

ORIGINAL ARTICLE

Allergen-Specific Immunotherapy and Biologics

A birch sublingual allergy immunotherapy tablet reduces rhinoconjunctivitis symptoms when exposed to birch and oak and induces IgG₄ to allergens from all trees in the birch homologous group

Peter Couroux¹ | Henrik Ipsen² | Brian Sonne Stage²  | Jakob Thomas Damkjær² | Maria Abildgaard Steffensen² | Anne Marie Salapatek¹ | Kaare Lund² | Peter Adler Würtzen²

¹Inflamax Research Limited, Mississauga, Ontario, Canada

²ALK, Hoersholm, Denmark

Correspondence

Peter Adler Würtzen, ALK, Hoersholm, Denmark.
Email: pawdk@alk.net

Funding information

The trial was sponsored by ALK, Hoersholm, Denmark, who assumes overall responsibility for the trial and has been involved in both trial design and conduct.

Correction added on 22 February 2019 after first online publication: the supporting information file has been updated in this version.

Abstract

Background: This randomized, double-blind trial was conducted to determine the optimal dose for clinical efficacy of the SQ tree SLIT-tablet. An environmental exposure chamber (EEC) was used to reduce variability of allergen exposure and allow investigation of symptom reduction towards different species from the birch homologous group in separate EEC sessions.

Methods: Eligible subjects (N = 219) were randomized to receive treatment with placebo or the SQ tree SLIT-tablet (2, 7, or 12 DU) for 24 weeks. EEC pollen challenges were conducted outside the birch pollen season and included four birch and two oak EEC sessions. The primary efficacy endpoint was the average allergic rhinoconjunctivitis (ARC) total symptom score (TSS) after 24 weeks of treatment.

Results: There was a statistically significantly lower TSS during the 24-week birch EEC session for 7 DU and 12 DU compared to placebo with relative differences of 24% (P = 0.03) and 25% (P = 0.02). For the 24-week oak EEC session, there was a statistically significant difference for 12 DU (24%, P = 0.03). IgE and IgG₄ measurements supported these findings and demonstrated cross-reactivity to all other species within the birch homologous group. Treatment was well-tolerated with the most frequently reported adverse reactions being the local reactions in the oral cavity of mild-to-moderate severity.

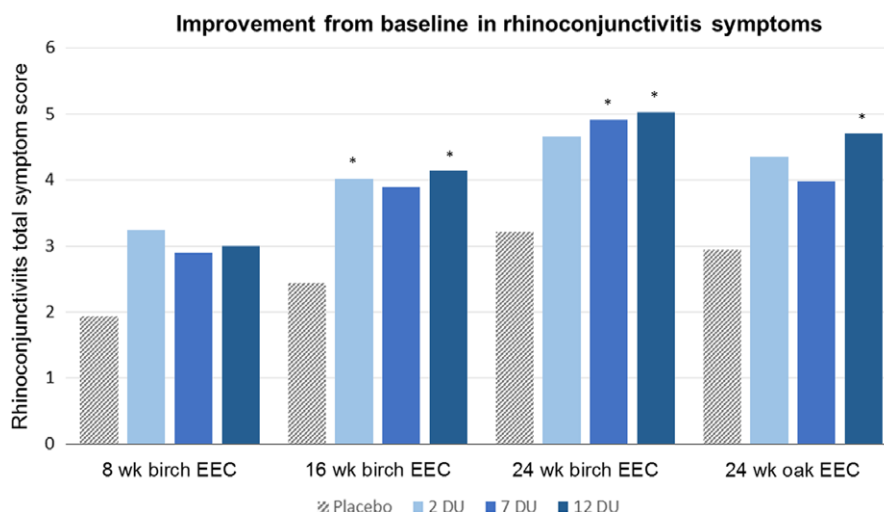
Conclusion: This trial demonstrates that the SQ tree SLIT-tablet reduce ARC symptoms triggered by birch or oak pollen. The optimal dose for further development was 12 DU. Clinical and immunological findings suggest that the tablet may be used to treat allergies to all species within the birch homologous group.

Abbreviations: AE, adverse event; AIT, allergy immunotherapy; ARC, allergic rhinoconjunctivitis; Bet v, betula verrucosa (birch); DU, developmental units; eCRF, electronic case report form; EEC, environmental exposure chamber; FAS, full analysis set; HDM, house dust mite; IgE-BF, IgE-blocking factor; IMP, investigational medicinal product; LME, linear mixed effects model; Que a, quercus alba (oak); slgE, serum IgE; slgG₄, serum IgG₄; SLIT-tablet, sublingual allergy immunotherapy tablet; SQ, SQ is a method of standardization; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TSS, total symptom score.

Correction added on 22 February 2019 after first online publication: the supporting information file has been updated in this version.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2018 The Authors. *Allergy* Published by John Wiley & Sons Ltd.



GRAPHICAL ABSTRACT

Treatment with the SQ tree SLIT-tablet, induced statistically significant reductions of rhinoconjunctivitis symptoms compared to placebo, in birch allergic subjects exposed to birch or oak pollen in an environmental exposure chamber. Statistically significant differences between 12 DU and placebo were reached after 16 weeks treatment and persisted until week 24 (end-of-trial). The reduction in rhinoconjunctivitis symptoms during oak pollen exposure, provides proof of concept for clinically relevant therapeutic cross-reactivity between birch and other trees in the birch homologous group, which was also supported by IgG₄ measurements. * $P < 0.05$; SLIT: sublingual allergy immunotherapy tablet; SQ: SQ is a method of standardisation; DU: development unit.

