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Available online April 16, 2018.
<https://doi.org/10.1016/j.jaci.2018.02.048>

Benefit of Bet v 1 contiguous overlapping peptide immunotherapy persists during first follow-up season



To the Editor:

Efficacy and long-term protection of subcutaneous allergen immunotherapy (AIT) to *Betulacea* pollens have been well demonstrated. To achieve long-term protection, treatment has to be classically maintained for 3 or more years.¹⁻³ We have approached the issues of AIT differently by providing a safe and short (2 months) immunotherapy protocol using long contiguous overlapping peptides (COPs, 5 subcutaneous injections).⁴⁻⁶ COP treatment significantly improved allergic rhinitis combined symptom and medication score (RSMS) in a recent double-blind, multinational, placebo-controlled phase IIb trial (acronym AN004T) after a first seasonal exposure to birch pollen (50 µg dose).⁵ Treatment with Bet v 1 COPs induced Bet v 1-specific IgG₄ levels,⁵ which remained elevated over the course of 4 seasons in a phase I/IIa clinical trial.⁶ One of the major interests of AIT is its capacity not only to induce tolerance to the allergen but also to act as a disease-modifying therapy. It was thus of the utmost importance to demonstrate the long-term persistence of the efficacy of this innovative AIT approach.

We therefore report here the clinical efficacy of the AN004T immunotherapy trial⁵ during a second seasonal exposure to birch pollen (study AN005T). One hundred ninety-six of 240 randomized subjects (81.7%) returned for a second birch pollen season evaluation without having received any additional Bet v 1 COP treatment or other AIT (see Fig E1 in this article's Online Repository at www.jacionline.org). Treatment allocation was maintained blind to subjects and clinical and laboratory investigators until the end of the second season. Without further Bet v 1 COP treatment before the 2014 birch season, daily recorded symptom scores indicated that subjects previously treated with Bet v 1 COPs continued to report improved RSMS in both treatment groups as compared with subjects who had received the placebo (see Fig E2 in this article's Online Repository at www.jacionline.org). Based on the per-protocol set, the difference in RSMS, the primary end point, reached significance in the Bet v 1 COP 50 µg group (mean RSMS improvement, 21%; adjusted mean difference from placebo of -0.200 with 95% confidence limit [CL], -0.375 to -0.025; $P = .03$), but not in the Bet v 1 COP 100 µg group (mean RSMS improvement, 18%; adjusted mean difference from placebo of -0.169 with 95% CL, -0.353 to 0.014; $P = .07$) (Fig 1, A). Similar results were obtained in the modified intent-to-treat set ($n = 189$, 50 µg group [adjusted mean difference from placebo of -0.162 with 95% CL, -0.325 to -0.001; $P = .05$] and 100 µg group [adjusted mean difference from placebo of -0.120 with 95% CL, -0.288 to 0.049; $P = .16$]).

Among secondary end points, subjective quality of life was assessed by Mini Rhinoconjunctivitis quality-of-life questionnaire

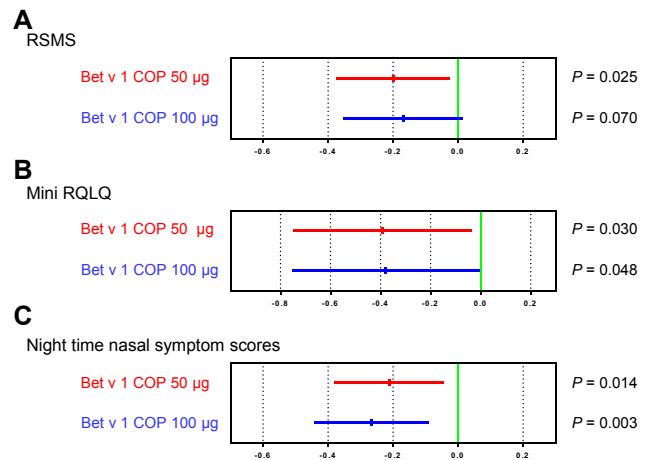


FIG 1. Differences from placebo for the Bet v 1 COP-treated groups: **A**, RSMS. **B**, MiniRQLQ score. **C**, NNSS. LS mean differences of the indicated scores are plotted relative to placebo (green line) for 50 µg (red) and 100 µg (blue) groups. 95% CLs are indicated as horizontal colored lines. Analyses were performed during the 2014 pollen season on the PP set. P values were determined by analysis of covariance with treatment and geographic region as independent factors in the model. LS, Least squares; MiniRQLQ, Mini Rhinoconjunctivitis quality-of-life questionnaire; NNSS, nighttime nasal symptom score; PP, per-protocol.

and both treatment groups had lower scores when compared with placebo and improvement reached statistical significance (Mini Rhinoconjunctivitis quality-of-life questionnaire improvement, 21% and 20%; adjusted mean difference from placebo of -0.394 and -0.379; $P = .03$ and $P = .05$, respectively) (Fig 1, B). Nighttime nasal symptom score, an assessment widely used to evaluate the severity of allergic rhinitis, was significantly improved (mean improvement, 31% and 39%; adjusted mean difference from placebo of -0.212 and -0.268; $P = .01$ and $P = .003$ for 50 µg and 100 µg groups, respectively) (Fig 1, C).

