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Lolium perenne peptide immunotherapy is well tolerated and elicits a protective B-cell response in seasonal allergic rhinitis patients

R. Mösges[1,2] | A. F. Koch[1] | E. Raskopf[1] | J. Singh[1] | K. Shah-Hosseini[1] | A. Astvatsatourov[1] | B. Hauswald[3] | Y. Yarin[3] | F. Corazza[4] | L. Haazen[5] | S. Pirotton[5] | S. Allekotte[1] | G. Zadoyan[1] | T. Legon[5] | S. R. Durham[6] | M. H. Shamji[6]

[1]Institute of Medical Statistics, Informatics and Epidemiology (IMSIE), Faculty of Medicine, University of Cologne, Cologne, Germany

[2]CRI—Clinical Research International Limited, Hamburg, Germany

[3]Department of Oto-Rhino-Laryngology, University Clinic Carl Gustav Carus, Technical University Dresden, Dresden, Germany

[4]Laboratory of Brussels University Hospitals, Laboratory of Translational Research, Brugmann University Hospital, ULB, Brussels, Belgium

[5]ASIT biotech s.a., Brussels, Belgium

[6]Immunomodulation and Tolerance Group, Allergy and Clinical Immunology, Respiratory Science Division, National Heart and Lung Institute, Imperial College, London, UK

Correspondence
Ralph Mösges, Institute of Medical Statistics, Informatics and Epidemiology (IMSIE), Faculty of Medicine, University of Cologne, Cologne, Germany.
Email: ralph@moesges.de

Funding information
ASIT biotech s.a., Brussels, Belgium

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Abstract

Background: Systemic allergic reactions are a risk for allergen immunotherapy that utilizes intact allergen preparations. We evaluated the safety, efficacy and immune mechanisms of short-course treatment with adjuvant-free *Lolium perenne* peptides (LPP) following a 6-week dose-escalation protocol.

Methods: In a prospective, dose-escalation study, 61 grass pollen–allergic patients received 2 subcutaneous injections of LPP once weekly for 6 weeks. Safety was assessed evaluating local reactions, systemic reactions and adverse events. The clinical effect of LPP was determined by reactivity to the conjunctival provocation test (CPT). Specific IgE, IgG_4 and blocking antibodies were measured at baseline (V1), during (V6) and after treatment (V8).

Results: No fatality, serious adverse event or epinephrine use was reported. Mean wheal diameters after injections were <0.6 cm and mean redness diameters <2.5 cm, independent of dose. Transient and mostly mild adverse events were reported in 33 patients. Two patients experienced a grade I and 4 patients a grade II reaction (AWMF classification). At V8, 69.8% of patients became nonreactive to CPT. $sIgG_4$ levels were higher at V6 (8.1-fold, $P < .001$) and V8 (12.2-fold, $P < .001$) than at V1. The sIgE:$sIgG_4$ ratio decreased at V6 (−54.6%, $P < .001$) and V8 (−71.6%, $P < .001$) compared to V1. The absolute decrease in IgE-facilitated allergen binding was 18% ($P < .001$) at V6 and 25% ($P < .001$) at V8.

Conclusion: Increasing doses of subcutaneous LPP appeared safe, substantially diminished reactivity to CPT and induced blocking antibodies as early as 4 weeks after treatment initiation. The benefit/risk balance of LPP immunotherapy remains to be further evaluated in large studies.

KEYWORDS
allergen, grass pollen peptides, safety, subcutaneous immunotherapy, tolerability

Abbreviations: AE, adverse event; AWMF, Association of the Scientific Medical Societies in Germany; CPT, conjunctival provocation test; FAB assay, IgE-facilitated allergen binding assay; Ig, immunoglobulin; ITT, intention to treat; LPP, *Lolium perenne* peptides formulated for subcutaneous injection; PP, per protocol; SCIT, subcutaneous immunotherapy; sIg, grass pollen–specific immunoglobulin; SR, systemic allergic reaction; V, visit.

1 | INTRODUCTION

Conventional allergen injection immunotherapy, based on the concept of tolerance induction,[1,2] involves the administration of incremental doses of the sensitizing allergen followed by monthly high-dose maintenance injections for several years.[3] This paradigm, which is the basis for a personalized medicine approach using "named patient products" that are still common in the United States and in parts of Europe, has been shattered during recent years. Regulatory agencies, such as the German Paul-Ehrlich-Institut and the European Medicines Agency, regard allergen preparations as therapeutics similar to synthesized molecules and recommend a classical product development pathway.[4] This concept requires evidence of an optimal therapeutic dose, which is defined within the framework of efficacy, tolerability and safety. In this regard, the standard procedures of developing medicines also have to be applied to immunotherapeutics containing the prevalent allergens of the homologous groups of trees, grasses and house dust mites.[5] After preclinical tests assuring quality and harmlessness in terms of toxicology, the 3 classical phases of the product development route apply.

