

ORIGINAL ARTICLE

Allergen-Specific Immunotherapy and Biologics

Molecular reactivity profiling upon immunotherapy with a 300 IR sublingual house dust mite tablet reveals marked humoral changes towards major allergens

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Abstract

Background: Molecular antibody reactivity profiles have not yet been studied in depth in patients treated by sublingual house dust mite (HDM) tablet immunotherapy. Humoral immune responses to a large panel of HDM mite allergens were studied using allergen microarray technology in a subset of clinically defined high and low responder patients from a double-blind placebo-controlled allergen-specific immunotherapy (AIT) trial using sublingual 300 IR HDM tablets.

Methods: Serum levels of IgE, IgG and IgG₄ to 13 *Dermatophagoides pteronyssinus* molecules were measured at baseline and after 1-year AIT, using allergen microarrays in 100 subjects exhibiting high or low clinical benefit.

Results: Der p 1, Der p 2 and Der p 23 were the most frequently recognized allergens in the study population. Patients with HDM-related asthma had significantly higher allergen-specific IgE levels to Der p 1 and Der p 23. No significant difference in the distribution of allergen sensitization pattern was observed between high and low responders. An increase in serum allergen-specific IgG and IgG₄ occurred upon AIT, in particular to allergens Der p 1, Der p 2 and Der p 23 ($p < 0.0001$).

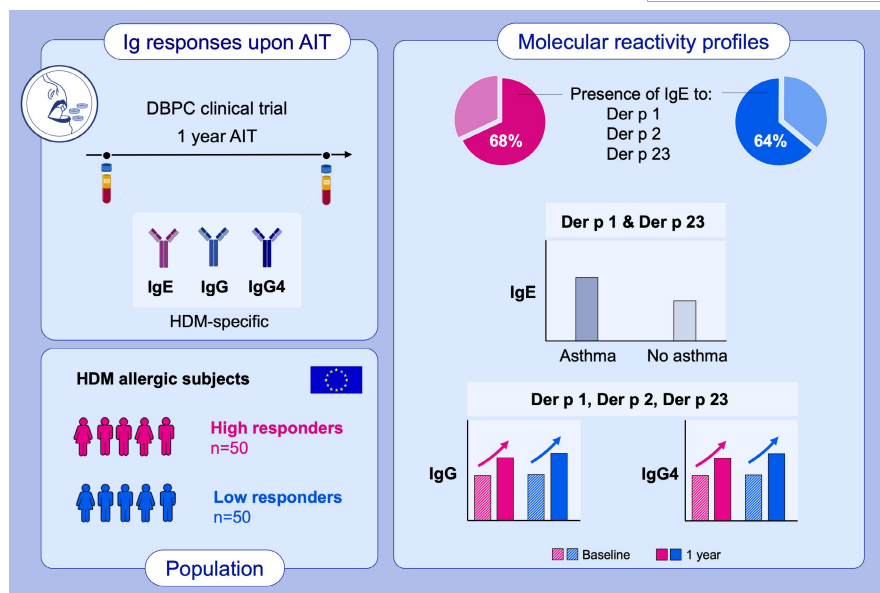
Conclusions: We confirm for our study population that Der p 1- and Der p 23-specific IgE levels are associated with asthma. IgE reactivity profiles were not predictive of sublingual AIT outcomes, with 300 IR tablets as efficacious in pauci- and multi-sensitized subjects. Our study is the first to demonstrate the induction of IgG and IgG₄ specific for the HDM allergens Der p 1, Der p 2 and Der p 23 by sublingual AIT.

KEYWORDS

allergen microarray, allergen-specific immunotherapy, house dust mite allergy, molecular reactivity profile, SLIT tablet

Ekaterina Potapova and Véronique Bordas-Le Floch contributed equally to the work.

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GRAPHICAL ABSTRACT

This study evaluated humoral responses to HDM allergen panel in a subset of clinically defined high and low responder patients from a DBPC AIT trial using sublingual HDM tablets. Der p 1, Der p 2 and Der p 23 were the most frequently recognized allergens in the study population. Patients with HDM-related asthma had significantly higher Der p 1- and Der p 23-specific IgE levels compared to non-asthmatic. Upon AIT, an increase in allergen-specific IgG and IgG4 was observed, in particular Der p 1-, Der p 2- and Der p 23-specific.

Abbreviations: AIT, allergen immunotherapy; DBPC, double-blind placebo-controlled; HDM, house dust mite; Ig, immunoglobulin

1 | INTRODUCTION

Dermatophagoides farinae and *Dermatophagoides pteronyssinus* house dust mites (HDM) are main potent causes of perennial respiratory allergies, affecting more than half a billion people worldwide.^{1,2} Allergen immunotherapy (AIT) to HDM is now well-established and recommended for treating allergic rhinitis with or without asthma.³ HDM have a complex allergen repertoire with at least 32 allergen groups officially registered as of today by the International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee (www.allergen.org) but prevalence of IgE sensitization and biological potency vary greatly among those allergens.^{4,5} Groups 1 and 2 are considered the most clinically relevant ones with a high prevalence of IgE sensitization (60%–100% in mite allergic patients) and a high potency (anti-group 1 and anti-group 2 IgE combined represent 50%–77% of total HDM-specific IgE).^{6–12} Der p 23 has been added as a new important and major allergen, with a high prevalence of IgE recognition but its allergenic potency is still debated.^{11–15}

Allergen microarrays allow large multiplex analysis of molecular allergen sensitization profiles.^{11,13,16–18} Accordingly, molecular IgE tests and allergen microarrays have become important tools for detection of allergen-specific IgE particularly in patients with complex IgE sensitization profiles.¹⁹ Furthermore, allergen microarrays are useful for the correct prescription of AIT by identifying the sensitizing allergen sources and for the monitoring of the effects of AIT.^{20,21} Besides offering the simultaneous testing of IgE and IgG antibody reactivity to multiple allergens, allergen microarrays are different from established IgE test systems such as the ImmunoCAP

because only very tiny amounts of allergen are used for detecting allergen-specific IgE which allows to visualize the effects of IgG antibodies capable of blocking IgE binding to allergens as it occurs during AIT.^{22,23} As such, reduction of IgE binding to micro-arrayed allergens in the course of AIT is associated with the development of blocking IgG antibodies which may be considered as a surrogate marker for AIT.^{24–26} Besides, the allergen microarrays have been proven to be useful for many other aspects in allergy. In particular, longitudinal studies in children from the MAS cohort using allergen arrays have revealed mite sensitization trajectories.¹⁶ In this cohort study, IgE sensitization patterns of certain children became broader and included other mid-tier or minor HDM allergens over time, beyond the three early sensitizers Der p 1, Der p 2 and Der p 23. In this study, mite allergens have been classified according to prevalence of IgE reactivity into group 'A' (Der p 1, Der p 2 and Der p 23; above 40%), 'B' (Der p 4, Der p 5, Der p 7, Der p 21; between 15% and 30%) and 'C' (Der p 11, Der p 14, Der p 15, Der p 18, Der p 37; below 10%).^{16,27} In the MAS birth cohort, mite sensitization began with molecules of the group 'A', then about 50% of the patients showed sensitization to group 'B' allergens and later about 20% of the patients developed the sensitization to the allergens of the group 'C', representing a typical sensitization pattern. High IgE levels to Der p 1 and Der p 23 have been investigated to be associated with asthma.^{11,12,16,27,28} Moreover, molecular polysensitization was associated with a higher risk of developing allergic rhinitis or asthma.^{16,27}

