ORIGINAL ARTICLE

Airway Diseases

WILEY Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

A randomized, double-blind, placebo-controlled, dose-finding trial with *Lolium perenne* peptide immunotherapy

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Abstract

Background: A novel subcutaneous allergen immunotherapy formulation (gpASIT+™) containing *Lolium perenne* peptides (LPP) and having a short up-dosing phase has been developed to treat grass pollen–induced seasonal allergic rhinoconjunctivitis. We investigated peptide immunotherapy containing the hydrolysate from perennial ryegrass allergens for the optimum dose in terms of clinical efficacy, immunogenicity and safety.

Methods: This prospective, double-blind, placebo-controlled, phase IIb, parallel, four-arm, dose-finding study randomized 198 grass pollen–allergic adults to receive placebo or cumulative doses of 70, 170 or 370 μg LPP. All patients received weekly subcutaneous injections, with the active treatment groups reaching assigned doses within 2, 3 and 4 weeks, respectively. Efficacy was assessed by comparing conjunctival provocation test (CPT) reactions at baseline, after 4 weeks and after completion. Grass pollen–specific immunoglobulins were analysed before and after treatment.

Results: Conjunctival provocation test (CPT) response thresholds improved from baseline to V7 by at least one concentration step in 51.2% (170 μg; $P = .023$), 46.3% (370 μg), and 38.6% (70 μg) of patients receiving LPP vs 25.6% of patients receiving placebo (modified per-protocol set). Also, 39% of patients in the 170-μg group became nonreactive to CPT vs 18% in the placebo group. Facilitated allergen-binding assays revealed a highly significant ($P < .001$) dose-dependent reduction in IgE allergen binding across all treatment groups (70 μg: 17.1%; 170 μg: 18.8%; 370 μg: 26.4%). Specific IgG_4 levels increased to 1.6-fold (70 μg), 3.1-fold (170 μg) and 3.9-fold (370 μg) (mPP).

Conclusion: Three-week immunotherapy with 170 μg LPP reduced CPT reactivity significantly and increased protective specific antibodies.

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1 | INTRODUCTION

Advances in allergen immunotherapy (AIT), particularly in subcutaneous (SCIT) and sublingual immunotherapy (SLIT), aim to further reduce safety concerns for severe systemic reactions (SRs) and anaphylaxis as well as to increase real-life effectiveness, particularly by improving compliance and acceptance among patients through shorter treatment with a more convenient product.[1] To achieve these goals, novel therapeutics have been developed to overcome the limitations of natural allergens' intrinsic features. Recent investigations on peptide immunotherapy focus on synthetic peptide immunoregulatory epitopes (SPIREs) containing T cell–reactive short peptides[2] and longer continuous overlapping peptides (COPs)[3] of up to 80 amino acids.[4] Sets of long COPs that encompass all potential T-cell epitopes without IgE conformations induce IgG_4 but also evoke late asthmatic responses at high concentrations.[4,5]

Mixtures containing grass allergens from the *Pooideae* subfamily have been shown to possess no advantage over single grass allergen extracts, which produced completely cross-reactive IgG_4 and were substituted for multiple grass subfamilies.[6,7] Perennial ryegrass (*Lolium perenne, L. perenne*) contains group 1, 2/3, 4, 5, 11, 12 and 13 allergens.[6] *Lolium perenne*, like the other members of the *Pooideae* subfamily, possesses strong cross-allergenicity, which is attributable to the high homology of groups 1, 2/3 and 5.[8] In this trial, different lengths of *L. perenne* peptides (LPPs) obtained from enzymatic hydrolysis were administered subcutaneously in a short up-dosing phase. We determined the optimum dose of LPP in terms of safety as well as clinical and immunological effects in patients with seasonal allergic rhinoconjunctivitis (SAR).

2 | METHODS

2.1 | Trial design

This randomized, parallel-group, double-blind, placebo-controlled, dose-finding trial was conducted at 23 outpatient study centres. Patients were screened in mid-August 2014, and enrolled participants completed the study by mid-November 2014 after having attended 7 visits (V1–V7). Conjunctival provocation test (CPT) responses and immunogenicity parameters of placebo were compared with those of 3 different cumulative peptide doses (70, 170 and 370 μg) administered postseasonally. Inclusion/exclusion criteria are reported in Table S1 in this article's Online Repository.

2.2 | Study medication

The adjuvant-free immunotherapy peptides used in this trial were extracted from whole ryegrass pollen by enzymatic digestion and formulated for subcutaneous injections according to good manufacturing practice requirements (see Online Repository Methods) as described by Shamji et al.[9] ASIT biotech s.a. (Brussels, Belgium) provided labelled LPP and placebo treatment kits (per visit and treatment number).

2.3 | Planned interventions and timing

Patients received 10 subcutaneous injections of placebo or of increasing doses of peptides at 5 visits (V2–V6) to participating study centres within 4 weeks. The first injection at each visit was given in one arm and, if no major local or systemic allergic reaction occurred within 30 minutes, the second injection was given in the other arm. Patients stayed at the study centre for another 30 minutes and were monitored closely. Injection volumes increased for all patients according to Table 1. Wheals and redness reactions were measured 30 minutes after each injection and recorded by the patient in a diary on the next 3 evenings. SRs were classified according to the German anaphylaxis guideline.[10] Investigators issued 3 tablets of rescue medication (cetirizine dihydrochloride, 10 mg per os, once daily) at each visit to all patients to relieve mild local reactions after injections if necessary.

