Efficacy and safety of sublingual tablets of house dust mite allergen extracts: Results of a dose-ranging study in an environmental exposure chamber

Michel Roux, MD,[a] Philippe Devillier, MD, PhD,[b] William H. Yang, MD,[c] Armelle Montagut, MSc,[a] Kathy Abiteboul, PharmD,[a] Agnès Viatte, MSc,[a] and Robert K. Zeldin, MD[a] *Antony and Suresnes, France, and Ottawa, Ontario, Canada*

Background: In a natural field study, sublingual tablets of house dust mite (HDM) allergen extracts (STG320) were efficacious in treating HDM-associated allergic rhinitis.
Objectives: We sought to assess the efficacy and safety of 3 doses of STG320 in an environmental exposure chamber.
Methods: In this randomized, double-blind study, adults with HDM-associated allergic rhinitis were given a daily sublingual tablet containing placebo or STG320 at a dose of 500IR, 300IR, or 100IR (IR, index of reactivity) for 6 months. Participants recorded their rhinitis symptoms during 4-hour HDM EEC challenges at randomization and months 1, 2, 4, and 6. The primary efficacy end point was the change from baseline to end of treatment in the area under the curve of the rhinitis total symptom score ($Ch_{BL}AUC_{RTSS\ 0\text{-}4h}$). Differences from the placebo group were analyzed by analysis of covariance. Adverse events (AEs) and routine safety parameters were recorded.
Results: A total of 355 subjects were randomized to 1 of 4 groups: 500IR (n = 93), 300IR (n = 86), 100IR (n = 89), or placebo (n = 87). The least squares mean differences from placebo in $Ch_{BL}AUC_{RTSS\ 0\text{-}4h}$ for the 500IR, 300IR, and 100IR groups indicated a dose-dependent effect, with reductions in symptom scores of 33%, 29%, and 20%, respectively. The most frequent AEs were throat irritation and oral pruritus. There were no reports of anaphylaxis or reports consistent with severe laryngopharyngeal disorders and no use of epinephrine. AEs leading to premature discontinuations were more common in the 500IR group.
Conclusions: A dose-dependent effect of sublingual HDM immunotherapy was demonstrated in this environmental exposure chamber study, supporting further development of this treatment. (J Allergy Clin Immunol 2016;138:451-8.)

Key words: *Allergen immunotherapy, allergic rhinitis, house dust mite, sublingual immunotherapy tablet, double-blind placebo-controlled trial, environmental exposure chamber, dose-ranging*

Among indoor allergies, house dust mite (HDM) allergy is the most prevalent and is associated with atopic dermatitis, perennial rhinitis, and asthma.[1] A diagnostic classification of patients with persistent rhinitis showed that 39% had allergic rhinitis and 52% of them were sensitized to HDM allergens.[2] HDM allergy is strongly associated with development, severity, and morbidity of asthma.[3] Mite avoidance measures are generally not effective.[4-6] Symptomatic medications such as antihistamines and corticosteroids provide partial or temporary relief but are not effective in all patients and are not disease-modifying.[7] In addition, pharmacotherapy may be associated with frequent side effects.

Recent studies and the GA^{2}LEN meta-analysis have provided evidence of efficacy and safety of sublingual immunotherapy in patients with HDM-associated allergic rhinitis and asthma.[8] In a randomized, double-blind, placebo-controlled, 1-year phase II/III study conducted under natural field conditions, treatment with 2 doses of sublingual allergen immunotherapy (AIT) tablets (referred to as STG320) containing freeze-dried extracts of the 2 most common allergenic mite species, *Dermatophagoides pteronyssinus* (*Der p*) and *Dermatophagoides farinae* (*Der f*), was efficacious and safe.[9,10] In the present study, the dose-dependent effect of STG320 was further evaluated with 3 doses in an environmental exposure chamber (EEC) as proposed in the European Medicines Agency's Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases.[11]

The EEC has been used extensively for dose-ranging and assessment of onset of action of symptomatic medications.[11-13] However, this tool has been underexploited in clinical trials of AIT[12,14] and until recently,[15] there have been only a few reports on HDM challenge in an EEC with small numbers of subjects.[16-18] In this multicenter study, HDM allergen challenge in an EEC was used to assess the dose-dependent effect and safety of AIT.

From [a]Stallergenes S.A., Antony; [b]UPRES EA 220, Clinical Research Unit, Foch Hospital, Suresnes; and [c]the Allergy and Asthma Research Centre, Ottawa.

This study was funded by Stallergenes S.A., France.

Disclosure of potential conflict of interest: M. Roux, A. Montagut, K. Abiteboul, A. Viatte, and R. K. Zeldin are employees of Stallergenes S.A. P. Devillier has received consulting fees from Stallergenes S.A. and has received honoraria for board membership, consultancy, lectures, and/or manuscript preparation from ALK, Almirall, Astra-Zeneca, Boehringer-Ingelheim, Chiesi, CLL Pharma, GlaxoSmithKline, Meda Pharma, Mundipharma, Novartis, Sandoz, Stallergenes S.A., and Teva. W. H. Yang has received research grants from Stallergenes S.A.

Received for publication June 3, 2015; revised February 2, 2016; accepted for publication March 2, 2016.

Available online May 6, 2016.

Corresponding author: Robert K. Zeldin, MD, Stallergenes S.A., Global Clinical Development Department, 6, rue Alexis de Tocqueville, 92183, Antony, France. E-mail: rzeldin@stallergenes.com.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.03.039

Abbreviations used

AE: Adverse event
AIT: Allergen immunotherapy
ARSS: Average Rhinitis Symptom Score
ARTSS: Average Rhinitis Total Symptom Score
AUC: Area under the curve
AVASS: Average Visual Analog Scale Score
Ch_{BL}: Change from baseline
EEC: Environmental exposure chamber
HDM: House dust mite
IR: Index of reactivity
RTSS: Rhinitis Total Symptom Score
TEAE: Treatment-emergent adverse event
VAS: Visual Analog Scale

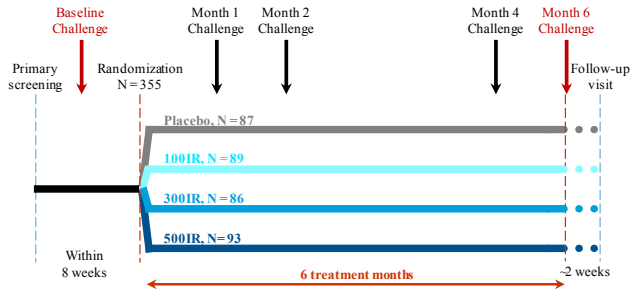

FIG 1. Trial design. After the primary screening based on clinical history of HDM-associated allergic rhinitis, eligible subjects underwent a baseline HDM allergen challenge in an EEC. Those with symptom scores above the predefined threshold were randomized to 1 of the 4 treatment arms, and underwent 4 additional allergen challenges during the 6 treatment months.