AIT is established as a safe and efficacious way for the causative treatment of IgE-mediated respiratory allergies.^{29,30} Changes occur in humoral immune responses of patient undergoing AIT and

encompass transient increase in allergen-specific IgE levels as well as induction of IgG₁ and IgG₄, the latter being considered as an effective compliance marker.^{31,32} However, there is currently no predictive marker of efficacy of AIT available. Recently, it has been proposed that certain forms of AIT may have a greater efficacy in patients exhibiting non-complex sensitization pattern, mostly restricted to major mite allergens.^{33,34} Herein, we describe for the first time the analysis of molecular patterns of IgE, IgG and IgG₄ reactivity subjects having received 300 IR HDM tablets in a phase III clinical trial. Specifically, the molecular antibody reactivity profiles were investigated in a subset of clinical high and low responders.

2 | METHODS

2.1 | Clinical samples

A subset of patients having completed the SL75.14 trial³⁵ were selected among the European *per protocol* group monosensitized to HDM and classified as high ($n = 50$) and low ($n = 50$) responders according to their relative change in Average Total Combined Score (ATCS), as previously described.³⁶ See supporting information for details in the online repository as well as in Table 1 and Figure S1.

2.2 | Immunoglobulin assays

All available serum samples were tested with MeDALL allergen chips which had been produced according to ImmunoCAP ISAC technology (Medical University of Vienna), as described previously,²² to detect IgE, IgG and IgG₄ levels specific to HDM allergen molecules Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 11, Der p 14, Der p 15, Der p 18, Der p 20, Der p 21, Der p 23 and Der p 37. Serum samples were used either undiluted for IgE or diluted 1:50 for IgG and IgG₄ measurements as previously described.²² Slides were washed with wash solution (PBS containing 1% Tween-20) and dried for 5 min, then 30 μ l of serum samples were added and incubated for 2 h. Slides were rinsed and washed with wash solution and dried again. Then, 30 μ l of either anti-human IgE, IgG or IgG₄ antibodies conjugated with fluorophore (DyLight™ 550-2xPEG NHS Ester) were added and incubated for 30 min. Slides were rinsed, washed, and dried a third time. Afterwards, the slides were scanned with CapitalBio LuxScan™ 10K/A confocal laser scanner and analyzed with the LuxScan software. IgG and IgG₄ were scanned at 560 PMT and IgE at 630 PMT. The obtained signal was expressed in fluorescence intensity and transformed into ISAC units (ISU) by matching and interpolating the samples outcomes against the curve obtained with the HDM calibrator in the same session. Results above 0.3 ISU for IgE and IgG and 0.1 ISU for IgG₄ were considered positive. Values below 0.01 were set to 0.01.

2.3 | ABC categorization

Allergen were categorized in groups A, B and C, with Der p 20 added to group C given the prevalence of IgE sensitization, then patterns were assigned.¹⁶ See Figures S1–S10 for details.

2.4 | Statistical analyses

Analyses were performed using GraphPad prism software version 9. Statistical differences were computed using non-parametric Wilcoxon and Mann–Whitney test for paired and non-paired values, respectively. p values below 0.05 were considered significant ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$).

3 | RESULTS

3.1 | Molecular reactivity patterns of HDM-allergic patients and allergen-specific IgE, IgG and IgG₄ levels at baseline

A subset of HDM-allergic subjects ($n = 100$) having participated in a phase III clinical study investigating the efficacy of AIT using a 300 IR HDM tablets was selected.³⁵ The subjects were classified either as high ($n = 50$) or low ($n = 50$) responders according to the relative change in clinical score. Study population characteristics are provided in Table 1. Serum samples were tested using allergen microarrays for IgE, IgG and IgG₄ specific for 13 HDM allergens.

We first analyzed the IgE reactivity patterns of patients according to the 3 groups and 'ABC' typical patterns previously defined given a spreading of allergen sensitization.¹⁶ In our study population, the mean number of recognized allergens was 3.92, with subjects reactive to 2–6 allergens representing 74% of the investigated patients (Figure 1A). As illustrated on Figure 1(B), the overall most prevalent allergens were the 3 major allergens, belonging to group A, Der p 2 (92%), Der p 1 (83%) and Der p 23 (82%). Prevalence of IgE sensitization to the other tested allergens was much lower (ie <35%) (Figure 1B). Altogether, prevalence of group B allergens ranged from 17% to 33% and prevalence of group C was below 7%, except for Der p 37 (19%). We then further analyzed the IgE sensitization to the three major allergens, Der p 1, Der p 2 and Der p 23. A first observation was that 98% of the patients were positive to either one of the 3 major allergens, irrespective of their sensitization to other allergens. Additionally, 66% of patients were sensitized to the three allergens while 27% were sensitized to 2 out of 3 (12% to Der p 1 and Der p 2; 5% to Der p 1 and Der p 23; 10% to Der p 2 and Der p 23) and 5% sensitized to only one of the 3 (4% to Der p 2 and 1% to Der p 23) (Figure 1C).

