

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

# Immunological effects and tolerability of a new fast updosed immunologically enhanced subcutaneous immunotherapy formulation with optimized allergen/adjuvant ratio

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## Keywords

adjuvant; immunology; short up dosing; subcutaneous immunotherapy; tolerability.

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## Abstract

**Background** Subcutaneous immunotherapy (SCIT) traditionally includes an up-dosing phase injecting increasing doses of allergen over a period of several weeks, followed by a maintenance phase. To establish shorter and more convenient up-dosing schedules, a fast updosed immunologically enhanced SCIT formulation has been developed by optimizing the allergen/adjuvant (aluminium hydroxide) ratio.

**Methods** In a randomized, controlled, parallel-group trial, patients with grass pollen induced rhinoconjunctivitis with/without asthma were treated with an immunologically enhanced SCIT formulation (AVANZ, ALK, Denmark). The trial included up dosing with five injections (300, 600, 3000, 6000 and 15 000 SQ+) injected either in weekly interval (Group 1) or in 3–4 days interval (Group 2) followed by two maintenance injections (15 000 SQ+), approximately 10 weeks treatment. The immunological effects (primary endpoint) and tolerability (secondary endpoint) of the up dosing schedules were evaluated.

**Results** Four hundred patients were treated (Group 1: 201, Group 2: 199). In both groups, an immunological response with statistically significant increases in levels of IgE-blocking factor, IgG<sub>4</sub> and IgE ( $P < 0.001$ ), was induced from baseline to end of trial. Most frequently reported adverse events were local injection site reactions such as injection site swellings (Group 1: 30% of patients, Group 2: 41% of patients). Other frequently reported adverse events included systemic reactions (Group 1: 21% of patients, Group 2: 33% of patients), primarily mild to moderate allergic rhinitis and urticaria.

**Conclusions** Fast updosed immunologically enhanced SCIT with an optimized allergen/adjuvant ratio induced significant immunological effects and had an acceptable safety profile. Clinical efficacy will be investigated in future clinical trials.

Classical subcutaneous immunotherapy (SCIT) is initiated by long up dosing phases with injections of increasing allergen doses over a period of several weeks. This is followed by maintenance phases where patients receive the maximum tolerated dose of allergen with each injection.

Subcutaneous immunotherapy products with nonmodified allergens have been widely used for decades and are docu-

mented to be efficacious and well tolerated (1). However, the use of classical SCIT is limited by the long up dosing phase, being inconvenient for patients and as well as for centres with large patient numbers. Hence, there is a need to develop new SCIT formulations with focus on compliance and convenience, while maintaining the clinical benefits of classical SCIT.

Aluminium hydroxide (alum) is widely used in SCIT for adsorption of allergens to induce slow release, to reduce adverse events and to increase effectiveness of treatment (2). Alum acts like an adjuvant, and even though used for decades (3, 4), the mode of action is still not completely understood. The effect has been attributed to various mechanisms such as prolongation of interaction between antigen and antigen-presenting cells (APCs), recruitment and activation of APCs because of induction of inflammation and facilitation of phagocytosis by APCs (5–8). More recently, it has been suggested that alum may exert its effect through danger signals via activation of the NLRP3 inflammasome complex (9–12).

The possibility of optimizing the allergen/alum ratio of traditional SCIT designated for shorter updosing regimens while maintaining the immunogenicity has been investigated. In mice, equivalent immunological efficacy has been demonstrated comparing a classical alum-adsorbed SCIT preparation with an extract of optimized allergen/alum ratio, but with reduced allergenicity (13). On the basis of these data, a new fast updosed immunologically enhanced SCIT formulation (AVANZ) has been developed allowing for administration of lower doses of allergen while maintaining the immunogenicity. The product is intended for fast up dosing followed by maintenance treatment every  $6 \pm 2$  weeks for 5 years.

The maintenance dose of this formulation has been reduced to 15 000 SQ+ as opposed to the recommended maximum maintenance dose of 100 000 SQ-U with classical SCIT (Alutard SQ), whereas the concentration of alum and other excipients are kept at the same level. These concentrations have shown a similar allergen-specific antibody response in mice (13). In addition, the reduced maintenance dose may justify the use of a shorter up dosing schedule.

This trial was conducted to examine immunological effects and tolerability of two different up dosing schedules of the immunologically enhanced SCIT formulation containing a mix of four sweet vernal grasses and rye.

