

Accepted Manuscript

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PII: S0091-6749(16)30255-X

DOI: [10.1016/j.jaci.2016.02.044](https://doi.org/10.1016/j.jaci.2016.02.044)

Reference: YMAI 12096

To appear in: *Journal of Allergy and Clinical Immunology*

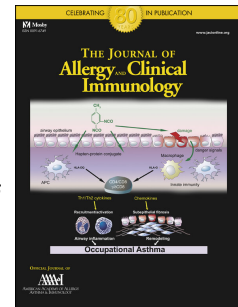
Received Date: 12 July 2015

Revised Date: 8 February 2016

Accepted Date: 19 February 2016

Please cite this article as: Spertini F, DellaCorte G, Kettner A, de Blay F, Jacobsen L, Jutel M, Worm M, Charlon V, Reymond C, Efficacy of two months of allergen specific immunotherapy with Bet v 1-derived contiguous overlapping peptides in patients with allergic rhinoconjunctivitis: results of a phase IIb study, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.02.044.

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Efficacy of two months of allergen specific immunotherapy with Bet v 1-derived contiguous overlapping peptides in patients with allergic rhinoconjunctivitis: results of a phase IIb study

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Funding source: The study was supported by Anergis SA (Route de la Corniche 9B,
1066 Epalinges, Switzerland).

Word count: 3291

Running title: COPs based Bet v 1 specific immunotherapy

ABSTRACT

Background: An immunotherapy formulation consisting in three contiguous overlapping peptides (COPs) derived from Bet v 1, the major birch pollen allergen, showed good clinical tolerability in a previous phase I/IIa clinical trial.

Objectives: To evaluate the efficacy and safety of allergen specific immunotherapy using two dose regimens of Bet v 1 COPs versus placebo in subjects with birch pollen allergic rhinoconjunctivitis.

Methods: A randomized, double-blind, placebo-controlled phase IIb clinical trial was performed to assess the efficacy of Bet v1 COPs immunotherapy during the 2013 birch pollen season. Prior to season, Bet v1 COPs (50 and 100 µg in aluminum hydroxide) or placebo (saline and aluminum hydroxide) were administered as five subcutaneous injections to 239 adults with allergic rhinoconjunctivitis to birch pollen. 25- or 50µg Bet v1 COPs were administered on day 1, and 50- or 100µg on days 8, 15, 29, and 57 respectively. Patients were monitored for adverse events during the treatment period, and assessed for combined rhinoconjunctivitis symptom and medication score (RSMS) as well as quality of life.

Results: In both Bet v1 COPs treated groups, RSMS improved, reaching statistical significance over placebo in the 50µg group (LS mean -0.23, 26% improvement, $p=0.015$). Both active groups showed significant improvement in quality of life and night time nasal symptoms scores, supporting the primary endpoint findings. Bet v1 COPs injections were well tolerated with a higher frequency of systemic adverse events in the 100µg group.

Conclusion: Two months of pre-seasonal immunotherapy with three COPs derived from Bet v 1 at 50 µg dose showed promising efficacy, small risk for systemic reactions, and immunomodulatory changes in this single season, dose-finding phase IIb trial in patients allergic to birch pollen.

Clinical implications: Immunotherapy with three contiguous overlapping peptides from Bet v 1 shows promise for use in allergen specific immunotherapy, providing a safe and effective treatment, considerably shorter than current AIT preparations.

CAPSULE SUMMARY

Two months of pre-seasonal, allergen-specific immunotherapy with 50µg dose of Bet v1 contiguous overlapping peptides provided symptom relief in allergic rhinoconjunctivitis subjects during the following birch pollen season.

Key words: allergen specific immunotherapy, birch pollen allergy, Bet v 1, contiguous overlapping peptides, synthetic peptides, aluminum hydroxide, IgG4, IgE, desensitization, clinical trial

Abbreviations: AE, adverse event; AIT, allergen immunotherapy; ANCOVA, analysis of covariance; ASMS, asthma symptom and medication score; CL, confidence limits; COPs, contiguous overlapping peptides; CpG, cytosine-phosphate-guanosine, FEV1, forced expiratory volume in the first second of expiration; LS, least squares (mean); MiniRQLQ[®], Mini Rhinoconjunctivitis Quality-of-Life Questionnaire; mITT, modified intent-to-treat; NNSS, night-time nasal symptom score; RMS, rhinoconjunctivitis medication score; RSMS, rhinoconjunctivitis symptom and medication score; RSS, rhinoconjunctivitis symptom score; SAE, serious adverse event; SPT, skin prick test; WAO, World Allergy Organization.

INTRODUCTION

The efficacy of subcutaneous allergen specific immunotherapy (AIT) to *Betulacea* has been well demonstrated in several clinical trials in humans.¹⁻⁴ AIT, including hymenoptera venom immunotherapy, provides long-term protection but requires a treatment period of 3 to 5 years. Unfortunately AIT is not devoid of safety issues, in particular when administered subcutaneously: allergic reactions are frequent particularly during ultra-rush therapies, with up to 30% of patients requiring treatment for allergic symptoms during the course of therapy.^{5, 6} As there are clearly still unmet needs in AIT, we designed a novel strategy, based on allergen derived peptides (named contiguous overlapping peptides, COPs), aiming to combine the proven efficacy of AIT with reduced treatment length and good safety.

The immunotherapy formulation tested in this study consists of three COPs derived from Bet v 1, and specifically developed for immunotherapy of birch pollen allergy. The three COPs together encompass the entire Bet v 1 sequence with overlapping sequences. Each COP of 49-71 amino acids length was selected for its inability to bind IgE in both *in vitro* and *in vivo* tests.⁷ A previous proof-of-concept study in adults with birch pollen allergic rhinoconjunctivitis showed that a 2-month treatment with Bet v 1 COPs was safe and resulted in an immune response characterized by a strong allergen-specific IgG4 induction.⁸ The current Phase IIb trial was designed to evaluate the efficacy and tolerability of two doses regimen of Bet v 1 COPs (50- and 100µg) in a multicenter clinical study in adults.

METHODS

Subjects

The study included subjects of 18 to 55 years of age with moderate to severe allergic rhinoconjunctivitis to birch pollen confirmed at screening by a rhinoconjunctivitis symptom score (RSS) ≥ 12 (table E1, online repository text), a history of anti-allergy medication use, a positive skin prick tests (SPT wheal ≥ 3 mm) to birch pollen extract (ALK Abello, Denmark) and Bet v 1 specific IgE ≥ 0.7 kU/L (ThermoFisher Scientific, Waltham, MA). Subjects were excluded who had FEV1 $< 80\%$ of predicted, chronic treatment for asthma, perennial allergies, positive SPT to Bet v 1 COPs or who were immunodepressed or who received AIT for birch or any allergen within the last 5 years. Pregnant or lactating women were also excluded. Sensitization of patient to other allergens is shown in table E10 together with other concomitant organ diseases.

Study design and treatment

This randomized, double-blind, placebo-controlled, parallel-group, Phase IIb trial (ClinicalTrials.gov reference number NCT01720251) was conducted in 23 centers in 7 European countries (Denmark, France, Latvia, Lithuania, Poland, Sweden, and Switzerland) in accordance with Good Clinical Practice, the CONSORT standards and the Oct. 2008 amended Declaration of Helsinki with approval of local Ethics Committees.

