SUPPORTING INFORMATION FOR PUBLICATION

Efficacy and safety of birch pollen allergoid subcutaneous immunotherapy: A 2-year double-blind, placebo-controlled, randomized trial plus 1-year open-label extension.

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CONTENTS

ADDITIONAL METHODOLOGY INFORMATION

Randomization

After a patient met all inclusion criteria and did not meet any exclusion criterion, they were assigned to a random (patient) number according to the randomization scheme/list, which was located at Allergopharma under blinded conditions and under lock and key.

The random allocation of treatments to patients was performed by the responsible randomization manager at Allergopharma according to standard operating procedures. The randomization schedule was kept in the department of Quality Management (QM) sealed and locked and was not accessible to the study team prior to termination of the trial.

Protocol Approval

The study protocol was approved by:

- Central Ethics Committees in Germany (principal Ethics Committee: Ärztekammer Schleswig-Holstein, AZ:III/EK 30/05(II), Bad Segeberg and others with advisory function)
- Respective Independent Ethics Committees of the participating countries:
  - Varsinais-Suomen-Sairaanhoitopiiri Eettisen TYKS hallintokeskus Viitenr.: Dnr 134/205, Turku, Finland
  - Regionala etiprövningsnämnden i Göteburg Ref. – No.345-05 Göteborg, Sweden
  - Komisja Biotyczna przy Akademii Medycznej, Warszawa, Poland.

Birch Pollen Allergy Documentation

Birch pollen allergy was documented on the basis of troublesome symptoms requiring medication during the preceding 2005 birch pollen season (baseline), Enzyme Allergo Sorbent Test/CAPACITY (EAST/CAP) [Pharmacia ThermoFisher Scientific] to birch pollen of class 2 or higher, positive Skin Prick Test, and positive Conjunctival Provocation Test with natural birch pollen extract (both Allergopharma GmbH & Co. KG).

Permitted Rescue Medication

Prior to start of the birch pollen season patients were issued permitted rescue medication: topical levocabastine nasal spray and eye drops (0.5 mg/mL each) for mild to moderate symptoms and loratidine/cetirizine tablets (10 mg) for treatment of more severe symptoms. For symptomatic treatment of asthma, patients received salbutamol (100 µg/puff) when necessary for lower airways symptoms. A short course of oral corticosteroids was only available at the discretion of the investigator. Asthmatic patients were allowed to use inhaled corticosteroids, maintained unchanged (up to 400 µg budesonide equivalent, in GINA grade II patients) during the birch pollen season. Rescue medication was scored as follows: levocabastine nasal spray 0.5 per puff; levocabastine eye drops 0.5 per drop; loratadine/cetirizine tablets 6 per 10 mg; oral corticosteroid 4 per 5 mg prednisolone/equivalent; salbutamol 1 per 100 µg; inhaled corticosteroids 6 per 400 µg budesonide/equivalent.

FULL LIST OF INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria

Patients meeting the following criteria were included:

- Had signed informed consent document.
- Male and female outpatients, 18 – 60 years.
- Patients with IgE-mediated, moderate to severe seasonal allergic rhinitis/rhinoconjunctivitis with or without bronchial asthma (GINA grade I and II), attributable to birch pollen allergens.
- Symptoms of allergic rhinoconjunctivitis against birch pollen allergens requiring medication during birch pollen season 2005.
- Positive Enzyme Allergo Sorbent Test/CAPACITY (EAST/CAP) to birch pollen class ≥ 2.
- Positive Skin Prick Test reaction to natural birch pollen allergens demonstrated by allergen wheal at least as large as histamine control reaction (histamine dihydrochloride 1.7 mg/mL corresponding to 0.1% histamine solution) and a negative control test (physiological saline solution). A positive histamine control reaction was to be demonstrated by wheal diameter ≥ 3 mm, a negative control test was to be demonstrated by wheal diameter < 3 mm.
- Proven clinical relevance of birch pollen allergy by positive Conjunctival Provocation Test result using natural birch pollen extract.
- For female patients: effective contraception and negative pregnancy test result.

