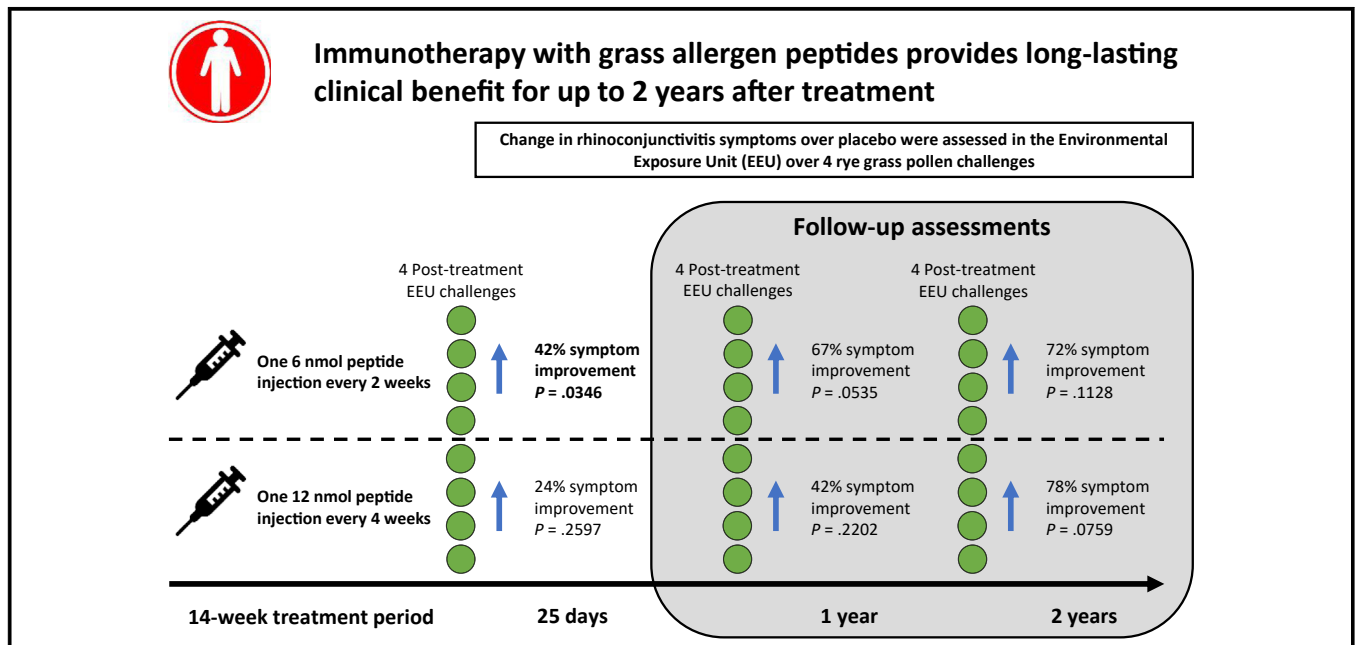


Persistence of the clinical effect of grass allergen peptide immunotherapy after the second and third grass pollen seasons



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GRAPHICAL ABSTRACT



Background: Grass allergen peptides are in development for the treatment of grass pollen-induced allergic rhinoconjunctivitis. A previous randomized, placebo-controlled study demonstrated that grass allergen peptides significantly improved total rhinoconjunctivitis symptom scores (TRSSs) after posttreatment challenge (PTC) to rye grass in an environmental exposure unit after 1 intervening grass pollen season (GPS1).

Objective: We sought to evaluate the efficacy/safety of 4 dosing regimens of grass allergen peptides after a second (GPS2) and third (GPS3) intervening GPS in the environmental exposure unit.

Methods: Eligible subjects who were randomized in the parent study (GPS1) during the first year of recruitment were invited to participate in GPS2 and GPS3, which took place 1 and 2 years

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options in Circassia Pharmaceuticals when the study was conducted. R. P. Hafner is employed by Circassia and is a director and shareholder of Circassia Pharmaceuticals. The rest of the authors declare that they have no relevant conflicts of interest.

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after treatment cessation, respectively. Participants were not treated further, and both participants and study personnel remained blinded. The primary efficacy end point was the change in mean TRSS (reported every 30 minutes) from GPS1 baseline to the follow-up PTC calculated across all time points over days 2 to 4 for GPS2 and across hours 1 to 3 over days 2 to 4 for GPS3. Secondary efficacy end points and safety were also assessed.

Results: One hundred twenty-two and 85 participants were enrolled in GPS2 and GPS3, respectively. A numerically greater, but not statistically significant improvement from baseline in mean TRSS at PTC was observed in the group receiving one 6-nmol intradermal injection every 2 weeks for 14 weeks group compared with the placebo at GPS2 (−6.0 vs −3.6, $P = .0535$) and GPS3 (−6.2 vs −3.6, $P = .1128$). Similar findings were observed for the group receiving one 6-nmol intradermal injection every 2 weeks for 14 weeks at GPS3 (−6.4 vs −3.6, $P = .0759$). No adverse safety signals were detected.

Conclusion: Treatment with grass allergen peptides led to an improvement in allergic rhinoconjunctivitis symptoms after 3 intervening GPSs, corresponding to up to 2 years off treatment. (J Allergy Clin Immunol 2020;145:610-8.)

Key words: Allergic rhinoconjunctivitis, allergen challenge, environmental exposure unit, synthetic peptide immunoregulatory epitope, peptide, immunotherapy, grass, synthetic peptide immunoregulatory epitope, allergic rhinitis

The pathophysiology of allergic rhinoconjunctivitis (ARC) includes IgE-mediated activation and degranulation of mast cells and basophils after inhalation of allergens to which a subject is sensitized. Subsequent symptoms include sneezing, rhinorrhea, nasal itching, congestion, and itchy, watery, red/burning eyes.¹ First-line treatments for ARC, including antihistamines and intranasal corticosteroids, only work to resolve ARC symptoms and not the underlying disease. Moreover, 61% of patients with ARC receiving pharmacologic therapy still report that their symptoms are not well controlled.² Specific allergen immunotherapy aims to modify the body's immunologic response to the sensitized allergen, providing long-term clinical benefit and altering the course of disease for treated subjects.³

For patients with ARC, grass pollen is a common environmental trigger of allergic symptoms. According to the third National Health and Nutrition Examination Survey (NHANES III), 26.9% and 18.1% of US respondents were sensitized to rye and Bermuda grass, respectively, a greater than 2-fold increase from NHANES II.⁴ NHANES 2005-2006 also reported that 19.5% and 15.0% of survey respondents had a positive specific IgE result to rye and Bermuda grass, respectively.⁵ In Canada the prevalence of grass sensitization using skin testing was 21.8% for timothy grass and 19.5% for Kentucky bluegrass across 6 provinces.⁶

Synthetic peptide immunoregulatory epitopes (SPIREs) were developed to reduce the likelihood of severe IgE-mediated adverse reactions associated with traditional whole allergen immunotherapy. SPIREs consist of short synthetic peptides (13-17 amino acids) derived from immunodominant T-cell epitopes of major allergens. The lack of reactive IgE epitopes serves to reduce IgE-dependent reactions during treatment.^{7,8} To date, SPIREs have been developed for cat, house dust mite (HDM), ragweed,