METHODS

Trial design

This randomized, double-blind, placebo-controlled study (Fig 1) was conducted at 8 centers in Canada (ClinicalTrials.gov no. NCT01527188). All allergen challenges in the EEC were performed at Cetero Research/PRACS Institute (Mississauga, Ontario). The study was approved by the appropriate institutional review boards and Health Canada and was performed according to Good Clinical Practices guidelines of the International Conference on Harmonization. Written informed consent was obtained from all subjects before study entry.

Participants were enrolled between December 2010 and September 2011 for 6 treatment months. Using a computer-generated randomization code (see details in this article's Online Repository at www.jacionline.org), eligible subjects were assigned 1:1:1:1 to take a daily dose of either placebo or HDM allergen extracts at doses (expressed in index of reactivity [IR], the in-house standardization unit) of 500IR, 300IR, or 100IR. Subjects, investigators, and all other study personnel remained blinded for the entire study.

Trial population

Subjects aged 18 to 55 years were selected in a 2-step process. First, they had to have at least a 1-year history of HDM-associated allergic rhinitis uncontrolled despite the use of symptomatic treatments, a positive skin prick test result (wheal diameter, >3 mm), and HDM-specific serum IgE level of 0.7 kU/L or more. Those with a Rhinitis Total Symptom Score (RTSS) of 6 or more (0-12 scale) for at least 2 of the 12 time points during the baseline allergen challenge were eligible for randomization.

Subjects sensitized to seasonal allergens were permitted to participate if the relevant allergy season was outside of the primary evaluation period. Subjects sensitized to other perennial allergens were excluded as were those with an FEV_1 value of less than 80% of predicted or subjects with asthma requiring treatment other than with inhaled beta-2 agonists, which could be used as needed.

Investigational treatment and rescue medication

The investigational products were sublingual tablets containing a 1:1 mixture of standardized extracts of *Der p* and *Der f* (STG320), at doses of 500IR, 300IR, or 100IR, or placebo tablets matched for size, shape, color, and taste. Allergen content of the study tablets measured by commercial ELISA kit (Indoor Biotechnologies, Va) was 22 to 23 μg Der p 1 and 99 to 102 μg Der f 1 per 500IR tablet, 14 to 17 μg Der p 1 and 53 to 62 μg Der f 1 per 300IR tablet, and 4 to 6 μg Der p 1 and 18 to 21 μg Der f 1 per 100IR tablet. Treatment consisted of sublingual administration of a single tablet daily for 6 months. Subjects were asked to keep the tablet under the tongue until complete disintegration. All subjects took the first dose of the investigational product under supervision at the trial site. Subsequent doses were self-administered at home. In the 500IR and 300IR groups, treatment was initiated with a dose-escalation phase, as previously described.[10]

Symptomatic treatment of rhinitis was permitted between visits, but predefined washout periods were to be respected before allergen challenges (see the Methods section in this article's Online Repository at www.jacionline.org). Outside of the allergen challenges, inhaled beta-2 agonists could be used as needed. Rescue medications were not permitted during the 4-hour allergen challenges unless prescribed by the investigator to treat an adverse event (AE).

Allergen challenge and trial measurements

The allergen challenge was carried out in the EEC, a sealed room in which milled whole bodies of *Der p* mites with a particle size range of 15 to 100 μm were aerosolized and delivered at a constant flux via turbulent airflow. The average concentrations of the HDM allergen in the chamber were monitored to measure the concentration of allergen particles to which subjects were exposed. These concentrations were maintained between 29 and 111 ng Der p $1/m^3$. In the published literature and experience at Cetero Research/PRACS Institute, aerosolization of Der p 1 allergen in this concentration range has been shown to induce both nasal and nonnasal symptoms in patients allergic to dust mite.[19]

Randomized subjects underwent five, 4-hour allergen challenges during the study: at baseline and after 1, 2, 4, and 6 treatment months (Fig 1). During the challenges, participants recorded the severity of their 4 nasal symptoms (rhinorrhea, nasal congestion, sneezing, and nasal pruritus) on a scale of 0 (no symptoms), 1 (mild), 2 (moderate) to 3 (severe). The RTSS is the sum of these 4 nasal symptom scores and thus ranges from 0 to 12.

In the EEC, subjects assessed the overall severity of their allergic symptoms on a 10-cm Visual Analog Scale (VAS), anchored from "absence of symptoms" to "very severe symptoms."[20,21]

For each challenge, rhinitis symptoms and the VAS score were recorded at 13 time points: once before entering the EEC, then every 15 minutes for the first 2 hours, and every 30 minutes for the last 2 hours. Because the intervals between time points were not equal, the area under the curve (AUC) of RTSSs was used to analyze the symptom scores in the EEC. In addition, the average score of each of the 4 individual rhinitis symptoms (Average Rhinitis Symptom Score [ARSS]), the RTSS (Average Rhinitis Total Symptom Score [ARTSS]), and the VAS score (Average Visual Analog Scale Score [AVASS]) were determined for each allergen challenge. Allergen challenge methods are further described in this article's Online Repository at www.jacionline.org.

The primary efficacy end point was the change from baseline (Ch_{BL}) to the end of treatment (ie, 6 months) in the AUC of the RTSS over the 4 hours of the challenge ($Ch_{BL}AUC_{RTSS\ 0\text{-}4h}$). This variable was also evaluated after 1, 2, and 4 months of treatment. The changes from baseline to the end of treatment in ARTSS ($Ch_{BL}ARTSS$), average individual rhinitis symptom scores ($Ch_{BL}ARSS_{0\text{-}4h}$), and AVASS ($Ch_{BL}AVASS_{0\text{-}4h}$) over the 4 hours of the challenge were reported as secondary efficacy end points. Previous studies with grass pollen have indicated that symptom scores increase rapidly at the start of an allergen challenge and plateau after approximately 2 hours.[14] Hence, the changes from baseline to the end of treatment for the last 2 hours of the allergen challenges were analyzed for the total rhinitis score (AUC of RTSS [$Ch_{BL}AUC_{RTSS\ 2\text{-}4h}$] and ARTSS [$Ch_{BL}ARTSS_{2\text{-}4h}$]), individual rhinitis scores (ARSS [$Ch_{BL}ARSS_{2\text{-}4h}$]), and the VAS score ($Ch_{BL}AVASS_{2\text{-}4h}$) as secondary end points.