Doses were adjusted as follows: if a wheal measuring 5-8 cm in diameter appeared within 30 minutes after an injection or if an SR grade I occurred, the same dose was repeated for the following injection. If the wheal diameter was >8 cm 30 minutes after an injection or if an SR grade II occurred, the dose was reduced by one step for the next injection. Patients were to be excluded from further participation in the treatment if an SAE or SRs grade III or IV occurred.

2.4 | Conjunctival provocation test

Conjunctival provocation tests (CPTs) were conducted[11] and recorded[12] as described before. The allergen extract ALK-lyophilized grass (ALK-Abelló, Wedel, Germany) was used in concentrations of 100, 1000 and 10 000 SQ-U/mL. CPT responses ≥ stage II according to the Riechelmann scale[11] were considered positive. If baseline CPT responses at V1 and V2 differed by one concentration stage, the higher concentration step was used for further analyses. CPTs were performed at baseline, V6 and V7. At V2 and V6, CPTs were conducted before the study medication was administered.

The CPT score was calculated as follows: 0 = no reaction at all, 1 = reaction at 10 000 SQ-U/mL, 2 = reaction at 1000 SQ-U/mL and 3 = reaction at 100 SQ-U/mL. To calculate the mean composite score, CPT scores of all grass allergen concentrations used in the individual tests were combined as described before.[13,14]

Conjunctival provocation test (CPT) results are a predictive surrogate marker for SAR severity, as reduced CPT reactivity after

TABLE 1 Cluster titration schedule

Visit (days)	1 (−d 35 to −d 2)	2 (d 1)	3 (d 8 ± 2)	4 (d 15 ± 2)	5 (d 22 ± 2)	6 (d 29 ± 2)	7 (d 36 ± 2)
CPT	X	X[a]				X[a]	X
Placebo group							
Inj. 1 (μg)		0	0	0	0	0	
Inj. 2 (μg)		0	0	0	0	0	
70 μg group							
Inj. 1 (μg)		5	10	20	0	0	
Inj. 2 (μg)		5	10	20	0	0	
170 μg group							
Inj. 1 (μg)		5	10	20	50	0	
Inj. 2 (μg)		5	10	20	50	0	
370 μg group							
Inj. 1 (μg)		5	10	20	50	100	
Inj. 2 (μg)		5	10	20	50	100	

CPT, conjunctival provocation test; IMP, investigational medicinal product.

[a]CPT was performed before the administration of the IMP.

preseasonal SLIT predicted significantly fewer seasonal SAR symptoms, less rescue medication use and an increased number of well days.[14]

2.5 | Study endpoints

2.5.1 | Efficacy endpoints

The primary efficacy endpoint was defined as the proportion of patients whose CPT reactivity to the different allergen extract concentrations decreased from baseline to V7. The secondary efficacy endpoints included the proportion of patients whose CPT reactivity to the different allergen extract concentrations decreased from baseline to V6, composite and CPT score reductions, as well as immunological changes.

2.6 | Immunological responses

Sera were collected from all patients at the screening visit (V1) and at the follow-up visit (V7, after finishing treatment). Immunoglobulin analyses measured grass pollen–specific IgG (sIgG), IgG$_4$ (sIgG$_4$) and IgE (sIgE) levels using the ImmunoCap® system (Pharmacia AB, Uppsala, Sweden).

The production of blocking antibodies was assessed using a functional assay.[15-17] Relative allergen-IgE complex binding to CD23 detected in the presence of patient and indicator serum was expressed as the percentage of binding observed in a reference condition with indicator serum only. The production of blocking antibodies was reflected by a decrease in complex binding.

2.7 | Statistics

The sample size was calculated under the assumption that a maximum of 40% of placebo group patients and 75% or more of the

actively treated patients would improve.[18] Given a 5% error and a power of 90%, Wilson's method estimated a group size of 46.

Statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA), and data were described in means and standard errors of the mean. P values vs placebo were obtained using the two-tailed Fisher's exact test or the two-tailed Mann-Whitney U test, with $P < .05$ considered as significant.

A group sequential analysis was conducted under the null hypothesis that there would be no difference between the treatment groups regarding the proportion of patients with a reduction in CPT reactivity to a certain concentration of grass pollen allergen between baseline and V7.

3 | RESULTS

3.1 | Demographic data and baseline values

Of 240 screened patients, 198 were randomized to the placebo, 70-, 170- and 370-μg groups (46, 50, 49 and 53 patients, respectively). Of those randomized, 192 patients received at least one dose of placebo or LPP and provided at least 2 evaluable CPT data sets to be included in the modified ITT set (mITT set). Patients who had completed the up-titration schedule without any per-protocol dose adjustment were analysed in the modified PP set (mPP set) (Figure 1). The exploratory immunogenicity parameters set (EIP set) consisted of patients who supplied at least one blood sample for exploratory immunogenic analyses (Figure 1).

