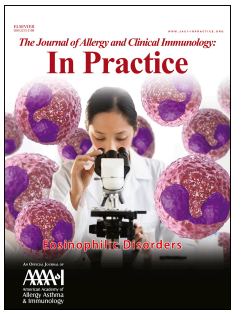
Journal Pre-proof



Treatment Effect of the Tree Pollen SLIT-Tablet on Allergic Rhinoconjunctivitis During Oak Pollen Season

Hendrik Nolte, MD, PhD, Susan Waserman, MD, Anne K. Ellis, MD, Tilo Biedermann, MD, Peter A. Würtzen, PhD

PII: S2213-2198(21)00163-X

DOI: https://doi.org/10.1016/j.jaip.2021.01.035

Reference: JAIP 3412

To appear in: *The Journal of Allergy and Clinical Immunology: In Practice*

Received Date: 12 October 2020

Revised Date: 21 December 2020

Accepted Date: 20 January 2021

Please cite this article as: Nolte H, Waserman S, Ellis AK, Biedermann T, Würtzen PA, Treatment Effect of the Tree Pollen SLIT-Tablet on Allergic Rhinoconjunctivitis During Oak Pollen Season, *The Journal of Allergy and Clinical Immunology: In Practice* (2021), doi: https://doi.org/10.1016/j.jaip.2021.01.035.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

Journal Pre-proof

Treatment Effect of the Tree Pollen SLIT-Tablet on Allergic Rhinoconjunctivitis During Oak Pollen Season

Hendrik Nolte, MD, PhD[1], Susan Waserman, MD[2], Anne K. Ellis, MD[3], Tilo Biedermann, MD[4], Peter A. Würtzen, PhD[5]

[1]ALK, Bedminster, NJ, USA; [2]Department of Medicine, McMaster University, Hamilton, ON, Canada; [3]Division of Allergy & Immunology, Department of Medicine, Queen's University, Kingston, ON, Canada; [4]Department of Dermatology and Allergology, Technical University of Munich, Munich, Germany; [5]ALK, Hørsholm, Denmark

Email addresses: Hendrik.Nolte@alk.net; waserman@mcmaster.ca; anne.ellis@kingstonhsc.ca; PeterAdler.Wuertzen@alk.net; tilo.biedermann@tum.de

Corresponding author:

Hendrik Nolte, MD, PhD
ALK
US Highway 135 Route 202/206
Bedminster NJ 07921
Telephone: (+1) 516 767 1800
Email: Hendrik.nolte@alk.net

Funding source: Funding for this study was provided by ALK, Hørsholm, Denmark. The study sponsor was involved in the study design, analysis and interpretation of the data, and the writing of the report. The decision to submit the manuscript was made by all authors.

Disclosures: H. Nolte and P.A. Würtzen are employees of ALK. S. Waserman has served as an advisor and or speaker for Aralez, AstraZeneca, CSL Behring, Shire, Novartis, Pediapharm, Pfizer, Merck, Sanofi, Glaxo Smith Kline, Takeda, Novartis, ALK-Abelló and Stallergenes. A.K. Ellis has participated in advisory boards for ALK-Abelló, AstraZeneca, Aralez, Bausch Health, Circassia Ltd, GlaxoSmithKline, Johnson & Johnson, Merck, Mylan, Novartis, Pediapharm and Pfizer, has been a speaker for ALK, Aralez, AstraZeneca, Boehringer-Ingelheim, CACME, Meda, Medexus, Mylan, Merck, Novartis, Pediapharm, Pfizer, The ACADEMY, and Takeda. Her institution has received research grants from AstraZeneca, Bayer, LLC, Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Sun Pharma, Merck, Novartis, Pfizer, Regeneron and Sanofi. She has also served as an independent consultant to Allergy Therapeutics, Bayer, LLC, Ora Inc., and Regeneron in the past. T. Biedermann has served as a consultant, received an honorarium as a speaker, or received research grants from ALK-Abelló, Celgene-BMS, Lilly Deutschland GmbH, Mylan, Novartis, Phadia-Thermo Fisher, Sanofi-Genzyme, and Regeneron.

Journal Pre-proof

Abstract

Background: Birch, alder, hazel, and oak are members of the birch homologous group based on cross-reactivity toward the birch pollen allergen Bet v 1. Theoretically, allergy to these tree pollens may be treated by immunotherapy with one representative allergen extract.

Objective: To evaluate post-hoc whether treatment of birch pollen-induced allergic rhinoconjunctivitis with a standardized tree sublingual immunotherapy (SLIT)-tablet containing birch pollen extract reduces symptoms and symptom-relieving medication use during the oak pollen season (OPS).

Methods: In a randomized, multinational, double-blind trial (EudraCT-2015-004821-15), 634 participants (12-65 years) received daily tree SLIT-tablet (12 SQ-Bet) or placebo before and during tree pollen season (alder/hazel plus birch pollen season [BPS]). Symptom-relieving medication was allowed. The primary endpoint was the average total combined score (TCS; sum of rhinoconjunctivitis daily symptom score [DSS] and daily medication score [DMS]) during BPS. Outcomes during the OPS (excluding overlapping BPS days) were analyzed post-hoc.

Results: Relative improvements in average TCS, DSS, and DMS with the tree SLIT-tablet versus placebo during the OPS were 25%, 22%, and 32%, respectively (all $p<0.001$). Significant correlations were observed between birch and oak sIgE at baseline ($r=0.86$) and between birch and oak IgG_4 after treatment ($r=0.72$). Oak sIgE and IgG_4 kinetics in response to tree SLIT-tablet treatment were similar to birch.

