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ORIGINAL ARTICLE

Allergen-Specific Immunotherapy and Biologics

First-in-human phase 2 trial with mite allergoids coupled to mannan in subcutaneous and sublingual immunotherapy

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Abstract

Background: Polymerized allergens conjugated to non-oxidized mannan (PM-allergoids) are novel vaccines targeting dendritic cells (DCs). Previous experimental data indicate that PM-allergoids are readily taken up by DCs and induce Treg cells. This first-in-human study was aimed to evaluate safety and to find the optimal dose of house dust mite PM-allergoid (PM-HDM) administered subcutaneously (SC) or sublingually (SL).

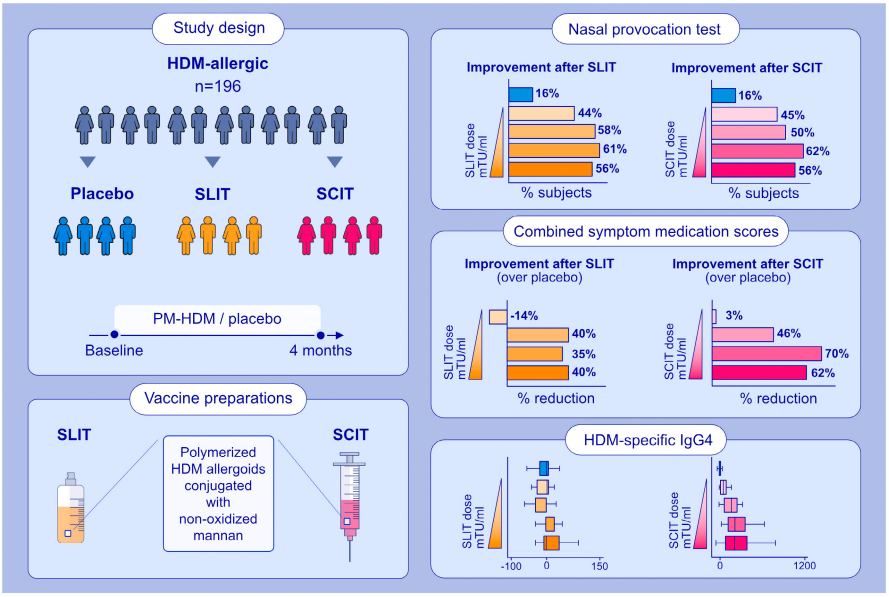
Abbreviations: AEMPS, Agencia Española del Medicamento y Productos Sanitarios; AIT, Allergen-specific ImmunoTherapy; ASCA, Anti-*Saccharomyces cerevisiae* Antibodies; AUC, Area Under the Curve; CSMS, Combined Symptoms and Medication Score; Df, *Dermatophagoides farinae*; Dpt, *Dermatophagoides pteronyssinus*; dMC, daily Medication Score; dSC, daily Symptom Score; EAACI, European Academy of Allergy and Clinical Immunology; EMA, European Medicines Agency; HDM, House Dust Mites; HEP, Histamine Equivalent Prick-test; IMP, Investigational Medicinal Product; NPT, titrated Nasal Provocation Tests; mTU, mannan-conjugated Therapeutic Units; PM, Polymerized allergens conjugated with Mannan; PM-HDM, PM-allergoids of HDM; SC, SubCutaneous; SCIT, SubCutaneous ImmunoTherapy; SD, Standard Deviation; SL, SubLingual; SLIT, SubLingual ImmunoTherapy.

Methods: In a randomized, double-blind, double-dummy, placebo-controlled trial, 196 subjects received placebo or PM-HDM at 500, 1000, 3000, or 5000 mannan-conjugated therapeutic units (mTU)/mL in 9-arm groups for 4 months. All subjects received 5 SC doses (0.5 ml each) every 30 days plus 0.2 ml SL daily. The primary efficacy outcome was the improvement of titrated nasal provocation tests (NPT) with *D. pteronyssinus* at baseline and at the end of the study. All adverse events and reactions were recorded and assessed. Secondary outcomes were the combination of symptom and medication scores (CSMS) and serological markers.

Results: No moderate or severe adverse reactions were reported. Subjects improving the NPT after treatment ranged from 45% to 62% in active SC, 44% to 61% in active SL and 16% in placebo groups. Statistical differences between placebo and active groups were all significant above 500 mTU, being the highest with 3000 mTU SL ($p = 0.004$) and 5000 mTU SC ($p = 0.011$). CSMS improvement over placebo reached 70% ($p < 0.001$) in active 3000 mTU SC and 40% ($p = 0.015$) in 5000 mTU SL groups.

Conclusions: PM-HDM immunotherapy was safe and successful in achieving primary and secondary clinical outcomes in SC and SL at either 3000 or 5000 mTU/ml.

