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Efficacy and tolerability of a house dust mite allergoid in allergic bronchial asthma: a randomized dose-ranging trial

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Aim: This multicenter randomized placebo-controlled double-blind clinical trial investigated which maintenance dose shows the optimal benefit–risk ratio for subcutaneous immunotherapy with a *Dermatophagoides pteronyssinus* allergoid preparation. **Objective:** To evaluate four maintenance doses of the allergoid preparation versus placebo. **Methods:** The late-phase reaction of the intracutaneous test was chosen as efficacy parameter and minimal dose of fluticasone required for asthma control. **Results:** A total of 146 adults with bronchial asthma were randomized. After subcutaneous immunotherapy, reductions in swelling size were greatest with 10,000 therapeutic units (TU). The 18,000 TU group showed the highest percentage of patients with fluticasone dose reduced to 0 µg/day. **Conclusion:** The optimal dose of allergoid for the investigation in a confirmatory trial with inhaled corticosteroid reduction is 18,000 TU.

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House dust mite (HDM) allergy is the most prevalent IgE-mediated indoor allergy and may be associated with bronchial asthma, rhinitis and/or rhinoconjunctivitis [1,2]. Allergen immunotherapy (AIT) is the only causative treatment for IgE-mediated allergy [2,3]. Subcutaneous allergen immunotherapy (SCIT) is the most common form of AIT used for this type of allergy worldwide.

Application of native allergens for AIT dates to over a century. Chemical modification of allergens was introduced to increase the safety profile of AIT by substantially reducing allergenicity, as judged by skin prick testing, provocation testing, histamine release from sensitized leukocytes and measurement of IgE-binding activity [4–7]. Immunogenic activity and T-cell reactivity are retained [7]. These features account for a reduced risk of IgE-mediated side reactions and the possibility of administration of higher doses of immunogen over a shorter time compared with native allergens.

The SCIT allergoid preparation under investigation is a modified formulation of the HDM *Dermatophagoides pteronyssinus*. For details of the production of this and other SCIT allergoid preparations, see the Supplementary Material and previous publications [4,8].

The efficacy and safety of SCIT with this allergoid preparation have been investigated in HDM-induced bronchial asthma, showing promising first results. The minimum inhaled corticosteroid (ICS) dose required for asthma control was significantly reduced during AIT-SCIT treatment with 6000 therapeutic units (TU) versus placebo in 65 pediatric patients [9].

The aim of the current Phase II trial was to determine the optimal dose for efficacy and tolerability of SCIT with the *D. pteronyssinus* allergoid preparation in adults with well-controlled allergic bronchial asthma and allergic rhinitis (AR) or allergic rhinoconjunctivitis induced by HDM. The results will serve the optimization of the following confirmatory trial. The trial also investigated whether the *D. pteronyssinus* SCIT preparation is able to induce *Dermatophagoides farinae*-specific IgG and IgG4 antibody responses.

Considering the positive outcome of previous studies, the change of the wheal size in intracutaneous test (ICT) measured after 6 h (late phase) assessed before and after AIT treatment was chosen as the primary end point in this study [10–12].

Methods

Trial design

This multicenter, randomized, double-blind and placebo-controlled dose-ranging trial with parallel groups at different doses of SCIT were conducted in 32 centers in Poland and six centers in Spain between April 2012 and April 2015.

Objective

To evaluate the efficacy and safety of four different maintenance doses (2000, 6000, 10,000 and 18,000 TU) of *D. pteronyssinus* allergoid preparation (Acaroid[®], Allergopharma GmbH & Co. KG, Reinbek, Germany) in adults with controlled allergic bronchial asthma and AR or allergic rhinoconjunctivitis caused by HDM.

Trial population

For inclusion, the following criteria were applied: patients aged 18–40 years with allergic bronchial asthma caused by HDM sensitization documented by skin prick test wheal for *D. pteronyssinus* ≥ 3 mm in diameter; immunoassay result for *D. pteronyssinus*-specific IgE > 1.5 kU/l; proven clinical relevance of HDM allergy by positive conjunctival provocation test with *D. pteronyssinus* allergens; peak expiratory flow (PEF) or forced expiratory volume in 1 s (FEV₁) $\geq 80\%$ of predicted normal.

Patients selection were based on: a confirmed asthma diagnosis, classified as ‘controlled’ according to GINA guidelines [13]; a history of ICS treatment with a fluticasone equivalent of up to a maximum dose of 500 $\mu\text{g}/\text{day}$; late-phase reaction (LPR) in ICT at baseline ≥ 20 mm in longest diameter.