Lolium perenne peptides immunotherapy has been shown to have limited IgE binding, basophil and mast cell reactivity and hence is considered as a safe alternative that can be administered at higher doses and for a shorter period to improve treatment adherence.[6] Here, we report a proof-of-concept study, which involved an up-dosing regimen with the primary aim to assess safety and to identify an individual maximum tolerated cumulative dose for patients with different statuses of allergen sensitization. Furthermore, we investigated the immunological effects and a surrogate parameter of clinical efficacy.[7]

2 | METHODS

2.1 | Approvals and ethics

This study (EudraCT number 2013-000056-18) was approved by the Independent Ethics Committee (IEC) of the Technical University of Dresden (IEC number EK 53032013) and by the Paul-Ehrlich-Institut (PEI) as competent federal authority in Germany. The study was conducted in accordance with local regulations, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (International Conference on Harmonisation (ICH)–Good Clinical Practices (GCP)) and the Declaration of Helsinki.[8]

2.2 | Study design

This open-label, prospective, dose-escalation study was performed in grass pollen-allergic patients outside of the grass pollen season. Patients were required to give written informed consent before being included in the study. Participants were required to have a medical history of moderate-to-severe seasonal allergic rhinoconjunctivitis during the grass pollen seasons of at least the 2 previous years. In addition, a positive skin prick test (wheal diameter ≥3 mm for a grass pollen mixture) and grass pollen–specific IgE (sIgE) antibodies >0.7 kU/L were necessary for study inclusion (see Data S1 for further inclusion and exclusion criteria).

The study was carried out in the outpatient allergy clinic of the Department of Oto-Rhino-Laryngology at the University Hospital Carl-Gustav-Carus (Dresden, Germany) and consisted of 8 visits: 1 screening visit (V1), 6-weekly treatment visits (V2–V7) and 1 follow-up visit (V8), which took place 1–2 weeks after V7. The patients received 12 subcutaneous injections of increasing doses of LPP in form of a cluster scheme.[9] At each treatment visit (V2–V7), 2 injections of equal peptide dose were administered 30 minutes apart, one in each arm. After the second injection, patients remained under observation at the trial site for a further 30 minutes. Dose escalation started with a total LPP dose of 10 μg administered at V2 and increased to 20 μg at V3, 40 μg at V4, 80 μg at V5, 140 μg at V6 and 200 μg at V7, resulting in a cumulative dose of 490 μg.

2.3 | Formulation of the *Lolium perenne* peptides (LPP)

Based on the extensive immunological cross-reactivity among grass pollens belonging to different species, *Lolium perenne* (*L. perenne*)–derived allergens are considered appropriate for the treatment of IgE-mediated grass pollen allergy in general.[10] Briefly, *L. perenne* proteins were extracted from a natural source to obtain a crude extract, which was then purified of nonprotein components. The proteins were then denatured and enzymatically hydrolysed to generate peptides of 1000–10 000 Da.[6] The LPP were supplied in ready-to-use vials with a ryegrass pollen peptide concentration of 100 μg/mL in 1.5 mL aqueous-buffered solution (pH 7.4). The formulation contained no adjuvant.

2.4 | Administration site reactions, safety considerations

Local reactions after subcutaneous immunotherapy (SCIT) are common and may be accompanied by swelling, tenderness and occasional discomfort. Early reactions >5 cm are an indication for dosage adjustment, and large late local reactions may be a source of discomfort and inconvenience to patients. For this reason, we made objective measurements of early skin reactions at 30 minutes and late reactions up to 48 hours. The local reaction diameters (wheal and redness at the injection site) were recorded by the investigator 30 minutes after the injections and by the patients after 8 hours and in the evenings of day 1 and day 2 after injection.

All local symptoms at the injection site other than wheal and redness or systemic AEs were reported as unsolicited AEs and were coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 16.1). Systemic allergic reactions (SRs) emerging after an injection were graded appropriately as stage I to stage IV

according to the recommendations of the Association of the Scientific Medical Societies in Germany (AWMF: Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V.).[11] According to the protocol, if a wheal diameter measuring 5–8 cm appeared within 30 minutes after an injection or if an SR grade I occurred (AWMF classification), the same dose had to be repeated at the following injection. If a wheal diameter was >8 cm 30 minutes after an injection or if an SR grade II occurred, the dose was reduced by one step at the next injection. Patients were to be excluded from further participation to study treatment if an SR grade III or IV occurred.