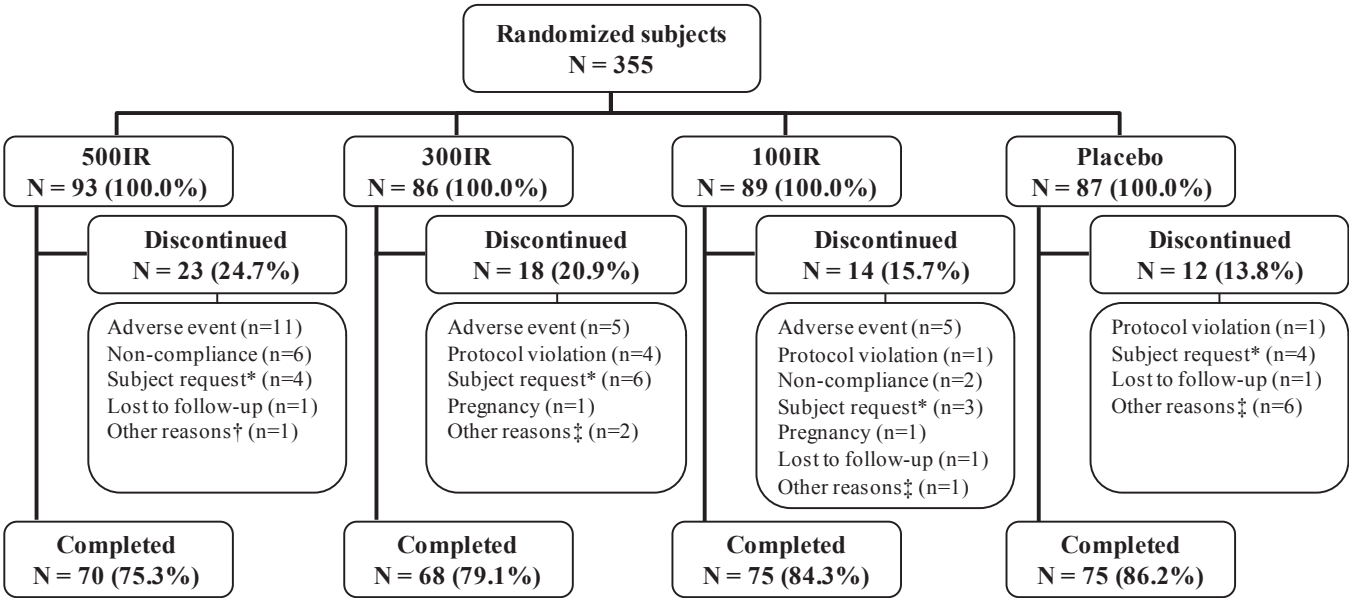

FIG 2. Subject disposition. *Recorded as withdrawal by subject. †Sponsor's decision to withdraw the subject. ‡Subject did not tolerate the chamber.

Blood samples were collected at baseline and after 2, 4, and 6 treatment months, before the allergen challenges. *Der p*– and *Der f*–specific serum IgE and IgG_4 levels were determined using the ImmunoCap Phadia Laboratory System (Phadia AB, Uppsala, Sweden).

Safety variables included AEs, which were monitored during the study and categorized according to MedDRA dictionary version 14.0. AEs occurring during the treatment period and up to 30 days after the last treatment administration are presented here. Treatment-emergent adverse events (TEAEs), drug-related TEAEs, and withdrawals due to TEAEs were identified in the different study groups. The occurrence of AEs in the peri-EEC period (ie, the day of and the day after an allergen challenge) was compared with that in the rest of the study. In addition, changes from baseline in vital signs, lung function, and routine laboratory tests were assessed.

Statistical methods

Assuming a type I error of 5% (2-sided, $\alpha = 0.05$) and a coefficient of variation of the primary end point of 45%, a sample size of 71 subjects per treatment group would have a power of 90% to detect a relative mean difference versus placebo of 22%. A 15% dropout rate was assumed. Therefore, the enrollment target was 84 subjects per group. Statistical analyses were performed using SAS software (version 9.1.3; SAS Institute Inc, Cary, NC). The threshold of statistical significance was set to a P value of less than .05, and all inferential tests were 2-sided.

Efficacy and safety were evaluated on all randomized subjects who received at least 1 dose of the assigned treatment. In this study, the efficacy set (Full Analysis Set) and the Safety Set were identical, and consistent with the intent-to-treat principle. The primary efficacy criterion was analyzed using analysis of covariance with treatment as the main effect and the baseline $AUC_{RTSS\ 0\text{-}4h}$ as covariate. Treatment comparisons were done with a step-down approach for the primary end point (first 500IR vs placebo, then 300IR vs placebo, then 100IR vs placebo) to maintain the overall type I error rate at 5%. Secondary efficacy variables were analyzed as per the primary efficacy criterion.

RESULTS

Subject disposition

A total of 355 subjects were randomized and constituted the Full Analysis Set: 500IR (n = 93), 300IR (n = 86), 100IR (n = 89), and placebo (n = 87); 288 (81%) completed the study (Fig 2).

Demographic and baseline disease characteristics were similar across the groups (Table I). The mean age was 32 years, the mean duration of allergic rhinitis was 17 years, about 76% were polysensitized, and about 13% had asthma.

Efficacy analysis

On the primary end point, the $Ch_{BL}AUC_{RTSS\ 0\text{-}4h}$ after 6 treatment months, a dose-dependent effect was observed with relative differences versus placebo of 33.2% for the 500IR group, 28.8% for the 300IR group, and 19.8% for the 100IR group. The difference between the 500IR and placebo groups was statistically significant ($P = .0427$) (Fig 3, *A*). The time course of nasal symptoms recorded during the HDM allergen challenge showed a biphasic curve, with an initial phase of increasing RTSSs (0-2 hours) followed by a phase where the RTSSs stabilized (2-4 hours), seen as a plateau (see Fig 4 for the baseline challenge and the 6-month challenge). For the last 2 hours of the challenge, when the symptoms were recorded every 30 minutes by the subject, the change from baseline in AUC_{RTSS} ($Ch_{BL}AUC_{RTSS\ 2\text{-}4h}$) and average RTSSs ($Ch_{BL}ARTSS_{2\text{-}4h}$) after 6 treatment months also correlated with the dose. For the $Ch_{BL}AUC_{RTSS\ 2\text{-}4h}$, the relative differences versus the placebo group were 42.0%, 40.9%, and 30.5% for the 500IR, 300IR, and 100IR groups, respectively (Fig 3, *B*). The relative differences from placebo in $Ch_{BL}ARTSS_{2\text{-}4h}$ for the 500IR, 300IR, and 100IR groups were 44.7%, 42.3%, and 31.3%, respectively. The differences were statistically significant for the 2 higher dose groups (Fig 3, *D*).

Also, compared with the placebo group, the relative difference in $Ch_{BL}ARTSS_{0\text{-}4h}$ for the 500IR group was statistically significant ($P = .0469$) after 6 months (Fig 3, *C*).