Exclusion criteria

Patients meeting any of the following criteria were excluded from the study:

Previous course of hyposensitisation against tree pollens or other allergens that are not known. Patients that had undergone an unsuccessful course of specific Immunotherapy with any allergen. Symptoms during birch pollen season related to or strong skin test positivity (wheal diameter ≥ diameter of the birch pollen wheal) to alder, hazel, poplar, elm, willow tree, beech, oak, ash, rape, *Dermatophagoides pteronyssinus, Dermatophagoides farinae, dog, cat, Aspergillus, Penicillium*.

- Clinically relevant rhinitis/rhinoconjunctival or respiratory symptoms related to other reasons that had not been clearly identified.
- FEV_1 < 80% of predicted normal (European Community for Coal and Steel [ECCS]).
- Moderate to severe bronchial asthma (GINA grade III and IV).
- Vasomotor, drug-induced or other kinds of non-allergic rhinitis/rhinoconjunctivitis.
- Febrile infections or inflammation of the respiratory tract at the time of inclusion.
- Irreversible secondary alterations of the reactive organ (emphysema, bronchiectasis etc.).
- Severe acute or chronic diseases, severe inflammatory diseases.
- Other severe generalized diseases (liver, kidneys, metabolic diseases, etc.).
- Autoimmune diseases, immune-defects including immunosuppression, immune complex induced immunopathies.
- Multiple sclerosis, active tuberculosis.
- Severe psychiatric and psychological disorders including impairment of cooperation (eg alcohol or drug abuse).
- Allergy treatment according to severity of symptoms with other than the following medication during the birch pollen season:
  - Levocabastine nasal spray/eye drops (0.5 mg/mL each), loratadine/cetirizine tablets (10 mg), salbutamol (100 µg/puff). Exacerbation treatment with a short course of oral corticosteroids. Unchanged basic treatment with inhaled corticosteroids up to 400 µg budesonide or equivalent was permitted. Treatment with other medication had to be stopped two weeks prior to birch pollen season.
- Any prophylactic and any treatment with anti-allergic medication in fixed (constant) dosage during the birch pollen season.
- Contraindications for application of adrenaline:
  - Severe acute or chronic symptomatic coronary heart disease.
  - Severe arterial hypertension.
- Treatment with ß-blockers.
- Pregnancy and lactation period.
- Female patients seeking to become pregnant.
- Concurrent participation in any other clinical study or participation in any other clinical study during the previous 30 days.
- Patients being in any relationship of dependence with the sponsor and/or with the investigator.
- Low compliance or inability to understand instructions/study documents.

SMS OF ACTIVE- AND PLACEBO-TREATED PATIENTS OF THE FAS AND FAS-NE SUBGROUP IN RELATION TO THE BIRCH POLLEN COUNT FOR ALL THREE TREATMENT YEARS (2006-2008).