Conclusions: The tree SLIT-tablet leads to significant improvement of rhinoconjunctivitis outcomes during the OPS, supporting the clinical relevance of immunologic cross-reactivity towards birch and oak allergens.

Trial registration: EudraCT-2015-004821-15

Journal Pre-proof

Highlights

What is already known about this topic?

Oak is a member of the birch homologous group and oak allergen is an important Bet v 1 cross-reactive tree pollen allergen, particularly in North America.

What does this article add to our knowledge?

The tree SLIT-tablet improves allergic rhinoconjunctivitis symptoms and reduces symptom-relieving medication use during oak pollen season in addition to birch pollen season and induces oak-specific immunologic responses.

How does this study impact current management guidelines?

The tree SLIT-tablet may be used to treat oak-related allergic rhinoconjunctivitis symptoms and reduce the need for symptom-relieving pharmacotherapy.

Keywords: allergic rhinitis; birch; cross-reactivity; IgE; IgG_4; oak; sublingual immunotherapy; tree

Abbreviations: AR/C, allergic rhinitis with or without conjunctivitis; BPS, birch pollen season; DMS, daily medication score; DSS, daily symptom score; EEC, environmental exposure chamber; SLIT, sublingual immunotherapy; TCS, total combined score; TPS, tree pollen season

Journal Pre-proof

Journal Pre-proof

Introduction

Allergen homologous groups are composed of allergens with similar biochemical properties. The allergens within a homologous group demonstrate extensive IgE cross-reactivity as a result of structural similarity.[1, 2] Alder, hazel, hornbeam, and oak are the original members of the birch homologous group based on allergen structural homology to the birch major allergen Bet v 1 and resulting IgE cross reactivity.[1] Chestnut and beech have also been added to the group.[2] Theoretically, allergy to these tree pollens may be treated by immunotherapy with one representative allergen extract.[3, 4]

Because of the cross-reactivity, individuals who are allergic to one allergen in the birch homologous group will often react to pollen from other members of the birch homologous group. Sensitivity to allergens in the birch homologous group is common in Europe and North America and is increasing in some Asian regions.[5-10] In the United States, birch and oak are found throughout the country, and oak is heavily distributed in the northeastern, eastern, and central regions.[11, 12] IgE-sensitization to oak in these regions is approximately 33% among patients with allergic rhinitis.[5, 6] Although oak trees are abundant in Europe,[13] oak allergens are not included in standard European skin prick test panels.

Oak trees are prolific pollinators.[12] Peak pollen counts can reach to approximately 1700 grains/m^3 in Europe[14] and over 2400 grains/m^3 in the United States.[15] In the United States, oak pollens constitute approximately one-third to one-half of all tree pollen in the northeastern and eastern regions.[6, 16] Peak oak pollen counts are significantly associated with daily over-the-counter allergy medication sales[17] and with an increase in asthma-related emergency department visits.[17-19] The clinical impact of oak pollen allergy has been found to match that of allergy to

Journal Pre-proof

trees that are not in the birch homologous group, such as maple, ash, sycamore/London planetree, and hickory.[17]

Sublingual immunotherapy (SLIT) tablets for the treatment of allergic rhinitis with or without conjunctivitis (AR/C) induced by grass, ragweed, and house dust mite allergens are approved and commercially available in Europe and North America. A tree SLIT-tablet containing standardized birch pollen extract is approved in Europe and Canada. Two phase 2 clinical trials of the tree SLIT-tablet have been conducted in individuals with birch pollen-induced AR/C.[20, 21] One of the trials was an environmental exposure chamber (EEC) dose-finding trial conducted in 219 adults living in Canada.[20] During the EEC sessions, participants were exposed to either birch or oak pollen. Although the tree SLIT-tablet contains only birch pollen extract, 24 weeks of treatment with the highest dose (12 SQ-Bet) resulted in a similar magnitude of improvement in rhinoconjunctivitis total symptom score after oak challenge as after birch challenge when compared with placebo (24% and 25%, respectively; $p<0.05$ vs placebo).[20] The tree SLIT-tablet also induced oak-specific immunologic responses.[20] A phase 3 field trial (TT-04) of the tree SLIT-tablet was conducted in Europe in participants with moderate-to-severe birch pollen-induced AR/C.[22] In the TT-04 trial, significant improvements relative to placebo in the rhinoconjunctivitis total combined score (TCS) were observed not only during the birch pollen season (BPS), but also during the alder/hazel pollen season. Considering the IgE cross-reactivity between birch and oak allergens and the beneficial effect of the tree SLIT-tablet in participants exposed to oak allergen in the EEC trial, it was hypothesized that the tree SLIT-tablet would also have a beneficial effect during natural oak pollen exposure. The objectives of the post-hoc analyses reported here were to evaluate whether treatment of birch pollen-induced AR/C with the tree SLIT-tablet reduced symptoms and symptom-relieving medication use during the oak pollen

Journal Pre-proof

139 season in the TT-04 trial and to characterize the oak-specific immunologic responses induced by
140 the tree SLIT-tablet.

141

142

Journal Pre-proof

Journal Pre-proof

Methods

Trial design and participants

TT-04 was a randomized, parallel-group, double-blind, placebo-controlled, multi-site, phase 3 field trial conducted in Europe; details and primary results have been previously described (EudraCT-2015-004821-15).[22] Briefly, participants (aged 12–65 years) with persistent moderate-to-severe birch pollen-induced AR/C with or without asthma received daily tree SLIT-tablets (12 SQ-Bet; ALK-Abelló, Hørsholm, Denmark) or placebo at least 16 weeks before and during the tree pollen season (TPS). One SQ-Bet corresponds to approximately 5 µg Bet v 1. The first dose was administered at the study site followed by a 30 minute observation.