## Materials and methods

This multicentre, randomized, parallel-group, phase II equivalence trial was initiated using two up dosing schedules with 3-step up dosing (300, 3000 and 15 000 SQ+); one with daily

injections and one with 3–4 days injection intervals. Shortly after initiation of the trial, the protocol was amended after occurrence of a number of delayed allergic reactions, and the 3-step up dosing schedules were changed to 5-step up dosing schedules (one with weekly injections and one with 3–4 days injection intervals). In this study, results of the amended up dosing schedules are reported.

Patients treated after amendment of the up dosing schedules were allocated (1 : 1) to one of the two up dosing schedules (Table 1) with the immunologically enhanced SCIT formulation (AVANZ, 30 000 SQ+/ml, ALK, Denmark) containing equal parts of *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense* and *Secale cereale*. Allergen extracts were standardized according to internal standards; the biological activity was determined as previously described (14, 15) and is expressed in immunologically enhanced standardized quality (SQ+) units.

In this trial, patients received five up dosing injections (300, 600, 3000, 6000 and 15 000 SQ+) and two maintenance injections (15 000 SQ+) as a short-term single course of preseasonal SCIT (approximately 10 weeks of treatment). The maintenance dose of 15 000 SQ+ contains 0.565 mg aluminium. Patients were treated according to an up dosing schedule with weekly injection interval (Group 1) or with 3–4 days injection interval (Group 2). The trial was conducted outside the grass pollen season (GPS) and included nine visits at the clinic (a screening visit, seven treatment visits and a follow-up visit); written informed consent was obtained before patients entered the trial, and the trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice.

Main inclusion criteria were the following: male and female patients 18–65 years of age, clinical history of grass pollen-induced allergic rhinoconjunctivitis of 2 years or more requiring treatment during GPS, lack of adequate relief with symptomatic medications during previous pollen season and positive skin prick test response to *Phleum pratense* (wheal diameter  $\geq 3$  mm).

Main exclusion criteria were the following:  $FEV_1 < 70\%$  of predicted value at screening, bronchial asthma (GINA step 3 or more), history of asthma exacerbation or emergency visit/admission because of asthma in previous 12 months, treatment with immunotherapy with grass pollen allergen within previous 5 years and history of anaphylactic shock.

**Table 1** Up dosing schedules

| Injection no | Phase       | Group 1<br>Up dosing with weekly injection interval |               | Group 2<br>Up dosing with 3–4 days injection interval |               |
|--------------|-------------|-----------------------------------------------------|---------------|-------------------------------------------------------|---------------|
|              |             | Dose                                                | Time          | Dose                                                  | Time          |
| 1            | Up dosing   | 300 SQ+                                             | Day 0         | 300 SQ+                                               | Day 0         |
| 2            |             | 600 SQ+                                             | Day 7         | 600 SQ+                                               | Day 3–4       |
| 3            |             | 3000 SQ+                                            | Day 14        | 3000 SQ+                                              | Day 7–8       |
| 4            |             | 6000 SQ+                                            | Day 21        | 6000 SQ+                                              | Day 10–11     |
| 5            | Maintenance | 15 000 SQ+                                          | Day 28        | 15 000 SQ+                                            | Day 14–15     |
| 6            |             | 15 000 SQ+                                          | +14 days      | 15 000 SQ+                                            | +14 days      |
| 7            |             | 15 000 SQ+                                          | +3 to 4 weeks | 15 000 SQ+                                            | +3 to 4 weeks |

Assessments of immunological effects included analysis of blood samples for the determination of specific IgE-blocking factor, specific IgE and specific IgG<sub>4</sub> against *Phleum pratense*. Blood samples were drawn at screening and at the follow-up visit (in total 2 × 5 ml).

The IgE-blocking factor was determined with the ADVIA Centaur immunoassay system (Siemens Medical Solutions, Diagnostics, Tarrytown, NY, USA) as previously described (16). The IgE-blocking factor is a dimensionless number that varies theoretically from 0 (no presence of any IgE-blocking components) to 1 (all IgE blocked from binding to allergen).

Specific IgE was determined using the ADVIA Centaur immunoassay as described in (17). Specific IgG<sub>4</sub> was determined using the ImmunoCAP method (Phadia AB, Uppsala, Sweden).

