JAMA | Original Investigation

Effect of 2 Years of Treatment With Sublingual Grass Pollen Immunotherapy on Nasal Response to Allergen Challenge at 3 Years Among Patients With Moderate to Severe Seasonal Allergic Rhinitis

The GRASS Randomized Clinical Trial

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IMPORTANCE Sublingual immunotherapy and subcutaneous immunotherapy are effective in seasonal allergic rhinitis. Three years of continuous treatment with subcutaneous immunotherapy and sublingual immunotherapy has been shown to improve symptoms for at least 2 years following discontinuation of treatment.

OBJECTIVE To assess whether 2 years of treatment with grass pollen sublingual immunotherapy, compared with placebo, provides improved nasal response to allergen challenge at 3-year follow-up.

DESIGN, SETTING, AND PARTICIPANTS A randomized double-blind, placebo-controlled, 3-parallel-group study performed in a single academic center, Imperial College London, of adult patients with moderate to severe seasonal allergic rhinitis (interfering with usual daily activities or sleep). First enrollment was March 2011, last follow-up was February 2015.

INTERVENTIONS Thirty-six participants received 2 years of sublingual immunotherapy (daily tablets containing 15 µg of major allergen Phleum p 5 and monthly placebo injections), 36 received subcutaneous immunotherapy (monthly injections containing 20 µg of Phleum p 5 and daily placebo tablets) and 34 received matched double-placebo. Nasal allergen challenge was performed before treatment, at 1 and 2 years of treatment, and at 3 years (1 year after treatment discontinuation).

MAIN OUTCOMES AND MEASURES Total nasal symptom scores (TNSS; range; 0 [best] to 12 [worst]) were recorded between 0 and 10 hours after challenge. The minimum clinically important difference for change in TNSS within an individual is 1.08. The primary outcome was TNSS comparing sublingual immunotherapy vs placebo at year 3. Subcutaneous immunotherapy was included as a positive control. The study was not powered to compare sublingual immunotherapy with subcutaneous immunotherapy.

RESULTS Among 106 randomized participants (mean age, 33.5 years; 34 women [32.1%]), 92 completed the study at 3 years. In the intent-to-treat population, mean TNSS score for the sublingual immunotherapy group was 6.36 (95% CI, 5.76 to 6.96) at pretreatment and 4.73 (95% CI, 3.97 to 5.48) at 3 years, and for the placebo group, the score was 6.06 (95% CI, 5.23 to 6.88) at pretreatment and 4.81 (95% CI, 3.97 to 5.65) at 3 years. The between-group difference (adjusted for baseline) was −0.18 (95% CI, −1.25 to 0.90; [P = .75]).

CONCLUSIONS AND RELEVANCE Among patients with moderate to severe seasonal allergic rhinitis, 2 years of sublingual grass pollen immunotherapy was not significantly different from placebo in improving the nasal response to allergen challenge at 3-year follow-up.

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Supplemental content

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The prevalence of allergic rhinitis in the United States has been estimated at 15% based on physician diagnosis and at 30% based on self-reported nasal symptoms.[1,2] Rhinitis has major effects on quality of life, sleep, and work and school performance.[3] Whereas antihistamines and topical nasal corticosteroids are effective,[4] community surveys suggest that approximately 60% of patients with allergic rhinitis do not respond adequately to these measures.[1] When avoidance of allergens is not feasible and patients have inadequate response to antiallergic medications or have bothersome adverse effects, allergen immunotherapy is a reasonable choice for treatment.[5] Subcutaneous immunotherapy is highly effective.[5,6] The sublingual route has emerged as an alternative treatment for seasonal allergic rhinitis.[7,8] Three years of continuous treatment with immunotherapy via either delivery method modifies the underlying course of the disease with long-term remission of symptoms for several years after stopping treatment.[9-11] It is unknown whether a shorter course of immunotherapy provides long-term benefits, while reducing overall costs, patient inconvenience, and adverse events.

The purpose of this study was to explore whether 2 years of immunotherapy with a grass pollen allergen sublingual tablet of proven efficacy induced persistent benefit 1 year after discontinuation (clinical tolerance).

Key Points

Question Does 2 years of grass pollen sublingual immunotherapy reduce symptoms after nasal allergen challenge at 3-year follow-up (1 year after discontinuation of treatment)?

Findings In this randomized clinical trial of 106 adults, 2 years of treatment with sublingual immunotherapy, compared with placebo, did not significantly reduce total nasal symptom scores after challenge at 3 years.

Meaning Among patients with moderate to severe seasonal allergic rhinitis, 2 years of sublingual grass pollen immunotherapy was not significantly different than placebo in improving the nasal response to allergen challenge at 3-year follow-up.