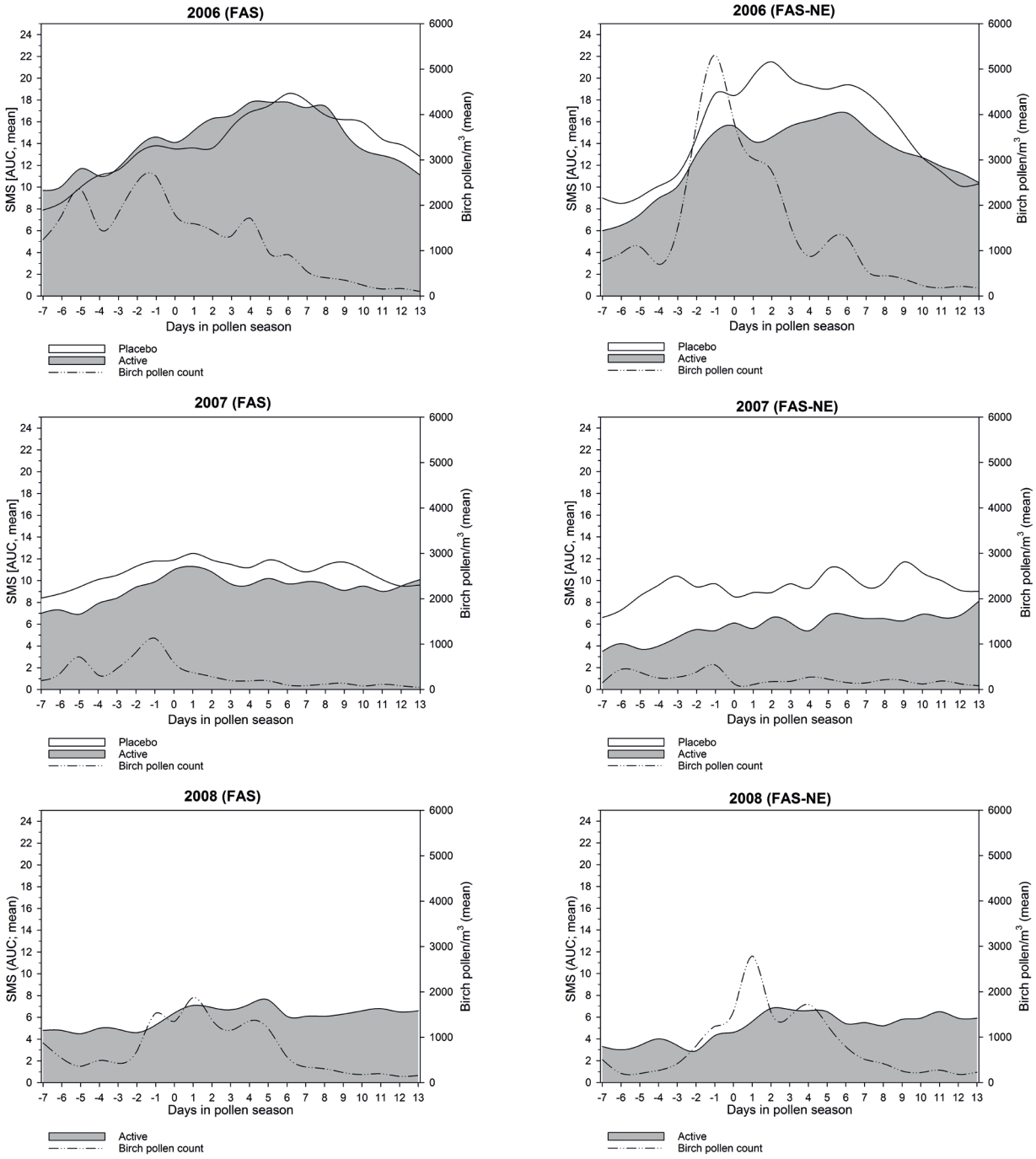

FIGURE S1: The figure shows the courses of SMS (AUC, mean) of the active- and placebo-treated group of the FAS and FAS-NE subgroup and the course of birch pollen counts (mean) of the study area (FAS) or north-east region (FAS-NE) during the 3 treatment years:
2006-2007: first and second treatment years; correspond to the double-blind, placebo-controlled phase of the study.
2007: year when the primary endpoint was evaluated.
2008: third treatment year, corresponding to the non-controlled, open label extension of the clinical trial.
AUC: area under the curve; days in pollen season: the evaluation period of the SMS was 7 days before and 14 days after the birch pollen peak count (day 0); FAS: Full Analysis Set (all patients of Germany, Poland, Sweden, Finland); FAS-NE: subgroup of patients of the north-east region (Finland, Sweden, and Poland without the most south-western center of Poland); SMS: Symptom Medication Score.

The Figure S1 indicates that the AUC of the SMS (mean) of the active-treated patients (FAS, FAS-NE) decreased from treatment year to treatment year (2006-2008). In the first year of treatment (2006), the highest pollen counts were measured, whereas in 2007 (year of the primary endpoint), the lowest pollen exposure during the study was detected. In 2007, due to the low pollen exposure, also the placebo-treated patients had comparably low SMS thus making it difficult to show a statistically significant difference in SMS between the two treatment groups. In the third treatment year (2008), despite higher pollen counts compared to 2007, the SMS of the active-treated patients had decreased further, suggesting an increased efficacy for the treatment.