Eligible participants had a positive skin prick test (≥3 mm wheal diameter) to birch, sIgE for Bet v 1 of ≥0.7 kU/L, and impaired health-related quality of life (determined by the presence of impairment for at least 1 Allergic Rhinitis and its Impact on Asthma quality of life items[23]) associated with birch pollen-induced AR/C. Participants with impaired lung function (FEV_1 <70% of predicted value after adequate pharmacologic treatment), uncontrolled asthma or recent severe asthma exacerbations were excluded. Polysensitized subjects were enrolled, however, patients with a history of symptomatic AR/C to allergen sources other than tree pollens from the birch homologous group that overlap the TPS were excluded. Open-label symptom-relieving medications (oral antihistamine, ocular antihistamine, and nasal corticosteroids) for allergic rhinoconjunctivitis symptoms were provided. An epinephrine prescription was not mandated.

Pollen season definitions

Based on published literature and clinical trials for grass pollen and weeds, a cut-off value of 10 pollen grains/m^3 air is a reasonable threshold for detecting symptoms and for detecting treatment

Journal Pre-proof

effects.[24-26] In contrast, several birch pollen studies have confirmed that for birch a higher threshold value of 30 birch grains/m^3 air is recommended for predicting the onset of clinical symptoms in most allergic patients.[27-30] For these reasons, 30 birch pollen grains/m^3 is a reasonable threshold for defining the start of the birch season in TT-04. Therefore, the BPS was defined as the first day of 3 consecutive days with at least 30 pollen grains/m^3 through the last day of the last of 3 consecutive days with at least 30 pollen grains/m^3. The relationship between alder/hazel and oak pollen counts and symptoms or allergenicity of these pollens is less established. Limited field data and EEC data show that alder/hazel and oak require lower pollen exposure to elicit symptoms. Thus, the threshold level to define the start of the alder/hazel and oak season were set at 10 grains/m^3. Because there was a large overlap between the alder and hazel seasons, the analyses were performed on the combined alder and hazel season.[22]

In 12 of the 24 pollen stations, there was overlap between the BPS and the oak pollen season. Therefore, a pure oak pollen season was analyzed, which excluded data from the days that overlapped with the BPS. Excluding all the overlapping BPS days rules out the possibility that the treatment benefits observed during the oak pollen season were because of a carry-over effect related to birch pollen.

Efficacy during a continuous TPS that included only hazel, alder, and birch pollen seasons was also evaluated post-hoc. The continuous TPS was defined as all days from the start of alder/hazel pollen season through the end of the BPS, including days with pollen counts below the predefined thresholds, thereby accounting for low-level exposure to other tree species in the birch homologous groups depending on the pollen station.

Assessments

Journal Pre-proof

Participants assessed 4 rhinitis symptoms (runny nose, blocked nose, sneezing, and itchy nose) and 2 conjunctivitis symptoms (gritty feeling/red/itchy eyes and watery eyes) on a scale of 0 to 3 in an electronic diary during the TPS. Participants also recorded symptom-relieving medication use. The primary endpoint was the average daily rhinoconjunctivitis TCS (maximum=38) during the BPS. The TCS was the sum of the rhinoconjunctivitis daily symptom score (DSS; maximum=18) and rhinoconjunctivitis daily medication score (DMS; maximum=20; **Table I**).

Blood samples for allergen-specific serum IgE (sIgE) and IgG_4 were collected at baseline and after 1, 4, 7, and 9 months of treatment. Serum sIgE and IgG_4 specific for Bet v and Que a were analyzed by ImmunoCAP (Phadia 250, Thermo Fischer Scientific, Uppsala, Sweden). The lower limit of quantitation for sIgE was 0.7 kU_A/L and for IgG_4 was 0.15 mg_A/L.

Statistical analysis

Average TCS, average DSS, and average DMS were analyzed post-hoc for all randomized subjects during the oak pollen season. Outcomes during the continuous TPS were analyzed post-hoc for the entire study population who had at least 1 diary entry during the BPS (FASBPS). These post-hoc analyses were not declared a priori in the original protocol. The average daily TCS was plotted by relative day from the BPS start together with the average daily pollen counts for alder, hazel, birch, and oak weighted by the number of participants at each pollen station. Efficacy outcomes were analyzed using a linear mixed-effect model, with the square root-transformed average score as a response variable, treatment group as fixed effect, and pollen station as random effect. Differential residual errors were specified for each treatment group. For the oak pollen season analysis, Rubin's multiple imputation strategy was used and missing values in both treatment groups were sampled from the observed data of the endpoint in the

Journal Pre-proof

placebo group. Statistical significance was set at 5% and all tests and 95% CI were 2-sided. SAS version 9.4 (SAS Institute, Cary, NC) software was used for the analysis.

Changes from baseline in sIgE and IgG_4 were analysed in a subset of 100 actively-treated and 100 placebo participants for birch and a subset of 60 actively-treated and 40 placebo participants for oak on log10 transformed measurements using a linear mixed-effects model using subjects as random factor (restricted maximum likelihood). Post-hoc comparisons were performed using Tukey's HSD. Cross-reactivity of sIgE (n=397, active and placebo, baseline) and IgG_4 (n=282, actively treated, end of treatment) between Bet v and Que a was analyzed by Pearson correlations. All sIgE and IgG_4 samples below the lower limit of quantitation were recoded to 0.35 kU_A/L and 0.075 mg_A/L, respectively.