KEYWORDS
allergoid, clinical trial, i, mmunotherapy, mannan, polymerized



GRAPHICAL ABSTRACT
This first-in-human study evaluates safety and optimal dose of PM-HDM in SCIT and SLIT. PM-HDM is safe and shows dose-dependent clinical efficacy outcomes in SCIT and SLIT. A dose-response in specific IgG4 to HDM is observed in SCIT, but not SLIT.
Abbreviations: AIT, allergy immunotherapy; PM-HDM, polymerized house dust mite allergoids conjugated with mannan; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy

1 | INTRODUCTION

Allergen-specific immunotherapy (AIT) has been shown to be effective in relieving symptoms, reducing medication use, and improving quality of life in patients with respiratory allergies.[1–3] This is thought to be due to the induction of a state of tolerance to specific allergens with long-lasting effects after discontinuation of treatment.[4,5]

Since Noon and Freeman's first description,[6] different ways to improve both safety and efficacy of AIT have been continuously sought. To this end, the modification of allergens to reduce their reactivity with IgE, the use of safer routes of administration, and the incorporation of different adjuvants to slow allergen release and/or stimulate a desired immune response have been considered.[7,8] In terms of safety, chemical modification of allergens (allergoids) can

reduce their allergenicity while sublingual immunotherapy (SLIT) may be a safer alternative than subcutaneous immunotherapy (SCIT).[9] On the contrary, immunomodulatory adjuvants can promote T cells to counteract pro-allergic Th2 responses and/or induce peripheral T cell tolerance to allergens, which is considered a key step for successful AIT.[10–12]

Chemical polymerization of allergens with glutaraldehyde is a well-established method to obtain hypoallergenic preparations.[9,13] Taking advantage of this agent, polymerized allergens conjugated to non-oxidized mannan (PM-allergoids) were obtained.[14] In addition of being hypoallergenic,[14,15] PM-allergoids target dendritic cells by binding to C-type lectin receptors through their mannan moiety, which are rapidly and efficiently taken up by these cells.[16,17] Preclinical data indicate that PM-allergoids induce tolerogenic dendritic cells and macrophages capable of promoting Treg-cell responses in vitro[15,18–20] and in vivo, whether administered subcutaneously (SC)[15,20] or sublingually (SL),[17] thus, with promising potential for AIT.[21]

A pilot study in veterinary medicine indicated that SC immunotherapy with PM-allergoids (*D. farinae*) was safe and successful in the treatment of canine atopic dermatitis.[22]

Here, we show the first-in-human trial with a PM-allergoid vaccine of house-dust mites (PM-HDM) aimed to search for the best concentration by dose escalation. Improvement in titrated nasal provocation tests (NPT) was used as the primary outcome. Both SC and SL routes were addressed using a double-dummy design.

2 | METHODS

2.1 | Trial design and ethics

The clinical trial was conducted in 13 Allergy Services located on the Mediterranean coast of Spain, a geographic area with a high prevalence of allergy to HDM.[23] It was a Phase 2 prospective, randomized, double-blind, placebo-controlled, double-dummy study with 9-arms aimed at finding the best dose in terms of safety and efficacy. Figure 1 shows the distribution of subject groups and the trial scheme.

The study was conducted in accordance with the Declaration of Helsinki[24] and the ICH Guideline on Good Clinical Practice.[25] It was approved by Ethics Committee of the Hospital La Fe (Valencia, Spain) and the Spanish Regulatory Authorities (AEMPS). All patients provided written informed consent. The trial was registered in EudraCT (2015–000820-27) and in ClinicalTrials.gov (NCT02661854).

2.2 | Sample size and subject population

Sample size was calculated based on the assumption that 15% of subjects in the placebo group and 60% of each group receiving active treatment will experience improvement. Assuming an alpha error of 0.05 and a power of 0.80, the number of subjects was 17 per group. Assuming dropouts, eligible subjects were allocated using a list generated by Random software in blocks of 20 patients, with 9 different treatments and stratified by center.

Two hundred and sixteen subjects were enrolled in the study, 196 initiated the treatment (107 males and 89 females) and received, at least, one dose of treatment (intention to treat population -ITT-). From these, 161 were evaluable for nasal provocation test (NPT) at baseline and at the end of the study (per protocol population—PP). The age ranged from 12 to 62 years. The CONSORT flow diagram is shown in Figure 2. The demographic characteristics of subjects are shown in Table 1 and Tables S1 and S2 (online supporting information).

All subjects had rhinitis/rhinoconjunctivitis, induced by allergic sensitization to *Dermatophagoides pteronyssinus* (Dpt) and *Dermatophagoides farinae* (Df). All subjects had positive skin reactions (wheal size >6 mm diameter) to Dpt and Df skin prick tests (Inmunotek, Alcalá de Henares, Spain) and specific IgE to HDM >10 kU/L (ImmunoCAP, Thermo-Fisher Scientific, Waltham, Massachusetts, USA). Subjects sensitized to pollens were allowed