Eligible subjects were randomized using computer generated random numbers to receive Bet v 1 COPs (AllerTTM, Anergis SA, Epalinges, Switzerland) 50- or 100 μ g doses or placebo, with half-dose delivered on day 1 and full dose administered on days 8, 15, 29, and 57. For

each injection, Bet v 1 COPs (an equimolar mix of three COPs encompassing the entire Bet v 1 sequence⁷) or placebo (saline) was mixed with an equal volume of adjuvant (2 mg/ml aluminum hydroxide in saline, Miltenyi Biotech GmbH, Teterow, Germany). Placebo containing Aluminum Hydroxide was used to prevent unblinding due to absence of local reaction in saline only. All injections (0.8 ml, 0.4 ml at day 1) were administered subcutaneously in the deltoid area of alternating arms, at least one month prior to the birch pollen season for the last one. Treatment was not administered to patients with hypotension (systolic blood pressure < 90 mmHg), fever (body temperature > 38°C), or FEV1 < 70% of predicted value, or within 1 hour after a heavy meal, intense physical exercise, or smoking. The expected birch pollen season was determined using past 10 years information from PollenInfo⁹ for each geographical region (France/Switzerland, Denmark/Sweden, Poland, and Latvia/Lithuania). Efficacy assessment was performed from days 25 to 35 prior to the expected pollen peak (V12), during the season (V13: 7 to 14 days after expected peak) and 2 weeks after the end of the season (V14). End of season was defined as the 42nd day after season start or the last day of 3 consecutive days with < 10 grains/m³, whatever comes first.

Concomitant medications

Symptomatic medications permitted during the treatment period (salbutamol inhaler, loratadine 10-mg tablets and prednisone 5-mg tablets) and those permitted during the birch pollen season (azelastine or levocabastine as nasal spray or ocular drops, loratadine 10-mg tablets, prednisone 5-mg tablets) were provided by the investigator to be used as needed. Nasal steroids were not allowed. Beta-blocking agents, angiotensin-converting enzyme

inhibitors, immunosuppressive medication, investigational drugs, vaccines or devices, and any other allergen specific immunotherapy were not allowed during the trial.

Efficacy assessments

Daily symptoms (RSS) and use of rescue medications (RMS), each on a 4-point scale, as well as weekly assessment of quality of life (using validated MiniRQLQ[®])¹⁰ were recorded on electronic diary (LogPad[®] PHT). The primary endpoint of efficacy was the daily RSMS combined score, namely a 1:1 combination of mean RSS and RMS (see Methods in Online repository).^{11, 12} Secondary and exploratory outcomes included MiniRQLQ[®] and a daily night-time nasal symptom score (NNSS, mean of night-time awakenings and nasal congestion scores on a 4-point scale). Other variables included “Well days” (days during which RMS is zero and RSS is ≤ 2), “Days with severe symptoms” (days during which at least 3 of the 6 symptoms of RSS are scored as severe), and “Subject’s global analysis of treatment efficacy” (Subject- rated treatment efficacy at study end compared to the previous season).

Safety assessments

Adverse events (AEs) were monitored for one hour after each injection. Late AEs were collected from the subjects’ self-description upon next visit 24 hours later. Final safety assessment was performed 28-31 days after the last injection. Immediate (within 30 minutes post-injection) or delayed (over 30 minutes post-injection) systemic allergic reactions were graded according to World Allergy Organization (WAO).¹³ Grade-3 or 4 systemic allergic reactions occurring within one hour after injection were reported as serious AEs (SAEs). Other SAEs were reported according to ICH E2A step 4 guideline (1994). Grade-3 reactions

occurring within one hour after injection led to study treatment discontinuation. Later grade-3 reactions and any grade-2 reaction resulted in a half-dose injection at the next visit.

Body temperature, vital signs, and FEV1 were measured prior to injection; vital signs were also measured at 30 minutes and FEV1 at 30 minutes, 1, 6 to 8, and 24 hours after each injection (FEV1 at 6 to 8 and 24 hours were self-recorded by the subjects). Standard physical examinations, clinical hematology / biochemistry tests, electrocardiography, and serum pregnancy tests were performed at screening and 1 month after treatment end.

Immunological evaluation

Five ml of blood was collected prior (V0, V1) and one month after Bet v 1 COPs or placebo administration (V11). Further samples were collected prior, in the midst and after the birch pollen season (V12, V13 and V14 respectively). Serum supernatant was collected and stored at -80°C. Antigen specific IgG4 and IgE concentration was determined by a direct ELISA as previously described¹³.

Statistical analysis

The combined RSMS obtained daily during the birch pollen season was defined as primary efficacy variable. The main analysis of the primary variable tested Bet v 1 COPs 100µg versus placebo within an analysis-of-covariance (ANCOVA) model, with treatment and predefined geographical regions as independent factors, using the modified intent-to-treat set (mITT, i.e., treated subjects who received at least four injections of randomized treatment and had at least eight valid daily diaries) with no imputation for missing values. mITT was preferred over ITT in analogy to vaccine clinical trials where a minimum number of

injections and days of exposure are necessary to evaluate efficacy. If the null hypothesis was rejected, the subordinate null hypothesis concerning Bet v 1 COPs 50µg would be similarly tested, thus preserving the overall trial-wise type-1 error (0.05, two sided). Data were summarized using model-derived least squares (LS) means with standard errors and 95% confidence limits (CL). Placebo-corrected treatment effects were summarized using LS mean from placebo, each with 95% CL and presented in forest plots.¹⁴ Secondary and exploratory variables were evaluated using mITT in a manner similar to the main analysis of the primary variable, with missing values imputed using Little and Rubin's multiple imputation procedure for the ANCOVA.¹⁵ The safety set included any patient receiving at least one injection (ITT set, according to CONSORT standards). Safety variables were summarized for all treated subjects (safety analysis set) and compared descriptively only.

RESULTS

Subjects

240 patients were randomized to 5 s.c. injections of either placebo or Bet v 1 COPs at 50- or 100 μ g dose within two weeks after screening (Figure 1). Two dropouts were recorded, namely one patient in the Bet v 1 COPs 50 μ g group did not receive treatment due to detection of hypertension after randomization, a second patient in the placebo group was lost to follow up after the fifth injection. The 239 treated subjects were Caucasians (51.9% women) from 18 to 55 years old. Baseline data were similar among the three treatment groups (Table I). Three patients on Bet v 1 COPs 50 μ g (4%) and 10 patients on Bet v 1 COPs 100 μ g (12%) discontinued treatment, 2 (2.8%) and 8 (9.8%) respectively resulting from treatment related AEs. In accordance with adjustment guidance, 10% of placebo, and 33% and 38% of the Bet v 1 COPs 50 μ g and Bet v 1 COPs 100 μ g patients respectively, received adapted lower doses. Thus, the mean cumulative doses were 203.2 and 391.8 μ g in the Bet v 1 COPs 50 μ g and 100 μ g groups respectively (compared with planned 225 and 450 μ g for full dose). Taken together, 239 treated subjects were assessed during the following birch pollen season, whether or not receiving the planned treatment course (Figure 1).

Efficacy

RSMS values in the placebo group paralleled the major pollen peaks during the birch season (Figure 2). Both 50- and 100 μ g groups showed reduced RSMS scores compared to placebo (Figure 3A and Table E2). Based on the modified intent-to-treat set (mITT, n=211), Bet v 1 COPs 50 μ g versus placebo revealed a LS mean treatment effect of -0.23 (mean RSMS

improvement of 26%), which was statistically significant ($p=0.015$). Bet v 1 COPs 100 μ g showed a non significant LS mean treatment effect of - 0.13 ($p=0.150$).

