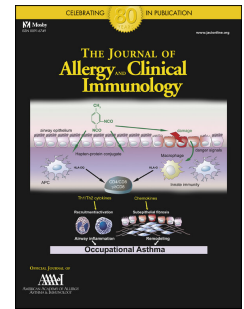


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Efficacy and Safety of Sublingual Tablets of House Dust Mite Allergen Extracts:
Results of a Dose-Ranging Study in an Environmental Exposure Chamber

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TITLE PAGE

Original Article

Efficacy and Safety of Sublingual Tablets of House Dust Mite Allergen Extracts: Results of a Dose-Ranging Study in an Environmental Exposure Chamber

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Abstract (250 words)

Background: In a natural field study, sublingual tablets of house dust mite (HDM) allergen extracts (STG320) were efficacious in treating HDM-associated allergic rhinitis.

Objective: To assess the efficacy and safety of three doses of STG320 in an environmental exposure chamber (EEC).

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 ($Ch_{BL}AUC_{RTSS\ 0-4h}$). Differences from the placebo group were analyzed by ANCOVA. Adverse events (AEs) and routine safety parameters were recorded.

Results: 355 subjects were randomized to one of four groups: 500IR (n=93), 300IR (n=86), 100IR (n=89), or placebo (n=87). The least squares mean differences from placebo in the $Ch_{BL}AUC_{RTSS\ 0-4h}$ 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.

Keywords: allergen immunotherapy; allergic rhinitis; house dust mite; sublingual immunotherapy tablet; double-blind placebo-controlled trial; environmental exposure chamber; dose-ranging.

Abbreviations used

AE: Adverse event

AIT: Allergen immunotherapy

ANCOVA: Analysis of covariance

ARSS: Average Rhinitis Symptom Score

ARTSS: Average Rhinitis Total Symptom Score

AUC: Area-under-the-curve

AVASS: Average Visual Analog Scale Score

Der f: *Dermatophagoides farinae*

Der p: *Dermatophagoides pteronyssinus*

EEC: Environmental exposure chamber

Ch_{BL}: Change from baseline

CI: Confidence interval

FAS: Full Analysis Set

FEV: Forced expiratory volume

HDM: House dust mites

IR: Index of reactivity

LS: Least squares

RTSS: Rhinitis Total Symptom Score

TEAE: Treatment-emergent adverse event

VAS: Visual Analog Scale

82

83 **Clinical Implications** (27 words)

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

87

88 **Capsule Summary** (26 words)

89 The results of this placebo-controlled, dose-ranging study conducted in an environmental
90 exposure chamber demonstrate a dose-dependent effect of STG320 sublingual
91 immunotherapy tablets of HDM allergen extracts.

INTRODUCTION

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 GA²LEN 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, one-year phase II/III study conducted under natural field conditions, treatment with two doses of sublingual allergen immunotherapy (AIT) tablets containing freeze-dried extracts of the two most common allergenic mite species, *Dermatophagoides pteronyssinus* (*Der p*) and *D. farinae* (*Der f*), (referred to as STG320) was efficacious and safe.^{9, 10} In the present study, the dose-dependent effect of STG320 was further evaluated with three 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

116 with small numbers of subjects.¹⁶⁻¹⁸ In this multicenter study, HDM allergen challenge in an
117 EEC was used to assess dose-dependent effect and safety of AIT.

METHODS

Trial design

This randomized, double blind, placebo-controlled study (Fig. 1) was conducted at eight centers in Canada (ClinicalTrials.gov No.: NCT01527188). All allergen challenges in the environmental exposure chamber (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 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 18–55 years of age were selected in a 2-step process. First, they had to have at least a one-year history of HDM-associated allergic rhinitis uncontrolled despite the use of symptomatic treatments, a positive skin prick test (wheal diameter, >3 mm), and HDM-specific serum IgE (≥ 0.7 kU/L). Those with a Rhinitis Total Symptom Score (RTSS) ≥ 6 (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 forced expiratory volume in one second (FEV₁) <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, USA) was 22–23 µg *Der p* 1 and 99–102 µg *Der f* 1 per 500IR tablet, 14–17 µg *Der p* 1 and 53–62 µg *Der f* 1 per 300IR tablet, and 4–6 µg *Der p* 1 and 18–21 µg *Der f* 1 per 100IR tablet. Treatment consisted of sublingual administration of a single tablet daily for six 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.

