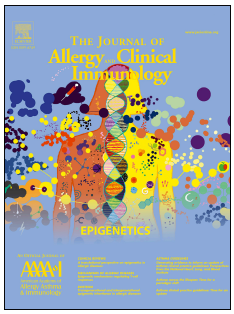
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Persistence of the clinical effect of grass allergen peptide immunotherapy after the second and third grass pollen season

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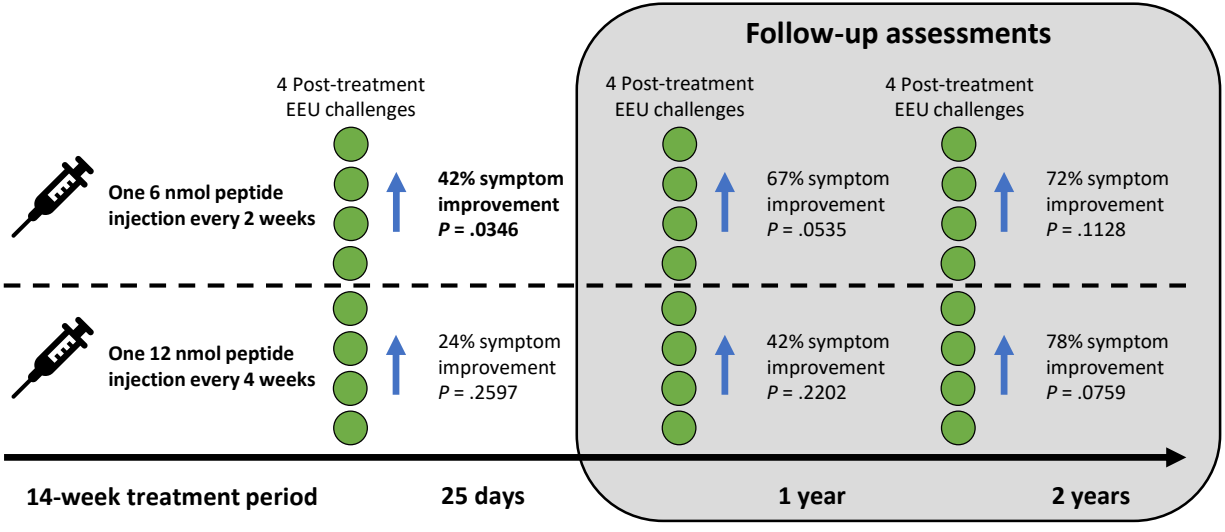
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Immunotherapy with grass allergen peptides provides long-lasting clinical benefit for up to 2 years after treatment

Change in rhinoconjunctivitis symptoms over placebo were assessed in the Environmental Exposure Unit (EEU) over 4 rye grass pollen challenges



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

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KA was employed by Adiga Life Sciences Inc and had share options in Circassia Pharmaceuticals plc when the study was conducted.

LMS and MWT reports no conflicts of interest.

RH is employed by Circassia Ltd and is a Director and shareholder of Circassia Pharmaceuticals plc.

SP was an employee of Circassia Ltd and had share options in Circassia Pharmaceuticals plc when the study was conducted.

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Abstract (current word count = 249)

Background

Grass allergen peptides are in development for the treatment of grass pollen-induced allergic rhinoconjunctivitis(ARC). A previous randomized, placebo-controlled study demonstrated grass allergen peptides significantly improved total rhinoconjunctivitis symptom scores(TRSS) following post-treatment challenges(PTC) to rye grass in an Environmental Exposure Unit(EEU) after one intervening grass pollen season(GPS1).

Objective

To evaluate the efficacy/safety of four dosing regimens of grass allergen peptides after a second(GPS2) and third(GPS3) intervening grass pollen season in the EEU.

Methods

Eligible individuals 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 after treatment cessation respectively. Participants were not treated further and both participants and study personnel remained blinded. The primary efficacy endpoint was the change in mean TRSS(reported every 30 minutes) from GPS1 baseline to the follow-up PTC, calculated across all

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time points over days 2-4 for GPS2, and across hours 1-3 over days 2-4 for GPS3. Secondary efficacy endpoints and safety were also assessed.

Results

122 and 85 participants were enrolled in GPS2 and GPS3 respectively. A greater improvement from baseline in mean TRSS at PTC was observed in the 8x6nmolQ2W group compared to placebo in GPS2(-6.0 vs. -3.6, *P*=.0535) and GPS3(-6.2 vs. -3.6, *P*=.1128). Similar findings were observed for the 4x12nmolQ2W group 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 ARC symptoms after three intervening grass pollen seasons, corresponding to up to 2 years off treatment.

Clinical trial registration: Clinicaltrials.gov Identifiers, NCT01923779 and NCT0229875

Clinical implications

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

Capsule summary

Eight intradermal injections of T cell epitope-based grass allergen peptides administered pre-seasonally improved grass pollen-induced rhinoconjunctivitis symptoms over placebo, lasting up to 2 years after the end of treatment.