For assessment of tolerability, all adverse events were collected and recorded. Adverse events were coded using the Medical Dictionary for Regulatory Activities version 11.0. Adverse drug reactions were defined as either early (occurring 0–60 min after injection) or delayed (occurring 1–24 h after injection). Delayed systemic reactions were classified according to the European Academy of Allergy and Clinical Immunology (EAACI) (18) as unspecific symptoms, urticaria, eczema, rhinoconjunctivitis, angioedema or asthma.

A global assessment of tolerability was also conducted. At the follow-up visit, patients and investigators were asked about tolerability, and opinions were graded as very good, good, moderate or bad.

A safety committee was established to ensure that tolerability was closely monitored during the trial. The safety committee convened every week and evaluated accessible tolerability data.

## Statistics

The primary endpoint was change from baseline in specific IgE-blocking factor against *Phleum pratense* at follow-up (1 week after last injection).

Sample size calculation was based on an equivalence test of the primary endpoint. The clinically relevant equivalence margin was set to 0.03. From clinical trials, the common standard deviation of changes from baseline of IgE-blocking factor was estimated to 0.09, and the true absolute difference between Group 1 and Group 2 is assumed to be 0. Using a significance level of 0.05 and a power of 0.9, at least 190 patients per schedule should complete the trial. Considering a small dropout rate, it was decided to include 200 patients per schedule.

Changes from baseline in specific IgE-blocking factor against *Phleum pratense* 1 week after last injection were tested for equivalence of the two up dosing schedules according to the principle of 'confidence interval inclusion' based on Satterthwaite's modified *t*-test with 0.03 as equivalence margin.

Changes in the specific IgG<sub>4</sub> and specific IgE against *Phleum pratense* 1 week after last injection were compared between the two up dosing schedules by means of Satterthwaite's *t*-test after logarithmic transformation.

All randomized patients, who received at least one injection, were evaluated as the full analysis set and were used for analysis. No imputation of data was carried out in case of missing data; all available data were used to its full extent. Tolerability of the two up dosing schedules was analysed by descriptive statistics.

## Results

A total of 473 patients were randomized, 14 patients withdrew before treatment, and 459 patients were treated. Of these, 59 patients were treated according to the initial 3-step up dosing schedules, which led to a number of delayed systemic reactions (primarily urticaria). The remaining 400 patients were treated according to two amended 5-step up dosing schedules (Table 1). Only data from these 400 patients are reported here.

The trial flow is presented in Fig. 1. Of the 400 patients, a total of 42 (10.5%) withdrew and of these 27 (6.8%) withdrew because of adverse events. There were more withdrawals in Group 2 than in Group 1.

The trial population included 202 (50.5%) men and 198 (49.5%) women. Patients were between 18 and 66 years (mean: 37 years) with a mean duration of grass pollen allergy of 16 years. Overall, patient characteristics were similar between groups (Table 2).

## Immunological effects

A statistically significant increase of IgE-blocking factor from baseline to follow-up was observed for both up dosing schedules ( $P < 0.001$ ; Fig. 2). Mean change from baseline in IgE-blocking factor in Group 1 was 0.32 with 95% confidence limits of [0.30; 0.35]; mean change in Group 2 was 0.36 with 95% confidence limits of [0.33; 0.39].