Abbreviations used

AE:	Adverse event
ANCOVA:	Analysis of covariance
ARC:	Allergic rhinoconjunctivitis
EEU:	Environmental exposure unit
GPS:	Grass pollen season
HDM:	House dust mite
ITT:	Intent to treat
LS:	Least squares
NHANES:	National Health and Nutrition Examination Survey
PTAE:	Posttreatment adverse event
PTC:	Posttreatment challenge
4x12Q4W:	One 12-nmol intradermal injection every 4 weeks for 14 weeks with placebo injections given in between to maintain blinding
8x6Q2W:	One 6-nmol intradermal injection every 2 weeks for 14 weeks
8x12Q2W:	One 12-nmol intradermal injection every 2 weeks for 14 weeks
SAP:	Statistical analysis plan
SCIT:	Subcutaneous immunotherapy
sIgG ₄ :	Allergen-specific IgG ₄
SLIT:	Sublingual immunotherapy
SPIRE:	Synthetic peptide immunoregulatory epitope
TNSS:	Total nasal symptom score
TNNSS:	Total nonnasal symptom score
TRSS:	Total rhinoconjunctivitis symptom score

and grass allergens, with efficacy evaluated in phase 2 and 3 trials.⁹⁻¹⁷ SPIREs are thought to induce allergen tolerance by targeting T-cell populations, leading to upregulation of allergen-specific regulatory T cells, specific T-cell anergy, and a shift from specific effector T_H2 cells to a T_H1 cell phenotype.¹⁸ Studies have also demonstrated the ability of SPIREs to reduce the expression of several T_H2-related genes in whole blood samples, which correlated with symptom improvement after treatment in participants with cat allergy.^{19,20}

Recently, a multicenter, phase 2, dose-finding study (NCT01385800) assessed the efficacy and safety of 3 intradermal regimens of grass allergen peptides using the environmental exposure unit (EEU), a validated challenge model approved by the US Food and Drug Administration to investigate the efficacy of new allergic rhinitis therapies.^{17,21} Participants with grass allergy were randomized to placebo or one of three 14-week treatment regimens: one 6-nmol intradermal injection every 2 weeks for 14 weeks (8x6Q2W), one 12-nmol intradermal injection every 4 weeks for 14 weeks with placebo injections given in between to maintain blinding (4x12Q4W), and one 12-nmol intradermal injection every 2 weeks for 14 weeks (8x12Q2W). All subjects received an intradermal injection at 2-week intervals, with placebo injections used where necessary to maintain blinding. We showed that after the peak of the grass pollen season (GPS; approximately 25 weeks after treatment initiation), only the 8 × 6 nmol group had significantly reduced mean total rhinoconjunctivitis symptom scores (TRSSs) compared with those in the placebo group during posttreatment challenges (PTCs) with grass pollen in the EEU.¹⁷ Other phase 2 studies demonstrated a persistent treatment benefit for both cat and HDM allergen peptides for up to 2 years after treatment cessation.^{9,10,12,13} Thus it was

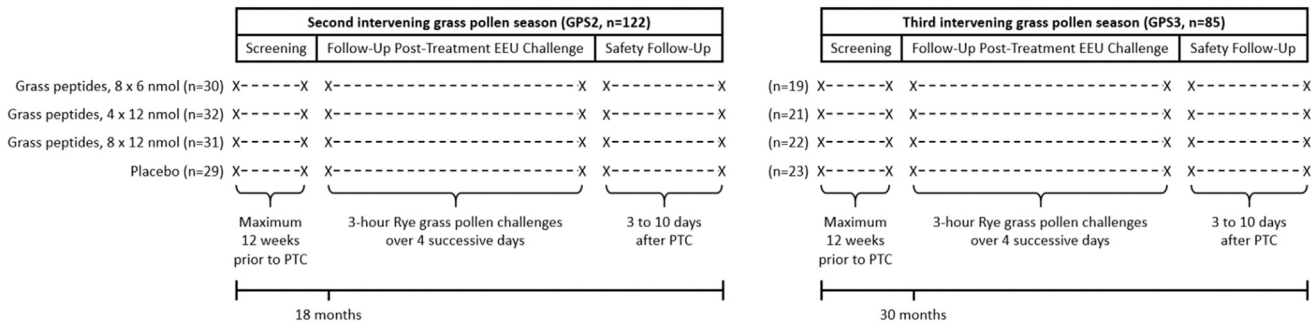


FIG 1. Overall study design.

hypothesized that treatment persistence might also be observed with grass allergen peptides. This report summarizes the findings from 2 blind follow-up studies assessing the efficacy of grass allergen peptides after a second and third intervening GPS (GPS2 and GPS3), corresponding to approximately 1 and 2 years off treatment, respectively.

METHODS

Study design

Both studies were follow-up assessments to a previously completed multicenter, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the continued efficacy and safety of grass allergen peptides and took place after 1 (GPS2) and 2 (GPS3) years off treatment. All study-related procedures, amendments, and documentation were reviewed and granted ethical approval by 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), the International Conference on Harmonisation guidelines on Good Clinical Practice, and all relevant local regulatory requirements. The [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01923779) identifiers are NCT01923779 and NCT02292875.

The clinical methodology for the parent study (GPS1) has been published elsewhere.¹⁷ Briefly, participants with grass allergy were randomized to one of three 14-week dosing regimens of grass allergen peptides completed before the start of GPS: 8x6Q2W, 4x12Q4W, and 8x12Q2W. Efficacy was evaluated 25 weeks after treatment initiation, corresponding to after the peak of GPS and before the start of the ragweed pollen season. Participants returned to the study site for the rye grass pollen PTCs conducted in the EEU over 4 successive days.

Both GPS2 and GPS3 were comprised of 3 study periods: screening visits, follow-up PTCs, and safety follow-up visits (Fig 1). Screening visits were conducted 12 or fewer weeks before the PTC. For GPS2, the PTC occurred approximately 1 year after the GPS1 PTC (18 months after treatment initiation). For GPS3, the PTC occurred approximately 2 years after the GPS1 PTC (30 months after treatment initiation). For the PTC, eligible participants attended four 3-hour rye grass pollen challenges held over 4 successive days in the EEU. The airborne grass pollen concentration was maintained at 3500 ± 500 grains/m³, which is consistent with GPS1. Safety follow-up visits were performed 3 to 10 days after the final EEU visit. Participants, study staff, and investigators directly involved in GPS2 and GPS3 remained blind to the treatment allocation schedule. No further immunotherapy treatments were administered during the follow-up studies.

Study participants

Participants who were randomized in the GPS1 study during the first year of recruitment (before the 2012 GPS), completed all treatment and PTC visits, and achieved a mean baseline TRSS of 8 or greater of 24 in GPS1 (calculated as the mean score of all time points across days 1-4 during the baseline challenge) were eligible to participate in GPS2 and GPS3. Participation in

GPS2 was not required for participation in GPS3 if all other inclusion criteria were met. Enrollment into the GPS2 study was performed before completion of GPS1.

The key exclusion criteria were unblinding of participants to their original treatment allocation, a history of grass pollen-induced asthma since participation in GPS1, a history of uncontrolled asthma or asthma requiring Global Initiative for Asthma step 2 or greater treatment (exercise-induced bronchospasm or controlled asthma not triggered by grass pollen and requiring only Global Initiative for Asthma step 1 treatment were acceptable), FEV₁ of less than 80% of predicted value, use of allergen immunotherapy treatments since completing GPS1, history of recurrent acute or chronic sinusitis, and pregnancy (current or planned). Participants for whom administration of epinephrine was contraindicated were also excluded. Additional inclusion and exclusion criteria are summarized in Table E1 in this article's Online Repository at www.jacionline.org.