2.5 | Reactivity to the conjunctival provocation test (CPT)

As a secondary endpoint, the CPT was used as a surrogate marker for the assessment of the clinical effects of the LPP treatment. The CPT was performed at V1, V6 and V8 using the allergen extract ALK-lyophilized SQ (ALK-Abelló, Hamburg, Germany) with standardized units (SQ-E/mL).[12] The stock solution consisted of 100 000 SQ-E/mL and was extemporaneously diluted to 10 000, 1000 and 100 SQ-E/mL. The test procedure was performed and evaluated according to the CPT protocol described by Riechelmann et al[13] and was considered positive if the response was stage II or higher. The CPT score was calculated as 0 = no reaction at all, 1 = reaction at 10 000 SQ-E/mL, 2 = reaction at 1000 SQ-E/mL and 3 = reaction at 100 SQ-E/mL.

2.6 | Allergen-specific immunoglobulin and blocking antibody production

Specific IgE and IgG_4 levels for 5 grasses (*Anthoxanthum odoratum*, *Lolium perenne*, *Phleum pratense*, *Secale cereale* and *Holcus lanatus*) were measured in serum at V1, V6 and V8 using the ImmunoCap® method (Pharmacia Diagnostics AB, Uppsala, Sweden). The IgG-associated blocking antibodies were measured by FAB assay[10,14,15] (see Data S1 for detailed description).

2.7 | Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows (version 22.0, IBM Corp., Armonk, NY, USA) and consisted of descriptive statistics including the mean and standard error of the mean (SEM). Changes in sIgE and $sIgG_4$ levels between V1 and V6 were analysed using the Friedman signed rank test, taking into account correction for multiple comparison. A $P < .05$ was considered providing the statistical significance threshold.

3 | RESULTS

3.1 | Demographic data and baseline values

Overall, 88 patients were screened. Of these, 20 patients did not fulfil the inclusion criteria or met at least one of the exclusion criteria, and 3 patients withdrew their consent (Figure 1). The intention-to-

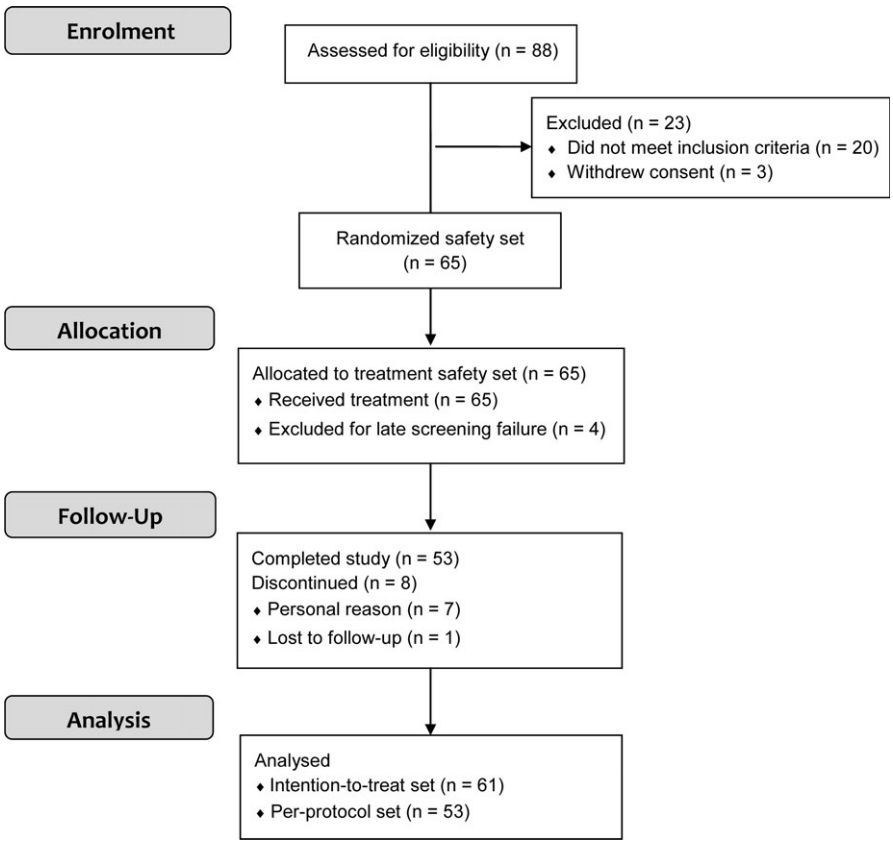

FIGURE 1 CONSORT flow chart of the study population

TABLE 1 Patient demographics

	Patients (N = 61)
Age (y), mean ± SD	36.0 ± 9.3 years
Gender, n (%)	
Male	40 (65.6)
Female	21 (34.4)
Disease duration (y), mean (range)	20.6 (3–58)
Grass pollen-specific IgE	
IgE serum level (kU/L), mean (range)	38.2 (1–101)
CAP class, mean (range)	4 (2–6)
Skin prick pest to grass pollen (mm), mean (range)	6.3 (4–15)