1 | INTRODUCTION

In Europe and North America, exposure to pollen from birch and other related trees may lead to development of respiratory allergic diseases such as allergic rhinoconjunctivitis (ARC). The prevalence of birch pollen sensitization in adults has been estimated to be more than 20% in some areas of central Europe, with a mean prevalence of 8% of the population in 13 developed countries worldwide.¹ Based on amino acid sequence homology of the major allergens and IgE cross-reactivity, five tree species (birch, alder, hornbeam, hazel, and oak) have been assigned to the same homologous group² termed the “birch homologous group”, with birch as the representative species. An additional two species (beech and chestnut) have been suggested as members of the birch homologous group.³ The relevance of birch pollen as a representative allergen source has been confirmed by in vitro IgE inhibition studies in which birch pollen extract almost completely inhibited human IgE binding to alder, hazel, hornbeam, and oak allergen extracts.⁴ Patients who are allergic to birch pollen may also experience symptoms in response to pollen from the other members of the birch group, which, due to successive seasons and geographical distribution of the birch group species, increases the burden of tree pollen allergy in terms of relevant seasons and regions.⁵

The SQ tree SLIT-tablet is being developed as allergy immunotherapy (AIT) for treatment of ARC induced by pollen from the birch group. Data from a phase I trial suggested that doses up to 12 development units (DU) have a tolerability profile suitable for at-home administration.⁶ Efficacy of the SQ tree SLIT-tablet (in doses from 0.5 to 12 DU) was evaluated in a phase II trial (EudraCT 2012-

000031-59) which was a randomized, double-blind, placebo-controlled trial in 637 adults and adolescents with moderate to severe ARC induced by birch.⁷ The tree pollen season, in which the phase II trial was conducted, was short and characterized by low birch pollen counts.⁸ Consequently, the trial did not show a dose response for the primary endpoint (daily symptom score during the birch pollen season). However, clinical data and IgG₄ measurements suggested that the optimal dose for clinical efficacy was above 4 DU.

The present trial was conducted to confirm the optimal dose for clinical efficacy using an environmental exposure chamber (EEC) to eliminate variability of allergen exposure in different trial sites or seasons.⁹⁻¹¹ While in the EEC, birch allergic subjects are expected to experience rhinoconjunctivitis symptoms similar to what is normally experienced during the BPS.¹² To investigate the clinical efficacy of the SQ tree SLIT-tablet towards other species from the birch homologous group, subjects were exposed to white oak pollen (*Quercus alba*; Que a) in separate EEC sessions. Immunological analyses (specific IgE, IgG₄, and IgE-blocking factor (IgE-BF)) were performed throughout the treatment period in order to characterize sensitization profiles and the ability of the SQ tree SLIT-tablet to induce immunological responses that cover all species in the birch homologous group.

2 | METHODS

2.1 | Ethics

The trial is identified by ClinicalTrials.gov Identifier NCT02481856. The trial was designed and conducted in accordance with the principles of

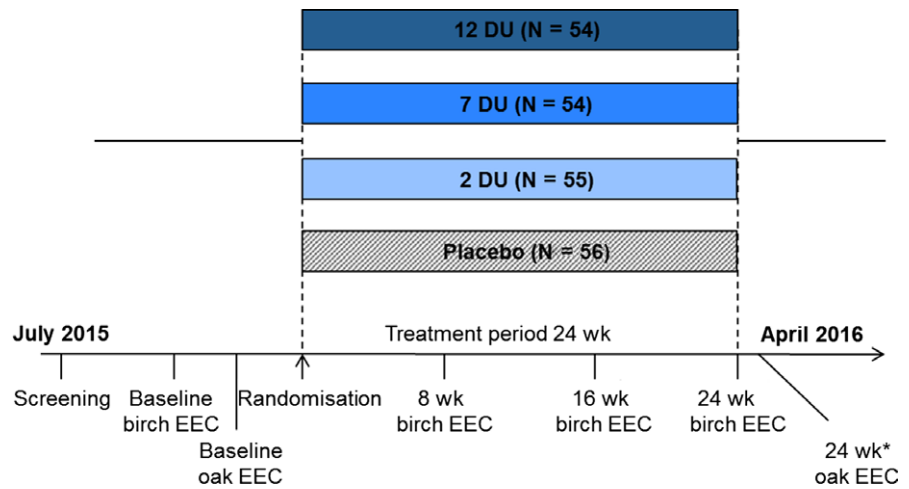


FIGURE 1 Trial design. *the 24 week oak environmental exposure chamber (EEC) visit was conducted 1-4 d after the 24 wk birch EEC [Colour figure can be viewed at wileyonlinelibrary.com]

the Declaration of Helsinki and its amendments¹³ and conducted in compliance with the principles of ICH Good Clinical Practice.¹⁴

2.2 | Trial design

The trial was a phase II, randomized, parallel-group, double-blind, placebo-controlled trial conducted in Canada using the Inflamm Research EEC (Mississauga, Ontario). Subjects were randomized equally to receive daily treatment with the SQ tree SLIT-tablet in doses of 2, 7, or 12 DU, or placebo. The EEC pollen challenge was conducted outside the birch pollen season and included four birch EEC sessions and two oak EEC sessions. The initiation of treatment with the investigational medicinal product (IMP) took place from August to October 2015, and subjects received IMP for 24 weeks. Birch EEC sessions were performed at baseline and after 8, 16, and 24 weeks of treatment, and oak EEC sessions at baseline and after 24 weeks of treatment (Figure 1).

2.3 | Trial population

The subjects eligible for the trial were adults (18-65 years) with moderate to severe rhinoconjunctivitis (with or without asthma) induced by pollen from the birch homologous group (confirmed by medical history, positive skin prick test to birch, and specific IgE to birch (*Betula verrucosa*) major allergen 1 (*Bet v 1*) (≥ 0.70 kU/L)) despite having received allergy pharmacotherapy during the two birch pollen seasons prior to trial entry. The eligibility criterion for the level of allergic rhinoconjunctivitis symptoms during the baseline birch EEC session was a score of at least seven on a TSS scale from 0 to 18.¹⁵ Subjects were excluded if they had a history of uncontrolled asthma, reduced lung function ($FEV_1 < 70\%$), or any other clinically relevant allergies expected to cause symptoms during the intervention period, which as judged by the investigators would act to confound the trial (see the Appendix S1 for further details on selection criteria).