Methods

Study Design

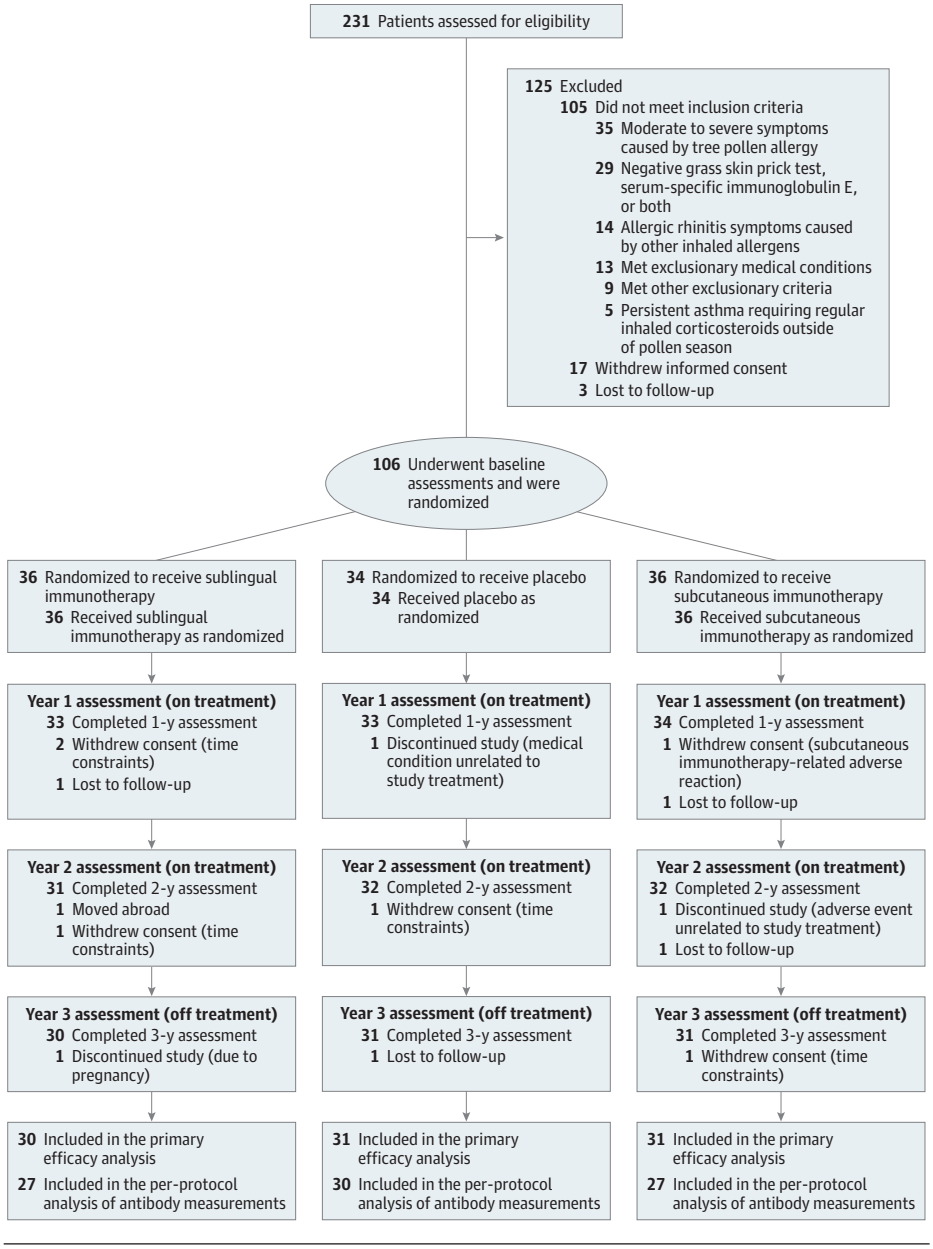
This was a randomized, double-blind, placebo-controlled single-center trial conducted over 4 years, March 2011-March 2015. The study was approved by the National Research Ethics Committee. All participants provided written informed consent. Inclusion criteria included age of 18 to 65 years, a minimum 2-year clinical history of moderate to severe grass-pollen–induced allergic rhinitis (causing interference with usual daily activities or sleep[3]), a positive skin prick test to grass pollen extract (wheal diameter ≥3 mm), elevated serum-specific immunoglobulin E (IgE ≥0.7 kU/L), and a positive nasal grass allergen challenge (total nasal symptom score [TNSS] ≥7 on a 12-point scale). Exclusion criteria included a history of moderate to severe symptoms on exposure to other overlapping seasonal or perennial allergens, a history of moderate to severe or uncontrolled asthma, severe anaphylaxis due to any cause, chronic sinusitis, other diseases of the immune system, and current smoking (see eMethod 1.1 in Supplement 1).

At screening, we collected demographic data that included self-reported race (according to fixed categories) as per National Institutes of Health requirements. Eligible participants (**Figure 1**) were randomized 1:1:1 to receive either sublingual allergen tablet immunotherapy with placebo injections, subcutaneous injection immunotherapy with placebo tablets or double-placebo tablets and injections. Subcutaneous immunotherapy was included as a positive control. Treatment assignment was by use of a central automated web-based randomization system (RhoRAND) that helped provide remote network backup and 24-hour support (eMethod 1.2.1 in Supplement 1). Clinical surrogate end points were collected at baseline, 1 and 2 years on treatment, and 3 years at 1 year after treatment discontinuation. Double-blinding was maintained for all participants and clinical and laboratory staff throughout the entire duration of the study (eMethod 1.2.2 in Supplement 1). The study protocol is provided in Supplement 2.

End Points

The primary end point was the nasal response to allergen challenge between sublingual immunotherapy and placebo at 3-year follow-up, 1 year after discontinuation of treatment.[12] This was defined as the equally weighted average of the TNSS per hour measured as the area under the curve (AUC) during the early response (0-1 hour) and late response (1-10 hours) after challenge (eMethod 1.3 and eMethod 1.4 in Supplement 1).[12,13] Secondary exploratory end points included change in peak nasal inspiratory flow (PNIF) after challenge (eMethod 1.4.1 and eMethod 1.4.2 in Supplement 1), seasonal weekly visual analog scale (VAS),[14] seasonal weekly Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ),[15] end-of-season global rhinitis severity scores (eMethod 1.6 in Supplement 1), seasonal medication use (eMethod 1.7 in Supplement 1), and early and late skin responses to intradermal allergen (eMethod 1.5 in Supplement 1).[16] Before the pollen season began, participants received a prespecified package containing tablets (desloratadine), nasal sprays (fluticasone propionate), and eyedrops (olopatadine). Both used and unused medication was returned to the investigators who measured the quantity of these medications used according to the amount that was returned. A composite rescue medication score was derived using an algorithm for each prescribed medication and the mean composite score in each treatment group was computed and compared (eMethod 1.7 in Supplement 1). For clinical outcomes in which anchor-based methods for determining the minimum clinically important difference (MCID) were not available, a distribution-based method was used[17] based on 50% of the standard deviation of baseline values from all randomized participants. For TNSS, the range was 0 (best) to 12 (worst) and the MCID was 1.08[17] (eMethod 1.3 in Supplement 1). For the change in PNIF after challenge, the observed range was −388 to 26.7 L/min and the MCID was 33.9 L/min[17] (eMethod 1.4.1 in Supplement 1). For the global evaluation of

Figure 1. Flow of Participants Randomized to Receive Sublingual Immunotherapy, Placebo, or Subcutaneous Immunotherapy



Eligibility and baseline assessments were completed from September 2011 to January 2012 (study completion for the last participant was February 2015).

The intent-to-treat (ITT) sample was defined as all randomized participants. If participants dropped out after randomization, they were invited to complete study assessments throughout the duration of the trial. The modified ITT population (those included in the primary efficacy analysis) included all randomized participants with an evaluable outcome. The per-protocol sample was defined as the ITT sample participants who remained in the study for at least 3 years and in whom the primary end point was assessed. Participants in the per-protocol sample had to be adherent with study medication (taking ≥50% of study medication for the study duration).

seasonal symptoms, the range was 0 to 18, and the MCID was 1.4.[17] For those clinical end points for which an MCID was available from published anchor-based methods, the range values for the Mini-RQLQ were 0 to 6 (MCID, 0.7)[15] and for the seasonal weekly VAS, the range was 0 to 10 cm (MCID, 1.0).[14]

Intervention

Freeze-dried grass pollen (*Phleum pratense*) sublingual tablets (Grazax [ALK])[10] or matched placebo sublingual tablets were self-administered daily for 2 years. Subcutaneous alum-adsorbed grass pollen immunotherapy (Alutard SQ grass pollen [ALK])[18] or matched placebo subcutaneous injections were given weekly for 15 weeks followed by monthly maintenance injections until 2 years. For immunotherapy protocols, see eMethod 1.8 (in Supplement 1).