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 15–100 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 non-nasal symptoms in dust mite allergic patients.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 four nasal symptoms (rhinorrhea, nasal congestion, sneezing and nasal pruritus) on a scale of 0 (no symptoms), 1 (mild), 2 (moderate) to 3 (severe). The Rhinitis Total Symptom Score (RTSS) is the sum of these four 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), ranging from “absence of symptoms” to “very severe symptoms.”^{20, 21}

For each challenge, rhinitis symptoms and 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. As 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 four individual rhinitis symptoms (ARSS), RTSS (ARTSS) and VAS 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 to the end-of-treatment (i.e., 6 months) in the AUC of the RTSS over the 4 hours of the challenge ($\text{Ch}_{\text{BL}}\text{AUC}_{\text{RTSS } 0-4\text{h}}$). This variable was also evaluated after 1, 2 and 4 months of treatment. The changes from baseline to the end-of-treatment in average RTSS ($\text{Ch}_{\text{BL}}\text{ARTSS}$), average individual rhinitis scores ($\text{Ch}_{\text{BL}}\text{ARSS}_{0-4\text{h}}$) and average VAS score ($\text{Ch}_{\text{BL}}\text{AVASS}_{0-4\text{h}}$), 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 two 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 [$\text{Ch}_{\text{BL}}\text{AUC}_{\text{RTSS } 2-4\text{h}}$] and ARTSS [$\text{Ch}_{\text{BL}}\text{ARTSS}_{2-4\text{h}}$]), individual rhinitis scores (ARSS [$\text{Ch}_{\text{BL}}\text{ARSS}_{2-4\text{h}}$]), and VAS score ($\text{Ch}_{\text{BL}}\text{AVASS}_{2-4\text{h}}$) as secondary end points.

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 IgG₄ were determined using the ImmunoCap Phadia Laboratory System[®].

Safety variables included adverse events (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 (i.e., 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, USA. The threshold of statistical significance was set to a p-value of less than 0.05, and all inferential tests were 2-sided.

Efficacy and safety were evaluated on all randomized subjects who received at least one dose of the assigned treatment. In this study, the efficacy set (Full Analysis Set, FAS) and the Safety Set were identical, and consistent with the Intent-To-Treat principle. The primary efficacy criterion was analyzed using analysis of covariance (ANCOVA) with treatment as the main effect and the baseline AUC_{RTSS 0-4h} 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 FAS: 500IR (n=93), 300IR (n=86), 100IR (n=89) and placebo (n=87). 288 (81%) completed the study (Fig. 2).

Demographics and baseline disease characteristics were similar across the groups (Table I).

The mean age was 32 years, 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 $Ch_{BL}AUC_{RTSS\ 0-4h}$ after six treatment months, a dose-dependent effect was observed with relative differences vs. 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 ($p=0.0427$) (Fig. 3A). The time course of nasal symptoms recorded during the HDM allergen challenge showed a biphasic curve with an initial phase of increasing RTSSs (0–2h), followed by a phase where the RTSSs stabilized (2–4h), seen as a plateau (Fig. 4 for the baseline challenge and the 6-month challenge). For the last two hours of the challenge, when the symptoms were recorded every 30 min by the subject, the change from baseline in AUC_{RTSS} ($Ch_{BL}AUC_{RTSS\ 2-4h}$) and average RTSSs ($Ch_{BL}ARTSS_{2-4h}$) after 6 treatment months also correlated with dose. For the $Ch_{BL}AUC_{RTSS\ 2-4h}$, the relative differences vs. the placebo group were 42.0%, 40.9% and 30.5% for the 500IR, 300IR and 100IR groups, respectively (Fig. 3B). The relative differences from placebo in $Ch_{BL}ARTSS_{2-4h}$ for the 500IR, 300IR, and 100IR groups were 44.7%, 42.3% and 31.3%, respectively. The differences were statistically significant for the two higher dose groups (Fig. 3D).

