

Treatment with grass allergen peptides improves symptoms of grass pollen-induced allergic rhinoconjunctivitis

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Background: Synthetic peptide immunoregulatory epitopes are a new class of immunotherapy to treat allergic rhinoconjunctivitis (ARC). Grass allergen peptides, comprising 7 synthetic T-cell epitopes derived from Cyn d 1, Lol p 5, Dac g 5, Hol l 5, and Phl p 5, is investigated for treatment of grass pollen-induced ARC.

Objective: We sought to evaluate the efficacy, safety, and tolerability of intradermally administered grass allergen peptides.

Methods: A multicenter, randomized, double-blind, placebo-controlled study evaluated 3 regimens of grass allergen peptides versus placebo in patients with grass pollen-induced allergy (18–65 years). After a 4-day baseline challenge to rye grass in the environmental exposure unit (EEU), subjects were randomized to receive grass allergen peptides at 6 nmol at 2-week intervals for a total of 8 doses (8x6Q2W), grass allergen peptides at 12 nmol at 4-week intervals for a total of 4 doses (4x12Q4W), or

grass allergen peptides at 12 nmol at 2-week intervals for a total of 8 doses (8x12Q2W) or placebo and treated before the grass pollen season. The primary efficacy end point was change from baseline in total rhinoconjunctivitis symptom score across days 2 to 4 of a 4-day posttreatment challenge (PTC) in the EEU after the grass pollen season. Secondary efficacy end points and safety were also assessed.

Results: Two hundred eighty-two subjects were randomized. Significantly greater improvement (reduction of total rhinoconjunctivitis symptom score from baseline to PTC) occurred across days 2 to 4 with grass allergen peptide 8x6Q2W versus placebo (−5.4 vs −3.8, respectively; $P = .0346$). Greater improvement at PTC also occurred for grass allergen peptide 8x6Q2W versus placebo ($P = .0403$) in patients with more symptomatic ARC. No safety signals were detected.

Conclusion: Grass allergen peptide 8x6Q2W significantly improved ARC symptoms after rye grass allergen challenge in an EEU with an acceptable safety profile. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

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

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Allergic rhinoconjunctivitis (ARC) is common and costly and has a negative effect on well-being and quality of life. In the third United States National Health and Nutrition Examination Survey (NHANES III; 1988–1994), 16% of respondents aged 6 to 59 years reported allergy-related nasal symptoms alone, 6% reported ocular symptoms alone, and 30% reported both nasal and ocular symptoms.¹ Pollen was the most common environmental trigger for combined nasal and ocular symptoms (60%); pollen-induced nasal symptoms (42%) and pollen-induced ocular symptoms (44%) occurred with similar frequency.¹

Fifty-four percent of the US population participating in NHANES III had a positive skin test result for at least 1 of 10 indoor or outdoor allergens; this included 27% with a positive skin test result for perennial rye grass.² NHANES 2005–2006 found that 45% of participants had positive serum IgE test results for at least 1 of 19 allergens.³ Positive IgE-specific test results for rye grass, ragweed, and tree pollens occurred in 20%, 16%, and 21% of NHANES respondents, respectively. For rye grass pollen sensitivity in the United States, NHANES respondents had the highest prevalence of positive IgE test results in the west (25%). The next highest prevalence of positive IgE test results for rye grass occurred in the south (20%), followed by the northeast (17%), and the Midwest (14%).³

Abbreviations used

AE:	Adverse events
AIT:	Allergen immunotherapy
ARC:	Allergic rhinoconjunctivitis
BC:	Baseline challenge
EEU:	Environmental exposure unit
mITT:	Modified intent-to-treat
NHANES:	National Health and Nutrition Examination Survey
4x12Q4W:	Grass allergen peptides at 12 nmol at 4-week intervals for a total of 4 doses
8x6Q2W:	Grass allergen peptides at 6 nmol at 2-week intervals for a total of 8 doses
8x12Q2W:	Grass allergen peptides at 12 nmol at 4-week intervals for a total of 4 doses
PEFR:	Peak expiratory flow rate
PNIF:	Postnasal inspiratory flow
PTC:	Posttreatment challenge
SCIT:	Subcutaneous immunotherapy
SLIT:	Sublingual immunotherapy
SPIRE:	Synthetic peptide immunoregulatory epitope
SPT:	Skin prick test
TEAE:	Treatment-emergent adverse event
TNSS:	Total nasal symptom score
TNNSS:	Total nonnasal symptom score
TRSS:	Total rhinoconjunctivitis symptom score

For patients with ARC, a significant reduction in ARC symptom scores is evident for several products for specific allergen immunotherapy (AIT) administered subcutaneously and with sublingual tablets.⁴ In addition, immunotherapy can reduce asthma development.⁵⁻¹⁰ Existing evidence also suggests that AIT can prevent the acquisition of new allergic sensitizations.^{11,12} Allergists have used AIT to treat allergies for more than 100 years.¹³ Subcutaneous immunotherapy (SCIT) was the first available mode of this treatment; sublingual immunotherapy (SLIT) tablets and drops have become available more recently in Europe, the United States, and Canada.¹⁴⁻¹⁶ Systemic allergic reactions are a risk for SCIT and SLIT because intact allergens in these treatments can cross-link IgE molecules on the surfaces of mast cells and basophils, causing activation and degranulation and resulting in systemic allergic reactions. Other limitations of SCIT and SLIT include marked local side effects (eg, injection-site swelling with SCIT, which can be prolonged, impressive, and dose limiting, and oral pruritus with SLIT),^{15,17-19} the risk of systemic allergic reactions (higher with SCIT than with SLIT¹⁶) along with the need to provide patients with “rescue” epinephrine in the United States, lengthy (≥3 years) treatment necessary to achieve disease modification,^{20,21} and the cost of long-term treatment.²²⁻²⁴ Systematic reviews and meta-analyses show grass SLIT and SCIT to have a modest treatment benefit for symptom and medication score reduction in patients with seasonal ARC.^{25,26}

Concerns in the AIT field include high levels of nonadherence (in one study 23% of patients continued SCIT and 7% of patients continued SLIT for a minimum period of 3 years)²⁷ and the belief that these forms of AIT are either too costly, too risky, too inconvenient, or ineffective.²¹ A new class of synthetic peptide immunoregulatory epitope (SPIRE),²⁸ which has targeted immunoregulatory properties and the potential to transform allergy treatment, is being developed to address issues of efficacy, safety, and adherence. Possible mechanisms for allergen SPIRE

therapy include induction of specific T-cell anergy, differentiation of naive T cells to regulatory T cells, immune deviation (ie, decreased T_H2/T_H1 ratio), and allergen-specific T_H2 cell deletion.²⁸⁻³⁰ Cyn d 1, Lol p 5, Dac g 5, Hol 1 5, and Phl p 5 grass allergen peptides, members of the SPIRE class, have been designed as a short course of treatment with reduced potential to cross-link IgE on the surface of mast cells and basophils. The allergenic molecules of grass pollens have been classified into 13 distinct groups based on their structure and biologic and immunologic properties. However, it has been demonstrated that major contributors to allergic sensitization and hence induction of symptoms are the allergens belonging to groups 1 and 5, both of which are contained in grass allergen peptides.³¹⁻³⁴ Group 1 allergens are recognized by approximately 95% of grass pollen-sensitive patients, followed by group 5 allergens, which are recognized by up to 85% of these patients.³²

Clinical development programs for antiallergic medications and AIT have included studies performed in environmental exposure units (EEUs)/allergen challenge chambers,³⁵⁻³⁹ which have preceded clinical trials in uncontrolled outdoor and indoor settings in which exposure to allergens (eg, pollens, animal salivary proteins, or glandular secretions) occurs naturally. Advantages of this model include control over all environmental variables, consistent allergen delivery, and the ability to conduct studies outside a specified pollen season.⁴⁰

The EEU used in the current grass allergen peptide study is located in Kingston General Hospital, Ontario, Canada, and allows for the reliable exposure of up to 140 participants to predetermined levels of allergen, which remain consistent regardless of outside weather conditions, to determine the efficacy and onset of action of new therapies for ARC.³⁶ The EEU setup, including location of chairs, feeder, fans, and Rotorod (IMS Health, Plymouth Meeting, Pa) sampling equipment, is illustrated in Fig E1 in this article's Online Repository at www.jacionline.org.

Grass allergen peptide, a SPIRE for grass allergy, is a combination of 7 T-cell epitope-based peptides (each of 10-18 amino acids) derived from major grass allergens.^{41,42} The purpose of the present study was to evaluate the efficacy, safety, and tolerability of 3 regimens of grass allergen peptides in subjects allergic to grass pollen after challenge with rye grass allergen in an EEU.