Subjective quality of life was significantly improved as indicated by lower average MiniRQLQ[®] scores in both groups treated with Bet v 1 COPs compared with placebo (LS means of -0.50 (-27%) and -0.48 (-26%) respectively for 50- and 100 μ g doses) (Figure 3B).

Night-time nasal symptoms score (NNSS) represents an additional assessment of efficacy since it may be less influenced by activity and/or awareness. Improvement in NNSS was also observed with both Bet v 1 COPs 50- and 100 μ g dosages (LS mean effect -0.23 (-37%) and -0.21 (-34%) respectively) (Figure 3C). Improvements were also observed in others secondary variables which included percent of “well days” and “days with severe symptoms” as well as “subjects’ global evaluation of treatment efficacy” (Tables E3, E4 and E5 respectively).

Immunogenicity

Repeated administration of Bet v 1 COPs led to significant increase in serum Bet v 1-specific IgG4 (Figure 4A) in all but a few patients (2 non-responders in the 50 μ g group and 4 in the 100 μ g group). The about 20-fold increase in specific IgG4 remained unchanged during the pollen season in both 50- and 100 μ g treated groups. Specific IgG4 were significantly increased compared to placebo in both treated groups after treatment (V11) as well as during and after pollen season (V13, V14) (Figure 4B). No significant difference in specific IgG4 level was observed between 50- and 100 μ g doses.

Bet v 1 specific IgE increased significantly during the pollen season in the placebo group (Figure 4C). Bet v 1 COPs treatment resulted in a 2 to 3-fold increase in specific IgE (V11) without further increase during the pollen season. The specific IgE increase in both 50- and 100µg groups was within the range of the increase in the placebo group after seasonal exposure (Figure 4C, left panel).

Safety

Most subjects had treatment-emergent AEs with an about 2-fold higher overall incidence and number of events in subjects on Bet v 1 COPs than on placebo (Table E6, online repository). Bet v 1 COPs local reactions at the administration site were mild and present to the same extent in all three treatment groups, most probably resulting from the presence of aluminum hydroxide (Figure 5A).

Three SAEs were reported, two of which were Bet v 1 COPs related, and resolved upon symptomatic treatment. The Bet v 1 COPs 50µg patient suffered from conjunctivitis, urticaria, tongue edema and oropharyngeal discomfort after the 5th injection, resolving with antihistamine, intramuscular epinephrine and oral steroid. The Bet v 1 COPs 100µg patient developed hand pruritus, face erythema, feeling of throat swelling and generalized urticaria after the 4th injection, resolving with antihistamine, intravenous steroids and inhaled beta-2 agonists. No WAO grade 3 systemic allergic reaction occurred within 30 minutes post-injection. Two patients, one in each active treatment group reported grade 3 delayed reactions occurring more than 60 minutes after the injection that resolved upon treatment. Most of the WAO Grade 1 and 2 systemic reactions, which occurred more frequently on Bet v 1 COPs than placebo, were mild to moderate and resolved spontaneously (Table E7).

Patients used multiple wordings to describe AEs. Systemic reactions were thus reassigned according to MedDRA 15.1 dictionary (Table E8). In Bet v 1 COPs treated patients (Figure 5B), upper respiratory track related AEs (cough, dyspnea and chest discomfort) seemed to be the more frequent, followed by skin reactions (pruritus, urticaria). Accordingly, patients self-tested FEV1 decrease of >30% occurred more frequently in Bet v 1 COPs 50- and 100µg than in placebo with most events occurring after the first two injections (Figure 5C, Table E9). No change in vital signs, clinical laboratory tests, or electrocardiography variables appeared related to Bet v 1 COPs treatment or dose.

DISCUSSION

For the first time, we show here that AIT based on COPs was efficient and of acceptable safety profile in a phase IIb field trials. The primary objective of this study was to assess the efficacy of COPs derived from the major birch pollen allergen Bet v 1 in improving combined rhinoconjunctivitis symptom and medication score (RSMS). Both Bet v 1 COPs 50- and 100µg doses improved RSMS as compared to placebo. Improvement of LS mean by 26% over placebo in the primary endpoint was significant in the Bet v 1 COPs 50µg group and above the clinically relevant efficacy limit of 20% generally admitted.¹² Despite a considerably shorter treatment period (only two months as opposed to 18 to 36 months), this improvement in RSMS is comparable to scores of other recent field based AIT trials.¹⁷⁻²⁰ The 100µg group showed almost the same rhinoconjunctivitis symptom score level, however failed to reach significance in RSMS. Upon post-hoc analysis, the high medication score observed in the Bet v 1 COPs 100µg group (Table E2) could be explained by sustained self-administration of oral corticosteroids (highest weight in the score) in five patients out of 82 for a prolonged period of time. Interestingly, the Bet v 1 COPs 100µg was undistinguishable from the 50µg group in both MiniRQLQ[®] and night time nasal symptom scores. Considering the limited statistical power of the study, both dosages may thus be considered equivalent in efficacy with no additional benefit from the higher dose as previously observed in another AIT studies based on peptides.²¹

Long synthetic peptides appeared safe in several vaccine trials^{22, 23} as well as in two AIT trials based on COPs derived from PLA2, the major allergen of bee venom, or from *Bet v 1* (Bet v 1 COPs) in a recent phase I/IIa study.^{8, 24} COPs were carefully selected among several

sets of peptides, leading to the choice of Bet v 1 COPs, a set of three long synthetic peptides with the lowest IgE binding capacity.⁷ The safety of COPs was clearly demonstrated in preclinical murine models, where the intraperitoneal injection of COPs did not induce allergic reactions in contrast to native allergen, and protected mice from anaphylaxis upon re-exposure.^{7, 25} In this study, like in the previous phase I/IIa, we show that most local AEs are related to aluminum hydroxide.⁸ The number of dropouts due to treatment related AEs was limited to 2.8% in the 50µg group, but reached 9.8% in the 100µg group, suggesting an improved clinical tolerance of the 50µg dose. The same trend was observed when comparing the frequency of systemic allergic reactions between the two dose groups, which nonetheless remained mild to moderate. Another Phase IIb study is currently in progress, which may help to better define the relationship between a series of lower Bet v 1 COPs dose levels and tolerability in the setting of an experimental pollen exposure chamber (EEC) model.

Administration of Bet v 1 COPs resulted in FEV1 drops $\geq 30\%$ in 6.5% of patients, most of them occurring late (> 3 hours) after the first or second injection. Such respiratory events were also observed in early clinical trials with short T cell epitope peptides as well as with longer allergen fragments.^{26, 27} The number and severity of FEV1 drops may have been overestimated in the present study, possibly due to self-measurement by the patient in the absence of medical supervision. However, none of the FEV1 drops led to SAE reporting, emergency room visit or even to a call to the investigator before the next scheduled visit (24 hours post-injection). Episodes of pulmonary symptoms like cough, shortness of breath or dyspnea were more frequent in the 100µg group (55% of the patients) and in the 50 µg group (35%) than in the placebo group (13%) indicating a probable relationship between Bet v 1

COPs and late respiratory systemic reactions. The mechanisms of these late allergic reactions are not fully understood, but most probably are dependent on T cell activation rather than on IgE binding, mast cell or eosinophil activation.^{28, 29} Such respiratory AEs also occurred in AIT trials based on major T cell epitope peptides, which are unable to bind IgE.²⁷ In the present trial as well as in previous peptide based AIT studies, FEV1 drops were mainly observed after the first injection to decrease thereafter, thus arguing again for early T cell activation followed by progressive T cell tolerance upon repeated injections.^{8, 27} Other non-lower respiratory tract AEs were mild and occurred also late after injection, including skin, upper respiratory tract and eye symptoms, once more suggesting T cell activation rather than IgE dependent mast cell activation. Finally, only 2 WAO-grade 3 events relatively moderate in severity were considered as SAE (a third one was unrelated to vaccine) since they occurred within 60 min of the injections. Taken together, this safety report is clearly distinct from earlier trials based on non selected allergen fragments or recombinant allergen³⁰ or restricted T cell epitope peptides²⁶ by the very limited number of immediate allergic reactions and SAE which were of moderate severity and which did not require hospitalization, although they were considered as SAE by protocol definition. All other AEs were mild to moderate in severity, and all resolved without medical intervention within a few hours.