Procedures

In this dose range finding (DRF) phase, a 1:1:1:1 randomization ratio for the four doses of SCIT and placebo were used. Randomization was performed independent centrally. The treatment schedule comprised of subcutaneous injections of Acaroid[®] or placebo, administered for approximately 7 months during the DRF phase, which was divided into a dose-escalation phase and a dose-maintenance phase. Patients and investigators were blinded to treatment. The randomization, dosing and assessment schedules are described in more detail in the Supplementary Material, including in Supplementary Tables 1 & 2 and Supplementary Figure 1.

ICT solution was always applied on the dorsal side of the forearm. The skin reaction was assessed using an optical device (Primos 3D, GF Messtechnik GmbH, Teltow, Germany) to objectively measure the wheal swelling area (LPR) and volume induced by the ICT solution of *D. pteronyssinus* allergen extracts of 5000 standardized biological unit per ml (SBE/ml) of *D. pteronyssinus* [10]. The camera provides a measurement with a height resolution of > 20 μm . Within the gauge field of $160 \times 100 \times 90$ mm the area and volume of the skin reaction can be calculated at inevitable time points [14].

In addition to measuring of the wheal size after ICT, efficacy was assessed by investigating the effects of SCIT on asthma control (using the Asthma Control Test [ACT]), lung function (morning PEF) and the serum concentrations of antibodies (IgE, IgG and IgG₄ specific to HDMs using ImmunoCAP [ThermoFisher Scientific, Uppsala, Sweden]) at screening and after SCIT. The ACT is a validated five-item patient questionnaire used to determine whether asthma symptoms are well controlled during the previous 4 weeks. Higher scores indicate better asthma control. During screening, the dose of fluticasone was reduced in predefined steps from 2×250 $\mu\text{g}/\text{day}$ to 2×100 $\mu\text{g}/\text{day}$ and 0 $\mu\text{g}/\text{day}$. The ACT was undertaken at each step. Each dose-step lasted for 6 weeks until uncontrolled conditions were reached and patients with controlled asthma without fluticasone were excluded from the ACT analyses. After SCIT, patients received the fluticasone dose at which their asthma was uncontrolled at baseline and subsequently undertook the ACT.

Outcomes

The primary efficacy end point was the absolute change of the swelling area of the LPR after ICT from baseline to after the course of SCIT.

Secondary end points were changes in the following variables from baseline to after SCIT: swelling area and volume 20 min (early-phase reaction [EPR]) after ICT; minimal ICS dose for asthma control; ACT score; morning PEF; concentrations of IgE, IgG, and IgG₄ specific to *D. pteronyssinus* and *D. farinae*.

The following safety measures were assessed: treatment-emergent adverse events (TEAEs), safety laboratory, vital signs, and lung function tests at screening and at the end of the trial. Systemic AEs were graded according to the WAO system [15].

Statistical methods

Primary and secondary efficacy end points were analyzed by analysis of covariance, with change of the parameter as the dependent variable, treatment and center as fixed effects and the baseline value of the parameter as covariable. Change of minimal ICT dose for asthma control was summarized as a continuous variable and in shift tables. Change in $\mu\text{g/day}$ and in dose steps were analyzed with the Mann–Whitney U and Mantel–Haenszel tests, respectively. Fisher's exact test was performed for categorical variables. p -values <0.05 were considered statistically significant.

The dose–response relationship of the four doses studied is performed in an exploratory manner and thus, no formal hypotheses are tested. It is assumed that 32 patients per treatment group will be sufficient to get valid information about the dose–response curve of the investigational treatment. Thus, it is planned, that 300 patients will be screened to randomize 160 patients to the five treatment groups.

All efficacy analyses were conducted using the full analysis set (FAS; patients who received ≥ 1 injection of trial medication or placebo and for whom an efficacy assessment was available) and per-protocol set (PPS; patients of the FAS without major protocol violations). Data for per-protocol set are not shown in this report. All safety analyses were conducted with patients who received ≥ 1 injection of trial medication or placebo.

Additional statistical methods are in the Supplementary Material.

Results

A total of 146 patients were randomized to the four active treatment groups and one placebo group (Figure 1); 91.1% of the 146 patients completed the trial (90.6% in the placebo group vs 83.9–100% in the active groups).