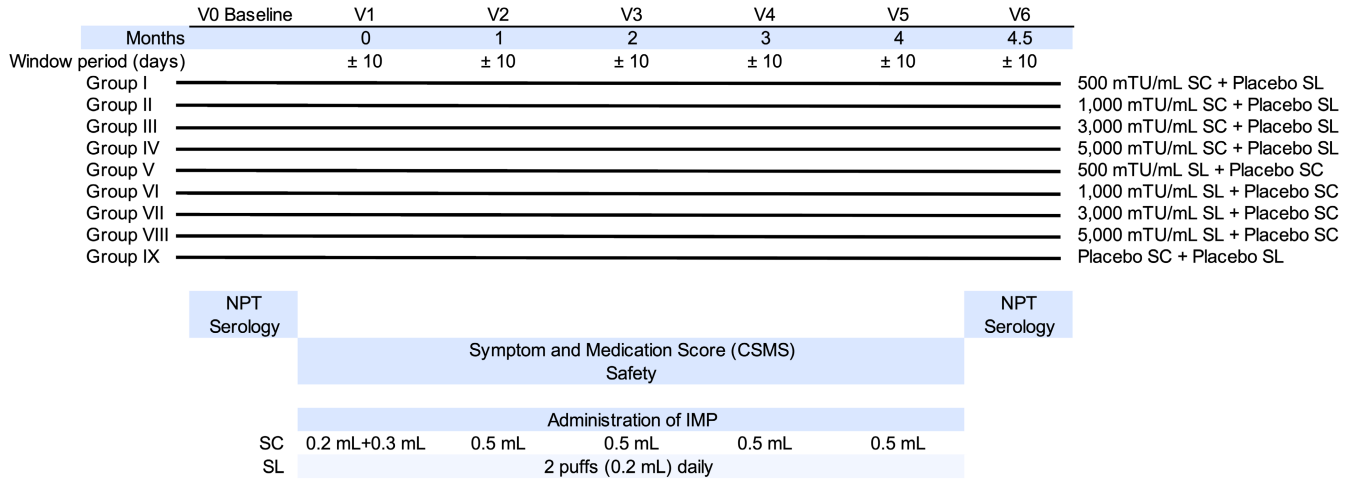


FIGURE 1 Distribution of groups and study schedule

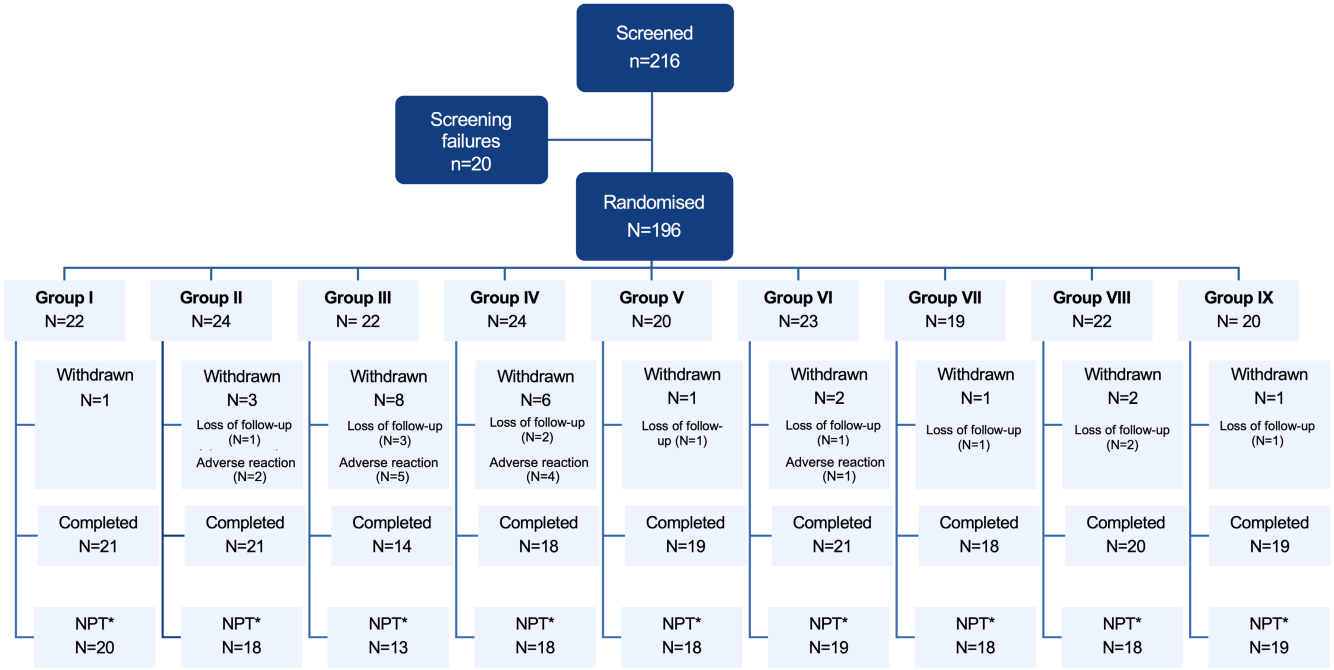


FIGURE 2 Consort diagram of the study population. NPT*: number of subjects with data of NPT al baseline (VO) and at the end (V6)

to be included if the pollen season did not coincide with the study period, while subjects with positive skin tests to perennial allergens other than mites were allowed if specific IgE was <0.7 kU/L. Table 1 shows the characteristics of these subjects in each group.