We also investigated the presence of IgG and IgG₄ directed to individual allergens before start of AIT. All patients tested had IgG directed to each of the tested allergen molecules except one which was negative for Der p 7 (data not shown). Focusing on IgG₄, the most frequently recognized allergens were, as for IgE, the 3 major

TABLE 1 Study population characteristics

	All	High responders	Low responders
<i>n</i>	100	50	50
Gender			
Female <i>n</i> (%)	37 (37%)	16 (32%)	21 (42%)
Male <i>n</i> (%)	63 (63%)	34 (68%)	29 (58%)
Age			
Mean (Min, Max)	27.76 (12–60)	28.76 (12–60)	26.76 (12–59)
SD	12.64	12.48	12.86
Median (Q1;Q3)	25 (16.25–36.75)	25 (18–42)	24.5 (14.75–35.25)
Asthma			
With <i>n</i> (%)	57 (57%)	33 (66%)	24 (48%)
Without <i>n</i> (%)	43 (43%)	17 (34%)	26 (52%)
IgE <i>D. pteronyssinus</i> at baseline			
Mean (Min, Max)	37.92 (2.58–443.19)	37.78 (3.01–443.19)	38.07 (2.58–226.20)
SD	63.82	73.33	53.41
Geometric mean (95% CI)	17.58 (13.95–22.15)	16.33 (11.65–22.89)	18.92 (13.64–26.23)
Median (Q1–Q3)	15.15 (6.49–37.34)	12.7 (5.913–38.8)	17.2 (8.065–36.88)
IgE <i>D. farinae</i> at baseline			
Mean (Min, Max)	32.62 (2.37–339.42)	33.86 (2.37–339.42)	31.39 (2.72–180.90)
SD	51.71	62.22	39.1
Geometric mean (95% CI)	16.56 (13.35–20.55)	15.43 (11.21–21.25)	17.77 (13.17–23.97)
Median (Q1–Q3)	14.78 (7.34–31.44)	10.77 (6.91–31.50)	16.5 (8.07–32.4)
IgG4 <i>D. pteronyssinus</i> at baseline			
Mean (Min, Max)	0.52 (0.035–7.23)	0.4221 (0.035–2.67)	0.6197 (0.035–7.23)
SD	0.869	0.49	1.125
Geometric mean (95% CI)	0.30 (0.25–0.37)	0.28 (0.22–0.36)	0.33 (0.25–0.44)
Median (Q1–Q3)	0.29 (0.16–0.49)	0.25 (0.15–0.46)	0.31 (0.17–0.51)
IgG4 <i>D. farinae</i> at baseline			
Mean (Min, Max)	0.49 (0.035–8.92)	0.35 (0.035–1.90)	0.64 (0.035–8.92)
SD	1.03	0.38	1.396
Geometric mean (95% CI)	0.25 (0.20–0.31)	0.23 (0.17–0.30)	0.28 (0.20–0.39)
Median (Q1–Q3)	0.23 (0.14–0.43)	0.23 (0.14–0.43)	0.23 (0.16–0.44)
Relative change from baseline in Average Total Combined Score			
Mean (Min, Max)	–42.77 (–100 to 88.24)	–84.71 (–100 to –68.18)	–0.83 (–31.16 to 88.24)
SD	46.48	10.63	25.73
Median (Q1–Q3)	–49.67 (–82.70 to –4.53)	–82.67 (–96.47 to –75.34)	–4.73 (–20.14 to 14.03)

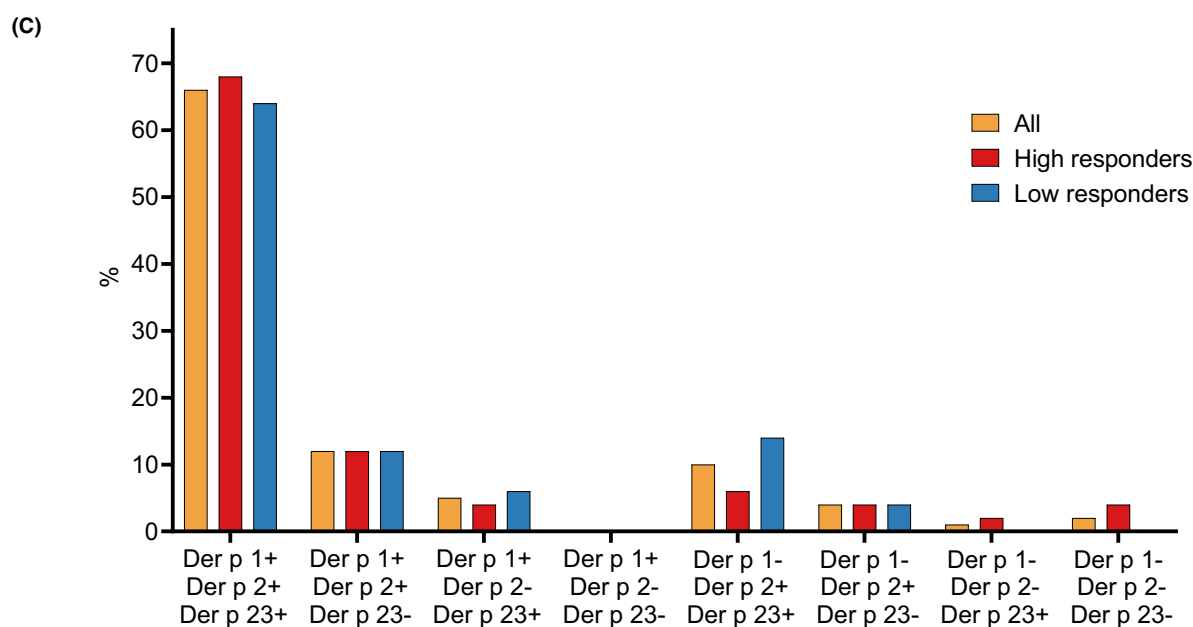
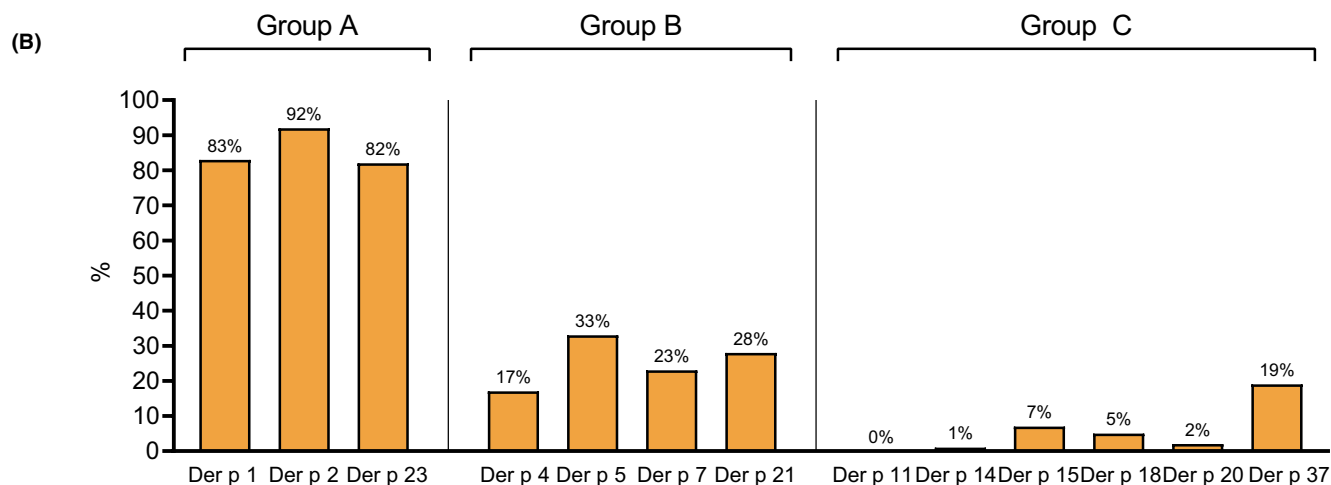
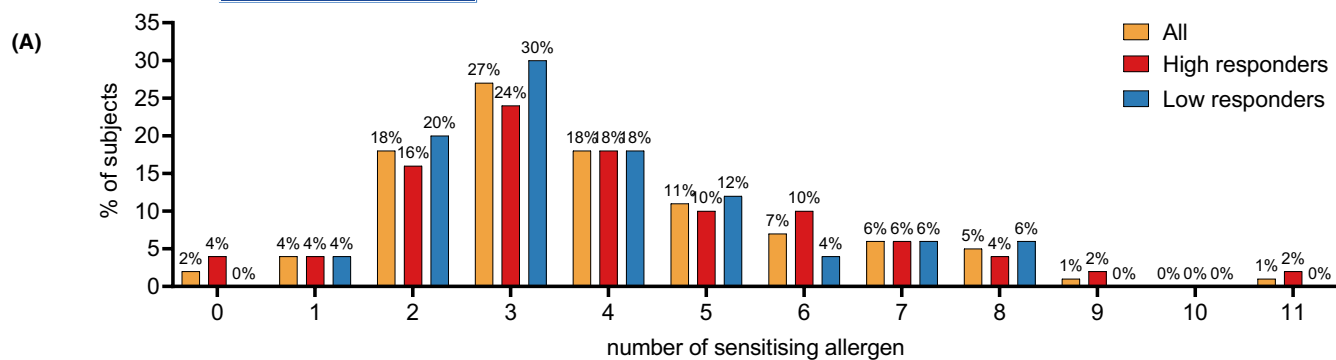
allergens with 92%, 74% and 63% of patients having IgE levels at baseline above the IgG₄ positivity threshold set at 0.1 ISU, for Der p 2, Der p 1 and Der p 23, respectively (data not shown).