Patients in the safety set showed a mean age of 36.9 years (Table 2). Mean duration of SAR and wheal size in the SPT for grass pollen were similar across all groups (Table 2). Specific IgE to grass pollen was significantly higher at baseline in the 70-μg ($P = .004$) and 370-μg groups ($P = .031$) than in the placebo group. Most patients had sIgE levels belonging to classes 3 and 4
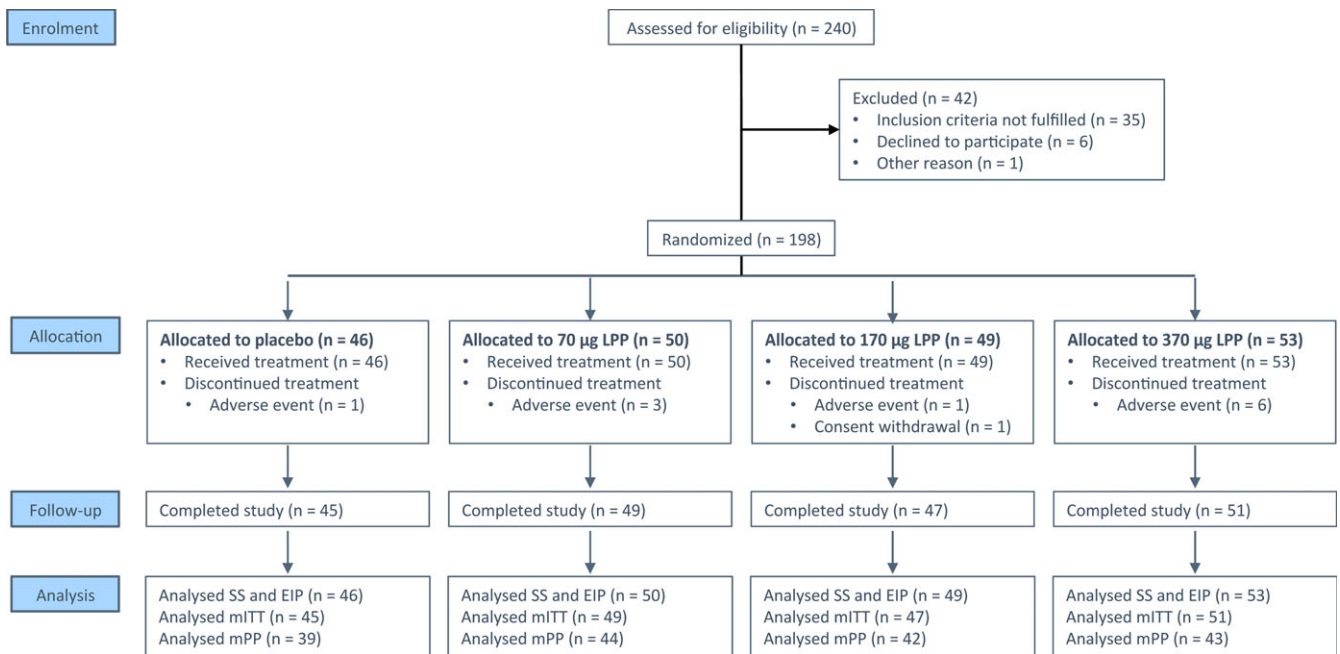
FIGURE 1 CONSORT flow diagram of the trial. EIP, exploratory immunological parameters; LPP, *Lolium perenne* peptide; mITT, modified intention to treat; mPP, modified per protocol; SS, safety set [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Baseline demographic data for the safety set

Characteristic	Placebo	70 µg	170 µg	370 µg	Total
Total (n)	46	50	49	53	198
Total (%)	23.2	25.3	24.8	26.8	100
Female (n)	27	22	20	22	91
Female (%)	58.7	44.0	40.8	41.5	46.0
Male (n)	19	28	29	31	107
Male (%)	41.3	56.0	59.2	58.5	54.0
Age (y), mean ± SEM	39.2 ± 1.97	35.1 ± 1.73	36.9 ± 1.58	36.6 ± 1.88	36.9 ± 0.90
Duration of SAR (y), mean ± SEM	18.7 ± 1.90	18.6 ± 1.76	20.6 ± 1.71	17.5 ± 1.73	18.8 ± 0.88
Asthmatic patients (%)	17.4	26.0	24.5	17.0	21.2
SPT grass pollen (mm), mean diameter ± SEM	7.5 ± 0.49	8.5 ± 0.56	7.8 ± 0.46	7.6 ± 0.38	7.9 ± 0.47
sIgE grass pollen (kU/L)[a], mean ± SEM	20.4 ± 4.31	29.5 ± 3.94	23.8 ± 4.25	29.5 ± 4.49	26.0 ± 2.13
CAP class grass pollen[a], mean ± SEM	3.2 ± 0.17	3.7 ± 0.15	3.5 ± 0.16	3.6 ± 0.18	3.5 ± 0.08

CAP, carrier polymer system; SAR, seasonal allergic rhinoconjunctivitis; SEM, standard error of the mean; SPT, skin prick test.
[a]As determined in the local laboratories.

(38.4% and 27.8%, respectively). One-fifth of the patients had asthma (Table 2).

3.2 | Reduction in CPT reactivity from baseline to V7

In the mPP set, exploratory analyses showed the most prominent decrease in CPT reactivity from baseline in the group receiving 170 µg, followed by those receiving 370 µg, 70 µg and placebo (Figure 2A). Similarly, in the mITT set CPT reactivity decreased from baseline to V7, the greatest decrease being observed in patients

receiving 170 µg, followed by those receiving 370 µg, 70 µg and placebo (Figure S1A).