2.3 | Treatments and schedules

The investigational medicinal product was PM-HDM (Inmunotek, Alcalá de Henares, Spain), which contains a 50% mixture of Dpt and Df polymerized allergoids conjugated with non-oxidized mannan derived from *Saccharomyces cerevisiae* as described.[14]

The concentrations used were 500, 1000, 3000, and 5000 mTU (mannan-conjugated Therapeutic Units)/mL, which corresponded to 0.8, 1.6, 5.0, and 8.3 μg/ml, respectively, of group 1 allergens (Der p 1 and Der f 1) extrapolated from the corresponding native extracts. Der p 1 and Der f 1 in the final product (PM-HDM) were confirmed by mass spectrometry.

Human serum albumin, sodium chloride, phenol, and water for injection were the excipients for active and placebo SC preparations. Glycerol, sodium chloride, artificial pineapple flavor, and water for injection were the excipients for active and placebo SL preparations.

The duration of the treatment was 4 months (Figure 1). For SC, a cluster administration (0.2 plus 0.3 ml in alternate arms separated by 30 minutes) was used the first day, followed by a monthly dose of 0.5 ml administered in the study center. SL preparations were delivered as a spray (two puffs of 0.1 ml each, daily under the tongue) outside of meals and held without swallowing for at least 1 min. The first dose was administered under supervision, and subsequent doses were self-administered at home. To assess the degree of compliance with the test medication, the volume of liquid remaining in the bottles at the end of treatment was measured.

2.4 | Outcome measures

2.4.1 | Primary outcome

Titrated specific nasal provocation test (NPT), defined as the threshold concentration of native Dpt allergen extract required to trigger a positive nasal response measured by acoustic rhinometry (Optomic, Madrid, Spain), was used as the primary outcome.

NPT was performed in an asymptomatic phase of the patient's disease at baseline (V0) and at the end (V6) of the study. It was performed according to the guidance of the Spanish Society of Allergy and Clinical Immunology.[26] The test was considered positive when the nasal cavity volume between 2 cm and 6 cm had a minimum variation of 25%.[26] The test was not performed if any of the following were present: (i) use of medication that could affect the test parameters (oral, or topical antihistamines, steroids, or antidepressants with antiallergic properties), (ii) presence of unstable asthma (peak-flow rate below 20% of normal values), (iii) sign or symptoms of allergic, viral, or infectious rhinitis 2 weeks before the nasal challenge tests, and (iv) positive reaction with the negative control (saline solution).

NPT were performed following standard procedures.[27] Once the solutions to be tested were tempered, nasal challenges were initiated using first the negative control (saline), followed by increasing concentrations (0.3, 1.0, and 3.0 HEP/ml) of Dpt NPT (Inmunotek) until a positive response was obtained. A subject was considered to improve when the concentration required to produce a positive NPT at V6 was at least one concentration higher than at V0.

TABLE 1 Characteristics of participants

(a) Subjects receiving active treatment by SC route						
Subcutaneous		**Placebo**	**500 mTU/ml**	**1000 mTU/ml**	**3000 mTU/ml**	**5000 mTU/ml**
n		19	22	21	14	18
Age	Median (Q1, Q3)	26 (17, 41)	22 (14, 32)	25 (15, 31)	21 (16, 28)	28 (17, 36)
	Age *p*-value[a]		0.236	0.460	0.274	0.695
Gender						
Female	*n* (%)	10 (52.6%)	12 (54.5%)	14 (66.7%)	4 (28.6%)	8 (44.4%)
Male	*n* (%)	9 (47.4%)	10 (45.5%)	7 (33.3%)	10 (71.4%)	10 (55.6%)
	p-value[b]		0.903	0.366	0.167	0.619
Sensitization status						
HDM monosensitized	*n* (%)	10 (52.6%)	6 (27.3%)	4 (19.0%)	5 (35.7%)	4 (22.2%)
With other sensitizations	*n* (%)	9 (47.4%)	16 (72.7%)	17 (81.0%)	9 (64.3%)	14 (77.8%)
	p-value[b]		0.097	0.026	0.335	0.057
(b) Subjects receiving active treatment by SL route						
Sublingual		**Placebo**	**500 mTU/ml**	**1000 mTU/ml**	**3000 mTU/ml**	**5000 mTU/ml**
n		19	19	21	18	20
Age	Median (Q1, Q3)	26 (17, 41)	27 (14, 36)	25 (13, 35)	32 (18, 38)	24 (18, 33)
	Age *p*-value[a]		0.750	0.341	0.892	0.645
Gender						
Female	*n* (%)	10 (52.6%)	10 (52.6%)	10 (47.6%)	13 (72.2%)	12 (60.0%)
Male	*n* (%)	9 (47.4%)	9 (47.4%)	11 (52.4%)	5 (27.8%)	8 (40.0%)
	p-value[b]		1.000	0.752	0.219	0.643
Sensitization status						
HDM monosensitized	*n* (%)	10 (52.6%)	8 (42.1%)	7 (33.3%)	5 (27.8%)	9 (45.0%)
With other sensitizations	*n* (%)	9 (47.4%)	11 (57.9%)	14 (66.7%)	13 (72.2%)	11 (55.0%)
	p-value[b]		0.516	0.217	0.124	0.634

[a]Mann–Whitney test vs Placebo.