Study assessments

During the PTC, 4 nasal symptoms, 4 nonnasal symptoms, and breathlessness symptoms were scored at 30-minute intervals using a 4-point categorical scale (0, none; 1, mild; 2, moderate; and 3, severe). The 4 nasal and 4 nonnasal scores were combined into the TRSS, which has a range of 0 (no symptoms) to 24 (all symptoms severe). Baseline symptoms were defined as matched symptom scores achieved during the baseline challenge in GPS1. The primary efficacy end point in GPS2 was the change from baseline in mean TRSS at all time points on days 2 to 4 of the follow-up PTC (month 18) compared with placebo. The primary efficacy end point in GPS3 was the change from baseline in mean TRSS at hours 1 to 3 on days 2 to 4 of the follow-up PTC (month 30) compared with placebo. For GPS3, hours 0 and 0.5 were excluded to focus on symptoms during the plateau phase.

Secondary efficacy end points included change from baseline in mean TRSS at all time points on days 1 to 4 (GPS2 and GPS3) and days 2 to 4 (GPS3), change from baseline in mean TRSS on days 2 to 4 of the follow-up PTC for participants with a mean baseline TRSS of 12 or greater, change from baseline in mean total nasal symptom score (TNSS; the sum of the 4 nasal symptoms), and change from baseline in mean total nonnasal symptom score (TNNSS; the sum of the 4 nonnasal symptoms). Exploratory assessments included mean change in rye grass-specific IgA, IgE, and IgG₄ levels at the safety follow-up visit. An additional subgroup analysis of the primary efficacy end point in GPS3 was conducted in participants who completed GPS1, GPS2, and GPS3.

Safety assessments in both follow-up studies included physical examinations, vital signs, incidence and severity of adverse events (AEs), FEV₁, peak expiratory flow rate, and breathlessness.

Statistical analyses

For both GPS2 and GPS3, efficacy analysis was based on the intent-to-treat (ITT) population, which included participants who completed at least 1 follow-up PTC assessment (≥ 1 of 4 EEU visits). Analysis of primary, secondary, and exploratory end points was conducted by using analysis of

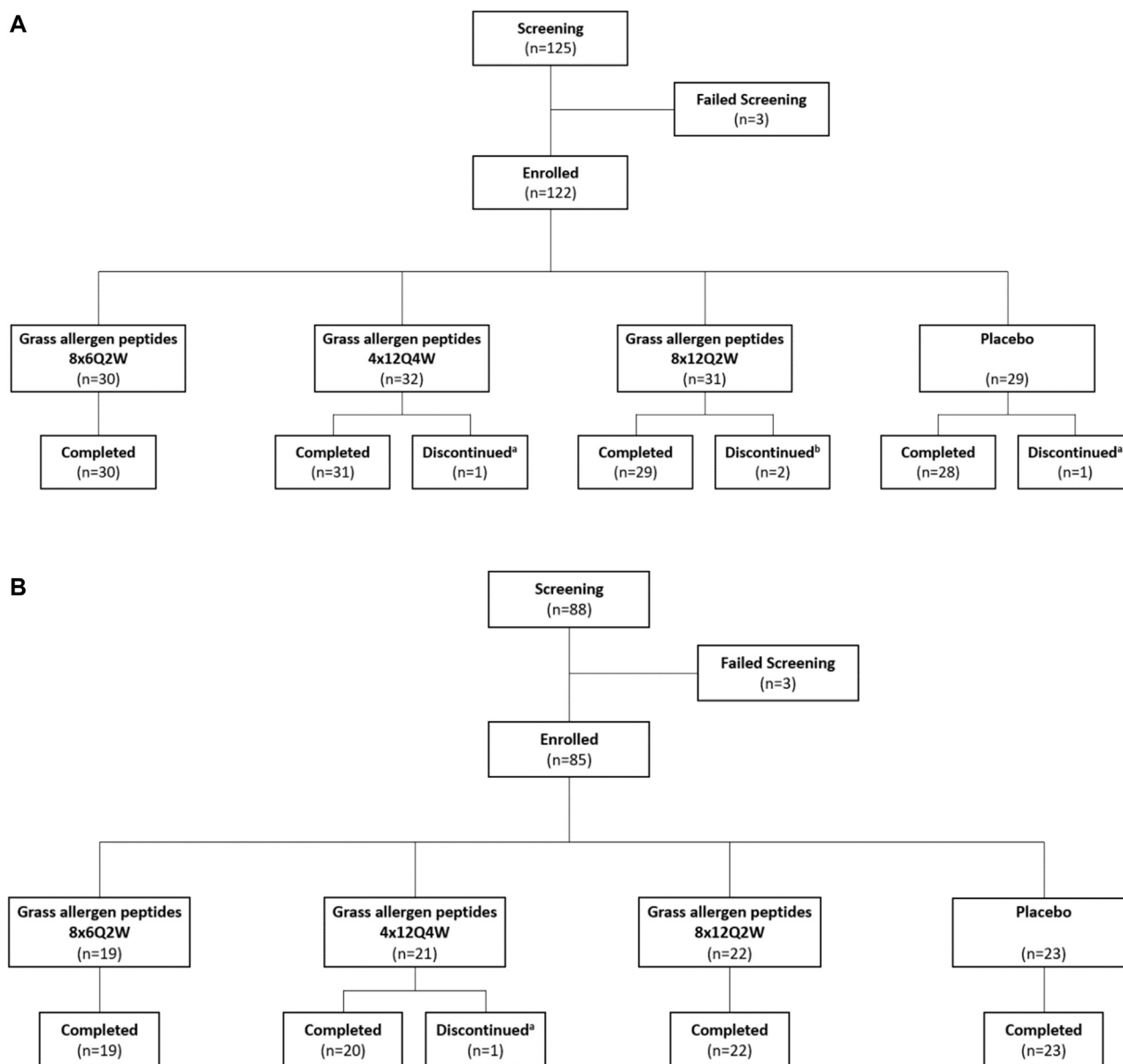


FIG 2. Participant disposition. All participants were randomized and dosed in the parent study approximately 18 months before the 1-year follow-up study (**A**) and approximately 30 months before the 2-year follow-up study (**B**). No further treatment was administered during follow-up studies. ^aOne subject withdrew because of schedule conflict. ^bOne subject moved abroad, and 1 subject withdrew consent.

covariance (ANCOVA). The ANCOVA model contained terms for the fixed effects of treatment and GPS1 baseline assessments (symptom scores and immunoglobulin levels) as the covariate. A 2-sided *P* value of less than .05 was taken as significant. Because both follow-up studies were exploratory in nature, adjustments for multiple comparisons were not made.

RESULTS

Participants' demographics

For GPS2, a total of 122 participants were enrolled, and 118 completed the study (Fig 2, A). Two of the enrolled participants withdrew because of schedule conflicts, 1 participant withdrew consent, and 1 participant moved abroad. For GPS3, a total of 85 participants were enrolled, and 84 completed the study (Fig 2, B). One participant withdrew because of a schedule conflict.

Participants were evenly distributed among the treatment groups for GPS2 (8x6Q2W, *n* = 30; 4x12Q4W, *n* = 32; 8x12Q2W, *n* = 31; and placebo, *n* = 29) and GPS3 (8x6Q2W, *n* = 19; 4x12Q4W, *n* = 21; 8x12Q2W, *n* = 22; and placebo, *n* = 23). Treatment groups were also generally well matched at baseline in terms of demographics (Table I). For GPS2, the 8x12Q2W group had fewer male subjects compared with the other treatment groups (32.3% vs 44.8% to 56.3%). The 4x12Q4W group in GPS3 had more male subjects compared with the other treatment groups (61.9% vs 27.3% to 36.8%).