Overall, the five groups were comparable with respect to demographic characteristics. As shown in Table 1, the mean $\pm$ SD age of the overall population was 27.5 ± 6.8 years. Almost half (47.9%) of the patients were female. Most groups were well balanced by sex, although women comprised 35.5 and 65.6% of the 18,000 TU and placebo groups, respectively (Table 1). Mean $\pm$ SD BMI was 24.46 ± 4.42 kg/m². Most patients were Caucasian (98.6%), had no pets (78.8%) and only 1.4% ($n = 2$) were smokers.

All patients had HDM allergic asthma and rhinitis symptoms (Table 1), which were present the whole year in 91.8 and 84.2% of patients, respectively. Most patients had conjunctivitis allergica (70.5%), whereas few patients (7.5%) reported atopic dermatitis; the five groups were well balanced with regard to allergic symptoms (Table 1). 11.6% ($n = 17$) of patients reported other allergic diseases, including allergies to animals and pollen.

While there was some variation between the groups regarding the dose of fluticasone needed to achieve asthma control at baseline, there were no statistically significant differences between the active groups versus the placebo group (Table 1). The 18,000 TU dose group tended to have higher total and HDM-specific IgE concentrations versus the placebo and other dose groups (Table 1). All patients had PEF or FEV₁ $>80\%$ of predicted normal (Table 1). All treatment groups were similar with respect to reactions to the different substances in the SPTs, including *D. pteronyssinus* and *D. farinae* (Supplementary Table 3).

Changes in wheal size

As shown in Figure 2 and Table 2, after SCIT there were statistically significant reductions in the swelling area of the LPR in all four-dose groups. Reductions were also seen in the EPR swelling area (secondary end point), with a statistically significant effect compared with placebo present for the 10,000 TU group (Table 2). The greatest reductions in the least square (LS) means of the swelling area were found in the 10,000 TU group (LPR - 10.83 cm²; EPR - 3.01 cm²) versus placebo (LPR + 6.41 cm², $p < 0.0001$; EPR - 0.79 cm², $p = 0.0063$).

Changes in asthma control

Over half (53.7%, $n = 73$) of the patients in the FAS already had asthma control without ICS before receiving SCIT and thus were not analyzed for asthma control end points.

Table 1. Patient characteristics at baseline in the dose range-finding phase – safety set.

Baseline characteristics	Placebo (n = 32)	2000 TU (n = 24)	6000 TU (n = 31)	10,000 TU (n = 28)	18,000 TU (n = 31)	Overall (n = 146)
Age, years:						
– Mean (SD)	28.3 (7.5)	25.4 (5.0)	27.5 (7.3)	27.9 (6.7)	28.0 (7.0)	27.5 (6.8)
– Min–Max	18–40	18–40	18–40	18–39	18–40	18–40
Sex, n (%): [†]						
– Female	21 (65.6)	12 (50.0)	14 (45.2)	12 (42.9)	11 (35.5%)	70 (47.9)
– Male	11 (34.4)	12 (50.0)	17 (54.8)	16 (57.1)	20 (64.5%)	76 (52.1)
Household pets, n (%):						
– At present	5 (15.6)	3 (12.5)	6 (19.4)	3 (10.7)	3 (9.7%)	20 (13.7)
– Formerly	3 (9.4)	2 (8.3)	3 (9.7)	1 (3.6)	2 (6.5%)	11 (7.5)
– No pets	24 (75.0)	19 (79.2)	22 (71.0)	24 (85.7)	26 (83.9%)	115 (78.8)
History of allergy symptoms, n (%):						
– Allergic asthma	32 (100.0)	24 (100.0)	31 (100.0)	28 (100.0)	31 (100.0)	146 (100.0)
– Rhinitis allergica	32 (100.0)	24 (100.0)	31 (100.0)	28 (100.0)	31 (100.0)	146 (100.0)
– Cough/sibilant rhonchi [‡]	24 (75.0)	19 (79.2)	24 (77.4)	19 (67.9)	26 (83.9)	112 (76.7)
– Conjunctivitis allergica [‡]	25 (78.1)	16 (66.7)	23 (74.2)	21 (75.0)	18 (58.1)	103 (70.5)
– Sneezing [‡]	21 (65.6)	17 (70.8)	22 (71.0)	19 (67.9)	24 (77.4)	103 (70.5)
– Atopic dermatitis [‡]	3 (9.4)	1 (4.2)	2 (6.5)	1 (3.6)	4 (12.9)	11 (7.5)
Fluticasone dose needed for asthma control, n (%): [‡]						
– 0 µg/day	17 (53.1)	12 (50.0)	17 (54.8)	19 (67.9)	14 (45.2)	79 (54.1)
– 200 µg/day	3 (9.4)	7 (29.2)	8 (25.8)	5 (17.9)	5 (16.1)	28 (19.2)
– 500 µg/day	12 (37.5)	5 (20.8)	6 (19.4)	4 (14.3)	12 (38.7)	39 (26.7)
Mean (SD) IgE, IU/ml [‡] :						
– <i>D. pteronyssinus</i>	21.1 (24.7)	22.79 (20.37)	28.8 (29.4)	20.7 (22.4)	33.4 (28.0)	25.6 (25.7)
– <i>D. farinae</i>	19.6 (22.6)	20.8 (18.6)	27.8 (26.4)	16.2 (17.9)	32.2 (29.1)	23.6 (24.1)
– Total	184.4 (195.5)	209.8 (168.8)	319.4 (459.4)	199.3 (163.6)	306.9 (417.9)	246.4 (318.4)
FEV ₁ in relation to predicted value, %:						
– Mean (SD) [‡]	99.7 (10.8)	98.4 (9.5)	98.7 (10.1)	100.5 (11.1)	96.9 (8.9)	98.9 (10.0)
– n (missing)	18 (14)	14 (10)	18 (13)	15 (13)	14 (17)	79 (67)
PEF in relation to predicted value, %:						
– Mean (SD) [‡]	98.1 (9.3)	102.4 (8.4)	97.7 (8.8)	97.4 (10.1)	97.0 (12.5)	98.3 (10.0)
– n (missing)	14 (18)	10 (14)	13 (18)	13 (15)	17 (14)	67 (79)