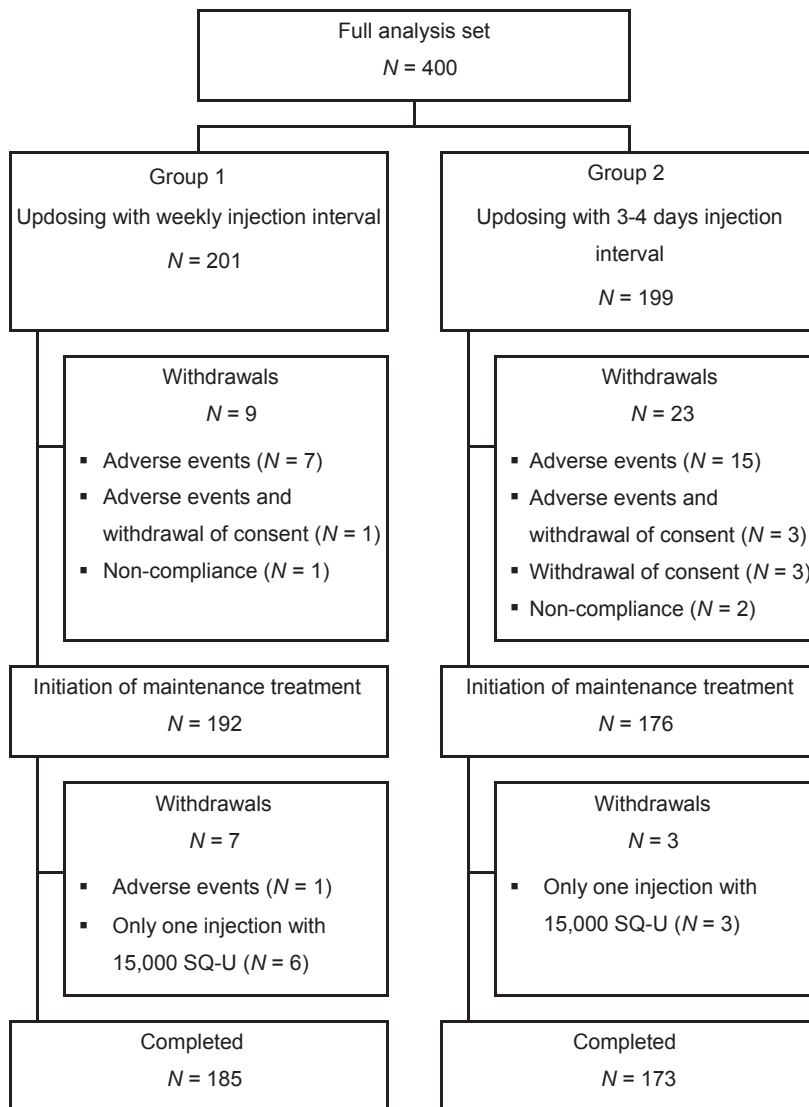
The increase in IgE-blocking factor was not statistically significantly different between Group 1 and Group 2 ( $P = 0.059$ ). However, equivalence between groups could not be confirmed as the estimated difference was not statistically significantly smaller than the equivalence margin of 0.03 ( $P = 0.62$ ).

Similarly, statistically significant increases from baseline to follow-up in IgG<sub>4</sub> and IgE were observed for both groups ( $P < 0.001$ ; Fig. 2). The difference between Group 1 and Group 2 was statistically significantly different from zero for changes from baseline in IgE ( $P = 0.0012$ ), but not for changes from baseline in IgG<sub>4</sub> ( $P = 0.1068$ ). The level of IgG<sub>4</sub> increased from baseline to follow-up by 382% in Group 1 and 464% in Group 2.

## Tolerability

Adverse events are summarized in Table 3. A total of 125 (62%) patients in Group 1 and 140 (70%) patients in Group 2 reported adverse events.

Adverse events were mainly related to the injection site (Table 4). The most frequently reported adverse event (injection site swelling) was reported more frequently by patients



N = Number of patients

**Figure 1** Trial flow diagram. N, number of patients.

in Group 2 (41%) than in Group 1 (30%). Pruritus and urticaria were also reported more frequently in Group 2 (9% and 11% of patients, respectively) as compared with Group 1 (4% and 6% of patients, respectively). Reports of other frequently reported adverse events were evenly distributed between the two updosing schedules.

Local reactions were reported by 36% of patients in Group 1 and by 47% of patients in Group 2, and systemic reactions were reported by 21% of patients in Group 1 and by 33% of patients in Group 2. The majority of reactions were nonserious, mild to moderate reactions evaluated to be related to the treatment.

The vast majority (92%) of the systemic reactions occurred within 1–24 h after injection (delayed reactions) and mainly included reactions classified by EAACI as unspecific symptoms (Group 1: reported by 9% of patients; Group 2: 20%

of patients), rhinoconjunctivitis (Group 1: 9% of patients; Group 2: 12% of patients) and urticaria (Group 1: 6% of patients; Group 2: 10% of patients). Only few patients experienced asthma-related delayed systemic reactions (Group 1: 3%; Group 2: 4%); none of these reactions were severe.

Two patients withdrew because of serious adverse events; one patient in Group 1 (cerebrovascular event, unlikely related to treatment), and one patient in Group 2 (11 local and systemic reactions, all related to treatment).