Key words

Allergic rhinoconjunctivitis, allergen challenge, Environmental Exposure Unit, SPIRE, peptide, immunotherapy, grass, synthetic peptide immuno-regulatory epitope, allergic rhinitis

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Abbreviations used

AE: Adverse event

AIT: Allergen immunotherapy

ARC: Allergic rhinoconjunctivitis

EEU: Environmental Exposure Unit

FEV_1: Forced expiratory volume in 1 second

GPS: Grass pollen season

Ig: Immunoglobulin

PEFR: Peak expiratory flow rate

PTAE: Post-treatment adverse event

PTC: Post-treatment challenge

SCIT: Subcutaneous immunotherapy

SPIRE: Synthetic peptide immuno-regulatory epitope

sIgG4: Allergen-specific IgG4

SLIT: Sublingual immunotherapy

Th2: T helper 2

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114 TNSS: Total nasal symptom score

115 TNNSS: Total non-nasal symptom score

116 TRSS: Total rhinoconjunctivitis symptom score

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Introduction

The pathophysiology of allergic rhinoconjunctivitis (ARC) includes immunoglobulin (Ig)E-mediated activation and degranulation of mast cells and basophils after inhalation of allergens to which an individual is sensitized. Subsequent symptoms include sneezing, rhinnorhea, 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 ARC patients on 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 individuals (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 (sIgE) 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 six provinces (6).

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To reduce the likelihood of severe IgE-mediated adverse reactions associated with traditional whole allergen immunotherapy, synthetic peptide immuno-regulatory epitopes (SPIREs) were developed. 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 serve to reduce IgE-dependent reactions during treatment (7,8). To date, SPIREs have been developed for cat, house dust mite (HDM), ragweed, 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 an upregulation of allergen-specific regulatory T cells, specific T cell anergy, and a shift from specific effector T helper 2 (Th2) cells to a Th1 cell phenotype (18). Studies have also demonstrated the ability of SPIREs to reduce the expression of several Th2-related genes in whole blood samples, which correlated with symptom improvement following treatment in cat-allergic participants (19,20).

Recently, a multicenter, phase 2, dose-finding study (NCT01385800) assessed the efficacy and safety of three intradermal regimens of grass allergen peptides using the Environmental Exposure Unit (EEU), a validated challenge model approved by the FDA to investigate the efficacy of new AR therapies (17,21). Grass-allergic participants were randomized to placebo or one of three 14-week treatment regimens; 8 x 6 nmol 2 weeks apart (8x6Q2W), 4 x 12 nmol 4 weeks apart (4x12Q4W), and 8 x 12 nmol 2 weeks apart (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 grass pollen season (approximately 25

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weeks after treatment initiation), only the 8 x 6 nmol group significantly reduced mean total rhinoconjunctivitis symptom score (TRSS) compared to placebo during the post-treatment grass pollen challenges (PTC) in the EEU (17). Other phase 2 studies demonstrated a persistent treatment benefit for both cat- and HDM-allergen peptides for up to two years after treatment cessation (9,10,12,13). Thus, it was hypothesized that treatment persistence may also be observed with grass allergen peptides. This report summarizes the findings from two blinded follow-up studies assessing the efficacy of grass allergen peptides after a second and third intervening grass pollen season (GPS2 and GPS3), corresponding to approximately 1 and 2 years off treatment respectively.

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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 one (GPS2) and two (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 identifiers are NCT01923779 and NCT02292875.

The clinical methodology for the parent study (GPS1) has been published elsewhere (17). Briefly, grass-allergic participants were randomized to one of three 14-week dosing regimens of grass allergen peptides completed prior to the start of grass pollen season: 8x6Q2W (one 6 nmol intradermal injection every 2 weeks for 14 weeks), 4x12Q4W (one 12 nmol intradermal injection every 4 weeks for 14 weeks with placebo injections given in between to maintain blinding), 8x12Q2W (one 12 nmol intradermal injection every 2 weeks for 14 weeks). Efficacy was evaluated 25 weeks after treatment initiation, corresponding to after the peak of grass

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pollen season and before the start of ragweed pollen season. Participants returned to the study site for the post-treatment rye grass pollen challenges conducted in the EEU over four successive days.

Both GPS2 and GPS3 were comprised of three study periods: screening visits, follow-up PTC, and safety follow-up visits (Figure 1). Screening visits were conducted ≤ 12 weeks before the PTC. For GPS2, the PTC occurred approximately one year after the GPS1 PTC (18 months after treatment initiation). For GPS3, the PTC occurred approximately two 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 four successive days in the EEU. The airborne grass pollen concentration was maintained at 3500 ± 500 grains/m^3, 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 blinded 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 grass pollen season), who completed all treatment and PTC visits, and achieved a mean baseline TRSS ≥ 8/24 in GPS1 (calculated as the mean score of all time points

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across Days 1 to 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 the completion of GPS1.

The key exclusion criteria were the 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 ≥ GINA Step 2 treatment (exercise-induced bronchospasm or controlled asthma not triggered by grass pollen and requiring only GINA Step 1 treatment were acceptable), FEV_1 < 80% of predicted, use of allergen immunotherapy treatments since completing GPS1, a history of recurrent acute or chronic sinusitis, pregnancy (current or planned). Participants for whom administration of epinephrine was contraindicated were also excluded. Additional inclusion and exclusion criteria are summarized in Tables E1.

Study assessments

During the PTC, four nasal, four non-nasal, and breathlessness symptoms were scored at 30-minute intervals using a 4-point categorical scale (0, none; 1, mild; 2, moderate; 3, severe). The four nasal and four non-nasal scores were combined into the TRSS which has a range of 0 (no symptoms) to 24 (all symptoms severe). Baseline symptoms were defined as the matched symptom scores achieved during the baseline challenge in GPS1. The primary efficacy endpoint in GPS2 was the change from baseline in mean TRSS at all time points on days 2 to 4 of the

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follow-up PTC (month 18) compared to placebo. The primary efficacy endpoint 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 to placebo. For GPS3, hours 0 and 0.5 were excluded to focus on symptoms during the plateau phase.