METHODS

Peptides

Grass allergen peptides contain 7 peptides, 3 conserved in group 1 allergens and 4 conserved in group 5 allergens across a range of different grasses, including rye, timothy, Kentucky bluegrass, orchard, velvet, canary, and Bermuda grasses. The individual peptide sequences were as follows: SGKAFGAMAKKGQED, FIPMKSSWGA, KSSWGAIWRID PKKPLK, KYDAYVATLLEALR, LKKAVTAMSEAEK, KKIPAGELQIIDKIDA, and KPEVKYAVFEAALTAKIT. The peptides were synthesized by Bachem (Bubendorf, Switzerland), according to current Good Manufacturing Practice; formulated, filled, and finished by Nova Laboratories (Leicester, United Kingdom), also according to current Good Manufacturing Practice; tested at Gen-Probe (Livingston, United Kingdom); and released in accordance with the EU Clinical Trials Directive.

Study design

This was a multicenter, randomized, double-blind, placebo-controlled, dose-ranging, parallel-group phase 2 dose-finding study to evaluate the

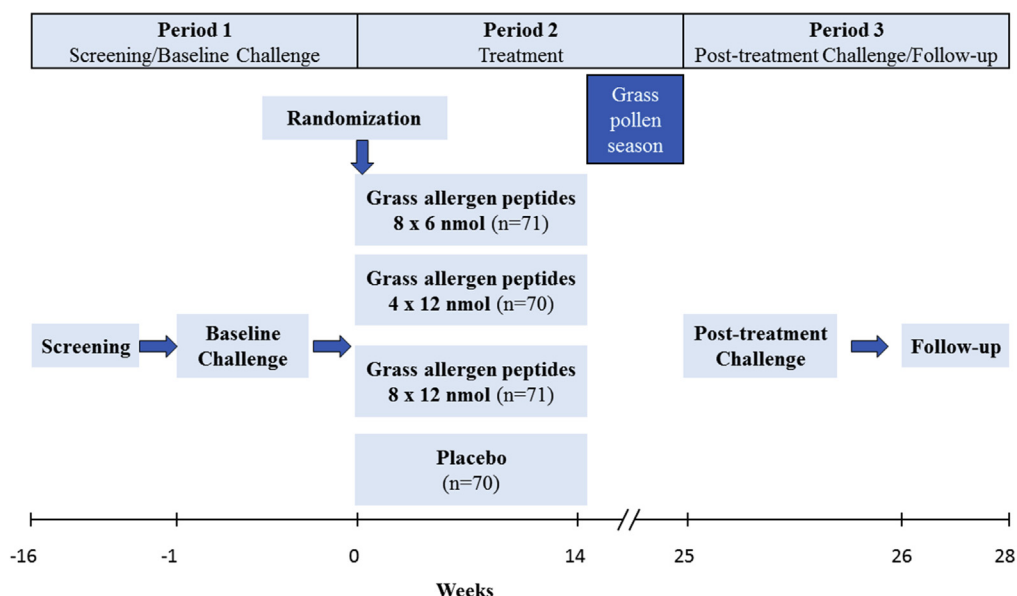


FIG 1. Overall study design.

efficacy, safety, and tolerability of 3 regimens of grass allergen peptides versus placebo in patients with a documented history of allergy to grass. The Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board granted written clearance for the study. The Research Ethics Board–approved informed consent form was signed by each subject before any study-related procedures were conducted. The study was conducted according to the principles of the Declaration of Helsinki, the International Conference on Harmonisation guidelines on Good Clinical Practice, and all relevant local regulatory requirements. The Clinicaltrials.gov identifier is NCT01385800.

A central EEU was used for baseline challenge (BC) and posttreatment challenge (PTC), with subjects recruited and treated at several sites. There were 3 study periods: (1) screening (≤ 16 weeks before randomization) and BC at 4 EEU visits on successive days over the period from November through February to avoid grass, ragweed, and tree pollen seasons; (2) treatment (8 visits that were 14 days apart over 14 weeks), which was completed before the start of the grass pollen season; and (3) PTC and follow-up (approximately 25 weeks after the first treatment administration), when the peak grass pollen season was over but the ragweed season had not yet begun (Fig 1).

Study subjects

All study sites were located in Canada: site 1A, Kingston General Hospital, Ontario; site 1B, Kanata, Ontario; site 1C, Oshawa, Ontario; and site 1D, Alpha Medical Research, Mississauga, Ontario. The study had 2 rounds of recruitment and treatment ahead of the 2012 and 2013 grass pollen seasons. See the [Methods](#) section in this article's Online Repository at www.jacionline.org (Protocol Amendment 03) for additional details. The first subject was enrolled in November 2011, and the last completed the study in July 2013.

Included were male or female subjects 18 to 65 years of age with a minimum 2-year documented history of ARC on exposure to grass, a positive skin prick test (SPT) response to rye grass pollen allergen (wheal diameter ≥ 3 mm larger than that elicited by the negative control), and a minimum qualifying score on at least 1 time point on days 3 and 4 of BC in the EEU for total rhinoconjunctivitis symptom score (TRSS; 10/24) and total nasal symptom score (TNSS; 6/12). All included subjects provided written informed consent and ensured their compliance with study requirements, and women of childbearing potential used an acceptable method of birth control throughout the study.

Excluded were subjects with a history of grass pollen–induced asthma (exercise-induced bronchospasm and controlled asthma not triggered by grass

pollen were acceptable), a history of anaphylaxis to grass pollen allergen, FEV₁ of less than 80% of predicted value, AIT in the last 12 months or grass AIT in the last 10 years, pregnancy (current or planned), and a history of recurrent acute sinusitis or chronic sinusitis; subjects in whom epinephrine was contraindicated were also excluded (see [Table E1](#) in this article's Online Repository at www.jacionline.org for additional exclusion criteria).

Study treatments

Subjects were randomized in a ratio of 1:1:1:1 to receive one of 3 regimens of grass allergen peptides or placebo administered intradermally: (1) grass allergen peptides at 6 nmol at 2-week intervals for a total of 8 doses (8x6Q2W); (2) grass allergen peptides at 12 nmol at 2-week intervals for a total of 8 doses (8x12Q2W); (3) grass allergen peptides at 12 nmol at 4-week intervals for a total of 4 doses (4x12Q4W; in this group placebo alternated with grass allergen peptides at 2-week intervals for a total of 8 doses to maintain blinding); and (4) placebo at 2-week intervals for a total of 8 doses (see [Table E2](#) in this article's Online Repository at www.jacionline.org for additional details). All treatments were administered over a 14-week period.

Study assessments

During the BC and PTC, rye grass allergen was distributed into the EEU by dispersion equipment to achieve consistent levels (mean hourly range of 3500 ± 500 grains/m³). Subjects spent 3 hours in the EEU exposed to rye grass allergen on 4 successive days during the BC and PTC. Four nasal and 4 nonnasal symptoms were assessed, each on a 4-point scale from 0 (none) to 3 (severe) every 30 minutes during rye grass exposure. Breathlessness was assessed the same way. In addition, peak nasal inspiratory flow (PNIF), peak expiratory flow rate (PEFR), and vital signs were measured. Blood samples were taken to measure rye grass–specific IgA, IgE, and IgG₄ before the start of treatment and at the follow-up visit; changes to concomitant medication and adverse events (AEs) were also recorded throughout the study. During the treatment period, subjects attended the research site for 8 treatment visits 14 days (± 2 days) apart, finishing before the start of the natural grass pollen season. The following assessments were carried out before and 1 hour after dosing: visual analog scale measuring both breathlessness and nasal symptoms, examination of injection site, PEFR, AEs, and vital signs. Women of childbearing potential were required to have a negative urine pregnancy test result before dosing was permitted. The PTC and follow-up occurred approximately 25 weeks after the first treatment administration,

when the peak grass pollen season was over but the ragweed season had not yet begun. See the [Methods](#) section and [Table E3](#) in this article's Online Repository at www.jacionline.org for additional details on study assessments.

Efficacy end points

The study's primary efficacy end point was the change from BC in mean TRSSs at all time points on days 2 to 4 of PTC for grass allergen peptide treatment groups versus placebo in the modified intent-to-treat (mITT) population, which included all subjects who had a mean TRSS of 8 or greater at all time points on all days of the BC and completed at least 1 PTC assessment in the EEU. Secondary efficacy end points were the TRSS change on all 4 days of PTC, the TNSS change on days 2 to 4 of PTC, the total nonnasal symptom score (TNNSS) change on days 2 to 4 of PTC, the TRSS change on days 2 to 4 of PTC for subjects with baseline TRSS of 12 or greater, and the TRSS change on days 2 to 4 of PTC for all subjects. Exploratory efficacy end points were changes at follow-up in the SPT response, PNIF, and rye grass-specific IgA, IgE, and IgG₄ levels. The [Methods](#) section in this article's Online Repository provides additional details on the secondary and exploratory efficacy variables.