Nasal and Intradermal Allergen Challenge

Nasal allergen challenge was performed before treatment, at 1-year follow-up, 2-year follow-up, and at 3-year follow-up (1 year after treatment discontinuation). Nasal challenge was performed[12] at 9 AM using Aquagen (ALK) *P pratense* (Timothy grass) extract and participants were observed for 10 hours (eMethod 1.4 in Supplement 1). Intradermal allergen challenge[16] was performed 1 hour after nasal challenge. The early skin response was recorded at 15 minutes and the late response at 8 hours (eMethod 1.5 in Supplement 1).

Table 1. Participant Demographic and Baseline Characteristics for Treatment of Moderate to Severe Seasonal Allergic Rhinitis

	Sublingual Immunotherapy (n = 36)	Placebo (n = 34)	Subcutaneous Immunotherapy (n = 36)
Age, mean (95% CI), y	34.1 (30.77 to 37.45)	32.8 (29.97 to 35.63)	33.7 (30.46 to 36.89)
Male sex, No. (%)	26 (72.2)	23 (67.6)	23 (63.9)
Race/ethnicity, No. (%)[a]			
Asian	5 (13.9)	4 (11.8)	2 (5.6)
Black	3 (8.3)	4 (11.8)	3 (8.3)
Chinese	0	1 (2.9)	1 (2.8)
Middle Eastern	1 (2.8)	0	0
Mixed	3 (8.3)	1 (2.9)	0
White	24 (66.7)	24 (70.6)	30 (83.3)
Skin prick test, mean (95% CI), mm	10.4 (9.31 to 11.52)	8.9 (7.80 to 10.09)	8.6 (7.43 to 9.85)
Grass serum immunoglobulin E, median (interquartile range), kU/L	16.8 (5.71 to 54.00)	18.6 (5.94 to 35.50)	10.8 (4.04 to 42.85)

[a] In the setting of this study (United Kingdom), participants who self-reported as black were predominantly from the Caribbean Islands or Africa. Therefore, the black population differed from that in the United States. Participants who self-reported as Asian were predominantly from the Indian subcontinent. Race/ethnicity categories were fixed and based on National Institutes of Health requirements.

Serum Immunoglobulins

Timothy grass pollen-specific IgE and specific IgG4 were quantified using the fluorescent enzyme immunoassay (CAP FEIA) system (Phadia).[19]

Adverse Event Recording

Adverse events were classified according to the *Medical Dictionary for Regulatory Activities* version 14.0 (MedDRA).[20] In view of the known frequent local symptoms that occur after both subcutaneous immunotherapy and sublingual immunotherapy, these symptoms were recorded as adverse events only if they were considered bothersome by participants (interfering with usual daily activities or sleep) or as described in eMethod 1.9 (in Supplement 1). Observed immediate systemic allergic reactions to immunotherapy injections (active or placebo) were recorded by study clinicians according to the World Allergy Organization (WAO) grading system for subcutaneous immunotherapy (eMethod 1.9.2 in Supplement 1).[21] The same system was applied to adverse reactions in response to sublingual immunotherapy (active or placebo) reported by participants at routine clinic visits. Observed responses to the first sublingual tablet taken were recorded by study clinicians.[22]

Statistical Analysis

The intent-to-treat (ITT) population included all randomized participants. The modified ITT population included all randomized participants with an evaluable end point. The per-protocol population included participants who were adherent with study medications (defined as taking ≥50% of study medication for the duration of the study), and who had an evaluable primary end point (eMethod 1.10 in Supplement 1). The primary analysis compared nasal challenge–induced TNSS AUC in sublingual immunotherapy to placebo at 3 years using an analysis of covariance model adjusted for baseline values. Participants in the ITT population with a missing primary end point had their data imputed using a regression model based on those participants within their randomized treatment group who had available TNSS AUC values (eMethod 1.3 in Supplement 1). Subcutaneous immunotherapy vs placebo was analyzed as a positive control. Subcutaneous immunotherapy vs sublingual immunotherapy was a secondary exploratory analysis, but the study was not powered on this comparison. Secondary outcomes were assessed using analysis of covariance or nonparametric methods where appropriate. Allergen-specific immunoglobulin data were assessed in the per-protocol population using linear mixed models adjusted for baseline. The study was powered at 90% to detect a standardized mean difference between sublingual immunotherapy and placebo groups of approximately 40% with a 15% dropout rate (eMethod 1.11 in Supplement 1). The threshold for significance was a *P* value of less than .05 (2-sided). Secondary outcomes were considered exploratory and not adjusted for multiple comparisons. Analyses were performed using JMP V11, SAS version 9.3 (SAS Institute Inc), and R version 3.2.4 (R Foundation for Statistical Computing). Analysis of data was performed at the end of the study with no interim analyses. The statistical analysis plan is provided in Supplement 3. Analysis data sets are available through the public website of the Immune Tolerance Network.[23]

Results

Participant Characteristics, Progression, and Adherence With Trial Medication

One hundred and six participants (mean age, 33.5 years; 34 women [32.1%]; 78 white [73.6%]) were enrolled and 92 (87%) completed the primary end point evaluation at 3 years (Figure 1 and **Table 1**). The 3 groups were similar in age, sex, and race. Adherence to injections was recorded by study staff: 100% of completed participants received more than 50% of their injections, 95% received more than 75%, and 82% received more than 90% throughout the 2-year treatment period. Adherence to sublingual medication was assessed by counting returned tablets: 91.3% of completed participants took more than 50% of study tablets (protocol adherent), 75.0% took more than 75%, and 46.7% took more than 90%.