3.2 | At baseline subjects with asthma showed IgE reactivity to more HDM allergens and had higher IgE levels to Der p 1 and Der p 23

Sensitization to Der p 1 and Der p 23 has been reported to be associated with a higher risk of asthma.^{15,16,28} We thus further explored

the IgE sensitization patterns and the distribution of IgE levels with regard to the presence of a concomitant asthma. As seen in Figure 2(A), we observed a trend for IgE sensitization to a broader allergen panel in asthmatic patients (mean 4.44 vs 3.52 in patients with and without asthma, respectively, $p = 0.0352$).

The percentage of patients with IgE directed to Der p 23 was higher in patients with concomitant asthma (data not shown). More specifically, IgE levels were significantly higher ($p = 0.0109$ and $p = 0.0051$, respectively) in patients with asthma for Der p 1 and Der p 23 (Figure 2B,D). This observation also extended to baseline levels of IgG₄ ($p = 0.0159$ and $p = 0.0009$, respectively;



	Der p 1+ Der p 2+ Der p 23+	Der p 1+ Der p 2+ Der p 23-	Der p 1+ Der p 2- Der p 23+	Der p 1+ Der p 2- Der p 23-	Der p 1- Der p 2+ Der p 23+	Der p 1- Der p 2+ Der p 23-	Der p 1- Der p 2- Der p 23+	Der p 1- Der p 2- Der p 23-
All	66 (66%)	12 (12%)	5 (5%)	0 (0%)	10 (10%)	4 (4%)	1 (1%)	2 (2%)
High responders	34 (68%)	6 (12%)	2 (4%)	0 (0%)	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Low responders	32 (64%)	6 (12%)	3 (6%)	0 (0%)	7 (14%)	2 (4%)	0 (0%)	0 (0%)

FIGURE 1 Prevalence of IgE sensitization in patients to individual mite allergens. Serum levels (ISU) of IgE to 13 *D. pteronyssinus* allergens have been measured with positivity threshold set to 0.3 ISU (A) Frequency of polysensitization in the whole study population ($n = 100$), high ($n = 50$) and low ($n = 50$) responders (B). Prevalence of IgE sensitization to individual allergens in the whole study population ($n = 100$). (C) Distribution of IgE sensitization to the major Der p 1, Der p 2 and Der p 23 major allergens in the whole study population ($n = 100$), high ($n = 50$) and low ($n = 50$) responders

Figure 2E,G).³⁷ For Der p 2, IgE levels tended to be higher in patients with asthma, but the difference was only significant for IgG₄ (Figure 2C,F).

Der p 37 has also recently been reported to be associated with asthma.³⁸ We thus analyzed the Der p 37 reactivity among patients with and without concomitant asthma. Patients sensitized to Der p 37 tended to have a more complex reactivity pattern (mean 5.3 other allergens vs 3.4, $p = 0.004$; data not shown). However, in this subgroup representing less than 20% of the study population, we did not observe a trend for asthma in Der p 37-sensitized patients, with 8 asthmatic patients sensitized to Der p 37 (42.1%) compared with 11 non-asthmatic (57.9%) patients (Figure S2). Such discrepancies are likely explained by differences in population characteristics (eg geographical origin, allergy comorbidities, polysensitization).

3.3 | Molecular IgE reactivity patterns at baseline does not predict clinical response to the 300 IR HDM tablet

We assigned 'ABC' patterns¹⁶ according to the IgE reactivity profiles of each patient at baseline and analyzed the distribution of patterns. A and AB profiles were the most frequent (45% and 28%, respectively) followed by ABC (18%) and AC (7%) (Figure 3). We then examined whether the molecular reactivity ABC patterns at baseline were predictive of clinical responses to immunotherapy. No significant difference was found between the subgroups of patients stratified as high and low responders regarding the ABC profiles (Figure 3). There was also no major difference in the number of sensitizing allergens between high and low responders (Figure 1A).