Secondary efficacy endpoints included the change from baseline in mean TRSS at all time points on days 1 to 4 (GPS2 and GPS3) and days 2 to 4 (GPS3), the change from baseline in mean TRSS on days 2 to 4 of the follow-up PTC for participants with a mean baseline TRSS ≥ 12, the change from baseline in mean total nasal symptom score (TNSS, the sum of the four nasal symptoms), and the change from baseline in mean total non-nasal symptom score (TNNSS, the sum of the four non-nasal symptoms). Exploratory assessments included the 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 endpoint 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), FEV_1, peak expiratory flow rate (PEFR), and breathlessness.

Statistical analyses

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For both GPS2 and GPS3, efficacy analysis was based on the intent-to-treat (ITT) population, which included participants who completed at least one follow-up PTC assessment (≥ one of four EEU visits). Analysis of the primary, secondary, and exploratory endpoints was conducted using an analysis of covariance (ANCOVA). The ANCOVA model contained terms for the fixed effects of treatment and GPS1 baseline assessments (symptom scores and Ig levels) as the covariate. A two-sided *P* value of < .05 was taken as significant. As both follow-up studies were exploratory in nature, adjustments for multiple comparisons were not made.

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Results

Participant demographics

For GPS2, a total of 122 participants were enrolled and 118 completed the study (Figure 2, A). Two of the enrolled participants withdrew due to schedule conflicts, one participant withdrew consent, and one participant moved abroad. For GPS3, a total of 85 participants were enrolled and 84 completed the study (Figure 2, B). One participant withdrew due to a schedule conflict. Participants were evenly distributed among the treatment groups for GPS2 (8x6Q2W, n=30; 4x12Q4W, n=32; 8x12Q2W, n=31; placebo, n=29) and GPS3 (8x6Q2W, n=19; 4x12Q4W, n=21; 8x12Q2W, n=22; placebo, n=23). The treatment groups were also generally well matched at baseline in terms of demographics (Table 1). For GPS2, the 8x12Q2W group had fewer males compared to the other treatment groups (32.3% vs. 44.8-56.3%). The 4x12Q4W group in GPS3 had more males compared to the other treatment groups (61.9% vs. 27.3-36.8%).

Primary efficacy assessment

At the 1-year follow-up, approximately 18 months after treatment initiation, the 8x6Q2W group demonstrated a trending reduction in mean TRSS compared to placebo (-6.0 vs. -3.6). The least squares (LS) mean treatment difference between the groups was -2.3 (95% confidence interval [CI], -4.65 to 0.04; *P* = .0535) (Table 2). Changes in mean TRSS for this group were greater than placebo across all time points over days 2 to 4 of the PTC, with the greatest change observed on

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day 4 (Figure 3). In contrast, the 4x12Q4W group showed a modest reduction in mean TRSS compared to placebo (LS mean treatment difference, -1.4; 95% CI, -3.77 to 0.88; *P* = .2202), with change being greater than placebo across all but one timepoint (Day 2, hour 3) (Figure 3). The 8x12Q2W group showed a reduction in mean TRSS comparable to that of placebo (LS mean treatment difference, 0.5; 95% CI, -1.83 to 2.91; *P* = .6521) (Table 2).

At the 2-year follow-up, approximately 30 months after treatment initiation, a greater reduction in mean TRSS compared to placebo was observed for both 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 3). 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 (Figure 4). Similar to the 1-year follow-up, reductions in mean TRSS during the PTC were comparable between 8x12Q2W and placebo (LS mean treatment difference, 1.2; 95% CI, -1.81 to 4.28; *P* = .4218) (Table 3).

Secondary efficacy assessments

Changes in mean TRSS in favor of the treatment (that were not statistically significant, however) were observed for both 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 (Table

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E2). Similar changes in mean TNSS and TNNSS 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 ≥ 12 were evaluated, reductions in mean TRSS similar to that of the primary analysis were observed for both 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 to placebo (Table E3).

At year 2, reductions in mean TRSS were observed for both 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 (Tables E4 and E5). A trending reduction in mean TNSS compared to placebo was observed in the 8x6Q2W group (-2.5 vs. -1.2, *P* = .1030). In contrast, a significant reduction in mean TNSS was observed for the 4x12Q4W group versus placebo (-3.0 vs. -1.2, *P* = .0224). Changes in TNNSS during the PTC were comparable between all three treatment groups and placebo (8x6Q2W, *P* = .1874; 4x12Q4W, *P* = .2660; 8x12Q2W, *P* = .2660). In participants with a baseline mean TRSS ≥ 12, significant reductions in mean TRSS were only observed in the 4x12Q4W group versus placebo (LS mean treatment difference, -4.4; 95% CI, -7.97 to -0.87; *P* = .0156) (Table E6). Changes in mean TRSS 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).

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317 **Exploratory and subgroup assessments**

318 After a second and third intervening grass pollen season, there were no significant changes in
319 rye grass-specific IgA, IgE, and IgG4 levels at follow-up compared to baseline in the treatment
320 groups compared to placebo. An additional analysis of the primary efficacy endpoint in GPS3
321 was performed in a subgroup of participants who completed GPS1, GPS2, and GPS3. A total of
322 54 participants completed both follow-up studies (8x6Q2W, n=17; 4x12Q2W, n=18; placebo,
323 n=19). The 8x6Q2W group showed improvement in mean TRSS compared to placebo across all
324 three grass pollen seasons. The LS mean treatment differences were -2.9 (*P* = .0754) at 25
325 weeks, -5.0 (*P* = .0035) at 18 months, and -3.4 (*P* = .0331) at 30 months after the start of
326 treatment (Table E7). Similar findings were observed for the 4x12Q4W group, with LS mean
327 treatment differences of -4.0 (*P* = .0158), -4.5 (*P* = .0080), and -4.1 (*P* = .0101) at 25 weeks, 18,
328 and 30 months respectively (Figure E1).

