

Lack of effect of Timothy grass pollen sublingual immunotherapy tablet on birch pollen–induced allergic rhinoconjunctivitis in an environmental exposure unit



Anne K. Ellis, MD, MSc, FRCPC ^{*,†,‡}; Mark W. Tenn, BHSc ^{†,‡}; Lisa M. Steacy, BSc [‡]; Daniel E. Adams, BSc [‡]; Andrew G. Day, MSc [§]; Terry J. Walker, BA [‡]; Hendrik Nolte, MD, PhD ^{||}

^{*} Department of Medicine, Queen's University, Kingston, Ontario, Canada

[†] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada

[‡] Allergy Research Unit, Kingston General Health Research Institute, Kingston, Ontario, Canada

[§] Kingston General Health Research Institute, Kingston, Ontario, Canada

^{||} ALK-Abello, Basking Ridge, New Jersey

ARTICLE INFO

Article history:

Received for publication November 15, 2017.

Received in revised form February 1, 2018.

Accepted for publication February 6, 2018.

ABSTRACT

Background: Timothy grass pollen allergen extract tablets (Grastek) are standardized sublingual immunotherapy tablets (SLIT-T) approved for the treatment of grass pollen–induced allergic rhinitis (AR) and conjunctivitis. Many grass allergic patients are also cosensitized to birch pollen. Whether Timothy grass SLIT-T can confer symptomatic benefits for birch pollen–induced AR symptoms is unknown.

Objective: To evaluate the treatment effect of Timothy grass SLIT-T for birch pollen–induced AR in participants sensitized to both grass and birch pollen using an environmental exposure unit (EEU).

Methods: This study was a phase 4, randomized, double-blind, placebo-controlled, parallel-group study that enrolled participants aged 18 to 65 years allergic to both timothy grass and birch pollen. After a baseline EEU birch pollen challenge, in which a minimum total nasal symptom score (TNSS) of 6 of 12 was required for enrollment, participants were randomized to receive Timothy grass SLIT-T or placebo taken once daily for 4 months. No confirmatory grass pollen challenge was performed. The primary end point was the change in TNSS averaged from assessments from hours 2 to 5 during the posttreatment birch pollen challenge compared with baseline. The secondary and exploratory end points included temporally identical changes in total ocular symptom score (TOSS), total rhinoconjunctivitis symptom score (TRSS), and individual symptom scores.

Results: The difference in TNSS reduction after 4 months of therapy between the Timothy grass SLIT-T and placebo group was not significant ($P = .83$). Reductions in TOSS ($P = .19$) and TRSS ($P = .67$) were also comparable between groups. Findings between groups for individual symptom scores were similar (all $P > .40$), except for watery eyes, in which symptom reduction was slightly better in the placebo arm ($P = .01$). Timothy grass SLIT-T was well tolerated, and no serious adverse effects occurred.

Conclusion: A bystander effect of grass SLIT-T on birch pollen–induced AR symptoms was not detected. Symptomatic benefits of grass SLIT-T are likely allergen specific.

Trial Registration: [ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT02394600.

© 2018 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Reprints: Anne K. Ellis, MD, MSc, FRCPC, Department of Medicine and Department of Biomedical and Molecular Sciences, Queen's University, Watkins D1, 76 Stuart St, Kingston, ON K7L 2V7, Canada; E-mail: ellisa@queensu.ca.

Disclosures: Dr Ellis reported participating on advisory boards for ALK-Abello, Circassia Ltd, GlaxoSmithKline, Johnson & Johnson, Merck, and Novartis and serving as a speaker for Aralez, AstraZeneca, Boehringer Ingelheim, Meda, Mylan, Merck, Novartis, Padiapharm, Pfizer, and Takeda. Her institution has received research grants from Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Sanofi, Sun Pharma, Merck, Novartis, and Pfizer. Dr Ellis is also a consultant to Bayer Inc. Ms Steacy and Messrs Adams, Day, and Walker are employees of Kingston General Hospital. Dr Nolte is employed by ALK-Abello. Mr Tenn has no conflicts of interest to disclose.

Funding Sources: This study was funded by Merck Canada through the Investigator Initiated Program.

Introduction

Allergic rhinitis (AR) is primarily a nasal inflammatory disorder that affects approximately 20% of Canadians and an estimated 1.4 billion people worldwide.^{1,2} Symptoms of sneezing, rhinorrhea, nasal congestion, and associated ocular symptoms are triggered on inhalation of relevant aeroallergens and greatly affect the quality of life of individuals with AR by interfering with work or school performance and disturbing sleep.³ Aside from allergen avoidance, primary treatments for AR include the use of antihistamines (preferably second-generation agents), intranasal corticosteroids, and/or, where available, combination intranasal antihistamine and

corticosteroid preparations. However, even with the use of available pharmacologic treatment, individuals with AR still experience lifestyle limitations and ongoing symptoms, with approximately 60% of patients reporting that their AR symptoms are not well controlled by pharmacologic therapy.¹ Allergen immunotherapy is the only treatment capable of modifying the disease processes involved in allergic diseases and provides long-term benefits for treated individuals.⁴

Timothy grass pollen allergen extract tablets (Grastek; previously called MK-7243) are standardized sublingual immunotherapy tablets (SLIT-T) that contains 2,800 bioequivalent allergy units of standardized timothy grass (*Phleum pratense*) pollen allergen extract. This SLIT-T product is approved by both the US Food and Drug Administration and Health Canada for the treatment of grass pollen-induced AR in the United States and Canada, respectively. The efficacy of Timothy grass SLIT-T has been recently evaluated in 3 large-scale, critical, multicenter clinical trials. These studies revealed the superiority of the Timothy grass SLIT-T over placebo in the treatment of grass allergy in both polysensitized (including tree sensitivity) children and adults when taken before and throughout the grass pollen season.^{5–7} In all 3 trials, a separation was noted in total combined scores (combined daily symptom and medication scores) between the SLIT-T-treated and placebo groups beginning well before the start of the protocol-defined grass pollen season (coincident with very low levels of grass pollen). The time of separation coincided with the peak of tree pollen season, suggesting that Timothy grass SLIT-T may provide symptomatic benefits when taken by individuals with birch pollen-induced AR in addition to grass pollen-induced AR.