[†]When analyzed using Fisher's exact test, comparing active groups versus placebo, no statistically significant differences were found except in sex in the 18,000 TU versus placebo group (two-sided $p = 0.0237$).

[‡]When analyzed using Mann–Whitney U test, comparing active groups versus placebo, no statistically significant differences were found except in *D. pteronyssinus* concentration in the 18,000 TU versus placebo group (two-sided $p = 0.0398$).

D. farinae: *Dermatophagoides farinae*; *D. pteronyssinus*: *Dermatophagoides pteronyssinus*; DRF: Dose range finding; FEV₁: Forced expiratory volume in 1 s; Ig: Immunoglobulin; Max: Maximum; Min: Minimum; N: Number of patient in the group; n: Number of patient with data; PEF: Peak expiratory flow; SD: Standard deviation; TU: Therapeutic unit.

Table 2. Summary of all end points.

End point (change from baseline)	Placebo (n = 29)	2000 TU (n = 24)	6000 TU (n = 30)	10,000 TU (n = 25)	18,000 TU (n = 28)
LPR (cm ²)	-6.41	7.98	6.30	10.83	8.64
EPR (cm ²)	0.79	1.15	1.99	3.01	0.91
ICS* (reduction by two steps)	20%	25%	23%	25%	69%
PEF* (l/min)	4.10	-52.09	-41.99	-87.56	-22.49
ACT* score	-2.94	-5.01	-5.45	-4.03	-5.20
IgG ₄ <i>D. pter.</i> (mg/l)	-0.03	-1.07	-2.44	-2.16	-3.04
IgG <i>D. pter.</i> (mg/l)	-0.54	-4.02	-6.47	-7.56	-9.38

ACT: Asthma Control Test; *D. pter.*: *Dermatophagoides pteronyssinus*; EPR: Early-phase reaction; ICS: Inhaled corticosteroid; IgG: Immunoglobulin G; LPR: Late-phase reaction; N: Number of patient with data; PEF: Peak expiratory flow; TU: Therapeutic unit.

Figure 1. Flow of patients in the dose range finding phase.

AE: Adverse event; DRF: Dose range finding; FAS: Full analysis set; N: Number of patient; TU: Therapeutic unit.

With regard to change in the minimal ICS (fluticasone) dose required for asthma control, the greatest and only statistically significant decrease was in the highest dose group, 18,000 TU, with a mean $\pm$ SD of -376.9 ± 200.6 $\mu\text{g/day}$ ($n = 13$) versus -100.0 ± 352.5 $\mu\text{g/day}$ with placebo ($n = 15$; $p = 0.0217$). Similarly, as shown in Table 3, the percentage of patients who had better asthma control after SCIT, and therefore could reduce their dose of fluticasone, was highest in the 18,000 TU group versus placebo (69.2 vs 20.0%; $p = 0.0160$). As shown in Table 3 and Figure 3, 84.6% of patients reduced their fluticasone dose to 0 $\mu\text{g/day}$ in the 18,000 TU group versus 40.0% in the placebo group ($p = 0.0238$). However, while average ACT scores increased in all dose groups, the only statistically significant ($p = 0.0432$) and greatest increase was in the 6000 TU group (LS mean $\pm$ SE, 4.83 ± 1.22 , $n = 9$) versus placebo (1.77 ± 1.08 , $n = 13$).