2.4 | Interventional medicinal product

The IMP was an oral lyophilisate (fast dissolving, freeze-dried formulation) for sublingual administration provided by ALK (Hoersholm,

Denmark). The drug substance used for the active doses (2, 7, and 12 DU) was an allergen extract derived from birch pollen. DU has been used as a standardized potency unit during the development programme and is based equally on the major allergen content (*Bet v 1*) and total allergenic activity. One DU corresponds to approximately $5 \mu\text{g}$ *Bet v 1*. The matrix used was identical to the other SQ SLIT-tablets (eg. grass, house dust mite, and ragweed). The placebo tablets were similar to the active IMP with regard to appearance, smell, and taste. The first dose was administered under medical supervision lasting at least 30 minutes after tablet intake. Subjects were instructed to take one sublingual tablet daily. Before the subjects attended each of the EEC sessions, a washout period ranging from 3 days (short acting antihistamines and nasal decongestants) to 120 days (eg. for glucocorticoids and, systemic depot formulations) was specified for relevant concomitant medication that interfered with the efficacy evaluations. If deemed necessary by the investigator, subjects could be provided with antihistamines or a β_2 -agonist inhaler after an EEC session. None of the patients needed oral corticosteroids after the EEC sessions.

2.5 | Allergen exposure

During the EEC sessions, subjects were exposed to airborne birch pollen at a density of 3500 ± 500 grains/ m^3 or to oak pollen in a density of 2000 ± 500 grains/ m^3 for six consecutive hours. The pollen levels used in the birch EEC sessions resembled what could be experienced during the peak season under natural exposure.⁸ Pollen counts were obtained using rotational impaction samplers every 30 minutes during the EEC sessions to ensure stable pollen exposure.

2.6 | Randomization

Each subject was randomly assigned to receive one of three active doses (2, 7, or 12 DU) or placebo. Randomization was performed according to an allocation schedule generated by a trial-independent statistician and was stratified by sites using block randomization. Details on the randomization can be found in the Appendix S1.

TABLE 1 Average TSS at 24 wk EEC

TSS (24 wk)	Treatment	N	Adjusted means [95% CI]	Absolute difference [95% CI]	Relative difference	P-value
TSS birch	Placebo	54	7.10 [6.05; 8.15]	-	-	-
	2 DU	52	5.66 [4.60; 6.72]	1.44 [-0.02; 2.90]	20%	0.054
	7 DU	48	5.42 [4.26; 6.58]	1.68 [0.15; 3.22]	24%	0.03
	12 DU	46	5.29 [4.21; 6.37]	1.81 [0.33; 3.28]	25%	0.02
TSS oak	Placebo	54	7.47 [6.20; 8.74]	-	-	-
	2 DU	52	6.05 [4.83; 7.28]	1.42 [-0.10; 2.93]	19%	0.07
	7 DU	47	6.43 [5.27; 7.59]	1.04 [-0.46; 2.53]	14%	0.17
	12 DU	46	5.70 [4.41; 6.99]	1.77 [0.18; 3.37]	24%	0.03

CI, confidence interval; EEC, environmental exposure chamber session; N, number of subjects attending the EEC session; TSS, total symptom score. *p*-values below 0.05 are indicated in bold.

2.7 | Endpoints and assessments

An electronic diary was issued to all subjects before every EEC session, and was used for assessment of rhinoconjunctivitis symptoms before and during the 6-hour exposure period in the chamber.

The primary efficacy endpoint was the average total symptom score (TSS) measured at 9 time points from hour 2 to 6 during the 6-hour birch EEC session after 24 weeks of treatment.

The average TSS for each subject was calculated as the average sum of the total nasal symptom score consisting of four symptoms (TNSS); runny nose, blocked nose, sneezing, and itchy nose and the total ocular symptom score (TOSS) consisting of two symptoms; gritty feeling/red/itchy eyes and watery eyes. Each of the six symptoms were measured on a scale from 0-3, where 0 corresponds to no symptoms and three corresponds to severe symptoms. Thus, the TSS had a range of 0-18.

At the randomization visit, subjects received an adverse event (AE) notebook to be completed in case the subject experienced any AEs during the trial. The AE notebook entries served as a basis for AE assessment. At all subsequent visits, the investigator reviewed and discussed data entered in the AE notebook with the subject, and entries fulfilling the requirement for an AE were entered into the eCRF.

2.8 | Immunological assessments

At each visit to the trial site, blood samples were collected for immunological assessments. The amount of allergen (Bet v and Que a) specific IgE and IgG₄ antibodies were measured by ImmunoCAP (Phadia 250, Thermo Fischer Scientific, Uppsala, Sweden) and IgE-BF was measured as described previously.¹⁶ The IgE-BF assay is a competition assay supplementing the data for IgG₄ and IgE titers with a readout reflecting the combined effect of IgE and non-IgE (IgG₄) changes. In addition, it has been shown to correlate to functional assays such as basophil activation test (BAT) and facilitated allergen presentation (FAP) (15).

For immunological cross-reactivity studies, specific serum IgE (sIgE, *pretreatment*) and IgG₄ (sIgG₄, *posttreatment*) to birch (Bet v), oak (Que a), hazel (Cor a), alder (Aln i), beech (Fag g), chestnut (Cas

s), hornbeam (Car b), and olive (Ole e) (as a negative control), were measured by standard ImmunoCAP. An overview of pollen sources used for the immunological assays and assays applied for the different treatment groups can be found in Table E1 and E2 of the Appendix S1.