The increase in IgG4 after AIT represents the only marker associated with successful immunotherapy.³¹ We confirm here the findings of our phase I/IIa trial, i.e. a vigorous induction of Bet v 1 specific IgG4 of about 20-fold one month after the treatment phase. IgG4 levels persisted elevated up to three years after Bet v 1 COPs treatment suggestive of

long-term immunological memory.⁸ These results are in sharp contrast with the absence of IgG4 induction observed in clinical trials based on short T cell epitopes.³² An interval of one month between the last injection and the start of the pollen season appears thus ideal to reach an optimal plateau of IgG4 serum level. No increase in IgG4 was observed in the placebo group, in contrast to IgE which increased in all groups in proportion similar to those observed during the pollen season.¹⁶

In conclusion, this study demonstrates for the first time that a short course of AIT with COPs efficiently immunomodulated the allergic response of patients with rhinoconjunctivitis during the first season post treatment, improving symptoms and quality of life, without inducing more safety concerns than conventional SCIT³³. Furthermore this approach induced a particularly strong IgG4 specific response, the hallmark of successful immunotherapy. Follow up studies will help to define the duration of protection, and possibly better characterize individuals with potential risk of late allergic reactions.

ACKNOWLEDGMENTS

The authors wish to thank **Karen Manchester, PhD** for editorial assistance with the manuscript and all investigators of AN004T study for their commitment to successfully recruit patients

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FIGURE LEGENDS

Fig. 1: Trial profile.

Flow diagram illustrating the numbers of subjects screened, randomized, treated, and evaluated during the study.

Fig. 2: RSMS and pollen exposure during the birch pollen season.

Start of birch pollen season (D0) was set as the first day of three consecutive days with at least 10 pollen grains/m³. Regional birch pollen air concentration was measured separately in Denmark and Sweden, Latvia/Lithuania, Poland and France/Switzerland (grey shaded areas). Daily RSMS scores (right axis) are reported as smoothed curves in green for placebo, red for Bet v 1 COPs 50µg and blue for Bet v 1 COPs 100µg.

Fig. 3: Differences from placebo of Bet v 1 COPs treated groups

LS mean differences of the indicated scores (panels A, B, C) are plotted relative to placebo (green line) for Bet v 1 COPs 50µg (red) and Bet v 1 COPs 100µg (blue). 95% CI limits are indicated as horizontal colored lines. The analyses were performed during the 2013 birch pollen season on the mITT set). P-values were determined by analysis of covariance (ANCOVA) with treatment and geographical region as independent factors in the model.

Fig. 4: Effect of treatment on levels of Bet v 1-specific IgG4 and IgE

Panel A: Betv1 specific IgG4 was measured by ELISA in serum from Bet v 1 COPs and placebo treated patients of the mITT set at pre-treatment (V0 and V1), 30 days post-treatment (V11), at peak of season (V13) and about 14 days post-season (V14).

Panel B: Fold increase over baseline in Bet v 1 specific IgG4 was calculated for each individual patient at the indicated visits. Medians (solid line) are displayed within solid boxes showing 25th to 75th percentiles. Whiskers expand to the 10th and 90th percentile and individual outliers are shown as colored dots.

Panel C: Fold increase over baseline in Bet v 1 specific IgE was calculated for each individual patient at the indicated visits and is displayed as indicated under panels A and B.

NS indicates $p > 0.05$; * $0.05 > p > 0.001$; ** $0.001 > p > 0.0001$; *** $0.0001 > p$ as determined by Wilcoxon signed rank test.

Fig. 5: Safety analysis, local and systemic AEs and FEV drops

Panel A: Frequency of treatment-emergent local AEs at the administration site stratified by clinical description and recorded on the safety set (any patient receiving at least one injection).

Panel B: All systemic AEs with frequency $\geq 5\%$ stratified by clinical description, recorded on the safety set.

Panel C: Frequency of self-tested FEV1 drops (Δ FEV1) of more than 30% stratified by injections 1 to 5.

Table 1 Demographics and baseline disease characteristics (all treated subjects)

Characteristics	Placebo (n = 79)	AllerT 50 µg (n = 78)	AllerT 100 µg (n = 82)
Sex (%), male / female	51.9 / 48.1	44.9 / 55.1	47.6 / 52.4
Age (years), mean ± SD range	34.1 ± 9.1 18–55	36.2 ± 9.9 19–54	36.4 ± 9.4 18–53
Race (%), Caucasian / other	100 / 0	100 / 0	100 / 0
Region (%)			
Denmark/Sweden	32.9	32.1	32.9
France/Switzerland	5.1	6.4	7.3
Latvia/Lithuania	24.1	25.6	25.6
Poland	38.0	35.9	34.1
RSS (mean ± SD) ≥overall median score (%)	14.3 ± 1.6 68.4	14.3 ± 1.7 64.1	14.4 ± 1.8 65.9
FEV1 (% of predicted), mean ± SD range	95.3 ± 10.5 77.9–123.0	95.3 ± 10.7 80.3–124.0	95.5 ± 9.7 78.2–124.9

FEV1, forced expiratory volume in the first second of expiration; RSS, rhinoconjunctivitis symptom score; SD, standard deviation.