All 3 Timothy grass SLIT-T clinical trials were conducted at multiple sites across North America.^{5–7} Inclusions of multiple study sites at disparate geographic locations may introduce potential confounding variables, such as differences in participant lifestyles and weather conditions among the sites. These variables could lead to variations in pollen exposure among study participants. Controlled allergen challenge facilities (CACFs), such as the environmental exposure unit (EEU), are internationally recognized and clinically validated models of AR that can be used to evaluate novel therapeutics for AR treatment.^{8,9} These facilities offer several advantages over natural allergen exposure studies. CACFs, such as the EEU, are capable of exposing a large group of participants to a controlled quantity of allergen under tightly regulated conditions. Confounding environmental variables, such as air quality, temperature, humidity, carbon dioxide levels, and allergen concentration in the air, can also be controlled, creating a unique environment suited for the evaluation of various antiallergic medications.⁸ For example, the EEU has been used previously to evaluate the efficacy of several nasal antihistamines and corticosteroids in participants with AR.^{10,11} More recently, the unit has also been used to evaluate the efficacy of a novel peptide-based immunotherapy treatment for grass pollen allergy.¹² The aim of the current study was to evaluate the effect of 4 months of treatment with Timothy grass SLIT-T on the symptoms of birch pollen-induced AR in participants with both timothy grass pollen-induced and birch pollen-induced AR using the EEU.

Methods

Study Participants

Adult participants, aged 18 to 65 years, were enrolled in the study and were required to have a documented or participant-reported 2-year minimum history of both birch pollen-induced and grass pollen-induced seasonal AR, a positive skin prick test response (defined as a wheal diameter ≥ 5 mm over negative control) to both birch (*Betula* species) and timothy grass (*Phleum pratense*) pollen

extracts within 12 months before enrollment, and a serum specific IgE (sIgE) level of 0.7 kU/L or higher to both birch and timothy grass.

The key exclusion criteria were active symptomatic perennial or non-AR; breastfeeding; pregnancy; clinically significant nasal polyps, nasal septal perforation, or nasal tract malformations; use of immunotherapy treatments that contained birch or grass pollen within the last 3 years; participation in a clinical trial within 30 days before screening; significant pulmonary disease and/or active asthma that required daily drug therapy (mild, intermittent asthma managed with short-acting β -agonist <3 times per week is allowed); any respiratory infection within 1 week before the baseline exposure visit; and use of restricted medication that may interfere with study measurements (eTable 1).

Allergen Challenge

Participants were exposed to European white birch pollen (*Betula pendula*, Greer Laboratories, Lenoir, North Carolina) in the EEU on the described challenge visits. The ventilation system provided the unit with 100% fresh filtered outdoor air, which was exhausted directly outside. Airborne birch pollen concentration in the unit was maintained at a mean (SD) of 3,500 (500) grains/m³ throughout the exposure period, with temperature (22°C–24°C) and relative humidity (40%) being tightly controlled. During exposure periods, pollen concentration was measured at 30-minute intervals using the 7 Rotorod samplers located throughout the unit (eFig 1). Pollen grains were captured on the spinning rods and manually counted using a microscope to assess airborne pollen concentration. Participants remained under constant supervision by medical staff, including physicians, a study nurse, and study coordinators, inside the unit at all times.

Study Design and Treatment Schedule

This was a phase 4, randomized, double-blind, placebo-controlled, single-center, parallel-group study conducted in the EEU at Kingston General Hospital, Kingston, Ontario, Canada, between November 2015 and April 2016. The study was reviewed and granted ethical clearance by the Queen's University Health Sciences and Affiliated Teaching Hospital Research Ethics Board. It was conducted in accordance with the Declaration of Helsinki (Somerset West Amendment 1996) and the International Council for Harmonisation Guideline on Good Clinical Practice.

The study was composed of a screening visit, a baseline exposure or qualifying visit, two 2-month treatment periods, and a posttreatment challenge (PTC) visit (Fig 1). Screening began in March 2015 and continued until November 2016. All nonscreening visits were conducted between November 2015 and mid-April 2016 (ie, outside all local pollen seasons).

Screening visits were conducted from –120 to –1 days to assess participant eligibility. Venous blood samples were drawn to determine sIgE levels to birch and timothy grass. If applicable, participants were required to revisit the research site to ensure adequate washout of restricted medications (eTable 1) before the baseline exposure or qualifying visit.

Participants returned for a 5-hour birch pollen challenge in the EEU during the baseline exposure or qualifying visit (day 1). Nasal and ocular symptoms, and peak nasal inspiratory flow were recorded by each participant at baseline (before challenge) and at 30-minute intervals during the allergen challenge. The severity of each symptom was rated on a scale of 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe) (eTable 2). Varying combinations of nasal and ocular symptoms comprised the total nasal symptom score (TNSS: sum of runny nose, nasal congestion, sneezing, and nasal itching), total ocular symptom score (TOSS: sum of gritty, itchy, or red eyes and

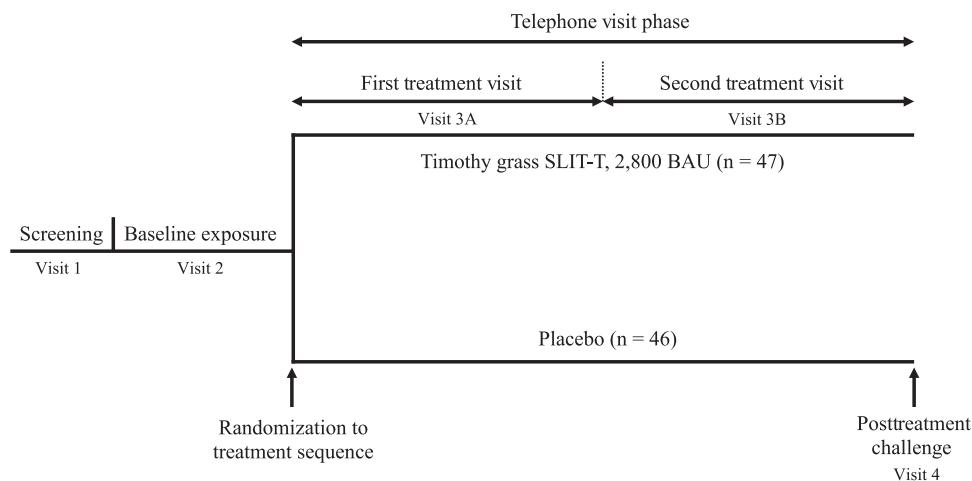


Figure 1. Study diagram. BAU indicates bioequivalent allergy units.

watery eyes), and total rhinoconjunctivitis symptom score (TRSS: sum of TNSS, TOSS, and itching of ear, palate, or throat). Participants were required to obtain a TNSS of 6 or higher within the first 2 hours of birch pollen exposure to proceed to the treatment and randomization phase. Those achieving the qualifying score remained in the EEU for an additional 3 hours of pollen exposure to establish a baseline plateau of symptoms for comparison at the PTC. No grass challenges were performed to confirm the history and IgE test results of grass allergy.

