

Dose-Dependent Immunological Responses after a 6-Month Course of Sublingual House Dust Mite Immunotherapy in Patients with Allergic Rhinitis

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Key Words

Dermatophagoides pteronyssinus · Efficacy · House dust mite · IgE-blocking factor · Immunotherapy · Safety · Sublingual immunotherapy

Abstract

Background: House dust mite (HDM) immunotherapy has proven efficacy in treating allergic rhinitis (AR) symptoms. This trial evaluated the dose-response relationship of SLITone^{ULTRA}® HDM mix based on immunological parameters and safety in subjects with moderate-to-severe HDM AR not controlled by symptomatic medication. **Materials and Methods:** A randomized, parallel-group, open-label, clinical trial compared 50/150/300 standard reactivity unit (SRU) doses of SLITone^{ULTRA}® HDM mix for 6 months. Subjects had moderate-to-severe HDM AR, positive skin prick and IgE against *Dermatophagoides pteronyssinus*/*Dermatophagoides farinae* (DP/DF). The primary end point was change from baseline in the IgE-blocking factor against DP after 6 months. Secondary end points measured changes in the IgE-blocking factor for DP at 3 months and for DF at 3 and 6 months, and in IgG₄ and specific IgE to DP/DF after 3 and 6 months. Tolerability was assessed through the evaluation of all adverse events (AEs). **Results:** A total of 219 subjects were random-

ized and 196 completed the trial. Dose effect was significant on DP IgE-BF after 6 months ($p = 0.018$). The change in the DP IgE-blocking factor at a 300-SRU dose was higher than at other doses after 3 ($p = 0.008$) and 6 ($p = 0.005$) months of treatment. Similar changes were observed for IgG₄ and allergen-specific IgE. The number of AEs increased with the dose and were mild-to-moderate, with no severe treatment-related AEs reported. The most frequent AEs were oral/tongue pruritus, mouth oedema and abdominal upper pain. **Conclusions:** Data showed a dose-response for immunological markers and safety with a better immunological response for 300 SRU. The highest dose (300 SRU daily) was considered as the optimal maintenance dose.

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Introduction

The increasing prevalence of allergic rhinitis (AR) has become a major health issue worldwide, currently affecting more than a fifth of the adult population in Western Europe [1, 2]. AR has an important influence on quality of life [3] and is an independent risk factor for other conditions such as asthma or sinusitis [4]. Indirect costs of AR are substantial, with estimates suggesting 3.5 million

lost workdays and 2 million lost schooldays per year in the USA alone [4].

House dust mites (HDM) are one of the most relevant aeroallergens [5], affecting 49% of subjects with AR in Western Europe [1]. AR due to HDM causes persistent and moderate-to-severe symptoms, so most patients cannot be totally controlled by medication alone [6]. Moreover, allergy avoidance is not possible to an extent that relieves patients from their symptoms [7, 8]. Allergen immunotherapy (AIT) is able to alter the natural course of the disease by modifying the immunological mechanisms responsible for allergic inflammation [9, 10] and is able to control symptoms by a subcutaneous or sublingual route with good tolerance [11]. Also, the risk of developing new allergen sensitizations and asthma seems to be reduced after treatment with AIT [12, 13–16]. HDM immunotherapy has proven efficacy in rhinoconjunctivitis in several double-blind placebo-controlled randomized trials performed in adults [17–20].

However, the clinical efficacy of AIT is often evaluated by subjective methods such as questionnaires or symptom score. The importance of identifying objective immunological markers has increased in recent years, since they could act as potential end points in phase-II dose-finding AIT trials, reducing sample size and costs, and also help in the selection of responders at an early stage during treatment or even prior to treatment initiation [21]. Some studies have revealed that the quantitative measurement of IgG₄, which has been classically linked to tolerance, may not be a valid surrogate of clinical efficacy [22], and that is the functionality of IgG₁/G₄, the parameter linked to sustained efficacy [23]. Other newer biomarkers such as the IgE-blocking factor reflect the ability of certain AIT-induced non-IgE antibodies and other soluble components to diminish the union of specific IgE to the allergen and subsequently decrease the effect of the antibody-allergen union. There is current and increasing evidence that variation of the serum IgE-blocking factor is linked to the clinical efficacy of AIT in several clinical trials, being the correlation variable among studies [24–28]. Changes in the IgE-blocking factor have been shown to correlate with clinical efficacy in subcutaneous immunotherapy with *Phleum pratense* [26]. This correlation in sera may explain 40% of efficacy (combined symptom and medication score). Moreover, these changes were also correlated to clinical efficacy in clinical trials performed with sublingual immunotherapy (SLIT) with grass [27] and HDM tablets [24].

The primary objective of the present study was to evaluate the dose-response relationship with regards to

changes in immunological parameters and safety for an SLIT immunotherapy drops product (SLITone^{ULTRA}® HDM mix) in adult subjects with moderate-to-severe HDM AR inadequately controlled by symptomatic medication. This immunological dose-response evaluation was based on changes in the IgE-blocking factor for the three dosage groups selected for this trial. As a secondary objective, the safety of SLITone^{ULTRA}® HDM mix was evaluated based on the number and severity of the adverse events (AEs) observed in the trial.

Methods

A randomized, multi-site, parallel group, open label, phase-II clinical trial was conducted in France (26 centres) and Spain (13 centres) between October 2012 and July 2013. The trial was reviewed by the appropriate ethics committees and performed following the recommendations of the Declaration of Helsinki (EudraCT No. 2012-002177-62). All subjects signed a written informed consent prior to their inclusion in the trial.