CAP, carrier polymer system; Ig, immunoglobulin; SD, standard deviation. Values are for the intention-to-treat (ITT) set.

treat analysis set (ITT set) included the patients who had completed at least one treatment visit and for whom the safety parameters had been documented. Four more patients had already received their first injection when a delayed laboratory analysis showed that they were ineligible. By consequence, they were excluded from further treatment. The safety population consisted of 65 patients, 61 of whom constituted the ITT set. The per-protocol set included 53 patients.

Patients in the ITT set had a medical history of seasonal allergy to grass pollen with a mean duration of 20.6 years (range: 3–58 years) (Table 1). Skin prick tests for grass pollen showed a mean wheal diameter of 6.3 mm (range: 4–15 mm). The mean sIgE level at baseline was 38.2 kU/L (range: 1.0–101.0 kU/L).

3.2 | Conjunctival provocation test (CPT)

The clinical effects of LPP were analysed using the surrogate parameter CPT. At baseline, a positive reaction was documented for 19.2% of patients at 100 SQ-E/mL, 53.8% of patients at 1000 SQ-E/mL and 26.9% of patients at 10 000 SQ-E/mL (Figure 2A). At V6, reactivity to the CPT was lower than that at baseline in 83% of patients. Most of the patients (67.3%) did not react at all to the CPT, and 25.0% of the patients reacted at the 10 000 SQ-E/mL level. At V8, CPT reactivity was decreased compared to baseline in 87.5% of the patients, and 69.8% of patients did not react to provocation at all. Fifteen patients (28.3%) reacted to the highest concentration of 10 000 SQ-E/mL, and only one patient (1.9%) reacted to 1000 SQ-E/mL. None of the patients reacted to 100 SQ-E/mL at V8. The improvement was also reflected by the change in the CPT score (Figure 2B). The mean CPT score was significantly lower at V6 ($P < .001$) and V8 ($P < .001$) than at V1. These findings are supported by the objective measurements of conjunctival redness using digital image analysis.[16] Figure 3 depicts the reduction in the proportion of red pixels in the region of interest as a sign of decreased conjunctival vasodilation.

3.3 | Safety

Fifty-three patients (86.9%) reached the target cumulative dose of 490 μg of LPP. No fatality or any other serious AE was reported during the study.

Thirty minutes after all LPP injections, mean wheal diameters at the injection site were well below the 5-cm threshold,[17] varying from 0.36 to 0.53 cm, independent of the LPP dose injected (5–100 μg) (Figure S1). The largest wheal diameter was 3.5 cm. Mean wheal diameters 8 hours after injections were somewhat larger (range: 0.43–0.75 cm) and decreased slightly 1 day after injections (range: 0–0.24 cm). Two days after injections, mean wheal diameters ranged from 0 to 0.12 cm. Only 4 patients experienced a wheal reaction greater than 5 cm at some time. Also, 97.8% of reactions reported in diary cards were mild (<5 cm), 2% were moderate (5–8 cm), and 0.2% were severe (>8 cm, with a maximum of 9.5 cm observed 8 hours after injection).

The mean redness diameters reported 30 minutes after injections were less than 2.3 cm throughout the study, with individual values

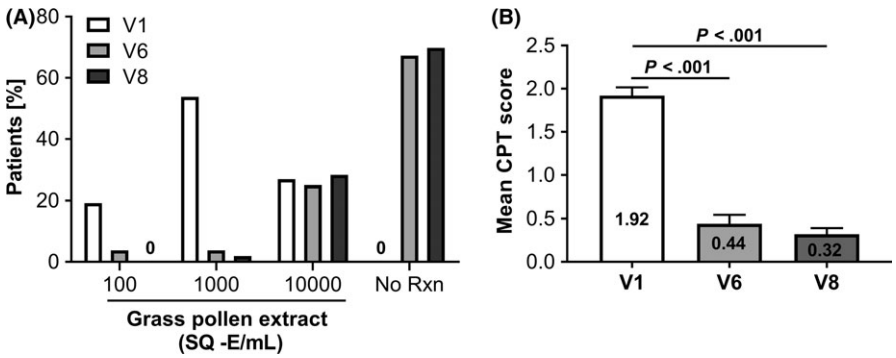

FIGURE 2 Effects of LPP immunotherapy on CPT reactivity and CPT scores (ITT set). (A) Proportion of reactive patients to CPT in response to 100, 1000 and 10 000 SQ-E/mL of grass pollen allergen extract at V1, V6 and V8. No Rxn represents patients nonreactive at 10 000 SQ-E/mL. Data are expressed as percentage of reactive patients. (B) Development of CPT score. Data are expressed as mean ± SEM, $P < .001$ compared to V1. CPT, conjunctival provocation test; ITT, intention to treat; LPP, *Lolium perenne* peptides formulated for subcutaneous injection; SEM, standard error of the mean; SQ-E, standardized quality units; V, visit