Overall, 54.7%, 50.9%, and 38.8% of subjects in Bet v 1 COP 50 µg, Bet v 1 COP 100 µg, and placebo groups, respectively, rated themselves improved from the previous season.

For the sake of comparison between AN004T and AN005T trials, Fig 2 summarizes results obtained in the same per-protocol set of both trials. Data showed strong consistency from first season to second season with an expected decrease in efficacy that nevertheless always remained 20% or more from placebo when significant (Fig 2, A-C).

Bet v 1-specific IgG₄ increased by 10- to 20-fold after Bet v 1 COP treatment and remained elevated during the first birch pollen season (AN004T).⁵ Before the second season (V15), Bet v 1-specific IgG₄ had decreased but remained elevated (2-3 times) compared with placebo and the pretreatment level (see Fig E3, A and B, in this article's Online Repository at www.jacionline.org). A small further increase in Bet v 1-specific IgG₄ was observed during the second pollen season in all 3 treatment groups (V16-V17). Antibody responses to COPs are shown in Fig E4 in this article's Online Repository at www.jacionline.org. Nearly all subjects had a positive skin prick test result to birch pollen extract at the end of the second season, regardless of the treatment group (93.9%, 96.8%, and 98.5% of subjects in the Bet v 1 COP 50 µg, Bet v 1 COP 100 µg, and placebo groups, respectively).

In conclusion, we present here the first successful phase IIb AIT trial based on COP reporting significant persistent clinical

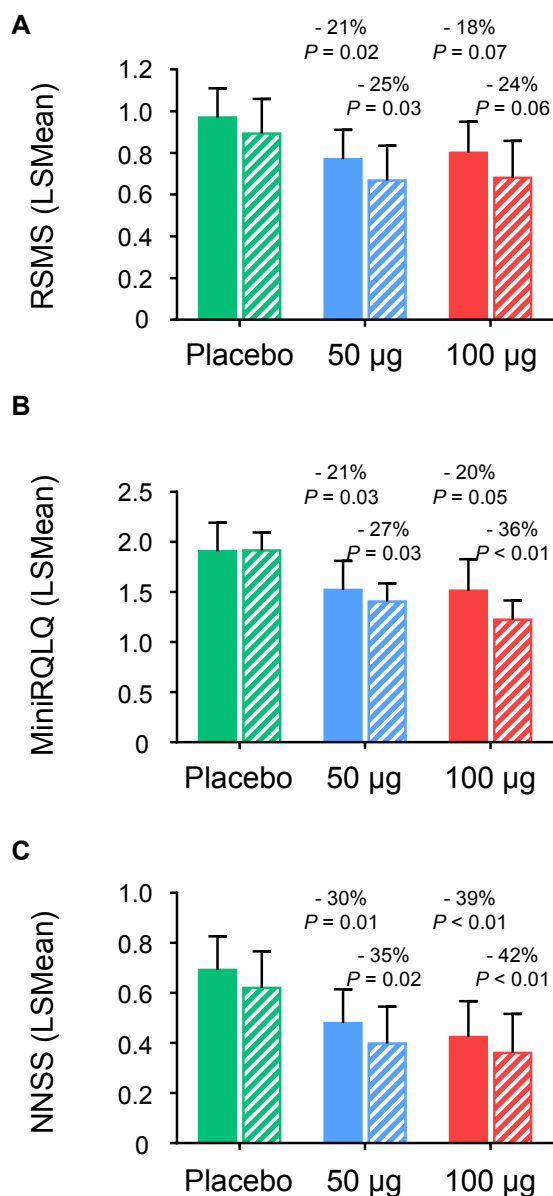


FIG 2. Comparison of RMS, MiniRQLQ score, and NNSS between first and second seasons after Bet v 1 COP treatment. **A**, RMS in the PP set. **B**, MiniRQLQ in the PP set. **C**, NNSS in the PP set. Results are presented as LS mean $\pm$ 95% CL (solid bars: second season; hatched bars: first season) with percent difference from placebo and P values (analysis of covariance) indicated. LS, Least squares; MiniRQLQ, Mini Rhinoconjunctivitis quality-of-life questionnaire; NNSS, nighttime nasal symptom score; PP, per-protocol.

improvement extending to at least 2 seasons posttreatment in subjects allergic to birch pollen. Clinical findings were associated with a durable elevation in Bet v 1-specific IgG₄ and no long-term changes in Bet v 1-specific IgE were observed. Of note, post hoc analysis of subjects who did not return to the present study revealed that 10 subjects in the 50 µg group not returning for evaluation were good responders during the first season (>50% reduction in RMS compared with placebo). In comparison with study AN004T (Fig 2), this may explain in part the decrease in efficacy level together with a natural decrease in overall tolerance after AIT. No such tendency was found for subjects in the 100 µg group or the placebo group.