2.9 | ImmunoCAP IgE (pretreatment) and IgG₄ (posttreatment) inhibition assays

For the inhibition assays, serum samples from 42 patients treated with 12 DU (with samples available from baseline and visit eight) were pre-incubated at 4°C overnight with tree pollen extract or PBS. The following day, the extent of residual IgE/IgG₄-binding against pollen extracts from Bet v, Que a, Aln i, Cor a, and Ole e (negative control) were measured by standard ImmunoCAP. Data were only included if antibody titer of the non-inhibited sample was ≥0.7 kU/L (sIgE) or ≥0.07 mgA/L (IgG₄) towards the individual trees.

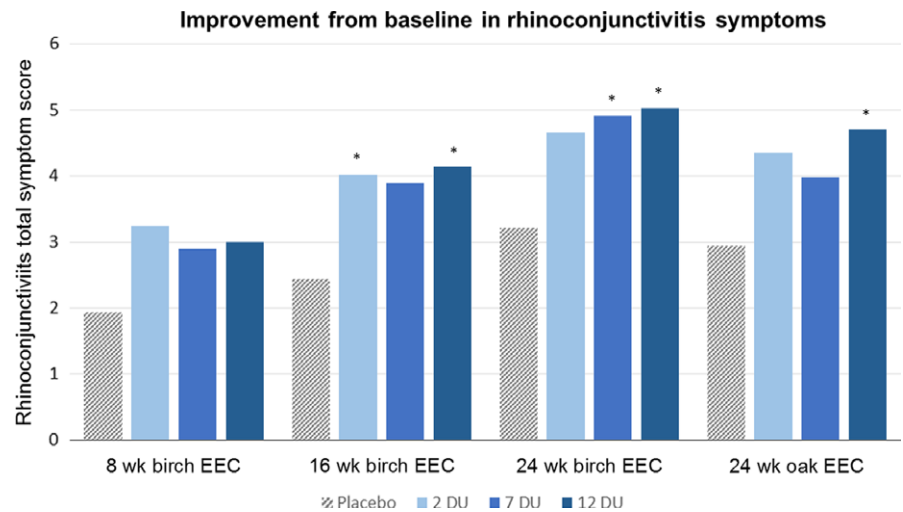
2.10 | Statistical methodology

The efficacy analyses were conducted based on all randomized subjects (full analysis set, FAS) who attended an EEC visit. Statistical tests were two-sided at a 5% significance level unless otherwise specified and confidence intervals were two-sided 95% confidence intervals. All statistical analyses were pre-defined in the statistical analysis plan prior to unblinding.

The primary endpoint (the average TSS during the 24 weeks birch EEC visit) was analyzed using a linear mixed effect (LME) model with the average TSS as the response variable, and treatment, visit, and their two-factor interaction as fixed class effects, the average TSS at baseline EEC as a fixed regression variable, and chamber cohort and subject as random class variables. No adjustments for multiplicity were performed.

The change from baseline of the immunological parameters, Bet v and Que a specific IgE, and IgG₄, were tested using the post hoc Tukey's HSD method. The log₁₀ transformed values were fitted to a linear repeated measures mixed model using subject ID as a random factor (restricted maximum likelihood).

FIGURE 2 Adjusted mean change from baseline in total symptom score during birch and oak environmental exposure chamber (EEC) sessions (FAS). The total symptom score is the sum of the total nasal symptom score (comprising four symptoms) and the total ocular symptom score (comprising two symptoms). Each of the symptoms are scored from 0-3 (no symptoms - severe symptoms), resulting in a scale from 0-18. *statistically significant change compared to placebo ($P < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]



Further details of the statistical methods including sample size calculation and the correlations for antibody response can be found in the Appendix S1.

3 | RESULTS

3.1 | Population

A total of 219 eligible subjects were randomized to blinded treatment with either the SQ tree SLIT-tablet (2, 7, or 12 DU) (N = 163) or placebo (N = 56). During the trial, 19 subjects (9%) discontinued (10 subjects due to AEs) and 200 (91%) subjects completed the trial. By definition, all randomized subjects were included in analyses based on FAS. Subject disposition is summarized in Figure E1 of the Appendix S1. The treatment groups were similar with regard to gender composition (47% female), ethnicity, race, smoking history, age, and BMI. Subject baseline demography is presented in Table E3 in the Appendix S1. In accordance with the inclusion criteria, all subjects were IgE sensitized to birch and had a medical history of birch pollen allergy. The majority of subjects also showed positive skin prick test towards pollen from other members of the birch homology group such as oak (75%) and alder (91%), as well as other perennial and seasonal allergens. 10% of the subjects suffered from asthma.

3.2 | Efficacy on rhinoconjunctivitis symptoms

The results of the statistical analyses of the TSS at the 24-weeks birch and oak EEC sessions are presented in Table 1.

Analysis of the primary endpoint revealed statistically significantly lower TSS during the 24-week birch EEC session for 7 and 12 DU compared to placebo with relative differences of 24% and 25%. The difference from placebo was numerically higher for 12 DU than for 7 DU although the overall magnitudes of the absolute and relative differences were comparable between the two doses. For the 24-week oak EEC session, a statistically significant difference from placebo was noted for the 12 DU dose (24%, $P = 0.03$).

Figure 2 shows the change from baseline in TSS at the 8, 16, and 24 week birch EEC sessions. For 12 DU, the difference from placebo was statistically significant at both the 16 and 24 weeks EEC session. The adjusted mean decrease from baseline in TSS at 24 weeks was 5.03 for 12 DU compared to 3.22 for placebo. A similar decrease from baseline in TSS for the oak EEC sessions was seen (4.71 for 12 DU compared to 2.94 for placebo).