Fig. 1

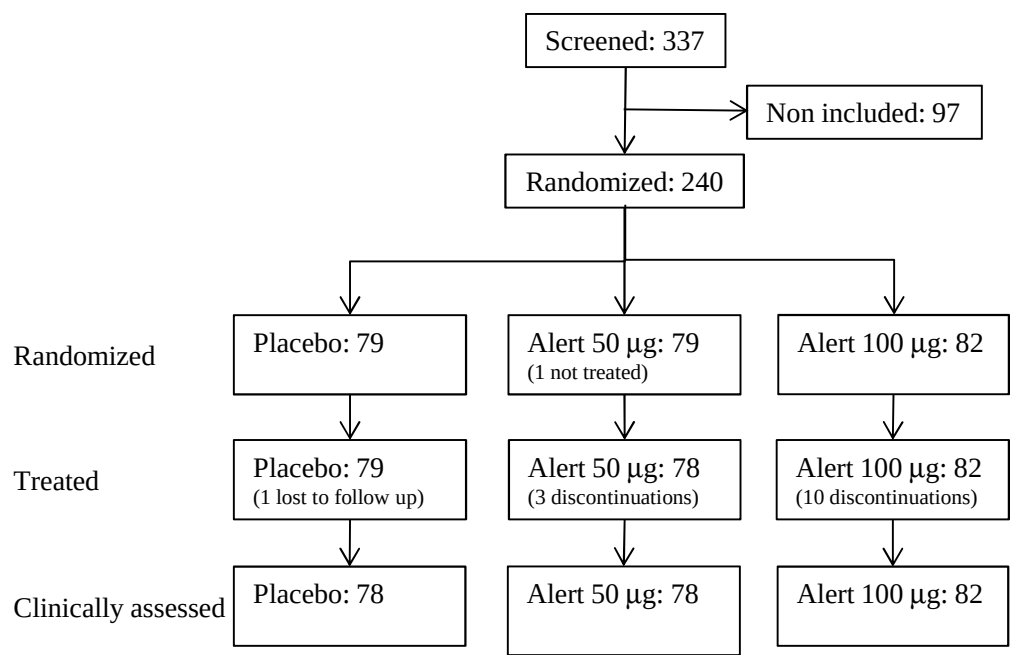


Fig. 2

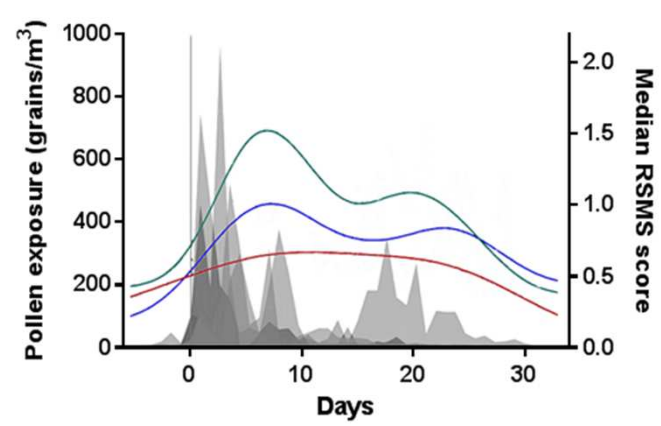
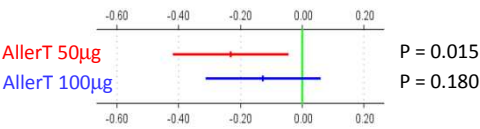
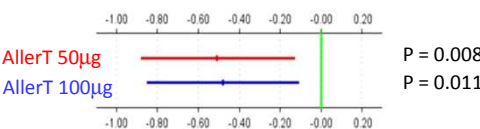


Fig. 3

A) RSMS



B) Mini RQLQ



C) Night time nasal symptom scores

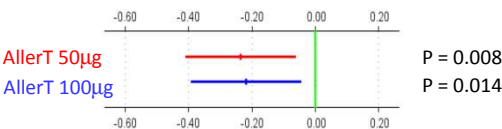


Fig. 4

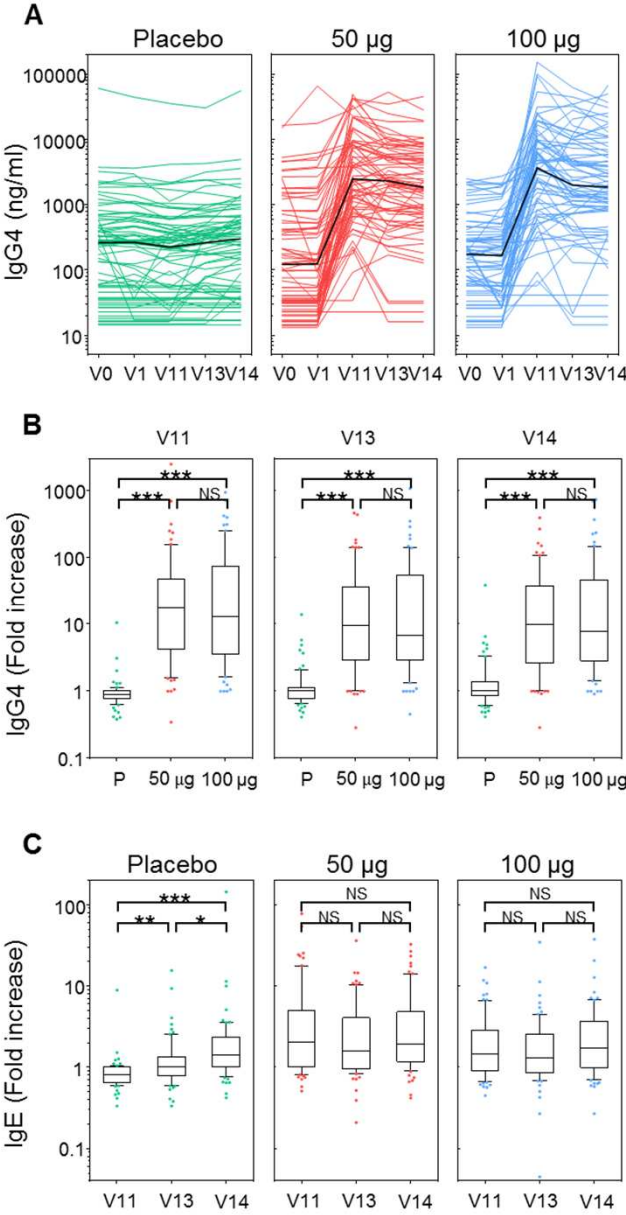
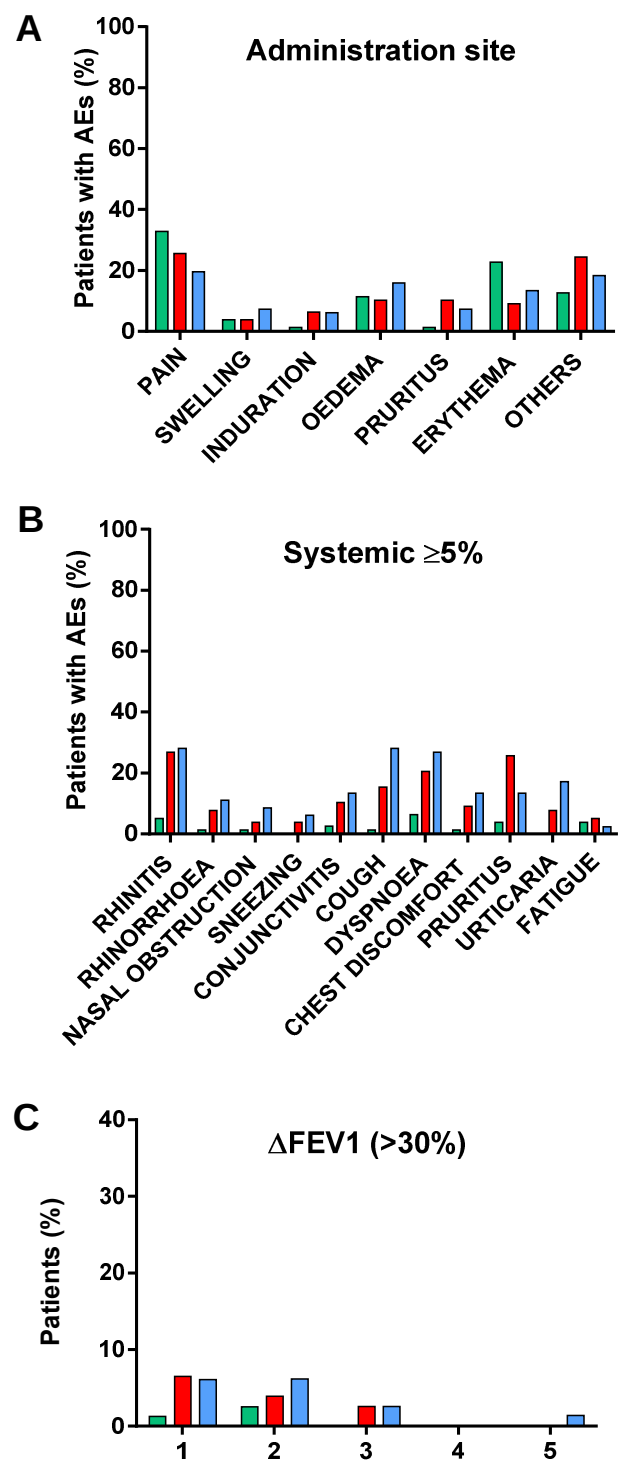


Fig. 5



ONLINE REPOSITORY

METHODS

- **Rhinoconjunctivitis Symptom Score (RSS)**

The RSS was determined based on subject's self-administered questionnaire filled out daily from Visit V1 to Visit V3.