329

330 **Safety**

331 For GPS2, a total of 123 post-treatment AEs (PTAEs) were reported with 60 participants
332 experiencing ≥ one PTAE (8x6Q2W, n=16; 4x12Q4W, n=14; 8x12Q2W, n=17; placebo, n=13)
333 (Table 4). The frequency of severe PTAEs was evenly distributed among the treatment groups
334 (8x6Q2W, n=3; 4x12Q4W, n=4; 8x12Q2W, n=5; placebo, n=3) and primarily involved nervous
335 system disorders (e.g. headaches). One participant in the 8x12Q2W group reported a severe AE

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(anaphylactic reaction secondary to a wasp sting) during the study. Of note, this e severe AE occurred 14 months after the last dose and was deemed unrelated to the 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 ≥ one PTAE (8x6Q2W, n=4; 4x12Q4W, n=5; 8x12Q2W, n=6; placebo, n=5) (Table 4). Severe PTAEs were reported by one participant in 8x6Q2W, one participant in 8x12Q2W, and two participants in the placebo group. These AEs were single cases of pain in extremities (both in placebo group), migraine (8x6Q2W), and excoriation (8x12Q2W). All reported PTAEs were considered unrelated to treatment.

In both follow-up studies, no PTAEs led to study withdrawal. No participant reported a > 30% reduction in PEFR during the follow-up PTC compared to screening.

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Discussion

The current study reports the findings from two follow-up studies which assessed the continued efficacy of grass allergen peptides after a second and third intervening grass pollen season. Efficacy was evaluated during rye grass pollen challenges in the EEU after the peak of the grass pollen season. The 8x6Q2W dosing regimen was associated with a reduction in grass-induced rhinoconjunctivitis symptoms compared to the placebo group, which trended towards 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 to 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.

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A numerically greater improvement in mean TRSS over placebo was observed for the 4x12Q4W group compared to the 8x6Q2W group (-2.8 vs. -2.6) at year 2. This contrasts with the 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 may also be attributable to the significant improvement in mean TRSS in the subgroup of participants with severe baseline symptoms (TRSS ≥ 12). As this subgroup represents 65% of the 4x12Q4W ITT population in GPS3, it is likely that these participants were driving the overall analysis.

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

A possible mechanism underlying the successful desensitization following AIT involves the induction of allergen-specific IgG4 (sIgG4) blocking antibodies. Conventional immunotherapy is

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associated with an increase in sIgG4 levels, with symptomatic improvement moderately correlated with the functionality of sIgG4 (24-29). However, sIgG4 levels remained unchanged following 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 49 to 71 amino acids in length induced robust increases in Bet v 1-sIgG4 levels after two 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 Th1/regulatory T cell-orientated immune responses and a shift away from allergen-specific effector Th2 cells and Th2 cytokine production (32-35).

Conventional subcutaneous and sublingual immunotherapy (SCIT and SLIT respectively) 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,

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immunotherapy with SPIREs may represent an alternative option towards tolerance, requiring fewer injections to that of 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, the frequency of systemic reactions (e.g. anaphylaxis) often occur during the up-dosing phase, where increasing doses of allergen are administered over a short time period until maintenance dose is achieved (49-51). 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 two follow-up studies presented here provide preliminary evidence demonstrating the persistent efficacy and safety of grass allergen peptides after three intervening grass pollen seasons. Four or eight intradermal injections of grass allergen peptides given 2 to 4 weeks apart led to a numerical reduction in grass-induced ARC symptoms, as evaluated in the EEU, lasting up to 2 years after treatment cessation when compared to placebo. Future studies are required to determine if a similar treatment effect using a 14-week AIT regimen with SPIREs can be observed in larger participant groups and in the field.

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643 **Figure Legends**

644

645 **Figure 1. Overall study design.**

646

647 **Figure 2. Participant disposition.** All participants were randomized and dosed in the parent
648 study approximately 18 months prior to the 1-year follow-up study (**A**) and approximately 30
649 months prior to the 2-year follow-up study (**B**). No further treatment was administered during
650 the follow-up studies. [a]One subject withdrew due to schedule conflict. [b]One subject moved
651 abroad and one subject withdrew consent.

652

653 **Figure 3. Improvement in TRSS after a second intervening grass pollen season.** The 8x6Q2W
654 (**A**) and 4x12Q4W (**B**) groups demonstrated a trending and nonsignificant improvement in TRSS
655 respectively compared to placebo approximately 18 months after treatment initiation.

656

657 **Figure 4. Improvement in TRSS after a third intervening grass pollen season.** Both 8x6Q2W (**A**)
658 and 4x12Q4W (**B**) groups demonstrated a trending improvement in TRSS respectively compared
659 to placebo approximately 30 months after treatment initiation.

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Tables

Table 1. Participant demographics.