The first treatment visit occurred 8 to 12 days after the baseline exposure or qualifying visit. Participants were randomized in a 1:1 ratio to receive Timothy grass SLIT-T or matching placebo (Merck, Kirkland, Quebec, Canada) according to a computer-generated allocation schedule. Both Timothy grass SLIT-T and placebo were supplied as fast-dissolving tablets to be placed under the tongue. Participants were monitored for 30 minutes after the first dose and were given a 2-month supply of study medication to be taken daily at home and at approximately the same time each day. An epinephrine autoinjector and take-home diary cards were also dispensed to document dosing and any adverse events (AEs). Two months after the first dose, participants returned and exchanged their used study drug packaging for another 2-month supply of study medication with the first dose directly observed for a minimum of 5 minutes. During the 4-month treatment phase, participants received weekly (for the first 4 weeks) and then monthly telephone calls from the research site staff. Instructions on taking the study medication were reviewed, and any AEs and concomitant medications were evaluated.

After the treatment phase, participants returned to the research site for a PTC visit, which was composed of a 5-hour birch pollen challenge in the EEU. A subsequent follow-up visit occurred thereafter for poststudy safety assessment to be completed (ie, vital signs, capture of AEs, and a physical examination).

Assessments

The primary, secondary, and exploratory end points were based on the change in symptoms scores from baseline to the PTC measured at 2, 2.5, 3, 3.5, 4, 4.5, and 5 hours. In particular, the primary outcome was the change in TNSS, the secondary end points were the changes in TOSS and TRSS, and the exploratory end points were the changes in mean individual symptom scores (runny nose, itchy nose, sneezing, nasal congestion, gritty, itchy, or red eyes, watery eyes, itchy ears, palate, or throat). Safety and tolerability were assessed through physical examinations, vital signs (blood pressure,

heart rate, respiration rate, and oral temperature), and incidence and severity of AEs.

Statistical Analysis

Since this was a pilot exploratory study to assess the potential for successful implementation of a future larger study, an a priori power calculation was not made. Specifically, because we had no prior estimate of minimally important or even plausible effect sizes, this study used a convenience sample of 100 individuals, assuming complete data would be captured on at least 43 individuals per arm. Such a sample size would provide 80% power at a 2-sided $\alpha = .05$ if the mean difference in the change in symptoms between arms was 61% of the SD of the within-patient change. Assuming a 14% dropout rate, up to 100 eligible participants were randomized to obtain complete data collection for at least 86 participants (43 in each treatment arm).

Efficacy and safety analyses included all randomized participants who took 1 dose or more of study medication and provided 1 efficacy measurement or more (full analysis set approach). The primary efficacy analysis of the primary, secondary, and exploratory end points was conducted using a linear mixed-effects model with random intercepts and time slopes to account for within-individual dependence attributable to repeated measurements and fixed effects for treatment. The treatment effect estimated the difference between groups in the change from baseline to follow-up in the mean of the symptom assessments at 2, 2.5, 3, 3.5, 4, 4.5, and 5 hours. All results from the mixed-effects model were verified using an independent *t* test approach, which was also used to estimate differences between groups of time specific changes. A 2-sided $P \leq .05$ was considered statistically significant. Adjustments for multiplicity of tests were not made. All statistical analyses were performed using SAS statistical software, version 9.4 (SAS Institute Inc, Cary, North Carolina).

Results

Participant Characteristics

After screening 226 individuals, a total of 93 participants were ultimately deemed eligible to participate and were randomized into the study (Fig 2). A large proportion of excluded individuals had insufficient sIgE levels to birch and/or timothy grass pollen. The mean (SD) age was 37.6 (10.7) years, and approximately 51.6% were female. Most participants were white (89.2%) followed by Asian (4.3%) (Table 1). Of the 93 participants, 87 completed the treatment period

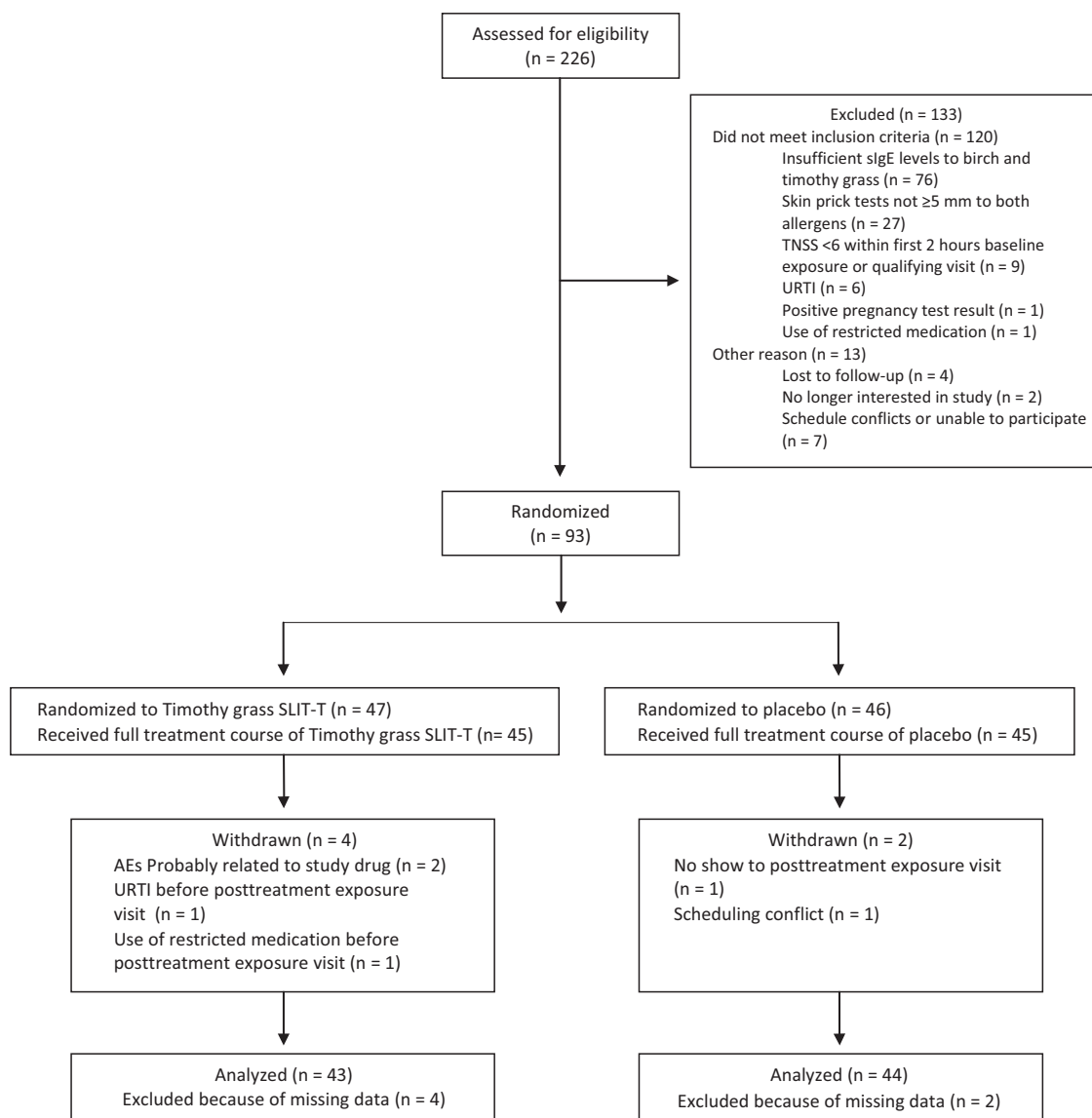


Figure 2. Screening, randomization, and follow-up. AE indicates adverse event; URTI, upper respiratory tract infection.