The RSS comprised 6 different symptoms from the nose and eyes (Table E1, online repository). The sum of the 6 symptom scores divided by 6 was used as the RSS (scale of 0 to 3).

All individual rhinoconjunctivitis symptoms were scored on a scale from 0 to 3 as follows:

0 = no symptoms

1 = slight symptoms

2 = moderate symptoms

3 = severe symptoms

- **Rhinoconjunctivitis Medication Score (RMS)**

The RMS was determined based on medications taken to treat rhinoconjunctivitis symptoms and recorded on a specific questionnaire by the subject on the same days as the RSS questionnaire.

The daily RMS was determined by assigning daily scores as follows:

0 = no medication

1 = subject took topical antihistamine

2 = subject took oral antihistamine

3 = subject took oral corticosteroids

The maximum daily score was 3. The daily score assigned for a given day is the highest score of any single medication administered that day.

The following medications were provided to subjects during the clinical assessment period of the trial:

- Antihistamine topical: azelastine for intra-nasal use
azelastine for ocular use
- Antihistamine oral: loratadine 10-mg tablets
- Corticosteroids oral: prednisone 5-mg tablets

If azelastine was unavailable in a given country, levocabastine nasal spray and ocular drops may be used instead.

If prednisone was unavailable in a given country, prednisolone may be used instead.

- **Combined Rhinoconjunctivitis Symptom and Medication Score (RSMS)¹**

The RSMS was calculated as the sum of the RSS and the RMS divided by 2.

¹ Nelson HS, Nolte H, Creticos P, Maloney J, Wu J, Bernstein DI. Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. *J Allergy Clin Immunol* 2011; 127:72-80, 80.e1-2.

Legends to Tables E2, E3, E4

Others variables included the “Well days” (days during which RMS is zero and RSS is ≤ 2), the “Days with severe symptoms” (Days during which at least 3 of the 6 symptoms of RSS are scored as severe), the “Subject’s global analysis of treatment efficacy” (Subject- rated treatment efficacy at study end compared to the previous season, from 0 for significantly improved to 6 for significantly worse).

Table E1: Rhinoconjunctivitis symptoms assessed during the birch pollen season

Symptom class	Symptoms
Nose symptoms	Runny nose
	Blocked nose
	Sneezing
	Itchy nose (might also affect eyes, throat or ears)
Eye symptoms	Gritty feeling / red / itchy eye
	Watery eye

Table E2: Summary of the average combined RSMS, RSS, and RMS during the birch pollen season (mITT set)

Characteristics	Placebo (n = 71)	AllerT 50 µg (n = 70)	AllerT 100 µg (n = 70)
Combined RSMS			
mean (95% CL)	0.87 (0.72, 1.01)	0.63 (0.50, 0.75)	0.73 (0.59, 0.87)
median (95% CL)	0.76 (0.54, 1.09)	0.44 (0.36, 0.56)	0.53 (0.40, 0.80)
LS mean ± SE	0.87 ± 0.07	0.64 ± 0.07	0.74 ± 0.07
p-value*		0.015	0.180
RSS			
mean (95% CL)	0.87 (0.72, 1.01)	0.65 (0.53, 0.76)	0.66 (0.54, 0.78)
median (95% CL)	0.75 (0.58, 0.92)	0.51 (0.42, 0.61)	0.53 (0.39, 0.79)
LS mean ± SE	0.86 ± 0.07	0.65 ± 0.07	0.66 ± 0.07
RMS			
mean (95% CL)	0.86 (0.69, 1.04)	0.61 (0.45, 0.76)	0.80 (0.61, 0.99)
median (95% CL)	0.76 (0.37, 1.18)	0.34 (0.16, 0.63)	0.55 (0.20, 0.91)
LS mean ± SE	0.88 ± 0.10	0.63 ± 0.09	0.82 ± 0.09

* p-values determined by analysis of covariance with treatment and geographical region as independent factors in the model.

ANCOVA, analysis of covariance, CL, confidence limits; RMS, rhinoconjunctivitis medication score; RSMS, rhinoconjunctivitis symptom and medication score; RSS, rhinoconjunctivitis symptom score; SE, standard error of the mean; (95% CL) 5-95% Confidence Limit

Table E3

Study AN004T

Analysis summary of percent of well days during the birch pollen season

Analysis Set: Modified Intent-to-Treat

	Placebo N=71	AllerT 50 µg N=70	AllerT 100 µg N=70
Over the birch pollen season			
n valid	71	70	70
LS mean	51.22	62.68	54.24
Standard error	4.88	4.83	4.83
TREATMENT EFFECT			
Mean		11.46	3.02
95% CL of mean		-0.93 , 23.85	-9.36 , 15.41
p-value ANCOVA		0.0696	0.6309
(95% CL) 5-95% Confidence Limit			

Table E4

Study AN004T

Analysis summary of percent of days with severe symptoms during the birch pollen season

Analysis Set: Modified Intent-to-Treat

	Placebo N=71	AllerT 50 µg N=70	AllerT 100 µg N=70
Over the birch pollen season			
n valid	71	70	70
LS mean	6.52	3.26	3.33
Standard error	1.70	1.68	1.68
TREATMENT EFFECT			
Mean		-3.26	-3.20
95% CL of mean		-7.57 , 1.05	-7.51 , 1.11
p-value ANCOVA		0.1375	0.1453
(95% CL) 5-95% Confidence Limit			

Table E5

Study AN004T

Analysis summary of subject's global evaluation of treatment efficacy collected at the end of the birch pollen season

Analysis Set: Modified Intent-to-Treat

	Placebo		AllerT 50 µg N=70		AllerT 100 µg N=70	
	n	%	n	%	n	%
SIGNIFICANTLY IMPROVED						
Total subjs	16	22.5%	22	31.4%	27	38.6%
MODERATELY IMPROVED						
Total subjs	20	28.2%	23	32.9%	17	24.3%
MILDLY IMPROVED						
Total subjs	15	21.1%	13	18.6%	10	14.3%
UNCHANGED						
Total subjs	15	21.1%	8	11.4%	6	8.6%
MILDLY WORSE						
Total subjs	4	5.6%	4	5.7%	6	8.6%
MODERATELY WORSE						
Total subjs	0		0		2	2.9%
SIGNIFICANTLY WORSE						
Total subjs	1	1.4%	0		2	2.9%
n						
Improved	51	71.8%	58	82.9%	54	77.1%
Exact 97.5% CLs*	58.2%, 83.1%		70.4%, 91.7%		63.9%, 87.4%	
TREATMENT EFFECT:						
Risk Ratio			1.15		1.07	
97.5% CLs			0.94, 1.42		0.86, 1.34	
p-value Fisher's exact test			0.1591		0.5632	

* Clopper-Pearson formula

Table E6: Summary of adverse events (all treated subjects)

Characteristics	Placebo (n = 79)	AllerT 50 µg (n = 78)	AllerT 100 µg (n = 82)
Number of treatment-emergent			
AE reports	267	479	513
AEs occurring ≤1 h post injection	9	26	36
Injection-site reaction reports	144	136	129
Systemic reaction reports**	42	253	296
Subjects (n [%]) with ≥1 treatment-emergent AE	59 (74.7)	70 (89.7)	70 (85.4)
Treatment-related AE	56 (70.9)	65 (83.3)	69 (84.1)
Injection-site reaction	50 (63.3)	46 (59.0)	44 (53.7)
Systemic reaction	21 (26.6)	50 (64.1)	58 (70.7)
Immediate *	—	3 (3.8)	6 (7.3)
WAO grade-3	—	2 (2.6)	2 (2.4)
SAE	—	1 (1.3)	2 (2.4)***
AE leading to treatment discontinuation	—	2 (2.6)	8 (9.8)
Subjects (n[%]) with ≥1 non-treatment-emergent AE†	23 (29.1)	20 (25.6)	25 (30.5)

* Immediate TEAEs occurred within 30 minutes of an injection; other AEs were defined as delayed.