Average morning PEF increased in all four-dose groups, reaching statistical significance ($p = 0.0165$) in the 10,000 TU group (LS mean $\pm$ SE, 87.56 ± 30.99 l/min, $n = 6$) versus placebo (-4.10 ± 20.90 l/min, $n = 13$). The LS means of morning PEF in relation to predicted value (%) also increased, by 3.51% in the placebo group, 8.91% in the 2000 TU group, 7.82% in the 6000 TU group, 12.71% in the 10,000 TU group and 4.29% in the 18,000 TU group, although no increases in the active groups were statistically significant versus placebo.

Changes in the concentrations of HDM-specific antibodies

As shown in Figure 4 and in Supplementary Table 5, statistically significant increases were detected in the concentrations of *D. pteronyssinus*- and *D. farinae*-specific IgG₄ concentrations in the active groups versus placebo,

Figure 2. Reduction in swelling area of the late-phase reaction in four dose groups. Changes in swelling area (cm²) of the (A) LPR and (B) EPR after SCIT – full analysis set. LPR was 6 h and EPR was 20 min after injection of the ICT solution. Two-sided analysis of covariance p-values were for comparisons between the active dose group and placebo. The bars show the least square means. The whiskers show the upper or lower limits of the 95% CIs. LPR: Late-phase reaction; n: Number of patient with data; SCIT: Subcutaneous allergen immunotherapy; TU: Therapeutic unit.

Table 3. Change of minimal asthma control dose after subcutaneous allergen immunotherapy – frequency table – full analysis set.

Reduction steps	Placebo (n = 29)		2000 TU (n = 24)		6000 TU (n = 30)		10,000 TU (n = 25)		18,000 TU (n = 28)	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Reduction by two steps [–2]	3/15	(20.0%)	3/12	(25.0%)	3/13	(23.1%)	2/8	(25.0%)	9/13	(69.2%)
Reduction by one step [–1]	6/15	(40.0%)	5/12	(41.7%)	8/13	(61.5%)	4/8	(50.0%)	2/13	(15.4%)
No change	3/15	(20.0%)	3/12	(25.0%)	2/13	(15.4%)	1/8	(12.5%)	2/13	(15.4%)
Increase by one step [+1]	3/15	(20.0%)	1/12	(8.3%)	0/13	(0.0%)	1/8	(12.5%)	0/13	(0.0%)
p-value [†]			0.5693		0.2129		0.6737		0.0160	
Reduction to 0 µg/day after AIT	6/15	(40.0%)	7/12	(58.3%)	10/13	(76.9%)	6/8	(75.0%)	11/13	(84.6%)
p-value [‡]			0.4495		0.0671		0.1930		0.0238	

Patients who were controlled without fluticasone before treatment and patients who were without any asthma control assessment post-treatment were excluded from the table.

[†]p-value from exact two-sided Mantel–Haenszel χ^2 test comparing the active dose group against placebo.

[‡]p-value from exact two-sided χ^2 test comparing the active dose group against placebo.

FAS: Full analysis set; N: Number of patient in the group; n/N: Number of patient with the applicable change of asthma control dose relative to the number of patient with evaluable change of asthma control dose; SCIT: Subcutaneous allergen immunotherapy; TU: Therapeutic unit.

except for a nonsignificant increase in *D. farinae*-specific IgG₄ in the 2000 TU group ($p = 0.0654$). Dose–response relationships were observed with both IgG₄ antibodies; the greatest increases were detected at 18,000 TU versus placebo ($p < 0.0001$, for both HDMs). Dose-dependent and statistically significant increases were also observed in

Figure 3. Percentages of patients without need for inhaled corticosteroids after SCIT. Percentages and numbers of patients without need for ICS after subcutaneous allergen immunotherapy – FAS. p-values are from the exact two-sided χ^2 test comparing the active dose group against placebo. FAS: Full analysis set; ICS: Inhaled corticosteroid; N: Number of patients in the group; n: Number of patients with data; TU: Therapeutic unit.

D. pteronyssinus- and *D. farinae*-specific IgG concentrations in all four active groups versus placebo. No clinically meaningful changes were detected in *D. pteronyssinus*- and *D. farinae*- specific IgE concentrations.

