

REVIEW

Molecular approaches to allergen-specific immunotherapy: Are we so far from clinical implementation?

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Abstract

Conventional allergen-specific immunotherapy (AIT), based on administrations of allergen extracts, represents up to now the unique protocol for the desensitization of allergic patients. Whereas the effectiveness of AIT was evidenced for the treatment of allergic rhinitis and allergic asthma, such strategy remains experimental for food allergies up to now. However, important issues are commonly associated with AIT as the quality of natural allergen extracts, the long duration and adverse side-effects which negatively affect successful desensitization together with the patient compliance. The rapid progression of molecular allergology made possible the quest of safer, shorter and more effective immunotherapeutic approaches. The aim of this review was to provide an update on these different innovative recombinant derivatives including their efficacy but also their limitations. Despite promising preclinical and early clinical studies, the absence of convincing data in large phase III trials precludes so far the translation of these immunotherapeutic candidates into the clinic.

KEYWORDS

allergen-specific immunotherapy, blocking antibodies, clinical trial, efficacy, hypoallergenic, recombinant derivatives, safety, Treg

1 | INTRODUCTION

Allergen-specific immunotherapy (AIT) is an immune tolerance-inducing treatment used for the desensitization of allergic patients. Conventional AIT is until now based on either subcutaneous (SCIT) or sublingual (SLIT) administrations of natural allergen extracts.¹ Although the clinical efficacy of AIT is well confirmed for the reduction in the symptoms associated with allergic rhinoconjunctivitis or asthma and venom allergy, oral AIT (OIT) for food allergy is not recommended yet as routine practice.² The immunological mechanisms leading to successful AIT remain to be fully elucidated but are so far characterized by the rapid desensitization of basophils/mast cells, the induction of regulatory T (Treg) and B (Breg) cells followed by the up-regulation of allergen-specific blocking IgG antibodies (particularly IgG4 isotype) and the decrease in allergen-specific IgE (the mechanisms of AIT have been extensively reviewed in Ref. ³). Several important problems can negatively impact on the success of AIT: (a)

the allergen extracts are actually mixtures of allergenic and non-allergenic contaminating proteins. Strikingly, the presence in extracts of non-allergenic substances such as phytoprostane, LPS, chitin or β -glucans was shown to promote and amplify the Th2-biased allergic response.⁴⁻⁶ Some clinically relevant allergens can be absent from these extracts (as for the major HDM allergen Der p 23). (b) IgE-mediated early side-effects and late T cell-mediated adverse events are often observed. (c) The development of immune tolerance requires frequent allergen administrations over two to five years, leading to poor patient compliance.^{7,8} The direct (allergen extracts) and indirect treatment (notably the frequent visit to physicians for SCIT) costs for complete SLIT/SCIT could lead as well to treatment discontinuation. As example, total cost of SLIT/SCIT can reach 4900-6300 CAD in Canada (2019), 3200-3800 EUR in Denmark (2016), respectively.^{9,10}

Thanks to the molecular and immunological characterization of clinically relevant allergens from different sources, several

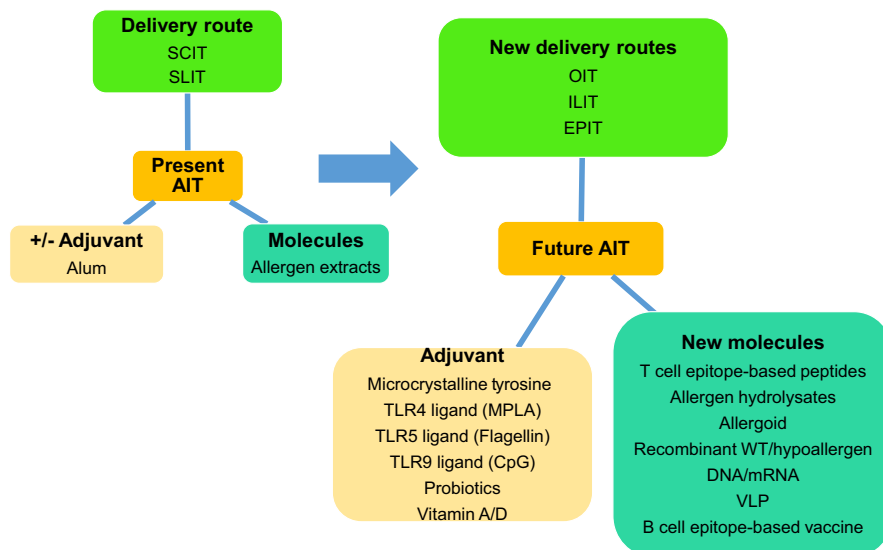


FIGURE 1 Design of safer and more effective AIT. In order to improve conventional allergen extract-based AIT, next-generation treatments need optimal combinations of non-allergenic molecules, adjuvant as well as ideal delivery route

innovative immunotherapeutic approaches were continuously designed to replace commonly used allergen extracts in the goal to improve the safety and efficacy of AIT while reducing the duration of treatment.