Taken together, improvements in scores after the second season for both primary and secondary end points were more than 20%, considered a clinically relevant threshold. This threshold compares favorably with data obtained in recent placebo controlled sublingual immunotherapy and subcutaneous allergen immunotherapy studies applying a similar scoring system.⁷

Additional information is available (see this article's [Methods](#), [Results](#), and [References](#) sections in the Online Repository at www.jacionline.org).

We thank all investigators of the AN005T study for their commitment to successfully recruit patients: Carsten Bindslev-Jensen, Dermato-Venerologisk og Allergiklinik, Klinisk Institut, Odense, Denmark; Tina Skjold, Lungemedicinsk Forskningsafdeling B, Bygning, Aarhus C, Denmark; Hans-Jørgen Malling, Allergiklinikken KAA-816, Hellerup, Denmark; Aurika Babjoni-seva, Center of Pulmonology and Allergology, Pauls Stradins Clinical University Hospital, Riga, Latvia; Svetlana Reznikova, The Centre of Investigation and Treatment of Allergic Diseases, Riga, Latvia; Viktorija Vevere, Viktorija's Vevere's Private Practice of Pneumology and Allergology, Rezekne, Latvia; Audra Blaziene, Vilnius University Hospital, Santakiskiu Klinikos Center of Pulmonology Allergology, Outpatient Department (610), Vilnius, Lithuania; Ruta Dubakiene, Allergy Clinic, Vilnius, Lithuania; Eduardas Razgauskas, Antakalnio affiliation of the Vilnius city Allergy Center Antakalnio, Vilnius, Lithuania; Brigita Sitkauskienė, Hospital of Lithuanian University of Health Sciences, Kaunas Clinics, Clinic of Pulmonology and Immunology Eiveniu 2, Kaunas, Lithuania; Małgorzata Bartkowiak-Emeryk, Alergotest ul, Sapiehy 2/4a 20-095, Lublin, Poland; Krzysztof Buczyłko, Niepubliczny Zakład Opieki Zdrowotnej Centrum, Alergologii ul. Kopernika 67/69, Łódź, Poland; Bernadetta Majorek-Olechowska, ALERGO-MED, Specjalistyczna Przychodnia Lekarska Sp. z o.o. ul. PCK 26, Tarnów, Poland; Ewa Sikorska, NZOZ Aler-med Specjalistyczna Opieka Medyczna ul, Wrocław, Poland; Barbara Tluczykont, NZOZ Przychodnia Lekarska Hipokrates Sp. z o.o. ul. Reymonta 42, Zabrze, Poland; Irena Wojciechowska, Centrum Alergologii IRMED Specjalistyczny Niepubliczny Zakład Opieki Zdrowotnej ul. Tyszkiewiczza 13 lok. U1, Warszawa, Poland; Piotr Kuna, SPZOZ Uniwersytecki Szpital Kliniczny nr 1 im. Norberta Barlickiego Uniwersytetu Medycznego w Łodzi Oddział Kliniczny Chorób Wewnętrznych, Astmy i Alergii ul. Łódź, Poland; Morgan Andersson, Department of Otorhinolaryngology, University Hospital Skåne, Lund, Sweden; Stig Rudblad, CRS, Clinical Research Support S-huset Södra Grev Rosengatan, Örebro, Sweden; and Sabina Rak, Lungmottagningen, Västermanlands sjukhus, Västerås, Sweden.

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The study was supported by Anergis SA (Route de la Corniche 9B, 1066 Epalinges, Switzerland), the developer of Bet v 1 COP.

Disclosure of potential conflict of interest: F. de Blay reports grants from Stallergenes-Greer and Chiesi and personal fees from ALK, Mundipharma, and Novartis, during the conduct of the study; and is a board member for Stallergenes-Greer, Novartis, ALK, Mundipharma, Medapharma, Boehringer, and Teva. M. Jutel reports lecture fees from ALK-Abello; lecture fees, advisory board, and clinical investigator fees from Allergopharma; lecture fees and clinical investigator fees from Stallergenes; advisory board and clinical investigator fees from Anergis; advisory board fees from Biomay and UCB; and clinical investigator fees from HAL, Astra-Zeneca, GSK, Novartis, Teva, Vectura, Tekada, Roche, Janssen, Medimmune, Allergy Therapeutics, Circassia, Leti, and Chiesi. L. Jacobsen and M. Worm received money for consulting fees and study preparation from Anergis SA. L. Jacobsen received money for study preparation from Anergis SA. M. Worm was paid for consultancy outside of this research by Anergis SA. A. Kettner, G. DellaCorte, V. Charlon, C. Reymond, and K. Simonsen are employees of Anergis SA and have stock options with this company. V. Charlon received travel/accommodations/meeting expenses from Anergis SA unrelated to this research. F. Spertini has stock/stock options with Anergis SA; received support for travel to meetings for the study or other purposes from Anergis SA; has patents (planned, pending, or issued) and received royalties from CHUV, Lausanne, CH; and is a funder of the company Anergis SA, which supported this study.