† A non-treatment-emergent AE occurred more than 28 days after the last injection; those occurring during the treatment period up to 28 days after the last injection were treatment emergent.

AE, adverse event; SAE, serious adverse event; WAO, World Allergy Organization.

** All non local reactions have been reported as "systemic"

*** One SAE was treatment unrelated

Table E7

Study AN004T
 Summary overview of treatment-emergent adverse event reports.
 Analysis Set: Safety

GRADE 1

		Placebo				AllerT 50 µg				AllerT 100 µg			
		N=79		Duration		N=78		Duration		N=82		Duration	
		n	(1)		(2)	n	(1)		(2)	n	(1)		(2)
AE reports		21	14	17.7%	173:26	73	36	46.2%	39:44	93	44	53.7%	162:34
Distribution by systemic allergic reaction:													
Attribution to injection:													
	Grade 1	21	14	17.7%	173:26	73	36	46.2%	39:44	93	44	53.7%	162:34
	1st injection	10	9	11.4%	3:58	19	17	21.8%	25:16	19	18	22.0%	31:10
	2nd injection	2	2	2.5%	0:45	16	16	20.5%	21:30	21	19	23.2%	34:44
	3rd injection	3	3	3.8%	101:00	14	14	17.9%	46:02	22	21	25.6%	24:02
	4th injection	4	4	5.1%	662:01	10	10	12.8%	14:45	14	13	15.9%	215:01
	5th injection	2	2	2.5%	325:00	14	12	15.4%	91:43	17	13	15.9%	603:27
Later than 28 days after last injection		-	-	-	-	-	-	-	-	-	-	-	-
GRADE 2													

GRADE 2

		Placebo				AllerT 50 µg				AllerT 100 µg			
		N=79		Duration		N=78		Duration		N=82		Duration	
		n			(2)	n			(2)	n			(2)
		(1)				(1)				(1)			
AE reports		21	10	12.7%	195:24	178	34	43.6%	31:26	200	44	53.7%	23:48
Distribution by systemic allergic reaction:													
	Grade 2	21	10	12.7%	195:24	178	34	43.6%	31:26	200	44	53.7%	23:48
SAEs		-	-	-	-	3	1	1.3%	-	-	-	-	-
Attribution to injection:													
	1st injection	6	4	5.1%	7:43	46	23	29.5%	28:05	71	32	39.0%	17:39
	2nd injection	9	5	6.3%	58:02	63	29	37.2%	18:55	61	31	37.8%	17:53
	3rd injection	1	1	1.3%	1127:59	36	21	26.9%	31:19	33	19	23.2%	11:16
	4th injection	1	1	1.3%	71:59	23	13	16.7%	17:02	19	12	14.6%	15:28
	5th injection	4	3	3.8%	583:45	10	5	6.4%	159:10	16	11	13.4%	109:17
Later than 28 days after last injection		-	-	-	-	-	-	-	-	-	-	-	-

GRADE 3

		Placebo				AllerT 50 µg				AllerT 100 µg			
		N=79				N=78				N=82			
		n	(1)	Duration	(2)	n	(1)	Duration	(2)	n	(1)	Duration	(2)
AE reports		-	-	-	-	2	2	2.6%	23:34	3	2	2.4%	13:56
Distribution by systemic allergic reaction:													
Grade 3		-	-	-	-	2	2	2.6%	23:34	3	2	2.4%	13:56
SAEs		-	-	-	-	1	1	1.3%	-	1	1	1.2%	-
Attribution to injection:													
1st injection		-	-	-	-	1	1	1.3%	45:00	1	1	1.2%	21:00
2nd injection		-	-	-	-	-	-	-	-	1	1	1.2%	17:00
3rd injection		-	-	-	-	-	-	-	-	-	-	-	-
4th injection		-	-	-	-	-	-	-	-	1	1	1.2%	3:49
5th injection		-	-	-	-	1	1	1.3%	2:08	-	-	-	-
Later than 28 days after last injection		-	-	-	-	-	-	-	-	-	-	-	-

(1) Number of reports – number of subjects reporting – percent of subjects reporting

(2) Mean Duration (hours:minutes)

Table E8

Study AN004T

Summary of treatment-emergent adverse events excluding unrelated to trial treatment by frequency