Safety measurements

AEs were recorded in a diary throughout the study. Treatment-emergent adverse events (TEAEs) are defined as AEs that first occurred or worsened in severity after initiation of treatment administration. Physical examinations were performed at screening and follow-up; blood pressure, pulse, temperature, and respiratory rate were recorded at all visits. Blood samples for hematology and serum biochemistry, as well as urine samples, were obtained at screening and follow-up. Blood samples for total IgE and tryptase levels were obtained at screening, and further samples for tryptase measurement were to be collected in the event of a severe allergic reaction.

FEV₁ was measured at screening and follow-up; PEFR was measured at BC, treatment visits, and PTC before and 3 hours after allergen exposure in the EEU. Subjective (visual analog scale) scores of breathlessness and nasal symptoms were collected at treatment visits immediately before dosing and 1 hour after dosing. Local reactions at the injection site were evaluated at treatment visits 1 hour after dosing.

Statistical analysis

The primary efficacy end point and all secondary efficacy analyses were based on the mITT population, who had a mean TRSS of 8 or greater at all time points on all days of the BC and completed at least 1 PTC assessment in the EEU. The mITT population was defined prospectively in a protocol amendment, which was approved by Health Canada after screening commenced but before any subjects were randomized. A sensitivity analysis was performed by repeating the primary end point analysis for all subjects who had at least 1 EEU visit at PTC but did not necessarily have a mean TRSS of 8 or greater at all time points on all days of the BC (ie, the full intent-to-treat population). A prespecified subgroup analysis was performed on the subpopulation of mITT subjects with a mean TRSS of 12 or greater at BC.

A detailed statistical analysis plan was finalized before database lock and study unblinding. Statistical significance was defined as a *P* value of .05 or less and was 2-tailed when appropriate. This study was exploratory in nature, and therefore there was no adjustment for multiplicity. Summary statistics included the number of subjects and the mean, SD, and median values for continuous variables; 95% CIs and *P* values were provided for differences between the active and placebo groups. Descriptive statistics were summarized by treatment group for continuous variables; frequency distribution is summarized for categorical variables. No formal inferential tests were performed on safety data.

For the primary efficacy end point, the mean TRSSs on days 2 to 4 of the BC and the PTC were calculated for each subject by using matched time points (ie, data available for both BC and PTC); the change in TRSS from the BC to the PTC was analyzed by using analysis of covariance. In addition, analysis of covariance was used to analyze the secondary and exploratory end points,

with the exception of the subject's global assessment, which used the Cochran-Mantel-Haenszel row mean scores statistic. A sample size of 65 evaluable subjects per arm was calculated to have 90% power to detect a 2.9-point difference between placebo and active treatment in TRSS at PTC compared with BC, assuming a common SD of 5.0 based on results from other SPIRE studies.

RESULTS

Subjects

A total of 282 subjects were randomized, and 266 completed the study ([Fig 2](#)). Treatment groups were generally well matched ([Table I](#)), although the 8x12Q2W group had fewer men (38%) than the other groups (50% to 59% men). Large variations existed in mean IgE levels at screening; all subjects had positive SPT responses for rye grass, 98% of subjects had positive SPT responses for timothy grass, and most were polysensitized to at least 1 other allergen.

Primary efficacy outcome

Mean symptom scores were lower at PTC than BC in all treatment groups. The reduction in the 8x6Q2W grass allergen peptide group was greater than the reduction in the placebo group (least squares mean difference, -1.6), and this difference was significant ($P = .0346$) in the mITT population ([Table II](#)).

Changes in this group were greater than changes with placebo at all time points across all 4 days of the PTC ([Fig 3, A](#)). The grass allergen peptide 4x12Q4W group also had greater TRSS changes than the placebo group at all but 1 time point across all 4 days of the PTC ([Fig 3, B](#)). The 8x12Q2W grass allergen peptide regimen was not different from placebo. For both the 8x6Q2W and 4x12Q4W regimens, the treatment effect was greatest on day 4 of the challenge, when the cumulative allergen challenge is greatest (see [Table E4](#) in this article's Online Repository at www.jacionline.org for mean TRSSs at baseline and PTC days 1-4).

The sensitivity analysis in the total population (full intent-to-treat population) confirmed the findings in the primary efficacy analysis, showing a significantly greater reduction in mean TRSS in all subjects for the grass allergen peptide 8x6Q2W group (-5.1) versus the placebo group (-3.8) at PTC ($P = .0448$).

Secondary efficacy outcomes

The improvement in mean TRSS from BC to PTC on all 4 days reflected the findings in the primary analysis. The reduction in TRSS was due to improvements in nasal (for 8x6Q2W and 4x12Q4W groups) and nonnasal (for 8x6Q2W group) symptoms. A significantly greater reduction in mean TNSS occurred for the grass allergen peptide 8x6Q2W group (-2.3) versus the placebo group (-1.6) at PTC ($P = .0447$). The mean TNSS reduction for the grass allergen peptide 4x12Q4W group (-2.2 ; $P = .0958$ vs placebo) was similar to the reduction in the grass allergen peptide 8x6Q2W group. In addition, a reduction in mean TNNSS was observed for the grass allergen peptide 8x6Q2W group (-3.1) versus the placebo group (-2.2) at PTC ($P = .0765$). No differences in mean TNNSSs from placebo occurred for the grass allergen peptide 4x12Q4W and 8x12Q2W groups (-2.4 and -2.0 , respectively).

For the mITT subpopulation with a baseline TRSS of 12 or greater, a greater reduction in mean TRSS occurred on days 2 to 4 for the grass allergen peptide 8x6Q2W group (-6.0) versus the

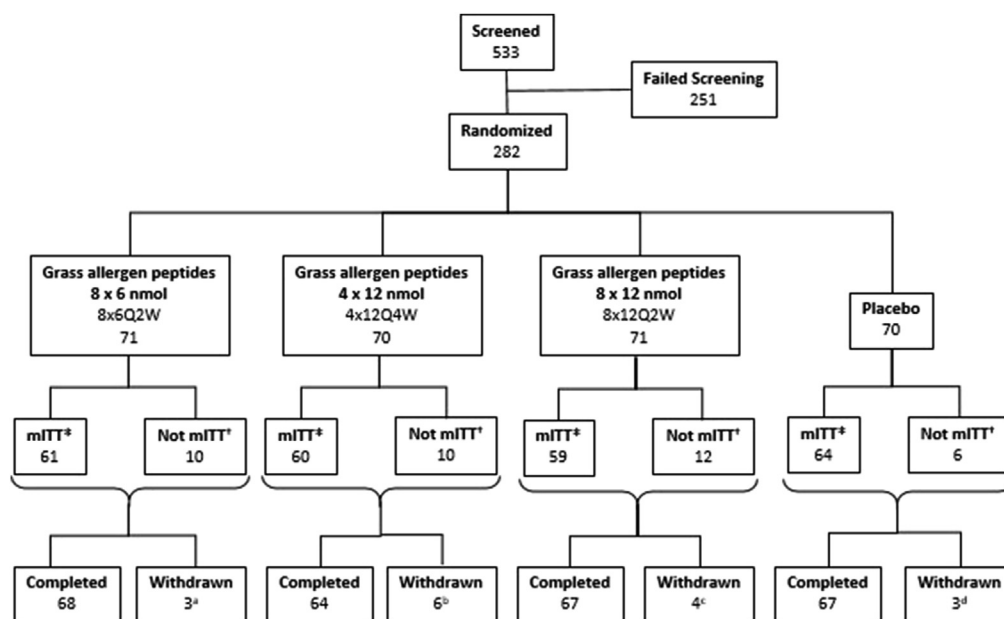


FIG 2. Subject disposition. *Had TRSS of 8 or greater. †Had TRSS of less than 8. ^aOne subject withdrew because of an AE, 1 subject withdrew consent, and 1 subject was lost to follow-up. ^bTwo subjects withdrew because of AEs, 1 subject was lost to follow-up, 2 subjects were withdrawn because of protocol deviations, and 1 subject withdrew because of relocation. ^cOne subject withdrew because of an AE, 1 subject withdrew because of pregnancy, 1 subject was withdrawn because of a protocol deviation, and 1 subject withdrew because of a schedule conflict. ^dTwo subjects withdrew because of AEs, and 1 subject withdrew consent.