For the primary efficacy variable, the $Ch_{BL}AUC_{RTSS\ 0\text{-}4h}$, the differences between active treatment and placebo numerically increased from 1 to 4 months of treatment for all groups and demonstrated a trend consistent with a dose-dependent effect,

TABLE I. Demographic and baseline characteristics—Full Analysis Set

Characteristic	500IR (N = 93)	300IR (N = 86)	100IR (N = 89)	Placebo (N = 87)
Age (y), mean ± SD	32.8 ± 9.33	32.5 ± 8.56	32.4 ± 10.09	31.3 ± 8.75
Sex: female, n (%)	44 (47.3)	45 (52.3)	47 (52.8)	47 (54.0)
Duration of AR (y), mean ± SD	19.2 ± 9.71	16.6 ± 9.12	16.6 ± 10.28	15.2 ± 9.76
FEV_1 (% predicted), mean ± SD	97.0 ± 13.02	97.3 ± 10.94	96.9 ± 10.87	95.0 ± 11.47
Asthma, n (%)	12 (12.9)	11 (12.8)	12 (13.5)	10 (11.5)
Polysensitized,* n (%)	69 (74.2)	70 (81.4)	68 (76.4)	64 (73.6)
Baseline $ARTSS_{0\text{-}4h}$, mean ± SD	6.21 ± 2.021	6.47 ± 2.092	6.56 ± 1.793	6.81 ± 2.352
Baseline $AVASS_{0\text{-}4h}$, mean ± SD	4.59 ± 1.725	4.78 ± 1.898	5.08 ± 1.662	5.03 ± 1.882

Continuous variables are reported as mean ± SD. Categorical variables are reported as n, number of subjects (%, percentage of subjects relative to *N*, number of participants in each treatment group in the Full Analysis Set). Range for ARTSS was 0 to 12 and for AVASS was 0 to 10.
AR, Allergic rhinitis.
*Sensitized to HDM allergen(s) and at least 1 of the other allergens tested based on skin prick testing.

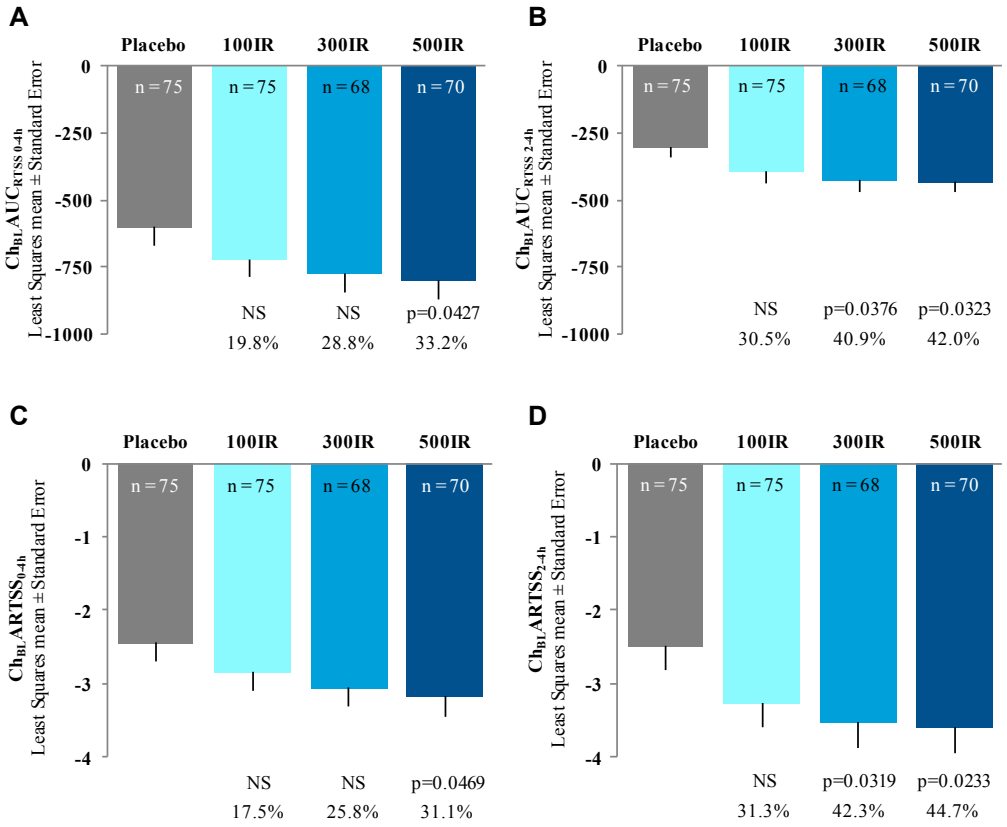

FIG 3. Treatment efficacy. Change from baseline (Ch_{BL}) to end of treatment (after 6 months) in the AUC of RTSSs for the full 4 hours (0-4 h) **(A)** or the last 2 hours (2-4 h) **(B)** of allergen challenge. Change from baseline (Ch_{BL}) in the ARTSSs for the full 4 hours (0-4 h) **(C)** or the last 2 hours (2-4 h) **(D)** of allergen challenge. *NS*, Not significant.

as did the $Ch_{BL}ARTSS_{0\text{-}4h}$ (see Table E2 in this article's Online Repository at www.jacionline.org).

The coefficients of variation of the average RTSS at 6 months (69.5% [500IR] to 57.6% [placebo]) were higher than those expected in an EEC.[14]

At the end of treatment, results of changes in the 4 individual rhinitis symptom scores were generally consistent with those of the RTSS. Results for the full 4 hours and the last 2 hours of the $Ch_{BL}AUC_{RTSS}$, the $Ch_{BL}ARTSS$, and changes in the individual rhinitis symptom scores are provided in this article's Online Repository (see Tables E2 and E3 in this article's Online Repository at www.jacionline.org).

Ocular symptoms were also assessed in this study and a positive trend favoring active treatment was observed (data not shown).

For the change from baseline in the average VAS score recorded during the 4 hours of the allergen challenge ($Ch_{BL}AVASS_{0\text{-}4h}$) after 6 treatment months, the relative differences versus placebo were 25.3%, 23.8%, and 14.1% for the 500IR, 300IR, and 100IR groups, respectively (Table II).