Journal Pre-proof

Journal Pre-proof

Results

In all, 634 individuals were randomized (tree SLIT-tablet=320; placebo=314) and analyzed during the oak pollen season; 575 participants were in the FASBPS population that was analyzed during the continuous TPS. In addition to birch, most participants were also sensitized to alder (92%), hazel (86%), and oak pollen (86%). The majority of subjects (76%) were polysensitized. The most common (non-tree) co-sensitizations were grass (51%), dog (37%), cat (32%) and house dust mites (23%). Other baseline demographics and disease characteristics have been previously described.[22] The mean duration of the oak pollen season was 24 days (range 5–49 days) and of the continuous TPS was 61 days (range 14–80 days). Average pollen counts were 284 grains/m^3 for birch, 100 grains/m^3 for alder, 40 grains/m^3 for hazel, and 66 grains/m^3 for oak.

Efficacy endpoints

The average TCS began to separate between the tree SLIT-tablet and placebo groups approximately 4 weeks before the start of the BPS (coinciding with the alder/hazel pollen seasons) and was maintained throughout the BPS and the oak pollen season (**Figure 1**). The most pronounced treatment effect on TCS was observed during the BPS when pollen counts were at their highest.

Relative improvements in the average TCS, DSS, and DMS with the tree SLIT-tablet versus placebo during the oak pollen season were 25%, 22%, and 32%, respectively, and for the continuous TPS were 35%, 32%, and 45% (all $p<0.001$; **Figure 2**). Details of the adjusted means and absolute differences in scores versus placebo are shown in **Table II**.

Immunology

Journal Pre-proof

The majority of the participants in the full study population demonstrated sIgE binding to oak at baseline (87%) and demonstrated serum IgG_4 binding to oak (70%) after tree SLIT-tablet treatment (**Figure 3**). There was a significant correlation between Bet v and Que a sIgE at baseline ($r=0.86$; $p<0.001$) and Bet v and Que a IgG_4 after tree SLIT-tablet treatment ($r=0.72$; $p<0.001$; **Figure 3**). In the participants treated with tree SLIT-tablet, oak (Que a) sIgE and IgG_4 over time had similar pharmacodynamics to those of birch (Bet v) (**Figure 4**). Statistically significant increases in Que a sIgE and IgG_4 with tree SLIT-tablet compared with placebo were observed starting at 1 month. Levels of Que a sIgE peaked at 1 month and then returned towards baseline whereas increases in IgG_4 plateaued after 1 month.

Journal Pre-proof

Journal Pre-proof

Discussion

In northern hemispheres, most tree allergic subjects are sensitized to pollen from birch and related trees, including oak. In this trial, participants reported symptoms from the beginning of February to the end of the tree pollen season in June and pollen counts were collected to assess the duration of the entire tree pollen season. The previously reported analyses of the alder/hazel[22] season and current post-hoc analysis of the oak pollen season (without birch) establish that the symptom burden extends beyond the birch season and tree pollen allergy may last for several months. Improvements in ARC symptoms and reductions in symptom-relieving medication use with the tree SLIT-tablet during the oak pollen season and the continuous TPS (which includes all days from the start of the alder/hazel season to the end of the BPS) were significant versus placebo and showed consistency across almost 3 months of exposure to allergens in the birch homologous group. The benefits observed during the oak pollen season, when overlapping days with the BPS were excluded, indicate a direct clinically relevant treatment effect of the tree SLIT-tablet on oak pollen allergy. Functionally, the immunologic cross-reactivity between birch and oak allergens was indicated by correlations between birch and oak-specific pre-treatment sIgE and post-treatment IgG_4, as well as modulation of oak-specific sIgE and IgG_4 after tree SLIT-tablet treatment. These results support the clinical relevance of immunologic cross-reactivity between birch and oak pollen homologous allergens. The results also suggest that use of the tree SLIT-tablet could simplify AIT in patients with birch and related tree pollen allergy since an initial skin prick test would cover both birch and oak in most cases, and birch extract could replace an allergen extract mix to treat birch and oak polysensitization.

The trends in TCS over the alder/hazel, birch, and oak pollen seasons are what one would expect to see in patients allergic to birch homologous allergens, with TCS peaks that generally

Journal Pre-proof

correspond to the peaks in pollen counts for each individual tree season. There was a slight delay of a few days between the TCS peaks and the pollen peaks that may be explained by an enhanced cumulative immunologic response induced by consistently high pollen exposure, which was then followed by an increase in symptoms. Evidence of a cumulative immunologic response to repeated high allergen exposure has been demonstrated in *in vivo* models of allergic rhinitis[31, 32] and a lag of 3 to 13 days in symptoms following natural hazel and alder pollen exposure has been previously documented.[33] After peaking, the TCS then decreased in between the individual tree pollen seasons. However, the TCS did not drop completely back down to the levels observed before the start of the alder/hazel season, despite a substantial decrease in average pollen levels. The reason for this is uncertain; however, a priming effect could explain the difference between average pollen levels and average TCS in the periods between the individual tree seasons. Participants in the trial could have been primed by alder and hazel pollen exposure, which led to a continued elevated TCS even after the drop in average pollen levels.[34] Furthermore, inflammation induced by allergens does not immediately resolve once the pollen levels drop and some level of symptoms may still be experienced.[35]

The magnitudes of improvement with the tree SLIT-tablet versus placebo on TCS (35%), DSS (32%), and DMS (45%) during the continuous TPS were similar to the previously reported improvements on TCS (37%), DSS (33%), and DMS (47%) during the TPS that included only days meeting the threshold pollen counts.[22] The improvements during the TPS were slightly lower than those observed during the BPS (40%, 37%, and 49%, respectively).[22] The magnitude of effect of the tree SLIT-tablet on TCS, DSS, and DMS during the oak pollen season was clinically meaningful (TCS >20%) with a pronounced reduction in symptom-relieving