The present review will describe the evolution of molecular AIT with a special focus on their evaluation in clinical trials. Moreover, as adjuvant composition and optimized delivery route frame the quality of immune responses to any antigen (Figure 1), the formulation with adjuvants together with innovative route of AIT administration will be discussed as well.

2 | NATURAL OR RECOMBINANT WILD-TYPE ALLERGENS

In different trials (Table 1), treatment with purified and standardized natural (n) or recombinant (r) major allergens significantly improved symptom and medication scores as well as reduced allergen-specific skin responses compared to placebo. Typically, up-regulation of specific IgG1 or IgG4 antibodies was accompanied by down-regulated allergen-specific IgE. Few local but not systemic adverse effects were observed along the studies. Unfortunately, only one phase II trial performed a head-to-head comparison between natural/recombinant allergen and corresponding extracts in the context of birch pollen allergy.¹¹ The clinical improvement obtained with natural or rBet v 1 was similar to that reached with the pollen extracts. However, these single molecule-based treatments did not induce new sensitizations whereas de novo IgE responses to Bet v 2 were measured in 10% of the patients treated with birch pollen extracts. SLIT protocol based on rBet v 1 administrations was similarly safe but promoted a modest significant reduction in average adjusted symptom scores, whatever the dose tested (12.5–50 µg).¹² The degree of adverse reactions observed at application sites, as oral pruritus and throat irritation, was similar to those previously observed from allergen extract-based SLIT. Of

note, a first phase II based on Mal d 1 apple allergen evidenced the safety and moderate efficacy of molecular SLIT in the context of food allergy.¹³

3 | T CELL EPIOTOPE PEPTIDES

Synthetic peptides displaying immunodominant T cell epitopes of major allergens but lacking IgE reactivity could trigger immune tolerance by different cellular mechanisms: induction of antigen-specific hyporesponsiveness (anergy), development of allergen-specific Th1 cells, and clonal deletion of allergen-specific Th2 cells together with the recruitment of Treg cells (Table 2). In contrast, such AIT candidates, through the absence of B cell epitopes, are poorly capable to modulate the antibody response. A first pilot study demonstrated that peptides derived from bee venom phospholipase A2 T cell epitopes successfully suppressed bee venom allergy in sensitized patients through T cell anergy.¹⁴ Later, different phase IIb trials showed that peptide immunotherapy was clinically effective in the context of cat, grass pollen or HDM allergy^{15–17} to significantly reduce total rhinoconjunctivitis symptom score (TRSS) in an environmental exposure chamber (EEC) model. Regrettably, these promising benefits were not reproduced in large phase III field study for cat or HDM allergy¹⁸ as both active treatment and placebo similarly reduced by 60% combined symptom and medication scores. For this reason, these peptide-based AIT programmes were discontinued.

Later, another approach consisted of contiguous overlapping peptides (COPs), carrying all the potential T cell epitopes from allergen without IgE binding activity. In a phase IIb dose-finding study, AIT with Bet v 1-derived COPs significantly reduced combined daily rhinoconjunctivitis symptom and medication scores compared with placebo group (around 26% improvement for the 50 µg group).¹⁹ Of note, these COPs elicited the production of Bet v 1-specific IgG4 antibodies. Bet v 1-specific IgE levels increased following COP treatment as well but remained unchanged during the pollen season.