TABLE I. Demographic data (safety population)

Characteristic	Grass allergen peptides, 8x6Q2W (n = 71)	Grass allergen peptides, 4x12Q4W (n = 70)	Grass allergen peptides, 8x12Q2W (n = 71)	Placebo (n = 70)
Sex, no. (%)				
Male	39 (54.9)	41 (58.6)	27 (38.0)	35 (50.0)
Female	32 (45.1)	29 (41.4)	44 (62.0)	35 (50.0)
Race, no. (%)				
White	62 (87.3)	63 (90.0)	65 (91.5)	66 (94.3)
Ethnicity, no. (%)				
Hispanic or Latino	1 (1.4)	3 (4.3)	1 (1.4)	1 (1.4)
Not Hispanic or Latino	66 (93.0)	65 (92.9)	69 (97.2)	64 (91.4)
Not reported	1 (1.4)	1 (1.4)	1 (1.4)	0
Unknown	3 (4.2)	1 (1.4)	0	5 (7.1)
Age (y)				
Mean ± SD	36.0 ± 10.2	34.1 ± 11.9	38.1 ± 12.5	35.2 ± 10.6
Range	18-61	18-64	19-64	18-58
Height (cm)				
Mean ± SD	170.6 ± 10.4	171.4 ± 10.6	168.3 ± 8.9	171.7 ± 9.4
Range	151-198	142-197	151-189	154-194
Weight (kg)				
Mean ± SD	85.61 ± 18.43	83.36 ± 22.17	80.43 ± 20.19	84.21 ± 20.69
Range	46.0-149.5	46.0-138.0	50.0-135.0	52.0-156.7
Total IgE (IU/mL)				
Mean ± SD	142.8 ± 174.1	311.5 ± 963.8	348.1 ± 697.4	267.3 ± 511.4
Median	69.7	98.4	117.2	84.5

placebo group (−4.0) at PTC ($P = .0403$, Fig 4). Reductions in TRSSs in this subgroup also occurred for the grass allergen peptide 4x12Qw4 (−5.8) and 8x12Q2W groups versus the placebo group ($P = .078$ and $P = .688$, respectively). As for the primary analysis, the treatment effect for 8x6Q2W and 4x12Q4W was greatest on day 4 of challenge, when the cumulative allergen challenge is greatest.

Exploratory assessments

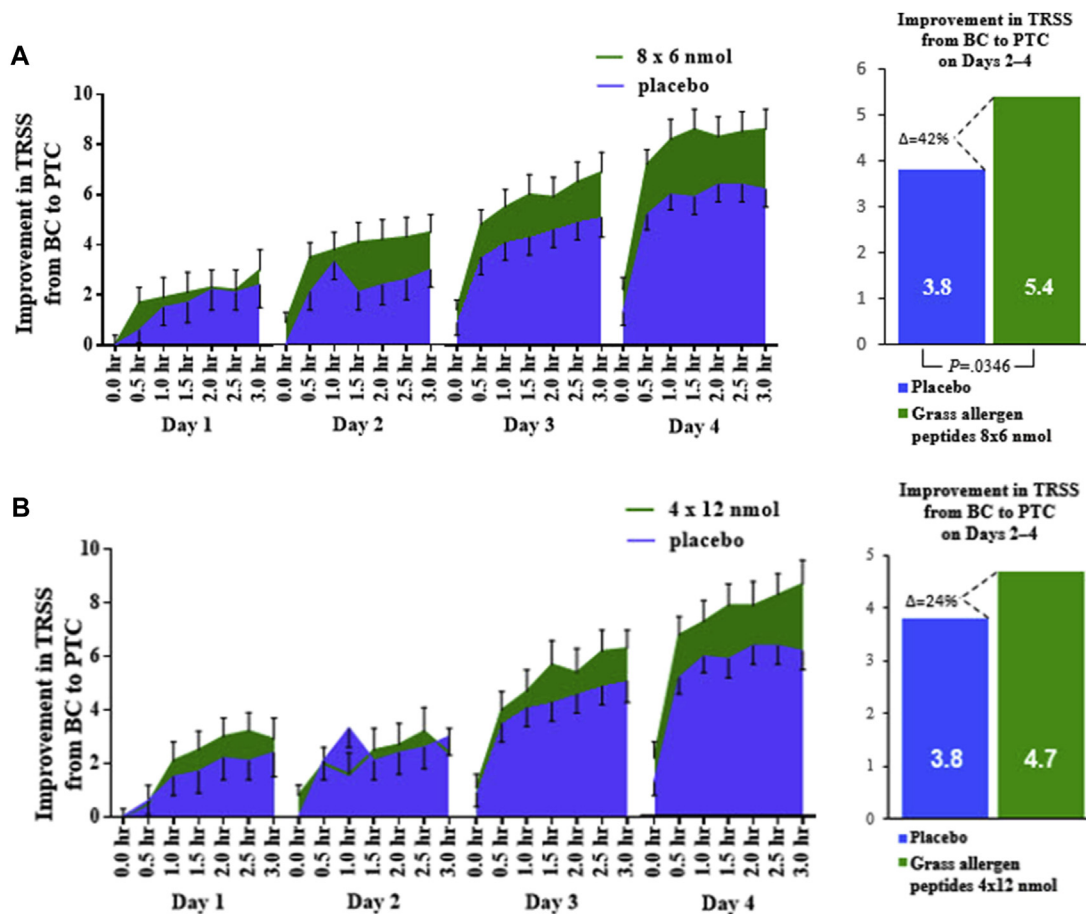
Differences in rye grass SPT responses at follow-up after the grass season were not significantly different for the grass allergen peptide groups versus the placebo group, although an increase in the area (wheal diameter, calculated as $\pi \cdot [\text{Wheal diameter}/2]^2$) of the cutaneous response to SPTs in the placebo group (27.4 mm²) was attenuated by grass allergen peptide 8x6Q2W

TABLE II. Change from baseline in TRSS on days 2 to 4 at 25 weeks (mITT population)

Population	No.	Mean	SD	Median	LS means	Difference between active treatment and placebo		
						LS means	95% CI	P value
Placebo	64	−3.8	4.0	−4.0	−3.8			
Grass allergen peptides 8x6Q2W	61	−5.4	4.4	−5.0	−5.4	−1.6	−3.08 to −0.12	.0346
Grass allergen peptides 4x12Q4W	60	−4.6	4.8	−4.7	−4.7	−0.9	−2.35 to 0.64	.2597
Grass allergen peptides 8x12Q2W	59	−3.3	3.9	−2.6	−3.3	0.6	−0.92 to 2.07	.4515

Baseline is defined as the matched average of symptom scores at all time points on days 2 to 4 of BC. PTC is defined as the matched average of symptom scores at all time points on days 2 to 4 of PTC. LS means, 95% CIs, and *P* values are based on analysis of covariance, with change from baseline as the dependent variable, treatment as a cofactor, and baseline mean TRSS as a covariate. *P* values are from the 2-sided test at a 5% level.

LS, Least squares.

**FIG 3.** A and B, Improvement in TRSS in the mITT. *n* = 63 at the PTC visit on day 4.

(4.7 mm²), 4x12Q4W (3.1 mm²), and 8x12Q2W (10.9 mm²). Data for changes in wheal areas from screening to follow-up demonstrated median changes of 0.00 mm² in the grass allergen peptide 8x6Q2W and 4x12Q4W groups, whereas the placebo and grass allergen peptide 8x12Q2W groups had median changes of 14.92 and 14.53 mm², respectively (see Fig E2 in this article's Online Repository at www.jacionline.org).

No consistent improvement in mean PNIF with grass allergen peptides versus placebo occurred on any day of PTC, although this appears to result from lower mean-matched PNIF values for grass allergen peptides compared with placebo at baseline before the EEU. No treatment-related changes from baseline in rye grass-specific IgA, IgE, and IgG₄ concentrations were observed (see Table E5 in this article's Online Repository at

www.jacionline.org for additional details). In the clinical global assessment measured at the final EEU session after PTC, 44% of subjects treated with grass allergen peptide 8x6Q2W and 41% of subjects treated with grass allergen peptide 4x12Q4W considered themselves improved or much improved during the PTC compared with the BC compared with 22% of placebo-treated subjects (*P* = .0094 and *P* = .0285, respectively; Table III).