Journal Pre-proof

medication use. The clinical effect determined by TCS appeared less than that of the BPS and continuous TPS and could be related to the relatively low average oak pollen exposure during the treatment year; mean oak pollen counts across 6 pollen stations in the US from 2001 to 2011 ranged from 62-581 grains/m^3 air.[15] A strong correlation between pollen counts and magnitude of clinical effect has been demonstrated for the grass SLIT-tablet.[36]

IgE cross-reactivity between birch and oak allergens is well documented[5, 37-39] and was confirmed by the high correlation between birch and oak-specific sIgE in the trial participants before tree SLIT-tablet treatment. A high correlation between birch and oak-specific IgG_4 was observed after tree SLIT-tablet treatment, which supports a cross-reactive birch/oak immunologic response. In accordance with previous observations from the tree SLIT-tablet EEC trial,[20] the clinical improvements associated with the tree SLIT-tablet during the oak pollen season were accompanied by modulation of oak-specific sIgE and IgG_4 that were pharmacodynamically similar to those of birch-specific sIgE and IgG_4. The finding that the IgG_4 change from baseline is generally higher for birch than for oak is to be expected because treatment-induced birch-reactive antibodies may have lower affinity towards the homologous, but not identical, allergens from oak. Furthermore, IgG_4 only binds the solid-phase allergen in the assay with one binding site,[40] which leads to a high sensitivity to minor differences between homologous allergens.

Interpretation of the results is limited by relatively low oak pollen counts and a short oak pollen season, which tends to reduce a measurable AIT treatment effect.[36] Geographical variation in oak pollen levels is also a potential limitation, since the trial was conducted at multiple sites across a broad geographic region without site selection based on a pre-specified oak pollen exposure. However, the results are based on multiple sites irrespective of the presence of an oak pollen

Journal Pre-proof

season, rather than one site with high or low oak pollen, which should minimize bias due to a random effect at one site. A possible practical limitation of the tree SLIT-tablet is treatment of tree-pollen allergic patients with non-birch–related tree pollen allergies, such as maple and elm.

These findings may have implications for patients with oak pollen allergy, since no approved SLIT-tablet specifically for oak pollen allergy is currently available in North America. However, an additional trial would be preferable to confirm and replicate the efficacy of the tree SLIT-tablet in patients monosensitized to oak allergen. The oak pollen season and continuous TPS together represent approximately a 3-month exposure to allergens in the birch homologous group during which the tree SLIT-tablet provides a clinical benefit.

Acknowledgments

Medical writing and editorial assistance were provided by Erin P. Scott, PhD, of Scott Medical Communications, LLC. This assistance was funded by ALK-Abelló, Hørsholm, Denmark.

The authors would like to acknowledge the contributions of investigators and the clinical trial team at ALK and thank the staff of the departments Bioanalytics, Immunology, and Immunochemistry for their expert technical assistance.

References

1. 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.
2. Guideline on allergen products: Production and quality issues. EMEA/CHMP/BWP/304831. Committee for Medicinal Products for Human Use. London, UK: European Medicines Agency 2008.
3. Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases. CHMP/EWP/18504. Committee for Medicinal Products for Human Use. London, UK: European Medicines Agency, 2008.
4. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, et al. Allergen immunotherapy: a practice parameter third update. J Allergy Clin Immunol 2011;127:S1-55.
5. Lin RY, Clauss AE, Bennett ES. Hypersensitivity to common tree pollens in New York City patients. Allergy Asthma Proc 2002;23:253-8.
6. Farnham JE, Vaida GA. A New Look At New England Tree Pollen. Allergy and Asthma Proceedings 1982;3:320-6.