and 6 discontinued the study. The reasons for study withdrawal include AEs (3 participants in the grass SLIT-T group), an upper respiratory tract infection before PTC exposure visit (1 participant in the grass SLIT-T group), use of restricted medication before PTC exposure visit (1 participant in the grass SLIT-T group), and scheduling

conflict or no show to PTC exposure visit (2 participants in the placebo group) (Fig 2). The 87 participants who took 1 dose or more of study drug and provided 1 postrandomization efficacy measurement or more were included in the primary efficacy analysis (n = 43 in the grass SLIT-T group and 44 in the placebo groups).

Table 1
Participant Characteristics

Characteristic	Placebo (n = 46)	Timothy grass SLIT-T (2,800 BAU) (n = 47)
Age, mean (SD), y	37.7 (10.9)	37.5 (10.8)
Female, No. (%)	23 (50)	23 (51)
Race, No. (%)		
Asian	1 (2)	3 (6)
Black or African American	2 (4)	0
White	40 (87)	43 (91)
White, American Indian or Alaskan Native	2 (4)	0
White, Black or African American	1 (2)	0
Missing	0	1 (2)

Abbreviations: BAU, bioequivalent allergy units; SLIT-T, sublingual immunotherapy tablet.

Efficacy Outcomes

After 4 months of treatment, mean symptom reductions during posttreatment hours 2 to 5 compared with baseline as estimated by the linear mixed-effects model were not significantly different between treatment groups for TNSS (estimated between-group difference, 0.1; 95% confidence interval [CI], −1.0 to 1.2; $P = .83$), TOSS (estimated between-group difference, −0.4; 95% CI, −1.1 to 0.2; $P = .19$), or TRSS (estimated between-group difference, −0.4; 95% CI, −2.3 to 1.5; $P = .67$) (Table 2). These findings were supported using independent t tests, which yielded between-group differences of 0.1 (95% CI, 1.0–1.3; $P = .81$) for TNSS, −0.5 (95% CI, −1.2 to 0.2; $P = .18$) for TOSS, and −0.4 (95% CI, −2.4 to 1.6; $P = .69$) for TRSS (eTable 3).

Mean reductions in TNSS were not significantly different between the grass SLIT-T- and placebo-treated groups at any of the time

Table 2Estimated Mean Decrease in Composite Symptom Scores From Baseline to Posttreatment Hours 2 to 5^a

Outcome	Placebo (n = 44)		Timothy grass SLIT-T (2,800 BAU) (n = 43)		Between-group difference	
	Mean (95% CI)	P value	Mean (95% CI)	P value	Mean (95% CI) ^b	P value
TNSS	2.2 (1.4 to 3.0)	<.001	2.3 (1.5 to 3.1)	<.001	0.1 (–1.0 to 1.2)	.83
TOSS	1.6 (1.1 to 2.0)	<.001	1.1 (0.6 to 1.6)	<.001	–0.4 (–1.1 to 0.2)	.19
TRSS	4.6 (3.3 to 6.0)	<.001	4.2 (2.8 to 5.6)	<.001	–0.4 (–2.3 to 1.5)	.67

Abbreviations: BAU, bioequivalent allergy units; CI, confidence interval; SLIT-T, sublingual immunotherapy tablet; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TRSS, total rhinoconjunctivitis symptom score.

^aResults were obtained using a mixed-effects model with random intercept and time slope by participant.

^bBetween-group differences were calculated as SLIT-T minus placebo.

Table 3Estimated Mean Decrease in Individual Symptom Scores From Baseline to Posttreatment Hours 2 to 5^a

Outcome	Placebo (n = 44)		Timothy grass SLIT-T (2,800 BAU) (n = 43)		Between-group difference	
	Mean (95% CI)	P value	Mean (95% CI)	P value	Mean (95% CI) ^b	P value
Running nose	0.6 (0.4 to 0.8)	<.001	0.7 (0.4 to 0.9)	<.001	0.0 (–0.3 to 0.4)	.80
Itchy nose	0.7 (0.5 to 1.0)	<.001	0.6 (0.4 to 0.9)	<.001	–0.1 (–0.4 to 0.3)	.61
Sneezing	0.3 (0.1 to 0.6)	.02	0.3 (0.1 to 0.6)	.009	0.0 (–0.3 to 0.4)	.84
Nasal congestion	0.5 (0.3 to 0.8)	<.001	0.7 (0.4 to 0.9)	<.001	0.1 (–0.2 to 0.5)	.42
Gritty, itchy red eyes	0.7 (0.5 to 1.0)	<.001	0.7 (0.5 to 1.0)	<.001	0.0 (–0.4 to 0.4)	.99
Watery eyes	0.8 (0.6 to 1.1)	<.001	0.4 (0.1 to 0.6)	.005	–0.5 (–0.8 to –0.1)	.01
Itchy ears, palate, throat	0.8 (0.5 to 1.1)	<.001	0.8 (0.5 to 1.1)	<.001	–0.1 (–0.5 to 0.4)	.74

Abbreviations: BAU, bioequivalent allergy units; CI, confidence interval; SLIT-T, sublingual immunotherapy tablet.

^aResults were obtained using a mixed-effects model with random intercept and time slope by participant.

^bBetween-group differences were calculated as SLIT-T minus placebo.

points (all $P > .30$). For the secondary end points, similar findings were observed at each time point for both TOSS (all $P > .15$) and TRSS (all $P > .40$) (Fig 3 and Fig 4).

For the exploratory end points, mean symptom reductions were not significantly different between treatment groups for all individual symptom scores (all $P > .40$), except watery eyes ($P = .01$, directionally favoring placebo) (Table 3). Independent t tests supported these findings (eTable 4).

The TNSS, TOSS, and TRSS were all significantly reduced at each time point during the PTC compared with the baseline challenge visit in both the grass SLIT-T (all $P < .001$) and placebo (all $P < .001$) groups (Fig 3 and Fig 4). Similar findings were observed in both

groups when comparing mean symptom reductions during post-treatment hours 2 to 5 vs baseline (TNSS, TOSS, TRSS, all $P < .001$) (Table 2). For individual symptom scores, estimated means from posttreatment hours 2 to 5 were significantly reduced vs baseline in the grass SLIT-T (all $P < .001$ except sneezing, $P = .009$, and watery eyes, $P = .005$) and placebo (all $P < .001$, except sneezing, $P = .02$) groups (Table 3).