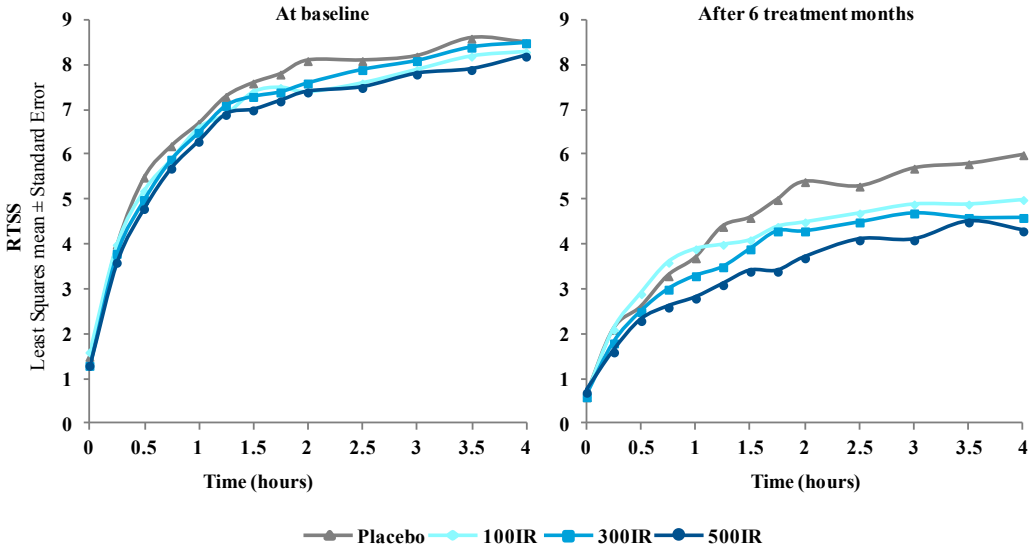

FIG 4. Assessment of RTSSs during HDM allergen challenge. Subjects were exposed to HDM allergens for 4 hours in the EEC. Rhinitis symptoms were recorded by the subjects at 13 time points, and RTSSs (range, 0-12) were derived at each time point.

Immune responses

At study entry, serum levels of *Der p*– and *Der f*–specific IgE and IgG_4 were similar across treatment groups (Fig 5). Within the first 2 treatment months, mite-specific serum IgE levels increased 5- to 7-fold in the 3 active treatment groups, and then gradually decreased, while remaining unchanged in the placebo group. Mite-specific serum IgG_4 levels increased over the treatment period in the 3 active treatment groups, and were little changed in the placebo group. For both these markers of immunologic activity, a dose-dependent effect was apparent at each time point.

Safety analysis

Overall, the incidence of TEAEs reported by the subjects was higher in the active groups (94%, 91%, and 97% in the 500IR, 300IR, and 100IR groups) compared with the placebo group (83%). The most frequent drug-related TEAEs were application site reactions such as oral pruritus, mouth edema, throat irritation, and ear pruritus (Table III). None of the 4 serious TEAEs reported during the treatment period (meningitis, convulsion, schizoaffective disorder, and nephrolithiasis) were considered related to the investigational product. There were no reports of anaphylaxis or reports consistent with severe laryngopharyngeal disorders or autoimmune disorders and no use of epinephrine.

The incidence of bronchospasm, asthma, and associated symptoms was higher during the peri-EEC periods than outside these periods (see Table E4 in this article's Online Repository at www.jacionline.org). The incidence of these TEAEs both during and outside the peri-EEC periods was similar across treatment groups.

Of the 355 randomized subjects, 20 (6%) withdrew from the study because of TEAEs: 11 in the 500IR group, 5 in the 300IR group, and 4 in the 100IR group. In addition, 2 subjects in the 300IR group, 1 in the 100IR group, and 6 in the placebo group withdrew because they did not tolerate the EEC challenge. The most frequent TEAEs leading to withdrawal were mouth edema, dyspnea, and cough. No significant changes were observed in

TABLE II. Change from baseline to end of treatment (month 6) in the average VAS scores for the full 4 hours and the last 2 hours of the allergen challenge—Full Analysis Set

			Average VAS scores: Difference from placebo			
Treatment	No.	LS means (SE)	LS mean difference	95% CI	*P* value	Relative LS mean difference* (%)
0-4 h of allergen challenge						
500IR	70	−2.64 (0.178)	−0.53	−1.02 to −0.05	.032	25.3
300IR	68	−2.61 (0.181)	−0.50	−0.99 to −0.01	.045	23.8
100IR	75	−2.41 (0.172)	−0.30	−0.78 to 0.18	NS	14.1
Placebo	75	−2.11 (0.172)	—	—	—	—
2-4 h of allergen challenge						
500IR	70	−3.12 (0.239)	−0.75	−1.40 to −0.09	.025	31.5
300IR	68	−3.04 (0.242)	−0.66	−1.32 to 0.00	.049	27.8
100IR	75	−2.87 (0.230)	−0.49	−1.13 to 0.15	NS	20.8
Placebo	75	−2.38 (0.230)	—	—	—	—

LS, Least squares; *No.*, number of subjects for whom data were available; *NS*, not significant; *SE*, standard error.
*Relative LS mean difference = 100 × (LS mean difference from placebo/LS mean placebo).

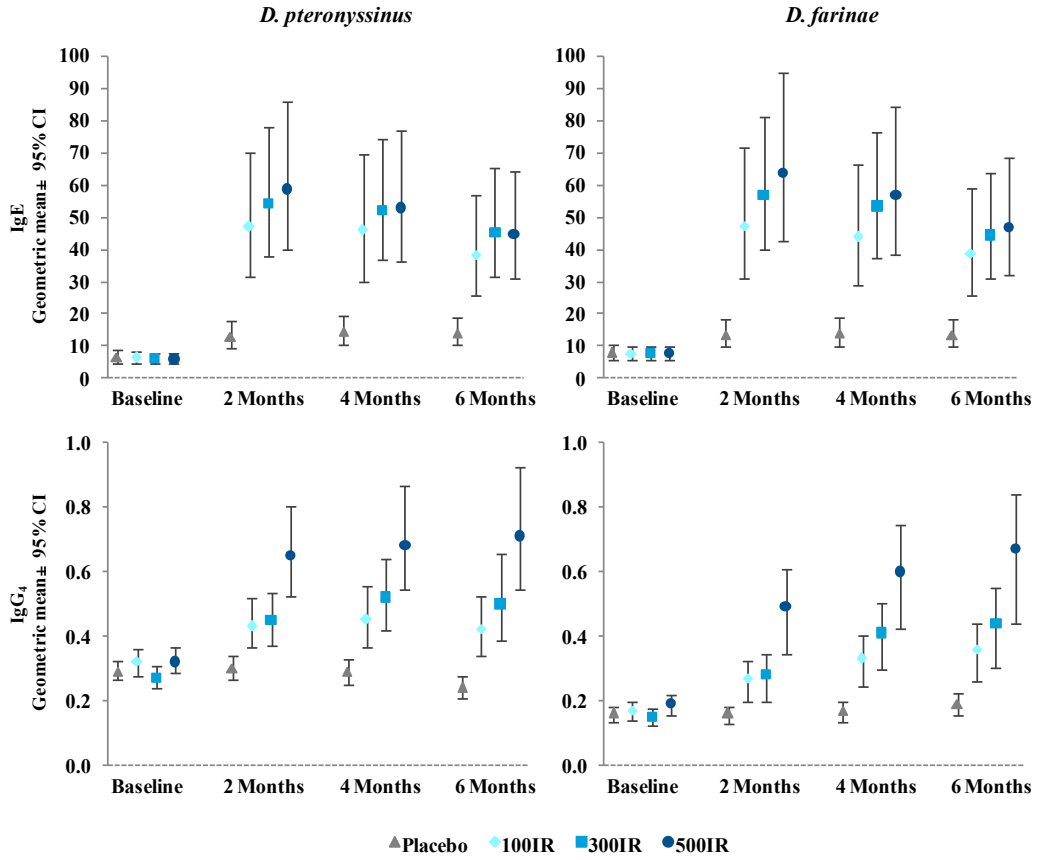

FIG 5. Immunological markers. Serum-specific IgE (top panels) and IgG_4 (bottom panels) levels for *D pteronyssinus* and *D farinae* at baseline and after 2, 4, and 6 months. Units: kU/L for IgE, μg/mL for IgG_4.

vital signs, laboratory tests, or spirometry parameters, except for transient decreases in FEV_1 during and immediately after the allergen challenges.