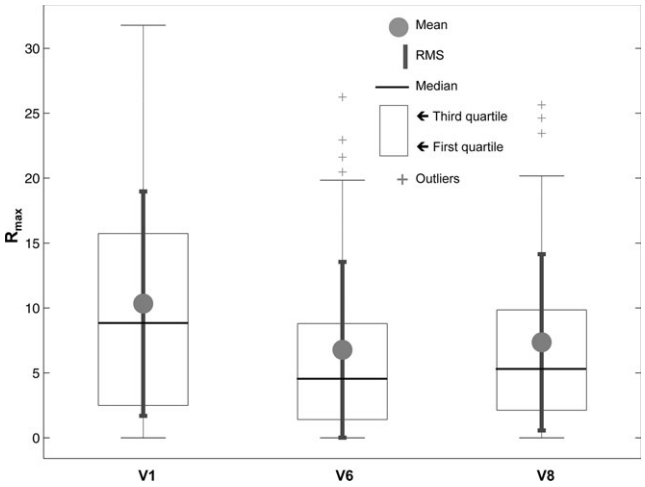

FIGURE 3 Digital analysis of the percentage of red pixels in the conjunctival region of interest (ROI) in patients undergoing conjunctival allergen challenge before (V1), during (V6) and after (V8) LPP immunotherapy (ITT set). R_{max} is the percentage of red pixels in the ROI following the maximum tolerated allergen concentration. ITT, intention to treat; LPP, *Lolium perenne* peptides formulated for subcutaneous injection; RMS, root mean square

ranging from 0 to 8.00 cm independent of the dose. Reactions were transient. Mean redness diameters increased slightly with time during the injection day (3.18 cm; maximum: 15.50 cm) and then decreased to less than 1.75 cm on day 1 and <0.5 cm on day 2 after injection.

Overall, AEs occurred in 33 patients. Furthermore, 83% of the AEs were considered related or possibly related to the study medication. The majority of AEs (96%) were classified as mild and 4% as moderate (one grade II reaction and one event of swelling of the upper arm were considered treatment-related). All AEs resolved spontaneously (32.1% of AEs) or with antihistamines and one case with a beta-agonist. No use of epinephrine or oral/IV steroid was reported. AEs related to treatment occurring in at least 5% of the patients are presented in Table S1. The most frequently reported AE was injection site pruritus (21.5% of patients).

Six patients (9.2%) experienced at least one SR (coded as hypersensitivity in Table S1). Two patients experienced an SR of grade I (AWMF classification), characterized by flushing of the face in the first patient and by pressure in the ears, facial erythema and itching of the mouth in the second patient. Four patients (6.2%) experienced SRs of grade II. The SRs included one case of facial flushing with nasal mucosa swelling (resolved with cetirizine and xylometazoline spray), one case of respiratory symptoms accompanied by eye redness (resolved with cetirizine) and one event of cough (treated with cetirizine). Another patient developed a first SR of grade II after receiving 10 μg of LPP (erythema, skin warmth and rhinorrhoea, treated with cetirizine). As defined in the protocol, the patient received 5 μg of LPP per injection at the next visit and tolerated it well. The patient experienced respiratory problems (SRs of grade II, treated with fenoterol) after receiving 10 μg of LPP, resulting in the discontinuation of treatment (as per protocol). All SRs occurred within 30 minutes after injection, except for one event of respiratory symptoms with eye redness that occurred 1 hour after injection, and all resolved within a few hours. No SR of grade III and IV was documented.

3.4 | Immunogenicity

Specific IgE levels to grass pollen were increased at V6 ($P < .001$) and at V8 ($P < .001$) compared to V1 (Figure 4A) but to a lesser extent than $sIgG_4$ serum levels (8.0-fold increase at V6 [$P < .001$] and 12.2-fold increase at V8 [$P < .001$]) (Figure 4B). Consequently, the $sIgE:sIgG_4$ ratio decreased from V1 to V6 ($P < .001$) and from V1 to V8 ($P < .001$) (Figure 4C). Treatment with LPP also induced a significant production of specific IgG ($P < .001$) but had no effect on IgA (data not shown). Serum inhibitory activity for IgE-FAB was assessed using the FAB assay. The values for the mean relative IgE-allergen complex binding to B cells were lower at V6 ($P < .001$) and at V8 ($P < .001$) than at V1. The absolute decreases for IgE-FAB were 18% (V6) and 25% (V8) (Figure 5A). Moreover, the change from baseline in the FAB value was strongly associated with specific IgG_4 at V6 (Spearman rank correlation coefficient: $r = .56$, $P < .001$) and V8 ($r = .45$, $P = .001$) (Figure 5B).

