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Double-blind, placebo-controlled, dose-ranging study of new recombinant hypoallergenic Bet v 1 in an environmental exposure chamber

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Keywords
dose-ranging; environmental exposure chamber; hypoallergenic Bet v 1; immunotherapy; recombinant folding variant.

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Abstract

Background: Recombinant allergens offer a tool for improving specific immunotherapy (SIT).
Objective: To find the optimal dose of a new hypoallergenic folding variant of recombinant Bet v 1 (rBet v 1-FV) as SIT for patients with birch pollen allergy.
Methods: Before SIT, thirty-seven adult patients were exposed for eight hours in an environmental exposure chamber (EEC) to birch pollen at an average concentration of 3500 ± 500 grains/m^3, then randomized to four maintenance dose groups of rBet v 1-FV and one placebo group: 20 μg (n = 7), 80 μg (n = 8), 160 μg (n = 7), 320 μg (n = 8), and placebo (n = 7). Patients were treated for 10 weeks with weekly injections and then re-exposed in the EEC. The optimal dose for SIT was assessed using efficacy results from the EEC, IgG responses, and tolerability.
Results: Thirty-six patients were evaluable for efficacy assessment. The total symptom score significantly decreased in all active groups compared with placebo (−18.8% for placebo patients; −71.9%, P = 0.0022 for 20 μg; −75.6%, P = 0.0007 for 80 μg; −81.8%, P = 0.0009 for 160 μg; −78.3%, P = 0.0003 for 320 μg). IgG_1 increased significantly in all active groups compared to placebo. All four active doses were well tolerated, no serious adverse event occurred; two Grade II reactions, according to EAACI classification, were observed, one in each of the 160- and 320-μg groups.
Conclusions: Considering efficacy, immunological response, and tolerability, a maintenance dose of 80 μg of rBet v 1-FV appears to be the ideal dose for allergen immunotherapy in birch pollen allergic patients.

In Central and Northern Europe, birch pollen allergy is one of the main pollen allergies, affecting nearly 20% of exposed population (1), and in some countries more than half of the patients undergoing allergy tests to inhalant allergens are sensitized to birch pollen (2). Double-blind placebo-controlled (DBPC) studies have demonstrated efficacy of birch pollen–specific immunotherapy (SIT) in the past (3–5): accordingly, birch pollen allergy is one of the main indications for SIT

Abbreviations
AE, adverse event; ANCOVA, analysis of covariance; AUC, area under the curve; Bet v, *Betula verrucosa*; cDNA, complementary DNA; CFDR, Canadian food and drug regulations; DBPC, double-blind, placebo-controlled; EAACI, European academy of allergology and clinical immunology; EAST, enzyme allergosorbent test; eCRF, electronic case report form; EEC, environmental exposure chamber; EMA, european medicine agency; FAS, full analysis set; GCP, good clinical practice; GMP, good manufacturing practice; ICH, international conference on harmonization; ICT, intracutaneous testing; IgE, immunoglobuline E; IgG_1, immunoglobuline G_1; IgG_4, immunoglobuline G_4; LS Means, least squares means; MedDRA, medical dictionary for regulatory activities; nBet v 1, natural Bet v 1; PP, per protocol population; rBet v 1-FV, folding variant of recombinant Bet v 1; rBet v 1, recombinant Bet v 1; SD, standard deviation; SE, standard error; SIT, specific immunotherapy; TEAE, treatment emergent adverse event; TNNSS, total non-nasal symptom score; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TSS, total symptom score; WHO, world health organization.