Journal Pre-proof

7. Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. Allergy 2009;64:1507-15.
8. Park HJ, Lim HS, Park KH, Lee JH, Park JW, Hong CS. Changes in allergen sensitization over the last 30 years in Korea respiratory allergic patients: a single-center. Allergy Asthma Immunol Res 2014;6:434-43.
9. Ahmed H, Ospina MB, Sideri K, Vliagoftis H. Retrospective analysis of aeroallergen's sensitization patterns in Edmonton, Canada. Allergy Asthma Clin Immunol 2019;15:6.
10. Chan-Yeung M, Anthonisen NR, Becklake MR, Bowie D, Sonia Buist A, Dimich-Ward H, et al. Geographical variations in the prevalence of atopic sensitization in six study sites across Canada. Allergy 2010;65:1404-13.
11. Rankin B. Presence of oak. Available at: http://www.radicalcartography.net/index.html?trees. Accessed November 14, 2019.
12. White JF, Bernstein DI. Key pollen allergens in North America. Ann Allergy Asthma Immunol 2003;91:425-35; quiz 35-6, 92.
13. European Atlas of Forest Tree Species. Luxembourg: Publication Office of the European Union, 2016. Available from: https://forest.jrc.ec.europa.eu/en/european-atlas/atlas-download-page/.
14. Grundstrom M, Adams-Groom B, Pashley CH, Dahl A, Rasmussen K, de Weger LA, et al. Oak pollen seasonality and severity across Europe and modelling the season start using a generalized phenological model. Sci Total Environ 2019;663:527-36.
15. Zhang Y, Bielory L, Georgopoulos PG. Climate change effect on Betula (birch) and Quercus (oak) pollen seasons in the United States. Int J Biometeorol 2014;58:909-19.
16. Kosisky SE, Marks MS, Nelson MR. Pollen aeroallergens in the Washington, DC, metropolitan area: a 10-year volumetric survey (1998-2007). Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 2010;104:223-35.
17. Ito K, Weinberger KR, Robinson GS, Sheffield PE, Lall R, Mathes R, et al. The associations between daily spring pollen counts, over-the-counter allergy medication sales, and asthma syndrome emergency department visits in New York City, 2002-2012. Environmental health : a global access science source 2015;14:71.
18. Dales RE, Cakmak S, Judek S, Coates F. Tree pollen and hospitalization for asthma in urban Canada. International archives of allergy and immunology 2008;146:241-7.
19. Darrow LA, Hess J, Rogers CA, Tolbert PE, Klein M, Sarnat SE. Ambient pollen concentrations and emergency department visits for asthma and wheeze. J Allergy Clin Immunol 2012;130:630-8.e4.
20. Couroux P, Ipsen H, Stage BS, Damkjaer JT, Steffensen MA, Salapatek AM, 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;74:361-9.
21. Makela MJ, Gyllfors P, Valovirta E, Steffensen MA, Gronager PM, Savolainen J, et al. Immunotherapy With the SQ Tree SLIT-tablet in Adults and Adolescents With Allergic Rhinoconjunctivitis. Clin Ther 2018;40:574-86.e4.
22. Biedermann T, Kuna P, Panzner P, Valovirta E, Andersson M, de Blay F, et al. The SQ tree SLIT-tablet is highly effective and well tolerated: Results from a randomized, double-blind, placebo-controlled phase III trial. Journal of Allergy and Clinical Immunology 2019;143:1058-66.e6.
23. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001;108:S147-334.
24. Chervinsky P, Philip G, Malice MP, Bardelas J, Nayak A, Marchal JL, et al. Montelukast for treating fall allergic rhinitis: effect of pollen exposure in 3 studies. Ann Allergy Asthma Immunol 2004;92:367-73.

Journal Pre-proof

25. Creticos PS, Maloney J, Bernstein DI, Casale T, Kaur A, Fisher R, et al. Randomized controlled trial of a ragweed allergy immunotherapy tablet in North American and European adults. J Allergy Clin Immunol 2013;131:1342-9 e6.
26. 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 e2.
27. Jantunen J, Saarinen K, Rantio-Lehtimaki A. Allergy symptoms in relation to alder and birch pollen concentrations in Finland. Aerobiologia 2012;28:169-76.
28. Viander M, Koivikko A. The seasonal symptoms of hyposensitized and untreated hay fever patients in relation to birch pollen counts: correlations with nasal sensitivity, prick tests and RAST. Clin Allergy 1978;8:387-96.
29. Taudorf E, Moseholm L. Pollen count, symptom and medicine score in birch pollinosis. A mathematical approach. Int Arch Allergy Appl Immunol 1988;86:225-33.
30. Caillaud D, Martin S, Segala C, Besancenot JP, Clot B, Thibaudon M. Effects of airborne birch pollen levels on clinical symptoms of seasonal allergic rhinoconjunctivitis. Int Arch Allergy Immunol 2014;163:43-50.
31. Eguíluz-Gracia I, Bosco A, Dollner R, Melum GR, Lexberg MH, Jones AC, et al. Rapid recruitment of CD14(+) monocytes in experimentally induced allergic rhinitis in human subjects. J Allergy Clin Immunol 2016;137:1872-81.e12.
32. Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. J Immunol 2000;165:4062-8.
33. Gokkaya M, Damialis A, Nussbaumer T, Beck I, Bounas-Pyrros N, Bezold S, et al. Defining biomarkers to predict symptoms in subjects with and without allergy under natural pollen exposure. J Allergy Clin Immunol 2020.
34. Fernández-González M, Álvarez-López S, González-Fernández E, Jesús Aira M, Rodríguez-Rajo FJ. Cross-reactivity between the Betulaceae family and fallout in the real atmospheric aeroallergen load. Sci Total Environ 2020;715:136861.
35. KleinJan A, McEuen AR, Dijkstra MD, Buckley MG, Walls AF, Fokkens WJ. Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of patients with hay fever after local allergen provocation. J Allergy Clin Immunol 2000;106:677-86.
36. Durham SR, Nelson HS, Nolte H, Bernstein DI, Creticos PS, Li Z, et al. Magnitude of efficacy measurements in grass allergy immunotherapy trials is highly dependent on pollen exposure. Allergy 2014;69:617-23.
37. Hauser M, Asam C, Himly M, Palazzo P, Voltolini S, Montanari C, et al. Bet v 1-like pollen allergens of multiple Fagales species can sensitize atopic individuals. Clin Exp Allergy 2011;41:1804-14.
38. 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;102:579-91.
39. Zetterstrom O, Fagerberg E, Wide L. An investigation of pollen extracts from different deciduous trees in patients with springtime allergy in Sweden. Acta Allergol 1972;27:15-21.
40. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clin Exp Allergy 2009;39:469-77.

Journal Pre-proof

449 **Table I.** Symptom and medication scoring.