Improvements were significantly greater in patients receiving 170 µg (P = .023 for the mPP set and P = .022 for the mITT set) than in those receiving placebo.

3.3 | Reduction in CPT reactivity from baseline to V6

At V6, which took place 1 week after the 170- and 370-µg groups reached a cumulative dose of 170 µg, the combined group analysis of
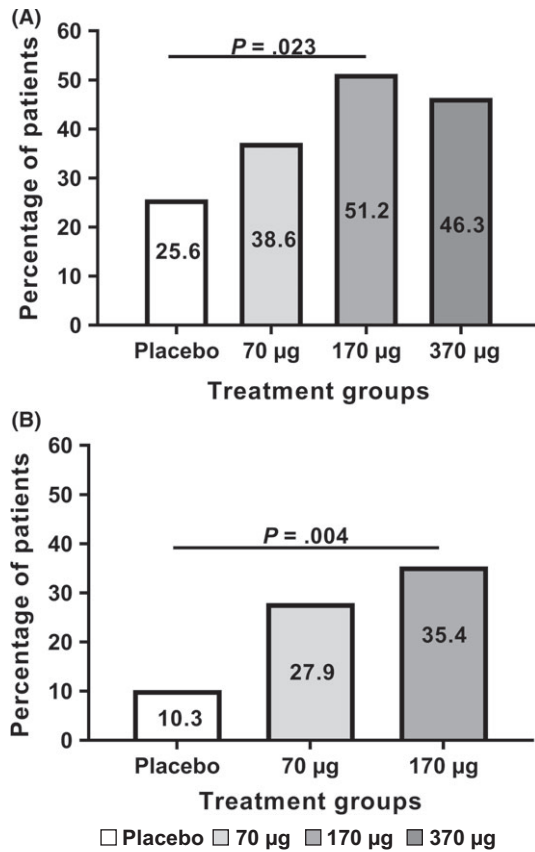
FIGURE 2 Percentages of patients (mPP set) whose reactivity in the CPT decreased from baseline to V7 (A) and V6 (B). Patients were treated with either placebo or increasing doses of LPP to reach cumulative doses of 70, 170 or 370 μg. The CPT was performed at screening, at V6 and at V7. At V7, all patients had reached the cumulative dose of their respective group. Data are presented as the percentages of patients with an improvement compared to baseline in the mPP set (placebo: n = 39; 70 μg: n = 43; 170 μg: n = 41; 370 μg: n = 41). At V6, patients allocated to the 170- and 370-μg groups had received 170 μg of LPP and were pooled for this analysis (n = 82). *P* values vs placebo were obtained using Fisher's exact test

CPT reactivity showed a significant decrease (mPP set: *P* = .004; mITT set: *P* = .008) in comparison with placebo (Figures 2B and S1B).

3.4 | Patients no longer reacting to conjunctival provocation

In the mPP set, the percentages of patients who no longer reacted to conjunctival provocation were 39.0% (370- and 170-μg groups), 27.9% (70-μg group) and 18.0% (placebo) after treatment completion (Figure 3). In the mITT set, the proportion of patients no longer reacting to conjunctival provocation at V7 was highest in the group receiving 170 μg and lowest in the placebo group (Figure S2).

3.5 | Mean composite scores

At baseline, mean composite scores[13,14] in the mPP set were similar across the groups: 0.35 (placebo), 0.39 (70 μg), 0.42 (170 μg) and
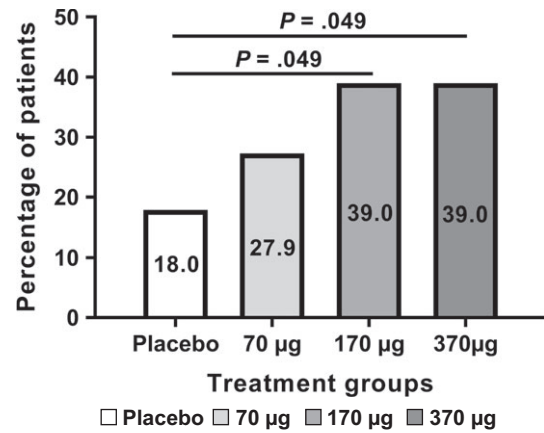

FIGURE 3 Percentages of patients in the mPP set (placebo: n = 39; 70 μg: n = 43; 170 μg: n = 41; 370 μg: n = 41) who became nonreactive to the highest allergen concentration used during the CPT after treatment (V7). Patients were treated with either placebo or increasing doses of LPP to reach cumulative doses of 70, 170 or 370 μg. *P* values vs placebo were obtained using Fisher's exact test

0.32 (370 μg). At V7, composite scores were significantly lower in the 170- and 370-μg groups than the score in the placebo group (*P* < .005) (Figure S3A). Similar results were obtained for the mITT set (Figure S4A).

3.6 | Mean CPT scores of conjunctival provocation analysis

In analogy to the composite scores, mean CPT scores (mPP) at baseline were similar across the groups: 1.28 (placebo), 1.26 (70 μg), 1.34 (170 μg) and 1.20 (370 μg). At V7, CPT scores were significantly lower in the 170- and 370-μg groups than the score in the placebo group (*P* < .015) (Figure S3B). Similar results were shown for the mITT set (Figure S4B).

3.7 | Immunological changes

An increase in sIgE levels was observed from baseline to V7 in the groups receiving LPP. At V7, these levels were significantly higher in the LPP groups than the sIgE level in the placebo group (*P* < .021) (Figure 4A, Table S2).