Also, compared with the placebo group, the relative difference in $\text{Ch}_{\text{BL}}\text{ARTSS}_{0-4\text{h}}$ for the 500IR group was statistically significant ($p=0.0469$) after 6 months (Fig. 3C). For the primary efficacy variable, the $\text{Ch}_{\text{BL}}\text{AUC}_{\text{RTSS } 0-4\text{h}}$, 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, as did the $\text{Ch}_{\text{BL}}\text{ARTSS}_{0-4\text{h}}$ (Table E2 in this article's Online Repository). 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 the changes in the four 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 $\text{Ch}_{\text{BL}}\text{AUC}_{\text{RTSS}}$, the $\text{Ch}_{\text{BL}}\text{ARTSS}$ and the changes in the individual rhinitis symptom scores are provided in the Online Repository (Table E2 and Table E3). 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 ($\text{Ch}_{\text{BL}}\text{AVASS}_{0-4\text{h}}$) after 6 treatment months, the relative differences vs. placebo were 25.3%, 23.8% and 14.1% for the 500IR, 300IR and 100IR groups, respectively (Table II).

Immune responses

At study entry, serum levels of *Der p*- and *Der f*-specific IgE and IgG₄ were similar across treatment groups (Fig. 5). Within the first 2 treatment months, mite-specific serum IgE increased 5- to 7-fold in the three active treatment groups, and then gradually decreased, while remaining unchanged in the placebo group. Mite-specific serum IgG₄ increased over the treatment period in the three active treatment groups, and was little changed in the placebo

group. For both of these markers of immunologic activity, a dose-dependent effect was apparent at each time point.

Safety analysis

Overall, the incidences of TEAEs reported by the subjects were higher in the active groups (94%, 91% and 97% in the 500IR, 300IR and 100IR groups) compared to 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 four 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 (Table E4). 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 due to TEAEs: 11 in the 500IR group, 5 in the 300IR group and 4 in the 100IR group. Additionally, 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 vital signs, laboratory tests or spirometry parameters, except for transient decreases in FEV₁ during and immediately after the allergen challenges.

DISCUSSION

This placebo controlled dose-ranging study, conducted using allergen challenges in an environmental exposure chamber, 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 the changes from baseline in the AUC of the RTSS and the average RTSS. 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 two 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 two 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 two hours of each challenge showed larger differences between each active treatment group and the placebo group than were observed across the full four hours of the challenges. Of note, in the EEC study reported by Nolte et al., the efficacy end points were analyzed during the last 4 hours of the 6-hour challenges (i.e., the plateau period).

Use of an EEC is particularly suitable for dose-ranging studies as 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 four 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 and colleagues,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.

A limitation of the study is the variability in rhinitis total symptom score between patients. 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 (i.e., 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 IgG₄, were dose-dependent. The increase in HDM-specific serum IgG₄ was apparent at two 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 regimen¹⁰ as well as those previously reported in response to treatment with tablets of HDM¹⁵ 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 adverse events. While the incidences of adverse reactions were similar across the active dose groups more subjects receiving the 500IR dose discontinued 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 environmental exposure chamber study. Based on the totality of the study results, the 300IR and 500IR doses will be further evaluated in natural field studies.