DISCUSSION

This placebo-controlled dose-ranging study, conducted using allergen challenges in an EEC, was designed to evaluate the effect of treatment with 500IR, 300IR, and 100IR sublingual tablets of HDM allergen extracts in adults with HDM-induced allergic rhinitis. After 6 months of treatment, a dose-dependent effect was observed on rhinitis symptoms as evidenced by changes from baseline in the AUC of the RTSS and the ARTSS. This effect was also reflected in the change from baseline in VAS scores, which conveys the subjects' overall assessment of the severity of their allergic symptoms during the allergen challenge. Typical symptoms of HDM-associated allergic rhinitis include nasal symptoms (rhinorrhea, sneezing, nasal obstruction, and pruritus) while ocular symptoms are more common in patients allergic to outdoor allergens such as pollens.[22] Nevertheless, in this study, ocular symptoms were also improved with the active treatment.

As described in 2 previous EEC studies conducted with grass pollen allergen (4-hour challenges) and HDM allergen (6-hour challenges),[14,15] the RTSS increased rapidly over the first 2 hours and then reached a plateau, indicating stabilization of the intensity of the allergic symptoms. The plateau period may be more consistent with real-life exposure to HDM and, therefore, is perhaps more clinically relevant. In the current study, efficacy analyses performed on data from the last 2 hours of each challenge showed larger differences between each active treatment group and the placebo group than were observed across the full 4 hours of the challenges. Of note, in the EEC study reported by Nolte et al,[15] the efficacy end points were analyzed during the last 4 hours of the 6-hour challenges (ie, the plateau period).

Use of an EEC is particularly suitable for dose-ranging studies because it allows assessment of symptoms under controlled and uniform exposure to the specific allergen, avoiding the variations observed with natural exposure and the confounding effects of other allergens and rescue medication usage on symptom scores.[11,12] Differences in the efficacy outcomes of the active treatment groups relative to the placebo group were larger in the current study than those observed in the natural field study,[10] perhaps due to more stringent subject selection including a baseline allergen challenge.

As previously observed in an exposure chamber study with 300IR 5-grass-pollen sublingual tablet,[14] subjects in the placebo group displayed a strong and persistent placebo effect at each of the 4 challenges. Such placebo effect has been attributed to interactions between the EEC trial subjects, and a potential impact of their discussions on symptoms scoring.[14] In contrast, in the HDM allergen chamber study conducted by Nolte et al,[15] subjects in the placebo group recorded worsening symptoms over the 24 weeks of the trial. This warrants further evaluation in future studies using this model.

TABLE III. Drug-related TEAEs occurring in at least 5% of the population of any treatment group—Safety Set

System Organ Class and Preferred Term	n (%)			
	500IR (N = 93)	300IR (N = 86)	100IR (N = 89)	Placebo (N = 87)
Subjects reporting ≥1 TEAE	87 (93.5)	78 (90.7)	86 (96.6)	72 (82.8)
Subjects reporting ≥1 drug-related TEAE	66 (71.0)	59 (68.6)	60 (67.4)	38 (43.7)
Gastrointestinal disorders	52 (55.9)	47 (54.7)	40 (44.9)	12 (13.8)
Oral pruritus	29 (31.2)	30 (34.9)	23 (25.8)	7 (8.0)
Edema mouth	20 (21.5)	19 (22.1)	16 (18.0)	0 (0.0)
Nausea	3 (3.2)	4 (4.7)	7 (7.9)	2 (2.3)
Paresthesia oral	6 (6.5)	3 (3.5)	3 (3.4)	1 (1.1)
Lip edema	8 (8.6)	3 (3.5)	1 (1.1)	0 (0.0)
Hypoesthesia oral	1 (1.1)	1 (1.2)	5 (5.6)	2 (2.3)
Respiratory, thoracic, and mediastinal disorders	45 (48.4)	42 (48.8)	35 (39.3)	24 (27.6)
Throat irritation	35 (37.6)	32 (37.2)	28 (31.5)	10 (11.5)
Oropharyngeal pain	6 (6.5)	6 (7.0)	4 (4.5)	4 (4.6)
Ear and labyrinth disorders	26 (28.0)	21 (24.4)	18 (20.2)	8 (9.2)
Ear pruritus	23 (24.7)	21 (24.4)	18 (20.2)	8 (9.2)
Nervous system disorders	5 (5.4)	4 (4.7)	7 (7.9)	6 (6.9)
Headache	5 (5.4)	2 (2.3)	4 (4.5)	6 (6.9)
Eye disorders	4 (4.3)	4 (4.7)	7 (7.9)	8 (9.2)
Eye pruritus	3 (3.2)	4 (4.7)	6 (6.7)	5 (5.7)

Drug-related *TEAEs* (AEs occurring during the treatment period and up to 30 d after the last treatment administration) were classified according to their System Organ Class and Preferred Term (MedDRA version 14.0).
n, Number of subjects with at least 1 event in the given Preferred Term; %, percentage of subjects with at least 1 event relative to *N*, number of participants in each treatment group in the Safety Set.

A limitation of the study is the variability in RTSS between subjects. This variability was higher than that previously reported in an exposure chamber study of sublingual immunotherapy for grass pollen allergy (40%-45%).[14] It is unclear whether this is due to differences in the allergens being evaluated (ie, grass pollen and HDM) or between the chambers.[23]

Sensitization to allergens other than the allergen being evaluated is often a confounding factor in natural field studies of AIT. In the present study, more than 75% of the randomized subjects were polysensitized. These subjects were eligible to participate in the study if they were not to be exposed to the allergens(s) in question during the time of their participation.