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Available online April 17, 2018.
<https://doi.org/10.1016/j.jaci.2018.01.052>

Thromboxane A₂ facilitates IL-17A production from Vγ4⁺ γδ T cells and promotes psoriatic dermatitis in mice



To the Editor:

Prostanoids are lipid mediators composed of prostaglandin (PG) D₂, PGE₂, PGF₂α, PGI₂, and thromboxane (Tx) A₂. Prostanoids exert their actions through their specific G-protein-coupled receptors,¹ and play important roles in various pathological conditions.¹

The involvement of prostanoids in the pathogenesis of psoriasis, an IL-17-mediated inflammatory skin disease,² has long been suspected, because various studies have reported elevation in prostanoids in psoriatic lesions,³ and several reports have indicated the efficacy of nonsteroidal anti-inflammatory drugs, which inhibit the production of prostanoids, for psoriasis.⁴ However, which prostanoids contribute to the development of psoriasis and how they do so have not been tested extensively

to date. To clarify the roles of prostanoids in psoriasis, we used an imiquimod (IMQ)-induced psoriasis model,⁵ and first measured the amount of each prostanoid in the psoriatic lesions by LC-MS/MS-based mediator lipidomics.⁶ Among the prostanoids, we found a significant increase in TxB₂, a stable metabolite of TxA₂ (see Fig 1, A, and Fig E1 in this article's Online Repository at www.jacionline.org). Consistently, real-time PCR analysis revealed increased mRNA expression of *Tbxas*, a synthase of TxA₂, and *Tbxar2* (TP), a receptor of TxA₂, in the psoriatic lesions (Fig 1, B). Furthermore, in human psoriatic lesions, mRNA expression of *TBXAS* and *TBXAR2* was significantly increased compared with that in healthy controls (Fig 1, C). These results prompted us to explore the role of TxA₂ and TP in psoriasis. Next, we applied TP-deficient mice⁷ to the psoriasis model. TP-deficient mice exhibited significantly reduced ear-swelling responses and inflammatory cell infiltration compared with wild-type (WT) mice (Fig 1, D and E). Flow cytometric analysis revealed significantly reduced numbers of neutrophils and dermal γδ T cells, which are composed of Vγ4⁺ and Vγ4⁻ subsets,⁸ in the skin of TP-deficient mice compared with WT mice in the psoriasis model (Fig 1, F), indicating that TxA₂-TP signaling plays proinflammatory roles in IMQ-induced psoriatic dermatitis.

IMQ induces cutaneous dendritic cells (DCs) to produce IL-23, which activates dermal γδ T cells to produce IL-17A and IL-22. To explore the molecular mechanisms by which TP signaling promotes psoriatic dermatitis, we examined the mRNA expression of these cytokines in the skin in this model. The mRNA expression of *Il23a* was comparable between WT and TP-deficient mice (Fig 2, A). The mRNA expression levels of *Il17a* and *Il22* were, however, significantly decreased in TP-deficient mice compared with those in WT mice (Fig 2, A). Because dermal Vγ4⁺ γδ T cells are the major cell population that produce IL-17A,⁸ we examined the production of IL-17A from Vγ4⁺ γδ T cells in TP-deficient mice by flow cytometry. The percentage of IL-17A-producing Vγ4⁺ γδ T cells in TP-deficient mice was significantly lower than that in WT mice, whereas the percentage of IL-17A-producing Vγ4⁻ γδ T cells and CD4⁺ T cells was equivalent between WT and TP-deficient mice (Fig 2, B, C, and data not shown).

On the basis of these results, we hypothesized that TxA₂ promotes the IL-17A production of Vγ4⁺ γδ T cells through TP. To examine this possibility, we next examined the expression of *Tbxar2* and *Tbxas* on Vγ4⁺ γδ T cells. We collected Vγ4⁺ γδ T cells, Vγ4⁻ γδ T cells, and MHC class II^{high} CD11c^{high} DCs from skin-draining lymph nodes (dLNs) of IMQ-treated WT mice, and examined the mRNA expression of *Tbxar2* and *Tbxas* by RT-PCR. Both *Tbxar2* and *Tbxas* were expressed in all cell populations (Fig 2, D). *Tbxar2* mRNA expression tended to be higher in Vγ4⁺ γδ T cells than in other cell populations, whereas *Tbxas* mRNA expression was much higher in DCs.