Primary efficacy assessment

At the 1-year follow-up, approximately 18 months after treatment initiation, the 8x6Q2W group demonstrated a

TABLE I. Participants' demographics

Characteristics	GPS2				GPS3			
	8x6Q2W group (n = 30)	4x12Q4W group (n = 32)	8x12Q2W group (n = 31)	Placebo group (n = 29)	8x6Q2W group (n = 19)	4x12Q4W group (n = 21)	8x12Q2W group (n = 22)	Placebo group (n = 23)
Sex, no. (%)								
Male	14 (46.7)	18 (56.3)	10 (32.3)	13 (44.8)	7 (36.8)	13 (61.9)	6 (27.3)	8 (34.8)
Female	16 (53.3)	14 (43.8)	21 (67.7)	16 (55.2)	12 (63.2)	8 (38.1)	16 (72.7)	15 (65.2)
Race, no. (%)								
White	24 (80.0)	28 (87.5)	27 (87.1)	27 (93.1)	17 (89.5)	18 (85.7)	19 (86.4)	22 (95.7)
Ethnicity, no. (%)								
Hispanic or Latino	0 (0.0)	3 (9.4)	1 (3.2)	0 (0.0)	0 (0.0)	1 (4.8)	1 (4.5)	0 (0.0)
Not Hispanic or Latino	27 (90.0)	29 (90.6)	29 (93.5)	26 (89.7)	16 (84.2)	19 (90.5)	20 (90.9)	21 (91.3)
Not reported	1 (3.3)	0 (0.0)	1 (3.2)	0 (0.0)	1 (5.3)	1 (4.8)	1 (4.5)	0 (0.0)
Unknown	2 (6.7)	0 (0.0)	0 (0.0)	3 (10.3)	2 (10.5)	0 (0.0)	0 (0.0)	2 (8.7)
Age (y)								
Mean $\pm$ SD	36.9 $\pm$ 9.1	36.3 $\pm$ 12.2	39.5 $\pm$ 12.2	36.2 $\pm$ 9.9	40.7 $\pm$ 9.9	40.5 $\pm$ 12.9	43.9 $\pm$ 12.7	38.3 $\pm$ 9.9
Range	22-61	20-64	21-63	22-55	25-64	23-66	24-65	27-58
Height (cm)								
Mean $\pm$ SD	167.4 $\pm$ 9.3	169.6 $\pm$ 10.3	167.1 $\pm$ 8.6	168.5 $\pm$ 7.8	165.9 $\pm$ 8.8	174.7 $\pm$ 10.5	165.6 $\pm$ 7.6	168.4 $\pm$ 8.2
Range	152-185	142-188	158-186	154-187	152-178	157-197	152-184	154-184
Weight (kg)								
Mean $\pm$ SD	89.8 $\pm$ 20.6	83.4 $\pm$ 20.6	80.7 $\pm$ 18.8	83.8 $\pm$ 20.3	90.8 $\pm$ 21.8	93.5 $\pm$ 23.4	79.5 $\pm$ 19.6	78.7 $\pm$ 13.8
Range	50-150	47-132	50-125	52-157	50-150	61-138	50-125	52-105

TABLE II. Primary efficacy assessment: Change from baseline in TRSS on days 2 to 4 at all time points (GPS2, ITT population)

Population	No.	Mean	SD	Median	Difference between active treatment and placebo		
					LS means	95% CI	P value
Placebo	28	-3.6	5.1	-3.1			
Grass allergen peptides, 8x6Q2W	30	-6.0	4.2	-5.6	-2.3	-4.65 to 0.04	.0535
Grass allergen peptides, 4x12Q4W	31	-5.1	4.6	-4.3	-1.4	-3.77 to 0.88	.2202
Grass allergen peptides, 8x12Q2W	29	-3.2	4.1	-2.8	0.5	-1.83 to 2.91	.6521

Baseline TRSS was defined as matched average values at all time points on days 2 to 4 of the baseline challenge at GPS1. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

trending reduction in mean TRSS compared with the placebo group (-6.0 vs -3.6). The least squares (LS) mean treatment difference between the groups was -2.3 (95% CI, -4.65 to 0.04 ; $P = .0535$; [Table II](#)). Changes in mean TRSSs for this group were greater than in the placebo group across all time points over days 2 to 4 of the PTC, with the greatest change observed on day 4 ([Fig 3](#)). In contrast, the 4x12Q4W group showed a modest reduction in mean TRSS compared with the placebo group (LS mean treatment difference, -1.4 ; 95% CI, -3.77 to 0.88 ; $P = .2202$), with change being greater than in the placebo group across all but 1 time point (day 2, hour 3; [Fig 3](#)). The 8x12Q2W group showed a reduction in mean TRSS comparable with that in the placebo group (LS mean treatment difference, 0.5 ; 95% CI, -1.83 to 2.91 ; $P = .6521$; [Table II](#)).

At the 2-year follow-up, approximately 30 months after treatment initiation, a greater reduction in mean TRSS compared with the placebo group was observed for both the 8x6Q2W (LS mean treatment difference, -2.6 ; 95% CI, -5.72 to 0.62 ; $P = .1128$) and 4x12Q4W (LS mean treatment difference, -2.8 ; 95% CI, -5.98 to 0.30 ; $P = .0759$) treatment groups ([Table III](#)). Both dosing regimens also showed greater changes in mean TRSS versus placebo across hours 1 to 3 on days 2 to 4 of the PTC ([Fig 4](#)). Similar to the 1-year follow-up, reductions in

mean TRSS during the PTC were comparable between the 8x12Q2W and placebo groups (LS mean treatment difference, 1.2 ; 95% CI, -1.81 to 4.28 ; $P = .4218$; [Table III](#)).

Secondary efficacy assessments

Changes in mean TRSS in favor of treatment (but that were not statistically significant) were observed for both the 8x6Q2W (LS mean treatment difference, -2.0 ; 95% CI, -4.20 to 0.22 ; $P = .0767$) and 4x12Q4W (LS mean treatment difference, -0.9 ; 95% CI, -3.14 to 1.24 ; $P = .3935$) groups at year 1 when all time points over days 1 to 4 of the PTC were evaluated (see [Table E2](#) in this article's Online Repository at www.jacionline.org). Similar changes in mean TNSSs and TNNSSs were also observed over days 2 to 4 of the PTC at year 1 for the 8x6Q2W (TNSS, -2.4 vs -1.3 , $P = .0678$; TNNSS, -3.6 vs -2.3 , $P = .0889$) and 4x12Q4W (TNSS, -2.2 vs -1.3 , $P = .1520$; TNNSS, -2.9 vs -2.3 , $P = .3920$) groups. When participants with a baseline mean TRSS of 12 or greater were evaluated, reductions in mean TRSSs similar to those of the primary analysis were observed for both the 8x6Q2W (LS mean treatment difference, -2.4 ; 95% CI, -5.19 to 0.39 ; $P = .0904$) and 4x12Q4W (LS mean treatment difference, -1.7 ; 95% CI, -4.45 to 1.13 ; $P = .2402$) treatment groups compared with the placebo group (see