Overall, the tolerability of the formulation was assessed to be good or very good by the majority of patients and investigators. In Group 2, the tolerability was assessed to be good or very good by 86% of patients and investigators, whereas the highest rate of tolerability was reported in Group 1, where 94% of patients and 96% of investigators assessed the tolerability to be good or very good.

**Table 2** Patient demographics

|                                              | Group 1<br>Updosing<br>with<br>weekly<br>injection<br>interval | Group 2<br>Updosing<br>with<br>3–4 days<br>injection<br>interval | Total        |
|----------------------------------------------|----------------------------------------------------------------|------------------------------------------------------------------|--------------|
| Number of patients                           | 201                                                            | 199                                                              | 400          |
| Age (years)                                  |                                                                |                                                                  |              |
| Mean (SD)                                    | 37.9 (11.1)                                                    | 35.0 (12.2)                                                      | 36.5 (11.7)  |
| Median                                       | 38.0                                                           | 34.0                                                             | 36.0         |
| Minimum–maximum                              | 18–66                                                          | 18–65                                                            | 18–66        |
| Gender, <i>n</i> (%)                         |                                                                |                                                                  |              |
| Male                                         | 97 (48.3%)                                                     | 105 (52.8%)                                                      | 202 (50.5%)  |
| Female                                       | 104 (51.7%)                                                    | 94 (47.2%)                                                       | 198 (49.5%)  |
| Ethnic origin, <i>n</i> (%)                  |                                                                |                                                                  |              |
| Caucasian                                    | 199 (99.0%)                                                    | 195 (98.0%)                                                      | 394 (98.5%)  |
| Other                                        | 2 (1.0%)                                                       | 4 (2.0%)                                                         | 6 (1.5%)     |
| History of rhinoconjunctivitis, <i>n</i> (%) |                                                                |                                                                  |              |
| Yes                                          | 201 (100.0%)                                                   | 199 (100.0%)                                                     | 400 (100.0%) |
| No                                           | –                                                              | –                                                                | –            |
| Duration of rhinoconjunctivitis (years)*     |                                                                |                                                                  |              |
| Mean (SD)                                    | 16.8 (11.4)                                                    | 15.9 (10.9)                                                      | 16.4 (11.1)  |
| Median                                       | 14.0                                                           | 13.0                                                             | 14.0         |
| Minimum–maximum                              | 2–50                                                           | 2–54                                                             | 2–54         |
| History of bronchial asthma, <i>n</i> (%)    |                                                                |                                                                  |              |
| Yes                                          | 65 (32.3%)                                                     | 45 (22.6%)                                                       | 110 (27.5%)  |
| No                                           | 136 (67.7%)                                                    | 154 (77.4%)                                                      | 290 (72.5%)  |
| Duration of bronchial asthma (years)†        |                                                                |                                                                  |              |
| Mean (SD)                                    | 12.5 (11.2)                                                    | 10.1 (8.5)                                                       | 11.5 (10.2)  |
| Median                                       | 10.0                                                           | 8.0                                                              | 9.0          |
| Minimum–maximum                              | 0–46                                                           | 1–29                                                             | 0–46         |

SD, Standard deviation.

\*Data from one patient in Group 1 are missing.

†Data from one patient in Group 2 are missing.

## Discussion

This trial was the first clinical trial conducted with a new fast updosed immunologically enhanced SCIT formulation. It was designed to investigate the immunological effects and tolerability of two different updosing schedules during a short-term single course of preseasonal SCIT.

The primary endpoint, IgE-blocking factor, assessed the effect of serum components competing with IgE for binding to allergen. Such components, including IgE-blocking antibodies of various isotypes, are known to be induced by immunotherapy (19–21).

Because of competitive inhibition, immunotherapy-induced allergen-specific non-IgE antibodies interfere with the binding of specific IgE to allergen. The degree of interference depends on the amount of competing components and the relative affinity of competing components compared to that of the

allergen-specific IgE in serum. Hence, a higher level of competing components and/or a higher affinity of these components will lead to fewer allergen molecules being captured by IgE. Because binding of allergen to IgE on mast cells and basophils is what causes immediate allergic symptoms, the reduction in IgE–allergen complexes on the surface of these cells is expected to lead to fewer symptoms.

The IgE-blocking factor analysis measured the combined effect of immunotherapy-induced non-IgE components and hence reflects the total reduction in IgE–allergen binding that occurs as a result of treatment.