Immune responses to treatment with STG320, measured as serum levels of HDM-specific IgE and IgG_4, were dose-dependent. The increase in HDM-specific serum IgG_4 level was apparent at 2 months, the first measurement after treatment initiation. Trends in these markers were similar to those observed in a natural field study of the same tablets, following the same dosing regimen[10] as well as those previously reported in response to treatment with tablets of HDM[15] and 5-grass.[14]

The favorable safety profile was consistent with that reported in a previous natural field study,[10] with application site reactions being the most frequent AEs. Although the incidence of adverse reactions was similar across the active dose groups, more subjects receiving the 500IR dose discontinued treatment in both studies.

Conclusions

The dose-dependent effect of STG320 sublingual immunotherapy tablets of HDM allergen extracts in treating HDM-associated allergic rhinitis was demonstrated in this EEC study. Based on the totality of the study results, the 300IR and 500IR doses will be further evaluated in natural field studies.

We thank all the participants, coordinators, and investigators for their participation. We acknowledge Dr Anne-Marie Salapatek and Dr Peter Couroux's contribution to the design and execution of the trial, and Anuradha Alahari and Josiane Cognet-Sicé for preparation of the manuscript.

Clinical implications: Results of this dose-ranging study are consistent with those of a previous study and support further development of this sublingual tablet for patients with HDM-associated allergic rhinitis.

REFERENCES

1. Thomas WR. House dust allergy and immunotherapy. Hum Vacc Immunother 2012;8:1469-78.
2. Rolla G, Guida G, Heffler E, Badiu I, Bommarito L, De Stefani A, et al. Diagnostic classification of persistent rhinitis and its relationship to exhaled nitric oxide and asthma: a clinical study of a consecutive series of patients. Chest 2007;131:1345-52.
3. Gaffin JM, Phipatanakul W. The role of indoor allergens in the development of asthma. Curr Opin Allergy Clin Immunol 2009;9:128-35.
4. Gotzsche PC, Johansen HK. House dust mite control measures for asthma: systematic review. Allergy 2008;63:646-59.
5. Nurmatov U, van Schayck CP, Hurwitz B, Sheikh A. House dust mite avoidance measures for perennial allergic rhinitis: an updated Cochrane systematic review. Allergy 2012;67:158-65.
6. Sheikh A, Hurwitz B, Nurmatov U, van Schayck CP. House dust mite avoidance measures for perennial allergic rhinitis. Cochrane Database Syst Rev 2010;CD001563.
7. Nathan RA. The burden of allergic rhinitis. Allergy Asthma Proc 2007;28:3-9.
8. Compalati E, Passalacqua G, Bonini M, Canonica GW. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. Allergy 2009;64:1570-9.
9. Baron-Bodo V, Batard T, Nguyen H, Frereux M, Horiot S, Harwanegg C, et al. Absence of IgE neosensitization in house dust mite allergic patients following sublingual immunotherapy. Clin Exp Allergy 2012;42:1510-8.
10. Bergmann KC, Demoly P, Worm M, Fokkens WJ, Carrillo T, Tabar AI, et al. Efficacy and safety of sublingual tablets of house dust mite allergen extracts in adults with allergic rhinitis. J Allergy Clin Immunol 2014;133:1608-14.e6.
11. European Medicines Agency. Committee for Medicinal Products for Human Use CHMP/EWP/18504/2006. Guideline on the clinical development of products for

specific immunotherapy for the treatment of allergic diseases, November 2008. Available at http://www.emea.europa.eu.

12. Devillier P, Le Gall M, Horak F. The allergen challenge chamber: a valuable tool for optimizing the clinical development of pollen immunotherapy. Allergy 2011;66:163-9.
13. Food and Drug Administration. Guidance for Industry. Allergic rhinitis: clinical development programs for drug products (draft guidance). U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); April 2000. Available at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
14. Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Devillier P, Montagut A, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. J Allergy Clin Immunol 2009;124:471-7, 477.e1.
15. Nolte H, Maloney J, Nelson HS, Bernstein DI, Lu S, Li Z, et al. Onset and dose-related efficacy of house dust mite sublingual immunotherapy tablets in an environmental exposure chamber. J Allergy Clin Immunol 2015;135:1494-501.e6.
16. Horak F, Toth J, Hirschwehr R, Marks B, Stubner UP, Jager S, et al. Effect of continuous allergen challenge on clinical symptoms and mediator release in dust-mite-allergic patients. Allergy 1998;53:68-72.
17. Ronborg SM, Mosbech H, Poulsen LK. Exposure chamber for allergen challenge: a placebo-controlled, double-blind trial in house-dust-mite asthma. Allergy 1997;52:821-8.
18. Stubner P, Zieglmayer R, Horak F. A direct comparison of the efficacy of antihistamines in SAR and PAR: randomised, placebo-controlled studies with levocetirizine and loratadine using an environmental exposure unit—the Vienna Challenge Chamber (VCC). Curr Med Res Opin 2004;20:891-902.
19. Patel D, Lee JS, Wilson D, Camuso N, Salapatek A. Repeated low-dose aerosolized dust mite allergen exposure in asthmatic and non-asthmatic dust mite allergic patients in an environmental exposure chamber induces specific asthma symptoms as well as allergic rhinoconjunctivitis symptoms. J Allergy Clin Immunol 2011;127:AB20(64).
20. Bousquet PJ, Combescure C, Klossek JM, Daures JP, Bousquet J. Change in visual analog scale score in a pragmatic randomized cluster trial of allergic rhinitis. J Allergy Clin Immunol 2009;123:1349-54.
21. Demoly P, Bousquet PJ, Mesbah K, Bousquet J, Devillier P. Visual analogue scale in patients treated for allergic rhinitis: an observational prospective study in primary care. Clin Exp Allergy 2013;43:881-8.
22. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008;63:8-160.
23. Rosner-Friese K, Kaul S, Vieths S, Pfaar O. Environmental exposure chambers in allergen immunotherapy trials: current status and clinical validation needs. J Allergy Clin Immunol 2015;135:636-43.

METHODS

Randomization

Eligible subjects were randomized 1:1:1:1 to 1 of the 4 treatment groups (500IR, 300IR, 100IR, or placebo) by using a computer-generated list (block size of 8) created by Cetero Research/PRACS Institute with the SAS System. Treatments were allocated to the study subjects chronologically with the next available treatment number ordered sequentially according to this randomization list.

Washout periods for symptomatic medications before an allergen challenge

The use of antihistamines, decongestants, intranasal and systemic corticosteroids, and other immunosuppressants was prohibited during predefined washout periods before and during the allergen challenge (Table E1).

Allergen challenge

The EEC is a specially designed room that provides a standardized, predetermined level of exposure to the specific allergen in a controlled environment, and the exposure is uniform throughout the chamber. Allergic responses of the study participants to HDM particles were recorded in terms of symptom scores, and treatment-induced changes in symptom severity were assessed during the study period. At each allergen challenge, subjects were exposed for 4 hours to the same concentration range of HDM particles.

RESULTS

Average symptom scores for the 4 rhinitis symptoms (ARSS)

The changes from baseline to the end of treatment in the average symptom scores for the 4 rhinitis symptoms (ARSS) were higher for the active treatment groups, and in general, increased with the dose (Table E3).

