data are the first to document a significant reduction in the primary outcome (SMS) in a double-blind, placebo-controlled study of SCIT using mixed birch and grass pollen extracts.

Of note, however, peak pollen counts were considerably lower for grass pollen than for birch pollen in both seasons, despite similar symptom scores for each pollen exposure (see Fig. 2). This finding is in agreement with other studies, including those with depigmented-polymerized extracts (8–10). Presumably patient exposure to allergen proteins and the threshold count for the development of symptoms for each pollen type depend on many factors including physicochemical characteristic of pollen grains.

For both allergens, active treatment induced a significant increase in allergen-specific IgG₄, as expected from studies of depigmented-polymerized extracts with single allergen (8–10). There was no correlation between either specific IgG₄ at the end of the study or increase in specific IgG₄ from baseline and SMS for 2010. No such correlation has been demonstrated for previous studies with conventional or modified immunotherapy extracts. Measurement of blocking activity (for allergen-IgE binding) of IgG induced by SCIT may be more closely related to clinical efficacy but further studies to assess this will be required (22).

The current study showed minimal side effects, and the ultra-rapid up-dosing achieved maintenance dose at the second visit without adverse events. Similar results from single allergen studies using depigmented-polymerized allergoids raise the possibility that in future, it may be safe to combine allergens without compromising dose (8–11). Further studies are required.

Here, we used combined symptom and medication score as the primary outcome measure, as suggested by guidelines and the European regulatory agency <http://www.emea.europa.eu/pdfs/human/ewp/1850406enfin.pdf> (15). However, rather than using a Likert scale to grade symptoms, we used a continuous VAS, as has been suggested (23), and as was shown to reflect severity in a large primary care population (14). We did not compare these methods of assessing symptom scores, but our data go some way towards validating the VAS to assess symptoms, at least when combined in the SMS. Medication scores were low and did not differ between groups in this study. This may reflect the scoring system used or recruitment of patients where pharmacological management had failed to control disease in previous years so that patients tended not to use these medications. The combination of continuous VAS and symptom score with discontinuous medication score to give a combined SMS has not previously validated. However, our data suggest that this approach may be useful in further studies.

Using median symptom and medication scores over the whole pollen season may result in low values because this includes days with low pollen counts when patients have few if any symptoms. This may have the effect of making disease load seem trivial and reduce the apparent efficacy of SCIT (2). We have previously analysed data for days with high pollen counts in a study of birch pollen alone (8). Here, because pollen counts differed between birch and grass, we examined the effect of treatment on days when placebo patients were symptomatic (13). One could argue that significant disease, requiring SCIT, should produce symptoms on most days of the pollen season, but experience shows that pollen counts are quite variable making measures that amplify treatment effect useful. This approach also allowed us to document efficacy for the individual birch and grass pollen seasons when placebo-treated patients were symptomatic, although SMS over the whole grass pollen season was not significantly different for active compared with placebo patients. Taken together, these results support defining the relevant days for (seasonal) analysis based on the disease burden of placebo patients as initially described by Frew et al. (13). This approach may be useful in large studies of SIT where collection of baseline year data is difficult.

In conclusion, we report significant but modest efficacy and excellent tolerability of mixed birch/grass pollen depigmented–polymerized allergen extracts for persistent rhinoconjunctivitis with or without asthma. This validates the use of combined allergen SCIT for polysensitized patients symptomatic to both allergens. The reduced IgE binding of these extracts may allow higher dose combinations for future studies to improve efficacy. Furthermore, the placebo-based analysis indicates that this approach may be useful in clinical trials in SIT.

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Conflicts of interest

O. Pfaar and L. Klimek have received research grants from ALK-Abello, Denmark; Allergopharma, Germany; Stallergenes, France; HAL, The Netherlands; Artu Biologicals, The Netherlands; Allergy-Therapeutics/Bencard, UK/Germany; Hartington, Spain; Lofarma, Italy; Novartis/LETI, Germany/Spain; Glaxo-Smith-Kline, UK/Germany; Essex-Pharma, Germany; Cytos, Switzerland; Curalogic, Denmark; Roxall, Germany; Biomay Austria; Thermo-Fisher, Germany; and MEDA Pharma GmbH, Germany, and/or they have served as advisors and on the speakers' bureaus for some of the aforementioned pharmaceutical.

O. Pfaar has received travel grants from HAL Allergy, Netherlands/Germany, and Allergopharma, Germany and is a consultant of HAL Allergy, The Netherlands. O. Pfaar is current chairman of the EAACI Interest Group Immunotherapy (IG IT). A. Sager is employee of the sponsor company LETI Pharma, Witten, Germany. D. S. Robinson is a paid consultant for Laboratorios LETI Unipersonal, Spain.

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Author contributions

T. Biedermann was coordinating investigator of the trial. O. Pfaar and L. Klimek participated in the trial as recruiting investigators. A. Sager was the sponsor's project manager of the trial and sponsor's medical responsible and was involved in the study design and conducted the trial. D.S. Robinson was involved in the study design. D.S. Robinson wrote the

first draft of the manuscript. O. Pfaar and A. Sager have furthermore contributed to the manuscript from the draft stage by reviewing and commenting as well as critically revis-

ing where applicable. As lead and corresponding author, O. Pfaar took overall responsibility for manuscript finalization and submission.

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