The results for the secondary endpoints on nasal and ocular symptom scores are shown in Table 2. Nasal symptoms (ie. TNSS) were significantly reduced compared to placebo for all three doses at the 24-week birch EEC session. For the 24-week oak EEC session, the TNSS reduction was significant for 12 DU. Eye symptoms TOSS were not significantly reduced relative to placebo for any dose, although the absolute differences were in favour of treatment.

3.3 | Immunological endpoints (IgE, IgG₄, IgE-BF)

As shown in Figure 3, the analyses of specific immunological parameters showed that 7 and 12 DU induced statistically significant increases from baseline in serum levels of both birch- and oak-specific IgG₄ compared to placebo whereas 2 DU induced a significant increase only for birch-specific IgG₄ (Figure 3A and B). The average birch- and oak-specific IgG₄ titers followed the same trend for all doses and all time points investigated. Similar results were seen for birch- and oak-specific IgE (Figure 3C and D) and Bet v IgE-BF (Figure 3E). Especially for IgE-BF a marked increase could be seen for 7 DU and 12 DU compared to 2 DU and placebo.

3.4 | Exploratory immunological analysis

IgE sensitization and the correlation between IgE titers towards individual trees of the birch homologous group is shown in Figure 4, top row. Serum samples collected at the indicated time points were analyzed for IgE and IgG₄ specific to birch homologous trees as well as olive (negative control) by ImmunoCAP. The data demonstrate a

Secondary Endpoints (24 wk)	Treatment	N	Adjusted means	Absolute difference [95% CI]	P-value
TNSS Birch EEC	Placebo	54	5.35 [4.60; 6.09]	-	-
	2 DU	52	4.25 [3.50; 5.00]	1.10 [0.05; 2.15]	0.04
	7 DU	48	3.79 [2.99; 4.59]	1.56 [0.47; 2.64]	0.01
	12 DU	46	3.93 [3.16; 4.70]	1.42 [0.35; 2.48]	0.01
TOSS Birch EEC	Placebo	54	1.74 [1.36; 2.11]	-	-
	2 DU	52	1.41 [1.03; 1.78]	0.33 [-0.18; 0.84]	0.20
	7 DU	48	1.60 [1.18; 2.03]	0.14 [-0.41; 0.68]	0.62
	12 DU	46	1.37 [0.98; 1.75]	0.37 [-0.14; 0.88]	0.16
TNSS Oak EEC	Placebo	54	5.62 [4.73; 6.51]	-	-
	2 DU	52	4.64 [3.80; 5.47]	0.98 [-0.10; 2.06]	0.07
	7 DU	47	4.62 [3.80; 5.43]	1.00 [-0.07; 2.08]	0.07
	12 DU	46	4.35 [3.45; 5.25]	1.27 [0.13; 2.42]	0.03
TOSS Oak EEC	Placebo	54	1.84 [1.36; 2.32]	-	-
	2 DU	52	1.42 [0.97; 1.86]	0.42 [-0.12; 0.97]	0.13
	7 DU	47	1.81 [1.40; 2.22]	0.03 [-0.50; 0.56]	0.90
	12 DU	46	1.40 [0.94; 1.86]	0.44 [-0.12; 1.01]	0.12

TABLE 2 Nasal and ocular symptom scores at 24 wk birch and oak EEC sessions. *p*-values below 0.05 are indicated in bold

CI, confidence interval; N, number of subjects attending the EEC session; TNSS, total nasal symptom score; TOSS, total ocular symptom score.

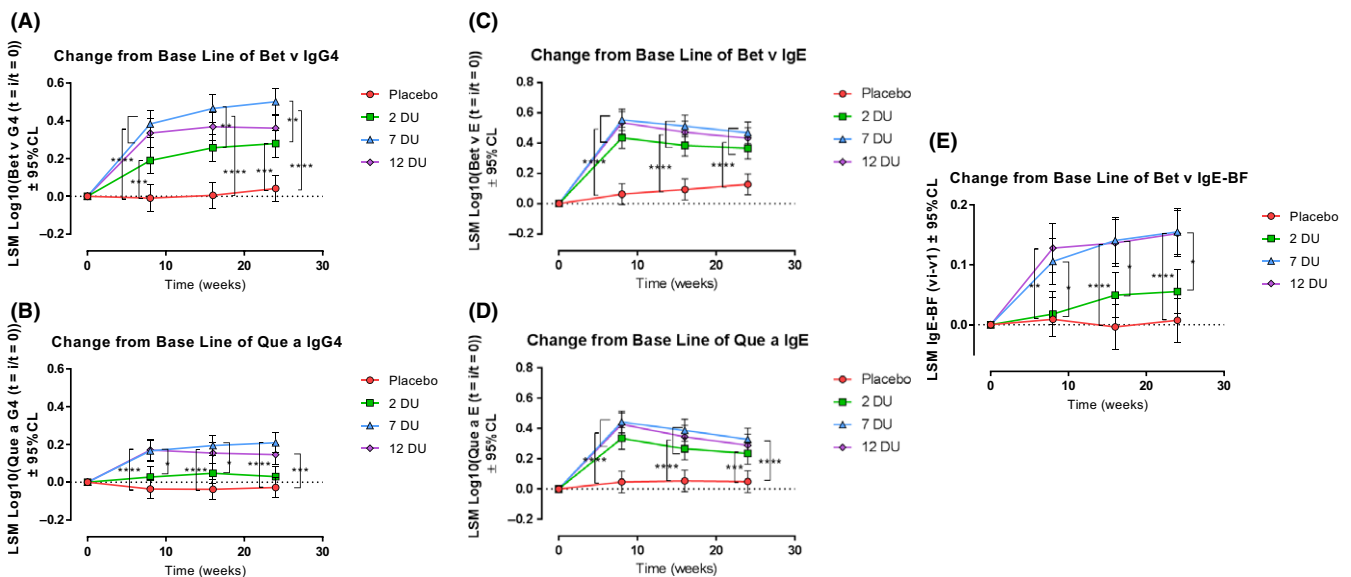


FIGURE 3 Serum samples collected at the indicated time points were analyzed for Bet v- or Que a-specific IgG₄ (A, B) and IgE (C, D) by ImmunoCAP and Bet v-specific IgE-BF (E) by AdviaCentaur. Data are represented as Least Squares Means (LSM) change from baseline of Bet v and Que a specific IgE *P*-values (corrected for multiple comparisons): <0.0001 = ****; <0.001 = ***; <0.01 = **; <0.05 = *. In the placebo group Bet v-specific IgE was significantly increased after 24 wk compared to baseline. No other changes were seen in the placebo group. Bet v = betula verrucosa (birch), Que a = quercus alba (oak) [Colour figure can be viewed at wileyonlinelibrary.com]