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(6). Among the identified and characterized birch pollen allergens, Bet v 1 by far represents the most important, being the only major allergen (7). It is recognized by IgE antibodies of 95% of tree pollen allergic patients and almost 60% of them react exclusively to Bet v 1 (8). One novel approach to perform SIT for birch pollen allergy utilizes the major allergen Bet v 1 produced by recombinant protein technology (8). Besides the use of the wild-type recombinant Bet v 1 (rBet v 1) (9, 10), several approaches have been used to construct modified Bet v 1 derivatives with hypoallergenic features (7, 11, 12). One approach produced a new stable rBet v 1 folding variant (rBet v 1-FV) that has lost the native conformation, while the primary sequence of the allergen is not disrupted and subsequently T-cell epitopes remain intact. (13) This altered conformation has the potential to hamper binding of Bet v 1-specific IgE, so that this molecule would represent a hypoallergenic form (13). Both features make the rBet v 1-FV an optimal molecule candidate for SIT (7, 13). In this phase II DBPC study, we evaluated the safety and efficacy of four doses of rBet v 1-FV in an environmental exposure chamber (EEC). The EEC has been used to evaluate the efficacy of symptomatic drugs and SIT and has been shown to be a robust model to evaluate the efficacy of allergy treatments (14–16). Due to reduced variability of data produced in the EEC, the outcomes of treatment can be determined with smaller sample sizes as compared with, for example, field based studies (17).

Material and methods

Patients

A total of 37 patients were consented and included in this study. Inclusion criteria were as follows: 18–65 years of age, history of allergic rhinitis to birch pollen during the last 2 years; birch pollen–specific IgE result (EAST) greater than 0.75 kU/l and positive skin prick test to birch pollen (wheal diameter of at least 4 mm); no relevant sensitizations to allergens interfering with birch pollen symptoms; no SIT with a tree pollen preparation in the previous 5 years.

Interventions

This was a randomized, DBPC, parallel group study. After a screening visit, patients were exposed before start with SIT for eight hours to airborne birch pollen (Allergon AB, Sweden) at an average concentration of 3500 ± 500 grains/m^3 in an EEC (CETERO RESEARCH, Mississauga, ON, Canada). A minimum Total Symptom Score (TSS) (at least 10 of maximum 24 on any 2 consecutive days following EEC exposure) was required to be randomized into the study. Patients were randomized to four rBet v 1-FV doses or placebo and received weekly subcutaneous injections for 10 weeks. Thereafter, patients attended a post-treatment EEC session.

Immunotherapy

The vaccine used in this trial was manufactured by Allergopharma GmbH & Co. KG according to the revised GMP guidelines of the WHO. Vials of trial medication contained 2.5 ml of sterile aluminum hydroxide suspension for subcutaneous injection. Placebo was the sterile aluminum hydroxide suspension alone. Active and placebo preparations were supplied by Allergopharma GmbH & Co. KG, Reinbek, Germany. Four different doses (with 20, 80, 160 and 320 μg in maintenance dose, leading to cumulative doses of 80, 320, 639 and 1270 μg) were compared with placebo. Table 1 shows the different up-dosing schedules and the resulting cumulative doses.

Dose-finding criteria

The finding of the optimal dose was based on the following criteria:

- Reduction of symptoms in the EEC, based on the change from baseline in area under the curve (AUC) for Total Symptom Score (TSS), Total Nasal Symptom Score (TNSS), Total Non-Nasal Symptom Score (TNNSS), and Total Ocular Symptom Score (TOSS);
- Post-treatment intracutaneous testing (ICT, Primary Endpoint);
- Change from baseline in specific IgG_1 and IgG_4 blood levels; and
- Safety and tolerability.

Efficacy measurement

Allergy symptoms were recorded using a severity rating scale after EEC exposure. The TNSS included congestion, rhinorrhea, nose itchiness, and sneezing; the TNNSS included itchy eyes, tearing eyes, red eyes, and itchy ears/palate, and the TOSS included itchy eyes, tearing eyes, and red eyes. Each symptom was assessed using a 0–3 rating scale (none, mild, moderate, and severe, respectively). The TSS is the sum of TNSS and TNNSS.

Intracutaneous testing

Intracutaneous testing was carried out 7 days after the last vaccine injection on the back of patients. ICT was performed with birch pollen extract (GREER® Laboratories, Inc., 639 Nuway Circle NE, P.O. Box 800, Lenoir, NC 28 645), positive control (0165 g/l histamine dihydrochloride), and negative control (physiological NaCl-solution). After 15 min and after 6 h, the early reaction and the late phase reaction, respectively, were evaluated taking into account the medium diameter of the erythema and the wheal measured.