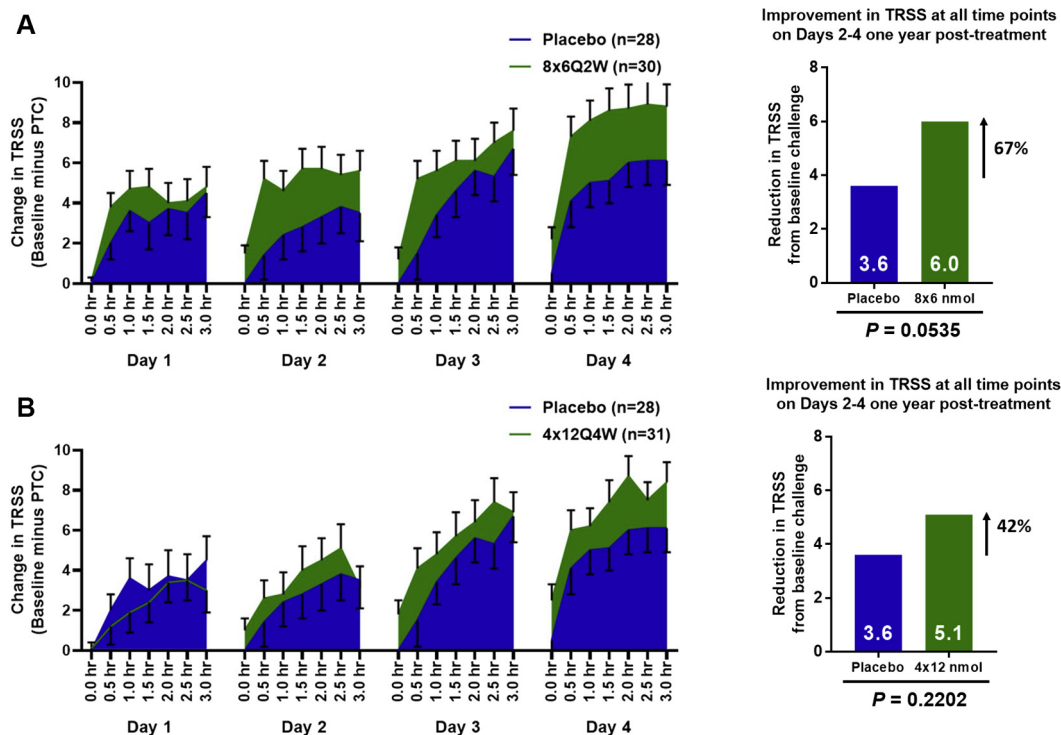


FIG 3. Improvement in TRSS after a second intervening GPS. The 8x6Q2W (**A**) and 4x12Q4W (**B**) groups demonstrated a trending and nonsignificant improvement in TRSSs, respectively, compared with the placebo group approximately 18 months after treatment initiation.

TABLE III. Primary efficacy assessment: Change from baseline in TRSS on days 2 to 4 at hours 1 to 3 (GPS3, ITT population)

Population	No.	Mean	SD	Median	Difference between active treatment and placebo		
					LS means	95% CI	P value
Placebo	23	-3.6	6.1	-1.9			
Grass allergen peptides, 8x6Q2W	19	-6.2	4.9	-5.6	-2.6	-5.72 to 0.62	.1128
Grass allergen peptides, 4x12Q4W	20	-6.4	5.0	-7.2	-2.8	-5.98 to 0.30	.0759
Grass allergen peptides, 8x12Q2W	22	-2.3	4.1	-2.5	1.2	-1.81 to 4.28	.4218

Baseline TRSS was defined as matched average values at hours 1 to 3 on days 2 to 4 of the baseline challenge at GPS1. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

Table E3 in this article's Online Repository at www.jacionline.org.

At year 2, reductions in mean TRSSs were observed for both the 8x6Q2W (days 1-4 at all time points, $P = .0740$; days 2-4 at all time points, $P = .0622$) and 4x12Q4W (days 1-4 at all time points, $P = .0673$; days 2-4 at all time points, $P = .0691$) groups and reflected the primary findings (see Tables E4 and E5 in this article's Online Repository at www.jacionline.org). A trending reduction in mean TRSS compared with placebo was observed in the 8x6Q2W group (-2.5 vs -1.2 , $P = .1030$). In contrast, a significant reduction in mean TRSS was observed for the 4x12Q4W group versus the placebo group (-3.0 vs -1.2 , $P = .0224$). Changes in TRSSs during the PTC were comparable between all 3 treatment groups and the placebo group (8x6Q2W, $P = .1874$; 4x12Q4W, $P = .2660$; and 8x12Q2W, $P = .2660$). In participants with a baseline mean TRSS of 12 or greater, significant reductions in mean TRSSs were only observed in the 4x12Q4W group versus the placebo group (LS mean treatment difference, -4.4 ; 95% CI, -7.97 to -0.87 ; $P = .0156$; see Table E6 in this

article's Online Repository at www.jacionline.org). Changes in mean TRSSs in favor of the treatment were observed in the 8x6Q2W group, but these did not reach statistical significance (LS mean treatment difference, -2.2 ; 95% CI, -5.68 to 1.34 ; $P = .2203$).

Exploratory and subgroup assessments

After a second and third intervening GPS, there were no significant changes in rye grass-specific IgA, IgE, and IgG₄ levels at follow-up compared with baseline in the treatment groups compared with the placebo group. An additional analysis of the primary efficacy end point in GPS3 was performed in a subgroup of participants who completed GPS1, GPS2, and GPS3. A total of 54 participants completed both follow-up studies (8x6Q2W, $n = 17$; 4x12Q2W, $n = 18$; placebo, $n = 19$). The 8x6Q2W group showed improvement in mean TRSSs compared with the placebo group across all 3 GPSs. The LS mean treatment differences were -2.9 ($P = .0754$) at 25 weeks, -5.0 ($P = .0035$) at 18 months,

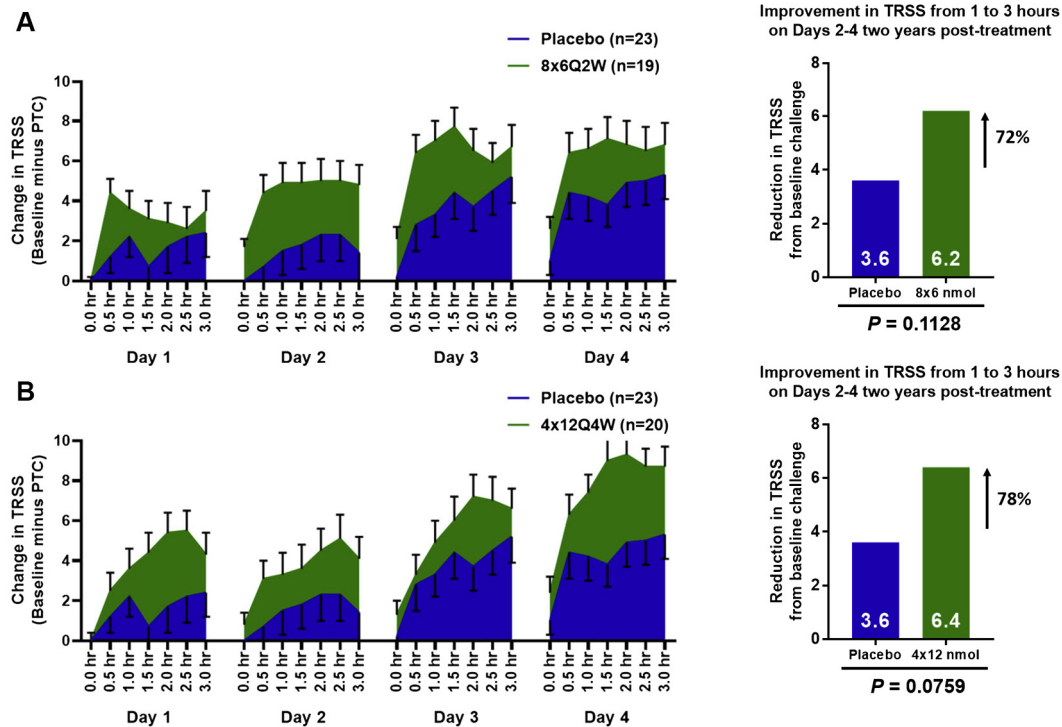


FIG 4. Improvement in TRSSs after a third intervening GPS. Both 8x6Q2W (A) and 4x12Q4W (B) groups demonstrated a trending improvement in TRSSs, respectively, compared with the placebo group approximately 30 months after treatment initiation.