374

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Table I. Demographics and Baseline characteristics - FAS

	500IR	300IR	100IR	Placebo
	(N = 93)	(N = 86)	(N = 89)	(N = 87)
Age (years)	32.8 (9.33)	32.5 (8.56)	32.4 (10.09)	31.3 (8.75)
Women, n (%)	44 (47.3)	45 (52.3)	47 (52.8)	47 (54.0)
Duration of AR (years)	19.2 (9.71)	16.6 (9.12)	16.6 (10.28)	15.2 (9.76)
FEV ₁ (% predicted)	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 _{0-4h}	6.21 (2.021)	6.47 (2.092)	6.56 (1.793)	6.81 (2.352)
Baseline AVASS _{0-4h}	4.59 (1.725)	4.78 (1.898)	5.08 (1.662)	5.03 (1.882)

Continuous variables are reported as Mean (*SD*, Standard Deviation). Categorical variables are reported as n, number of subjects (%), percentage of subjects relative to *N*, number of participants in each treatment group in the *FAS*, Full Analysis Set).

IR, Index of reactivity; *AR*, Allergic rhinitis; *ARTSS*, Average Rhinitis Total Symptom Score (range 0-12); *AVASS*, Average Visual Analog Scale Score (range 0-10).

*Sensitized to HDM allergen(s) and at least one of the other allergens tested based on skin prick testing.

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 - FAS

Treatment	no.	LS Means (SE)	Average VAS scores: Difference from placebo				Relative LS Mean difference*(%)
			LS Mean difference	95% CI	P value		
0-4 hours 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 hours 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)	—	—	—		—

CI, Confidence interval; FAS, Full Analysis Set; IR, Index of reactivity; LS, Least squares; no., Number of subjects for whom data were available; NS, Not significant; SE, Standard Error

* Relative LS mean difference = $100 \times (\text{LS mean difference from Placebo} / \text{LS mean Placebo})$

Table III. Drug-related treatment-emergent adverse events occurring in at least 5% of population of any treatment group – Safety Set

System Organ Class	500IR (N = 93)	300IR (N = 86)	100IR (N = 89)	Placebo (N = 87)
Preferred Term	no. (%)	no. (%)	no. (%)	no. (%)
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)
Oedema 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)
Paraesthesia oral	6 (6.5)	3 (3.5)	3 (3.4)	1 (1.1)
Lip oedema	8 (8.6)	3 (3.5)	1 (1.1)	0 (0.0)
Hypoaesthesia 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)

470 Drug-related *TEAEs*, Treatment-emergent adverse events (AEs occurring during the treatment
471 period and up to 30 days after the last treatment administration) were classified according to
472 their System Organ Class and Preferred Term (MedDRA version 14.0).
473 *IR*, Index of reactivity; *no.*, Number of subjects with at least one event in the given preferred
474 term; %, percentage of subjects with at least one event relative to *N*, number of participants in
475 each treatment group in the Safety Set.

476

Figure legends

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 environmental exposure chamber. Those with symptom scores above the pre-defined threshold were randomized to one of the four treatment arms, and underwent four additional allergen challenges during the 6 treatment months.

IR, Index of reactivity.

Fig. 2. Subject disposition.