Immunological response

IgG and IgE levels were measured before and after treatment. Measurements were taken as described before (18).

Safety assessment

Serious adverse events (SAEs), treatment emergent adverse events (TEAEs), vital signs, and clinical laboratory parameters

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were evaluated. AEs were monitored and coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 10.1, http://meddramsso.com). Systemic reactions were graded according to the EAACI grading system (6).

Blinding and randomization

This was a double-blind study in which the identity of a patient's treatment was unknown to the patient and the study personnel. Eligible patients were assigned a randomization number based on the randomization list: this list was generated with an equal number of patients assigned to each treatment and randomization numbers assigned sequentially.

Statistics

Analyses of symptom scores were based on the AUC of patient-rated TSS, TNSS, TNNSS, and TOSS before and after SIT. The trapezoidal rule was applied in the calculation of AUC using the times from 1.0 to 8.0 h in the EEC. An analysis of covariance (ANCOVA) was performed with change of the AUC as the dependent variable and baseline as a covariate and treatment as a fixed effect. The least squares means (LSMeans) for each treatment, the LSMeans of treatment differences together with the corresponding 95%-confidence interval and the *P*-value are presented. No adjustment of the significance level for multiple comparisons was made. An analysis of covariance (ANCOVA) was performed with change from baseline

Table 1 r Bet v 1-FV dosing schedule

			rBet v 1-FV							
			20 μg		80 μg		160 μg		320 μg	
Injection number	Date	Injected volume (ml)	Strength* (μg/ml)	Dose (μg)	Strength* (μg/ml)	Dose (μg)	Strength* (μg/ml)	Dose (μg)	Strength* (μg/ml)	Dose (μg)
1	7 (+7) after V2	0.15	A1	0.19	B1	0.75	C1	0.75	D1	0.75
2	7 (+7)	0.30	A1	0.38	B1	1.5	C2	2.25	D2	2.25
3	7 (+7)	0.60	A1	0.75	B1	3	C3	6	D3	6.75
4	7 (+7)	1.00	A1	1.25	B1	5	C3	10	D4	20
5	7 (+7)	0.10	A2	2.5	B2	10	C4	20	D5	40
6	7 (+7)	0.20	A2	5	B2	20	C4	40	D5	80
7	7 (+7)	0.40	A2	10	B2	40	C4	80	D5	160
8	7 (+7)	0.80	A2	20	B2	80	C4	160	D5	320
9	7 (+7)	0.80	A2	20	B2	80	C4	160	D5	320
10	7 (+7)	0.80	A2	20	B2	80	C4	160	D5	320
Dose†			80.06		320.25		639		1269.75	

*A1 = 1.25; A2 = 25.00; B1 = 5.00; B2 = 100.00; C1 = 5.00; C2 = 7.50; C3 = 10.00; C4 = 200.00; D1 = 5.00; D2 = 7.50; D3 = 11.25; D4 = 20.00; D5 = 400.00.
†Cumulative dose in μg.

Table 2 Baseline demographic data (safety set)

	rBet v 1-FV				
Parameter	Placebo (*n* = 7)	20 μg (*n* = 7)	80 μg (*n* = 8)	160 μg (*n* = 7)	320 μg (*n* = 8)
Race					
Asian	1 (14.3%)	1 (14.3%)	1 (12.5%)	1 (14.3%)	1 (12.5%)
Black/African-American	1 (14.3%)	1 (14.3%)	3 (37.5%)	2 (28.6%)	2 (25.0%)
White	4 (57.1%)	5 (71.4%)	3 (37.5%)	4 (57.1%)	4 (50.0%)
Multiple	1 (14.3%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Gender					
Female	3 (42.9%)	5 (71.4%)	3 (37.5%)	4 (57.1%)	4 (50.0%)
Male	4 (57.1%)	2 (28.6%)	5 (62.5%)	3 (42.9%)	4 (50.0%)
Age (years)					
n	7	7	8	7	8
Mean ± SD	35.6 ± 7.2	39.6 ± 14.7	31.4 ± 8.4	39.6 ± 12.7	40.0 ± 10.3
Median	39.0	43.0	32.5	41.0	42.5
Min, Max	24, 42	20, 61	21, 43	21, 61	25, 52

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in specific IgG_1 and IgG_4 as the dependent variable and baseline as a covariate and treatment as a fixed effect.