Study Group and Eligibility Criteria

The subjects eligible for the trial were males or females ≥ 18 years, with moderate-to-severe persistent AR caused by HDM with or without asthma, whose rhinitis symptoms persisted despite having received symptomatic treatment for at least 1 year prior to trial entry. The level of rhinitis severity at baseline was assessed for 7 consecutive days using four nasal symptoms (sneezing, rhinorrhea, nasal itching and nasal blockage) measured on a scale of 0–3. Subjects included in the trial were required to have a rhinitis symptom score ≥ 6 on at least 1 of the days or a score of 5 with one of the symptoms evaluated as being severe (3 points) on the worst day in this 7-day baseline period. Subjects were also required to have a positive skin prick test (SPT) to *Dermatophagoides pteronyssinus* (DP) and/or *Dermatophagoides farinae* (DF) extract ≥ 3 mm (ALK-Abelló, Denmark) and positive IgE to DP/DF ≥ 0.7 kU/L. Subjects with asthma must have had their symptoms controlled at least 3 months prior to their inclusion in the trial. Uncontrolled asthma was defined as ≥ 3 of the following items evaluated in the last month: daytime symptoms more than twice/week, any limitations of activities, any nocturnal symptoms/awakenings, the need for reliever treatment more than twice/week and peak flow or FEV₁ $< 80\%$ predicted or personal best. Female participants had to have a negative pregnancy test and a willingness to practice appropriate contraceptive methods until the end of the trial.

The main exclusion criteria were as follows: reduced lung function (FEV₁ $< 70\%$ of the predicted value), history of uncontrolled asthma (< 3 months before screening), treatment with HDM immunotherapy in the last 5 years, major conditions of the oral cavity (oral lichen planus with ulcerations, severe oral mycosis or tumours), systemic diseases and the use of certain medications (antidepressant/antipsychotic drugs, catecholamine-*o*-methyltransferase inhibitors, immunotherapy with other allergens, monoamine oxidase inhibitors, oral cromolyn sodium, systemic immunosuppressive/glucocorticosteroid treatment).

Trial Design

A total of 219 subjects were randomized in the trial to receive active treatment with SLITone^{ULTRA}® (single-dose presentation of Osiris® in France) HDM mix (50% DP/50% DF) with 3 maintenance doses (50, 150 or 300 standard reactivity units, SRU) in a 1:1:1 ratio. Patients received the treatment for 6 months, and the final concentrations of 150 and 300 SRU were achieved following an up dosing scheme of 5 days per dose: the group of 150 SRU took 50 SRU for 5 days and then up dosed to 150 SRU, and the group of 300 SRU took 50 SRU for 5 days, 150 SRU for 5 days and then up dosed to 300 SRU.

The patients were evaluated at the screening visit (visit 1), at the randomization visit (visit 2) and 3 months (visit 3) and 6 months (visit 4) after starting the treatment. At visit 1 after the informed consent was signed, the patients were examined for eligibility, recording demographic data and body measurements, relevant medical history, allergy, asthma and/or atopic dermatitis history, smoking history and previous/concomitant medication. Patients also underwent a physical examination, pregnancy test, FEV₁ measurement and SPTs with DP, DF and a panel of relevant allergens that included pollens, epithelia and moulds. A blood sample was taken for specific IgE measurement against DP and DF and for immunological baseline testing (see Immunological Evaluation). At visit 2, the inclusion criteria were reviewed (specific IgE >0.7 kU/l and rhinitis score ≥6), and if the criteria were fulfilled, then the patient was randomized and the treatment was given to the participant. Follow-up visits at 3 months (visit 3) and 6 months (visit 4) were performed, where blood samples for immunological testing were taken, and at visit 4 a physical examination, pregnancy test, FEV₁ measurement and SPTs with DP and DF were repeated.

AEs were recorded at all visits. Serious adverse effects (SAEs) had to be communicated within 24 h after the event. One week after visit 4, a phone interview was performed in order to assess possible AEs not recorded at visit 4. The number of used and unused immunotherapy single-dose containers and all empty packaging material were collected from all participants for assessment of compliance. Treatment compliance was calculated as follows: (number of doses taken/days between visit 2 and 4) × 100. Good compliers were defined as those subjects who took >80% and <120% of the treatment.

End Points and Assessments

The primary end point in this study was the pre- to posttreatment change after 6 months in the IgE-blocking factor for DP for the three dosage groups used. As a secondary end point, safety and tolerability was evaluated for all the doses through the evaluation of all AEs. Other assessments were physical examination, vital signs, FEV₁, and subject-rated global evaluation of rhinitis symptoms, asthma symptoms and atopic dermatitis after the treatment (much better, better, the same, worse, much worse), as well as immunological assessments (IgE-blocking factor for DP, IgG₄ and specific IgE).

Immunological Evaluation

To assess the immunological response to the treatment, blood samples were taken for the determination of specific IgG₄, IgE-blocking factor and specific IgE to DP and DF at visit 1 (baseline), visit 3 (3 months after starting AIT) and visit 4 (6 months after starting AIT).

Specific IgE and IgG₄ Assay

The determination of specific IgG₄ and IgE to DP and DF was performed using the ImmunoCAP system (Thermo Fisher, Waltham, Mass., USA). Serum samples for IgG₄ determination were diluted 1:50 before analysis.

IgE-Blocking Factor Assay

This assay measures the effect of serum allergen-specific IgE-blocking components induced by SLIT drops. This method is a competition assay based on two determinations of IgE: the total amount of IgE that binds to allergen in the absence of competing components (T) and the amount of IgE that binds to allergen in the presence of competing components (S). The results are reported as 1 – (S/T); the IgE-blocking factor varies from 0 (no presence of IgE-blocking components in serum) to 1 (all allergen-specific IgE antibodies are blocked from binding to allergen in serum). For the two IgE determinations, the ADVIA Centaur immunoassay system was used, as described previously [28, 29]. The results obtained before treatment initiation were compared with the results obtained at 3 and 6 months after active HDM SLIT drop treatment (50/150/300 SRU).