Equivalence of the change from baseline of IgE-blocking factor between Group 1 and Group 2 could not be demonstrated with respect to the prespecified equivalence margin of 0.03. However, both groups induced an immunological response in terms of statistically significant increases in the levels of IgE-blocking factor, IgG<sub>4</sub> and IgE.

The increase in IgG<sub>4</sub> levels of 382–464% from baseline to follow-up and the pattern of these immunological changes are in line with previous reports where SCIT has shown increases in allergen-specific IgG<sub>1</sub> and IgG<sub>4</sub> (22–24). IgG antibodies are believed to play an important role in the mechanisms of immunotherapy, as induction of these antibodies blocks the allergen–IgE interaction (25, 26). Because IgE is a prerequisite of allergic symptoms, a reduction in the proportion of IgE bound to allergen may result in a concomitant reduction in symptoms. Whether the increase in IgE-blocking antibodies observed in this trial results in adequate clinical effects and reduction in rhinoconjunctivitis symptoms remains to be demonstrated in further clinical trials.

The increase in IgG antibodies suggests retained immunogenicity of the formulation. Data from this trial thereby seem to confirm observations in mice (13), where similar effects, that is reduction in allergenicity and retained immunogenicity, have been shown by optimization of the allergen/alum ratio of SCIT with nonmodified allergens.

As the difference in change from baseline between Group 1 and Group 2 is very small and as both groups induced a significant immunological response, the choice of updosing schedule for the new immunologically enhanced SCIT formulation should be based on tolerability.

In routine SCIT, there is an inherent risk of systemic allergic reactions (18). In this trial, the immunologically enhanced SCIT formulation was shown to have an acceptable safety profile applying a 5-step updosing schedule. Systemic reactions (mostly mild to moderate events) were reported by 21% of patients in Group 1 and by 33% of patients in Group 2. Generally, the pattern of adverse events observed during the trial was in line with previous trials using nonmodified, alum-adsorbed grass pollen allergens (22, 27).

The updosing schedule with weekly injection interval (Group 1) had a better tolerability profile with fewer local and systemic reactions and fewer withdrawals because of adverse events than the updosing schedule with 3–4 days injection interval (Group 2). The updosing schedule with weekly injection interval is therefore considered to be the preferred updosing schedule.

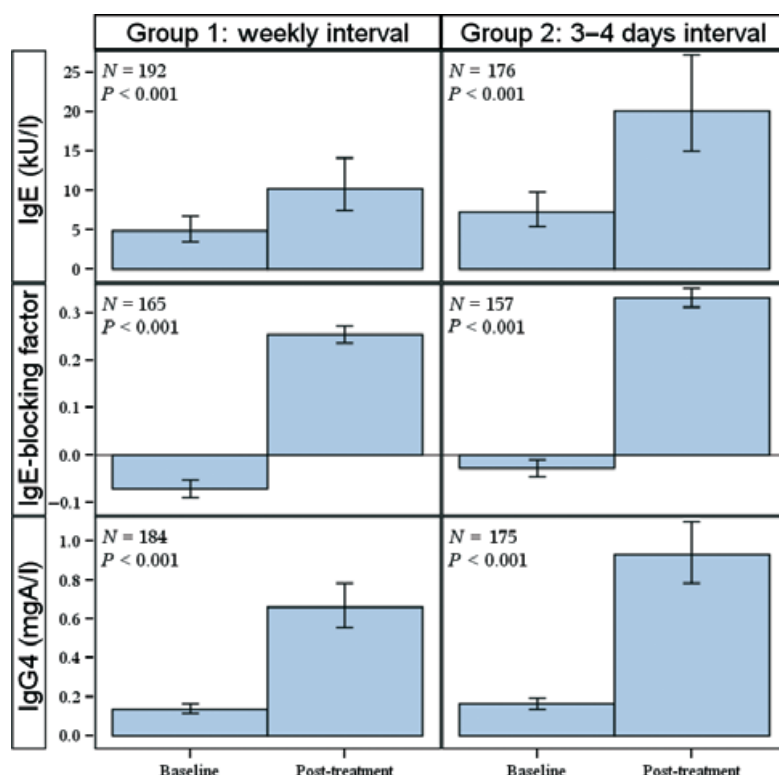


**Figure 2** Immunological Results. *N*, number of patients with measurements; 95% confidence limits; *P*-value for test of a statistical significant difference between baseline and post-treatment.