Analysis Set: Safety

System Organ Class / Preferred Term	Placebo N=79 n %	AllerT 50 µg N=78 n %	AllerT 100 µg N=82 n %
ALL SYSTEM ORGAN CLASSES			
Total subjs with at least one AE	56 70.9%	65 83.3%	69 84.1%
Total number of AEs	112	228	255
RHINITIS	4 5.1%	21 26.9%	23 28.0%
DYSPONOEA	5 6.3%	16 20.5%	22 26.8%
INJECTION SITE PAIN	26 32.9%	20 25.6%	16 19.5%
COUGH	1 1.3%	12 15.4%	23 28.0%
INJECTION SITE REACTION	10 12.7%	19 24.4%	15 18.3%
PRURITUS	3 3.8%	20 25.6%	11 13.4%
INJECTION SITE OEDEMA	9 11.4%	8 10.3%	13 15.9%
URTICARIA	-	6 7.7%	14 17.1%
CONJUNCTIVITIS	2 2.5%	8 10.3%	11 13.4%
INJECTION SITE ERYTHEMA	18 22.8%	7 9.0%	11 13.4%
CHEST DISCOMFORT	1 1.3%	7 9.0%	11 13.4%
RHINORRHOEA	1 1.3%	6 7.7%	9 11.0%
INJECTION SITE PRURITUS	1 1.3%	8 10.3%	6 7.3%
INJECTION SITE INDURATION	1 1.3%	5 6.4%	5 6.1%
NASAL OBSTRUCTION	1 1.3%	3 3.8%	7 8.5%
INJECTION SITE SWELLING	3 3.8%	3 3.8%	6 7.3%
SNEEZING	-	3 3.8%	5 6.1%
FATIGUE	3 3.8%	4 5.1%	2 2.4%
RHINITIS ALLERGIC	-	2 2.6%	4 4.9%
NASAL CONGESTION	1 1.3%	3 3.8%	1 1.2%
ASTHMA	-	1 1.3%	3 3.7%
NASOPHARYNGITIS	-	1 1.3%	3 3.7%
OCULAR HYPERAEMIA	-	2 2.6%	2 2.4%
PRURITUS GENERALISED	1 1.3%	1 1.3%	2 2.4%
MUSCULOSKELETAL PAIN	3 3.8%	1 1.3%	1 1.2%
CHILLS	1 1.3%	1 1.3%	1 1.2%
LACRIMATION INCREASED	1 1.3%	1 1.3%	1 1.2%
MYALGIA	1 1.3%	-	2 2.4%
OROPHARYNGEAL PAIN	1 1.3%	2 2.6%	-
THROAT IRRITATION	1 1.3%	1 1.3%	-
WHEEZING	1 1.3%	1 1.3%	1 1.2%
BODY TEMPERATURE INCREASED	-	1 1.3%	1 1.2%
CHEST PAIN	-	2 2.6%	-
CONJUNCTIVITIS ALLERGIC	-	2 2.6%	-
ERYTHEMA	-	-	2 2.4%
HEADACHE	-	2 2.6%	-
INJECTION SITE URTICARIA	-	2 2.6%	-
MALaise	-	1 1.3%	1 1.2%
PYREXIA	-	1 1.3%	1 1.2%
RESPIRATORY DISORDER	-	-	2 2.4%
SEASONAL ALLERGY	-	1 1.3%	1 1.2%
DYSPHONIA	1 1.3%	1 1.3%	-
FORCED EXPIRATORY VOLUME DECREASED	1 1.3%	-	1 1.2%
NAUSEA	1 1.3%	1 1.3%	-
PAIN IN EXTREMITY	1 1.3%	-	1 1.2%
PRURIGO	1 1.3%	-	1 1.2%
RASH PAPULAR	1 1.3%	1 1.3%	-
AGITATION	-	1 1.3%	-
APHONIA	-	1 1.3%	-
ASTHENIA	-	1 1.3%	-
BLISTER	-	-	1 1.2%
DERMATITIS ALLERGIC	-	1 1.3%	-
DISCOMFORT	-	-	1 1.2%
DIZZINESS	-	1 1.3%	-
DRY EYE	-	-	1 1.2%
DRY THROAT	-	-	1 1.2%
FACE OEDEMA	-	1 1.3%	-
FREEZING PHENOMENON	-	1 1.3%	-
HAEMATOMA	-	-	1 1.2%
HYPERSENSITIVITY	-	-	1 1.2%
INCREASED UPPER AIRWAY SECRETION	-	1 1.3%	-
INFLUENZA LIKE ILLNESS	-	-	1 1.2%
INJECTION SITE PAPULE	-	-	1 1.2%
LIP SWELLING	-	-	1 1.2%
MYOSITIS	-	-	1 1.2%
OBSTRUCTIVE AIRWAYS DISORDER	-	1 1.3%	-
OEDEMA MOUTH	-	1 1.3%	-
ORAL ALLERGY SYNDROME	-	1 1.3%	-
OROPHARYNGEAL DISCOMFORT	-	1 1.3%	-
PALPITATIONS	-	-	1 1.2%
PARAESTHESIA ORAL	-	1 1.3%	-
PHARYNGITIS	-	1 1.3%	-
RASH	-	1 1.3%	-
RASH MACULO-PAPULAR	-	1 1.3%	-
RASH PRURITIC	-	1 1.3%	-
STOMATITIS	-	-	1 1.2%
SWELLING	-	1 1.3%	-
SWELLING FACE	-	-	1 1.2%
TONGUE OEDEMA	-	1 1.3%	-
EYE IRRITATION	1 1.3%	-	-
FORCED EXPIRATORY VOLUME ABNORMAL	1 1.3%	-	-
HEPATIC ENZYME INCREASED	1 1.3%	-	-
HYPOAESTHESIA ORAL	1 1.3%	-	-
INJECTION SITE NODULE	1 1.3%	-	-
OEDEMA PERIPHERAL	1 1.3%	-	-

Table E9

Study AN004T

Occurrence of any decrease greater than 30% in FEV1 between pre- and post-injection measurements

Analysis Set: Safety

	Placebo		AllerT 50 µg		AllerT 100 µg	
	N=79		N=78		N=82	
	n	%	n	%	n	%
After 1st injection	n=79		n=77		n=82	
Total subjs with at least one abnormality	1	1.3%	5	6.5%	5	6.1%
Total number of abnormalities	1		5		6	
After 2nd injection	n=79		n=77		n=81	
Total subjs with at least one abnormality	2	2.5%	3	3.9%	5	6.1%
Total number of abnormalities	2		3		5	
After 3rd injection	n=79		n=76		n=77	
Total subjs with at least one abnormality	-		2	2.6%	2	2.6%
Total number of abnormalities	-		2		2	
After 4th injection	n=79		n=76		n=75	
Total subjs with at least one abnormality	-		-		-	
After 5th injection	n=79		n=75		n=72	
Total subjs with at least one abnormality	-		-		1	1.4%
Total number of abnormalities	-		-		1	

subjs = subjects

Table E10

Study AN004T

Summary of previous and concomitant diseases by system organ class

(Selection for organs related to allergy)

Analysis Set: Safety

System Organ Class / Preferred Term	Placebo N=79 n %	Allert 50 µg N=78 n %	Allert 100 µg N=82 n %
ALL SYSTEM ORGAN CLASSES			
Total subjs with at least one disease	79 100%	78 100%	82 100%
Total number of diseases	194	215	209
IMMUNE SYSTEM DISORDERS			
Total subjs with at least one disease	74 93.7%	75 96.2%	78 95.1%
Total number of diseases	112	109	120
SEASONAL ALLERGY (Other than birch)	70 88.6%	69 88.5%	71 86.6%
ORAL ALLERGY SYNDROME	21 26.6%	21 26.9%	24 29.3%
ALLERGY TO ANIMAL	9 11.4%	10 12.8%	16 19.5%
FOOD ALLERGY	4 5.1%	5 6.4%	6 7.3%
HOUSE DUST ALLERGY	2 2.5%	1 1.3%	1 1.2%
ASTHMA	2 2.5%	1 1.3%	-
ANTIPHOSPHOLIPID SYNDROME	-	1 1.3%	-
DRUG HYPERSENSITIVITY	-	-	1 1.2%
HYPERSENSITIVITY	-	-	1 1.2%
RUBBER SENSITIVITY	-	1 1.3%	-
CONJUNCTIVITIS ALLERGIC	2 2.5%	-	-
DERMATITIS ALLERGIC	1 1.3%	-	-
MYCOTIC ALLERGY	1 1.3%	-	-
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
Total subjs with at least one disease	13 16.5%	14 17.9%	12 14.6%
Total number of diseases	14	15	13
ASTHMA	12 15.2%	11 14.1%	11 13.4%
NASAL SEPTUM DEVIATION	-	3 3.8%	1 1.2%
ADENOIDAL HYPERTROPHY	-	-	1 1.2%
ASTHMA EXERCISE INDUCED	-	1 1.3%	-
BRONCHITIS CHRONIC	1 1.3%	-	-
NASAL POLYPS	1 1.3%	-	-
EYE DISORDERS			
Total subjs with at least one disease	6 7.6%	11 14.1%	10 12.2%
Total number of diseases	6	11	10
CONJUNCTIVITIS ALLERGIC	6 7.6%	10 12.8%	10 12.2%
CHORIORETINOPATHY	-	1 1.3%	-
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Total subjs with at least one disease	9 11.4%	3 3.8%	6 7.3%
Total number of diseases	9	3	7
DERMATITIS ATOPIC	3 3.8%	-	2 2.4%
ACNE	1 1.3%	-	1 1.2%
ANGIOEDEMA	1 1.3%	1 1.3%	-
URTICARIA	1 1.3%	-	1 1.2%
PRURITUS	-	1 1.3%	-
ROSACEA	-	-	1 1.2%
SCAR	-	1 1.3%	-
SEBORRHOEIC DERMATITIS	-	-	1 1.2%
URTICARIA CHRONIC	-	-	1 1.2%
ECZEMA	3 3.8%	-	-