high level of correlation between IgE reactivity towards birch and each of the additional four birch homologous group species. The high level of correlation in IgE titers ranged between $r = 0.98$ (birch and alder) and $r = 0.83$ (birch and oak). There was a very weak correlation between birch and olive (the negative control) ($r = 0.47$). A strong correlation between IgE titers was also seen for beech ($r = 0.91$), but not for chestnut ($r = 0.37$) (see Figure E3 Appendix S1 for details).

The correlations between the concentration of treatment-induced IgG₄ (measured at visit eight, posttreatment) specific towards birch, and each of the birch homologous group species, and olive (negative control) are shown in Figure 4, bottom row. In the majority of patients, treatment-induced IgG₄ reacted with all birch homologous group species to the same extent as to birch, with the strong correlations ranging between $r = 0.95$ (birch and alder) and $r = 0.78$ (birch and oak). For olive, no significant correlation with Bet

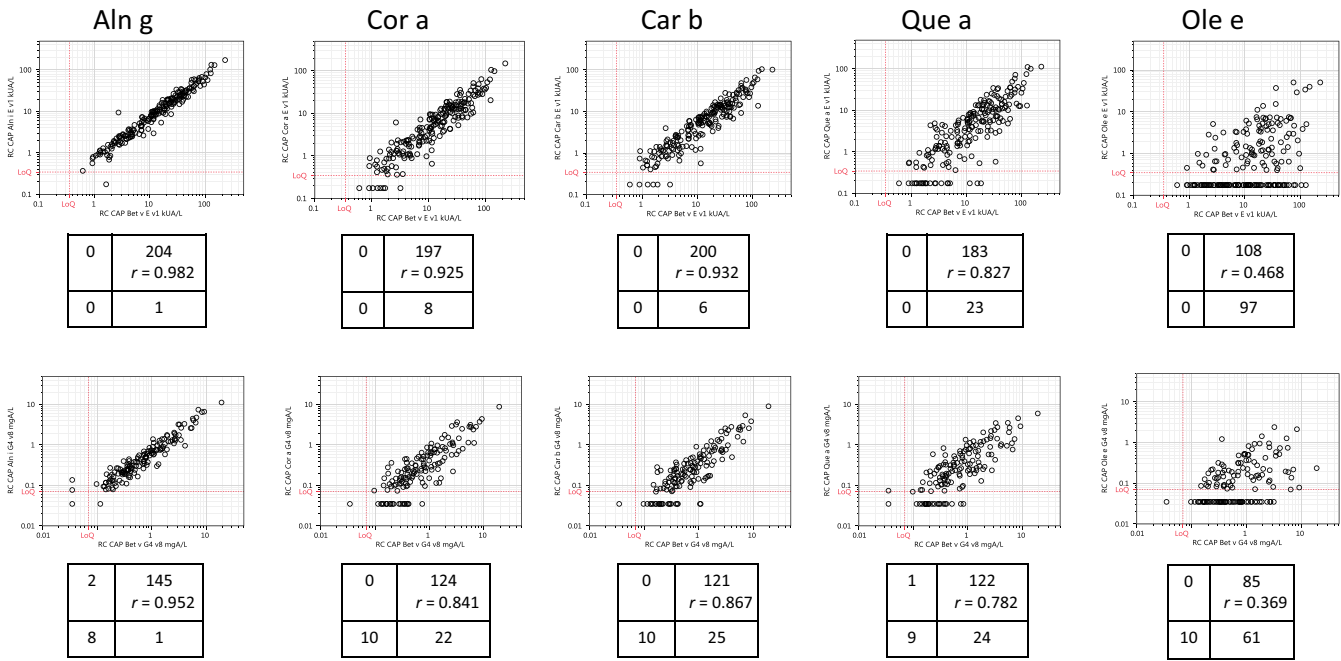


FIGURE 4 Correlation of antibody titers with birch for birch homologous tree species. Serum samples were analyzed for IgE (upper row, baseline, all patients) and IgG₄ (lower row, v8, 2, 7, 12 DU treated patients) specific to birch, birch homologous trees or olive (negative control) by ImmunoCAP. X-axis: Bet v CAP, y axis: homologous tree CAP. Tables indicate sample distribution above and below lower level of quantification and *r*-values (Pearson correlation) for the samples above threshold on both axes. Aln g: Alder; Cor a: hazel; Car b: beech; Que a: oak; Ole e: olive. [Colour figure can be viewed at wileyonlinelibrary.com]

v specific IgG₄ was found (*r* = 0.37). A strong correlation between IgG₄ titers was also seen between birch and beech (*r* = 0.81), but not for birch and chestnut (*r* = 0.15) (see Figure E3 Appendix S1 for details). Inhibition experiments showed that in the majority of the patients, alder-, hazel-, and oak-specific IgE and IgG₄ were inhibited by more than 80% by birch allergen extract clearly demonstrating that the IgE and treatment-induced IgG₄ are cross-reacting antibodies that react broadly with species from the birch homologous group (not shown). See Appendix S1 for additional details.