Statistical Analysis

The statistical analysis was performed in all subjects randomized in the trial following the intent-to-treat principle (full analysis set). Continuous variables were described as mean, median, standard deviation, minimum and maximum. Categorical variables were described as number and percentage of all subjects.

The primary end point of the trial was the change in the IgE-blocking factor for DP from baseline to visit 4 (6 months of treatment). The analysis was based on the full analysis set population. In the efficacy analysis, the last observation carried forward method was used for the imputation of missing values. In the primary analysis, change in the IgE-blocking factor for DP was assessed using analysis of covariance (ANCOVA) including the dose group as factor and baseline IgE-blocking factor as covariable. The results of the analysis were summarized by mean, median, standard deviation, minimum and maximum. The same methodology was used in the other efficacy secondary end points.

Clinical differences between doses were assessed using the Fisher exact test, grouping the results in two categories: positive response (much better + better) and non-positive response (the same + worse + much worse), and the Mann-Whitney test was used to compare differences between non-grouped categories (much better, better, the same, worse, much worse). All statistical analyses were performed with SAS software, version 9.2 (SAS Institute).

Results

Recruitment Results

A total of 251 subjects were initially recruited for the study. A complete flowchart of the study is shown in figure 1. Of those subjects recruited, 32 subjects were not randomized due to screening failure (specific IgE to DP/DF <0.7 kU/l or other eligibility criteria not fulfilled). Sev-

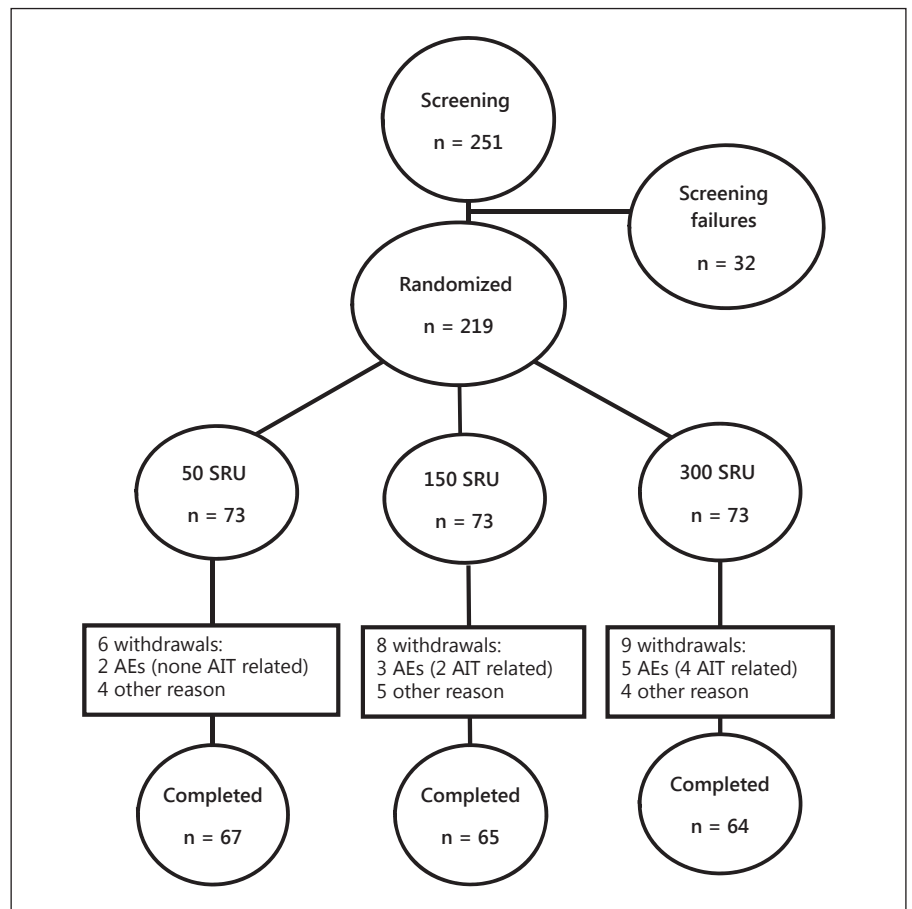


Fig. 1. Flowchart showing total number of patients recruited, screening failures, randomized patients and withdrawals in each dose group.

enty-three subjects were randomized in each dose group ($n = 219$). In the group taking 50 SRU, there were 6 withdrawals (none of which were due to AIT-related AEs). There were 8 withdrawals (2 due to AIT-related AEs) in the group taking 150 SRU and 9 withdrawals in the group with 300 SRU (4 due to AIT-related AEs). None of these AEs were severe.

Demographic Data

The full analysis set comprised 219 randomized subjects. Demographic and selected baseline characteristics are summarized in table 1. All parameters were similar between treatment groups, and baseline body characteristics were within the normal range. One hundred and one subjects were male (46%), 93% Caucasian, with a mean age of 32 ± 9 years. Mean FEV₁ as a percentage of the predicted value was 101% for the group taking 50 SRU, 100% for the group with 150 SRU and 98% for the group with 300 SRU. All subjects had rhinitis, with a similar length of disease (mean time of rhinitis: 10 years) and

Table 1. Demographic data of the participants at baseline (screening visit) grouped by study dose

	50 SRU	150 SRU	300 SRU	All
Male	32 (44)	34 (47)	35 (48)	101 (46)
Female	41 (56)	39 (53)	38 (52)	118 (54)
Caucasian	70 (96)	67 (92)	66 (90)	203 (93)
Other ethnic origin	3 (4)	6 (8)	7 (10)	16 (7)
Age, years	32 ± 10	32 ± 9	32 ± 10	32 ± 10
Height, cm	169 ± 9	170 ± 10	169 ± 9	169 ± 9
Weight, kg	69 ± 14	69 ± 14	68 ± 13	69 ± 14
Systolic BP, mm Hg	120 ± 13	119 ± 12	116 ± 14	118 ± 13
Diastolic BP, mm Hg	71 ± 9	71 ± 8	69 ± 9	70 ± 9
Heart rate, bpm	72 ± 9	72 ± 8	74 ± 11	73 ± 9

Values are n (%) or means $\pm$ SD, as appropriate.