Safety

Across all treatment groups, 80% or more of subjects reported at least 1 TEAE; treatment-related TEAEs were reported by 20% of subjects in the placebo group, 30% of subjects in the grass

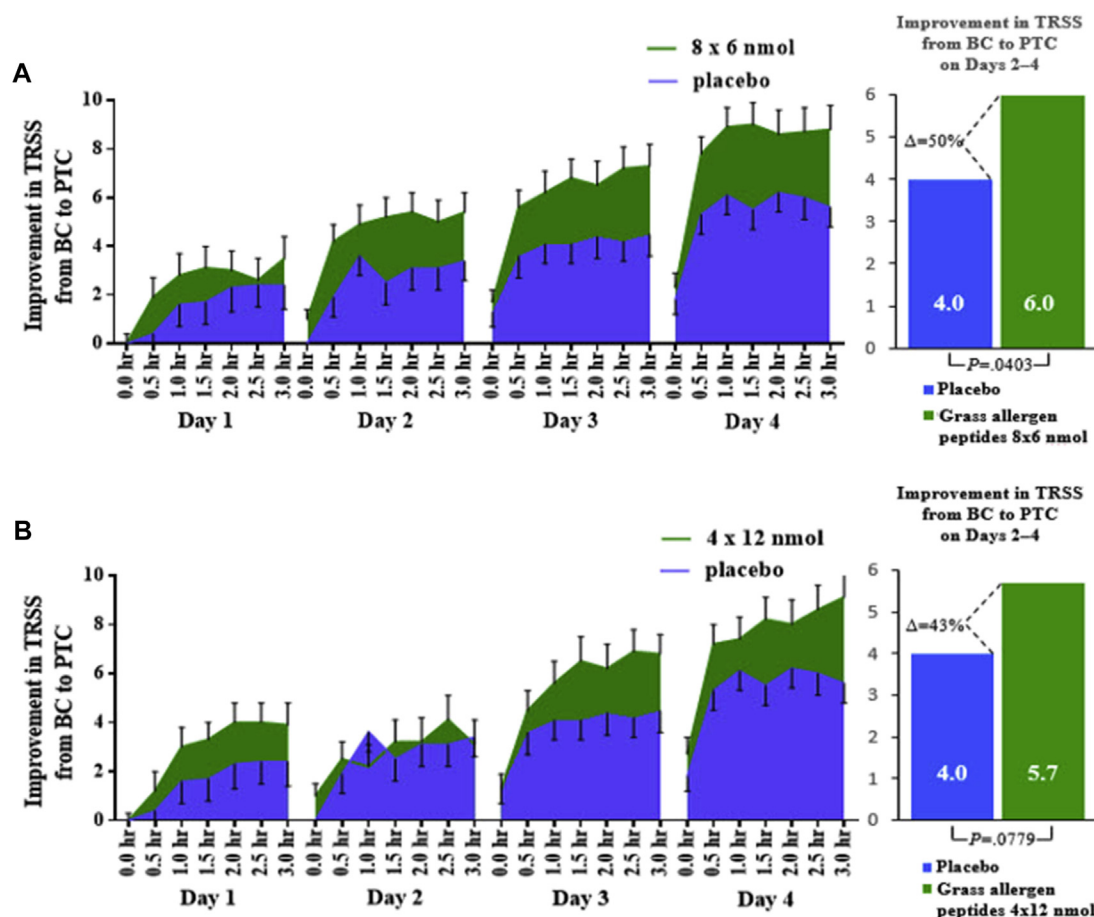


FIG 4. A and B, Improvement in TRSSs in subjects with TRSSs of 12 or greater. $n = 46$ at the PTC visit on day 4.

TABLE III. Summary of patient global assessment: Much improved and slight improvement/worsened (mITT population)

Treatment regimen	Category		
	Much improved*	Slightly improved/worsened†	P value (treatment vs placebo)
Placebo, no. (%)	14 (22.2)	49 (77.8)	—
Grass allergen peptides 8x6Q2W, no. (%)	27 (44.3)	34 (55.7)	.0094
Grass allergen peptides 4x12Q4W, no. (%)	24 (40.7)	35 (59.3)	.0285
Grass allergen peptides 8x12Q2W, no. (%)	10 (17.5)	47 (82.5)	.5240

Percentages are out of the number of subjects with a patient global assessment response in each treatment group. P values are from a Cochran-Mantel-Haenszel test.

*Much improved = very much better or much better.

†Slightly improved/worsened = little better, no change, little worse, much worse, or very much worse.

allergen peptide 8x6Q2W group, and 37% of subjects in both the grass allergen peptide 4x12Q4W and grass allergen peptide 8x12Q2W groups (Table IV).

Four grass allergen peptide-treated subjects withdrew because of TEAEs, one of which (headache in a grass allergen peptide 4x12Q4W subject) the blinded investigator considered to be treatment related. Two placebo-treated subjects withdrew because of TEAEs; the TEAEs (diarrhea and pyrexia) of 1 patient were considered to be treatment related by the blinded investigator. Only 2 subjects (in the grass allergen peptide 8x6Q2W group) had severe AEs, neither of which were considered to be treatment related; approximately one third of subjects in each group (including placebo) reported a severe

TEAE, which was defined by the protocol as any AE that resulted in interference with usual activities of daily living.

The proportion of subjects reporting severe AEs was similar in each treatment group and the placebo group, and there was no particular pattern of severe AEs that might have indicated a treatment concern. No subjects reported hypersensitivity AEs that would be consistent with a grade 3 or higher systemic allergic reaction according to the World Allergy Organization grading scheme.⁴³ The number of injection-site reactions was relatively low, although these reactions reported as AEs were more common (7% to 17% of subjects) in subjects receiving grass allergen peptides compared with subjects receiving placebo (1%). These reactions were mild and self-limiting and accounted for the

TABLE IV. Summary of TEAEs and serious AEs (safety population)

	Grass allergen peptides 8x6Q2W (n = 71), no. (%)	Grass allergen peptides 4x12Q4W (n = 70), no. (%)	Grass allergen peptides 8x12Q2W (n = 71), no. (%)	Placebo (n = 70), no. (%)
Subjects with ≥ 1 TEAE	62 (87.3)	61 (87.1)	65 (91.5)	56 (80.0)
Subjects with TEAEs that led to withdrawal	1 (1.4)	2 (2.9)	2 (2.8)*	2 (2.9)
Subjects with severe AEs	2 (2.8)†	0	0	0
Subjects with TEAEs related‡ to study drug	21 (29.6)	26 (37.1)	26 (36.6)	14 (20.0)
Subjects with severe TEAEs	25 (35.2)	23 (32.9)	26 (36.6)	24 (34.3)
Deaths	0	0	0	0

Subjects were asked to record every AE they experienced on diary cards throughout the study, and these were collected by the investigator at each visit. This might explain differences in the rate of AEs observed in this study compared with some other AIT studies.

*One of the 2 subjects in the 8x12Q2W group was withdrawn from the study because of pregnancy, which was also recorded as an AE.

†Not considered related to treatment.

‡Related = possibly, probably, or highly probably related to investigational product.

difference between placebo and grass allergen peptides in the number of subjects who had AEs related to treatment. No subject in the study experienced a TEAE of anaphylaxis or suspected anaphylaxis, and there were no reports of asthma related to treatment during the study; no deaths occurred in this study. There were no treatment-related findings in the assessments of safety laboratory tests, vital signs, pulmonary function tests, or the assessments of breathlessness and nasal congestion.

DISCUSSION

This is the first reported study evaluating the efficacy of grass allergen peptides, a new SPIRE for grass allergy. In the mITT population the 8x6Q2W regimen was associated with a clinically meaningful (ie, the patient is perceptive of the improvement)⁴⁴ and statistically significant reduction in rhinoconjunctivitis symptoms versus placebo in the EEU on challenge with rye grass allergen. The effect was primarily due to a significant improvement in nasal symptoms and a trend toward an improvement in nonnasal symptoms. The 4x12Q4W regimen also showed evidence of a treatment effect. The percentage improvements in TRSS scores presented here for 8x6Q4W versus placebo are substantiated by the 95% CIs, which are tabulated and consistent with World Allergy Organization recommendations from Canonica et al.⁴⁵ As mentioned, the treatment effect for both the 8x6Q2W and 4x12Q4W regimens was greatest on day 4 of the PTC, when the cumulative allergen challenge in the EEU was greatest. The EEU model facilitates sensitive measurement of symptom scores and treatment effects as they relate to precisely regulated levels of pollen exposure. This is not always possible with otherwise well-designed randomized, placebo-controlled field studies because pollen exposure varies widely in the natural environment and EEU studies can complement the findings of randomized controlled field studies.³⁶

Although there are clear differences between symptom scores in those receiving the 4x12Q4W regimen and placebo on days 1, 3, and 4 of the challenge, there was no difference on day 2. It might be that this lack of an effect on day 2 of the challenge is a chance finding resulting from the relatively small number of subjects in each treatment group. It is noteworthy that the treatment effect is similar in the 8x6Q2W and 4x12Q4W treatment groups on days 3 and 4 of challenge. Of note, however, only the 8x6Q2W arm was statistically significant for the primary end point. That both the 8x6Q2W and 4x12Q4W treatment regimens are effective is further supported by the statistically

significant improvement in the global impression of change for both of these treatment arms. Furthermore, the patient global assessment of response in the final EEU session identified more than 40% of subjects in the 8x6Q2W and 4x12Q4W treatment groups who perceived themselves to be much improved, which supports a clinically meaningful treatment effect with SPIRE therapy. In addition, no subjects reported hypersensitivity AEs consistent with a grade 3 or higher systemic allergic reaction,⁴³ and this suggests a favorable safety profile of SPIRE therapy.