Nevertheless, the graphs in the Figure S1 also demonstrate that the courses of SMS of active- and placebo-treated patients of the FAS and FAS-NE subgroup and the course of birch pollen counts do not match well in all 3 treatment years. It is important to note that, in all 3 treatment years, after the peak birch pollen count, the course of SMS of active- and placebo-treated patients of the FAS and FAS-NE and the course of birch pollen counts develop independently from each other: the SMS of active- and placebo-treated patients for the FAS and FAS-NE remained unchanged or even continued to rise despite the birch pollen counts continuously dropping to low numbers. Taking as example the 2006 FAS: the SMS increases after the peak pollen count, reaches its peak a week later, and stays high for up to 2 weeks despite low birch pollen counts. This phenomenon was also observed in the 2004 birch pollen study by Khinchi et al. and its authors called it a carry-over effect. Nevertheless, this has not been observed in grass pollen studies, which usually show similar curve progressions for pollen counts and patients' SMS indicating a dependency of the SMS on the pollen counts (Corrigan et al. 2006, Varney et al. 1991).

A possible explanation for this phenomenon could be interfering allergens causing symptoms in and around the birch pollen season; therefore, factors such as vegetation and climate would determine regional differences. For example, in Scandinavia the pollen seasons of the most important allergens are separated; therefore, there may be less interfering allergens than in the southern and western regions considered in this study (Germany and the most south-western center of Poland). In the year of assessment of the primary endpoint (2007), the flowering seasons of oak, plane tree, ash tree, beech, and hornbeam were unusually close together and interfered with the birch pollen season in several regions in Germany (see Figure S3). The northern and eastern parts of Europe showed less interference with other pollen species during the birch pollen season than the western and southern regions of Europe. Of note, patients sensitized to these pollen types were included in the study.

Another probable explanation for the effect of high SMS after the pollen peak and at low birch pollen counts could be that data of birch pollen counts measured by pollen traps may not reflect the actual birch pollen exposure of the individual patients.

IMMUNOLOGICAL PARAMETERS

In the FAS, median IgG_4 (Figure S2A) and IgG_1 (Figure S2C) levels increased significantly ($p < 0.001$) in favor of the active treatment group after the first preseasonal treatment course, followed by a further significant ($p < 0.001$) increase in the second year, and continued to rise in the third year of treatment. Median IgG_4 and IgG_1 levels of the placebo group remained largely unchanged during the entire study period. In the FAS-NE subgroup, the changes in the IgG_4 and IgG_1 levels were similar to those in the FAS (Figure S2B and S2D). In the FAS and FAS-NE, there was some variation in median IgE levels over the 3-year treatment period, although these changes were only minor in both treatment groups (Figure S2E and S2F).