3.5 | Safety

Table E4 of the Appendix S1 provides an overview of the main safety data. During the course of the trial, 191 subjects (87% of FAS) reported 839 AEs. The proportion of subjects with AEs assessed as being possibly related to the IMP was higher in the active treatment groups (71%–91%) compared to placebo (41%). 99% of reported AEs were mild or moderate in severity. The most frequently reported IMP-related AEs represented local reactions related to the IMP administration site (See Figure E2 of the Appendix S1 for details). In the active treatment groups, the three most frequently reported AEs were throat irritation (35%–59% of subjects), tongue pruritus (22%–39% of subjects), and ear pruritus (22%–31% of subjects), and these showed a dose response relationship in terms of number of subjects reporting the AE. SAEs were reported by three subjects (1%) (PTs: tibia fracture (2 DU), glioblastoma (7 DU), and depression (2 DU)); none of these were assessed as related to the IMP.

4 | DISCUSSION

This randomized double-blind, placebo-controlled phase II trial with the SQ tree SLIT-tablet, showed statistically significant reductions of ARC as well as rhinitis symptoms in birch allergic subjects exposed to birch or oak pollen in an EEC. Statistically significant differences between 12 DU and placebo were reached after 16 weeks of treatment and persisted until week 24 (end of trial). No significant treatment effect was shown for conjunctivitis symptoms alone; however, the level of conjunctivitis symptoms was generally low in all treatment groups.

An immune modulating effect of the SQ tree SLIT-tablet was confirmed by induction of statistically significant changes from baseline in serum levels of both birch and oak specific IgE and IgG₄ for all active treatment groups compared to placebo. The Bet v-specific immunological changes observed during the SQ tree SLIT-tablet treatment (IgG₄, IgE-BF) are similar to those observed for SQ grass SLIT-tablet, where sustained clinical effect has been demonstrated for up to 2 years after end of treatment,^{17,18} which suggests a durable treatment effect of SQ tree SLIT-tablet with continued daily treatment.

The SQ tree SLIT-tablet was generally well tolerated in all administered doses. The most frequently reported IMP-related AEs were mild or moderate in severity, and most were local reactions related to the sublingual administration of the SQ tree SLIT-tablet, which was expected from prior trials conducted with the grass, ragweed and HDM SLIT-tablets.^{19–26} Generally, tolerability during the trial was similar to observations from prior trials with the SQ tree SLIT-

tablet suggesting that doses up to 12 DU has a tolerability profile suitable for self-administration, provided that the first dose is administered under medical supervision.

A particularly important finding from the current trial is the reduction in ARC symptoms during oak pollen exposure, which provides proof of concept for clinically relevant therapeutic cross-reactivity between birch and oak. Since the trial subjects were selected based on birch sensitization it remains an open question whether patients recruited in an area completely devoid of birch trees would have a similar sensitization profile and response to the treatment. The possibility that clinically relevant AIT coverage with the SQ tree SLIT-tablet extends to the entire birch homologous group is supported by the very high level of immunological cross-reactivity between birch and all the birch homologous group species. This is further substantiated by the finding that treatment with the SQ tree SLIT-tablet demonstrated a significant and clinically relevant symptom reduction upon exposure to oak pollen despite oak being a birch homologous group member that did not show the highest level of immunological cross-reactivity to birch among the group members. Taken together, the demonstrated IgE and IgG₄ cross-reactivity supports the use of birch as a representative for the birch homologous group, as also suggested by Lorenz² on basis of structural homology between Bet v 1 and homologous PR10 proteins and antibody binding data. The IgE and IgG₄ data provided here also demonstrate immunological cross reactivity between birch and beech at the same level as between the birch homologous group species. This indicates that beech may be considered along with the original five birch homologous group species, as suggested by the European Medicines Agency, EMA³. This trial was carried out using an EEC, leading to some inherent limitations, especially concerning the reproducibility in an outpatient setting during natural pollination. Existing EEC chambers have been technically validated and it is widely acknowledged that EEC trials eliminate variations in pollen exposure and other potentially confounding environmental conditions.⁹ EEC trials are thus optimal for dose-finding trials and for obtaining information about the onset of efficacy of AIT, while in-field trials remain essential to measure efficacy during an entire pollen season³. Consequently, a double blind, placebo-controlled, in-field phase III trial has been initiated with the SQ tree SLIT-tablet (12 DU) (EudraCT: 2015-004821-15).

In conclusion, the current trial demonstrates that the SQ tree SLIT-tablet reduce rhinoconjunctivitis symptoms triggered by birch pollen. Results from oak EEC exposure and IgE and IgG₄ measurements suggest that the use of the SQ tree SLIT-tablet may be extended to treatment of ARC induced by all other species within the birch homologous group. The combined evidence from reduction of birch and oak induced rhinoconjunctivitis symptoms during pollen exposure in an EEC suggests that the optimal dose for further development is 12 DU.

ACKNOWLEDGMENTS

The trial was funded by ALK. In this context, the authors thank the clinical trial team at ALK for clinical project management, operational

oversight, safety monitoring, data management, statistics, medical writing, and bioanalytics.

CONFLICTS OF INTEREST

Dr. Couroux reports grants from ALK, during the conduct of the study. Dr. Ipsen, Mr. Stage, Dr. Steffensen, Dr. Damkjær, Dr. Lund and Dr. Wurtzen was employed by ALK at the time of trial conduct. Dr. Wurtzen and Dr. Lund own stocks in ALK. Dr. Salapatek has nothing to disclose.

AUTHOR CONTRIBUTIONS

Couroux P, Salapatek AM and Stage BS participated in the trial design and conduct. All authors contributed to data analysis and/or interpretation as well as to preparing and critically reviewing the manuscript. All authors have approved the manuscript and agrees to be accountable for all aspects of the work.