Safety

Timothy grass SLIT-T was generally well tolerated by participants with no deaths, severe swellings, systemic allergic reactions, epinephrine use, or serious AEs reported during the treatment period. Two participants from the grass SLIT-T group withdrew from the study because of an AE.

A total of 209 AEs were reported in total, with 1 or more AEs reported in 68 participants (30 in the placebo group and 38 in the grass SLIT-T group). Eleven participants (24%) in the placebo group and 14 participants (30%) participants in the grass SLIT-T group experienced severe AEs (as per participant self-report) (Table 4).

Of the 93 participants, 27 reported AEs that were assessed by the investigator as being probably or definitely treatment related (Table 4). Three of the 52 AEs (6%) occurred in the placebo group and 49 (94%) in the grass SLIT-T group. The most commonly reported treatment-related AE was itchy throat (1 case in the placebo group and 9 in the grass SLIT-T group), followed by itchy ears and tongue (none in the placebo group and 5 cases each in the grass SLIT-T group) (eTable 5).

Discussion

In this pilot evaluation, we endeavored to determine through a randomized, double-blind, placebo-controlled trial whether treating patients who have both birch pollen-induced and grass pollen-induced AR with Timothy grass SLIT-T would have any effect on their

Table 4

Summary of AEs

AE	No. (%)	
	Placebo (n = 46)	Timothy grass SLIT-T (2,800 BAU) (n = 47)
Any relation		
Total AEs ^a	71	138
Participants with AEs	30 (65)	38 (81)
Participants with severe AEs	11 (24)	14 (30)
Participants discontinued because of AEs	0 (0)	2 (4)
Possibly, probably, or definitely treatment related		
Total AEs ^a	9	79
Participants with AEs	7 (15%)	32 (68)
Participants with severe AEs	0 (0)	4 (9)
Participants discontinued because of AEs	0 (0)	2 (4)
Probably or definitely treatment related		
Total AEs ^a	3	49
Participants with AEs	3 (7)	24 (51)
Participants with severe AEs	0	2 (4)
Participants discontinued because of AEs	0 (0)	2 (4)

Abbreviations: AE, adverse event; BAU, bioequivalent allergy units; SLIT-T, sublingual immunotherapy tablet.

^aTotal AEs does not count the same AE within a participant more than once.

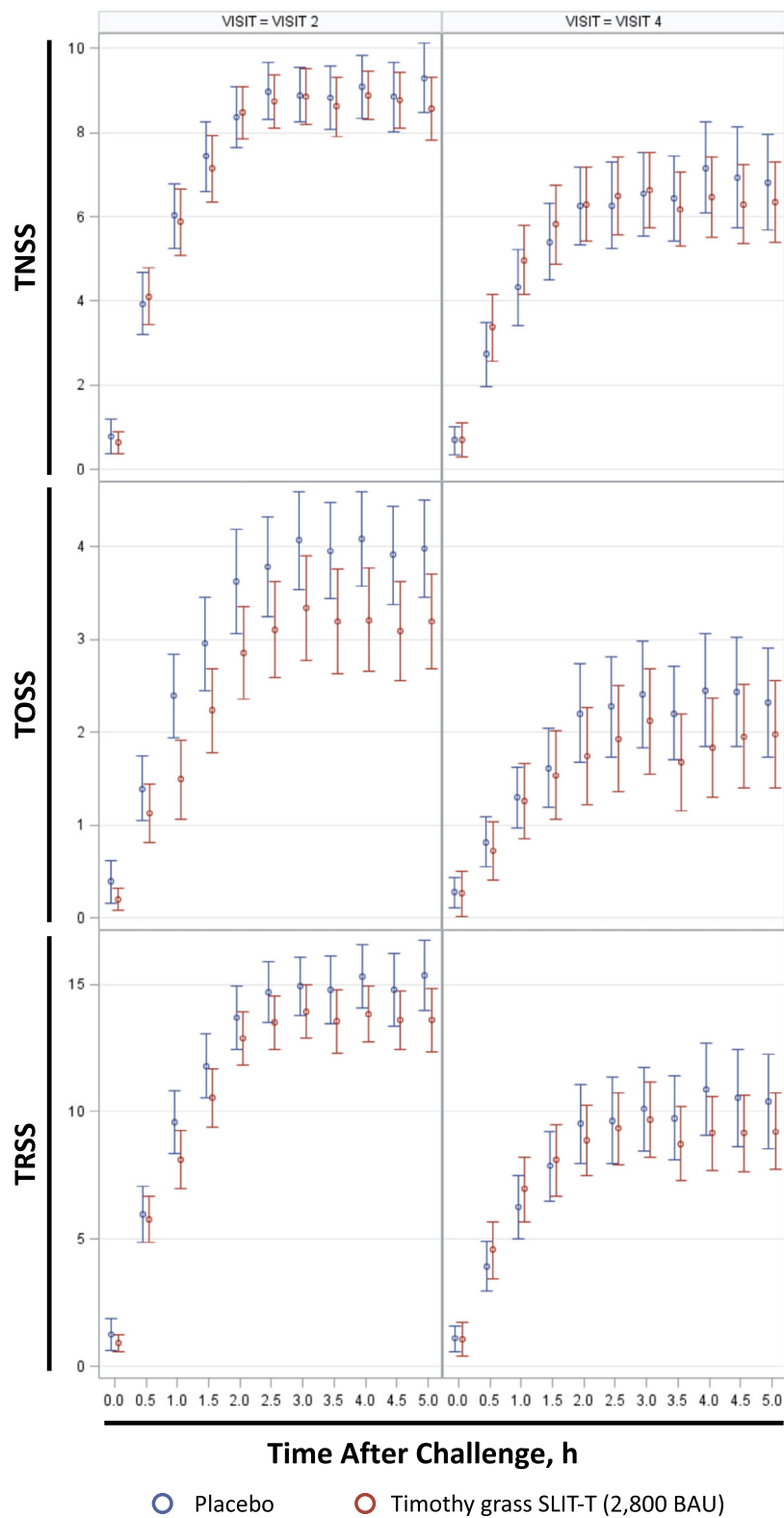


Figure 3. Measurement of allergic rhinoconjunctivitis symptoms before and after Timothy grass SLIT-T treatment. The total nasal symptom score (TNSS), total ocular symptom score (TOSS), and total rhinoconjunctivitis symptom score (TRSS) were consistently reduced at all time points during the posttreatment challenge (visit 4) compared with the baseline challenge visit (visit 2) in the Timothy grass SLIT-T (2,800 BAU) group. Similar findings were observed in the placebo group. Data are presented as means (95% confidence intervals).