[b]Chi-square test vs. Placebo.

2.4.2 | Secondary outcomes

Combined symptoms and medication scores (CSMS) and immunogenicity were considered secondary outcomes.

CSMS

Nasal/ocular symptoms and medication intake were recorded daily on a diary card for each patient and evaluated at each hospital visit. Nasal/ocular symptoms were itchy nose, nasal congestion, runny nose, sneezing, and itchy/red eyes. Each symptom was scored on a Likert scale from 0 to 3: 0 (no symptoms), 1 (mild; symptoms present but not bothersome), 2 (moderate; annoying symptoms but not disabling or intolerable), and 3 (severe; disabling and/or intolerable symptoms).[28] The mean daily symptom score (dSS) was the sum of all individual scores divided by the number of symptoms. The daily nasal/ocular medication score (dMS) was on a scale of 0 to 3. 0 value: no medication use; 1 value: use of oral and/or topical (eyes and/or nose) non-sedating antihistamines; 2 value: use of intranasal corticosteroids with/without non-sedating antihistamines; and 3 value: use of oral corticosteroids with/without intranasal corticosteroids.[29,30]

Daily CSMS was the sum of dSS and dMS, as recommended by World Allergy Organization[29] and EAACI.[30] The results were expressed as the area under the curve (AUC) of the CSMS during the entire study period (4 months). The score obtained in each group was compared with the score obtained in the placebo group. The percentage of reduction of CSMS of each group related to placebo was calculated as 100 – (CSMS of each group/CSMS of placebo group).

Immunogenicity

Serological determinations were performed at V0 and at V6. Total IgE and specific IgE for Dpt and Df (Immulite® 2000 XPi, Siemens, Germany), as well as specific IgG and IgG4 antibodies for each mite (UniCAP® 250, Thermo Fisher, Spain) were measured. Anti-*S. cerevisiae* antibodies (ASCAs), IgG and IgA, were also determined (Alegria® Orgentec, Palex Medical, Spain).

TABLE 2 Titrated Nasal Provocation Test results (primary outcome)

mTU/ml	Route of administration of the active treatment	Number of subjects	Number of subjects with improvement	Number of subjects without improvement	% of subjects that improved	*p*-Value	Effect size[c]	Interpretation effect size
Placebo		19	3	16	16%			
500	SC	20	9	11	45%	0.048[a]	-0.316	Moderate
500	SL	18	8	10	44%	0.057[a]	-0.314	Moderate
1000	SC	18	9	9	50%	0.026[a]	-0.365	Moderate
1000	SL	19	11	8	58%	0.007[a]	-0.436	Relatively strong
3000	SC	13	8	5	62%	0.020[b]	-0.473	Relatively strong
3000	SL	18	11	7	61%	0.004[a]	-0.467	Relatively strong
5000	SC	18	10	8	56%	0.011[a]	-0.416	Relatively strong
5000	SL	18	10	8	56%	0.011[a]	-0.416	Relatively strong

[a]Chi-square test vs. Placebo.
[b]Fisher's exact test vs. Placebo.
[c]Phi coefficient.

2.5 | Safety

Safety was assessed throughout the study by recording all adverse events and all adverse reactions. These were classified as immediate when the onset was during the first 30 minutes after the administration and delayed afterward.[31] Local SC reactions were quantified by measuring the diameter of the induration. Immediate SC reactions with a diameter less than 5 cm and delayed reactions less than 10 cm were considered clinically irrelevant.[32] Systemic reactions were graded according to the EAACI Position Paper.[31]

2.6 | Statistical methods

Statistical analyses were performed with SAS v9.4 software (Cary, North Caroline, USA). Appropriate parametric and nonparametric tests were performed for all variables.

The per-protocol population were the 161 subjects who received all doses and completed the study without any major protocol deviations and were used for primary outcome assessment. The intention-to-treat (ITT) analysis included the 196 subjects who received at least one dose of active treatment or placebo. ITT was used for the safety assessment and for comparative analysis of secondary outcomes. Summary statistics are shown as frequency (%) for categorical data and median with corresponding interquartile range (Q1 and Q3) or mean ± standard deviation (SD) or 95% confidence interval (CI) for continuous data, according to the normal distribution analyzed by Shapiro–Wilk test. Chi-square or Fisher's exact tests were used to analyze the number of subjects who improved in the primary outcome and Phi Coefficient was calculated to assess and to interpret the effect size.[33,34] For comparative statistics, nonparametric tests were used (Mann–Whitney U-test for unpaired data, Wilcoxon test for paired data). Estimate of location shift (Hodges–Lehmann) was calculated for the differences of CSMS between each group and placebo. The threshold for statistical significance was set at a $p < 0.05$.