Ethical conduct of the study

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, consistent with good clinical practices and the applicable regulatory requirements (Division 5 of the Canadian Food and Drug Regulations (CFDR). Written informed consent was obtained from all patients before applying any study procedures. The study and the informed consent form were reviewed and approved in writing by a central Institutional Review Board (IRB Services, Aurora, Ontario, Canada).

Results

Participant populations

Thirty-seven patients were randomized to five groups: placebo (n = 7), 20 μg (n = 7), 80 μg (n = 8), 160 μg (n = 7), 320 μg (n = 8) and included in the safety population. Thirty-six patients who had at least one post-treatment efficacy measurement were included in the full analysis set (FAS). Table 2 reports baseline demographic data of FAS patients. Thirty-two patients completed the study without major protocol deviations: placebo (n = 6), 20 μg (n = 6), 80 μg (n = 6), 160 μg (n = 6), 320 μg (n = 8) and were included in the per protocol analysis population (PP).

An overview of the subjects is provided in Fig. 1.

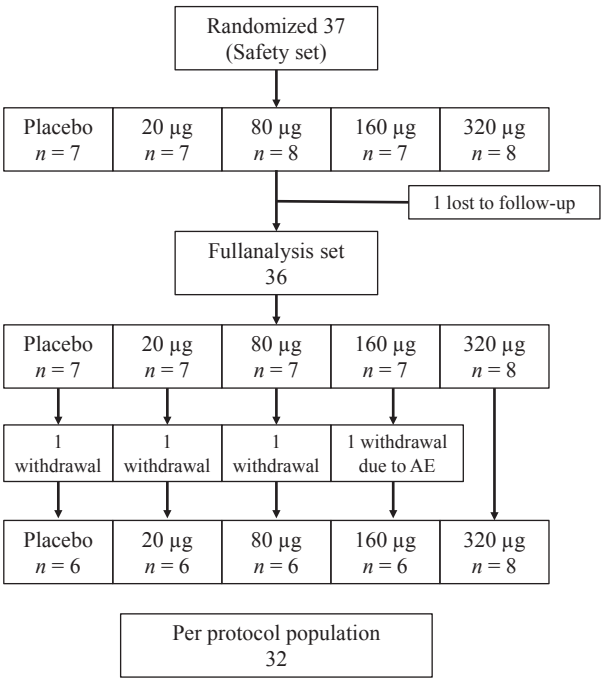


Figure 1 Overview of trial subjects.

Dose-finding criteria

Symptom scores

A statistically significant decrease of the TSS, TNSS, TNNSS and TOSS AUC was observed for all active treatment groups compared with placebo. Mean changes for these assessments are shown in Fig. 2.

Post-treatment intracutaneous testing

Wheal sizes after 6 h did not show significant differences between active treatments and placebo. Median values were 0 for all groups, because more than half of the patients showed no wheals at the 6-hour time point. Only 8 of the 32 patients from the 5 different treatment arms showed an ICT reaction. Therefore, calculating means and SD did not give meaningful values and results are not interpretable.

Immunological response

A statistically significant increase from baseline IgG_1 was observed for all of the active treatments when compared with placebo (20 μg: P = 0.0009, 80 μg: P = 0.0067, 160 μg: P = 0.0114, and 320 μg: P = 0.0419; two-sided ANCOVA P-values) (Fig. 3). No significant increase in specific IgG_4 was seen in any of the four doses compared with placebo. This might be due to the observation that IgG_4 is produced later than IgG_1 (19), and that the duration of treatment lasted only 10 weeks.