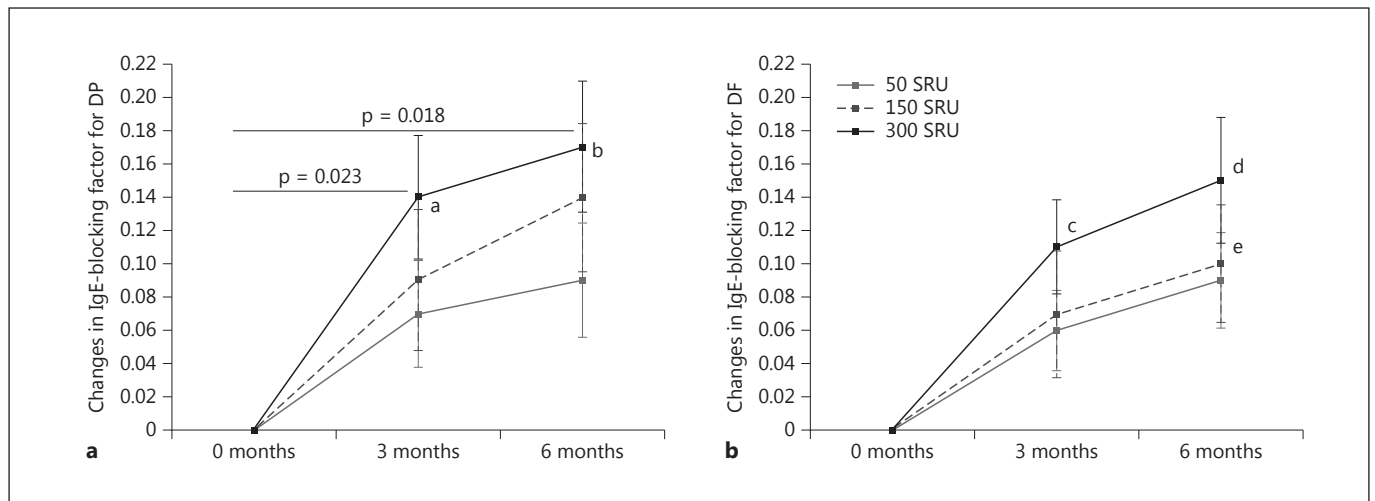


Fig. 2. **a** Changes in IgE-blocking factor for DP from baseline to visit 3 (3 months) and visit 4 (6 months; primary end point). ^a $p = 0.008$: 50 vs. 300 SRU at 3 months; ^b $p = 0.005$: 50 vs. 300 SRU at 6 months. **b** Changes in IgE-blocking factor for DF from baseline to visit 3 (3 months) and 4 (6 months). ^c $p = 0.023$: 50 vs. 300 SRU at 3 months; ^d $p = 0.006$: 50 vs. 300 SRU at 6 months; ^e $p = 0.033$: 150 vs. 300 SRU at 6 months.

with similar severity for all groups (60% moderate, 40% severe rhinitis symptoms). The level of rhinitis severity at baseline based on the symptom score on the worst day showed no differences among groups (mean total nasal symptom score = 9).

Regarding other associated diseases, the presence of asthma was similar in the three groups (40% in the group taking 50 SRU, 30% in the group with 150 SRU and 46% in the group with 300 SRU, mostly mild), and there was also a similar frequency of conjunctivitis (50%) and atopic dermatitis (10%). All subjects had positive SPT to HDM (DP or DF). Other relevant allergens were cat (42% of subjects sensitized), grass (32%) and *Olea europaea* (20%). Levels of specific IgE at baseline showed that almost 50% of the study subjects had IgE levels of class 3–4 (3.5–50 kU/l), and the distribution in classes was similar in all groups except for the subjects with 300 SRU, where 25% of the group had allergen-specific IgE class 5 (50–100 kU/l).

Dose-Response Effects in IgE-Blocking Factor and Secondary Immunological End Points after 3 and 6 Months of Immunotherapy

The primary end point of the trial was the change in the IgE-blocking factor for DP from baseline to visit 4. As shown in figure 2a, a dose-response was observed in the change from baseline to 6 months of treatment in the IgE-blocking factor for DP ($p = 0.018$).

Secondary immunological end points comprised evaluation of changes in the DP IgE-blocking factor after 3 months of treatment, changes in the DF IgE-blocking factor after 3 and 6 months of treatment, and changes in DP and DF IgG₄ and IgE after 3 and 6 months of treatment (fig. 2–4). In figure 2a, variations in the DP IgE-blocking factor after 3 months of treatment were observed in a similar way as that observed in the primary end point ($p = 0.023$), with a significant treatment effect in the higher dose group (50 vs. 300 SRU at 3 months, $p = 0.008$, and at 6 months, $p = 0.005$). A similar response was also observed in the DF IgE-blocking factor after 3 months ($p = 0.023$) and 6 months of treatment ($p = 0.006$) comparing the groups taking 50 versus 300 SRU, and also the groups taking 150 versus 300 SRU after 6 months ($p = 0.033$; fig. 2b).

The response to IgG₄ for DP is similar to that observed for the blocking factor, with significant increases at 3 and 6 months of treatment ($p = 0.001$ and $p = 0.003$, respectively) when the groups with 50 and 300 SRU were compared and at 6 months between the groups with 50 versus 150 SRU ($p = 0.04$; fig. 3a). For IgG₄ to DF, significant differences were also observed when the groups with 50 and 300 SRU were compared at 3 and 6 months ($p = 0.002$ and $p = 0.001$, respectively; fig. 3b).