TABLE IV. Summary of PTAEs and SAEs for GPS2 and GPS3 (safety population)

Characteristics	GPS2				GPS3			
	8x6Q2W group (n = 30)	4x12Q4W group (n = 32)	8x12Q2W group (n = 31)	Placebo group (n = 29)	8x6Q2W group (n = 19)	4x12Q4W group (n = 21)	8x12Q2W group (n = 22)	Placebo group (n = 23)
No. of PTAEs	38	19	44	22	8	8	8	8
Subjects with ≥ 1 PTAE	16 (53.3)	14 (43.8)	17 (54.8)	13 (44.8)	4 (21.1)	5 (23.8)	6 (27.3)	5 (21.7)
Subjects with PTAEs that led to withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with severe AEs	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with PTAEs related to study drug	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with severe PTAEs	3 (10.0)	4 (12.5)	5 (16.1)	3 (10.3)	1 (5.3)	0 (0.0)	1 (4.5)	2 (8.7)
Deaths	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

PTAEs were defined as AEs that first occurred or worsened in severity after informed consent in GPS2 and GPS3.

and -3.4 ($P = .0331$) at 30 months after the start of treatment (see Table E7 in this article's Online Repository at www.jacionline.org). Similar findings were observed for the 4x12Q4W group, with an LS mean treatment differences of -4.0 ($P = .0158$), -4.5 ($P = .0080$), and -4.1 ($P = .0101$) at 25 weeks and 18 and 30 months respectively (see Fig E1 in this article's Online Repository at www.jacionline.org).

Safety

For GPS2, a total of 123 posttreatment adverse events (PTAEs) were reported, with 60 participants experiencing 1 or more PTAEs (8x6Q2W, $n = 16$; 4x12Q4W, $n = 14$; 8x12Q2W, $n = 17$; placebo, $n = 13$; Table IV). The frequency of severe PTAEs was evenly distributed among the treatment groups (8x6Q2W, $n = 3$; 4x12Q4W, $n = 4$; 8x12Q2W, $n = 5$; placebo, $n = 3$)

and primarily involved nervous system disorders (eg, headaches). One participant in the 8x12Q2W group reported a severe AE (anaphylactic reaction secondary to a wasp sting) during the study. Of note, this severe AE occurred 14 months after the last dose and was deemed unrelated to treatment, with the participant making a full recovery. No PTAEs were considered related to treatment.

For GPS3, a total of 32 PTAEs were reported, with 20 participants experiencing 1 or more PTAEs (8x6Q2W, $n = 4$; 4x12Q4W, $n = 5$; 8x12Q2W, $n = 6$; placebo, $n = 5$; Table IV). Severe PTAEs were reported by 1 participant in the 8x6Q2W group, 1 participant in the 8x12Q2W group, and 2 participants in the placebo group. These AEs were single cases of pain in the extremities (both in the placebo group), migraine (8x6Q2W group), and excoriation (8x12Q2W group). All reported PTAEs were considered unrelated to treatment.

In both follow-up studies, no PTAEs led to study withdrawal. No participant reported a greater than 30% reduction in peak expiratory flow rate during the follow-up PTC compared with screening.

DISCUSSION

The current study reports findings from 2 follow-up studies that assessed the continued efficacy of grass allergen peptides after a second and third intervening GPS. Efficacy was evaluated during rye grass pollen challenges in the EEU after the peak of the GPS. The 8x6Q2W dosing regimen was associated with a reduction in grass-induced rhinoconjunctivitis symptoms compared with the placebo group, which trended to significance up to 2 years off treatment. Similar reductions in symptoms were also observed with the 4x12Q4W dosing regimen at the 2-year follow-up. These findings are comparable with those of previous phase 2 trials, which reported a continued treatment benefit up to 2 years after the start of treatment for SPIREs derived from HDM and cat allergens^{9,10,12,13} and up to 1 year after treatment initiation for peptides derived from birch pollen.^{22,23}

Consistent with the parent study, an apparent inverted dose-response threshold (based on cumulative exposure) was observed, with both 48-nmol regimens of grass allergen peptides outperforming the 96-nmol regimen at follow-up. Although previously reported immunotherapy peptide products have performed similarly,^{22,23} the reasons for these observations are not clear. A larger sample size would be required to compare clinical efficacy across different dosing regimens.

A numerically greater improvement in mean TRSS over placebo was observed for the 4x12Q4W group compared with the 8x6Q2W group (−2.8 vs −2.6) at year 2. This contrasts with results from year 1 (−1.4 vs −2.3) and the parent study (−0.9 vs −1.6), which demonstrated the opposite finding.¹⁷ Alongside the lower sample size, this finding might also be attributable to the significant improvement in mean TRSS in the subgroup of participants with severe baseline symptoms (TRSS ≥ 12). Because this subgroup represents 65% of the 4x12Q4W ITT population in GPS3, it is likely that these participants were driving the overall analysis.

Although improvements in mean TRSS were numerically greater in the 8x6Q2W group compared with the placebo group during both follow-up assessments, they only trended toward significance. Compared with the parent study, this is likely attributable to the loss of power caused by the small number of participants in the 2 follow-up studies. The 1- and 2-year follow-ups were not powered *a priori* and were reliant on the number of subjects prepared to return for additional EEU sessions, which are time consuming and potentially uncomfortable for the subjects. Although all eligible participants were invited to attend both follow-up studies, most who declined the invitation did so for logistical reasons (ie, schedule conflicts, had been started on another immunotherapy regimen, or had moved out of the region).

A possible mechanism underlying the successful desensitization after allergen immunotherapy involves induction of allergen-specific IgG₄ (sIgG₄) blocking antibodies. Conventional immunotherapy is associated with an increase in sIgG₄ levels, with symptomatic improvement moderately correlated with the functionality of sIgG₄.^{24–29} However, sIgG₄ levels remained unchanged after grass allergen peptide treatment at both the 1- and 2-year follow-ups. This is likely due to the short length of the peptides (<20 amino acids) and lack of IgE binding.³⁰

These findings have been observed in previous SPIRE products.³¹ In a recent phase 2b trial, birch-derived peptides of 49 to 71 amino acids in length induced robust increases in Bet v 1–sIgG₄ levels after 2 intervening birch pollen seasons, thereby illustrating the effect of peptide length on antibody production.²³ Although the precise mechanism of action of SPIREs is unclear, previous studies show it to be dependent on T_H1/regulatory T cell-orientated immune responses and a shift away from allergen-specific effector T_H2 cells and T_H2 cytokine production.^{32–35}

Conventional subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) often must be continued for at least 3 years to achieve persistent treatment effects beyond the year of their initial administration.^{36–41} The prolonged treatment regimens are burdensome and inconvenient for patients.⁴² A retrospective analysis of real-life compliance to conventional immunotherapy in 6486 patients showed that 20% of SCIT users and 62% of SLIT users discontinued their treatment 1 year after treatment initiation.⁴³ Another retrospective study cited injection frequency and inconvenience as the main reasons for stopping treatment among SCIT and SLIT users, respectively.⁴⁴ Although results from the current study showed a trending improvement in symptoms over placebo 2 years after treatment initiation, immunotherapy with SPIREs might represent an alternative option toward tolerance, requiring fewer injections than SCIT. Fully powered studies will be required to evaluate this potential further.