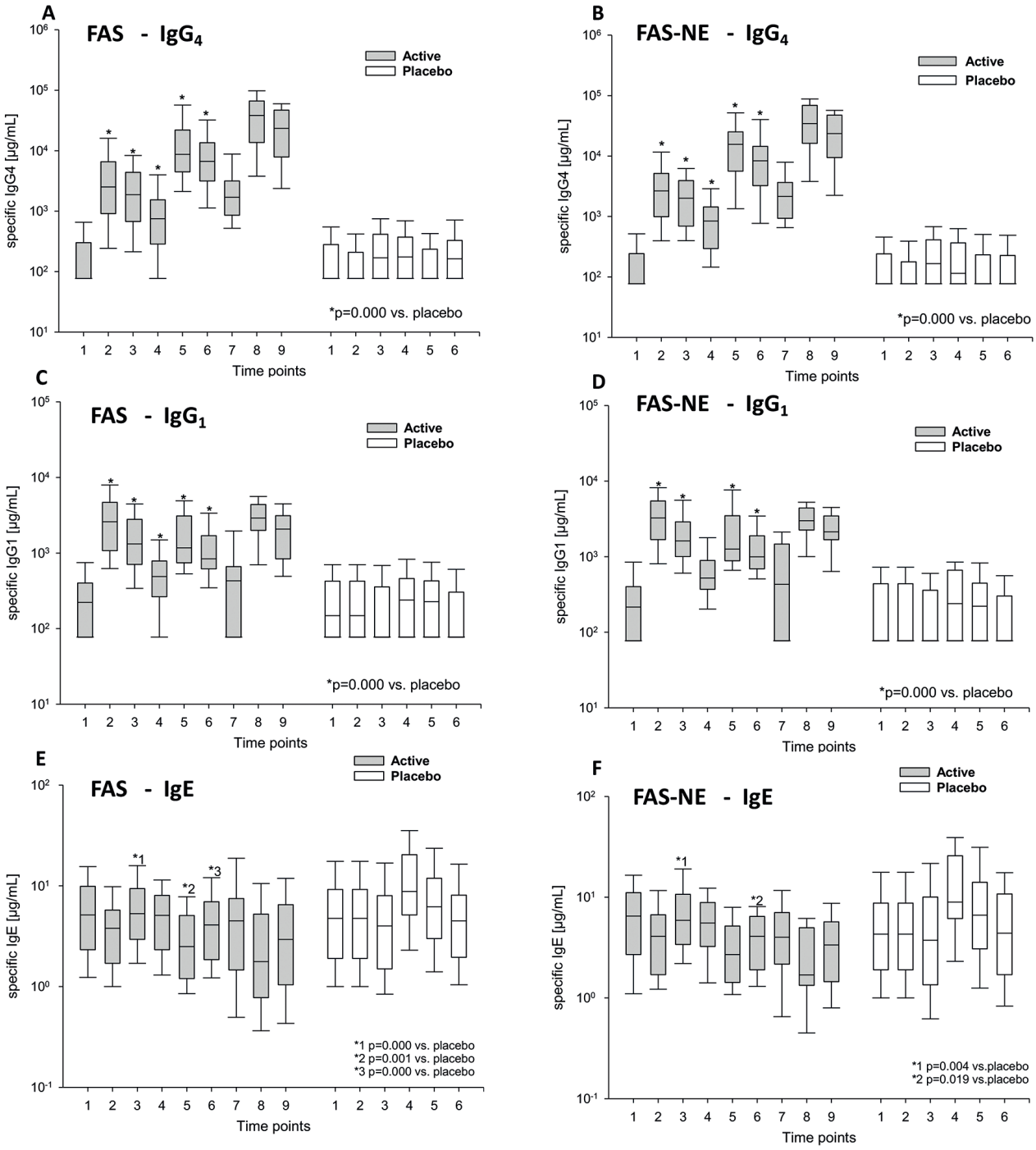

FIGURE S2: Courses of birch pollen-specific IgG_4, IgG_1, and IgE levels of the FAS and FAS-NE subgroup. The boxes show the 25th percentile (bottom) the 75th percentile (top); error bars show the 10th percentile (bottom) and the 90th percentile (top). Blood samples were obtained as follows: (1) screening visit in 2005, before 1st preseasonal treatment; (2) after 1st preseasonal treatment cycle, before pollen season in 2006; (3) after 1st pollen season 2006; (4) before 2nd treatment cycle; (5) after 2nd preseasonal treatment cycle in 2007; (6) after 2nd pollen season in 2007; (7) before 3rd treatment cycle; (8) after 3rd preseasonal treatment cycle before pollen season in 2008; (9) after 3rd pollen season in 2008. FAS: Full Analysis Set; FAS-NE: FAS north-east subgroup (patients of centers in Sweden, Finland, and Poland, excluding the most south-western center in Poland).