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

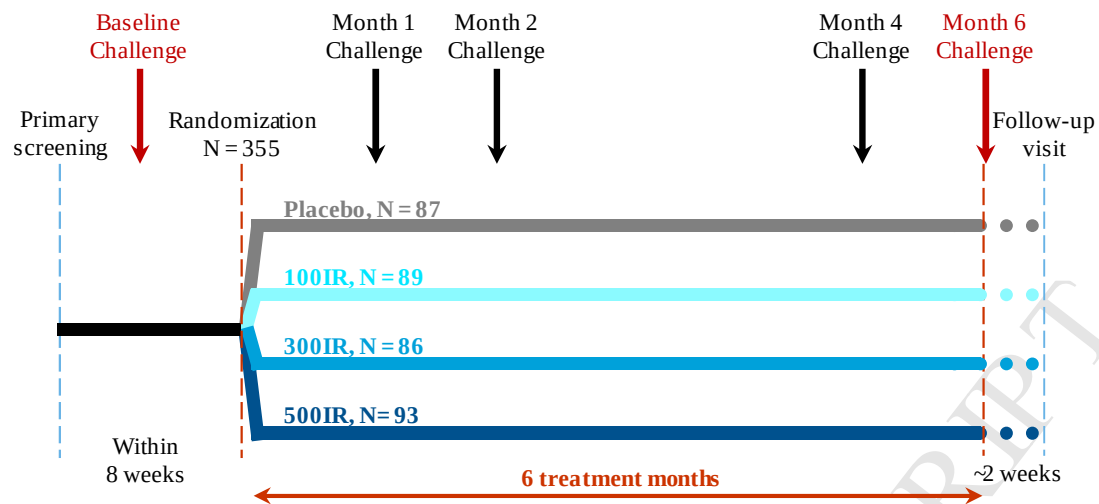
Fig. 3. Treatment efficacy. Change from baseline (Ch_{BL}) to end-of-treatment (after 6 months) in the area-under-the-curve (AUC) of Rhinitis Total Symptoms Scores (RTSS) for the full 4 hours (0–4h) (**A**) or the last 2 hours (2–4h) (**B**) of allergen challenge; Change from baseline in the Average Rhinitis Total Symptoms Scores (ARTSS) for the full 4 hours (0–4h) (**C**) or the last 2 hours (2–4h) (**D**) of allergen challenge. *NS*, Not significant.

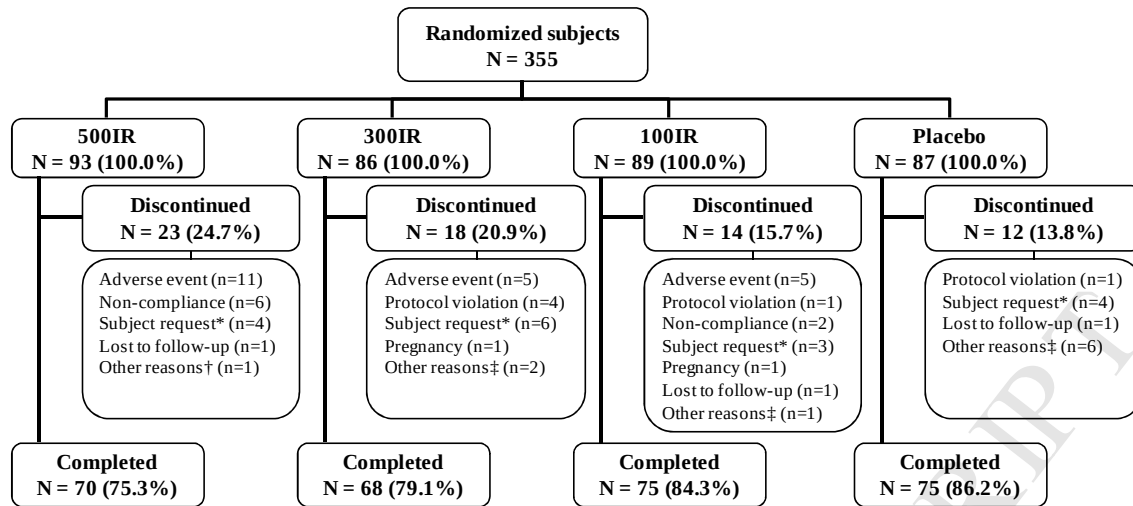
Fig. 4. Assessment of Rhinitis Total Symptom Scores during HDM allergen challenge.