Grass pollen–specific IgG levels also increased in the LPP groups from V1 to V7. At V7, sIgG levels were significantly higher in the LPP groups than the level in the placebo group (*P* < .009). Specific IgG levels in the placebo group remained unchanged (Figure 4B, Table S2).

Grass pollen–specific IgG₄ levels increased from V1 to V7 in the LPP groups but remained unchanged in the placebo group. At V7, these levels were significantly higher in the LPP groups than the sIgG₄ level in the placebo group (*P* < .001) (Figure 4C, Table S2).
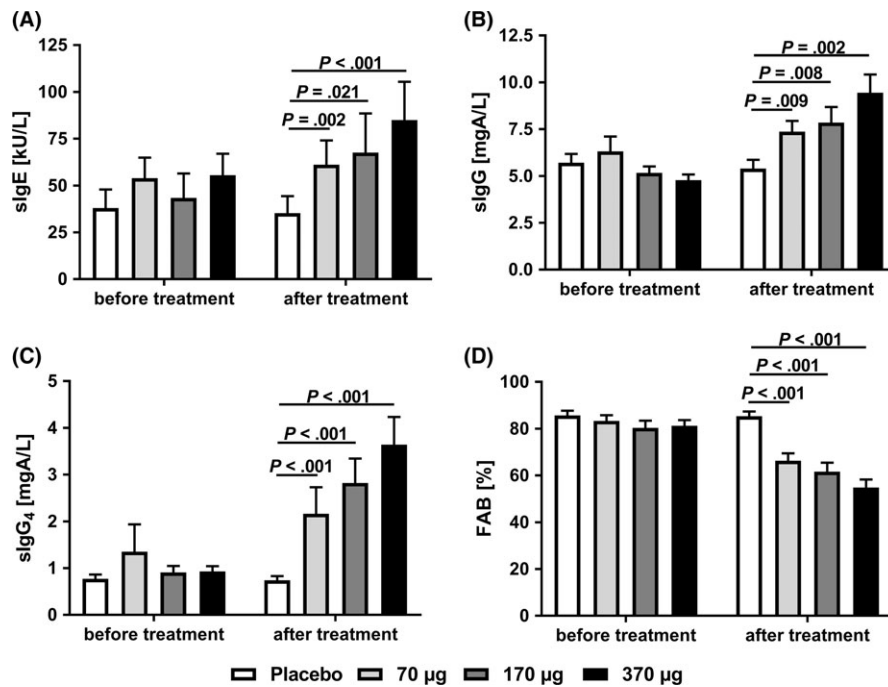
FIGURE 4 Immunogenicity before and after treatment in the EIP set. (A), Grass pollen–specific IgE (sIgE), (B) grass pollen–specific IgG (sIgG), (C) grass pollen–specific IgG_4 (sIgG$_4$) and (D) facilitated allergen binding (FAB). Patients were treated with either placebo or increasing doses of LPP to reach cumulative doses of 70, 170 or 370 μg. Data are presented as mean ± SEM; P values vs placebo were obtained using the Mann-Whitney U test

Blocking antibodies were induced in a dose-response manner. The inhibitory effect was evident in the significant difference in allergen binding at V7 observed between all 3 treatment groups and placebo ($P < .001$): 26.4% (370 μg), 18.8% (170 μg), and 17.1% (70-μg group). No change was observed in the placebo group (Figure 4D, Table S2).

A significant correlation was observed between the induction of specific IgG_4 and the induction of blocking antibodies in all LPP-treated groups but not in placebo recipients. The correlation remained significant when considering the whole treated population (Figure S5). Moreover, a modest but significant correlation was observed between CPT reduction in reactivity after treatment and blocking antibodies (Spearman $r = .1849$, $P = .011$). However, within individual groups, that is placebo, 70-, 170- and 370-μg groups, no correlations were observed between blocking antibodies and the reduction in CPT reactivity. Moreover, no correlation was found between the reduction in CPT reactivity and the IgG_4:IgE ratio at V7.

3.8 | Safety and clinical tolerability

There were no reports of SRs grade III or IV, anaphylactic reactions requiring the use of epinephrine or fatalities. Twenty-six SRs, most of which were grade I and of mild severity, occurred in 22 participants. In 89.9% of the patients, no SR occurred at all.

Mean wheal diameters 30 minutes after injection varied from 0.02 to 0.1 cm in the placebo group and from 0.07 to 0.83 cm in patients receiving LPP. There was neither a clinically relevant nor a

statistically significant increase in wheal size when the LPP dose increased (Figure S6). Patients recorded decreasing wheal diameters and redness from the evening of injections to the following 2 days. Use of antihistamines was rare.

Unsolicited treatment-emergent adverse events (TEAEs, n = 156) were reported by 75 patients (36.9%) (Table S3). Of these TEAEs, 78.2% were classified as mild. Four patients (3.8%) had severe AEs: 2 with grade II reactions (one being reported as an SAE based on prophylactic hospitalization overnight), one with a severe local reaction, and one with an AE unrelated to treatment. A total of 178 patients (70 μg: 94.0%; 170 μg: 89.8%; 370 μg: 77.4%) completed up-titrations per schedule. Twenty actively treated patients (10.1%) did not reach their group's full cumulative dose. Of those, 11 (5.6%) patients discontinued treatment following an AE, and one patient withdrew consent for personal reasons (Table S4). Fifteen patients (7.6%), including 2 patients in the placebo group, underwent 19 dose adjustments (Table S3); 2 of these 15 patients discontinued the trial permanently. The safety and tolerability profile of the asthmatic patients (21.2%) matched that of the nonasthmatic population.