4 | DISCUSSION

In this study, we show for the first time that the use of hydrolysates of LPP escalated to a maximum cumulative dose of 490 μg was well tolerated (86.9% of the patients reached this target dose), eliciting only minimal early local and late skin responses. There were a few, mostly mild, systemic reactions to these high-dose allergen injections that required either no treatment or responded well to oral antihistamines and, in a single case, inhaled fenoterol. No serious adverse drug reactions, no anaphylactic reactions and no use of epinephrine were reported throughout the study. The overall good safety profile can be explained by the *ex vivo* characteristics of LPP (limited IgE binding, basophil and mast cell reactivity).[6] There was a marked and consistent reduction in the immediate conjunctival response to whole allergen extract and an increase in functional specific IgG antibodies.

Local reactions at the injection site represent a relevant parameter when addressing the safety of grass pollen SCIT. Thirty minutes after the injections, all mean wheal diameters were mild (below the 5-cm threshold)[17] and transient, highlighting the overall good safety profile of the LPP treatment administered in such a cluster schedule. Wheals were accompanied by a small area of redness. Mean wheal and redness diameters increased slightly 8 hours after the injection, and a few patients reported moderate/severe local reactions. Although inconvenient, delayed (6–12 hours) local reactions are not regarded as an increased risk for SRs.[18] The safety and tolerability of the LPP treatment appear to be better than those reported for an ultra-short up-dosing scheme with a 6-grass mix and rye allergen

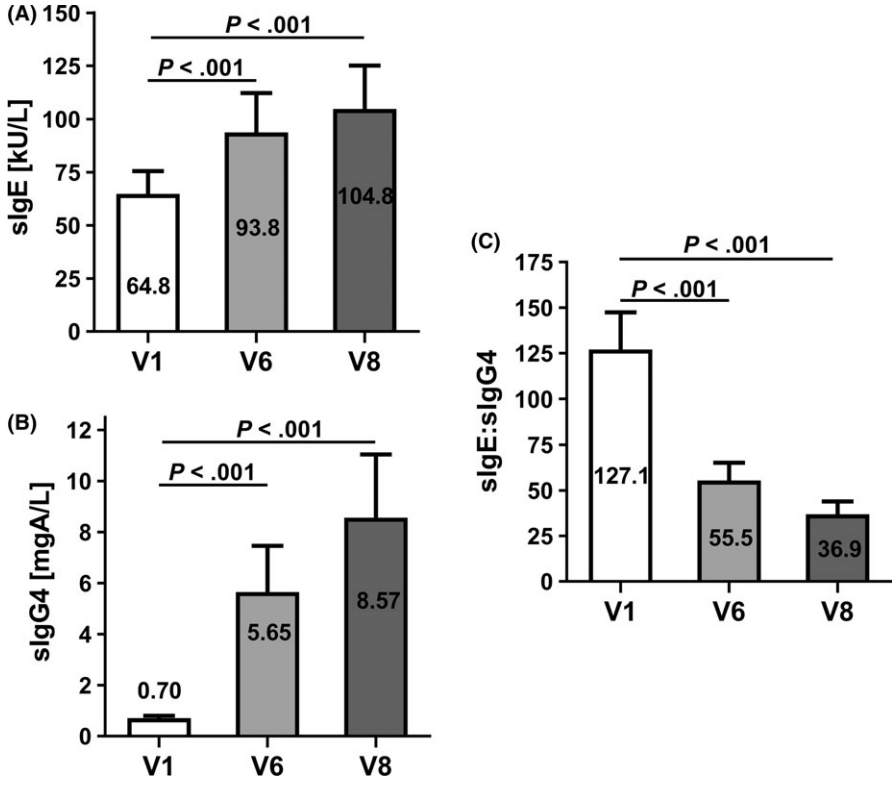

FIGURE 4 sIgE, sIgG$_4$ and sIgE:sIgG$_4$ ratio responses following LPP immunotherapy. (A) Induction of grass pollen–specific IgE; (B) sIgG$_4$ mean levels; and (C) sIgE:sIgG$_4$ ratio from V1 to V8 in patients treated with increasing doses of grass pollen peptides (ITT set). Data are presented as mean ± SEM, $P < .001$ compared to V1. Ig, immunoglobulin; ITT, intention to treat; LPP, *Lolium perenne* peptides formulated for subcutaneous injection; SEM, standard error of the mean; sIg, specific immunoglobulin; V, visit