Characteristics	GPS2				GPS3			
	8x6Q2W (n=30)	4x12Q4W (n=32)	8x12Q2W (n=31)	Placebo (n=29)	8x6Q2W (n=19)	4x12Q4W (n=21)	8x12Q2W (n=22)	Placebo (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 ± SD	36.9 ± 9.1	36.3 ± 12.2	39.5 ± 12.2	36.2 ± 9.9	40.7 ± 9.9	40.5 ± 12.9	43.9 ± 12.7	38.3 ± 9.9
Range	22-61	20-64	21-63	22-55	25-64	23-66	24-65	27-58
Height (cm)								
Mean ± SD	167.4 ±	169.6 ±	167.1 ± 8.6	168.5 ±	165.9 ±	174.7 ±	165.6 ± 7.6	168.4 ±

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	9.3	10.3		7.8	8.8	10.5		8.2
Range	152-185	142-188	158-186	154-187	152-178	157-197	152-184	154-184
Weight (kg)								
Mean ± SD	89.8 ± 20.6	83.4 ±20.6	80.7 ± 18.8	83.8 ± 20.3	90.8 ± 21.8	93.5 ± 23.4	79.5 ± 19.6	78.7 ±13.8
Range	50-150	47-132	50-125	52-157	50-150	61-138	50-125	52 -105

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

					Difference between active treatment and placebo		
Population	**No.**	**Mean**	**SD**	**Median**	**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

666

667 14-week dosing regimens: 8x6Q2W (one 6 nmol intradermal injection every 2 weeks for 14
668 weeks), 4x12Q4W (one 12 nmol intradermal injection every 4 weeks for 14 weeks with placebo
669 injections given in between to maintain blinding), 8x12Q2W (one 12 nmol intradermal injection
670 every 2 weeks for 14 weeks). Baseline TRSS was defined as the matched average values at all
671 time points on days 2 to 4 of the baseline challenge at GPS1. Results were obtained using an
672 analysis of covariance model containing terms for the fixed effects of treatment and baseline
673 mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least
674 squares.

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675 **Table 3.** Primary efficacy assessment: Change from baseline in TRSS on days 2 to 4 at hours 1 to
676 3 (GPS3, ITT population).

					Difference between active treatment and placebo		
Population	**No.**	**Mean**	**SD**	**Median**	**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

677

678 14-week dosing regimens: 8x6Q2W (one 6 nmol intradermal injection every 2 weeks for 14
679 weeks), 4x12Q4W (one 12 nmol intradermal injection every 4 weeks for 14 weeks with placebo
680 injections given in between to maintain blinding), 8x12Q2W (one 12 nmol intradermal injection
681 every 2 weeks for 14 weeks). Baseline TRSS was defined as the matched average values at
682 hours 1 to 3 on days 2 to 4 of the baseline challenge at GPS1. Results were obtained using an
683 analysis of covariance model containing terms for the fixed effects of treatment and baseline
684 mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least
685 squares.

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686 **Table 4.** Summary of PTAEs and SAEs for GPS2 and GPS3 (safety population).

Characteristics	GPS2				GPS3			
	8x6Q2W (n=30)	**4x12Q4W (n=32)**	**8x12Q2W (n=31)**	**Placebo (n=29)**	**8x6Q2W (n=19)**	**4x12Q4W (n=21)**	**8x12Q2W (n=22)**	**Placebo (n=23)**
Number of PTAEs	38	19	44	22	8	8	8	8
Subjects with ≥ one 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)

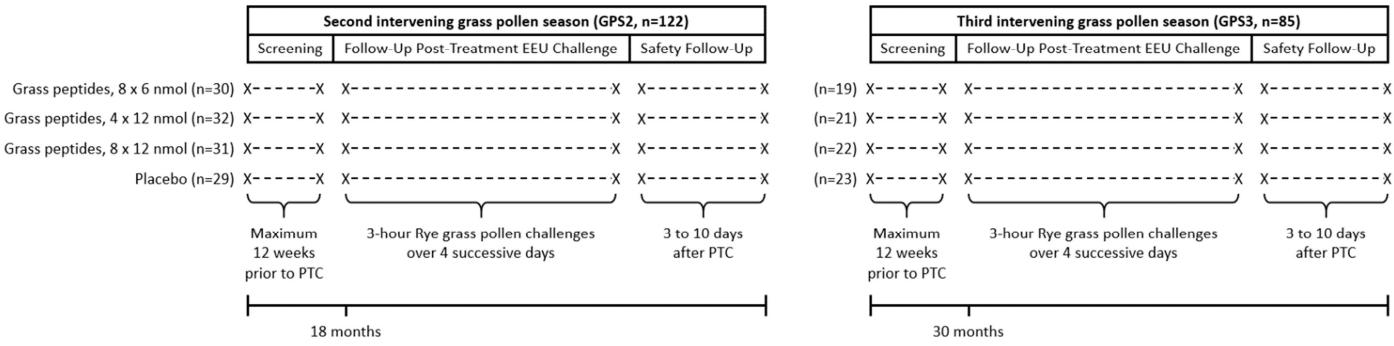
687

688 PTAEs were defined as AEs that first occurred or worsened in severity following informed

689 consent in GPS2 and GPS3. PTAE, post-treatment adverse event; SAE, severe adverse event.