BIRCH AND INTERFERING POLLEN GRAPHS IN RELATION TO THE SMS OF ACTIVE- AND PLACEBO-TREATED PATIENTS IN THE 2ND TREATMENT YEAR (2007) PER COUNTRY

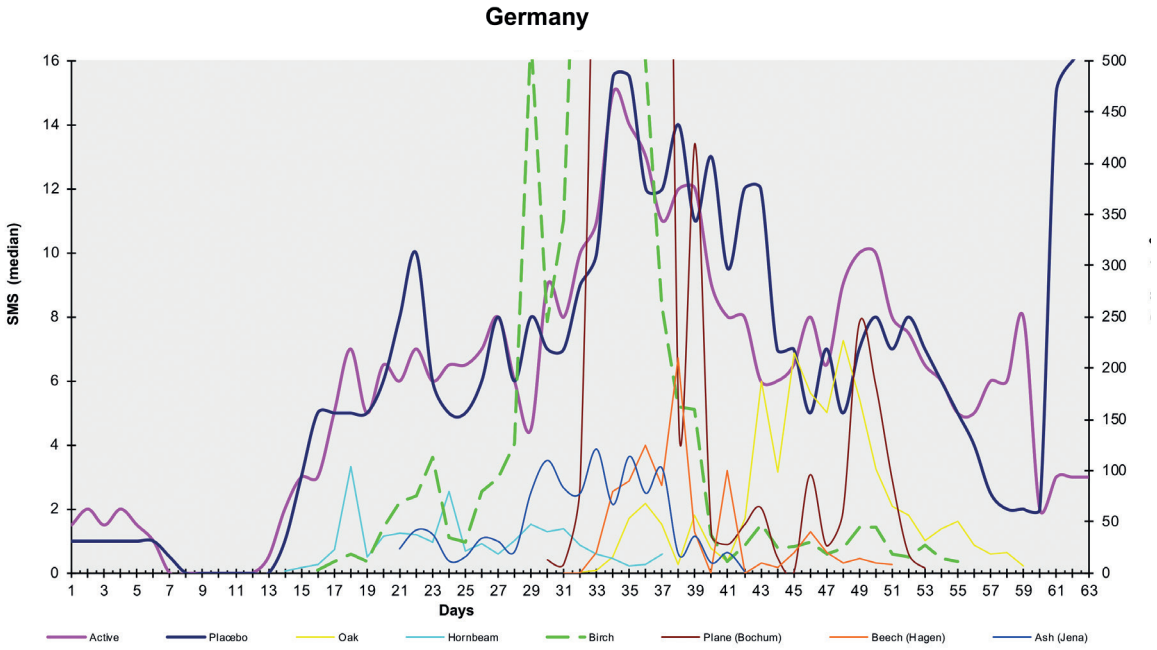

FIGURE S3 GERMANY: Pollengraphs of birch and interfering allergens are shown in relation to the SMS (median) of all active- or placebo-treated patients in Germany. The locations of exemplary pollen stations are listed in brackets (Bochum, Hagen, Jena). SMS: Symptom Medication Score; days: days of patients' diary phase; pollen/m^3: mean count over all stations of the country if not counts of a particular pollen station have been evaluated.