Safety and tolerability

None of the thirty-seven patients included in the safety population reported a SAE during this study. The majority of AEs related to study drug were injection site reactions. Table 3 shows the prevalence of patients reporting TEAEs. The two highest dose groups (160 μg and 320 μg) reported the highest incidence of drug-related TEAEs (71.4 and 87.5% respectively), while the lowest incidence was observed in the 80 μg group (37.5%). No EAACI grade III or IV reactions were reported (Table 4). The only two Grade II reactions occurred in the two highest dose groups, one in each group. The patient, in the 160 μg group, was withdrawn due to trouble breathing and chest pain. Both symptoms were considered mild in intensity and resolved rapidly after treatment discontinuation. In the 320 μg group, one patient experienced urticaria which was considered mild, related to study drug and resolved with no action taken.

Discussion

The aim of this study was to find the optimal dose of a new hypoallergenic folding variant of recombinant Bet v 1 intended for SIT. Overall, efficacy results of TSS showed that all active treatment doses provided significantly more symptom relief than placebo. However, when just TNSS was evaluated, a trend toward an inferior treatment effect at the lowest dose was observed. Higher doses of 80, 160, and 320 μg were effectively indistinguishable on the basis of efficacy alone. The difference of the LSMeans between the 20

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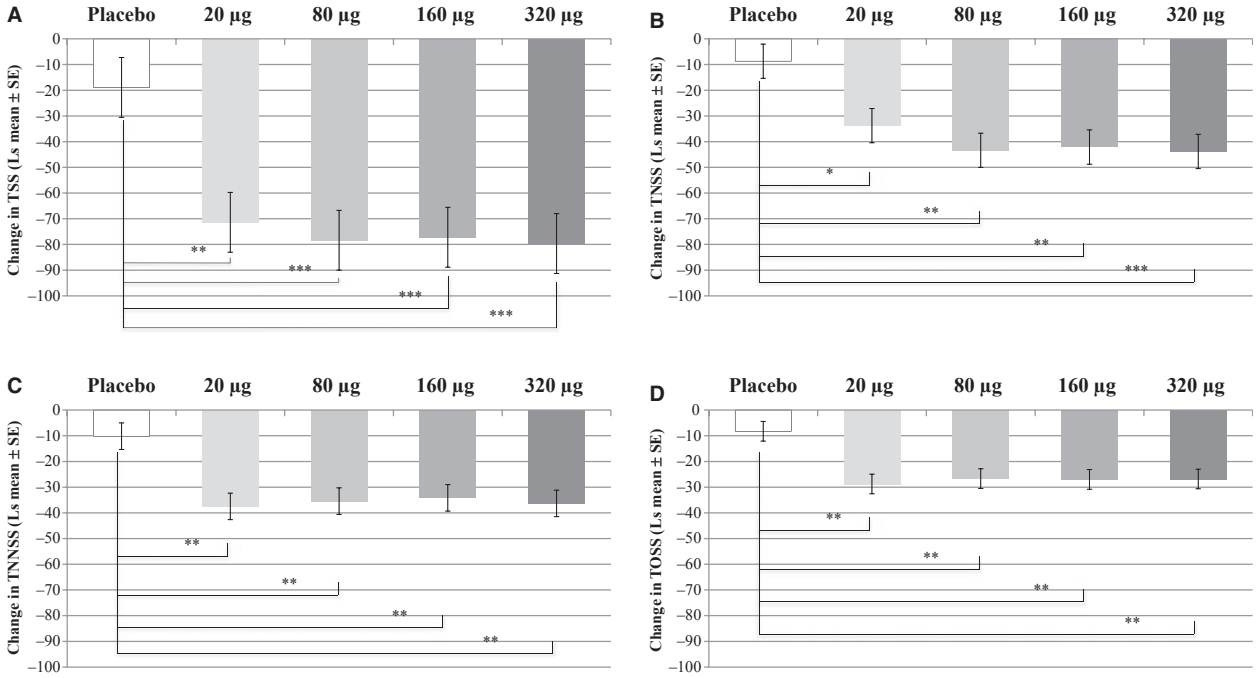