We next examined the effect of a TP agonist⁷ on IL-17A production from Vγ4⁺ γδ T cells *in vitro*. The cell suspensions from skin dLNs were stimulated with recombinant IL-23 either with or without pretreatment with a TP agonist (I-BOP). Then, the expression of intracellular IL-17A from Vγ4⁺ γδ T cells was analyzed by flow cytometry. Although the TP agonist alone did not exert a significant effect on the IL-17A production, it significantly enhanced the IL-23-induced IL-17A production (Fig 2, E), suggesting that TxA₂-TP signaling promotes psoriatic dermatitis by facilitating IL-17A production from Vγ4⁺ γδ T cells. To confirm the *in vivo* significance, we examined the

METHODS

Subjects

This exploratory study (ClinicalTrials.gov identifier NCT02143583, AN005T) included 196 subjects from the previously published randomized, double-blind, placebo-controlled, parallel-group, phase IIb trial (ClinicalTrials.gov identifier NCT01720251, AN004T) (Fig E1).^{E1} Reasons recorded for not participating in study were as follows: refusal to participate ($n = 14$), travel during season or moved away ($n = 7$), no contact ($n = 6$), specific immunotherapy started ($n = 5$), no efficacy ($n = 3$), pregnancy ($n = 2$), no reason given ($n = 2$), site not participating ($n = 1$), prohibited medication ($n = 1$), and screening failure ($n = 1$). Clinical and laboratory blinding was maintained until subjects finished the second season analysis.

Study design

This long-term follow-up of a phase IIb study^{E1} was conducted at 23 centers in 7 European countries (Denmark, France, Latvia, Lithuania, Poland, Sweden, and Switzerland) in accordance with Good Clinical Practice and the October 2008 amended Declaration of Helsinki with approval of local ethics committees.

Clinical assessment for each subject was performed at visit V15 (continued numbering from AN004T trial), before the birch pollen season (25–35 days before the expected peak of the birch pollen season), V16, during the birch pollen season (7–14 days after the expected peak of the birch pollen season), V17, and after the birch pollen season (within 2 weeks from the actual end of the birch pollen season, defined as the earliest between the 42nd day after the start of the season and the last day of the last occurrence of 3 consecutive days with a regional pollen count of ≥ 10 grains/m³). Mean pollen counts for each center were as follows: 732, 732, 768, 647, 509, and 687 grains/m³ (Denmark/Sweden); 90 and 221 grains/m³ (France/Switzerland); 865, 31, 1157, 865, 1261, and 922 grains/m³ (Latvia/Lithuania); and 32, 392, 392, 292, 286, 487, and 382 grains/m³ (Poland), respectively. The same independent official pollen monitoring sources involved in AN004T were used to monitor pollen counts by region and to obtain current pollen exposure data. Dates of the visits in the various regions were based on the long-term average birch pollen exposure flowchart on www.polleninfo.org for the region of the subject's site. At each visit, adverse events and concomitant treatments were recorded and a blood sample was collected for determination of serum IgE and IgG₄. Physical examinations (visits 15 and 17), skin prick tests (SPTs) with Bet v 1 COPs and birch pollen extract (visit 17), and a subject global evaluation of treatment efficacy (visit 17, ie, the last visit) were also performed. Between visits, subjects completed the subject diary (symptoms and medication score, RSMS) on a daily basis and a quality-of-life questionnaire (Mini Rhinoconjunctivitis quality-of-life questionnaire) weekly.

Medication

Specified commercially available antiallergy rescue medications could be prescribed by the investigator as instructed according to protocol. Use of these medications was to be recorded in the daily diaries.

The antiallergy medications permitted during the clinical assessment period (ie, over the birch pollen season) were antihistamine (azelastine, alternative levocabastine) nasal spray, antihistamine (azelastine) ocular drops, oral antihistamine (loratadine 10 mg tablets), or oral corticosteroids (prednisone 5 mg tablets, alternative prednisone). Topical corticosteroids were not allowed.

Efficacy assessment

The primary end point of efficacy was the combined RSMS, calculated as the arithmetic mean of the rhinoconjunctivitis symptom score and rhinoconjunctivitis medication score recorded on a 4-point scale. RSMS, Mini-RQLQ score,^{E2} and nighttime nasal symptom score were recorded in an electronic diary (LogPad PHT) as previously described.^{E1}