Table 2. Total Nasal Symptom Score (Weighted 10-Hour Area Under the Curve)

	Unadjusted Model			Adjusted Model, Mean Difference (95% CI)		
	Sublingual Immunotherapy	Placebo	Subcutaneous Immunotherapy	Sublingual Immunotherapy vs Placebo	Subcutaneous Immunotherapy vs Placebo	Sublingual Immunotherapy vs Subcutaneous Immunotherapy
Baseline						
No.	36	34	36			
Mean (95% CI)	6.36 (5.76 to 6.96)	6.06 (5.23 to 6.88)	6.10 (5.32 to 6.89)			
Median (range)	6.39 (2.8 to 9.8)	5.95 (2.4 to 11.5)	6.38 (1.9 to 10.1)			
Year 1 (Exploratory)						
No.	33	33	34			
Mean (95% CI)	3.94 (3.31 to 4.58)	4.63 (3.84 to 5.42)	3.05 (2.50 to 3.60)	−0.75 (−1.71 to 0.22)	−1.60 (−2.49 to −0.71)	0.84 (0.09 to 1.60)
Median (range)	3.47 (0.8 to 8.2)	3.97 (0.9 to 10.4)	2.70 (0.9 to 6.9)			
P value				.13	<.001	.03
Year 2 (Exploratory)						
No.	31	32	32			
Mean (95% CI)	3.70 (2.85 to 4.56)	5.07 (4.16 to 5.97)	2.96 (2.21 to 3.71)	−1.42 (−2.61 to −0.22)	−2.11 (−3.22 to −1.01)	0.68 (−0.36 to 1.73)
Median (range)	2.92 (0.7 to 8.1)	5.01 (0.6 to 9.8)	1.99 (0.5 to 9.2)			
P value				.02	<.001	.20
Year 3 (Primary Outcome)						
No.	30	31	31			
Mean (95% CI)	4.55 (3.67 to 5.43)	4.82 (3.90 to 5.74)	3.96 (3.21 to 4.71)	−0.30 (−1.52 to 0.92)	−0.90 (−1.96 to 0.16)	0.58 (−0.46 to 1.63)
Median (range)	4.44 (1.1 to 11.0)	4.57 (0.8 to 11.2)	3.76 (0.9 to 10.6)			
P value				.62	.10	.27
Year 3 (Primary Outcome Imputed), ITT Population						
No.	36	34	36			
Mean (95% CI)	4.73 (3.97 to 5.48)	4.81 (3.97 to 5.65)	3.89 (3.25 to 4.54)	−0.18 (−1.25 to 0.90)	−0.94 (−1.88 to 0.01)	0.73 (−0.17 to 1.62)
Median (range)	4.74 (1.1 to 11.0)	4.71 (0.8 to 11.2)	3.71 (0.9 to 10.6)			
P value				.75	.053	.11

Abbreviations: AUC, area under the curve; ITT, intent to treat; TNSS, Total Nasal Symptom Score.

P values, 95% CIs, and mean differences were calculated using an analysis of covariance model adjusted for pretreatment baseline TNSS (scale 0 [best] to 12 [worst]) AUC at the 0.05 level of significance. The weighted 10-hour AUC was calculated as the (early-phase response [0-1 h] /1 + late-phase response [1-10 h] /9). Values were imputed for participants in the ITT population with missing primary end point data (performed within treatment group using participants who had available TNSS AUC values). Specifically, a linear regression line and 95% CIs were fit where year 3 values were regressed on TNSS AUC values at time t (*t* = baseline, year 1, or year 2). Within each treatment group, a missing year 3 TNSS AUC value was imputed as the value predicted from the linear regression line. The primary end point was also calculated in the modified ITT population.

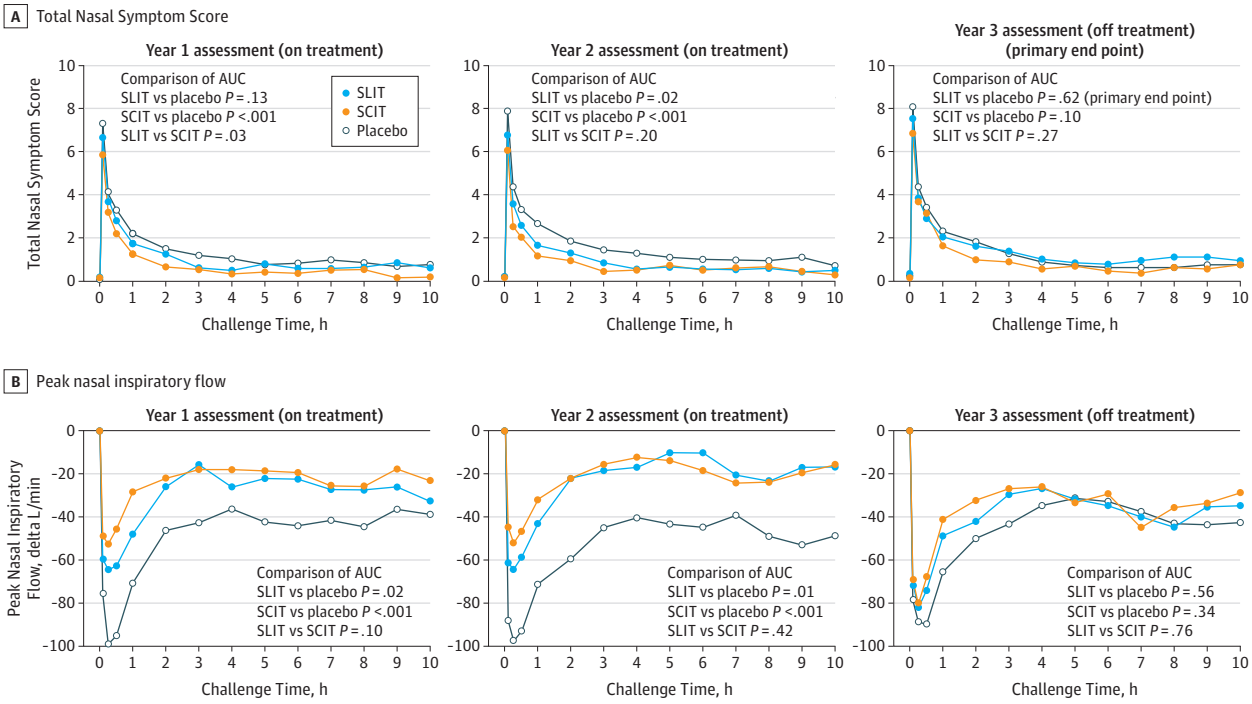
Primary Outcome

At 3-year follow-up, 1 year after completing treatment, nasal allergen-induced TNSS in the sublingual immunotherapy group did not differ from placebo. In the ITT population, the TNSS AUC at year 3 was as follows: for sublingual immunotherapy, 4.73 (95% CI, 3.97 to 5.48); for placebo, 4.81 (95% CI, 3.97 to 5.65). The adjusted mean difference was −0.18 (95% CI, −1.25 to 0.90), equivalent to −1.7% compared with placebo (*P* = .75) (**Table 2**). In the modified ITT population, the mean TNSS AUC for sublingual immunotherapy was 4.55 (95% CI, 3.67 to 5.43) and for placebo, it was 4.82 (95% CI, 3.90 to 5.74). The between-group difference adjusted for baseline was −0.30 (95% CI, −1.52 to 0.92), equivalent to −5.6% for sublingual immunotherapy compared with placebo (*P* = .62) (**Figure 2** and Table 2). Subcutaneous immunotherapy (positive control for the study) had a mean TNSS AUC of 3.96 (95% CI, 3.21 to 4.71), a difference from placebo of −0.90 (95% CI, −1.96 to 0.16), equivalent to −17.8% compared with placebo (*P* = .10). Baseline (pretreatment) TNSS AUC values for all 3 groups were as follows: sublingual immunotherapy, 6.36 (95% CI, 5.76 to 6.96); placebo, 6.06 (95% CI, 5.23 to 6.88); and for subcutaneous immunotherapy, 6.10 (95% CI, 5.32 to 6.89).