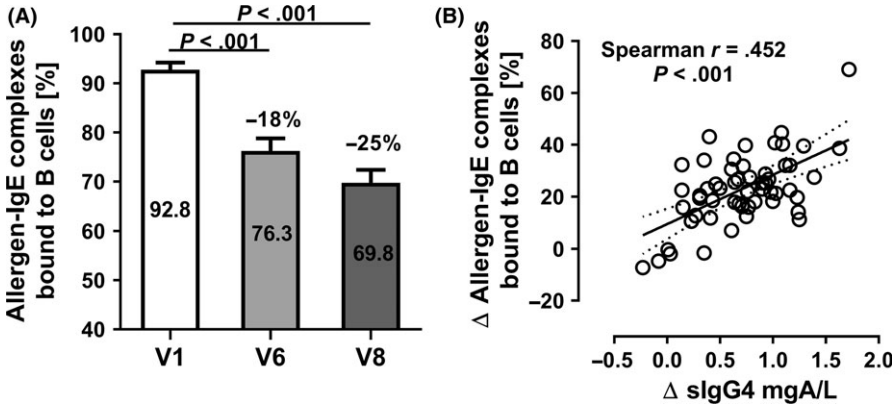

FIGURE 5 LPP peptide immunotherapy induces functional antibody responses that are closely associated with IgG$_4$ (ITT set). (A) Ag-IgE complexes binding to B cells were assessed by IgE-FAB assay using 0.3 μg/mL Phl p at V1, V6 and V8. Data are expressed as mean ± SEM, $P < .001$ compared to V1. (B) The change from baseline for Ag-IgE complexes binding to B cells was closely correlated with the change from baseline for sIgG$_4$. Spearman rank correlation coefficient $r = .452$, $P < .05$ was considered significant. Ag, allergen; FAB, facilitated allergen binding; Ig, immunoglobulin; ITT, intention to treat; LPP, *Lolium perenne* peptides formulated for subcutaneous injection; Phl p, *Phleum pratense*; SEM, standard error of the mean; V, visit

extract performed in-season.[19] In that study, wheal diameters ranging from 5 to 20 cm (11.9%) and even larger than 20 cm (0.3%) were observed 30 minutes after injection. The high local reactivity reported by the authors could be explained by the fact that product administration was performed during the pollen season.[20,21]

The analysis of the occurrence of SRs (9.2% of patients and 0.9% of injections, no grade III or grade IV SR) also suggests that LPP has a safety profile comparable to that of conventional SCIT, although a direct comparison is difficult due to differences in study designs and populations. A Cochrane meta-analysis of data on SCIT products used in 51 clinical studies showed that SRs occurred in 19% of patients.[2]

In a randomized, dose-ranging, safety study performed with recombinant *Phleum* grass pollen allergens absorbed to aluminium hydroxide, treatment-related SRs were reported in 16% of all patients and in 2.2% of all injections.[22,23] As observed with LPP, Klimek et al[22] did not detect a relation between the frequency of the SR and the increase in the injected dose. LPP may also have a safer profile than that of the Alutard® grass pollen product, as SRs were reported for 32.5% of patients treated with 100 000 SQ-U Alutard®

and for 21.2% patients in the 10 000 SQ-U group. Moreover, 9 non-life-threatening reactions of grade III (according to the EAACI classification) were observed.[24] In a more recent study, 7.1% of the patients experienced SRs following an ultra-short up-dosing schedule with Alutard SQ® up to the maintenance dose of 10 000 SQ-U.[19] SRs were also observed in 5.6% of the placebo group patients. The frequency of SRs reported by these authors is similar to the one observed here with LPP; however, no information on the severity of the SRs can be deduced from their study.[19] As mentioned earlier, SRs occurred in 0.9% of all injections in the present study. This is much lower than the frequency observed with the depot allergoid Purethal® (4.3%)[25] or with other SCIT products (reviewed in Ref[26,27]) and similar to the data reported after a conventional administration schedule with a 5-grass recombinant allergen mixture (0.98%).[28]

Although these data showed an overall good safety profile for LPP, they were obtained in a limited number of patients and need to be confirmed in larger studies.

Besides assessing the safety of LPP treatment, the CPT surrogate marker was used to follow up on the clinical status of the patients. Conjunctival provocation has been shown to be a reliable method for diagnosing allergy and for evaluating the clinical effect of immunotherapy products, especially if digital image analysis is used.[7,16] A decrease in CPT reactivity from baseline was observed in 83% (V6) and 87.5% (V8) of patients. In addition, most of the patients (67.3%) no longer reacted at all to the CPT at V6. No further reduction in CPT reactivity was found at V8 (69.8%). The objective assessment of conjunctival vasodilation using digital image analysis was in line with the findings of the investigators. These data are similar to those of Klimek et al,[22] although the studies are not directly comparable due to the different CPT provocation solutions and methodology used.