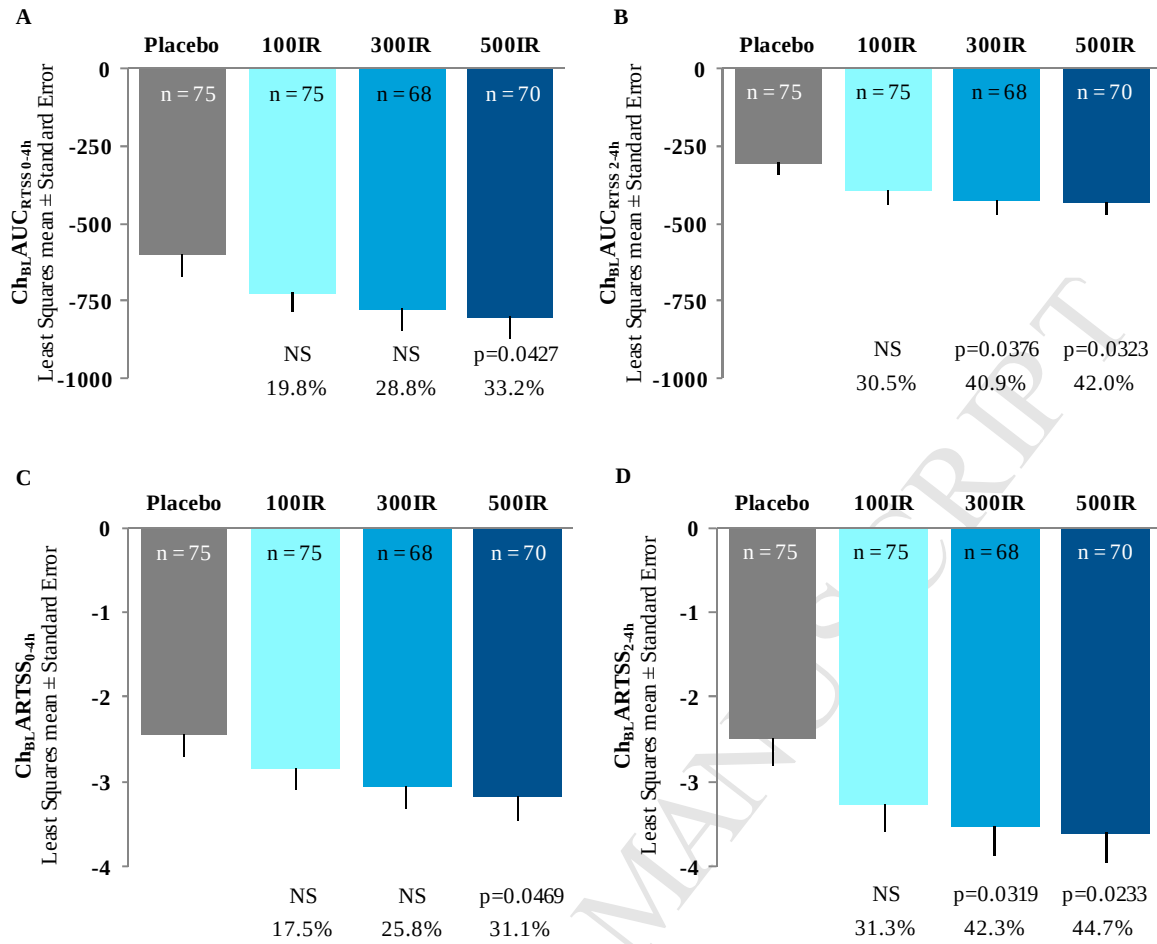
Subjects were exposed to HDM allergens for 4 hours in the EEC. Rhinitis symptoms were recorded by the subjects at 13 time points, and Rhinitis Total Symptom Scores (RTSS, range 0–12) were derived at each time point.