**Table 3** Summary of adverse events

|                             | Group 1<br>Updosing with weekly<br>injection interval<br><i>N</i> = 201 |          | Group 2<br>Updosing with 3–4 days<br>injection interval<br><i>N</i> = 199 |          | Total<br><i>N</i> = 400 |          |
|-----------------------------|-------------------------------------------------------------------------|----------|---------------------------------------------------------------------------|----------|-------------------------|----------|
|                             | <i>n</i> (% <i>n</i> )                                                  | <i>e</i> | <i>n</i> (% <i>n</i> )                                                    | <i>e</i> | <i>n</i> (% <i>n</i> )  | <i>e</i> |
| All adverse events          | 125 (62%)                                                               | 604      | 140 (70%)                                                                 | 608      | 265 (66%)               | 1212     |
| Severity                    |                                                                         |          |                                                                           |          |                         |          |
| Mild                        | 99 (49%)                                                                | 350      | 112 (56%)                                                                 | 403      | 211 (53%)               | 753      |
| Moderate                    | 70 (35%)                                                                | 228      | 71 (36%)                                                                  | 174      | 141 (35%)               | 402      |
| Severe*                     | 14 (7%)                                                                 | 26       | 14 (7%)                                                                   | 31       | 28 (7%)                 | 57       |
| Seriousness                 |                                                                         |          |                                                                           |          |                         |          |
| Nonserious                  | 124 (62%)                                                               | 601      | 140 (70%)                                                                 | 597      | 264 (66%)               | 1198     |
| Serious                     | 2 (<1%)                                                                 | 3        | 1 (<1%)                                                                   | 11       | 3 (<1%)                 | 14       |
| Relation to IMP             |                                                                         |          |                                                                           |          |                         |          |
| Possible                    | 47 (23%)                                                                | 188      | 65 (33%)                                                                  | 208      | 112 (28%)               | 396      |
| Probable                    | 72 (36%)                                                                | 270      | 92 (46%)                                                                  | 292      | 164 (41%)               | 562      |
| Unlikely                    | 65 (32%)                                                                | 146      | 51 (26%)                                                                  | 108      | 116 (29%)               | 254      |
| Type of AE                  |                                                                         |          |                                                                           |          |                         |          |
| Local reaction              | 73 (36%)                                                                | 302      | 94 (47%)                                                                  | 255      | 167 (42%)               | 557      |
| Systemic reaction           | 42 (21%)                                                                | 109      | 66 (33%)                                                                  | 191      | 108 (27%)               | 300      |
| Other                       | 76 (38%)                                                                | 193      | 66 (33%)                                                                  | 162      | 142 (36%)               | 355      |
| Event leading to withdrawal |                                                                         |          |                                                                           |          |                         |          |
| No                          | 122 (61%)                                                               | 587      | 131 (66%)                                                                 | 542      | 253 (63%)               | 1129     |
| Yes                         | 9 (4%)                                                                  | 17       | 18 (9%)                                                                   | 65       | 27 (7%)                 | 82       |

*N*, number of patients (safety set); *n*, number of patients with events; (%*n*), per cent of patients in treatment group (updosing schedule) of safety set with events; *e*, Number of events.

\*Severe events primarily included events related to the injection site (reported by 8 patients in Group 1 and 7 patients in Group 2) and the respiratory system (reported by 7 patients in Group 2).