Immunological evaluation

Bet v 1 COP-specific IgG₄ and IgE were quantified as described.^{E1} Briefly, the antigen-specific IgG₄ and IgE serum concentrations were determined by a direct ELISA. Bet v 1 (Biomay AG, Vienna, Austria) or Bet v 1 COPs were coated on microtiter plates (Nunc Immobilizer Amino Plates, Thermo Fisher Scientific Inc, Waltham, Mass) and incubated with test serum samples. Bound IgG₄ was located using biotinylated antihuman IgG₄ (G17-4, BD Biosciences, San Jose, Calif) or IgE (MHE-18, Biologend, San Diego, Calif), and the bound secondary antibody was quantified with horse radish peroxidase-conjugated streptavidin (BD Biosciences) and 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich, Buchs, Switzerland). OD at 450 nm was determined (with a reference filter set to 630 nm) using an Epoch Microplate Spectrophotometer (Biotek Instruments, Winooski, Vt). Specific IgG₄ or IgE concentrations in test samples were calculated using a standard curve obtained by coating the first 2 rows of the same microtiter plate with an anti-IgG₄ (ME-154, Abcam, Cambridge, UK) or IgE (Le27, NBS-C, Vienna, Austria) antibody, incubating with increasing amounts of human IgG₄ (Abcam) or IgE (SUS-11, NBS-C), and detecting said IgG₄ or IgE with the same secondary antibody used for detection of IgG₄ or IgE binding to the allergen. Curve fitting of mean OD values from serial dilutions of IgG₄ and IgE was done with 4-parameter logistic nonlinear regression model. Results were reported from the standard curve and expressed as concentration (ng/mL). Fold increase was calculated as ratio from antibody levels before treatment.

Skin prick test

SPTs with birch pollen extract were performed only at the end of the trial, at visit 17. For the SPT with birch pollen extract, commercially available solutions of birch pollen extract (ALK, Hørsholm, Denmark or, in Latvia and Lithuania, Stallergenes, Antony, France), histamine solution (positive control), and saline (negative control) were used. Each solution was applied directly to separate areas of skin, which were subsequently pricked using the usual disposable pricking device.

Statistical methods

Primary efficacy variable was daily RSMS obtained during birch pollen season. Comparisons between each Bet v 1 COP group and placebo were performed with an analysis of covariance model, with treatment and geographical location of center as fixed effects ($P < .05$ considered nominally significant).

An analysis in the per-protocol set ($n = 153$) was performed for this exploratory study's evaluation as well as an analysis in the modified intent-to-treat population (all randomized subjects who have received at least 4 injections and recorded at least 8 days of symptoms and medication use during the pollen second season or all days in case the season was only 2–8 days). In the per-protocol set were included all subjects from the AN004T who received at least 4 injections of Bet v 1 COPs and who did not violate either the AN004T or AN005T protocol in a way that might affect the evaluation of the primary variable, that is, subjects without major protocol deviation (forbidden medications intake before or during birch season or travel during the birch pollen season outside of the birch pollination area for more than 7 consecutive days).

No null hypothesis was set and no sample size calculation was performed for this exploratory study.

Anti-Bet v 1 IgG₄ and IgE levels and the changes from baseline were analyzed descriptively. The placebo-corrected treatment effect was summarized using median differences from placebo with corresponding 95% CL. Immunology data were also graphically presented by individual subject and summarized in box and whisker plots at each time point. P values for comparisons among treatments were from the 2-tailed Mann-Whitney test comparing ranks. P value of .05 was set as limit of significance.

Safety, baseline, and other variables were summarized in the safety set (all enrolled subjects who received at least 1 injection, intention-to-treat set according to Consort standards) and compared descriptively (Table E1).

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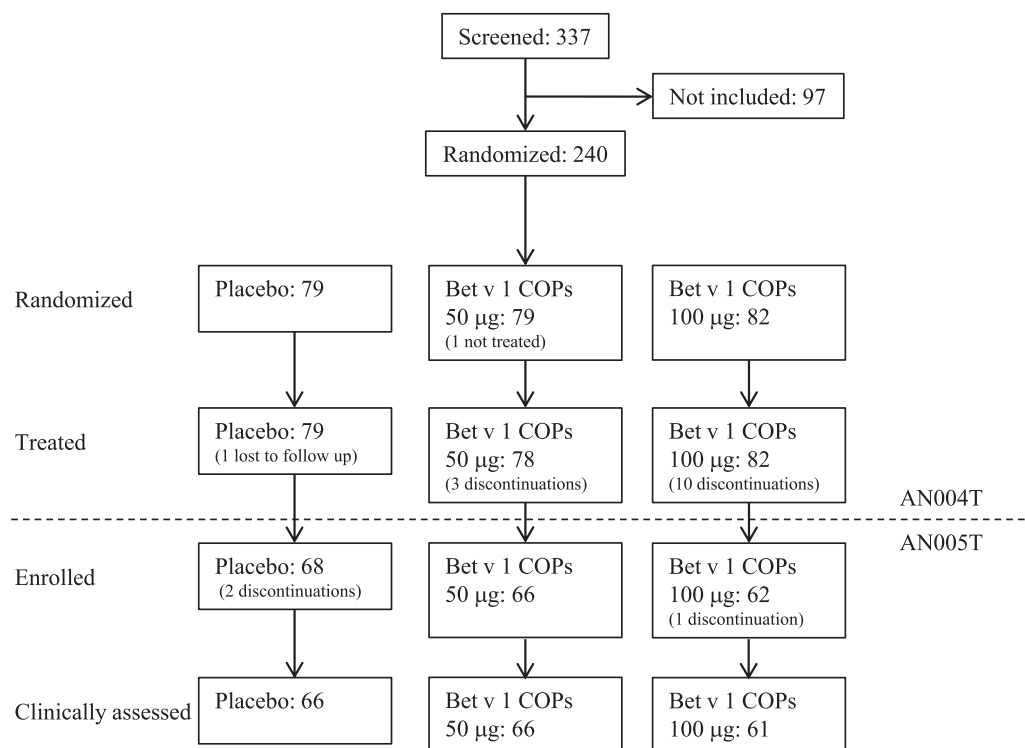