We noted an increasing difference between the active groups relative to the placebo group because the subjects progressed from day 1 to day 4 in the PTC phase. This had been observed previously in the Patel et al⁴⁶ Fel d 1 SPIRE therapy and likely relates to the effect of ongoing continuous allergen exposure allowing for initial placebo responses “fall-off”; treatment-related differences can more easily emerge.

It is intriguing that the 2 treatment arms that administered the same cumulative dose of grass allergen peptides (48 nmol), irrespective of whether they were divided into 4 or 8 administrations, resulted in a treatment effect, whereas administration of a higher dose (96 nmol) did not. Classic pharmacology leads to an expectation that a higher dose will result in a greater treatment effect until a plateau is reached. However, multiple hormesis-like bell-shaped dose-response relationships in which a drug can stimulate a positive effect on the immune system at one concentration and inhibit the immune system at a higher concentration have been described in the literature. A review article has identified such a bell-shaped dose response for more than 90 immune response-related end points induced by more than 70 endogenous agonists, more than 100 drugs, and more than 40 environmental contaminants.⁴⁷ The finding in the present study that increasing the cumulative dose does not lead to a greater treatment effect is consistent with the findings of other groups developing immunotherapy products incorporating peptide or peptide-like moieties. Evaluation of the birch peptide AllerT (Anergis SA, Lausanne, Switzerland) in a dose-ranging phase 2 study demonstrated a 50- μ g dose of the product resulted in a 30% reduction in median symptom scores in subjects allergic to birch pollen, whereas a 100- μ g dose only improved symptoms by 19%.⁴⁸ Similarly, a dose of 170 μ g of *Lolium perenne* whole allergen extract, which was hydrolyzed to create peptides, resulted in significant improvement in allergic reactivity in a conjunctival provocation test, whereas neither a lower (70 μ g) nor a higher (370 μ g) dose was different from placebo.⁴⁹ The bell-shaped response curve noted in the current study could

have been anticipated based on previous evaluations of peptide-based immunotherapies.⁵⁰

Complete or significant sequence homology exists between MHC class II-binding epitopes in grass allergen peptides and equivalent epitopes from many common grasses, including rye, timothy, Bermuda, orchard, velvet, Kentucky bluegrass, and canary. Indeed, although subjects were exposed to rye grass in the EEU, this is not a predominant grass allergen in the Kingston area; native grass pollens in the Kingston area include timothy, orchard, and sweet vernal.⁵¹ The ability of grass allergen peptides to show a treatment effect using rye grass pollen as the allergen in a population routinely exposed to other grasses is considered a positive indicator of the ability of the product to demonstrate a treatment effect in the field setting with multiple grass pollen exposure. This hypothesis needs to be tested in field studies.

SLIT treatments are widely used in Europe, but SCIT remains the form of AIT used most extensively in the United States. Although a recent systematic review found only 1 well-controlled head-to-head comparison of SLIT versus SCIT (in birch pollen allergy), the reviewers' indirect comparisons of 17 SCIT and 11 SLIT trials could not consistently demonstrate superior reductions of symptom and medication scores with SCIT over SLIT. The review also found that although AEs (mainly local) were common in both the SCIT and SLIT trials, the SCIT trials had a higher incidence of severe systemic reactions than the SLIT trials (19% and 3%, respectively).⁵²

Although AIT treatments with whole allergens, such as SCIT and SLIT, can induce IgE and IgG/IgG₄ changes,^{53,54} such changes are less apparent with AIT when using short peptides, such as SPIREs, including grass allergen peptides. One study with peptides from phospholipase A₂ bee venom allergen showed a modest and transient increase in IgG₄ levels.⁵⁵ An earlier study with bee venom peptides showed that during and shortly after treatment, allergen-specific IgE and IgG levels did not change. However, shortly after subjects were given a wild bee sting challenge (approximately 30 µg of allergen), IgG₄ levels increased significantly.⁵⁶ The currently available data from a study of SPIRE for cat allergy do not support IgG and IgE modification,⁵⁷ even though these treatments are clinically effective. The results from this study show an improvement in symptom scores without an increase in IgG₄ levels. Mechanistically, this might be because the SPIRE peptides are too short to bind to immunoglobulin on the surfaces of B cells. Studies have shown that AIT with peptide mixtures can decrease inflammatory cytokine levels,^{55,58-60} and IL-10 (an anti-inflammatory cytokine) levels appear to be increased when blood lymphocytes respond to allergen after AIT with peptide mixtures.^{55,59}

In the present study of grass allergen peptides, efficacy was observed in the primary mITT population and similarly in a subgroup with higher TRSSs at baseline. This observation is consistent with results from an analysis of 3 AIT studies by Howarth et al,⁶¹ which examined symptom improvements in subjects with low, medium, and high symptom scores. The analysis conducted in the subgroup of the mITT population with a mean TRSS of 12 or greater at the baseline EEU challenge was defined prospectively in the protocol before any subjects being recruited into the study and was intended to allow the treatment effect in more symptomatic subjects to be investigated. The greater treatment effect in more symptomatic subjects is an important observation, given that the target population for AIT

treatment consists of subjects with moderate-to-severe ARC symptoms.

The effectiveness and safety of T-cell epitope-based AIT has also been demonstrated for the treatment of cat allergy,^{46,62} including the finding of long-term effectiveness of cat allergen T-cell epitopes.⁶³ The present study found that pretreatment over 14 weeks before the start of the grass pollen season results in a treatment effect that can be determined in the EEU after the peak of the natural grass pollen season. This finding, despite no dosing during the pollen season, suggests the treatment effect can be sustained over the 9-week period between the end of treatment and the PTC, even though natural exposure to pollen over this period might have increased the allergen load before pollen exposure in the EEU during the PTC. This was a phase 2, dose-finding study to identify dosages of grass allergen peptides to test in a larger study population in a phase 3 trial. Based on our findings, the phase 3 trial program should seek to enroll subjects with more moderate-to-severe symptoms and evaluate the 8×6Q2W dosage. It will be important to follow subjects enrolled in the present grass allergen peptide study to examine the persistence of beneficial effects of this new AIT treatment after a second grass pollen season.

In conclusion, this trial demonstrated the efficacy, convenience, and safety of grass allergen peptides. Eight intradermal administrations of grass allergen peptides at 6 nmol significantly improved ARC symptomatology 25 weeks after treatment initiation, as evaluated in the EEU, and after an intervening grass pollen season. There was also evidence of efficacy with the same cumulative dose administered as 4 doses over the same period. The effect was similar in a subgroup with more severe ARC symptoms at baseline, which is important given that the target population for AIT consists of subjects with moderate-to-severe symptoms. The same cumulative dose divided into 4 administrations of 12 nmol also showed evidence of biologic activity. This study indicates a potential for SPIRE to be as effective as SCIT and SLIT in the treatment of allergic rhinitis; findings will obviously need to be replicated in a traditional phase III multicenter trial.

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Clinical implications: Short courses of treatment with T-cell epitope-based peptides of grass allergens alleviate symptoms of ARC.

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1 ONLINE REPOSITORY (SUPPLEMENT)

2 METHODS

3 Baseline challenge

- 4 1. Symptoms assessed: 1) four nasal symptoms (runny nose, sneezing, blocked nose, itchy
5 nose); 2) four non-nasal symptoms (itchy eyes, watery eyes, red/burning eyes, itchy ears,
6 palate or throat); each individual symptom scored on a scale of 0 (absent) to 3 (severe);
7 maximum combined scores: TNSS (4x3=12), TNNSS (4x3=12), TRSS (8x3=24 [TNSS +
8 TNNSS])

9 Secondary efficacy endpoints

- 10 1. Change from BC in the mean TRSS at all time points on all four days of PTC for grass
11 allergen peptides treatment groups vs placebo
- 12 2. Change from BC in the mean TNSS at all time points on days 2–4 of PTC for grass
13 allergen peptide treatment groups vs placebo
- 14 3. Change from BC in the mean total non-nasal symptom score (TNNSS) at all time points on
15 days 2–4 of PTC for grass allergen peptide treatment groups vs placebo
- 16 4. Change from BC in the mean TRSS at all time points on days 2–4 of PTC for grass
17 allergen peptide treatment groups vs placebo for subjects whose baseline TRSS was ≥ 12
- 18 5. Change from BC in the mean TRSS at all time points on days 2–4 of PTC for grass
19 allergen peptide treatment groups vs placebo for all subjects.

20 Exploratory efficacy endpoints

Mean change from baseline in the area (wheal size) of the SPT response at follow-up; mean change in PNIF; mean change from baseline in concentrations of rye grass-specific IgA, IgE, and IgG₄ at follow-up.

Protocol amendments

Protocol Amendment 01(September 28, 2011) clarified that subjects could use cream containing $\leq 1\%$ hydrocortisone as long as the affected area covered less than 10 % of the body and clarified the use of psychoactive drugs. A small number of other clarifications and corrections of inconsistencies were also addressed. No subjects had been randomized at the time of this amendment.