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Figure 1



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Figure 2

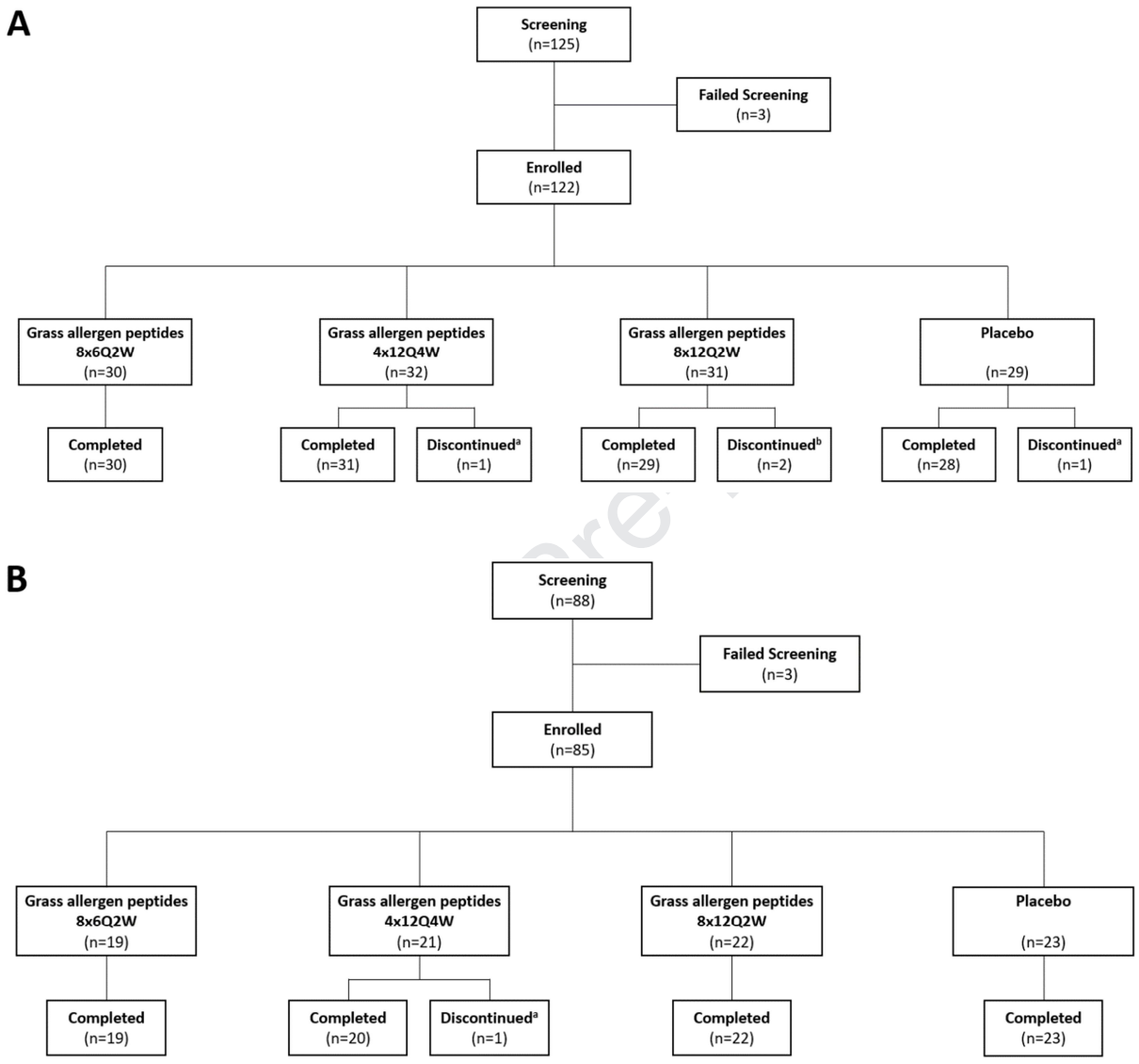
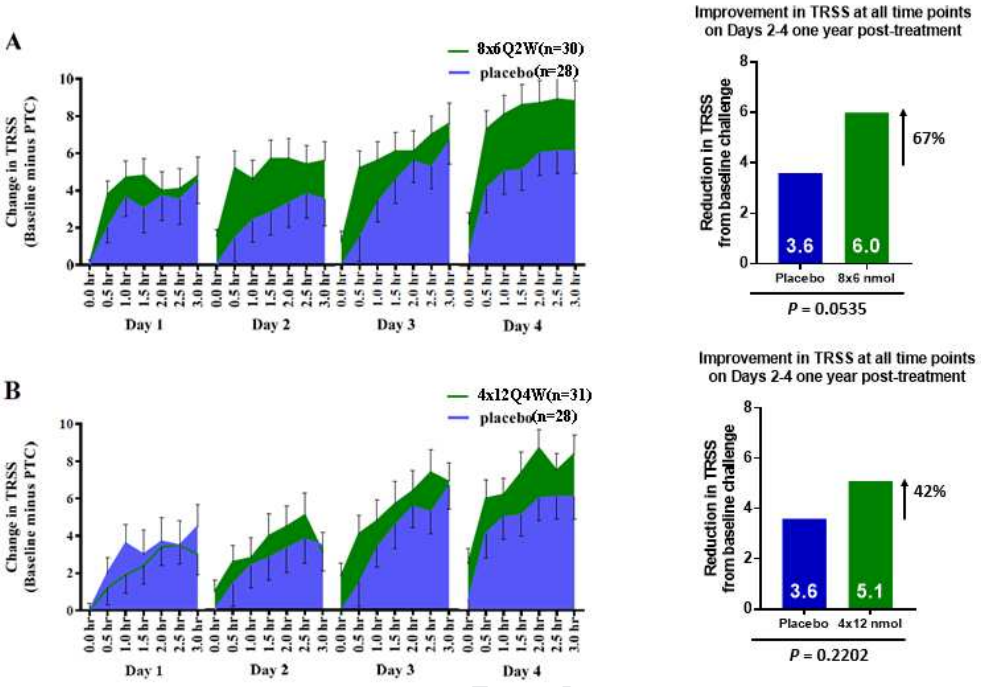
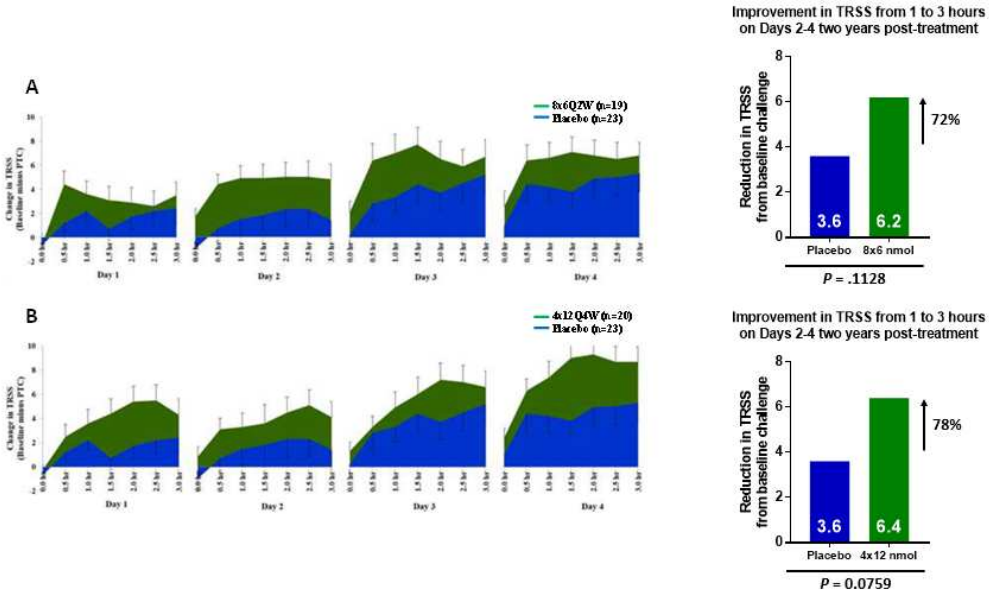