FIG E1. Trial profile. Flow diagram illustrating number of subjects screened, randomized, treated, enrolled, and evaluated for this study.

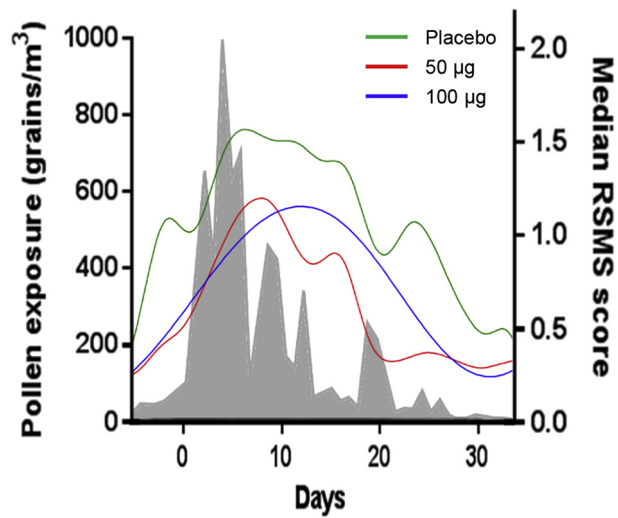


FIG E2. RSMS and pollen exposure during the pollen season. Start of pollen season (0) was set as the first of 3 consecutive days with at least 10 pollen grains/m³. Air pollen measures for all regions were pooled (gray area). Daily RSMSs (right axis) are reported as smoothed curves.