Safety

In total, 178 TEAEs were reported by 48.6% (n = 71) of patients, including 71 trial medication-related TEAEs reported by 18.5% (n = 27) of patients. Except for local reactions, all doses were comparable to placebo with respect to occurrence of trial medication-related TEAEs (Table 4). More patients in the 18,000 TU dose group (n = 10) experienced trial medication-related local reactions versus the 10,000 TU (n = 4), 6000 TU (n = 6), 2000 TU (n = 4) and placebo groups (n = 0; Table 4), although all these reactions resolved, were mild or moderate in intensity and were not treatment-limiting.

Only one patient discontinued treatment due to a TEAE (ulcerative colitis; Figure 1). This patient was in the placebo group, and the TEAE was nonserious and not related to trial medication.

Six patients reported a treatment-related systemic reaction, two patients each in the placebo, 6000 TU and 18,000 TU groups: one anaphylactic reaction (WAO grade 2) in the 18,000 TU group and five hypersensitivity reactions (WAO grade 1) including cough, AR and allergic conjunctivitis symptoms. All systemic reactions were mild or moderate in intensity. One adult male patient (aged 30 years) experienced an anaphylactic reaction involving symptoms of urticaria, dyspnea and weakness during the early maintenance phase following the third injection of 18,000 TU. The event was treated with compress-ice pack and oral doses of antihistamine, inhaled β -agonist and intramuscular corticosteroids and resolved completely the same day. The patient completed the trial with a dose reduction to once 6000 TU and 10,000 TU and three further injections of 18,000 TU without any new local or systemic reaction. This anaphylactic reaction was the only serious TEAE related to trial medication. Five other serious TEAEs, not related to treatment, were reported for four patients (postoperative hernia and appendicitis in the placebo group, asthma exacerbation in the 2000 TU group and varicose vein and umbilical hernia in one patient in the 18,000 TU group).

Laboratory measurements, vital signs and lung function tests did not reveal relevant differences between the different dose and placebo groups (data not shown).

Figure 4. Changes in concentrations of allergen-specific IgG₄. Changes in concentrations (mg/l) of IgG₄ specific to (A) *D. Pteronyssinus* and (B) *D. farinae* after SCIT – FAS. Two-sided ANCOVA p-values were for comparisons between the active dose group and placebo. The bars show the LS means. The whiskers show the upper or lower limits of the 95% CIs. *D. pteronyssinus*: *Dermatophagoides pteronyssinus*; *D. farinae*: *Dermatophagoides farinae*; FAS: Full analysis set; n: Number of patient with data; SCIT: Subcutaneous allergen immunotherapy; TU: Therapeutic unit.

Subgroup analysis

A subgroup analysis was performed for the primary end point in asthma patients planned to include in the following confirmatory Phase III trial. In this DRF patients with mild asthma without requirement of perennial ICS were included as well. Therefore, those patients have been excluded in the subgroup analysis of the primary end point. According to this subgroup analysis the biggest effect has been found for the 18,000 TU dose group for the primary end point and confirmed the result of the clinical end point (fluticasone dose reduction) chosen for the confirmatory trial as presented in Tables 2 and 5.

Discussion

According to the primary end point the change of the swelling area 6 h after ICT injection, 10,000 TU shows the highest statistically significant effect after SCIT for adults with HDM-allergic bronchial asthma and rhinitis. The

Table 4. Treatment-emergent adverse events related to trial medication – safety set.

System organ class preferred term	Placebo (n = 32)		2000 TU (n = 24)		6000 TU (n = 31)		10,000 TU (n = 28)		18,000 TU (n = 31)	
	n (%)	E	n (%)	E	n (%)	E	n (%)	E	n (%)	E
Overall	2 (6.3)	2	4 (16.7)	7	6 (19.4)	13	4 (14.3)	19	11 (35.5)	30
General disorders & administration site conditions	0	–	4 (16.7)	7	6 (19.4)	11	4 (14.3)	18	10 (32.3)	26
Injection site erythema	0	–	1 (4.2)	1	3 (9.7)	6	4 (14.3)	13	3 (9.7)	8
Injection site edema	0	–	1 (4.2)	2	0	–	1 (3.6)	2	3 (9.7)	5
Injection site pain	0	–	0	0	0	–	0	–	1 (3.2)	1
Injection site papule	0	–	1 (4.2)	1	0	–	0	–	0	–
Injection site pruritus	0	–	1 (4.2)	1	1 (3.2)	2	0	–	2 (6.5)	2
Injection site swelling	0	–	2 (8.3)	2	2 (6.5)	3	1 (3.6)	3	5 (16.1)	10
Vascular disorders	0	–	0	–	0	–	0	–	1 (3.2)	1
Hypertension	0	–	0	–	0	–	0	–	1 (3.2)	1
Immune system disorders	2 (6.3)	2	0	–	2 (6.5)	2	0	–	2 (6.5)	2
Hypersensitivity	2 (6.3)	2	0	–	2 (6.5)	2	0	–	1 (3.2)	1
Anaphylactic reaction	0	–	0	–	0	–	0	–	1 (3.2)	1
Investigations	0	–	0	–	0	–	0	–	1 (3.2)	1
Heart rate increased	0	–	0	–	0	–	0	–	1 (3.2)	1
Cardiac disorders	0	–	0	–	0	–	1 (3.6)	1	0	–
Palpitations	0	–	0	–	0	–	1 (3.6)	1	0	–