3 | RESULTS

3.1 | Primary endpoint

Table 2 and Figures S1 and S2 show the number of subjects who improved in NPT at the end of study in each treatment group and the comparison of each group with placebo. Most active groups experienced a significant ($p < 0.05$) improvement in NPT as compared to placebo. The best outcome was recorded with 3000 mTU/ml in both the SL group (61%; $p = 0.020$) and SC group (62%; $p = 0.004$). No better figures were obtained by increasing to 5000 mTU/ml in either SL or SC groups (56%; $p = 0.011$, in both cases). The lowest concentration (500 mTU/ml) also increased the number of subjects who improved without reaching significance with respect to the placebo in SL group (44%; $p = 0.057$). The effect size was scored as "relatively strong"[33,34] for the 1000 (SL), 3000 (SC and SL), and 5000 (SC and

SL) mTU/ml. Pairwise comparison of NPT (Chi-square/Fisher's exact test) between each group of subjects receiving SC or SL active treatment was non-significant (not shown).

3.2 | Secondary endpoints

3.2.1 | CSMS

Table 3 and Figures S3 and S4 show the mean daily AUC values and the comparison of each group with placebo. Subjects receiving 500 mTU/ml (SL or SC) did not show significant differences versus placebo. The greatest differences versus placebo were found in the SC groups with 3000 and 5000 mTU/ml ($p = 0.001$), with a mean reduction over placebo of 70% and 62%, respectively. For the SL groups, the greatest difference was found with 5000 mTU/ml ($p = 0.015$), with a mean reduction over placebo of 40%. The estimate of location shift (Hodges–Lehman) was relevant for all concentrations above 500 mTU/ml. Pairwise comparison of CSMS (Mann–Whitney test, Tables S7 and S8) shows that, besides significant differences between placebo and each active SC and SL group, there are differences in subjects receiving SC active treatment between 500 mTU/ml and 3000 and 5000 mTU/ml.

3.2.2 | Immunogenicity

At baseline, there were no significant differences in serum levels of specific IgE, IgG, and IgG4 for Dpt and Df between subjects in the active and placebo groups (Tables S5, S6, and S11). At the end of the study (V6), the levels of specific IgE remained without significant variations, except in the SC 1000 mTU/ml group, which experienced a slight increase for Dpt and Df. In contrast, specific IgG4 increased steadily in all active SC groups reaching above eightfold at V6 in the 3000 mTU/mL group (Table S6). As can be seen in the same Table, this was not the case in any of the active SL groups, with specific IgG4 remaining at baseline values. Specific IgG for both Dpt and Df showed the same trend observed for IgG4 although with less marked increases (Figures S6–S13). Pairwise comparison of specific IgG4 levels against Dpt and Df (Mann–Whitney test, Tables S7 and S8) shows that, besides significant differences between placebo and each active SC group, there are differences between 500 mTU/ml and 1000, 3000, and 5000 mTU/ml.

No significant variations in IgG-ASCA or IgA-ASCA levels were observed at V6 in either group as compared with baseline values (Tables S10 and S11, Figures S14–S17).

3.3 | Safety

Sixty-nine side reactions were reported in 43 subjects out of a total of 196. Of these, 66 reactions were related to active SC, 43 local in 32 subjects and 23 systemic in 10 subjects. No grade III or grade IV systemic reactions were observed. Most systemic reactions occurred in the groups receiving SC active treatment, most of them delayed ($n = 18$), 13 of grade I and 5 of grade II. Most local side reactions (27 delayed and 16 immediate) also occurred in this group. Table 4 shows the number of all systemic and local reactions. Full description of all adverse events and all adverse reactions are in online supporting information (Tables S12–S29).

There were 23 subjects who discontinued treatment. Of these, 8 were due to loss to follow-up, 1 due to pregnancy, 2 due to subject's own decision, and 12 due to side reactions. These were 1 delayed systemic reaction (cough and headache) in 1 subject receiving active SL 1000 mTU/ml and the other reactions occurred in subjects receiving active SC: 5 were delayed local at the injection site and 1 was a yellow staining of the teeth in a subject receiving active SC 3000 mTU/ml; 5 delayed systemic (one was decreased appetite) and 1 delayed local and systemic. Coincidentally, the group of subjects receiving the active treatment of 3000 mTU/ml SC had the most dropouts ($n = 8$, including 1 due to pregnancy and 2 by subject's own decision). Table S30 shows the list of dropouts.

TABLE 3 Area under the curve (AUC) of the combined symptom medication scores (CSMS) (secondary outcome)

mTU/ml	Route of administration of the active treatment	Median (Q1, Q3)	*p*-Value[a]	Improvement over placebo	Estimate of location shift[b]	95% Confidence limits[b]
Placebo		136.0 (101.5, 226.5)				
500	SC	131.5 (51.8, 281.0)	0.275	3%	39.25	−38.50, 98.00
500	SL	154.8 (32.5, 209.8)	0.334	−14%	36.00	−36.00, 100.00
1000	SC	73.0 (21.8, 194.3)	0.042	46%	65.75	9.00, 119.25
1000	SL	81.5 (29.3, 203.0)	0.051	40%	64.50	2.00, 117.25
3000	SC	41.1 (16.8, 100.0)	0.001	70%	96.13	57.75, 142,75
3000	SL	88.5 (13.9, 173.5)	0.031	35%	70.38	12.00, 125,25
5000	SC	51.5 (19.0, 92.3)	0.001	62%	90.50	50.75, 141,50
5000	SL	81.5 (54.5, 169.8)	0.015	40%	55.38	14.50, 103.75

[a]Mann–Whitney test vs. Placebo.