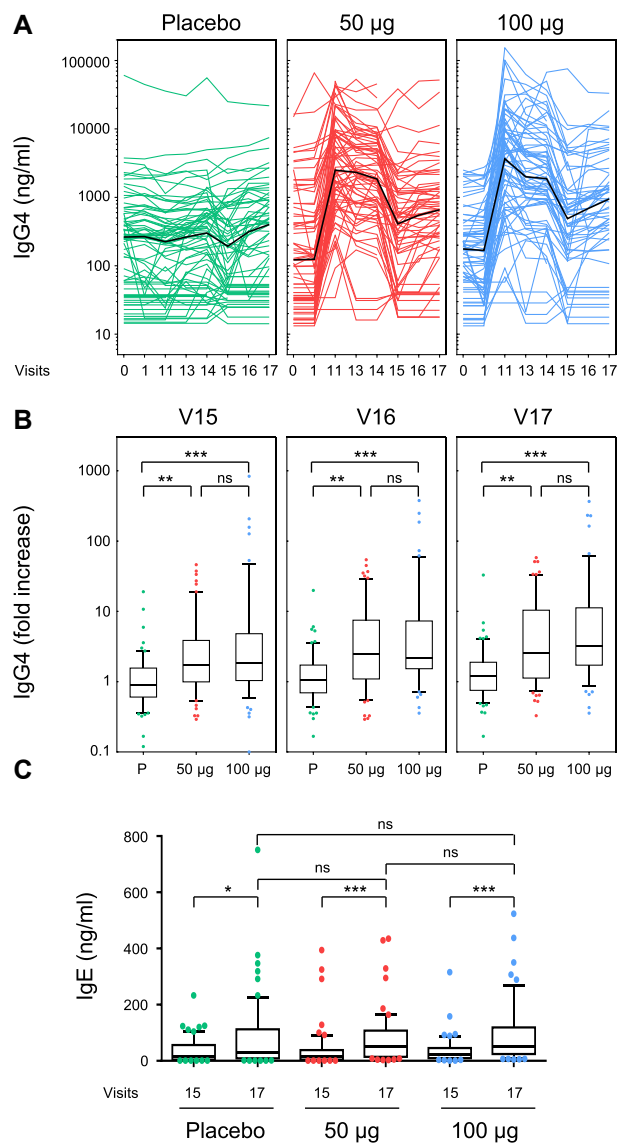


FIG E3. Bet v 1-specific IgG₄ and IgE levels assessed over the course of 2 birch seasons. **A**, Bet v 1-specific IgG₄ levels were measured by ELISA in the mITT set in individual subjects before treatment (V1), during first season (V11-V14), and before (V15), during (V16), and after (V17) second season. **B**, Fold increase over baseline of Bet v 1-specific IgG₄ was calculated for each subject at indicated visits. Results are displayed as box and whiskers plots with median and 25th to 75th percentile as box and whiskers expanding to 10th to 90th percentile and individual outliers as colored dots. *ns*, Not significant ($P > .05$), $^* .05 > P > .001$, $^{**} .001 > P > .0001$, and $^{***} .0001 > P$ as determined using the 2-tailed Mann-Whitney test comparing ranks. **C**, Bet v 1-specific IgE before (V15) and after season (V17). Results presented as in Fig E3, *B*. *mITT*, Modified intent-to-treat.

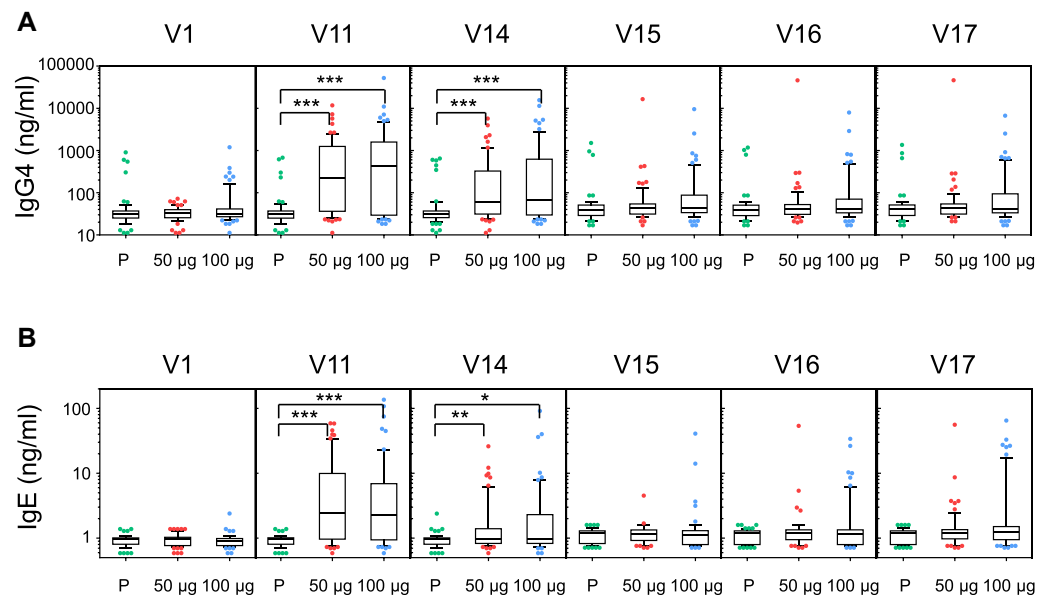


FIG E4. Bet v 1 COP-specific IgG₄ and IgE levels assessed during AN004T and AN005T clinical trials. Visits: before treatment (V1), after treatment before season (V11), after 2013 season (V14), before 2014 season (V15), and during (V16) and after (V17) 2014 birch pollen season. **A**, Bet v 1 COP-specific IgG₄ levels were measured by ELISA in the mITT set in individual subjects. **B**, Bet v 1 COP-specific IgE levels were measured same as IgG₄. Results are displayed as box and whiskers plot with median and 25th to 75th percentile as box and whiskers expanding to 10th to 90th percentile and individual outliers as colored dots. Only significant differences are indicated. *mITT*, Modified intent-to-treat. *.05 > *P* > .001, **.001 > *P* > .0001, and ***.0001 > *P* as determined using the 2-tailed Mann-Whitney test comparing ranks.

TABLE E1. Demographic and baseline disease characteristics (enrolled subjects)

Characteristic	Placebo (n = 68)	Bet v 1 COPs 50 µg (n = 66)	Bet v 1 COPs 100 µg (n = 62)
Sex (%), male/female	50.0/50.0	43.9/56.1	51.6/48.4
Age (y)			
Mean ± SD	35.5 ± 9.3	37.9 ± 9.4	39.0 ± 9.0
Range	20-56	21-55	22-55
Race (%), white/other	100/0	100/0	100/0
Region (%)			
Denmark/Sweden	30.9	30.3	33.2
France/Switzerland	4.4	4.5	5.1
Latvia/Lithuania	26.5	27.3	26.5
Poland	38.2	37.9	35.2
RSS, mean ± SD*	5.3 ± 4.6	4.8 ± 3.8	5.7 ± 4.9

RSS, Rhinoconjunctivitis symptom score.

*Allergy symptoms since the previous season recorded at visit before the pollen season.