For specific IgE to DP, a dose-response during the first 3 months of treatment was observed in the comparison of 50 versus 300 SRU ($p = 0.03$) and in 150 ver-

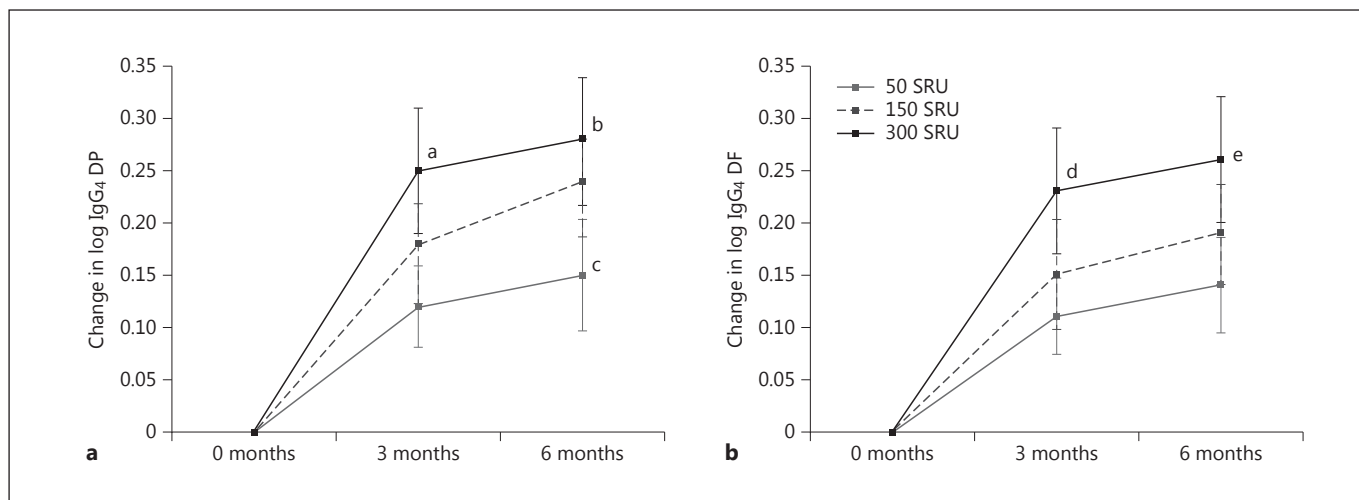


Fig. 3. a Changes in IgG₄ to DP from baseline to visit 3 (3 months) and 4 (6 months). ^a $p = 0.001$: 50 vs. 300 SRU at 3 months; ^b $p = 0.003$: 50 vs. 300 SRU at 6 months; ^c $p = 0.039$: 50 vs. 150 SRU at 6 months. **b** Changes in IgG₄ to DF from baseline to visit 3 (3 months) and 4 (6 months). ^d $p = 0.002$: 50 vs. 300 SRU at 3 months; ^e $p = 0.001$: 50 vs. 300 SRU at 6 months.

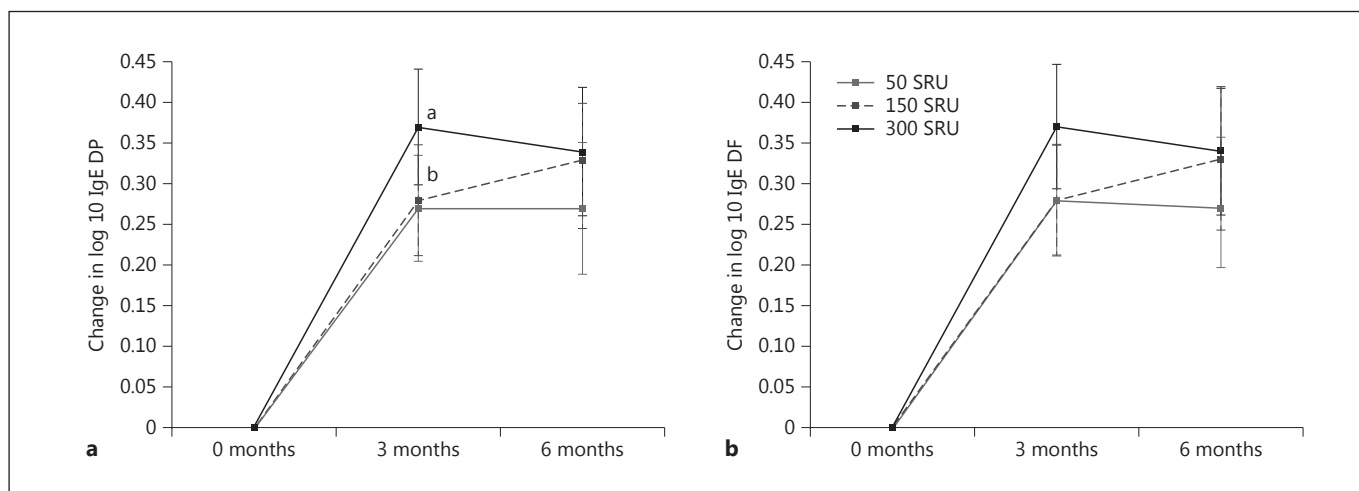


Fig. 4. a Changes in IgE to DP from baseline to visit 3 and 4. ^a $p = 0.03$: 50 vs. 300 SRU at 3 months; ^b $p = 0.04$: 150 vs. 300 SRU at 3 months. **b** Changes in IgE to DF from baseline to visit 3 and 4.

sus 300 SRU ($p = 0.038$), with no significant changes for other doses and no changes observed in the IgE to DF (fig. 4a, b).