Individual Symptoms	**Severity**	**Score**	**Maximum Daily Score**
Runny nose	0=No symptoms 1=Mild symptoms 2=Moderate symptoms 3=Severe symptoms	0–3	3
Blocked nose		0–3	3
Sneezing		0–3	3
Itchy nose		0–3	3
Gritty feeling/red/itchy eyes		0–3	3
Watery eyes		0–3	3
	Maximum rhinoconjunctivitis daily symptom score (DSS)		**18**
Symptomatic Medication	**Maximum Daily Dose**	**Score/Dose Unit**	**Maximum Daily Score**
Desloratadine tablet, 5 mg	1 tablet	6	6
Olopatadine eye drops, 1 mg/ml	2 drops in each eye	1.5	6
Mometasone nasal spray, 50 µg/spray	2 sprays in each nostril	2	8
	Maximum rhinoconjunctivitis daily medication score (DMS)		**20**
	Maximum total combined symptom and medication score (TCS)		**38**

450

451

452

453

454

455

Journal Pre-proof

Journal Pre-proof

Table II. Post-hoc analysis of average TCS, DSS, and DMS during the oak pollen season and continuous TPS. The oak pollen season excludes all days that overlapped with the birch pollen season. The continuous TPS includes all days from the start of alder/hazel pollen season through the end of birch pollen season.

	Treatment Group	No. Observed (No. Imputed)	Adjusted Mean	Absolute Difference (placebo-tree SLIT-tablet [95% CI])	Percentage Relative to Placebo (95% CI)	P Value
Oak pollen season						
TCS	Placebo	225 (89)	4.80			
	Tree SLIT-tablet	211 (109)	3.59	1.21 (0.32, 2.11)	25 (8, 41)	<0.001
DSS	Placebo	225 (89)	2.34			
	Tree SLIT-tablet	211 (109)	1.82	0.52 (0.07, 0.98)	22 (4, 39)	<0.001
DMS	Placebo	225 (89)	1.76			
	Tree SLIT-tablet	211 (109)	1.19	0.57 (0.06, 1.07)	32 (6, 54)	<0.001
Continuous TPS						
TCS	Placebo	292	5.96			
	Tree SLIT-tablet	283	3.88	2.09 (1.27, 2.90)	35 (23, 45)	<0.001
DSS	Placebo	292	2.89			
	Tree SLIT-tablet	283	1.98	0.91 (0.53, 1.29)	32 (20, 42)	<0.001
DMS	Placebo	292	2.48			
	Tree SLIT-tablet	283	1.36	1.13 (0.63, 1.62)	45 (29, 59)	<0.001

DMS, daily medication score; DSS, daily symptom score; SLIT, sublingual immunotherapy; TCS, total combined score; TPS, tree pollen season.

Journal Pre-proof

Journal Pre-proof

Figure Legends

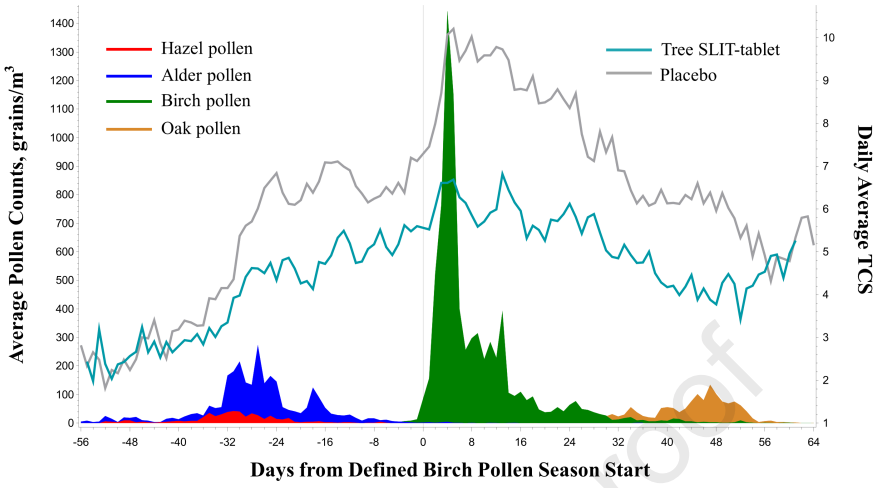
Figure 1. Pollen count and average total combined score (TCS) relative to season start weighted by the number of participants at each pollen station. SLIT, sublingual immunotherapy.

Figure 2. Rhinoconjunctivitis total combined score (TCS), daily symptom score (DSS), and daily medication score (DMS) during A) the oak pollen season and B) the continuous tree pollen season. The oak pollen season excludes all days that overlapped with the birch pollen season. The continuous tree pollen season includes all days from the start of alder/hazel pollen season through the end of birch pollen season. SE, standard error; SLIT, sublingual immunotherapy.

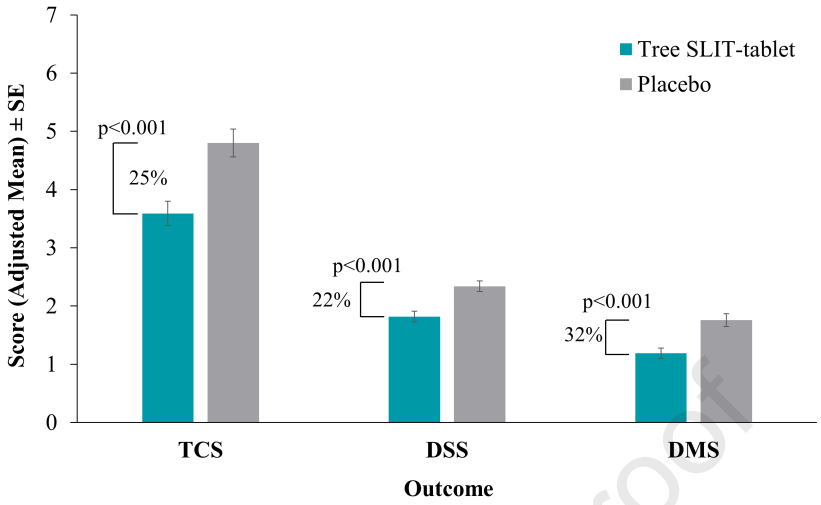
Figure 3. Correlation between A) birch (Bet v) IgE and oak (Que a) serum IgE at baseline and B) birch (Bet v) serum IgG_4 and oak (Que a) IgG_4 at tree SLIT-tablet treatment. Graphs indicate Pearson correlation r-values and the percentage of samples above the lower limit of quantitation (LLQ). Both correlations were statistically significant ($p<0.001$). V2=baseline, V6=end of treatment.