Protocol Amendment 02 (November 9, 2011 extended the study to multicenter to enable recruitment, dosing, and follow-up at additional centers, with subjects attending a central EEU to ensure consistent challenge. It also enabled subjects with controlled asthma not triggered by grass pollen to participate in the study. Completion of clinical study NCT01198613 with a similar product (ragweed specific peptides) provided the basis for adjustments to the analysis of the study including the introduction of the primary analysis being conducted in a mITT population with mean baseline TRSS >8 . Further, based on findings from NCT01198613 the 3-nmol dose arm was removed from the current study. A small number of other clarifications and corrections of inconsistencies were also addressed. No subjects had been randomized at the time of this amendment.

Protocol Amendment 03 (June 1, 2012) permitted continuation of the study after the 2012 ragweed season for a second year of recruitment and also allowed for subjects that dropped out during the first year of the study to be replaced in the second year. It also stated that a blinded

analysis of the variance would be performed for subjects enrolled in the first year of the study to determine whether it was necessary to continue to the enrolment of 280 subjects. It further clarified that baseline challenge would take place during the November to February period so as to avoid ragweed and tree pollen seasons. A small number of other clarifications and corrections of inconsistencies were also addressed. A total of 196 subjects had been randomized at the time of this amendment.

Method of assigning subjects to treatment groups

Those subjects who successfully completed Period 1 Screening/Baseline Challenge were randomized to receive one of the three regimens of grass allergen peptides or placebo. The ratio of grass allergen peptides (each of 3 regimens): placebo injected subjects was 1:1:1:1.

Randomization was performed by POI using a computer-generated algorithm with a block size of four.

Randomization was stratified according to whether a subject was a high or low responder in the EEU during Baseline Challenge. Subjects scoring a mean TRSS of <14 during Baseline Challenge were classified as a low responder and subjects scoring a mean TRSS of ≥14 during Baseline Challenge were classified as a high responder.

Randomization was achieved by the unblinded pharmacist (or designee) allocating the next numerical medication pack available within predesignated randomization blocks for high and low responders. The randomization number issued was recorded in the eCRF. The unblinded pharmacist or designated qualified team member also recorded the subject screening number against the allocated randomization number in the Drug Accountability Record and on the unblinded randomization list.

• Symptomatic seasonal ARC and inability to complete BC and PTC outside the local pollen season • Significant allergy to animal dander that could not be avoided during the study period • Inability to tolerate BC in the EEU
• Use of the following therapies (during specified periods prior to screening) - o Beta-blockers - o Alpha-adrenoceptor blockers - o Depot corticosteroids (90 d) - o Tricyclic antidepressants (14 d) - o Monoamine oxidase inhibitors (14 d) - o Psychoactive drugs (except for stable doses of nontricyclic antidepressants or occasional use of benzodiazepine or zopiclone for night sedation) - o Corticosteroids; systemic, dermatologic, inhalational, intranasal, or ocular (30 d) - o Antihistamines other than loratadine (7–10 d) - o Anticholinergics (7 d) - o Alpha-adrenergic agonists (7 d) - o Cromones (14 d) - o Leukotriene inhibitors (10 d)
• History of significant recurrent acute sinusitis requiring antibiotic treatment (2 episodes/y for the last 2 y) • History of chronic sinusitis, with sinus symptoms lasting more than 12 wk • Symptoms of a clinically relevant illness within 6 wks prior to screening • Any clinically relevant abnormalities detected on physical examination • Vital signs outside normal limits • Laboratory values outside normal ranges • Significant history of alcohol or drug abuse • History of immunopathologic diseases that could have interfered with the study results • Previous participation in this or another grass allergen peptide study

- History of severe drug allergy, severe angioedema, or anaphylactic reaction to food
- Received treatment with an investigational drug within 3 mos prior to study screening
- Unable to communicate or to understand the requirements of the study
- History of any significant disease or disorder that may have put the subject at risk or influenced the results of the study or the subject's ability to participate in the study
- Known history of positive test results for hepatitis B, hepatitis C, human immunodeficiency virus, or tuberculosis

67 Table E2. Treatment regimens for grass allergen peptides and placebo

Visit		3A	3B	3C	3D	3E	3F	3G	3H
Week		0	2	4	6	8	10	12	14
Regimen	Dose								
1	8 x 6 nmol	X	X	X	X	X	X	X	X
2	8 x 12 nmol	X	X	X	X	X	X	X	X
3	4 x 12 nmol	X	P	X	P	X	P	X	P
4	Placebo	P	P	P	P	P	P	P	P

68 *P*, placebo administration; *X*, grass allergen peptide administration.

69 Note: Placebo was administered on weeks 2, 6, 10, and 14 to maintain blinding for regimen 3.

70 **Table E3.** Schedule of assessments

Study Period	Screening/BC		Treatment	Post-treatment	
Visit	Screening	Baseline EEU	Treatment	PTC EEU	Follow-up
Informed consent and eligibility	X				
Demography and medical history	X				
Physical examination	X				X
Vital signs	X	X	X	X	X
SPT for panel of allergens	X				
SPT for rye grass allergen	X				X
Blood sample for total IgE	X				
Blood sample for rye grass-specific IgA		X			X
Blood sample for rye grass-specific IgE		X			X
Blood sample for rye grass-specific IgG ₄		X			X
FEV ₁	X				X
PEFR		X	X	X	
VAS breathlessness			X		
VAS nasal symptoms			X		
Diary for symptom scores and PNIF		X		X	
Patient global assessment				X	
Blood sample for hematology/biochemistry	X				X
Urine dipstick	X				X
Blood sample for tryptase	X		X*		
Urine pregnancy test (WOCBP)	X		X		X
Concomitant medication	X	X	X	X	X

Dosing			X		
Examination of the injection site			X	X	
Recording of AEs	X	X	X	X	X

*Only collected in the event of a suspected severe allergic reaction (anaphylaxis).

Screening consisted of one or two visits to the clinic, at the investigator's discretion. Follow-up took place 3–10 days after last EEU session. SPT was performed prior to first EEU session, if not performed within prior 6 mo. A blood sample for anti-peptide antibodies was only taken if local reactions to the peptides were noted at the previous visit and also at the final treatment visit for any subjects who had a reaction noted previously. PEFR was measured before and at the end of each EEU session, before each treatment visit, 1 h after dosing before subject was discharged from the unit. Patient global assessment was done only after the final PTC visit on day 4. Baseline samples for tryptase analysis were stored in a –80°C freezer and were only analyzed in the event of a suspected anaphylactic response at a treatment visit. Examination of the injection site included digital photography for any clinically significant dermal responses other than mild to moderate erythema or wheals.

AE, adverse event; *BC*, baseline challenge; *EEU*, environmental exposure unit; *FEV₁*, forced expiratory volume in one second; *Ig*, immunoglobulin; *PEFR*, peak expiratory flow rate; *PNIF*, post-nasal inspiratory flow; *PTC*, post-treatment challenge; *WOCBP*, women of child-bearing potential.

87 **Table E4. Summary of Mean TRSS at Baseline and PTC Days 1–4**

		Grass allergen peptides 8x6Q2W	Grass allergen peptides 4x12 nmol	Grass allergen peptides 8x12 nmol
	Placebo	2 wks apart	4 wks apart	2 wks apart
Study Period	(N=64)	(N=61)	(N=60)	(N=59)
Baseline, mean (SD)	13.6 (3.2)	13.5 (3.2)	13.4 (3.0)	13.8 (3.1)
PTC, mean (SD)	10.4 (4.8)	9.0 (4.6)	9.5 (4.5)	11.1 (4.1)

88 *PTC*, post-treatment challenge; *TRSS*; total rhinitis symptom score.