3.4 | Changes in antibody responses upon AIT with 300 IR HDM tablets

We investigated the changes in allergen-specific IgG, IgG₄ and IgE levels occurring upon immunotherapy. A clear significant ($p < 0.0001$) increase in IgG and IgG₄ levels was observed for Der p 1, Der p 2 and Der p 23 but also for the mid-tier allergen Der p 4 and some minor allergens Der p 15, Der p 18 and Der p 37 (Figures 4A–F and 5A–F, Figures S3A–H, S4A–L, and S5A–H and S6A–L). Regarding the changes in allergen-specific IgG and IgG₄ levels no significant difference was observed between high and low responders after 1 year of immunotherapy.

Focusing on allergen-specific IgE, an increase is barely detectable for most of the allergen tested except for Der p 1 in high

responders and for Der p 4 and Der p 37 in the whole population as well as in high and low responders (Figure 4G–I, Figures S3I–L and S4M–R). We also observed in a few patients, an appearance after treatment of IgE against allergens that were not recognized at baseline, suggesting limited neosensitization upon AIT (Figure 5G–I, Figures S5I–L and S6M–R). In most cases, it was counterbalanced by concomitant increase of IgG₄ (Figure 5J–L, Figures S7 and S8).

4 | DISCUSSION

Multiplex molecular IgE testing has proven valuable to decipher the reactivity repertoire of allergic patients and a promising tool for precision medicine in allergology.³⁹ Cross-sectional and longitudinal studies using such tools have highlighted molecular spreading of IgE reactivity pattern and higher risk of asthma and other allergic comorbidities for poly-sensitized patients. It has been recently suggested that selecting patients according to their molecular profile of IgE reactivity to HDM allergens may enhance outcomes of AIT.^{33,34} In the present study, we took advantage of a Phase III clinical trial with 300 IR HDM tablets to measure at baseline and after a 1-year treatment in a subset of 100 patients exhibiting high or low clinical benefit, serum levels of IgE, IgG and IgG₄ to 13 molecular *D. pteronyssinus* allergens using allergen microarrays. We thus assessed impact of reactivity patterns on the outcome of AIT, monitored changes in immune responses occurring upon AIT and examine influence of asthma on IgE reactivity.

Overall, the prevalences of IgE sensitization to the 13 HDM allergens tested at baseline in our study cohort are in agreement with certain other studies, as well as the reactivity pattern, and the number of allergens with IgE reactivities.^{7–9,11–13,16,27,28,33,40,41} Der p 1, Der p 2 and Der p 23 allergens were the most recognized molecules with 98% of patients tested exhibiting IgE reactivity to at least 1 of these 3 allergens. The prevalence of Der p 23 IgE sensitization in our cohort was notably in the upper range. However, in our study, only 1 patient showed IgE reactivity to Der p 23 without sensitizations to Der p 1 and Der p 2, but yet reacted to Der p 5. This rate was lower than the 5% or even higher described elsewhere.^{38,42,43} This might be due to chosen positive cut-off or to the allergen panel tested. Furthermore, it has been shown that IgE reactivity profiles to different HDM allergen molecules may vary in different populations mainly for Der p 4, Der p 7, Der p 15, Der p 18 and Der p 23,¹¹ the latter (ie Der p 23) being more prevalent in Europe than in North America especially Canada. This study testing allergen-specific IgE in serum samples from 685 HDM allergic subject confirmed that Der p 23 was a frequent sensitizer with a prevalence of 64% with 2.3% of the studied population monosensitized to Der p 23.¹¹