E: Number of TEAE; N: Number of patient in the group; n: Number of patient with TEAE; TEAE: Treatment-emergent adverse event; TU: Therapeutic unit.

Table 5. Changes in intracutaneous test late-phase reactivity for the subgroup analysis population eligible for the clinical end point inhaled corticosteroid-reduction.

Intracutaneous test late-phase reactivity in cm ²	Placebo (n = 15)	2000 TU (n = 12)	6000 TU (n = 13)	10,000 TU (n = 8)	18,000 PNU (n = 13)
LPR cm ²	-8.16	6.36	6.40	8.66	9.91
p-value		0.0065	0.0050	0.0047	0.0004

Two-sided ANCOVA p-values were for comparisons between the active dose group and placebo.

LPF: Late-phase reaction; n: Number of patient in the group; TU: Therapeutic unit.

10,000 TU dose was associated with the greatest reduction in the LPR swelling area compared with placebo, which was statistically significant ($p < 0.0001$). The Secondary end point analyses of EPR swelling area, and an increase in morning PEF supported the result of the primary end point.

But the clinical end point, change in the minimal ICS dose required for asthma control after SCIT, is in favor of 18,000 TU. The 18,000 TU dose demonstrated the highest efficacy for ICS reduction, as shown by the percentage of patients with fluticasone dose reduction (by two steps, 69.2 vs 20.0% with placebo) and the percentage of patients without need for ICS after SCIT (84.6 vs 40.0% with placebo). Clinical efficacy was supported by induction of the highest concentrations of *D. pteronyssinus*- and *D. farinae*-specific IgG₄ in the 18,000 TU group compared with all other doses ($p < 0.0001$ vs placebo for both antibodies). Further the subgroup analysis for the primary end point with patients of at least moderate asthma defined according to their requirement of ICS supports these findings.

Double-blind, placebo-controlled, DRF trials of SCITs have been published in a limited number over the last decade. Thus, the optimal doses are not well established [16]. The dose-dependent efficacy in the current double-blind, placebo-controlled, randomized DRF trial is comparable to previous findings in DRF trials of other SCIT preparations for HDM allergy. In one of the first HDM DRF trials, published in 1993, dose-dependent reductions in medication (such as ICS) and PEF scores were detected in adults with asthma treated with allergen extracts of *D. pteronyssinus* for 24 months [17]. In a recently published 12-month trial, dose-dependent reductions in nasal reactivity and increases in *D. pteronyssinus*-specific IgG and IgG₄ were detected in adults with allergic rhinoconjunctivitis treated with a mixed SCIT preparation of *D. pteronyssinus* and *D. farinae* [18].

According to the European Medicines Agency (EMA) and the Position Paper by the European Academy of Allergy and Clinical Immunology, provocation tests and/or clinical parameters can be used as primary end points in DRF trials [2,19]. Evidence from various randomized, controlled trials suggest that particularly the LPR of the ICT can resemble the responses of clinical end points to AIT [20–23]. Correlation of the ICT LPR with rhinoconjunctivitis symptom scores and medication scores has been reported for grass pollen AIT [24]. The EMA encourages the simultaneous use of different end points, which may not correlate with each other, to assess the effects of treatment on asthma, a multidimensional disease [25]. It is a strength of the current trial that several different end points were explored to identify an optimal dose. It is also an advantage that a new photographic method was used to objectively measure the LPR, as the results of LPR assessments are usually subject to high inter- and intrapersonal variations [13]. However, the current trial does have potential limitations. The male:female ratios were different in the placebo versus 18,000 TU group. Sensitization to *D. pteronyssinus* may differ by sex [26]. However, in general, the groups were well balanced with regards to allergy-related parameters that could affect the outcomes, such as history of allergy symptoms and HDM-specific IgE concentrations. In addition, the trial was limited by sample size, which was not planned to show statistically significant differences between the different active doses for the efficacy end points. In particular, the steroid sparing effect of AIT was investigated in a subset of 63 patients who had asthma control at baseline when treated with fluticasone (i.e., 73 patients without need of fluticasone for asthma control at baseline were excluded from this analysis). The results of the current trial will be utilized for the planning of a larger pivotal trial, to gather more information on the benefit-risk ratio and to find the optimal vaccine dose for the treatment of patients with asthma, rhinitis or rhinoconjunctivitis induced by HDM exposure.