We acknowledge the limitation of this study due to the lack of a placebo arm. Nonetheless, these results clearly suggest a significant therapeutic effect can be observed after only 4 weeks of treatment and the administration of a cumulative dose of 150 μg of LPP.

As a secondary outcome, the immunological effect of LPP treatment was analysed by measuring sIgE and $sIgG_4$ levels as well as the production of functional blocking antibodies. Specific IgE increased during the course of the study (1.7-fold). Some studies postulated that an increase in sIgE levels following SCIT might be associated with an increased risk of systemic AEs.[29,30] With regard to the ratio of SRs to injections and the small (although statistically significant) increase in sIgE levels presented here, the number of SRs was not higher than that reported in other studies.[2,19-28]

As reported previously,[6] we observed a stronger induction of $sIgG_4$ as early as 4 weeks after the initiation of treatment, with a further increase following treatment completion. This resulted in a shift of the sIgE:$sIgG_4$ ratio towards the induction of tolerance. These data, especially the induction of $sIgG_4$, concur with those of previous studies investigating conventional and short-course adjuvant-containing SCIT and sublingual immunotherapy.[31-33] The desirable immunological effects, which are similar to those of conventional long-term SCIT treatment, elucidate the mechanisms of action of LPP. Moreover, to our knowledge, LPP is the first peptide preparation which induces the production of blocking antibodies against allergens as measured by a functional bio-cellular assay.[15] No other studies performed with peptides have demonstrated the induction of protective blocking antibodies following treatment.[34,35]

In summary, the short-course cluster treatment with the adjuvant-free LPP formulation investigated here showed an overall good safety and tolerability profile, with virtually no local pain, discomfort or swelling following injections being reported. LPP also elicited a positive immune effect and a clinical effect in seasonal allergic rhinitis patients. Larger studies are of course needed to confirm the safety and efficacy of cluster treatment with LPP, starting with a randomized, double-bind, placebo-controlled dose-finding study. However, the favourable data reported here suggest that LPP might lead to improved patient compliance and ultimately to improved efficiency.

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CONFLICT OF INTEREST

In adherence with the definition of authorship set forth by The International Committee of Medical Journal Editors, it is declared that this study is work for hire supported by ASIT biotech s.a., Brussels, Belgium (sponsor). LH is consultant to ASIT biotech s.a., reports receiving fees, and SP is employee of ASIT biotech s.a. SP and TL are shareholders of ASIT biotech s.a. FC received an honorarium from ASIT biotech s.a. for his work contributed here. RM reports personal fees from ALK-Abelló, personal fees from Allergy Therapeutics, personal fees from Allergopharma, grants and personal fees from Bencard, grants and personal fees from ASIT biotech s.a., personal fees from Bayer, personal fees from GSK, grants from HAL, personal fees from Johnson & Johnson, grants and personal fees from Lofarma, personal fees from MSD, personal fees from Menarini, personal fees from Faes, personal fees from Novartis, personal fees from Leti, grants from Optima, nonfinancial support from Greer, nonfinancial support from Roxall, grants from AIPrevent, personal fees from Servier, personal fees from Stada, grants and personal fees from Stallergènes, personal fees and nonfinancial support from UCB, grants from Ursapharm, grants from Bitop, grants from Hulka, nonfinancial support from Atmos, grants and personal fees from Arthrocare, personal fees from Hexal, personal fees from Meda, personal fees from Ohropax, outside the submitted work; RM is a member of the guidelines task force of the German Academy of Otorhinolaryngology, he is the chairman of ISCOANA, the International Standardization Committee of the European Rhinologic Society (ERS) and board member of the ENT Section of the European Academy of Allergy, Asthma and Clinical Immunology (EAACI). SRD reports grants from the ITN, NIAID, Regeneron, ASIT biotech s.a., ALK; nonfinancial support from ALK; personal fees from Anergis, Circassia,

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AUTHOR CONTRIBUTIONS

RM, SP, MHS, TL, SA and GZ conceptualized and delineated the research hypotheses. BH and YY performed the clinical study; MHS, SRD and FC performed the experimental work. JS developed the eCRF, and KS performed statistical analyses on all clinical and mechanistic data. AA developed the digital analysis method and analysed the CPT images. RM, MHS, SP, AFK, ER, SA, SRD and GZ participated in the discussions of data analysis and interpretation and contributed to the manuscript. The manuscript was finalized by RM, MHS, LH and SP with the assistance of all authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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