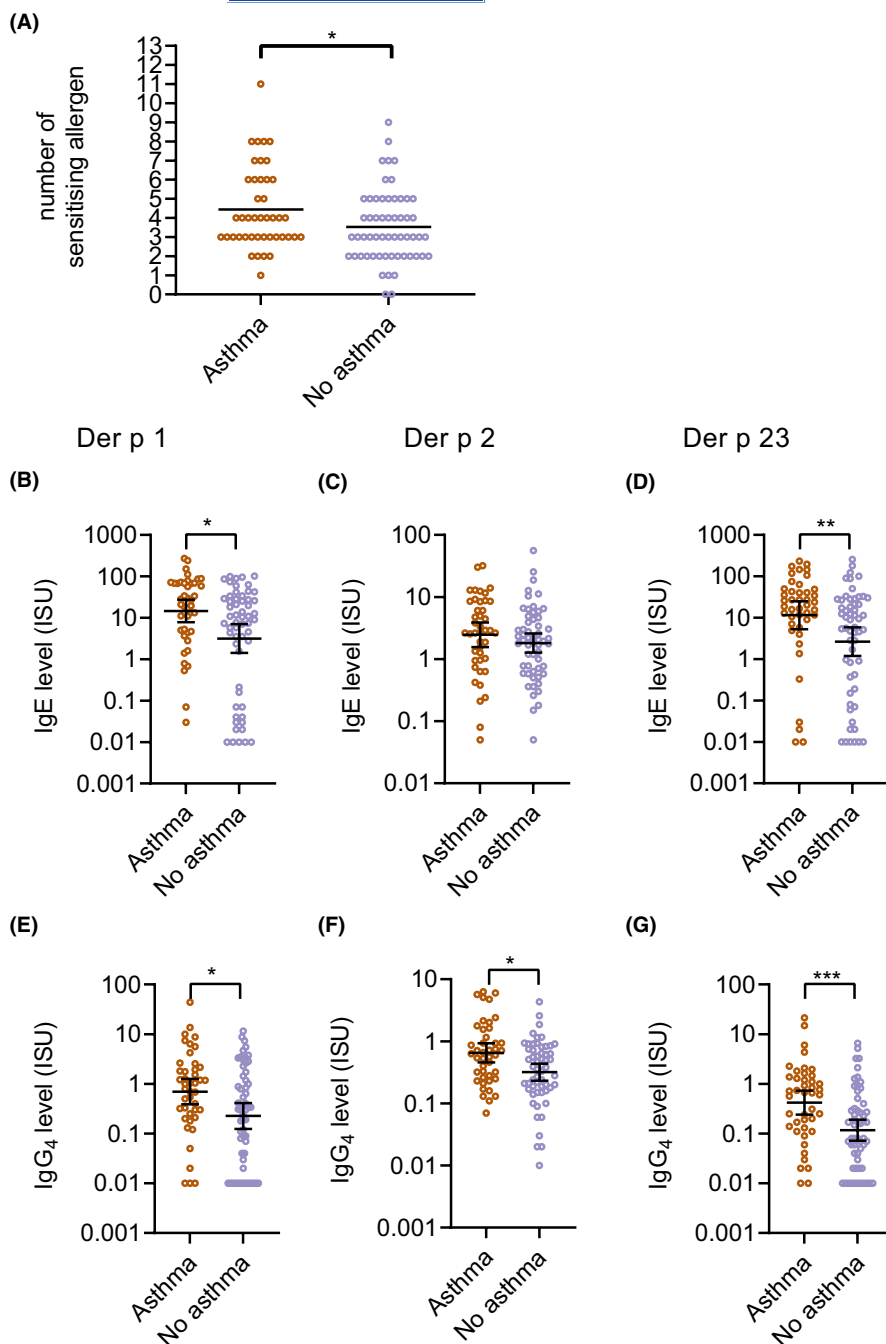


FIGURE 2 IgE and IgG₄ levels specific for Der p 1, Der p 2 and Der p 23 according to asthma status. (A) Number of sensitizing allergens in patients with and without concomitant asthma. (B–G) Serum levels (ISU, geometric mean with 95% CI) of (B–D) IgE and (E–G) IgG₄ to Der p 1 (left panel), Der p 2 (middle panel) and Der p 23 (right panel) measured in subjects with ($n = 43$) or without ($n = 57$) concomitant asthma at baseline. Statistical significance was determined using non-parametric Mann–Whitney with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Regarding the other allergens, our results were mostly in agreement with published data for group B as mid-tier allergens and group C as minor allergens with low prevalence of sensitization.^{11,13,33,34} Noticeably, prevalence for mid-tier allergens (Der p 4, Der p 5, Der p 7 and Der p 21) was lower (between 17 and 33%) when compared to other European cohorts (between 28% and 42% in Muddaluru et al¹¹; between 12.5% and 42% in Rodríguez-Domínguez et al³⁴ and between 44% to 61% in Chen et al³³), thus confirming a potential heterogeneity for mid-tier allergens depending on studied populations.

Recently, a study in 99 mite allergic patients showed a prevalence of sensitization to Der p 37 of 28%, which was higher than for other allergens in group C and furthermore showed the association with asthma in a higher rate of Der p 37-sensitized patients among