IgE-mediated adverse reactions during treatment can lead to discontinuations of both SCIT and SLIT. Particularly for SCIT, systemic reactions (eg, anaphylaxis) often occur during the up-dosing phase, during which increasing doses of allergen are administered over a short time period until the maintenance dose is achieved.^{45–47} Immunotherapy regimens using peptides lack an up-dosing phase, possibly requiring fewer injections to achieve tolerance. Treatment with grass allergen peptides was well tolerated in the parent and follow-up studies, with no clinically significant reductions in pulmonary function demonstrating the absence of late-onset safety signals with this immunotherapy.

In summary, the 2 follow-up studies presented here provide preliminary evidence demonstrating the persistent efficacy and safety of grass allergen peptides after 3 intervening GPSs. Four or 8 intradermal injections of grass allergen peptides given 2 to 4 weeks apart led to a numeric reduction in grass-induced ARC symptoms, as evaluated in the EEU, lasting up to 2 years after treatment cessation when compared with the placebo group. Future studies are required to determine whether a similar treatment effect using a 14-week allergen immunotherapy regimen with SPIREs can be observed in larger participant groups and in the field.

Clinical implications: A short 14-week treatment regimen of grass allergen peptides alleviated ARC symptoms for up to 2 years.

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METHODS

Primary efficacy end point

GPS2. The primary efficacy end point was the change from GPS1 baseline challenge in mean TRSS at all time points on days 2 to 4 of the follow-up PTC in the grass allergen peptide treatment groups compared with the placebo group.

GPS3. The primary efficacy end point was change from GPS1 baseline challenge in mean TRSS on days 2 to 4 at the 1- to 3-hour time points of the follow-up PTC in grass allergen peptide treatment groups compared with the placebo group.

Secondary efficacy end points

- Change from GPS1 baseline challenge in mean TRSS using all time points on days 2 to 4 of GPS3 follow-up PTCs in grass allergen peptide treatment groups compared with the placebo group.
- Change from GPS1 baseline challenge in mean TRSS using all time points on days 1 to 4 of GPS1, GPS2, and GPS3 follow-up PTCs in the grass allergen peptide treatment groups compared with the placebo group.
- Change from GPS1 baseline challenge in mean TNSS at all time points on days 2 to 4 of the GPS1, GPS2, and GPS3 follow-up PTCs in the grass allergen peptide treatment groups compared with the placebo group.
- Change from GPS1 baseline challenge in mean TNNSS at all time points on days 2 to 4 of the GPS1, GPS2, and GPS3 follow-up PTCs in the grass allergen peptide treatment groups compared with the placebo group.
- Change from GPS1 baseline challenge in mean TRSS using all time points on days 2 to 4 of the GPS1, GPS2, and 1- to 3-hour time points of the GPS3 follow-up PTCs in the grass allergen peptide treatment groups compared with the placebo group for subjects whose GPS1 baseline TRSS was 12 or greater.

Exploratory efficacy end points

- Mean change from baseline in concentrations of rye grass-specific IgA, IgE, and IgG₄ at follow-up.

Change to the conduct of the studies

There were no protocol amendments and no changes to the conduct of GPS2.

For GPS3, although included as a safety parameter in the protocol synopsis, use of a visual analogue scale for assessment of breathlessness was not otherwise specified in the protocol or statistical analysis plan (SAP), and these data were not collected. Breathlessness was instead scored using a 4-point scale.

Changes to planned analyses

There were no protocol amendments and no changes to the planned analysis of GPS2.

For GPS3, the following changes from the methods/statistical analyses outlined in the protocol were specified in the final SAP, which was finalized before database lock:

- An additional analysis to support the primary efficacy end point was included whereby the change in mean TRSS across the 1- to 3-hour time points recorded on days 2 to 4 was to be measured from GPS1 baseline to GPS1 and GPS2 follow-up PTCs for those subjects who had participated in all of these evaluations. This extra analysis enabled a comparison to be made between the findings in the current study and the findings in the same cohort of subjects in previous years.
- Urine dipstick results specified in the SAP in error were not collected in this study.
- Breathlessness was included as an efficacy assessment in the protocol in error and was subsequently included as a safety assessment in the SAP.

The following changes were made to analyses outlined in the protocol and SAP:

- Change in mean TRSS across the 1- to 3-hour time points recorded on days 2 to 4 from GPS1 baseline to GPS1 and GPS2 and GPS3 PTCs was not performed for the ITT or per-protocol populations, as originally outlined. Instead, this analysis was performed by using an ITT additional subpopulation of subjects who were enrolled in GPS1, GPS2, and GPS3.
- The LS mean difference and *P* value for the change from GPS1 baseline challenge in mean TRSS (for subjects whose GPS3 baseline TRSS was ≥ 12), TNSS, and TNNSS relative to placebo treatment was not analyzed across days 2 to 4 at all time points, as originally outlined. It was determined that these analyses would not meaningfully contribute to interpretation of the study.
- A repeated-measures analysis of matched mean change in TRSS from GPS1 baseline to GPS1 and GPS3 follow-up PTCs over days 1 to 4 at all time points was performed for all subjects. This analysis enabled differences in response across the various study time points to be determined.
- It was specified in the SAP that nonparametric procedures were to be used for analysis of changes in TRSSs, immunoglobulin levels (grass specific IgA, IgE, and IgG₄), and skin prick test responses if ANCOVA assumptions were not met; however, this was not required.

In addition, grass-specific IgA, IgE, and IgG₄ levels and skin prick tests responses were included as efficacy assessments in the SAP in error; these were collected as pharmacodynamic assessments.

Method of assigning subjects to study groups

Subjects were assigned to the same treatment groups as in GPS1. In GPS2 and GPS3 subjects retained their subject numbers from GPS1, such that the appropriate treatment regimens from GPS1 could be linked to this study. None of the subjects were told of their treatment allocation in GPS1. No treatment beyond that received in GPS1 was given.

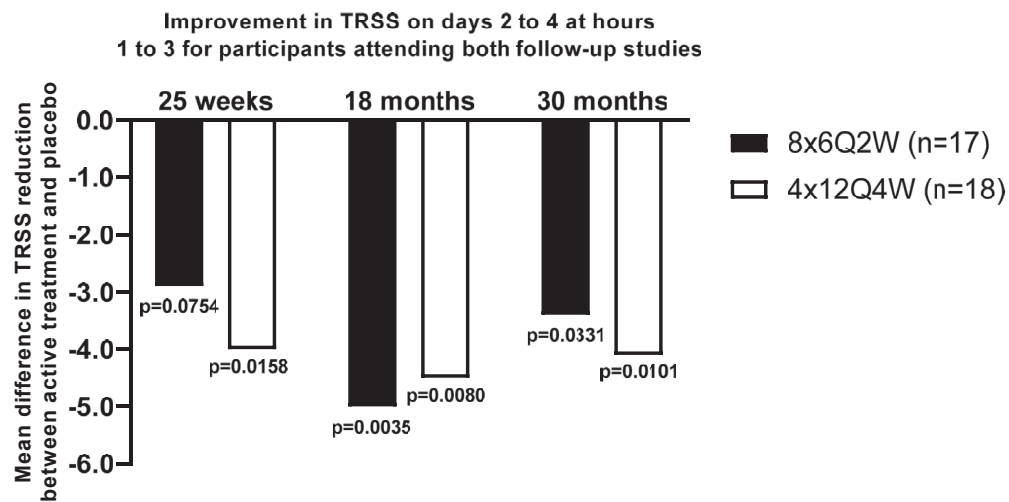


FIG E1. Persistent improvement in TRSSs after 3 consecutive GPSs. In both the 8x6Q2W and 4x12Q4W groups, participants attending the parent study and both 1- and 2-year follow-up studies demonstrated an improvement in TRSS compared with the placebo group.