ORCID

Brian Sonne Stage  <http://orcid.org/0000-0001-8351-8080>

REFERENCES

- Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. *Allergy*. 2007;62:301-309.
- Lorenz AR, Luttkopf D, May S, Scheurer S, Vieths S. The principle of homologous groups in regulatory affairs of allergen products - a proposal. *Int Arch Allergy Immunol*. 2009;148:1-17.
- EMA. Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases. CHMP/EWP/18504/2006[Final]. 2008.
- Niederberger V, Pauli G, Gronlund H, Froschl R, Rumpold H, Kraft D, et al. Recombinant birch pollen allergens (rBet v 1 and rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen: a quantitative IgE inhibition study with sera from different populations. *J Allergy Clin Immunol*. 1998;102(4 Pt 1):579-591.
- Mothes N, Horak F, Valenta R. Transition from a botanical to a molecular classification in tree pollen allergy: implications for diagnosis and therapy. *Int Arch Allergy Immunol*. 2004;135:357-373.
- Birk AO, Andersen JS, Villesen HH, Steffensen MA, Calderon MA. Tolerability of the SQ tree SCIT tablet in adults. *Clin Ther*. 2017;39:1858-1867.
- Makela MJ, Gyllfors P, Valovirta E, Steffensen MA, Grønager PM, Savolainen J, et al. Immunotherapy with the SQ tree SLIT-tablet in adults and adolescents with allergic Rhinoconjunctivitis. *Clin Ther*. 2018;40:574-586.
- Yli-Panula E, Fekedulegn DB, Green BJ, Ranta H. Analysis of airborne betula pollen in Finland; a 31-year perspective. *Int J Environ Res Public Health*. 2009;6:1706-1723.
- Rosner-Friese K, Kaul S, Vieths S, Pfaar O. Environmental exposure chambers in allergen immunotherapy trials: current status and clinical validation needs. *J Allergy Clin Immunol*. 2015;135:636-643.
- Hohlfeld JM, Holland-Letz T, Larbig M, Lavae-Mokhtari M, Wierenga E, Kapsenberg M, et al. Diagnostic value of outcome measures following allergen exposure in an environmental challenge chamber

- compared with natural conditions. *Clin Exp Allergy*. 2010;40:998-1006.
11. Devillier P, Le GM, Horak F. The allergen challenge chamber: a valuable tool for optimizing the clinical development of pollen immunotherapy. *Allergy*. 2011;66:163-169.
 12. Inflammex. Validation of birch environmental exposure chamber - Internal Data. 2006.
 13. World Medical Association. Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. 2013. Adopted by the WMA General Assembly in Helsinki (1964) and as amended by the WMA General Assembly.
 14. ICH. ICH Harmonised Tripartite Guideline Topic E6 (R1): Guideline for Good Clinical Practice. 1996.
 15. Horak F, Ziegelmayer P, Ziegelmayer R, Lemell P, Devillier P, Montagut A, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol*. 2009;124:471-477.
 16. Wurtzen PA, Lund G, Lund K, Arvidsson M, Rak S, Ipsen H. A double-blind placebo-controlled birch allergy vaccination study II: correlation between inhibition of IgE binding, histamine release and facilitated allergen presentation. *Clin Exp Allergy*. 2008;38:1290-1301.
 17. Durham SR, Emminger W, Kapp A, Colombo G, De Monchy JG, Rak S, et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol*. 2010;125:131-138.
 18. Durham SR, Emminger W, Kapp A, De Monchy JG, Rak S, Scadding GK, et al. SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J Allergy Clin Immunol*. 2012;129:717-725.
 19. Blaiss M, Maloney J, Nolte H, Gawchik S, Yao R, Skoner DP. Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. *J Allergy Clin Immunol*. 2011;127:64-71.
 20. Dahl R, Kapp A, Colombo G, De Monchy JG, Rak S, Emminger W, et al. Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2006;118:434-440.
 21. Maloney J, Bernstein DI, Nelson H, Creticos P, Hebert J, Noonan M, et al. Efficacy and safety of grass sublingual immunotherapy tablet, MK-7243: a large randomized controlled trial. *Ann Allergy Asthma Immunol*. 2014;112:146-153.
 22. Maloney J, Durham S, Skoner D, Dahl R, Bufe A, Bernstein D, et al. Safety of sublingual immunotherapy Timothy grass tablet in subjects with allergic rhinitis with or without conjunctivitis and history of asthma. *Allergy*. 2015;70:302-309.
 23. Nelson HS, Nolte H, Creticos P, Maloney J, Wu J, Bernstein DI. Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. *J Allergy Clin Immunol*. 2011;127:72-80.
 24. Nolte H, Hebert J, Berman G, Gawchik S, White M, Kaur A, et al. Randomized controlled trial of ragweed allergy immunotherapy tablet efficacy and safety in North American adults. *Ann Allergy Asthma Immunol*. 2013;110:450-456.
 25. Nolte H, Amar N, Bernstein DI, Lanier BQ, Creticos P, Berman G, et al. Safety and tolerability of a short ragweed sublingual immunotherapy tablet. *Ann Allergy Asthma Immunol*. 2014;113:93-100.
 26. Emminger W, Hernandez MD, Cardona V, Smeenk F, Fogh BS, Calderon MA, et al. The SQ house dust mite SLIT-tablet is well tolerated in patients with house dust mite respiratory allergic disease. *Int Arch Allergy Immunol*. 2017;174:35-44.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Couroux P, Ipsen H, Stage BS, et al. A birch sublingual allergy immunotherapy tablet reduces rhinoconjunctivitis symptoms when exposed to birch and oak and induces IgG₄ to allergens from all trees in the birch homologous group. *Allergy*. 2019;74:361-369.
<https://doi.org/10.1111/all.13606>