asthmatic patients than in non-asthmatic patients.³⁸ In our study population, we observed a prevalence of Der p 37 (19%) which was also higher than expected from the initial categorization¹⁶ but we did not observe such imbalance in IgE reactivity to Der p 37 between patients with and without asthma. Altogether and as discussed above, these discrepancies might be explained by differences in some characteristics of the study populations, including geographical origin, allergy comorbidities, polysensitization, as our study has been notably focused on Europe-wide distributed patients monosensitized to HDM.^{11,35} Together, the diversity of HDM sensitization profiles observed in allergic subject highlight the value of the molecular allergen profiling approach for treating patient with the right therapeutic option and administration scheme.

FIGURE 3 Molecular IgE reactivity profiles. IgE reactivity to 13 HDM allergens was categorized as group A (Der p 1, Der p 2 and Der p 23), B (Der p 4, Der p 5, Der p 7, Der p 21) and C (Der p 11, Der p 14, Der p 15, Der p 18, Der p 20, Der p 37). A group was considered positive if IgE levels were above 0.3 ISU for at least one molecule of the group. Patterns are shown for the whole study population ($n = 100$), high ($n = 50$) and low responders ($n = 50$)

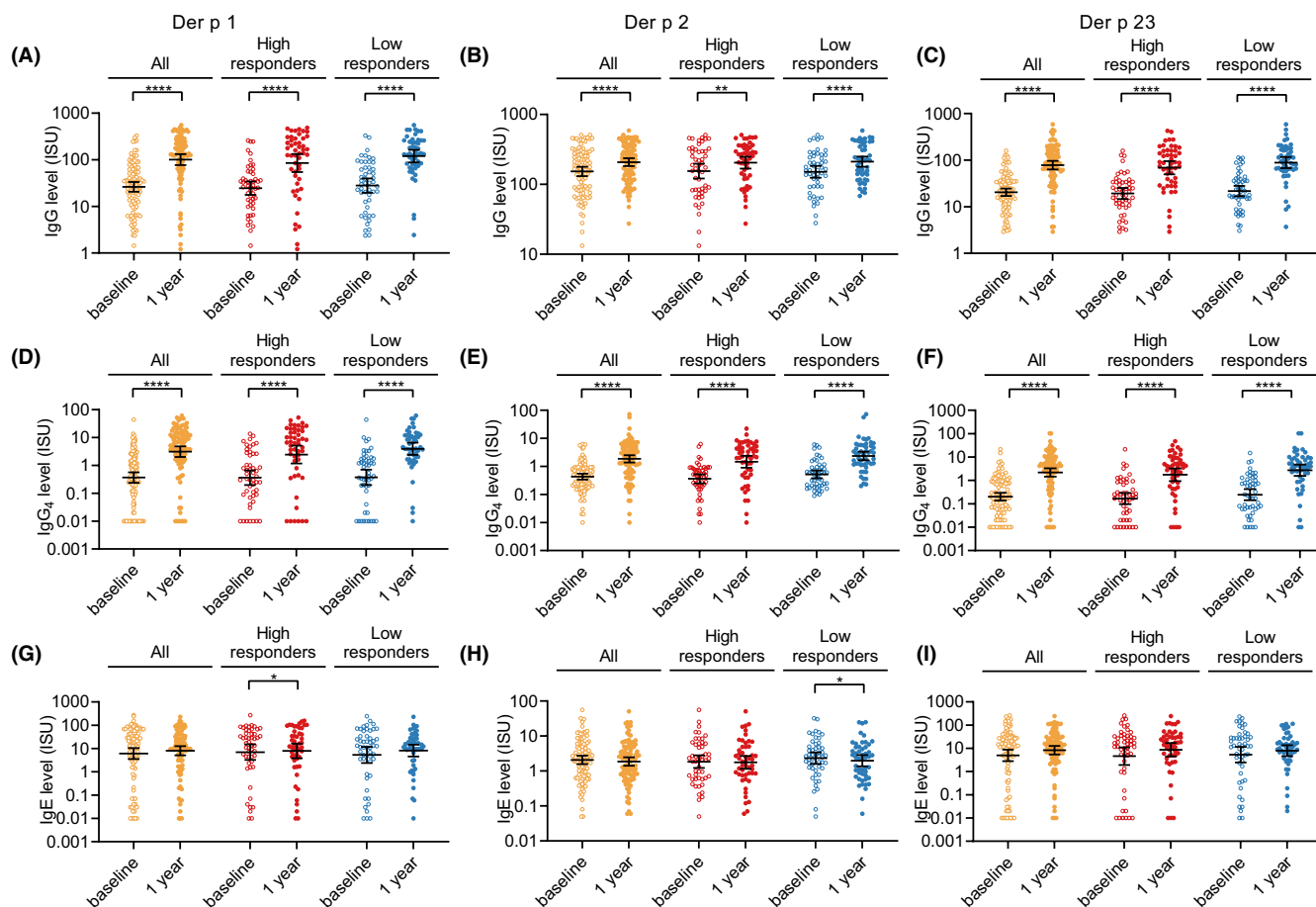
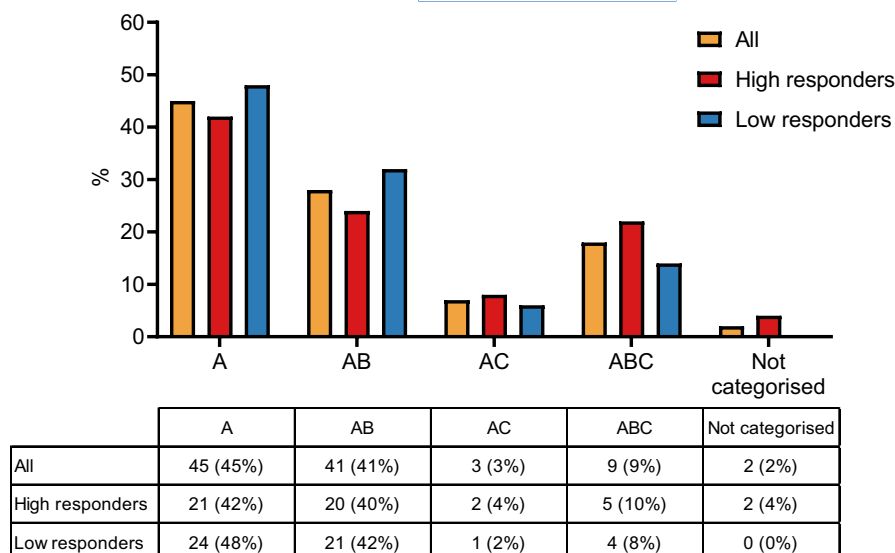