[b]Hodges–Lehmann estimate vs. Placebo.

4 | DISCUSSION

Here, we show the results of the first-in-human trial with PM-HDM in a multicenter, randomized, double-blind, double-dummy, placebo-controlled study. Subjects with HDM allergic rhinitis, with or without asthma, were recruited from the Mediterranean coast of Spain, where allergy to HDM is highly prevalent.[23] In this area, the natural exposure of the HDM is perennial with slight annual variations that depend more on the outside temperature than on the indoor humidity.[35]

The study was designed following the recommendations for a phase 2 dose-finding studies of AIT from the EMA[36] and the EAACI.[37] Although CSMS is preferred as the primary outcome, it is accepted the use of NPT as primary outcome in these studies.[36] NPT outcomes allow the inclusion of polysensitized subjects when no significant interference with the test is expected. Among the different ways to assess nasal patency, acoustic rhinometry was chosen because it is quick, easy to perform, requires little cooperation, and has been standardized.[26,27,38–41] Moreover, it provides a reproducible and objective measure of nasal congestion, one of the most difficult symptoms to improve in allergic rhinitis.[42]

The trial results indicated a clear dose–response relationship of the investigational product for the primary and secondary efficacy outcomes up to the concentration of 3000 mTU/ml as compared with placebo. Thus, the maximum effect was achieved using concentrations of either 3000 or 5000 mTU/ml, regardless of the SC or SL route of administration. However, at any given concentration, the cumulative dose during the entire treatment period was almost 10 times higher by the SL route due to its daily administration schedule. Regarding the magnitude of the effect by both routes, similar results were found for the primary outcome (~45% subjects improved NPT in both groups) but not apparently for the secondary outcome (up to 70% of global improvement in CSMS for the active SC group compared to placebo, and 37% for the active SL compared to placebo too). Although the study was underpowered to observe differences between the active groups, a better CSMS outcome with SC vs. SL is consistent with the few head-to-head studies comparing SLIT vs. SCIT with HDM.[43,44] In a post hoc analysis, combining the 3000 and 5000 mTU/ml active groups of each route to increase the sample size, the differences between the active SC and SL for CSMS were still not significant, although they were close ($p = 0.057$ Mann–Whitney (Figure S5, online supplementary information). Therefore, it appears that the concentration of PM-HDM to achieve maximum effect is strikingly similar by SC or SL route, in contrast to the idea that, in general, a higher concentration of allergen doses is required in SLIT vs SCIT for successful immunotherapy.[45] One might consider whether this is a reflection of a higher performance of PM-allergoids versus conventional native allergens used for SL delivery, for example, better uptake of PM-allergoids by oral myeloid cells.[17]

The differences between the SC and SL routes were remarkable when considering the IgG response to Dpt and Df. A clear increase in specific IgG4 (up to eightfold above baseline) could only be observed in the SC groups. This should not be surprising since IgG responses occur to a much lesser extent in SLIT than in SCIT, and are only barely achieved in short-term studies like this at very high allergen doses.[46] However, whether this could be a reflection of underdosage in the current study cannot be ruled out and should be addressed in confirmatory studies. When assessing whether the IgG4 (or IgG) response in subjects from SC groups could be related to improvement in CSMS, no correlation was found (data not shown), in line with the notion that the mere presence of IgG4 antibodies is not sufficient for successful AIT.[47,48] Whatever the case, the improvement in NPT and CSMS observed in subjects with active SL in the current trial occurred without a significant change in specific serum IgG, as has also been reported in other SLIT studies, particularly with HDM.[49,50] Serum levels of specific IgA were negligible in these subjects (data not shown). Nasal IgA, however, which has recently been shown to be induced mainly in SLIT,[51] was not assessed. Given the apparent lack of antibody response in the SL groups, it would be interesting to address whether an IgA response in nasal fluid can be observed in further studies. On the contrary, serum specific IgE did not change significantly from baseline values with the optimal concentrations (i.e., 3000 or 5000 mTU/ml), regardless of the route of administration used, which may be in contrast to the early increase generally associated with native allergens[52] or even allergoids.[53]

No major safety issues were reported. The number of moderate–severe adverse reactions in the active SC groups was like the reported for glutaraldehyde-polymerized mite allergoids in aluminum hydroxide in normal clinical conditions.[54] PM-HDM was not adsorbed onto a particulate matter (e.g., aluminum hydroxide), thought to mitigate systemic effects by maintaining the allergen at the injection site.[55] In this regard, it should be noted that PM-allergoids show a much lower diffusion rate than native allergens or polymerized allergens not coupled to mannan.[14] No grade III or IV systemic reactions were reported in subjects in the active SC groups, while grade I or II reactions, mostly of the delayed type. Interestingly, all grade II systemic reactions were in the group receiving SC 1000 mTU/ml, not in groups receiving higher concentrations. Regarding local SC reactions, the majority were mild and occurred with the first injections. However, 6 delayed reactions were severe and led to withdrawal from the trial.