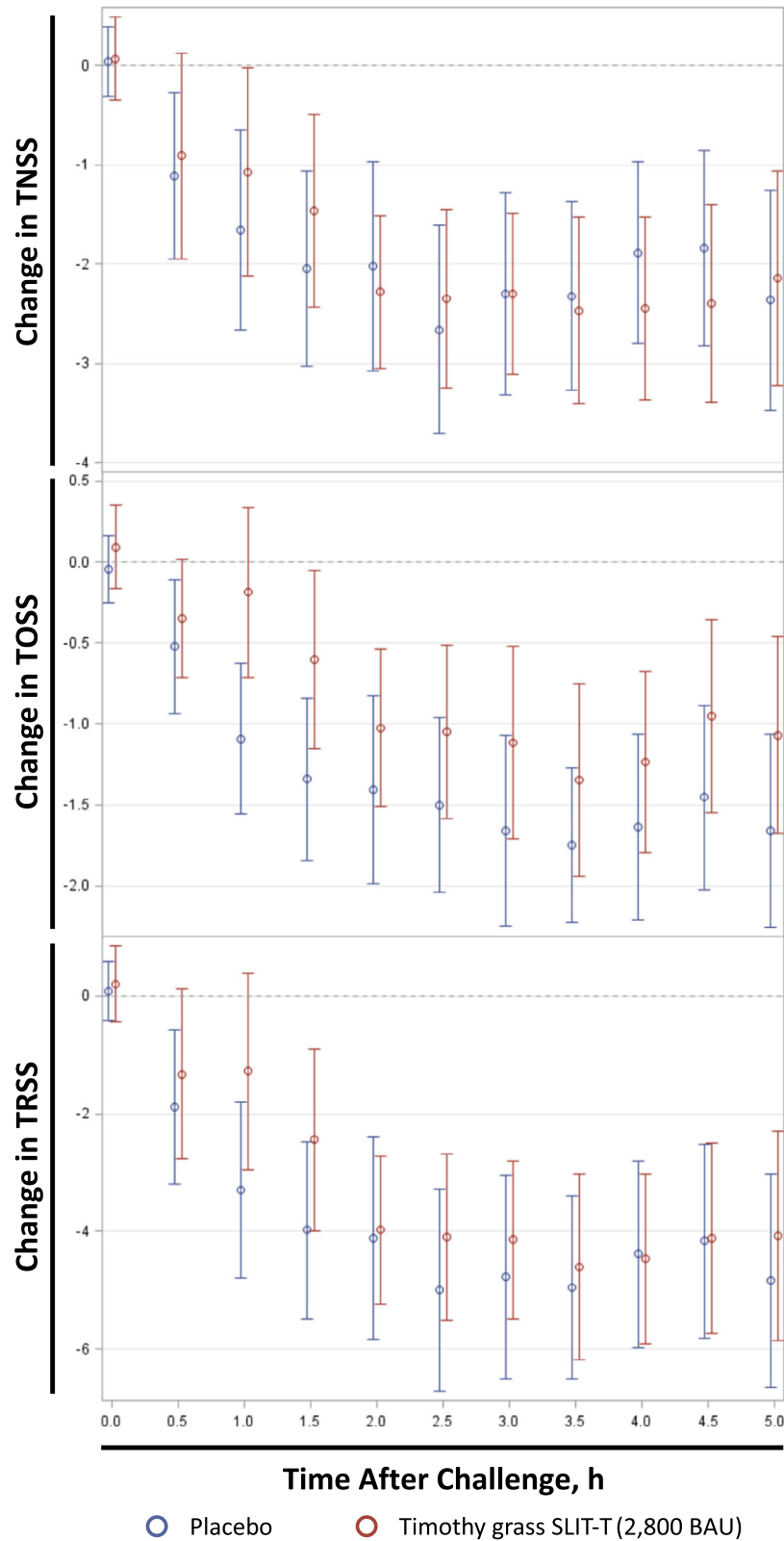


Figure 4. Change in allergic rhinoconjunctivitis symptoms before and after Timothy grass SLIT-T treatment. Within-group differences (posttreatment minus baseline) for total nasal symptom score (TNSS), total ocular symptom score (TOSS), and total rhinoconjunctivitis symptom score (TRSS) were negative (indicating an improvement in nasal and ocular symptoms after treatment) at all time points in the Timothy grass SLIT-T (2,800 BAU) group. Similar findings were observed in the placebo group. Data are presented as means (95% confidence intervals).

symptoms of birch-induced AR. The study was conducted out of season using a well-validated CACF (the EEU) to ensure that the AR symptoms participants experienced were antigen specific, that is, only attributable to birch pollen exposure in the controlled environment of the EEU. On the basis of this small pilot study, it does not appear that the Timothy grass SLIT-T will affect birch-induced AR if a patient is cosensitized.

The rationale for conducting this study was 2-fold. The pivotal phase 3 trials that demonstrated the efficacy of Timothy grass SLIT-T in treatment of grass-induced AR (in which approximately 70% of individuals were sensitized to tree pollen) found initial separation in symptom scores between the active and the placebo arms before the beginning of the actual grass pollen season.^{5–7} In many of the centers where these studies were conducted, this represented an active tree pollen season, and the Eastern United States and Canada have birch as a predominant tree allergen at that time of year. Thus, the hypothesis was generated that perhaps there was a mild effect of Timothy grass SLIT-T treatment on tree pollen-induced AR symptoms as well. The second main consideration was that polysensitization is one of the main reasons allergy practitioners are reluctant to treat grass allergic patients with sublingual grass monotherapy. Thus, if we could detect any signal of immunologic crossover, this would be of great benefit to both patients and prescribers.

Others have looked at the concept of treating one specific allergic sensitivity and evaluating changes in allergic responses to a different antigen. A study has been conducted in 20 adults with perennial rhinoconjunctivitis with exacerbation of symptoms during the grass pollen season and had immediate skin and conjunctival sensitivity and increased sIgE levels to both *Dermatophagoides farinae* and *P. pretense*.¹³ Participants were randomly assigned to timothy or *D. farinae* subcutaneous immunotherapy (SCIT) (with a target maintenance dose of 100 µg major allergen) for 3 years. Conjunctival provocation testing (CPT) and sIgG tests to both allergens were performed annually. Seven individuals in each arm completed the trial, with all patients in the timothy extract group achieving a target maintenance dose of 100 µg, but far fewer achieved target concentration in the dust mite group. After 3 years of immunotherapy, the timothy CPT threshold concentration had increased 16-fold in timothy-treated patients ($P < .05$; between-group change, $P < .05$), and the change in dust mite CPT threshold increased 13.8-fold for the dust mite-treated patients. Importantly, no changes were seen in CPT thresholds from timothy challenges in those treated with house dust mite immunotherapy, and only very modest improvements (3.7-fold increase) in *D. farinae* CPT threshold were noted after 3 years of timothy immunotherapy. The authors concluded that the study provided convincing evidence that SCIT is allergen specific, which echoes the findings of the current study. This specificity of SCIT has been shown by others as well, but of note, these other studies were all conducted with nonstandardized extracts and were not placebo-controlled.^{14,15}

This study has several limitations. Because this was a pilot study, it was difficult to determine a full power calculation for a sample size estimate, given that we did not have an expectation of exactly what degree of benefit Timothy grass SLIT-T may have for birch pollen-induced symptoms. We also lacked a positive timothy grass challenge control because of time restraints of the local off-season. We recognize that it would have been ideal to have had a baseline and PTC to grass pollen in the EEU to confirm the efficacy of Timothy grass SLIT-T for grass pollen-induced symptoms in this population because we know that individuals with grass allergy develop symptoms of AR during grass pollen challenge in the EEU,¹⁶ but as mentioned the duration between the end of ragweed season and the start of local birch pollen season could not accommodate both grass and birch challenges before and after treatment with appropriate washout periods.