89 **Table E5. Changes from baseline in rye grass-specific IgA, IgE, and IgG₄ concentrations**

Parameter (Unit)	Study Period	Statistics	Grass allergen peptides			
			Placebo (N=64)	2 wks apart (N=61)	4 wks apart (N=60)	2 wks apart (N=59)
IgA (mg/L)	Screening	n	64	61	60	58
		Mean (SD)	0.96 (0.53)	1.17 (0.78)	1.16 (0.78)	1.01 (0.62)
		Median	0.50	1.14	1.12	0.76
		Min, Max	0.50, 2.29	0.50, 3.85	0.50, 4.62	0.50, 2.94
	Follow-up	n	60	60	57	56
		Mean (SD)	0.97 (0.53)	1.20 (0.75)	1.15 (0.78)	0.98 (0.56)
		Median	0.76	1.18	1.14	0.76
		Min, Max	0.50, 2.30	0.50, 3.98	0.50, 4.62	0.50, 2.56
	Change from Screening	n	60	60	57	56
		Mean (SD)	0.02 (0.24)	0.01 (0.28)	−0.04 (0.33)	−0.01 (0.27)
		Median	0.00	0.00	0.00	0.00
		Min, Max	−0.52, 1.08	−0.96, 0.93	−0.70, 0.88	−0.61, 0.94
	% Change from Screening	n	60	60	57	56

			Grass allergen peptides 8x6Q2W	Grass allergen peptides 4x12 nmol	Grass allergen peptides 8x12 nmol	
			Placebo	2 wks apart	4 wks apart	2 wks apart
Parameter (Unit)	Study Period	Statistics	(N=64)	(N=61)	(N=60)	(N=59)
IgE (kU/L)		Mean (SD)	6.82 (38.61)	6.50 (34.27)	2.85 (40.62)	4.18 (39.15)
		Median	0.00	0.00	0.00	0.00
		Min, Max	−50.98, 216.00	−42.11, 186.00	−58.33, 176.00	−54.95, 188.00
	Screening	n	64	61	60	58
		Mean (SD)	15.81 (23.94)	8.444 (17.14)	9.337 (14.47)	10.679 (18.88)
		Median	5.16	1.27	2.33	3.46
		Min, Max	0.05, 100.00	0.05, 95.40	0.05, 54.20	0.05, 90.80
	Follow-up	n	60	60	57	56
		Mean (SD)	22.65 (27.79)	13.87 (22.45)	17.10 (22.20)	19.45 (25.74)
		Median	10.55	3.21	4.94	7.04
		Min, Max	0.05, 100.00	0.05, 100.00	0.05, 83.00	0.05, 100.00
	Change from Screening	n	60	60	57	56.
		Mean (SD)	7.35 (11.01)	5.30 (9.93)	7.45 (10.92)	8.46 (11.83)
		Median	3.29	1.24	2.19	3.09
		Min, Max	−25.70, 46.40	−17.90, 42.30	−5.70, 52.00	−1.54, 52.82

Parameter (Unit)	Study Period	Statistics	Placebo	Grass allergen peptides 8x6Q2W	Grass allergen peptides 4x12 nmol	Grass allergen peptides 8x12 nmol
			(N=64)	2 wks apart (N=61)	4 wks apart (N=60)	2 wks apart (N=59)
	% Change from Screening	n	60	60	57	56
		Mean (SD)	562.71 (2738.52)	153.34 (227.68)	145.22 (197.32)	128.29 (178.99)
		Median	84.67	100.00	84.93	72.13
		Min, Max	-34.68, 20300.00	-62.15, 1345.45	-50.00, 900.00	-92.77, 1006.38
IgG ₄ (mg/L)	Screening	n	64	61	60	58
		Mean (SD)	0.17 (0.27)	0.17 (0.37)	0.20 (0.36)	0.16 (0.19)
		Median	0.11	0.05	0.05	0.05
		Min, Max	0.05, 1.84	0.05, 2.58	0.05, 1.99	0.05, 1.04
	Follow-up	n	60	60	57	56
		Mean (SD)	0.23 (0.27)	0.23 (0.46)	0.27 (0.48)	0.21 (0.22)
		Median	0.15	0.10	0.11	0.14
		Min, Max	0.05, 1.38	0.05, 3.33	0.05, 2.58	0.05, 0.99
	Change from Screening	n	60	60	57	56
		Mean (SD)	0.05 (0.15)	0.054 (0.13)	0.07 (0.16)	0.07 (0.12)

			Grass allergen peptides 8x6Q2W	Grass allergen peptides 4x12 nmol	Grass allergen peptides 8x12 nmol	
			Placebo (N=64)	2 wks apart (N=61)	4 wks apart (N=60)	2 wks apart (N=59)
Parameter (Unit)	Study Period	Statistics				
		Median	0.01	0.00	0.00	0.00
		Min, Max	−0.46, 0.78	−0.19, 0.75	−0.06, 1.06	−0.06, 0.62
	% Change from Screening	n	60	60	57	56
		Mean (SD)	52.69 (137.86)	51.26 (99.39)	30.43 (54.44)	53.07 (83.44)
		Median	1.43	0.00	0.00	0.00
		Min, Max	−50.00, 960.00	−61.54, 480.00	−30.00, 216.67	−54.55, 316.67
Ig,			immunoglobulin.			

91 **Figure E1.** Environmental Exposure Unit (EEU)* Kingston General Hospital, Ontario, Canada.

92 *Capacity: from 10 to 140 subjects (303 m²)

93 **Figure E2. Change in rye grass wheal area**

94 Calculation of the IQR provides wider boundaries around the median than do the Q1 and Q3
95 boxes. Data points which fall outside the IQR-calculated boundaries are determined to be
96 outliers.

97 *IQR*, interquartile ratio $([Q3 - Q1] \cdot 1.5)$; *Q1*, first quartile; *Q3*, third quartile.

98

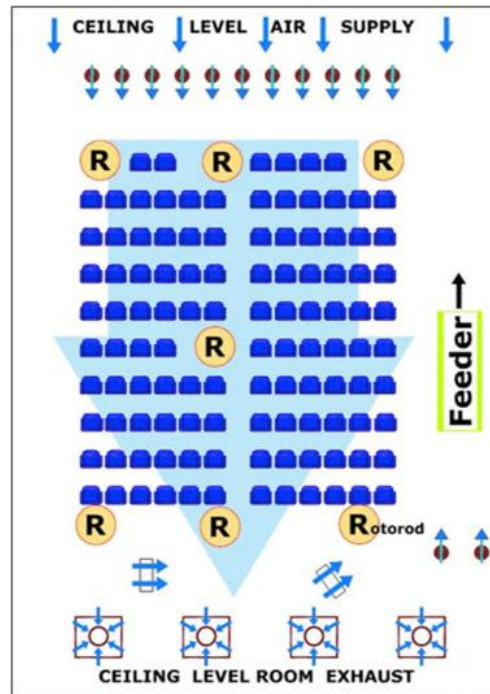


Fig E1.

Anne K. Ellis, Charles W. Frankish, Robyn E. O'Hehir, Kristen Armstrong, Lisa Steacy, Mark Larché, Roderick P. Hafner

Treatment with grass allergen peptides improves symptoms of grass pollen–induced allergic rhinoconjunctivitis

Journal of Allergy and Clinical Immunology, 2017, Available online 21 February 2017

<http://dx.doi.org/10.1016/j.jaci.2016.11.043>

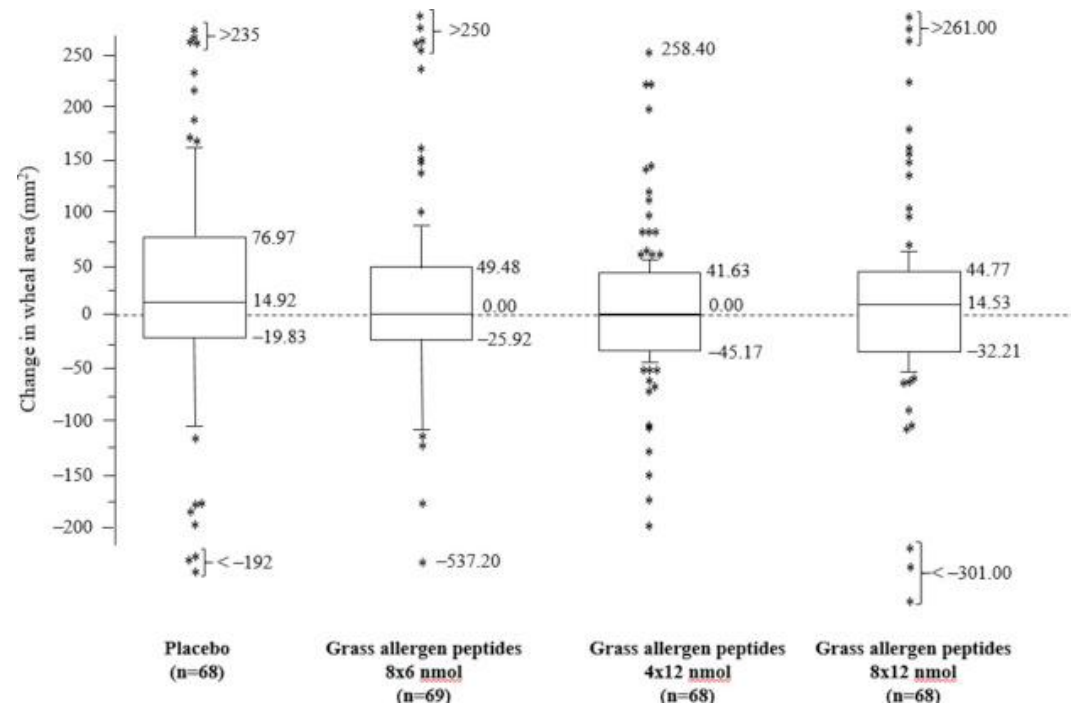


Fig E2.

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