**Table 4** Adverse events reported in  $\geq 5\%$  of patients

| System organ class/Preferred term                    | Group 1<br>Updosing with weekly<br>injection interval<br>N = 201 |     | Group 2<br>Updosing with 3–4 days<br>injection interval<br>N = 199 |     | Total<br>N = 400 |     |
|------------------------------------------------------|------------------------------------------------------------------|-----|--------------------------------------------------------------------|-----|------------------|-----|
|                                                      | n (%n)                                                           | e   | n (%n)                                                             | e   | n (%n)           | E   |
| Eye disorders                                        |                                                                  |     |                                                                    |     |                  |     |
| Conjunctivitis allergic                              | 24 (12%)                                                         | 26  | 19 (10%)                                                           | 20  | 43 (11%)         | 46  |
| General disorders and administration site conditions |                                                                  |     |                                                                    |     |                  |     |
| Injection site erythema                              | 19 (9%)                                                          | 31  | 11 (6%)                                                            | 17  | 30 (8%)          | 48  |
| Injection site pain                                  | 12 (6%)                                                          | 15  | 6 (3%)                                                             | 7   | 18 (5%)          | 22  |
| Injection site pruritus                              | 34 (17%)                                                         | 87  | 39 (20%)                                                           | 75  | 73 (18%)         | 162 |
| Injection site swelling                              | 61 (30%)                                                         | 150 | 81 (41%)                                                           | 146 | 142 (36%)        | 296 |
| Nervous system disorders                             |                                                                  |     |                                                                    |     |                  |     |
| Headache                                             | 16 (8%)                                                          | 36  | 12 (6%)                                                            | 14  | 28 (7%)          | 50  |
| Respiratory, thoracic and mediastinal disorders      |                                                                  |     |                                                                    |     |                  |     |
| Asthma                                               | 11 (5%)                                                          | 12  | 7 (4%)                                                             | 21  | 18 (5%)          | 33  |
| Cough                                                | 6 (3%)                                                           | 9   | 11 (6%)                                                            | 18  | 17 (4%)          | 27  |
| Rhinitis allergic                                    | 27 (13%)                                                         | 30  | 24 (12%)                                                           | 30  | 51 (13%)         | 60  |
| Skin and subcutaneous tissue disorders               |                                                                  |     |                                                                    |     |                  |     |
| Pruritus                                             | 9 (4%)                                                           | 12  | 17 (9%)                                                            | 25  | 26 (7%)          | 37  |
| Urticaria                                            | 13 (6%)                                                          | 15  | 21 (11%)                                                           | 23  | 34 (9%)          | 38  |

N, number of patients; n, number of patients with events; (%n), per cent of patients in treatment group (updosing schedule) of safety set with events; e, number of events.

The majority of the frequently reported adverse events were judged to be mild or moderate in severity. Frequently reported adverse events judged to be severe were the following: allergic conjunctivitis (2 patients in Group 2), event related to the injection site (13 patients in Group 1, 8 in Group 2), headache (1 patient in Group 1), event related to the respiratory system (4 patients in Group 2) and urticaria (3 patients in Group 2).

In conclusion, this trial has shown that it is possible to modify classical SCIT by developing formulations allowing for shorter and more convenient updosing schedules. The investigated immunologically enhanced SCIT formulation provided, in a 5-step updosing schedule with weekly injection intervals, significant immunological effects and has an acceptable safety profile. Clinical efficacy with this fast updosed immunologically enhanced SCIT formulation will be further investigated in future clinical trials.

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### Author contributions

K. Jung was coordinating investigator of the trial and was represented in the safety committee established to monitor the tolerability during the trial. O. Pfaar, E. Decot and L. Klimek participated in the trial as recruiting investigators. H. Wolf was the sponsor's project manager of the trial and was involved in the design and conduct of the trial. E. Wüstenberg was the sponsor's medical responsible and was involved in the design and conduct of the trial. J. Kleine-Tebbe was represented in the safety committee established to monitor the tolerability during the trial as an allergy expert. All authors have furthermore contributed to the manuscript from the draft stage by reviewing and commenting as well as critically revising where applicable.

### Conflict of interests

The investigators received remuneration from ALK for their time spent with each patient, all trial procedures and staff fees in conjunction with the ICH Guideline for Good Clinical Practice. K. Jung has received honoraria from ALK for being coordinating investigator in Germany and for educational presentations. O. Pfaar has received research grants from ALK, Allergopharma, Stallergenes, HAL, Artu Biologicals, Allergy-Therapeutics/Bencard, Hartington, Lofarma, Novartis/Leti, Glaxo-Smith-Kline, Essex-Pharma, Cytos, Curalogic and Roxall. He has also served as advisor and on the

speakers' bureaus for some of the above-mentioned pharmaceutical companies. E. Decot has received honoraria from ALK for educational presentations. L. Klimek has received honoraria for lecturing, performing clinical trials and medical advice from ALK, Allergopharma, Bencard, Boehringer Ingelheim, HAL, Hartington, Leti, Lofarma, Curalogic, Glaxo-Smith-Kline, MSD/Essex Pharma, Novartis, Optima

Pharma and Phadia. H. Wolf and E. Wüstenberg are employees of ALK. J. Kleine-Tebbe has received honoraria from ALK for safety board membership and educational presentations. The authors have indicated that they have no other conflicts of interest with regard to the content of this article.

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