Figure 4. Least square means fold change from baseline for birch (Bet v) and oak (Que a) A) serum IgE and B) serum IgG_4. $p<0.001$ vs placebo at all timepoints except baseline. Error bars indicate 95% confidence intervals.

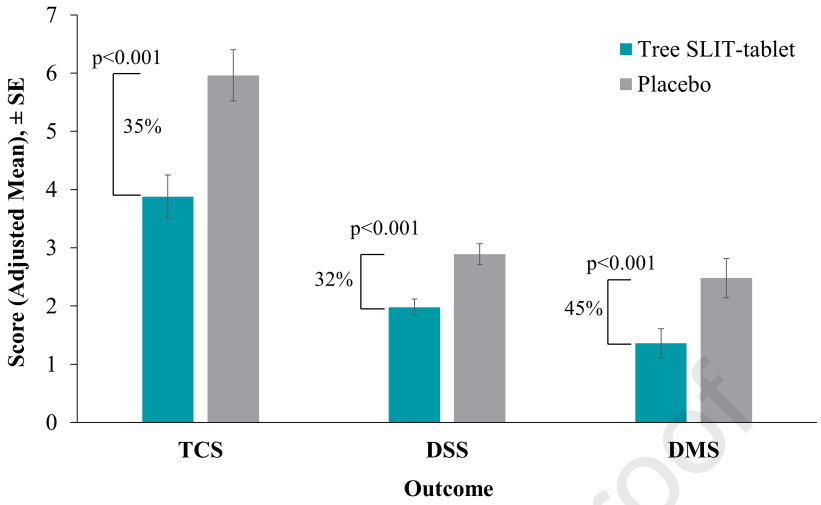
Journal Pre-proof



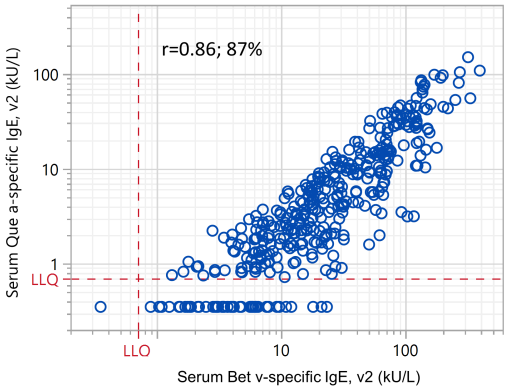
Journal Pre-proof



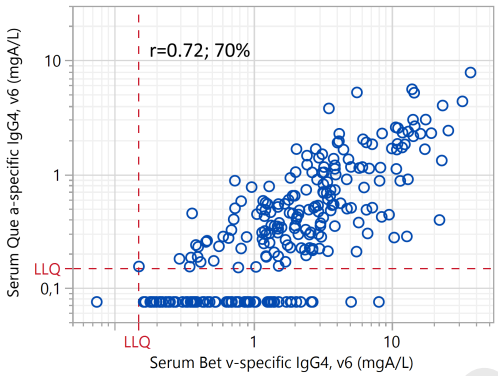
Journal Pre-proof



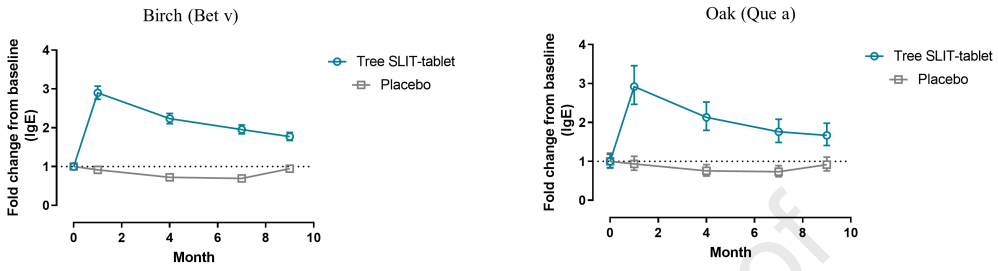
Journal Pre-proof



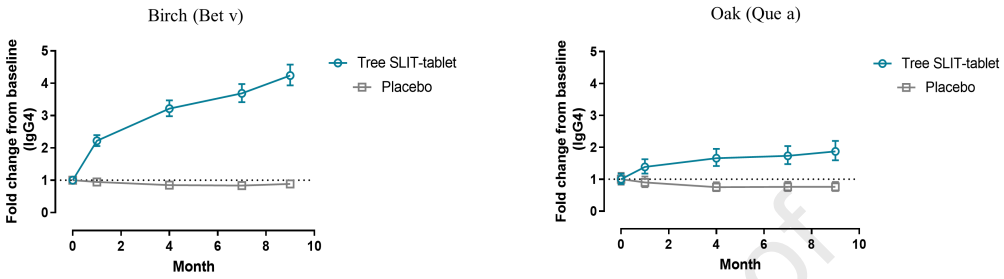
Journal Pre-proof



Journal Pre-proof



Journal Pre-proof



Journal Pre-proof