Secondary Exploratory Outcomes

At year 3 (1 year after discontinuation of treatment), allergen-induced reduction from prechallenge baseline in PNIF (Figure 2 and eTable 1 in Supplement 1), expressed as the 0- to 10-hour AUC, did not differ from placebo with either form of immunotherapy. Similarly at year 3, no benefit from either form of immunotherapy was observed in the weekly seasonal mini-RQLQ and VAS symptom scores (**Figure 3**; eTable 2a and eTable 2b in Supplement 1) or in global evaluations of rhinitis severity compared with placebo. (eFigure and eTable 2c in Supplement 1). Pollen season medication use was assessed by count of returned packages (both used and unused). Approximately 90% of participants returned some medication, whereas complete returns were obtained from 47% to 70% of participants throughout the 3 years (eTable 3a in Supplement 1). No significant differences in total rescue medication scores between the 3 groups were observed at year 3 after 1 year off

Figure 2. Time Course of Changes After Nasal Allergen Challenge for Total Nasal Symptom Scores and Peak Nasal Inspiratory Flow



Data are reported as mean values for all participants. Total Nasal Symptom Scores (TNSS) and peak nasal inspiratory flow (PNIF) were analyzed in the modified intent-to-treat population of 34 sublingual immunotherapy (SLIT) participants, 33 placebo participants, and 33 subcutaneous immunotherapy (SCIT) participants at year 1; 31 SLIT participants, 32 placebo participants, and 32 SCIT participants at year 2; and 30 SLIT participants, 31 placebo participants, and 31 SCIT participants at year 3. A, A higher TNSS indicates a higher burden of symptoms during the nasal challenge (range, 0 [best] to 12 [worst]). Mean scores for the TNSS are reported in Table 2. The *P* values compare the TNSS area under the curve (AUC) between treatment groups (calculated using an analysis of covariance [ANCOVA] model at at the 0.05 level of significance adjusted for pretreatment baseline AUC measures [minimal clinically important difference for this measure within a participant was 1.08]).[17] B, A larger change in PNIF indicates a higher burden of symptoms during the nasal challenge. Change (liters/min) was defined relative to the 0 time point in the challenge. Mean values for the change in PNIF are reported in eTable 1 (in Supplement 1). The *P* values compare the delta PNIF AUC between treatment groups (calculated using an ANCOVA model at the 0.05 level of significance adjusted for pretreatment baseline AUC measures [minimal clinically important difference for this measure within a participant was 33.9]).[17]

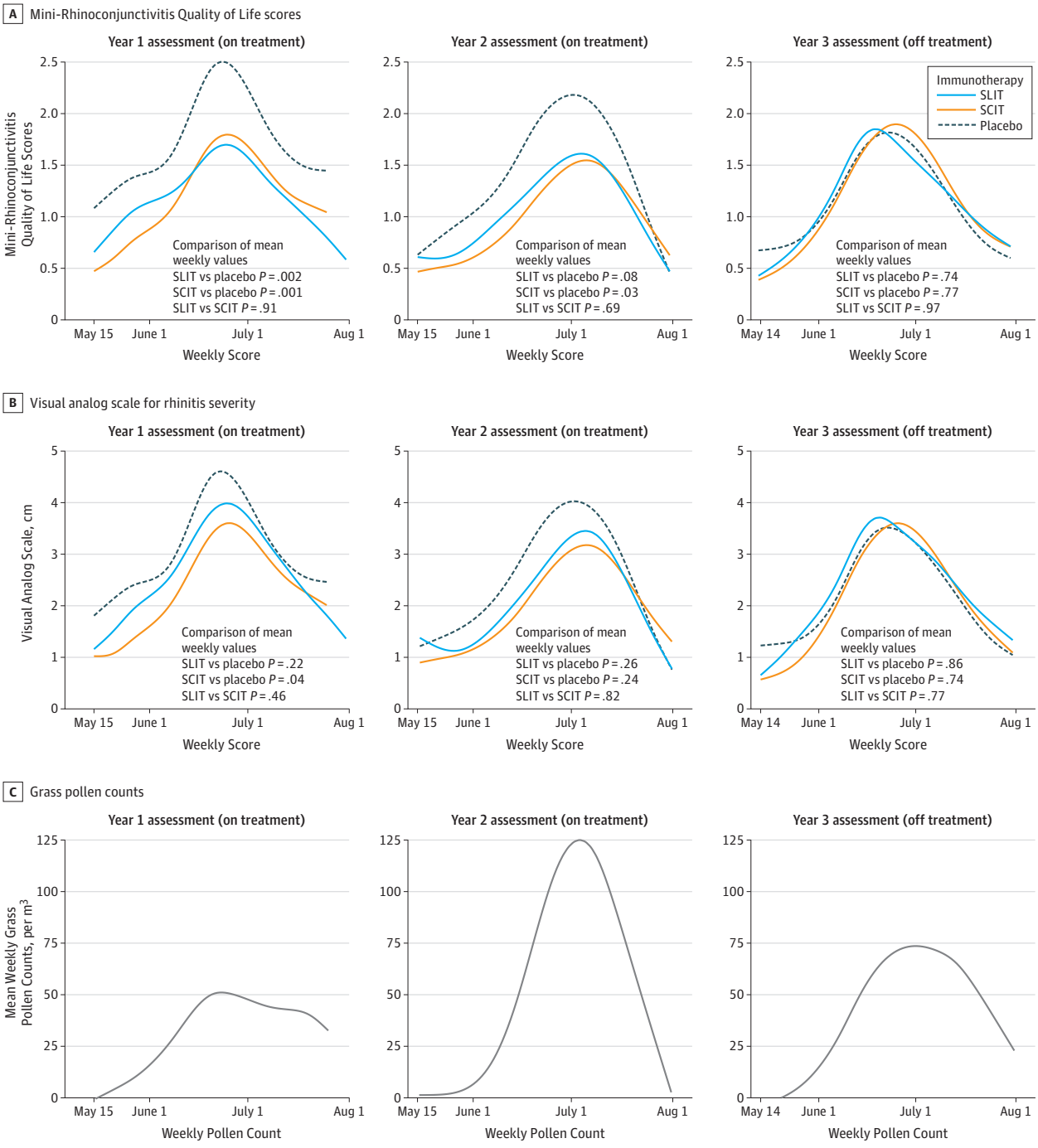
therapy (eTable 3b in Supplement 1). In contrast, both sublingual and subcutaneous immunotherapy groups had lower early skin response and lower late skin response to allergen than placebo at year 3 after 1 year off therapy (**Figure 4**; eTable 4a and eTable 4b in Supplement 1). Serum allergen-specific IgE did not differ between sublingual immunotherapy and placebo at year 3 but was significantly lower in the subcutaneous immunotherapy group than the other 2 groups (Figure 4 and eTable 5a in Supplement 1). Allergen-specific IgG4 in serum was significantly higher with both forms of immunotherapy compared with placebo at year 3 (Figure 4 and eTable 5b in Supplement 1).