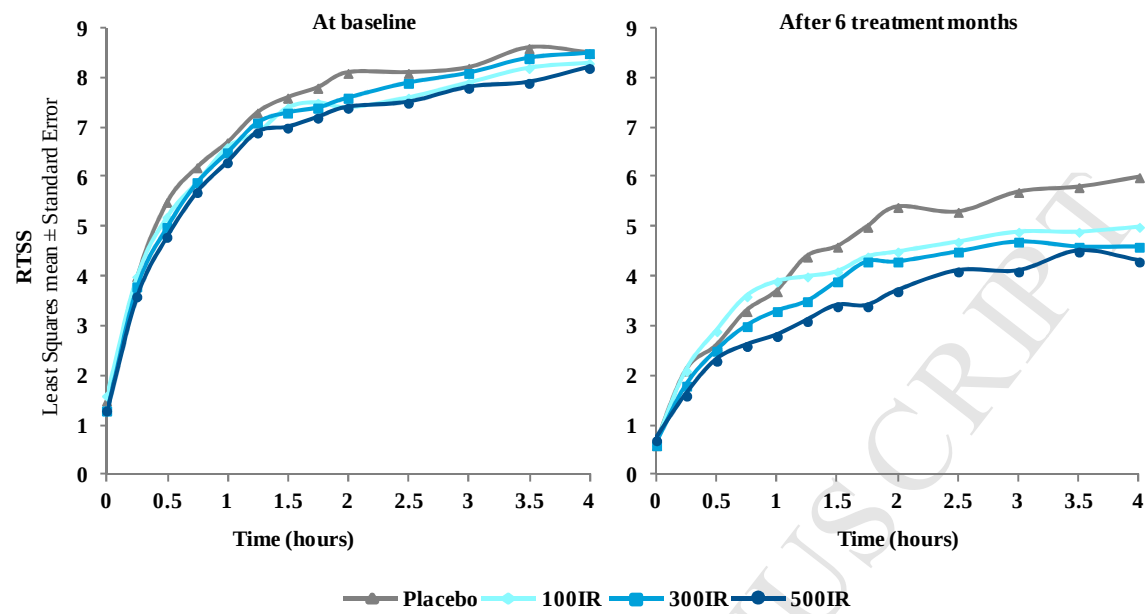
501 **Fig. 5. Immunological markers.** Serum-specific IgE (top panels) and IgG₄ (bottom panels)
502 levels for *D. pteronyssinus* and *D. farinae* at baseline and after 2, 4, and 6 months. [Units:
503 kU/L for IgE, mcg/mL for IgG₄]

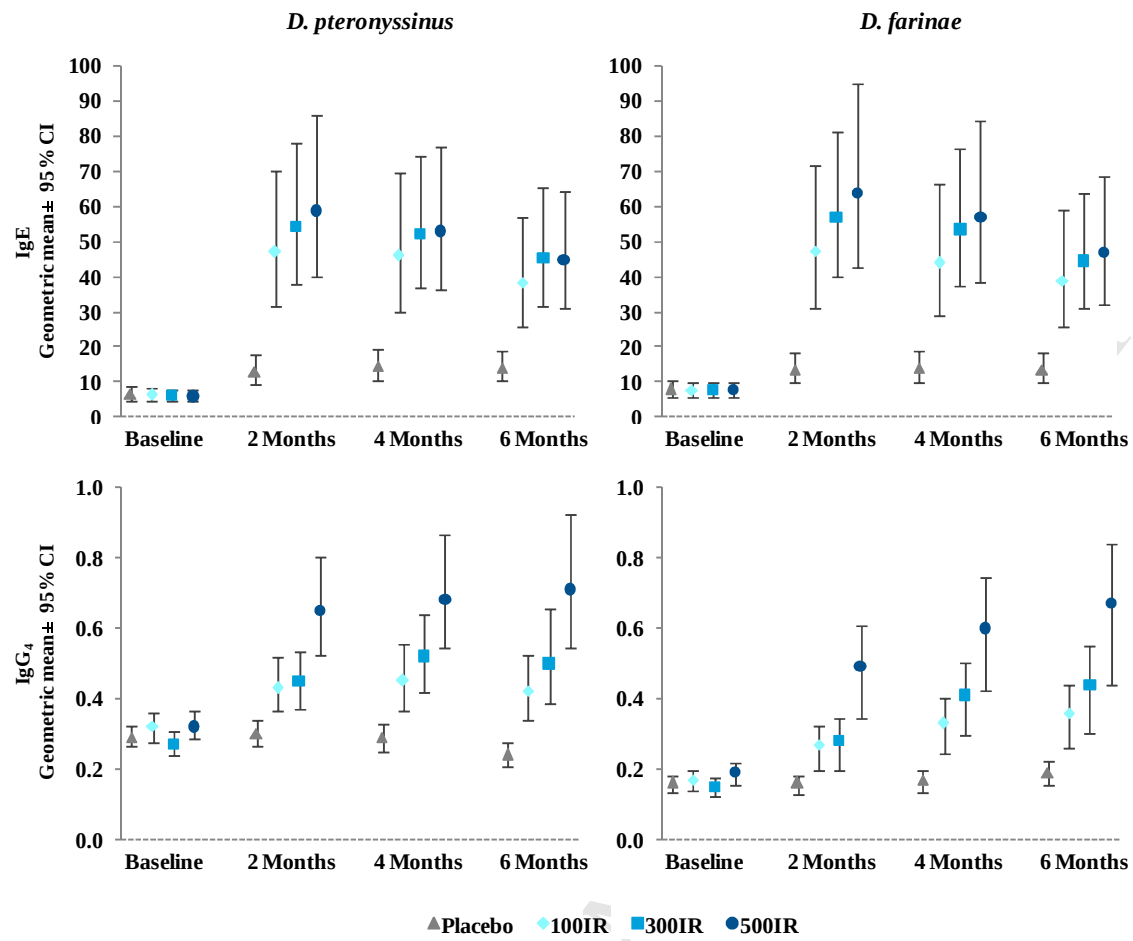
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TITLE PAGE