A
Screening
(n=125)
Failed Screening
(n=3)
Enrolled
(n=122)
Grass allergen peptides
8x6Q2W
(n=30)
Grass allergen peptides
4x12Q4W
(n=32)
Grass allergen peptides
8x12Q2W
(n=31)
Placebo
(n=29)
Completed
(n=30)
Completed
(n=31)
Discontinued[a]
(n=1)
Completed
(n=29)
Discontinued[b]
(n=2)
Completed
(n=28)
Discontinued[a]
(n=1)
B
Screening
(n=88)
Failed Screening
(n=3)
Enrolled
(n=85)
Grass allergen peptides
8x6Q2W
(n=19)
Grass allergen peptides
4x12Q4W
(n=21)
Grass allergen peptides
8x12Q2W
(n=22)
Placebo
(n=23)
Completed
(n=19)
Completed
(n=20)
Discontinued[a]
(n=1)
Completed
(n=22)
Completed
(n=23)

Figure 3



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Figure 4



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ONLINE REPOSITORY (SUPPLEMENT)

METHODS

Primary Efficacy Endpoint

GPS2: The primary efficacy endpoint was the change from GPS1 baseline challenge in mean total rhinoconjunctivitis symptom score (TRSS) at all time points on days 2 to 4 of the follow-up PTC in grass allergen peptides treatment groups compared with placebo.

GPS3: The primary efficacy endpoint was the 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 peptides treatment groups compared with placebo.

Secondary efficacy endpoints

- Change from GPS1 baseline challenge in mean TRSS using all time points on days 2 to 4 of GPS3 follow-up PTC in grass allergen peptides treatment groups compared with placebo.
- Change from GPS1 baseline challenge in mean TRSS using all time points on days 1 to 4 of GPS1, GPS2, and GPS3 follow-up PTC in grass allergen peptides treatment groups compared with placebo.
- Change from GPS1 baseline challenge in mean total nasal symptom score (TNSS) at all time points on days 2 to 4 of the GPS1, GPS2, and GPS3 follow-up PTC in grass allergen peptides treatment groups compared with placebo.
- Change from GPS1 baseline challenge in mean total non-nasal symptom score (TNNSS) at all time points on days 2 to 4 of the GPS1, GPS2, and GPS3 follow-up PTC in grass allergen peptides treatment groups compared with placebo.

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- Change from GPS1 baseline challenge in mean TRSS using all time points on days 2 to 4 of the GPS1, GPS2, and the 1- to 3-hour time points of the GPS3 follow-up PTC in grass allergen peptides treatment groups compared with placebo. for subjects whose GPS1 baseline TRSS was ≥12.

Exploratory efficacy endpoints

- Mean change from baseline in concentrations of rye grass-specific immunoglobulin (Ig)A, IgE, and IgG4 at follow-up.

Changes to the conduct of the studies

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

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 the planned analyses

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

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 endpoint 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 PTC for those subjects who had participated in all of these evaluations. This extra analysis enabled a comparison to be made

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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 the analyses outlined in the protocol and SAP:

- The 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 PTC was not performed for the intent-to-treat (ITT) or per-protocol (PP) populations as originally outlined. Instead, this analysis was performed using an ITT additional subpopulation of subjects who were enrolled in GPS1, GPS2, and GPS3.
- The least squares 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 the interpretation of the study.
- A repeat measures analysis of matched mean change in TRSS from GPS1 baseline to GPS1 and GPS3 follow-up PTC 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.

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- It was specified in the SAP that nonparametric procedures were to be used, for the analysis of changes in TRSS, immunoglobulin levels (grass specific IgA, IgE and IgG4) and skin prick testing response, if ANCOVA assumptions were not met; however, this was not required.

In addition, grass specific IgA, IgE and IgG4 levels and skin prick testing 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.

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Supplemental Figure Legend

Figure E1. Persistent improvement in TRSS after three consecutive grass pollen seasons. In both 8x6Q2W and 4x12Q4W groups, participants attending the parent study and both 1- and 2-year follow-up studies demonstrated an improvement in TRSS compared to placebo.