4 | DISCUSSION

The aim of this study in patients with allergic rhinitis was to establish an optimal dose in terms of clinical effect, clinical tolerability and safety of increasing doses of LPP when administered by subcutaneous injections. The clinical effect was assessed using the CPT,

which has been shown to be a reliable surrogate marker in the diagnosis of SAR and in the prediction of allergic rhinoconjunctivitis symptoms during the season in patients treated with preseasonal SLIT tablets.[19,20]

This dose-finding study showed the largest reduction in reactivity to CPT after 4 bilateral injections over 3 weeks and at a cumulative dose of 170 µg. Higher doses did not improve clinical effectiveness, making the cumulative dose of 170 µg the optimum dose. A clinical meaningful benefit was noted, as 39% of participants became completely tolerant to allergen challenge after the short treatment course employed in this study. Other recent SCIT studies have shown that short treatment phases comprising only a few weeks can have clinical and immunological effects.[21-24]

Moreover, 51.2% of patients in the 170-µg group (mPP set) showed significantly higher CPT response thresholds than those in the placebo group. By comparison, Riechelmann et al[25] reported decreased CPT reactivity in 51.0% of patients after 1 year of glutaraldehyde-modified house dust mite SCIT. Jutel et al[1] reported reductions in CPT reactivity that were not significantly different from those of placebo-treated patients after 6 weeks of recombinant grass pollen SCIT (n = 54, P = .081).

Our findings suggest most prominently in the mPP set that a plateau of dose-response effect is reached at a cumulative dose of 170 µg LPP. Similarly, *Felis domesticus* 1 (Fel d 1) SPIRE findings showed a better effect on late-phase allergic skin reactions with 3 nmol than with 12 nmol of peptides. Results of trials investigating COPs demonstrated greater improvements in rhinoconjunctivitis symptom scores using 5 injections with 50 µg than with 100 µg (P = .015).[4,26] Klimek et al[27] reported the highest percentages of CPT response threshold improvements (90% of patients) in the group receiving lower doses of a recombinant 5-grass pollen SCIT (40 µg) compared to 50% improvement under placebo (P = .466).

In comparison with other studies, a limited placebo effect was observed. In the mPP set at V7, 25.6% of placebo group patients showed less CPT responsiveness and 18.0% showed no reaction. However, another trial reported that 30.0% of patients receiving placebo showed an increase in their CPT threshold concentrations.[28] Fifty per cent of control group patients in the study by Klimek et al exhibited decreased CPT reactivity after receiving placebo. The authors argued that post-treatment reprovocations at threshold doses from baseline (without prior up-dosing and less cumulative allergen) were a possible cause.[27] Jutel et al[1] were unable to demonstrate significantly different CPT results between placebo and actively treated groups, with 53.8% of the placebo group patients having higher CPT response thresholds. Hüser et al[29] reported the highest number: 64.3% of placebo group patients showed reduced post-treatment CPT reactivity. Composite scores confirmed that no desensitization occurred in the placebo group of our study. In fact, conjunctival allergic inflammation under provocation (represented by the composite score) increased from baseline.

The positive effect on immunological serum parameters underscores the clinical effect of LPP. Facilitated allergen binding (FAB) assays revealed a dose-dependent induction of blocking antibodies parallel to that of $sIgG_4$ (Table S2).

A study comparing SCIT and SLIT in terms of immunogenicity deduced that the maximum changes in sIg and blocking antibodies were reached after 3 months of treatment. Facilitated allergen binding (FAB) inhibition after 1 month was less than 5% for SCIT and nonexistent for SLIT.[30] Nevertheless, in our study, LPP immunotherapy led to FAB inhibition that after 4 weeks was 26.4% greater than that at baseline (370-µg group). Shamji et al[16] observed FAB inhibitions of 24.70% ± 1.79% after double the treatment time (8 weeks) with 100 000 SQ grass pollen SCIT (n = 108).

Cumulative doses higher than 170 µg had no additional clinical benefits but increased immunological surrogate markers in this study. It remains unclear whether the greater induction of blocking antibodies and higher $sIgG_4$ levels in the 370-µg group would offer clinical benefits in the long run. The discrepancy in the dose-response curves of CPT and immunological parameters could have been the result of multiple factors, including (i) a difference in the kinetics of clinical and immunological effects and (ii) the reduction in CPT reactivity being considered a local response compared to the systemic production of serum-specific antibodies.