At the end of the first year of treatment, subcutaneous immunotherapy, but not sublingual immunotherapy, improved TNSS AUC when compared with placebo. At the end of the second year, both forms of immunotherapy performed better than placebo (Figure 2 and Table 2). For the seasonal mini RQLQ and global severity evaluations, both forms of immunotherapy showed improvement over placebo after the first and second years of treatment (Figure 3; eFigure, eTable 2a, and eTable 2c in Supplement 1). Results for other secondary exploratory outcomes at years 1 and 2 of treatment can be summarized as follows: sublingual immunotherapy but not subcutaneous immunotherapy was associated with decreased use of seasonal rescue medications (eTable 3b in Supplement 1); both sublingual immunotherapy and subcutaneous immunotherapy resulted in decreased early (15-minute) and late (8-hour) skin responses to intradermal allergen challenge at 1 and 2 years (Figure 4; eTable 4a and eTable 4b in Supplement 1); for allergen-specific IgE, sublingual immunotherapy resulted in increased values over placebo whereas subcutaneous immunotherapy had the opposite effect (Figure 4 and eTable 5a in Supplement 1); and for IgG4, both forms of treatment resulted in increases over placebo (Figure 4 and eTable 5b in Supplement 1).

Adverse Events

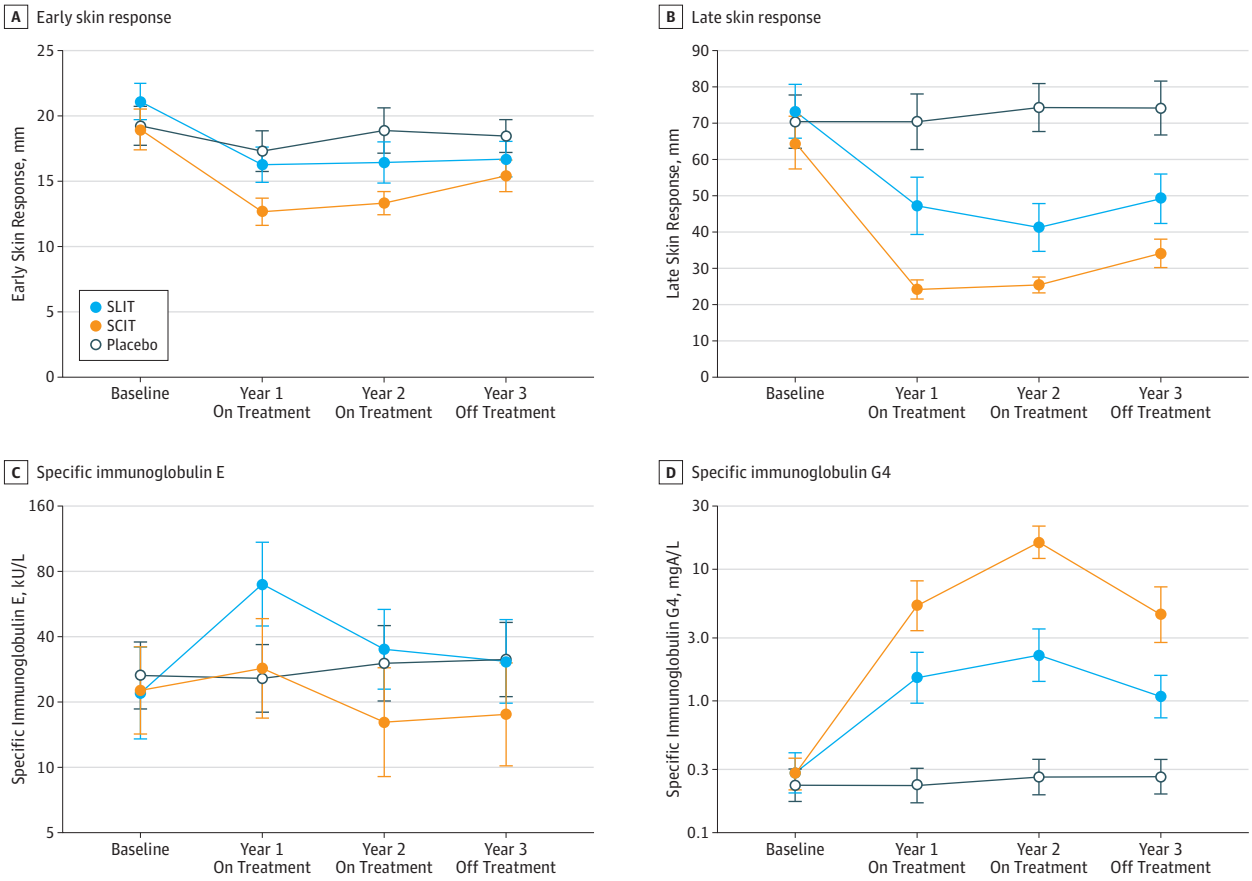
A total of 553 adverse events were recorded, of which 116 were related to study participation. All adverse events are shown in eTable 8 in Supplement 1. No serious treatment-related adverse events were recorded. Adverse events were higher in the subcutaneous immunotherapy group. Adverse events with significant differences between groups are shown in **Table 3**. Seventeen participants in the subcutaneous immunotherapy

Figure 3. Time Course of Weekly Seasonal Rhinitis Quality of Life Scores and Rhinitis Severity