Figure 2 Changes from baseline AUC in clinical efficacy scores in FAS population (LSmean ± SE) and *P*-values between active treatment and placebo. The results are based on $n = 6$ patients in the placebo group, $n = 6$ patients in the 20 µg, $n = 6$ patients in the 80 µg, $n = 6$ patients in 160 µg, and $n = 8$ patients in 320 µg group. (A) Total Symptom Score (TSS); (B) Total Nasal Symptom Score (TNSS); (C) Total Non Nasal Symptom Score (TNNSS); (D) Total Ocular Symptom Score (TOSS). $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

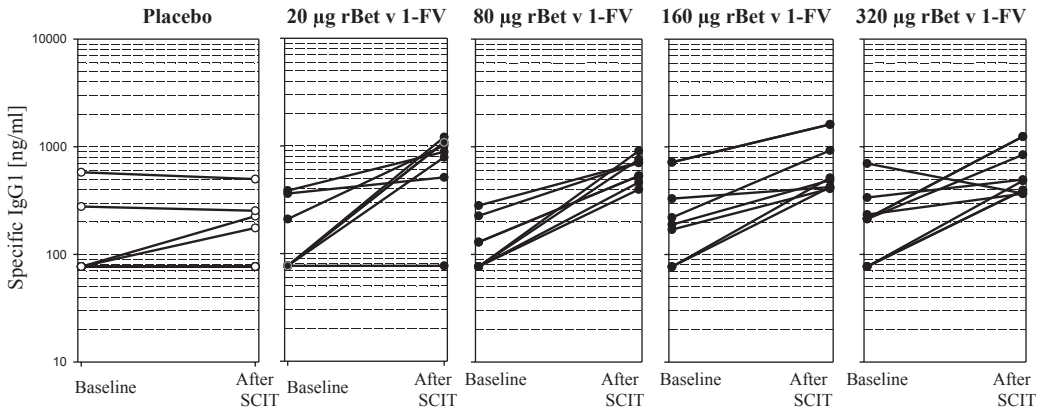


Figure 3 Changes from baseline in specific IgG_1 levels in FAS population for the treatment groups receiving either placebo, 20, 80, 160, or 320 µg rBet v1-FV maintenance dose.

and the 80 µg group is about 10 points (whereas the standard error is less than 7 points). Only very small differences of about 1 point were observed between the 80 and the 160 as well as the 320 µg group. The changes from baseline TSS AUC ranged from 71.9 to 81.8% proving the high clinical efficacy and relevance of all active doses; notably, we could observe a significant effect despite the low number of randomized patients.

All four doses were well tolerated, with a higher AE incidence with higher doses (160 µg and 320 µg). The two Grade II reactions occurred in the two higher doses (160 and 320 µg). When considering all TEAEs, as reported in Table 3, the dose of 80 µg appears to be the best tolerated dose. For assessment of safety, especially of rare events, higher patient numbers may be considered useful. However, ethical problems with patient exposure need to be considered

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Table 3 Overall summary of treatment emergent adverse events (TEAEs) (FAS population)

	Statistics	Placebo (N = 7)	20 μg (N = 7)	80 μg (N = 8)	160 μg (N = 7)	320 μg (N = 8)
Any TEAEs	*n* (%)	5 (71.4%)	4 (57.1%)	4 (50.0%)	5 (71.4%)	7 (87.5%)
	E	10	19	13	19	43
Any drug-related TEAEs	*n* (%)	3 (42.9%)	4 (57.1%)	3 (37.5%)	5 (71.4%)	7 (87.5%)
	E	4	18	10	13	35
Any TEAEs leading to withdrawal	*n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
	E	0	0	0	2	0
Local TEAEs (general disorders/admin. site conditions)	*n* (%)	3 (42.9%)	4 (57.1%)	3 (37.5%)	4 (57.1%)	5 (62.5%)
	E	4	16	10	9	32

N, Number of patients in the specified group; *n*, Number of patients with TEAE; %, Percentage based on *n*; E, Number of TEAEs.