TABLE E1. Inclusion and exclusion criteria

Inclusion criteria

Subjects who fulfilled the following criteria were eligible for enrollment into the study:

- previously randomized into GPS1 and completed all treatment visits and PTCs during calendar year 2012;
- mean TRSS at baseline challenge in GPS1 of ≥ 8 (calculated as the mean of all TRSS time points on day 1 to 4 of the baseline EEU visit);
- willing and able to provide written informed consent; and
- willing and able to comply with the study requirements.

If the subject was of childbearing potential, she had to practice an acceptable form of birth control and produce a negative urine pregnancy test result. A female subject could be included without a negative urine pregnancy test result if she could document that she was surgically sterile or at least 2 years postmenopausal.

Exclusion criteria

Subjects were excluded from the study based on the following criteria:

- were informed of the treatment received in GPS1;
- had grass pollen-induced asthma since the start of their participation in GPS1;
- currently had asthma symptoms that were either uncontrolled or required Global Initiative for Asthma step 2 or higher treatment (Note: A diagnosis of isolated exercise-induced bronchospasm or controlled asthma not triggered by grass pollen and controlled by GINA step 1 treatment did not constitute an exclusion criterion.);
- had $FEV_1 < 80\%$ of predicted value;
- received allergen immunotherapy since completing participation in GPS1 (other than treatment in GPS1);
- had contraindications for epinephrine administration (eg, subjects with acute or chronic symptomatic coronary heart disease or severe hypertension);
- had a history of significant recurrent acute sinusitis defined as 2 episodes per year for the last 2 years, all of which required antibiotic treatment;
- had a history of chronic sinusitis defined as sinus symptoms lasting > 12 weeks that included ≥ 2 major factors or 1 major factor and 2 minor factors (Major factors were defined as facial pain or pressure, nasal obstruction or blockage, nasal discharge or purulence or discolored postnasal discharge, purulence in nasal cavity, or impaired or loss of smell. Minor factors were defined as headache, fever, halitosis, fatigue, dental pain, cough, and ear pain, pressure, or fullness.);
- had symptoms of a clinically relevant illness that in the investigator's opinion would have made the subject's participation in the study unsafe or influenced the collection and/or outcome of efficacy data within 6 weeks before the screening visit;
- were pregnant, lactating, or planning a pregnancy during the study;
- had any clinically relevant abnormalities detected on physical examination;
- had vital signs (blood pressure, pulse rate, respiratory rate, and body temperature) that were outside normal limits unless the abnormality was considered not to be of clinical relevance by the investigator;
- received treatment with an investigational drug within 30 days before the screening visit or were currently participating in another clinical study; and
- had a significant disease, disorder, or inability to communicate since completing GPS1, which might have either put the subject at risk because of participation in the study or influenced the results of the study or the subject's ability to participate in the study.

GINA, Global Initiative for Asthma.

TABLE E2. Secondary efficacy assessment: Change from baseline in TRSS on days 1 to 4 at all time points (GPS2, ITT population)

Population	No.	Mean	SD	Median	Difference between active treatment and placebo		
					LS means	95% CI	P value
Placebo	28	−3.4	4.9	−2.6			
Grass allergen peptides, 8x6Q2W	30	−5.4	3.9	−5.2	−2.0	−4.20 to 0.22	.0767
Grass allergen peptides, 4x12Q4W	31	−4.4	4.4	−4.2	−0.9	−3.14 to 1.24	.3935
Grass allergen peptides, 8x12Q2W	29	−2.6	3.8	−3.4	0.9	−1.32 to 3.13	.4233

Baseline TRSS was defined as matched average values at all time points on days 1 to 4 of the baseline challenge at GPS1. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

TABLE E3. Subgroup analysis: Change from baseline in TRSS on days 2 to 4 at all time points for participants with a mean baseline TRSS of 12 or greater (GPS2, ITT population)

Population	No.	Difference between active treatment and placebo		
		LS means	95% CI	P value
Placebo	20			
Grass allergen peptides, 8x6Q2W	25	−2.4	−5.19 to 0.39	.0904
Grass allergen peptides, 4x12Q4W	25	−1.7	−4.45 to 1.13	.2402
Grass allergen peptides, 8x12Q2W	25	0.4	−2.34 to 3.23	.7513

Baseline TRSS was defined as matched average values at all time points on days 2 to 4 of the baseline challenge at GPS1. The subgroup population included participants with an unmatched (ie, all available data were used) mean baseline TRSS of 12 or greater. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

TABLE E4. Secondary efficacy assessment: Change from baseline in TRSS on days 1 to 4 at all time points (GPS3, ITT population)

Population	No.	Mean	SD	Median	Difference between active treatment and placebo		
					LS means	95% CI	P value
Placebo	23	−2.6	4.6	−1.5			
Grass allergen peptides, 8x6Q2W	19	−4.8	3.8	−4.7	−2.2	−4.67 to 0.22	.0740
Grass allergen peptides, 4x12Q4W	21	−4.7	4.1	−5.4	−2.2	−4.63 to 0.16	.0673
Grass allergen peptides, 8x12Q2W	22	−1.5	3.1	−1.8	1.0	−1.30 to 3.40	.3784

Baseline TRSS was defined as matched average values at all time points on days 1 to 4 of the baseline challenge at GPS1. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

TABLE E5. Secondary efficacy assessment: Change from baseline in TRSS on days 2 to 4 at all time points (GPS3, ITT population)

Population	No.	Mean	SD	Median	Difference between active treatment and placebo		
					LS means	95% CI	P value
Placebo	23	−3.0	5.3	−1.7			
Grass allergen peptides, 8x6Q2W	19	−5.5	4.2	−5.6	−2.5	−5.22 to 0.13	.0622
Grass allergen peptides, 4x12Q4W	20	−5.4	4.2	−6.0	−2.5	−5.10 to 0.20	.0691
Grass allergen peptides, 8x12Q2W	22	−2.0	3.3	−2.2	1.0	−1.61 to 3.53	.4605

Baseline TRSS was defined as matched average values at all time points on days 2 to 4 of the baseline challenge at GPS1. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

TABLE E6. Subgroup analysis: Change from baseline in TRSS on days 2 to 4 at hours 1 to 3 for participants with a mean baseline TRSS of 12 or greater (GPS3, ITT population)

Population	No.	Difference between active treatment and placebo		
		LS means	95% CI	<i>P</i> value
Placebo	16			
Grass allergen peptides, 8x6Q2W	14	−2.2	−5.68 to 1.34	.2203
Grass allergen peptides, 4x12Q4W	13	−4.4	−7.97 to −0.87	.0156
Grass allergen peptides, 8x12Q2W	15	−2.2	−5.69 to 1.21	.1990

Baseline TRSS was defined as matched average values at hours 1 to 3 on days 2 to 4 of the baseline challenge at GPS1. The subgroup population included participants with an unmatched (ie, all available data were used) mean baseline TRSS of 12 or greater. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

TABLE E7. Subgroup analysis: Change from baseline in TRSS on days 2 to 4 at hours 1 to 3 for participants enrolled in the original trial and both follow-up studies (ITT population)

Population	No.	LS mean difference between active treatment and placebo					
		25 wk*	<i>P</i> value	18 mo*	<i>P</i> value	30 mo*	<i>P</i> value
Grass allergen peptides, 8x6Q2W	17	−2.9	.0754	−5.0	.0035	−3.4	.0331
Grass allergen peptides, 4x12Q4W	18	−4.0	.0158	−4.5	.0080	−4.1	.0101

Baseline TRSS was defined as matched average values at hours 1 to 3 on days 2 to 4 of the baseline challenge at GPS1. Results were obtained by using an ANCOVA model containing terms for fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values.

*Time after first dose of grass allergen peptides.