While successful AIT correlates with higher IgG_4 levels, they might not be a prerequisite for clinical efficacy.[31] Phase IIa/IIb trials using COPs from the Bet v 1 (*Betula verrucosa*, birch allergen) sequence showed an up to 40-fold increase in IgG_4 levels, measured 85 days after the first injection, and a 20-fold increase in IgG_4 after 60 days of treatment.[32] There were no significant differences in specific IgG_4 levels between the low- and high-dose birch COP-treated patients.[4]

The absence of a significant correlation between blocking antibodies and CPT scores in individual treated groups in this study may be explained by the fact that the CPT data were categorical and in a low range (−1, 0, 1, 2, 3) while those of specific IgG_4 and FAB were continuous. Blocking antibodies have been shown to correlate with clinical response following long-term treatment.[16]

The tolerability and safety of the peptide treatment are as important as its clinical or immunological efficacy. The safety profile of the peptides used in this study was comparable to that of conventional SCIT: a Cochrane meta-analysis of SCIT showed that 19% of patients experience SRs.[33] Frew et al[34] reported up to 25.6% early SRs during 8-week conventional grass allergen SCIT. In a study using recombinant grass allergens, 7 of 62 randomized patients (11.3%) had SRs, corresponding to 0.96% of 731 active treatment injections and 0.47% of 1,479 overall injections during 10 weeks of up-dosing and a dose maintenance phase of 2 subsequent pollen seasons.[1] Another study using a mixture of 5 recombinant grass pollens reported SRs in 16% of the patients (8/50). This corresponded to 2.21% of all injections, assuming that patients had received 13 dose-escalation injections with maximum doses of up to 20, 40, 80 and 120 µg in 2-6 months.[27]

This trial showed a lower percentage rate for SRs than all the above trials, with 10.1% of the patients experiencing such events. Although that figure corresponds to 1.36% of all injections, it is

important to note that this trial followed a rapid up-titration protocol, which is more likely to elicit SRs than dose maintenance phases having longer up-dosing schedules. Most TEAEs occurred at doses below 100 µg and no late SRs were observed.

As for the tolerability in terms of local reactions, all patients in our study reported mild local erythema and wheals at the injection site within the first 30 minutes at least once (Figure S6). Good clinical tolerability of grass pollen carrier-based fusion proteins was shown in 60 patients with almost no immediate wheal reactions and no positive late-phase skin reactions after 48 hours of atopy patch testing.[35]

5 | CONCLUSION

Three-week treatment with an adjuvant-free formulation of LPP significantly reduced CPT reactivity in grass pollen–sensitized SAR patients. It appeared to have a more positive impact on FAB inhibition and sIgG$_4$ production than did conventional SCIT, and it also gave rise to lower sIgE levels. Seasonal clinical efficacy and safety of LPP during natural allergen exposure are currently being investigated in a large phase-III clinical trial (EudraCT number: 2015-002105-11).

AUTHOR CONTRIBUTIONS

RM and EMK contributed equally to this work and share first authorship. RM, SP, SRD, TL and MHS conceptualized and/or contributed to the research hypothesis and study design. RM, LS and GZ coordinated the clinical study. MHS, EMK and SRD conducted the experimental work. JS developed the eCRF, and KS and AA performed the statistical analyses. RM, EMK, ER, LS, SP, TL, GZ and MHS participated in the discussions of data analysis and interpretation. RM, SP, LH, EMK, ER and MHS finalized the manuscript. All authors critically revised the drafted and gave final approval of the submitted version of the manuscript.

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REFERENCES

1. Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol*. 2005;116:608-613.
2. Couroux P, Patel D, Armstrong K, Larché M, Hafner RP. Fel d 1-derived synthetic peptide immuno-regulatory epitopes show a long-term treatment effect in cat allergic subjects. *Clin Exp Allergy*. 2015;45:974-981.
3. Pellaton C, Perrin Y, Boudousquié C, et al. Novel birch pollen specific immunotherapy formulation based on contiguous overlapping peptides. *Clin Transl Allergy*. 2013;3:17.
4. Spertini F, DellaCorte G, Kettner A, et al. Efficacy of 2 months of allergen-specific immunotherapy with Bet v 1-derived contiguous overlapping peptides in patients with allergic rhinoconjunctivitis: results of a phase IIb study. *J Allergy Clin Immunol*. 2016;138:162-168.
5. O'Hehir RE, Prickett SR, Rolland JM. T Cell Epitope Peptide Therapy for Allergic Diseases. *Curr Allergy Asthma Rep*. 2016;16:14.
6. Gangl K, Niederberger V, Valenta R. Multiple grass mixes as opposed to single grasses for allergen immunotherapy in allergic rhinitis. *Clin Exp Allergy*. 2013;43:1202-1216.
7. Hejl C, Wurtzen PA, Kleine-Tebbe J, Johansen N, Broge L, Ipsen H. Phleum pratense alone is sufficient for allergen-specific immunotherapy against allergy to Pooideae grass pollens. *Clin Exp Allergy*. 2009;39:752-759.
8. Weber RW. Patterns of pollen cross-allergenicity. *J Allergy Clin Immunol*. 2003;112:229-239.
9. Shamji MH, Ceuppens J, Bachert C, et al. Lolium perenne peptides for the treatment of grass pollen allergy: a randomized, double-blind, placebo-controlled clinical trial. *J Allergy Clin Immunol*. 2017; http://www.jacionline.org/article/S0091-6749(17)31572-5/fulltext.
10. Ring J, Beyer K, Biedermann T, et al. Guideline for acute therapy and management of anaphylaxis — S2 Guideline of DGAKI, AeDA, GPA, DAAU, BVKJ, ÖGAI, SGAI, DGAI, DGP, DGPM, AGATE and DAAB. *Allergo J Int*. 2014;23:96–112.
11. Riechelmann H, Epple B, Gropper G. Comparison of conjunctival and nasal provocation test in allergic rhinitis to house dust mite. *Int Arch Allergy Immunol*. 2003;130:51-59.