Table 4 Summary of drug-related adverse events by EACCI grading (safety population)

Severity	Placebo (n = 7)	20 μg (n = 7)	80 μg (n = 8)	160 μg (n = 7)	320 μg (n = 8)
Grade I	0 (0.0%)	1 (14.3%)	0 (0.0%)	1 (14.3%)	1 (12.5%)
Grade II	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	1 (12.5%)
Grade III	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Grade IV	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Counts reflect numbers of patients reporting one or more adverse events that map to the MedDRA system organ class/preferred term. At each level of summarization (system organ class or preferred term), patients reporting more than one adverse event are counted only once (under the greatest reported EAACI). *n*, Number of patients in the specified group; %, Percentage based on *N*.

and in this specific situation have been deemed relevant as such high doses in connection with steep dose increases have never been administered before. Taken together, these data suggest that a maintenance dose of 80 μg would yield the best risk-benefit profile.

The ICT testing was technically defined 'primary end point' in the original study protocol. Only in confirmative studies is up-front determination of a primary end point required. The EMA guideline (20) states that for dose-ranging studies, provocation tests (e.g. conjunctival, nasal, or bronchial provocation or allergen exposure in allergen challenge chambers) may be used as primary end points. However, for this nonconfirmative phase II study, no stipulation of a primary end point was essential. Post-treatment intracutaneous tests did not show any difference between the active doses and placebo. The methodological approach may have been flawed due to a number of factors including use of test material from another supplier, with an allergen concentration which was probably too low to elicit 6 h or even 24 h wheals and use of the back instead of the forearm for ICT testing. All of this might have resulted in an inappropriate ICT.

The four allergen doses induced marked immunological responses, demonstrated by a significant increase in specific IgG_1 compared with the placebo group, with no notable differences between the doses.

Recombinant DNA technology enables the production of allergens equivalent to their natural counterparts, while offering genetically modified allergen variants with reduced IgE reactivity and maintained or enhanced immunogenicity (21). The new hypoallergenic folding variant of rBet v 1 used in this trial shows a random coil structure clearly distinguishable from the secondary structure of the native molecule, demonstrated by circular dichroism spectroscopy (13). Immunoassay inhibition tests, basophil activation, and skin testing demonstrated the hypoallergenic properties, whereas T-cell reactivity was comparable with that of the wild-type molecule (13, 21). As high doses are important for the success of SIT but carry the risk of undesirable side effects, the use of recombinant hypoallergenic variants can enhance the safety and the efficacy of this treatment (7, 21). The doses administered in our study are the highest Bet v 1 doses ever administered; in the study by Pauli et al. (10), the maintenance dose was 15 μg/month, while the highest dose administered by Purohit et al., using recombinant Bet v 1 hypoallergenic fragments, was 80 μg of protein (22). Due to the hypoallergenic characteristics of rBet v 1-FV, we could safely reach very high maintenance doses in just 8 injections, starting with a high dose and with a more rapid, threefold increase at every injection as shown in Table 1.

The clinical effects of the four doses of rBet v 1-FV in this study were evaluated in an EEC, a useful tool to expose allergic patients to relevant allergens in a controlled indoor setting. An EEC has been previously used for evaluating the efficacy of pharmacotherapies for seasonal allergic rhinitis (23–25), but only a few studies have employed an EEC in SIT investigations (26–28). The use of an EEC facilitates proper control and standardization of the quantitative allergen exposure. Further advantages are a) easing the dose-finding process, b) avoiding the confounding effects of rescue medications, and c) using smaller numbers of subjects than would be required in natural-exposure studies (17). The use of an EEC is now accepted by

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the European Medicine Agency (EMA) in dose-finding studies (20).

In summary, considering the symptom relief shown in the EEC, the immunological and the safety features, a maintenance SIT dose of 80 μg of the new rBet v 1-FV offers the best risk/benefit ratio for treating patients with allergic rhinoconjunctivitis due to birch pollen.

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Author contributions

DH participated in designing the study, especially dosing considerations, and drafted the manuscript; WM participated in designing the study, especially the dosing scheme, and contributed to the coordination of study management; AN conceived the dosing considerations, participated in designing of study, and gave interpretation of the data; AMS participated in designing the study and coordinated the study management at Cetero Research. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflict of interest with the publication of this article.

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