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Supplemental Tables

Table E1. Inclusion and exclusion criteria.

Inclusion Criteria
Subjects who fulfilled the following criteria were eligible for enrolment into the study: • Previously randomized into GPS1 and completed all treatment visits and PTC during calendar year 2012. • Mean TRSS at the baseline challenge in GPS1 was ≥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. • 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. A female subject could be included without a negative urine pregnancy test if she could document that she was surgically sterile or at least 2 years postmenopausal.

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Exclusion Criteria

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

- Informed of the treatment received in GPS1.
- Had developed grass pollen-induced asthma since the start of their participation in GPS1.
- Currently suffering from asthma that was either uncontrolled or requiring Global Initiative for Asthma (GINA) 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.
- FEV1 <80% of predicted.
- Allergen immunotherapy since completing participation in GPS1 (other than treatment in GPS1).
- Administration of epinephrine was contraindicated (eg, subjects with acute or chronic symptomatic coronary heart disease or severe hypertension).
- History of significant recurrent acute sinusitis, defined as two episodes per year for the last two years, all of which required antibiotic treatment.
- Had developed a history of chronic sinusitis, defined as sinus symptoms lasting >12 weeks that included two or more major factors or one major factor and two 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.

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- Symptoms of a clinically relevant illness, which 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 six weeks prior to screening visit.
- Were pregnant, lactating, or planning a pregnancy during the study.
- Any clinically relevant abnormalities detected on physical examination.
- Vital signs (blood pressure, pulse rate, respiratory rate, 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 prior to the screening visit, or were currently participating in another clinical study.
- Had developed 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.

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

					Difference between active treatment and placebo		
Population	**No.**	**Mean**	**SD**	**Median**	**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 the matched average values at all time points on days 1 to 4 of the baseline challenge at GPS1. Results were obtained using an analysis of covariance model containing terms for the fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least squares.

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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 ≥ 12 (GPS2, ITT population).

		Difference between active treatment and placebo		
Population	**No.**	**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 the 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 (i.e. all available data were used) mean baseline TRSS ≥ 12. Results were obtained using an analysis of covariance model containing terms for the fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least squares.

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

					Difference between active treatment and placebo		
Population	**No.**	**Mean**	**SD**	**Median**	**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 the matched average values at all time points on days 1 to 4 of the baseline challenge at GPS1. Results were obtained using an analysis of covariance model containing terms for the fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least squares.

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

					Difference between active treatment and placebo		
Population	**No.**	**Mean**	**SD**	**Median**	**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 the matched average values at all time points on days 2 to 4 of the baseline challenge at GPS1. Results were obtained using an analysis of covariance model containing terms for the fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least squares.

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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 ≥ 12 (GPS3, ITT population).

		Difference between active treatment and placebo		
Population	**No.**	**LS means**	**95% CI**	***P* 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 the 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 (i.e. all available data were used) mean baseline TRSS ≥ 12. Results were obtained using an analysis of covariance model containing terms for the fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least squares.

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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).

		Least squares mean difference between active treatment and placebo					
Population	**No.**	**25 weeks**[†]	***P* value**	**18 months**[†]	***P* value**	**30 months**[†]	***P* 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 the matched average values at hours 1 to 3 on days 2 to 4 of the baseline challenge at GPS1. Results were obtained using an analysis of covariance model containing terms for the fixed effects of treatment and baseline mean TRSS as the covariate. Data are presented as LS means, 95% CIs, and *P* values. LS, Least squares.

[†] Time after the first dose of grass allergen peptides.

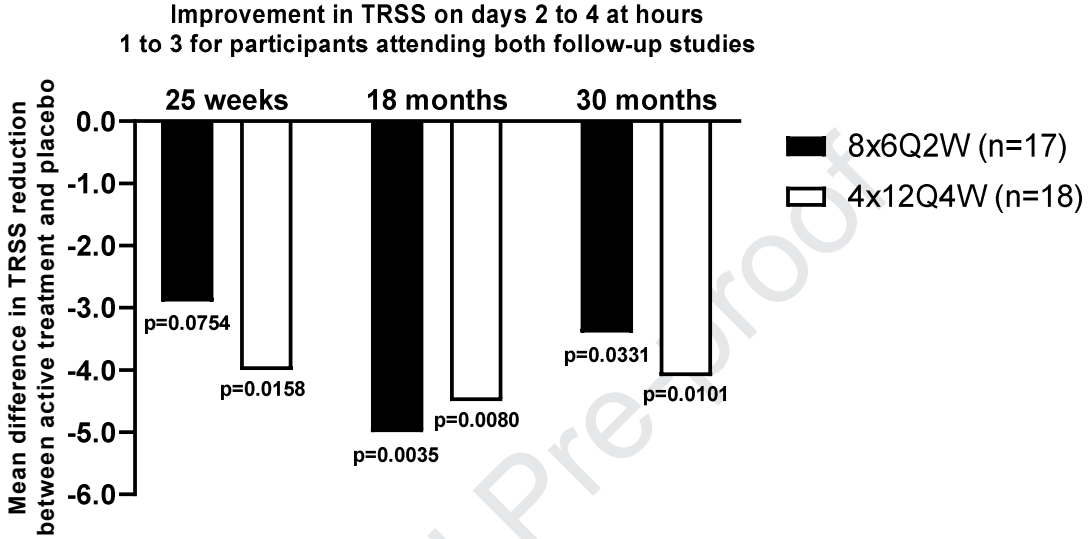
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Supplemental Figures

Figure E1



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