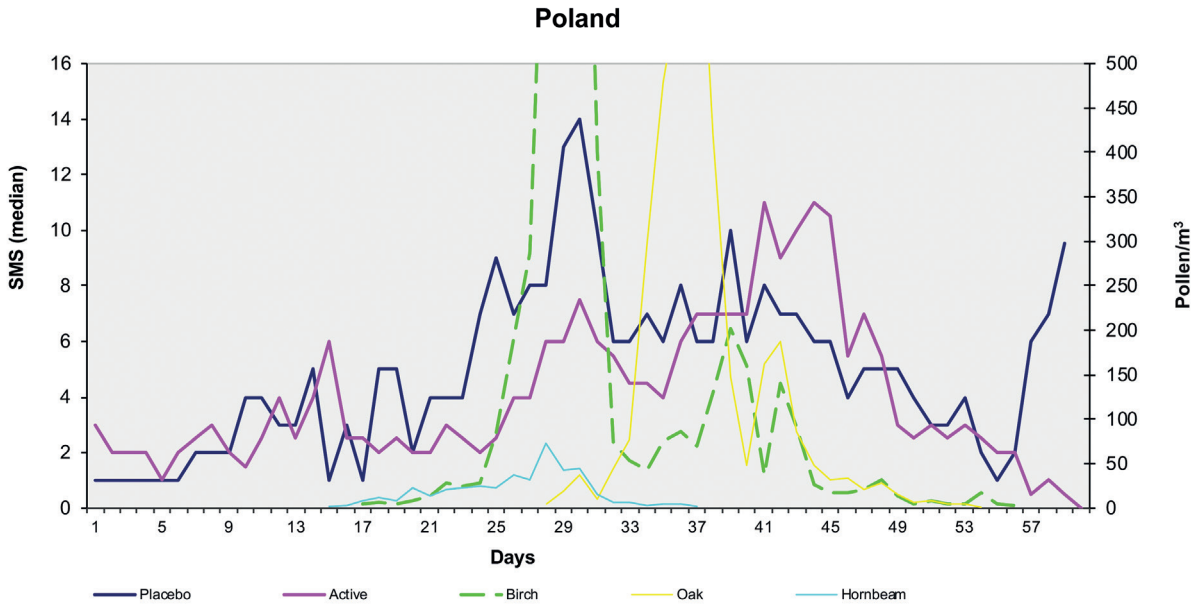

FIGURE S3 POLAND: Pollengraphs of birch and interfering allergens are shown in relation to the SMS (median) of all active- or placebo-treated patients in Poland. SMS: Symptom Medication Score; days: days of patients' diary phase; pollen/m^3: mean counts over all stations of the country.

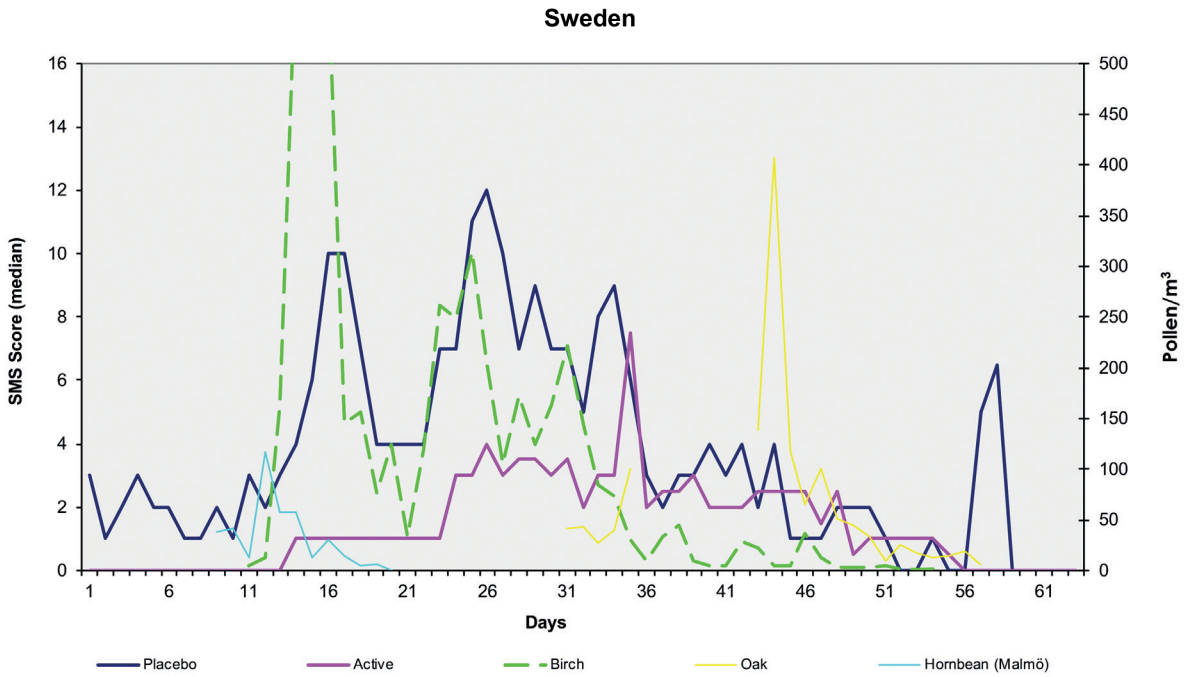

FIGURE S3 SWEDEN: Pollengraphs of birch and interfering allergens are shown in relation to the SMS (median) of all active- or placebo-treated patients in Sweden. The location of exemplary pollen stations are listed in brackets (Malmö). SMS: Symptom Medication Score; days: days of patients' diary phase; pollen/m^3: mean counts over all stations of the country if not counts of a particular pollen station have been evaluated.