The number of adverse events in the active SL groups was remarkably low, with only two grade I systemic reactions and one mild immediate local reaction. This contrast markedly with the very frequent local reactions reported in SLIT, especially in studies with HDM tablets. This may reflect notable differences in local mucosal allergen concentration, allergenicity, and/or other intrinsic properties of mite extracts between both products. In this regard, the maximum quantity of group 1 allergen was 8 μg/ml (1.6 μg/dose) and was sprayed in a large sublingual mucosal surface and, in addition, PM-allergoids have very low capacity to activate mast cells by IgE-dependent mechanisms,[15] the main triggers of oral reactions in SLIT.[56]

TABLE 4 Adverse reactions

A. Systemic reactions

Systemic

SC mTU/ml	Type	*n* Grade I	% per injection	*n* Grade II	% per injection	*n* Grade III	% per injection	*n* Grade IV	% per injection	*n* Total	% per injection
500	Immediate	0	0	0	0	0	0	0	0	0	0
	Delayed	0	0	0	0	0	0	0	0	0	0
1000	Immediate	0	0	0	0	0	0	0	0	0	0
	Delayed	2	2	5	4	0	0	0	0	7	5
3000	Immediate	1	1	0	0	0	0	0	0	1	1
	Delayed	5	4	0	0	0	0	0	0	5	4
5000	Immediate	4	4	0	0	0	0	0	0	4	4
	Delayed	6	5	0	0	0	0	0	0	6	5
	Global	18	4	5	1	0	0	0	0	23	5

SL mTU/ml	Type	*n* Grade I	% per subject	*n* Grade II	% per subject	*n* Grade III	% per subject	*n* Grade IV	% per subject	*n* Total	% per subject
500	Immediate	0	0	0	0	0	0	0	0	0	0
	Delayed	0	0	0	0	0	0	0	0	0	0
1000	Immediate	0	0	0	0	0	0	0	0	0	0
	Delayed	1	5	0	0	0	0	0	0	1	1
3000	Immediate	1	5	0	0	0	0	0	0	1	1
	Delayed	0	0	0	0	0	0	0	0	0	0
5000	Immediate	0	0	0	0	0	0	0	0	0	0
	Delayed	0	0	0	0	0	0	0	0	0	0
	Global	2	2	0	0	0	0	0	0	2	0

B. Local reactions

Local

SC mTU/ml	Type	*n*	% per injection	SL mTU/ml	Type	*n*	% per subject
500	Immediate	3	2	500	Immediate	0	0
	Delayed	2	2		Delayed	0	0
1000	Immediate	4	3	1000	Immediate	0	0
	Delayed	9	7		Delayed	0	0
3000	Immediate	3	3	3000	Immediate	0	0
	Delayed	11	9		Delayed	0	0

TABLE 4 (Continued)

B. Local reactions								
Local								
SC mTU/ml	Type	*n*	% per injection	SL mTU/ml	Type	*n*	% per subject
5000	Immediate	6	5	5000	Immediate	1	5
	Delayed	5	4		Delayed	0	0
	Global	43	9		Global	1	1

The investigational product is formulated with allergoids coupled to non-oxidized mannan derived from *S. cerevisiae*.[14] Partially oxidized mannan has been used in cancer vaccines with a long safety record in several clinical trials.[57,58] Antibody responses to mannan in vaccinated patients were not reported in those trials. Here, we did not observe significant variations in serum IgG-ASCA or IgA-ASCA levels in the study groups. This is consistent with our own studies in rabbits immunized with PM-allergoids indicating a low immunogenicity of mannan to induce antibody responses (unpublished results), and with other studies performed in mice.[59]

In conclusion, this first-in-human trial shows that PM-HDM is safe and successful in achieving primary and secondary clinical efficacy outcomes by both SL and SC routes. Either 3000 or 5000 mTU/ml are adequate concentrations to go further into a Phase 3 clinical trial for SC administration, while a Phase 2/3 with an additional higher concentration is being considered for the SL route.

AUTHOR CONTRIBUTIONS

MC, AN, JLS, and RC conceived and/or designed the clinical trial. AN, AM, MN, EI, DJ, SC, C P-F, JM, A de M, RA, DE-Q, JF, LM, MA, CA, and AF carried out the clinical trial. NC carried out the statistical analysis and MC participated in the discussion and interpretation of this analysis. AN, RC, JLS, and MC participated in the discussions of data analysis and interpretation and wrote the manuscript. SdP collaborated with writing the manuscript. The manuscript was finalized with the assistance of all authors.

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CONFLICT OF INTEREST

AN received fees of Astra-Zeneca, Merck, Novartis. DE-Q received fees of Chiesi, Sanofi, Stallèrgenes-Greer, Astra-Zeneca, Glaxo-Smith-kline, Roxall Medicine, and Novartis. AM, MN, EI, DJ, SC, C P-F, JM, A de M, RA, JF, LM, MA, CA, and AF declare no conflict of interest. RC and SdP are employees of Inmunotek SL. JLS and MC are shareholders of Inmunotek SL.

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SUPPORTING INFORMATION

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