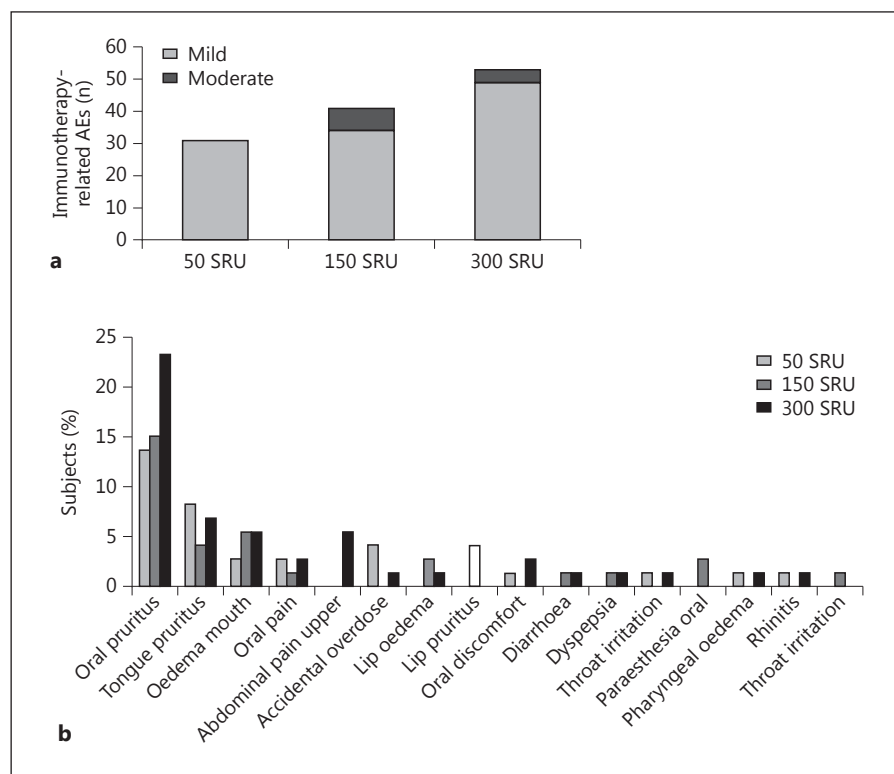
Compliance and Safety Results

A total of 73 subjects were randomized in each dose group ($n = 219$; fig. 1). After 6 months of treatment, 67 subjects finished the trial in the group taking 50 SRU, 65 in the group with 150 SRU and 64 in the group with 300

SRU ($n = 196$). The average time of treatment was 178, 174 and 173 days in the groups with 50, 150 and 300 SRU, respectively. Compliance to treatment was similar, having a good compliance in $>80\%$ of subjects in all dose groups.

Safety and tolerability of the treatment was assessed by the AEs reported for each dose group. Three SAEs, none of them related to the AIT treatment, were reported in the trial: 1 in the screening phase, 1 for 50 SRU and 1 for 150

Fig. 5. a Number and severity of immunotherapy-related AEs in the three dose groups. **b** Percentage of subjects with immunotherapy-related AEs grouped by symptoms.



SRU. Discontinuations due to AIT-related AEs comprised 2 subjects from the group taking 150 SRU and 4 from the group with 300 SRU. The frequency of AIT-related AEs for the groups taking 50, 150 and 300 SRU was 31.5, 35.6 and 47.9% of subjects, respectively. All the AIT-related AEs in the group with 50 SRU were mild, and up to 83% in the group with 150 SRU and 93% in the group with 300 SRU (fig. 5a). There were no severe local reactions compromising the airways or systemic reactions (anaphylactic reaction/shock, systemic allergic reaction). The most frequent immunotherapy-related AEs were oral pruritus (13.7, 15.1 and 23.3% of subjects), tongue pruritus (8.2, 0 and 6.8%), mouth oedema (0, 5.5 and 5.5%) and abdominal upper pain (0, 0 and 5.5%) in the groups with 50, 150 and 300 SRU, respectively (fig. 5b). The total number of AIT-related AEs and their severity decreased during the treatment period, affecting less than 10% of all patients after 3 months of treatment (fig. 6). When these reactions were stratified by dose, the number of AIT-related AEs increased with the dose, although the frequency of AEs in the group with 300 SRU decreased after the fourth month of treatment (6%) compared to the lower dose, where the percentage of AEs was stable until the end of the trial (frequency of AEs: 11%).

Clinical Response to Immunotherapy Treatment

After 6 months of treatment, the subjects rated the evolution of their symptoms as follows: much better, better, the same or worse. This rate was performed for rhinitis symptoms, and also for asthma and atopic dermatitis.

As shown in figure 7, the subject-rated global assessment for rhinitis supported the results from the immunological assessments, the dose of 300 SRU being superior to 50 and 150 SRU in terms of improvement of symptoms (50 vs. 300, $p = 0.006$; 50 vs. 150, $p = 0.948$; 150 vs. 300, $p = 0.015$). No significant differences between the doses were observed for the global assessment for asthma and atopic dermatitis, perhaps due to a lower number of subjects with these conditions ($p > 0.05$, data not shown).