FIGURE 4 IgG, IgG₄ and IgE levels (ISU) before and after 1 year of AIT. Scatter plots of serum levels of (A–C) IgG, (D–F) IgG₄ and (G–I) IgE to Der p 1 (left panel), Der p 2 (middle panel) and Der p 23 (right panel) at baseline and after immunotherapy in the whole study population ($n = 100$), high ($n = 50$) and low ($n = 50$) responders. Results are expressed in ISU and geometric means with 95% CI for the whole study population, high responders ($n = 50$) and low responders ($n = 50$). Statistical significance between the paired time points was determined using non-parametric Wilcoxon test with * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$

A significant increase of *D. pteronyssinus*-specific IgE was observed in our cohort (Figure S9), in agreement with the literature reporting a transient increase in IgE levels upon AIT.^{11,44,45} This

increase was not detected for most of the molecular allergens tested using allergen microarray likely due to the binding of competing IgG and IgG₄,^{33,34} since the levels of allergen-specific total IgG and

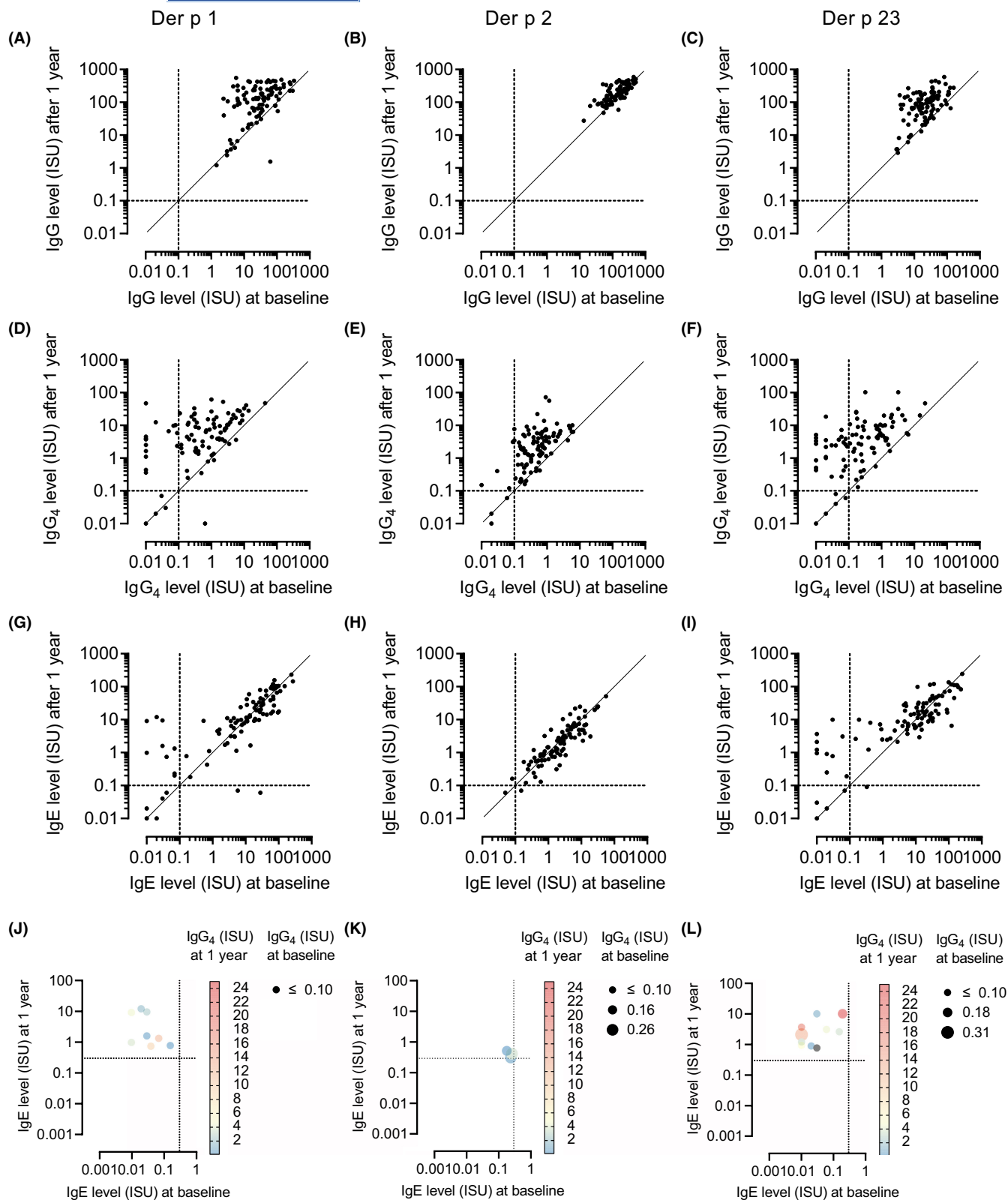


FIGURE 5 Evolution of IgG, IgG₄ and IgE levels before and after 1 year of AIT. Scatter plots of serum levels (ISU) of (A–C) IgG, (D–F) IgG₄ and (G–I) IgE to Der p 1 (left panel), Der p 2 (middle panel) and Der p 23 (right panel) at baseline and after immunotherapy for the whole study population ($n = 100$). Positivity thresholds were 0.3 and 0.1 for IgE/IgG and IgG₄ levels, respectively (dotted lines). Plain lines represent identity lines. (J–L) Bubble plots focusing on patients whose IgE levels switched over the 0.3 ISU IgE positivity threshold after 1-year immunotherapy. IgE levels at baseline and after 1 year are plotted on x and y axes, respectively. Bubble size is representative of IgG₄ level at baseline while colour is representative of IgG₄ level after 1 year

IgG₄ notably increased after AIT.^{44,45} Furthermore, we observed a trend for allergen-specific IgE decrease in subjects with boosted allergen-specific IgG/IgG₄ levels (Figure S10).

No major change was observed in prevalence of IgE reactivity after 1 year treatment. In agreement with previous studies reporting limited neosensitizations in patients undergoing AIT,^{33,34,46,47} we observed in the present study, a few patients with IgE levels rising above the 0.3 ISU positivity threshold. In most cases, the induction of IgE was counterbalanced by an increase of IgG/IgG₄. Our results do not strongly support de novo sensitization but rather more likely reflect natural variations with IgE levels fluctuating around positive cut-off values. A second explanation may be that the induction of IgE is due to natural exposure and evolution of reactivity pattern. A third one may be that such increase in IgE levels corresponds to a boost of pre-existing responses which could not properly be detected by our method, as previously reported.⁴⁸

Previous studies reported a trend for simple pattern to better respond to AIT.^{33,34} In our study, no significant difference appear between high and low responders suggesting that the complexity of IgE reactivity profiles was not predictive of the clinical response. Multiple reasons could account for the different findings between studies. The treatments administered differed in composition, route of administration, and degree of efficacy. The allergen repertoire of the AIT extracts used, both from qualitative and quantitative aspects, can possibly result in mismatches with the profile of the patients. Last, different extent of mite allergen avoidance during the trial among high and low responders may have significantly contributed to different disease progression trajectories within our study population, thus masking the putative impact of the patient's IgE molecular sensitization profile and the response to sublingual allergen immunotherapy. However, we were not able to measure the concentration of mite allergens in the patient's home during the treatment period to exclude this possibility.

Induction of IgG and IgG₄ responses to major allergens was seen in both high and low responders upon AIT. However, the observation that low responders also increase Der p 1, Der p 2 and Der p 23-specific IgG/IgG₄ antibody responses is somehow intriguing. One explanation, in line with our recent study showing a differential immune behaviour between high and low responders, is that the immune history of patients and intrinsic characteristics of their immune system impede optimal responses to standard protocol of AIT. Adapting treatment modalities by increasing the dose using a drop AIT platform might be an option taking into account patient's reactivity threshold, the latter agreeing with personalized practices already observed among physicians (our unpublished data, Ref. [49]).

The clinical relevance of the Der p 23 allergen is still a matter of debate. Previous studies of subcutaneous or sublingual AIT studies have shown limited even no response to Der p 23.^{33,34,50} Our study provides new insights showing that strong IgG and IgG₄ immune responses to Der p 23 occurred upon sublingual AIT with 300 IR HDM tablets, thus indicating a sufficient content of biologically active Der p 23 in the tablet to trigger an immune response. Besides, Der p 23 has been reported to be associated with asthma.¹⁵

Its role in the physiopathology of allergic asthma has been studied in a longitudinal analysis of the IgE reactivity profile in more than 700 German participants. Der p 23 was found to be among the most early recognized molecules.^{16,27} Furthermore, early IgE sensitization to Der p 23 and also to Der p 1, but not to Der p 2, was significantly associated with a higher risk of asthma appearing at school age. A recent real-life study conducted in 519 Italian patients confirms that Der p 23 is associated with asthma, particularly in Der p 23 mono-sensitized patients.²⁸ Herein, we confirm higher levels of IgE to Der p 1 and Der p 23 in asthmatic patients and extend this observation to IgG₄, representing an IgG subclass which is also controlled by IL-4.³⁷ Together from all these observations, we may expect Der p 23 to be clinically relevant for patients exhibiting high sensitization or monosensitized to Der p 23 (up to 5% of HDM allergic patients^{13,41,43}) but also patients sensitized to Der p 1 and Der p 23 or Der p 2 and Der p 23 (respectively 5% and 10% of our study cohort).

In summary, our study indicate that molecular reactivity profile is not the only factor predicting AIT efficacy. Markedly, strong humoral responses to Der p 1, Der p 2 and Der p 23 occur in mostly all patients undergoing 1-year sublingual AIT. Our data also suggest that IgG/IgG₄ responses to Der p 23 may be particularly beneficial for patients mainly sensitized to this allergen. Altogether, our finding paves the way for a better understanding of the biological effect of sublingual AIT and provide converging arguments favouring personalized medicine for allergy treatment.

AUTHOR CONTRIBUTIONS

LM and VBLF designed the research. LM, VBLF, PM, RV and SV supervised the research. EP, TS, HJH performed the experiments. LM, VBLF, PM, RV and WC analyzed the data. LM and VB wrote the paper. PM, RV and WC revised the paper.

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CONFLICT OF INTEREST

Co-authors (LM and VBLF) are employees at Stallergenes Greer. GWC reports having received in the last 3 years research grants as well as lecture or advisory board fees from Alk-Abello, Allergy Therapeutics, Anallergo, Hal Allergy and Stallergenes Greer. PM reports grants and personal fees from TPS Software Production, outside the submitted work. RV has received research grants from HVD Life-Sciences, Vienna, Austria, WORG Pharmaceuticals, Hangzhou, China, Hipp, Pfaffenhofen, Germany and from Viravaxx AG, Vienna, Austria. He also serves as consultant for Viravaxx AG and WORG Pharmaceuticals.

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SUPPORTING INFORMATION

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