TABLE E1. Prohibited medications and washout periods before allergen challenge

Prohibited medication	Washout period (d)
Decongestants (oral/nasal/ocular)	3
Short-acting antihistamines (ocular/topical/oral/nasal)	3
Long-acting antihistamines (ocular/topical/oral/nasal), such as cetirizine and fexofenadine	7
Loratadine and desloratadine	10
Over-the-counter cough and cold preparations or sleep aids containing antihistamines	10
Cromolyn, nedocromil, or lodoxamide (intranasal, ocular, or oral)	7
Leukotriene receptor antagonists or 5-LO inhibitors	7
Inhaled/oral/intranasal anticholinergics	7
All inhaled, intranasal, ocular, ear administered, and moderate to high-dose topical steroids	14
All oral steroids	30
Anti-IgE therapy	60

LO, Lipoxygenase.

TABLE E2. Change from baseline to treatment months 1, 2, 4, and 6 in the $AUC_{RTSS\ 0\text{-}4h}$ and in the $ARTSS_{0\text{-}4h}$ (summary statistics)—Full Analysis Set

		$AUC_{RTSS\ 0\text{-}4h}$				$ARTSS_{0\text{-}4h}$			
Time point	**Variable**	**500IR (N = 93)**	**300IR (N = 86)**	**100IR (N = 89)**	**Placebo (N = 87)**	**500IR (N = 93)**	**300IR (N = 86)**	**100IR (N = 89)**	**Placebo (N = 87)**
Baseline	Mean ± SD	1602.5 ± 497.62	1672.2 ± 522.21	1680.4 ± 457.82	1753.2 ± 576.91	6.21 ± 2.021	6.47 ± 2.092	6.56 ± 1.793	6.81 ± 2.352
Month 1	Mean Ch_{BL}	−397.3	−453.8	−378.6	−450.5	−1.59	−1.84	−1.58	−1.86
Month 2	Mean Ch_{BL}	−547.6	−553.2	−530.9	−480.3	−2.19	−2.22	−2.21	−1.91
Month 4	Mean Ch_{BL}	−710.9	−667.9	−633.3	−523.7	−2.80	−2.64	−2.55	−2.09
Month 6	Mean Ch_{BL}	−738.1	−786.4	−711.9	−639.4	−2.93	−3.10	−2.85	−2.59

N, Number of participants in each treatment group in the Full Analysis Set.

TABLE E3. Change from baseline to month 6 in the ARSSs for the last 2 hours of the allergen challenge—Full Analysis Set

Treatment group	No.	LS mean (SE)	Difference from placebo			Relative LS mean difference* (%)
			LS mean difference	95% CI	*P* value	
Rhinorrhea						
500IR	70	−0.92 (0.100)	−0.35	−0.62 to −0.08	.012	61.1
300IR	68	−0.88 (0.101)	−0.31	−0.58 to −0.04	.027	54.2
100IR	75	−0.85 (0.096)	−0.28	−0.55 to −0.01	.040	49.1
Placebo	75	−0.57 (0.096)				
Nasal congestion						
500IR	70	−1.00 (0.100)	−0.25	−0.52 to 0.03	NS	32.5
300IR	68	−0.99 (0.101)	−0.23	−0.51 to 0.04	NS	30.5
100IR	75	−0.94 (0.096)	−0.19	−0.45 to 0.08	NS	24.7
Placebo	75	−0.76 (0.096)				
Nasal pruritus						
500IR	70	−0.95 (0.097)	−0.31	−0.58 to −0.04	.023	48.3
300IR	68	−0.88 (0.099)	−0.23	−0.50 to 0.03	NS	36.3
100IR	75	−0.79 (0.094)	−0.15	−0.41 to 0.11	NS	23.1
Placebo	75	−0.64 (0.094)				
Sneezing						
500IR	70	−0.72 (0.100)	−0.23	−0.50 to 0.05	NS	45.9
300IR	68	−0.77 (0.101)	−0.28	−0.55 to 0.00	.049	56.3
100IR	75	−0.67 (0.096)	−0.18	−0.45 to 0.09	NS	37.3
Placebo	75	−0.49 (0.096)				

LS, Least squares; *No.*, number of subjects for whom data are available; *NS*, not significant; *SE*, standard error.
*Relative LS mean difference = 100 × (LS mean difference from placebo/LS mean placebo).

TABLE E4. AEs of asthma and associated symptoms in the peri-EEC periods and outside the peri-EEC periods—Safety Set

	n (%)							
	Peri-EEC periods				Outside the peri-EEC periods			
System Organ Class Preferred Term	**500IR (N = 93)**	**300IR (N = 86)**	**100IR (N = 89)**	**Placebo (N = 87)**	**500IR (N = 93)**	**300IR (N = 86)**	**100IR (N = 89)**	**Placebo (N = 87)**
Subjects reporting ≥1 AE of asthma or associated symptom	34 (36.6)	38 (44.2)	40 (44.9)	39 (44.8)	15 (16.1)	14 (16.3)	19 (21.3)	14 (16.1)
Respiratory, thoracic, and mediastinal disorders	30 (32.3)	32 (37.2)	37 (41.6)	35 (40.2)	14 (15.1)	14 (16.3)	17 (19.1)	14 (16.1)
Bronchospasm	25 (26.9)	22 (25.6)	33 (37.1)	29 (33.3)	1 (1.1)	0 (0.0)	4 (4.5)	4 (4.6)
Cough	4 (4.3)	8 (9.3)	10 (11.2)	7 (8.0)	7 (7.5)	10 (11.6)	11 (12.4)	9 (10.3)
Dyspnea	7 (7.5)	8 (9.3)	7 (7.9)	6 (6.9)	7 (7.5)	1 (1.2)	5 (5.6)	4 (4.6)
Asthma	3 (3.2)	1 (1.2)	3 (3.4)	0 (0.0)	0 (0.0)	4 (4.7)	2 (2.2)	1 (1.1)
Bronchial hyperreactivity	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Wheezing	1 (1.1)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.1)
Investigations	4 (4.3)	7 (8.1)	8 (9.0)	5 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Forced expiratory volume decreased	4 (4.3)	7 (8.1)	8 (9.0)	5 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
General disorders and administration site conditions	4 (4.3)	6 (7.0)	5 (5.6)	4 (4.6)	1 (1.1)	0 (0.0)	4 (4.5)	1 (1.1)
Chest discomfort	4 (4.3)	6 (7.0)	5 (5.6)	4 (4.6)	1 (1.1)	0 (0.0)	4 (4.5)	1 (1.1)

n, Number of subjects with at least 1 event in the given Preferred Term; %, percentage of subjects with at least 1 event relative to *N*, number of participants in each treatment group in the Safety Set.