Discussion

AR affects subjects of all ages worldwide, causing an important impairment in work/school performance, increasing the onset of significant comorbidities and causing important health care costs and a decrease in the quality of life [30]. HDM are one of the most common sources of indoor allergens, with a high prevalence in coastal

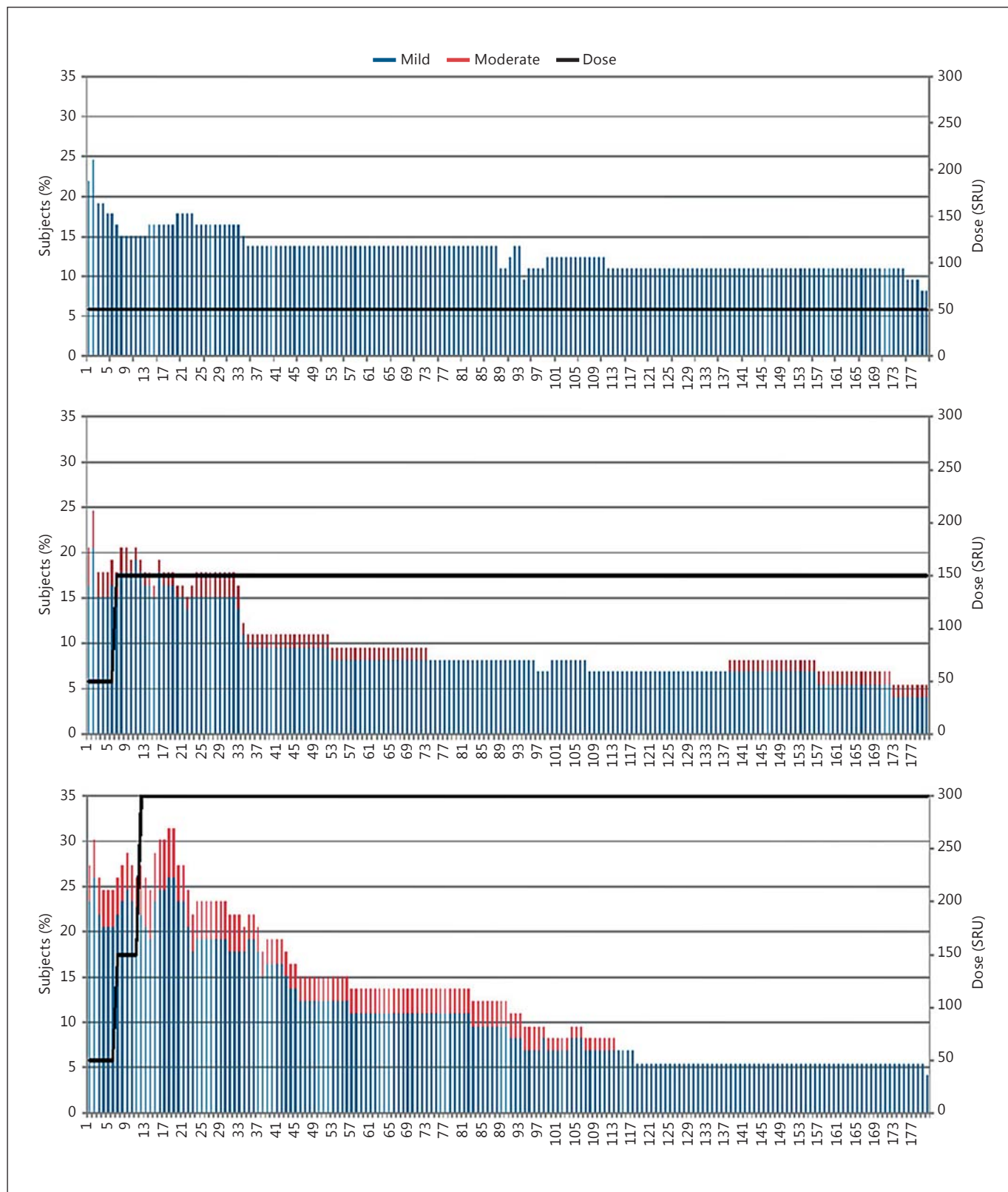
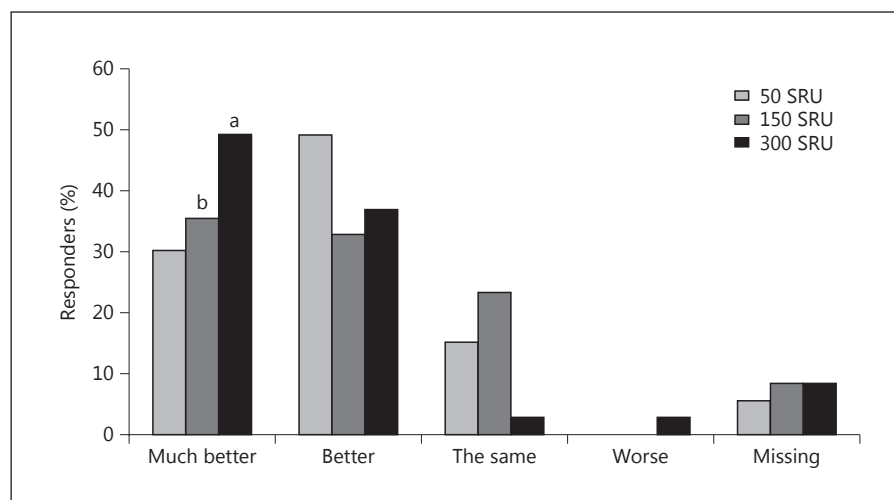


Fig. 6. Daily percentage of subjects with AEs during the treatment, separated by dose group. **a** 50 SRU. **b** 150 SRU. **c** 300 SRU. Mild AEs are pictured in light grey (or blue) and moderate AEs are pictured in dark grey (or red).

Fig. 7. Subject-rated global assessment for rhinitis grouped by categories (much better, better, the same or worse) and dose group. ^a $p < 0.05$: 50 vs. 300 SRU; ^b $p < 0.05$: 150 vs. 300 SRU.



areas and a major trigger of perennial rhinitis and asthma symptoms. The use of SLIT with HDM extracts has proven efficacy in adults with HDM-related rhinitis, inducing a reduction of symptoms and medication use [21, 31].