Data are mean weekly values for all participants. The curves were smoothed using a cubic spline smoothing function (using a set of third-degree polynomials spliced together such that the resulting curve is continuous and smooth at the splices [knot points]). The estimation was calculated by minimizing an objective function (a combination of the sum of squares error and a penalty for curvature integrated over the curve extent).[24] The *P* values compare the mean values between treatment groups and were calculated using an analysis of covariance model at the 0.05 level of significance adjusted for pretreatment baseline measures. The Mini-Rhinoconjunctivitis Quality of Life Questionnaire (Mini-RQLQ) and a visual analog scale (VAS) were analyzed in the modified intent-to-treat population of 33 sublingual immunotherapy (SLIT) participants, 33 placebo participants, and 34 subcutaneous immunotherapy (SCIT) participants at year 1; 28 SLIT participants, 31 placebo participants, and 30 SCIT participants at year 2; 27 SLIT participants, 30 placebo participants, and 29 SCIT participants at year 3. A, Mini-RQLQ values range from 0 to 6 (higher values indicate participants with more troublesome nose, eye, and other symptoms affecting regular activities resulting in a lower quality of life). The minimal clinically important difference for this measure within a participant is 0.7 (eTable 2a in Supplement 1).[15] B, VAS values range from 0 to 10 cm (higher values indicate participants with worse hay fever symptoms). The minimal clinically important difference for this measure within a participant is 1.0 cm (eTable 2b in Supplement 1).[14] C, Mean weekly grass pollen counts per cubic meter from a single site in Islington, London were provided by the Met Office (UK national weather service).

Figure 4. Time Course of Early and Late Skin Responses to Intradermal Allergen, Changes in Serum Grass Pollen Allergen–Specific Immunoglobulin E, and Immunoglobulin G4



Data are once-yearly mean values for all participants. Early skin response was recorded at 15 minutes and late skin response was recorded at 8 hours. Skin responses were analyzed using analysis of covariance with adjustment for baseline values in the modified intent-to-treat population of 33 sublingual immunotherapy (SLIT) participants, 33 placebo participants, and 34 subcutaneous immunotherapy (SCIT) participants at year 1; 31 SLIT participants, 32 placebo participants, and 32 SCIT participants at year 2; 30 SLIT participants, 31 placebo participants, and 31 SCIT participants at year 3 (eTable 4a and eTable 4b in Supplement 1). Serum allergen–specific immunoglobulin parameters were analyzed in the per-protocol population of 27 SLIT, 30 placebo, and 27 SCIT participants at all time points using a linear mixed model with adjustment for baseline values. Serum allergen–specific immunoglobulin responses were plotted after log transformation for normalization of these variables. The per-protocol sample included participants who were adherent with study medications (taking ≥50% of study medication for study duration) and who had an assessment of the primary end point. All comparisons for skin and serum allergen–specific immunoglobulin responses between treatment groups at years 1, 2, and 3 have *P* values less than .01, with the following exceptions: early skin responses between SLIT and SCIT at year 2 (*P* = .02) and year 3 (*P* = .94), specific immunoglobulin E between SLIT and placebo at year 2 (*P* = .04) and year 3 (*P* = .32), and between SCIT and placebo at year 1 (*P* = .10) (eTable 5a and eTable 5b in Supplement 1).

group (47.2%) experienced hypersensitivity episodes following injections compared with 1 (2.8%) in the sublingual immunotherapy group and 4 (11.8%) in the placebo group. Dyspepsia was reported by 8 (22%) participants who received active sublingual immunotherapy compared with none in the subcutaneous immunotherapy group and 1 (2.9%) in the placebo group. Episodes of dyspepsia (specifically mild heartburn or indigestion), were short lived and were either not treated or self-treated with antacids or antihistamines. No participant in the sublingual immunotherapy group withdrew due to adverse events.

Events occurring in the first hour following administration of the first sublingual tablet (active or placebo) under supervision were also recorded (eTable 6 in Supplement 1); none of the 106 participants had a systemic allergic reaction and 11 of the 106 (10 in the active sublingual group and 1 in the subcutaneous immunotherapy group) reported a mild local reaction.

Systemic allergic reactions after subcutaneous immunotherapy were graded according to the World Allergy Organization classification (eTable 7a in Supplement 1).[21] A total of 41 systemic reactions after active injections occurred in 19 participants. The majority were mild: 31 grade 1 of which 8 were early (0-60 minutes), 19 were delayed (after 1 hour), and in 4, the timing was undefined; 8 were grade 2 (2 early, 6 delayed); and 2 were grade 3 (1 early, 1 delayed [at 2 hours]). In participants with grades 1 and 2 reactions, symptoms resolved with no treatment or with oral antihistamines. In grade 3 reactions, adrenaline was used with prompt response. The same classification was used for participant-reported systemic

Table 3. Adverse Events[a]

System Organ Class Preferred Term	Sublingual Immunotherapy (n = 36)	Placebo (n = 34)	Subcutaneous Immunotherapy (n = 36)	Total (n = 106)	*P* Values		
					Sublingual Immunotherapy vs Placebo	Subcutaneous Immunotherapy vs Placebo	Subcutaneous Immunotherapy vs Sublingual Immunotherapy
Total No. of AEs	163	174	216	553	.63	.05	.01
Total No. of related AEs	34	18	64	116	.02	<.001	.004
Participants with ≥1 AE, No. (%)	34 (94.4)	33 (97.1)	35 (97.2)	102 (96.2)	>.99	>.99	>.99
Immune system disorders, No. (%)	5 (13.9)	9 (26.5)	20 (55.6)	34 (32.1)	.24	.02	<.001
Hypersensitivity	1 (2.8)	4 (11.8)	17 (47.2)	22 (20.8)	.19	.002	<.001
Gastrointestinal disorders, No. (%)	13 (36.1)	8 (23.5)	8 (22.2)	29 (27.4)	.30	>.99	.30
Dyspepsia	8 (22.2)	1 (2.9)	0	9 (8.5)	.03	.49	.005

Abbreviations: AE, adverse event; CRF, case report form.

[a] AEs were reported by observing the participant, questioning the participant in an objective manner, or receiving an unsolicited complaint from the participant. Any reactions occurring in the clinic due to procedures, administration of subcutaneous immunotherapy, or the first administration of sublingual immunotherapy were recorded on related CRFs and entered into the electronic database. Reactions occurring outside the clinic to either subcutaneous or sublingual immunotherapy or other AEs were assessed by study staff at clinic visits and recorded on related CRFs and entered into the electronic database. Adverse events for this trial have been coded according to international *Medical Dictionary for Regulatory Activities* classification. Only system organ classes and preferred terms with a significant *P* value are reported. *P* values for participant-level analyses (percentages) were computed using Fisher Exact Tests. *P* values for event-level analyses (total number of events) were computed using a Poisson regression model comparing the person-year adjusted event rates between each treatment group. Hypersensitivity indicates systemic reactions after subcutaneous immunotherapy, which included mostly mild reactions such as itchy eyes or nose, blocked nose, runny nose, sneezing, itchiness or rash; 2 events with shortness of breath, and sensation of throat closure resulted in administration of epinephrine. There were no serious treatment-related AEs.

reactions after administration of sublingual immunotherapy tablets (eTable 7b in Supplement 1). Of 18 reported reactions, 16 were grade 1, and 2 were grade 2. Although these events strictly fulfilled criteria for World Allergy Organization systemic reactions, they all consisted of local or upper gastrointestinal symptoms. All were transient and mild and none required adrenaline or resulted in participant withdrawals.