Original Article

Efficacy and Safety of Sublingual Tablets of House Dust Mite Allergen Extracts: Results of a Dose-Ranging Study in an Environmental Exposure Chamber

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METHODS

Randomization

Eligible subjects were randomized 1:1:1:1 to one of the four 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.

Wash-out 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 environmental exposure chamber (EEC) is a specially designed room that provides a standardized, pre-determined 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 four rhinitis symptoms (ARSS)**

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

1 **Table E1. Prohibited medications and washout periods before allergen challenge**

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

2

Table E2. Change from baseline to treatment months 1, 2, 4 and 6 in the AUC_{RTSS 0-4h} and in the average RTSS_{0-4h} (Summary statistics) – FAS

	AUC _{RTSS 0-4h}				Average RTSS _{0-4h}			
	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)
Mean Changes from Baseline								
Month 1	-397.3	-453.8	-378.6	-450.5	-1.59	-1.84	-1.58	-1.86
Month 2	-547.6	-553.2	-530.9	-480.3	-2.19	-2.22	-2.21	-1.91
Month 4	-710.9	-667.9	-633.3	-523.7	-2.80	-2.64	-2.55	-2.09
Month 6	-738.1	-786.4	-711.9	-639.4	-2.93	-3.10	-2.85	-2.59

AUC, Area-under-the-curve; IR, Index of reactivity; N, Number of participants in each treatment group in the FAS, Full Analysis Set; RTSS, Rhinitis Total Symptom Score; SD, Standard Deviation

9 **Table E3. Change from baseline to Month 6 in the average rhinitis symptom scores**
 10 **(ARSS) for the last 2 hours of the allergen challenge - FAS**

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)				

- 12 *CI*, Confidence interval; *FAS*, Full Analysis Set; *IR*, Index of reactivity; *LS*, Least squares;
- 13 *no.*, Number of subjects for whom data are available; *SE*, Standard Error
- 14 * Relative LS mean difference = $100 \times (\text{LS mean difference from Placebo} / \text{LS mean Placebo})$

15 **Table E4. Adverse events of asthma and associated symptoms in the peri-EEC periods and outside the peri-EEC periods - Safety Set**

System Organ Class Preferred Term	Peri-EEC periods				Outside the peri-EEC periods			
	500IR (N = 93)	300IR (N = 86)	100IR (N = 89)	Placebo (N = 87)	500IR (N = 93)	300IR (N = 86)	100IR (N = 89)	Placebo (N = 87)
	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)
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)

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17 *AE*, Adverse event; *EEC*, Environmental exposure chamber; *IR*, Index of reactivity; *no.*, Number of subjects with at least one event in the given

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