Although there have been instances in which results from phase 2 EEU-type studies led to different results in phase 3 field trials,¹⁷ this has not been the case with any of the SLIT products currently available on the North American market^{18,19}; therefore, we believe that Timothy grass SLIT-T would not be beneficial for birch-induced AR symptoms in a phase 3 outpatient evaluation either.

The placebo effect was also notable in this study. Placebo-treated participants reduced their mean TNSS by 2.2 compared with baseline, matching the reduction seen in grass SLIT-T-treated participants. This effect, however, is not an observation unique to the current study; it is often noted in other allergen immunotherapy studies conducted in CACFs. For example, in a study that evaluated a short course of ragweed SCIT in an environmental exposure chamber, participants assigned to receive placebo reduced their mean TRSS by 4.47 compared with baseline.²⁰ This finding has also been seen in outpatient field trials that evaluated various antiallergic therapies.^{21,22} Of note, however, the fact that the responses to placebo and active therapies are mirrored during the baseline and end-of-study challenges suggests that the typical placebo effect is not happening because there was absolutely no effect of Timothy grass SLIT-T on birch challenges in both the active and placebo groups. This finding suggests that part of the placebo effect noted is actually more of a statistical regression to the mean. Finally, the improvement in the placebo group alleviates concerns that those participants receiving active treatment were not masked to their randomization based on AEs and the placebo group did not have assumptions of response based on the lack of the same. Regardless, the findings highlight the critical importance of including a placebo arm in evaluations of a therapy for AR, which primarily rely on subjective measures to assess efficacy and are subjected to regression to the mean.

Importantly, this study confirmed the safety of Timothy grass SLIT-T in participants with an impressive degree of sensitivity to grass pollen as manifested by a skin prick test diameter of at least 5 mm and an sIgE level of at least 0.7 kU/L. Only 2 AEs led to treatment discontinuation, and there were no episodes of severe swellings or epinephrine use.

In conclusion, this exploratory pilot evaluation of using a grass pollen-specific SLIT treatment (Grastek) for patients with cosensitization to grass and birch allergen as a treatment specifically for symptoms of birch pollen-induced AR symptoms does not support a nonspecific immunologic effect of SLIT. Grass SLIT is thus allergen specific and should be prescribed accordingly for those patients whose rhinitis and conjunctivitis symptoms are clearly most bothersome during the grass pollen season.

Acknowledgments

We acknowledge the contributions of Jenny Thiele, Omid Aghamirian, and Silvia Walker to the successful conduct of this trial.

Supplementary Data

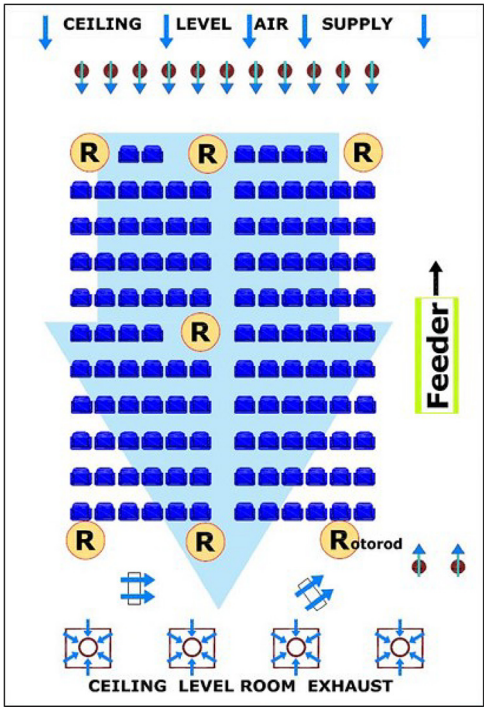
Supplementary data related to this article can be found at <https://doi.org/10.1016/j.anai.2018.02.003>.

References

- [1] Keith PK, Desrosiers M, Laister T, Schellenberg RR, Waserman S. The burden of allergic rhinitis (AR) in Canada: perspectives of physicians and patients. *Allergy Asthma Clin Immunol*. 2012;8:7.
- [2] Schwindt CD, Settupane R. Allergic rhinitis (AR) is now estimated to affect some 1.4 billion people globally and continues to be on the rise [editorial]. *Am J Rhinol Allergy*. 2012;26:S-S1.
- [3] Katelaris CH, Sacks R, Theron PN. Allergic rhinoconjunctivitis in the Australian population: burden of disease and attitudes to intranasal corticosteroid treatment. *Am J Rhinol Allergy*. 2013;27:506–509.
- [4] Jutel M, Agache I, Bonini S, et al. International consensus on allergy immunotherapy. *J Allergy Clin Immunol*. 2015;136:556–568.