Discussion

This study, by use of nasal allergen challenge, demonstrated that in patients with moderate to severe seasonal allergic rhinitis, treatment for 2 years with grass pollen sublingual immunotherapy was not sufficient to achieve an allergic response improvement at 3-year follow-up. Previous randomized placebo-controlled trials demonstrated that 3 years of continuous therapy with either sublingual immunotherapy or subcutaneous immunotherapy resulted in long-term clinical efficacy with decreases in seasonal symptoms and use of antiallergic medications that persisted for at least 2 years after discontinuation[9-11] (3 years for subcutaneous immunotherapy[11]). Subcutaneous immunotherapy has been used for more than 100 years,[6,25] but in recent years, the sublingual route has been shown to be an effective and safer alternative.[7] International guidelines regarding immunotherapy recommend a minimum of 3 years of treatment[7,26] with both delivery methods. If a 2-year regimen had demonstrated long-term benefits in addition to efficacy, this could have represented cost savings in terms of clinical resources and improved convenience for the patient. Because this was not observed, clinicians should be advised to follow established guidelines that recommend at least 3 years treatment.

The World Allergy Organization has recommended (empirically) a 20% difference from placebo as the minimum clinically meaningful difference for seasonal outcomes in immunotherapy trials.[27] Previous randomized clinical trials of both the sublingual and subcutaneous allergen immunotherapies used in the current trial demonstrated a 30% difference from placebo in seasonal symptoms.[6,18] In a previous cross-sectional study, a 45% reduction in TNSS and 54% improvement in PNIF following allergen challenge was shown in grass pollen immunotherapy-treated patients compared with untreated patients with seasonal rhinitis.[13] Therefore, this trial was powered to detect a difference of 40% between either form of immunotherapy and placebo. There was no significant decrease in the primary outcome of TNSS at 1 year following withdrawal of treatment (5.6% for sublingual immunotherapy compared with placebo and 17.8% for subcutaneous immunotherapy). Nonetheless, in secondary exploratory outcomes, both treatments were superior to placebo as shown by significant reductions for sublingual immunotherapy (27.0%) and subcutaneous immunotherapy (41.6%) at 2 years (Table 2).

There are limitations to this study. First, daily symptom diary records were not used during the grass pollen season. However, nasal allergen challenge was used as a surrogate[12] for seasonal outcomes, thereby allowing reproducible exposure to grass pollen allergen in a controlled environment while avoiding the high season-to-season variability to natural pollen exposure. A previous study showed a significant correlation between TNSS and reductions in PNIF after challenge and seasonal symptoms.[13] Second, the study was not designed to compare 2 vs 3 years sublingual immunotherapy, and therefore, this study cannot determine whether 3 years of therapy would have been sufficient to produce long-term benefits. Given that previous studies have consistently shown long-term benefits when therapy is discontinued after 3 years,[9-11,28] this trial was designed to address the question whether 2 years of treatment were adequate. For sublingual immunotherapy,

47% of participants took more than 90% of doses over the 2-year period compared with 82% for subcutaneous immunotherapy. In addition, although both treatments were administered using double-blind methods with both sublingual and subcutaneous placebos, it is possible that the occurrence of local reactions in a proportion of participants after both forms of immunotherapy may have compromised blinding. For this reason, all nasal challenges and skin tests were performed by 1 individual (G.W.S.) who was not involved in the clinical immunotherapy protocol or in seasonal assessments.

Secondary exploratory seasonal outcomes were in accord with the observed lack of effect of both treatment modalities on nasal challenge at 3 years. Seasonal outcomes and response to nasal challenge were also consistent in showing improvement while on treatment at years 1 and 2 (Figure 2 and Figure 3; eTable 1, eTable 2a, eTable 2b, and eTable 2c in Supplement 1). The comparative efficacy of the 2 routes of immunotherapy is unknown. Previous systematic reviews have relied on indirect comparisons[29-31] with few head-to-head randomized trials of natural allergen exposure.[32]

This study was not powered to detect differences between active treatments. However, at year 1, subcutaneous immunotherapy was more effective than sublingual immunotherapy in reducing TNSS after challenge; conversely, the use of seasonal rescue medication was lower for sublingual immunotherapy compared with subcutaneous immunotherapy. These data highlight the need for a head-to-head clinical trial of sublingual and subcutaneous immunotherapy during natural pollen exposure using seasonal outcomes. Sublingual immunotherapy was associated with a transient increase in allergen-specific IgE at year 1, whereas after subcutaneous immunotherapy, specific IgE levels were unchanged at year 1 and decreased during years 2 and 3 when compared with placebo. These findings are in agreement with previous studies although the mechanisms are unknown.[10,33] Changes in serum allergen-specific IgG4[16,34] paralleled suppression of allergen-induced early and late skin responses. These immunologic changes, although reduced, persisted at year 3 (Figure 4). Together with the accompanying suppression of early and late skin responses, these effects could be regarded as early indicators of effective clinical tolerance, which has previously been convincingly documented following 3 years immunotherapy via both routes.[9-11]

Almost all adverse reactions to sublingual immunotherapy were isolated, mild, transient, local oral or upper gastrointestinal symptoms (eTable 7b in Supplement 1). None of these reactions required acute medical intervention or resulted in withdrawal from the trial. Although these adverse reactions were recorded as systemic according to the World Allergy Organization grading for subcutaneous immunotherapy (eMethods 1.9.2. in Supplement 1),[21] this may not be appropriate given the proximity of the symptoms to the site of sublingual immunotherapy administration. These results are consistent with the safe self-administration of sublingual immunotherapy as reported in systematic reviews[8,30] and large controlled trials.[35,36] Subcutaneous immunotherapy was associated with expected systemic reactions[21] including 2 grade 3 reactions requiring adrenaline. This emphasizes the need for close observation in a specialist setting for subcutaneous immunotherapy (eTable 7a in Supplement 1).

Conclusions

Among patients with moderate to severe seasonal allergic rhinitis, 2 years of sublingual grass pollen immunotherapy was not significantly different from placebo in improving the nasal response to allergen challenge at 3-year follow-up.

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