The steroid sparing effect of SCIT will be used as the end point in the subsequent pivotal trial. The EMA recommends the investigation of the steroid sparing effect as a possible primary end point for Phase III studies to evaluate the efficacy of AIT in patients with allergic bronchial asthma [25]. Reduction of ICS while maintaining asthma control as the end point for AIT is based on the concept of disease control recommended by asthma guidelines [27,28]. The steroid sparing effect of SCIT and sublingual immunotherapy (SLIT) has been investigated in a number of double-blind, placebo-controlled trials [29–31] in adults as well as in children in an open-label trial [9].

Although the biggest efficacy for most of the end points was observed at 10,000 TU and 18,000 TU in the current study, efficacy could also be demonstrated at 6000 TU. In the 6000 TU group versus placebo, reductions in LPR swelling area reached statistical significance ($p < 0.05$), as did induction of *D. pteronyssinus*- and *D. farinae*-specific IgG ($p = 0.0002$ in both instances) and IgG₄ concentrations ($p < 0.0001$ in both instances) and improvements in asthma control as shown using the ACT questionnaire ($p = 0.0432$). These results are comparable to efficacy outcomes in previous trials of the same SCIT using 6000 TU as the maintenance dose, including reductions in skin and nasal reactivity in adults with rhinoconjunctivitis during a 2-year double-blind, placebo-controlled phase [32] and the steroid sparing effect in the aforementioned open-label trial following 2 years of SCIT in children with asthma [9]. These beneficial clinical effects were accompanied by the induction of *D. pteronyssinus*-specific IgG₄ levels [9,32].

All vaccine doses tested in this trial were well tolerated. Except for mild or moderate local reactions, which were more frequent at higher doses, all doses were comparable to placebo with respect to occurrence of trial medication-related TEAEs. Systemic reactions were rare and observed with similar incidence in the active and placebo groups. Laboratory measurements, vital signs and lung function tests did not reveal relevant differences between the different dose and placebo groups.

Conclusion

Considering all efficacy end points, including objective assessment of the ICT-induced LPR with a new photographic technique and the safety profile of all treatment groups, the HDM allergoid at the dose of 6000 TU and above has been shown to be safe and efficacious. However, the optimal dose might be even higher. Taking into account the subgroup analysis of the patients with at least moderate asthma the 18,000 TU group showed the highest reduction in the intracutaneous LPR and ICS reduction. These efficacy results of the dose of 18,000 TU was supported by the highest serum specific antibody responses. Compared with the lower doses the 18,000 TU showed more local reactions, which were still within the normal range for SCIT. Taking into account, that patients with at least moderate asthma, are in the focus of the development program in the indication of asthma, 18,000 TU will be further investigated in a confirmatory trial to gather more information on the benefit–risk ratio of this treatment.

Summary points

- Subcutaneous allergen immunotherapy (SCIT) with four increasing doses of a *Dermatophagoides pteronyssinus* allergoid was investigated in a multicenter randomized placebo-controlled double-blind clinical trial.
- The primary end point for efficacy was the change in late-phase response measured 6 h after intracutaneous injection of allergen assessed at baseline and after SCIT.
- Major secondary end point was the change of the minimal inhaled corticosteroid dose necessary for asthma control assessed at baseline and after SCIT.
- All four active groups showed a statistically significant reduction of the late-phase response after intracutaneous testing as compared with *placebo*.
- The highest dose shows the best efficacy for the inhaled corticosteroid reduction and most other end points compared with lower doses.
- Treatment was shown to be safe at all doses tested.
- The highest dose of 18,000 TU will further be investigated to treat allergic asthma induced by house dust mites.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: <https://www.futuremedicine.com/doi/suppl/10.2217/imt-2018-0087>

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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