Classical clinical efficacy end points in AIT are based on determinations of symptom severity and medication use. However, the use of immunological end points in clinical trials has been increasing in recent years as promising biomarkers in AIT [10, 17]. These immune response determinations include the measurement of allergen-specific IgE and blocking components to allergen IgE binding.

In this study, SLITone^{ULTRA}® HDM mix induced clear dose-related changes in the immune response to allergen, with a progressive increase over time in the IgE-blocking factor for DP, which was selected as the primary end point of the trial. The response observed in this primary end point after 6 months of treatment was also detected at 3 months after the beginning of the AIT, with a significant treatment effect observed in the dose of 300 SRU but not in 50 and 150 SRU. Thus, based on the immunological data of the primary end point, 300 SRU was the optimal tested dose for maintenance treatment, which is currently used in daily clinical practice. A limitation of the results was that the increase of the IgE-blocking factor at the dose of 300 SRU was relatively modest (maximum increase 0.16, which corresponds to a 15% decrease in IgE binding). However, these values are similar to those obtained with other SLIT with HDM (e.g. HDM SLIT-tablets) [24]. Also, no plateau was reached in this trial, so it may be possible that a higher dose could have produced a higher immunological response.

The IgE-blocking factor assay measures the effect of sera components competing with IgE for binding to the allergen and has been used in several clinical trials with AIT treatment containing pollen [28, 32–37] and HDM [24, 25], finding a significant yet highly variable correlation with clinical efficacy in some of these studies. In the case of pollen extracts, several dose-finding trials using oral AIT with grass have demonstrated a dose-response relationship in the magnitude of the induced early IgE-blocking factor increase [28, 32–34]. In a trial conducted with the 75,000 SQ-T dose (GRAZAX®), a progressive induction of an increase in the IgE-blocking factor was observed during a 3-year treatment period and reached a maximum peak [35]. Treatment cessation resulted in a progressive decrease in the changes in the IgE-blocking factor (Δ IgE-blocking factor) up to 2 years later. However, a statistically significant difference between the Δ IgE-blocking factor in the active versus the placebo treatment groups was still present. In the case of HDM AIT, a study performed with a subcutaneous DP extract (Alutard SQ DP) showed that the treatment induced an increase in the IgE-blocking factor from baseline to 1 year of treatment in contrast to placebo treatment, with the difference between active and placebo in the Δ IgE-blocking factor being statistically significant for all 3 years ($p < 0.0001$) [25]. In a trial performed with HDM SLIT (tablets), the changes from baseline to 1 year of treatment in the IgE-blocking factor against both HDM species (DP/DF) were statistically significantly correlated with the primary efficacy end point (reduction in the use of inhaled corticosteroids from baseline to 1 year). In the present study, the trends in the IgE-blocking factor for DF

were similar to those observed in the previous HDM SLIT-tablet trial [24].

The results of the previous trials and the present study therefore demonstrate that the magnitude of the IgE-blocking factor response is dose dependent and has a good overall correlation with the clinical end points, although still more studies are needed in order to fully validate this assay as a biomarker of effect. It is noteworthy that no placebo group was included in the present study, but this trial design was based on data obtained from previous studies that showed the change in the IgE-blocking factor was minimal in the placebo group during the conduct of the trial [24, 25, 28, 32–34].

Regarding other immunological parameters, specific IgE levels to DP increased in all dose groups after 3 months of treatment, followed by a slight decrease for the group with 300 SRU and with no variation 50 and 150 SRU. This induced dose-related change in the immune response to the allergen was in agreement with an induction of immune tolerance. In the case of the IgG₄ responses, these were similar to those observed for the blocking factor, where after 3 and 6 months there was a significant dose effect of treatment, especially in 300 SRU.

The secondary objective of the trial was to evaluate the safety and tolerability of SLITone^{ULTRA}® HDM mix, based on the number and severity of AEs observed in the trial. All doses of SLITone^{ULTRA}® HDM mix were safe and well tolerated. There were no AIT-related SAEs or severe events in the trial such as systemic reactions or severe local reactions compromising the airways. The frequency of subjects who discontinued the treatment due to AEs was low in all groups. The most common AEs were localized in the mouth and/or lips such as oral and/or tongue pruritus, and mouth or lip oedema. More than 80% of all AEs were mild, and did not result in discontinuation of the treatment. The total number of AIT-related AEs in the whole trial increased with the dose, but interestingly, the frequency of AEs in the group with 300 SRU decreased after the fourth month of treatment (6%) compared to 50 SRU, where the percentage of AEs was stable until the end of the study (frequency of AEs: 11%). This fact may possibly be due to the development of tolerance in the highest dose group.

In terms of clinical improvement, measured by global symptom assessment by the patient at the end of the treatment, the results showed a superiority of the treatment group with 300 SRU. Thus, patients taking 300 SRU experienced the most significant immunological changes in the IgE-blocking factor and better clinical improvement. Therefore, the main end point (objective) of the trial was

obtained, but the open-label design of this trial may have had an influence on the assessment of global improvement and also on the safety report.

In conclusion, the results demonstrate that SLITone^{ULTRA}® HDM mix AIT had a dose-dependent effect on several immunological end points. These induced dose-related changes in the immune response to allergen, with an increase over time in the inhibition of IgE allergen binding. Also, these immunological changes had a similar dose-response relationship with the clinical outcomes in terms of improvement of symptoms. SLITone^{ULTRA}® was well tolerated and safe in adult subjects with moderate-to-severe HDM rhinitis. Based on these results, 300 SRU was considered the optimal dose for maintenance.

In the future, more investigations are needed to confirm these observations and to validate the assay of blocking components to IgE allergen binding for the purpose of finding the optimal doses in terms of clinical efficacy and acceptable safety profile in dose-finding trials investigating allergen-specific immunotherapy products.

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