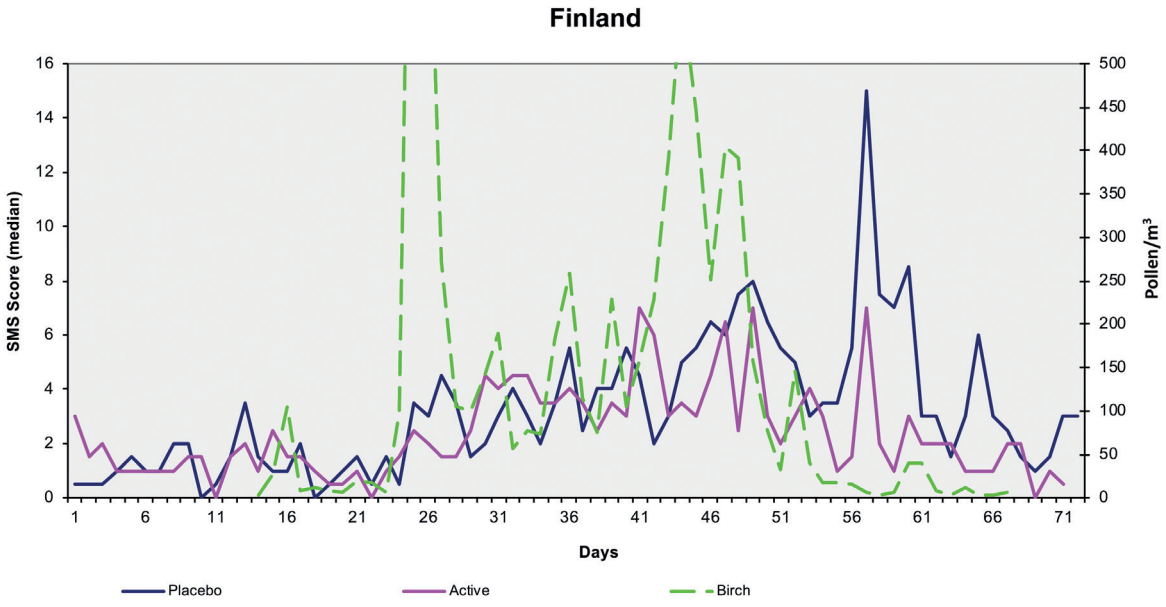

FIGURE S3 FINLAND: Pollengraphs of birch and interfering allergens are shown in relation to the SMS (median) of all active- or placebo-treated patients in Finland. SMS: Symptom Medication Score; days: days of patients' diary phase; pollen/m^3: mean count over all stations of the country.

The pollengraphs presented above show the overlap with the birch pollination of the hornbeam, beech, oak, ash tree, and plane tree 2007 pollen seasons in Germany, Poland, Sweden, and Finland (2007 was the year when the primary endpoint was evaluated). In the example presented (Bochum, pollen measuring station in Germany) it can be observed that high amounts of plane pollen were measured on the days around the birch pollen peak (coinciding with the SMS evaluation period, which was 7 days before and 14 days after the peak pollen day). In certain days, plane pollen was 4-5 times more abundant than birch pollen (1100 pollen/m^3 birch pollen vs 4160 pollen/m^3 plane tree pollen; 808 pollen/m^3 birch pollen vs 5408 pollen/m^3 plane tree pollen; 262 pollen/m^3 birch pollen vs 1528 pollen/m^3 plane tree pollen). Of note, in 2007 the average birch pollen peak counts in the investigated countries (for all centers) were similar: 1364 pollen/m^3 in Germany, 1141 pollen/m^3 in Poland, 1119 pollen/m^3 in Finland, and 1030 pollen/m^3 in Sweden.

The graphs show that, in the north-eastern countries, the pollen seasons are better separated and that there are less interfering allergens compared to Germany.

SUPPORTING LITERATURE

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