- [5] 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, e1–4.
- [6] 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, e1–2.
- [7] Maloney J, Bernstein DI, Nelson H, 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, e2.
- [8] Ellis AK, North ML, Walker T, Steacy LM. Environmental exposure unit: a sensitive, specific, and reproducible methodology for allergen challenge. *Ann Allergy Asthma Immunol*. 2013;111:323–328.
- [9] North ML, Soliman M, Walker T, Steacy LM, Ellis AK. Controlled allergen challenge facilities and their unique contributions to allergic rhinitis research. *Curr Allergy Asthma Rep*. 2015;15:11.
- [10] Ellis AK, Zhu Y, Steacy LM, Walker T, Day JH. A four-way, double-blind, randomized, placebo controlled study to determine the efficacy and speed of azelastine nasal spray, versus loratadine, and cetirizine in adult subjects with allergen-induced seasonal allergic rhinitis. *Allergy Asthma Clin Immunol*. 2013;9:16.
- [11] Ellis AK, Steacy LM, Joshi A, Bhowmik S, Raut A. Efficacy of the novel nasal steroid S0597 tested in an environmental exposure unit. *Ann Allergy Asthma Immunol*. 2016;117:310–317.
- [12] Ellis AK, Frankish CW, O'Hehir RE, et al. Treatment with grass allergen peptides improves symptoms of grass pollen-induced allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2017;140:486–496.
- [13] Dreborg S, Lee TH, Kay AB, Durham SR. Immunotherapy is allergen-specific: a double-blind trial of mite or timothy extract in mite and grass dual-allergic patients. *Int Arch Allergy Immunol*. 2012;158:63–70.
- [14] Norman PS, Lichtenstein LM. The clinical and immunologic specificity of immunotherapy. *J Allergy Clin Immunol*. 1978;61:370–377.
- [15] Winther L, Malling HJ, Mosbech H. Allergen-specific immunotherapy in birch- and grass-pollen-allergic rhinitis, II: side-effects. *Allergy*. 2000;55:827–835.
- [16] Ellis AK, Steacy LM, Hobsbawn B, Conway CE, Walker TJ. Clinical validation of controlled grass pollen challenge in the Environmental Exposure Unit (EEU). *Allergy Asthma Clin Immunol*. 2015;11:5.
- [17] Circassia Announces Top-Line Results from Cat Allergy Phase III Study—Press Releases. Circassia. <http://www.circassia.com/media/press-releases/circassia-announces-top-line-results-from-cat-allergy-phase-iii-study/>. Accessed January 22, 2018.
- [18] Horak F, Ziegelmayer P, Ziegelmayer R, 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, e1.
- [19] Nolte H, Maloney J, Nelson HS, et al. Onset and dose-related efficacy of house dust mite sublingual immunotherapy tablets in an environmental exposure chamber. *J Allergy Clin Immunol*. 2015;135:1494–1501, e6.
- [20] Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. *J Allergy Clin Immunol*. 2014;133:121–129, e1–2.
- [21] Carr W, Bernstein J, Lieberman P, et al. A novel intranasal therapy of azelastine with fluticasone for the treatment of allergic rhinitis. *J Allergy Clin Immunol*. 2012;129:1282–1289, e10.
- [22] Casale TB, Korenblat PE, Meltzer EO, Yen K, Bhatnagar A. Nasal carbon dioxide for the symptomatic treatment of perennial allergic rhinitis. *Ann Allergy Asthma Immunol*. 2011;107:364–370.

Supplementary Data



eFigure 1. Schematic of the Environmental Exposure Unit.

eTable 1
Restricted Medications and Required Washout Periods

Medication	Washout period before challenge visits
Topical, oral, or nasal decongestants	≥48 h
Short-acting histamine ₁ -antihistamines, cold and cough or sleep aids that contain antihistamines	≥3 d
Long-acting histamine ₁ -antihistamines and ocular allergy drugs	≥5 d
Leukotriene modifiers, nasal corticosteroids, tricyclic antidepressants, monoamine oxidase inhibitors, and β-blockers	≥14 d
Inhaled or systemic corticosteroids (except hydrocortisone ≤1% limited to ≤10% of total body surface area)	≥21 d
Immunosuppressive or anti-IgE therapy	≥60 d
Allergen immunotherapy (duration ≥3 mo) that contains birch or grass	≥3 y

eTable 2
Rating Scale for Seasonal Allergic Rhinoconjunctivitis Symptoms

Score	Grade	Guideline
0	None	No sign or symptom is evident
1	Mild	Sign or symptom clearly present but minimal awareness; easily tolerated
2	Moderate	Definite awareness of sign or symptom that is bothersome but tolerable
3	Severe	Sign or symptom that is hard to tolerate; causes interference with activities during the challenge session

eTable 3Confirmatory *t* Test Comparing Mean Change in Composite Symptom Scores Between 2 and 5 Hours From Baseline to Posttreatment^a

Outcome	Placebo (95% CI) (n = 44)	2,800 BAU (95% CI) (n = 43)	Between-group difference (95% CI) ^b	P value
TNSS	2.2 (1.3 to 3.0)	2.3 (1.5 to 3.1)	0.1 (1.0 to 1.3)	.81
TOSS	1.6 (1.1 to 2.1)	1.1 (0.6 to 1.6)	−0.5 (−1.2 to 0.2)	.18
TRSS	4.6 (3.1 to 6.1)	4.2 (2.9 to 5.5)	−0.4 (−2.4 to 1.6)	.69

Abbreviations: BAU, bioequivalent allergy units; CI, confidence interval; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TRSS, total rhinoconjunctivitis score.

^aResults were obtained from independent *t* tests.^bBetween-group differences were calculated as 2,800 BAU minus placebo.**eTable 4**Confirmatory *t* test Comparing Mean Change in Individual Symptom Scores Between 2 and 5 Hours From Baseline to Posttreatment^a

Outcome	Placebo (95% CI) (n = 44)	2,800 BAU (95% CI) (n = 43)	Between-group difference (95% CI) ^b	P value
Running nose	0.6 (0.4 to 0.9)	0.7 (4 to 0.9)	0.1 (−0.3 to 0.4)	.72
Itchy nose	0.7 (0.5 to 1.0)	0.6 (0.4 to 0.9)	−0.1 (−0.4 to 0.3)	.59
Sneezing	0.3 (0.0 to 0.6)	0.3 (0.1 to 0.6)	0.0 (−0.4 to 0.4)	.84
Nasal congestion	0.5 (0.3 to 0.8)	0.7 (0.5 to 0.9)	0.1 (−0.2 to 0.5)	.42
Gritty, itchy red eyes	0.7 (0.5 to 1.0)	0.7 (0.5 to 1.0)	0.0 (−0.4 to 0.4)	.92
Watery eyes	0.8 (0.6 to 1.1)	0.4 (0.1 to 0.7)	−0.4 (−0.8 to −0.1)	.02
Itchy ears, palate, throat	0.8 (0.5 to 1.2)	0.8 (0.5 to 1.0)	−0.1 (−0.5 to 0.4)	.76

Abbreviations: BAU, bioequivalent allergy units; CI, confidence interval; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TRSS, total rhinoconjunctivitis score.

^aResults were obtained using a mixed-effects model with random intercept and time slope by participant.^bBetween-group differences were calculated as 2,800 BAU minus placebo.**eTable 5**

Number of Participants With Adverse Events Considered Probably or Definitely Related to Treatment

Adverse event	Placebo (n = 46)	2,800 BAU (n = 47)
Abdominal cramps	0	1
Burning		
Nose	0	1
Throat	0	1
Cough	0	1
Dry mouth	1	0
Irritation lingual frenulum	0	1
Irritation of the throat and ear	0	1
Itch		
Ear	0	5
Mouth	0	2
Nose	0	2
Palate	1	3
Skin	0	1
Sublingual	0	2
Throat	1	9
Tongue	0	5
Nausea	0	1
Numbness of lower lip	0	1
Parasthesias		
Palate	0	1
Tongue	0	3
Sensation of throat or swelling	0	1
Shortness of breath	0	1
Swelling		
Facial	0	1
Lower lip	0	1
Sublingual	0	2
Throat	0	2