12. Dogan S, Astvatsatourov A, Deserno TM, et al. Objectifying the conjunctival provocation test: photography-based rating and digital analysis. *Int Arch Allergy Immunol*. 2014;163:59-68.

13. Astvatsatourov A, Reydelet Y, Mösges R. Photodocumentation of Allergic Severity under Conjunctival Provocation. *Stud Health Technol Inform*. 2015;213:11-14.

14. Astvatsatourov A, Mösges R. Image-Based Assessment of Allergic Inflammation under Conjunctival Provocation. *Stud Health Technol Inform*. 2015;213:15-18.

15. James LK, Shamji MH, Walker SM, et al. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol*. 2011;127:509-516.

16. Shamji MH, Ljørring C, Francis JN, et al. Functional rather than immunoreactive levels of IgG_4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy*. 2012;67:217-226.

17. Shamji MH, Layhadi JA, Scadding GW, et al. Basophil expression of diamine oxidase: a novel biomarker of allergen immunotherapy response. *J Allergy Clin Immunol*. 2015;135:913-921.

18. Mösges R. Hydrolyzed grass pollen proteins for allergen immunotherapy. In: 14th International Paul-Ehrlich-Seminar, Allergen Products for Diagnosis and Therapy: Regulation and Science; 2014; Bad Nauheim, Germany: Paul-Ehrlich-Institute; 2014. p. 12.

19. Kruse K, Gerwin E, Eichel A, Shah-Hosseini K, Mösges R. Conjunctival provocation tests: a predictive factor for patients' seasonal allergic rhinoconjunctivitis symptoms. *J Allergy Clin Immunol Pract*. 2015;3:381-386.

20. Fauquert JL, Jedrzejczak-Czechowicz M, Rondon C, et al. Conjunctival allergen provocation test: guidelines for daily practice. *Allergy*. 2017;72:43-54.

21. Subiza J, Feliú A, Subiza JL, Uhlig J, Fernández-Caldas E. Cluster immunotherapy with a glutaraldehyde-modified mixture of grasses results in an improvement in specific nasal provocation tests in less than 2.5 months of treatment. *Clin Exp Allergy*. 2008;38:987-994.

22. Peñas A, García-González M, Cruz MJ, et al. Observational study of the safety of a cluster schedule for subcutaneous immunotherapy in a pediatric population. *J Investig Allergol Clin Immunol*. 2013;23:63-65.

23. Pfaar O, Wolf H, Klimek L, Schnitker J, Wüstenberg E. Immunologic effect and tolerability of intra-seasonal subcutaneous immunotherapy with an 8-day up-dosing schedule to 10,000 standardized quality-units: a double-blind, randomized, placebo-controlled trial. *Clin Ther*. 2012;34:2072-2081.

24. Moreno C, De San Pedro BS, Millán C, Panizo C, Martín S, Florido F. Exploratory study of tolerability and immunological effect of a short up-dosing immunotherapy phase with a standardised allergen extract derived from pollen of Olea europaea. *Clin Transl Allergy*. 2015;5:27.

25. Riechelmann H, Schmutzhard J, van der Werf JF, Distler A, Kleinjans HA. Efficacy and safety of a glutaraldehyde-modified house dust mite extract in allergic rhinitis. *Am J Rhinol Allergy*. 2010;24:e104-109.

26. Worm M, Lee HH, Kleine-Tebbe J, et al. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy. *J Allergy Clin Immunol*. 2011;127:89-97.

27. Klimek L, Schendzielorz P, Pinol R, Pfaar O. Specific subcutaneous immunotherapy with recombinant grass pollen allergens: first randomized dose-ranging safety study. *Clin Exp Allergy*. 2012;42:936-945.

28. Larché M. Update on the current status of peptide immunotherapy. *J Allergy Clin Immunol*. 2007;119:906-909.

29. Hüser C, Dieterich P, Singh J, et al. A 12-week DBPC dose-finding study with sublingual monomeric allergoid tablets in house dust mite-allergic patients. *Allergy*. 2017;72:77-84.

30. Aasbjerg K, Backer V, Lund G, et al. Immunological comparison of allergen immunotherapy tablet treatment and subcutaneous immunotherapy against grass allergy. *Clin Exp Allergy*. 2014;44:417-428.

31. Allekotte S, Shamji MH, Zadoyan GV, et al. Facilitated Allergen Binding (FAB) Is a Meaningful Immunological Biomarker for Monitoring Immediate Clinical Efficacy in Short-Term Peptide Allergen Immunotherapy. *J Allergy Clin Immunol*. 2016;137:AB403.

32. Spertini F, Perrin Y, Audran R, et al. Safety and immunogenicity of immunotherapy with Bet v 1-derived contiguous overlapping peptides. *J Allergy Clin Immunol*. 2014;134:239-240.

33. Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database of Systematic Reviews*. 2007, Issue 1. Art. No.: CD001936. https://doi.org/10.1002/14651858.CD001936.pub2.

34. Frew AJ, Powell RJ, Corrigan CJ, Durham SR; UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2006;117:319-325.

35. Niederberger V, Marth K, Eckl-Dorna J, et al. Skin test evaluation of a novel peptide carrier-based vaccine, BM32, in grass pollen-allergic patients. *J Allergy Clin Immunol*. 2015;136:1101-1103.

SUPPORTING INFORMATION

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