TABLE 1 Natural/recombinant wild-type allergens

Target	Molecule	Route (number of injection/ application)	Phase (number of patients)	Length of treatment	Primary outcome	Secondary outcome	References
Birch pollen	rBet v 1, nBet v 1, birch pollen allergen extract	SCIT (36)	II (134)	12 weekly injections and monthly maintenance doses for 2 y	Reduction (~ 50%) in RH for rBet v 1, nBet v 1 and birch extract vs placebo ($P = .0002$, $.0006$, $.0024$, respectively) Reduction (~ 63%-70%) in RMS for rBet v 1, nBet v 1 and birch extract vs placebo ($P = .0011$, 0.0025 and $.0063$, respectively)	Reduction (~ 50%) in skin reactivity for the 3 active groups vs placebo ($P < .0001$) Increase in Bet v 1-specific IgG1, IgG4 and IgG2 in the 3 active groups	11
Birch pollen	rBet v 1 tablet	SLIT (150)	II (483)	4 mo pretreatment (before pollen season) to 1 wk after the pollen season	Reduction (~ 17%) in AAdSS for rBet v 1 vs placebo ($P < .03$) Reduction (~ 30%) in ARMS for rBet v 1 vs placebo ($P < .001$)	–	12
Birch pollen-associated apple allergy	rMal d 1	SLIT (120)	II (60)	4 mo	Reduction of oral symptoms with rMal d 1 vs placebo (70% vs 42%, $P = .001$)	Reduction in skin reaction with rMal d 1 vs placebo ($P = .022$) Enhanced IgG4/IgE ratios in rMal d 1-treated group vs placebo ($P = .012$).	13
Grass pollen	rPhl p 1, rPhl p 2, rPhl p 5a, rPhl p 5b and rPhl p 6	SCIT (median number 25)	I (62)	21 mo	Reduction (38.5%) in CSMS for active vs placebo ($P = .051$, non-significant)	Improvement in RQLQ for active group vs placebo ($P = .024$)	99
Grass pollen	rPhl p 1, rPhl p 2, rPhl p 5a, rPhl p 5b and rPhl p 6	SCIT (13)	II (50)	2-4 mo	Safety: systemic reactions for 16% of patients on active treatment	Improvement (67%-90%) in CPT for active group but also for placebo (50%, $P = .047$) Increase in IgG4 level for active group vs placebo ($P < .01$). No change in specific IgE	100
Fungus	nAlt a 1	SCIT (32)	I (111)	2 years	Reduction in CSMS (63%) for high-dose treatment vs placebo ($P = .046$)	Reduction in skin reaction with nAlt a 1 vs placebo ($P < .05$) Increase in sIgG4 in treated groups vs placebo ($P = < .05$)	101

Abbreviations: AAdSS, Average Adjusted Symptom Score; CPT, Conjunctival Provocation Test; CSMS, Combined Symptom and Medication Score; RH, Rhinoconjunctivitis Symptoms; RMS, Rescue Medication Score; RQLQ, Rhinitis Quality-of-Life Questionnaire; SMS, Symptom Medication Score.

TABLE 2 T cell and B cell epitopes

Target	Molecule	Route (number of injection/ application)	Phase (number of patients)	Length of treatment	Primary outcome	Secondary outcome	References
Grass pollen	Cyn d 1, Lol p 5, Dac g 5, Hol I 5, and Phl p 5 T cell epitope peptides	Intradermal (4 or 8)	II (282)	14 wk	Reduction (42%) in TRSS for 8x6Q2W vs placebo ($P = .0346$)	Reduction (43%) in TNSS and TNNSS for 8x6Q2W vs placebo ($P = .0447$ and $P = .0765$ respectively).	15
Cat	Fel d 1 T cell epitope peptides	Intradermal (4 or 8)	II (202)	14 wk	Reduction in TRSS for 4x6Q2W vs placebo ($P = .0104$).	Reduction in TNSS and TOSS for 4x6Q2W vs placebo ($P = .02$ and $P = .012$ respectively).	16
Birch pollen	Bet v 1 derived contiguous overlapping peptides (COPs)	SCIT (5)	IIb (239)	2 mo	Reduction (26%) in combined RSMS for 50 μ g dose vs placebo ($P = .015$)	Reduction (~27%) in MiniRQLQ score for active groups vs placebo ($P = .011$) Reduction (~35%) in NNSS for active group vs placebo ($P = .014$)	19
Bee Venom	bee venom phospholipase A2 peptides mapping the full allergen sequence	SCIT (12)	I (16)	70 d	T cell tolerance occurring after day 42 in active groups vs placebo ($P < .01$)	–	102
Ragweed	Amb a 1 T cell epitope peptides	SCIT (no data)	II (275)	No data	Reduction in TRSS for active group vs placebo ($P < .05$)	–	103
HDM	T cell epitope peptides from major HDM allergens	Intradermal (4)	II (50)	16 wk	Reduction (~35%) in CPT score for 3nM dose vs placebo ($P = .0257$) Reduction (~39% and 51%) in EPSR and LPSR respectively, for 3nM dose vs placebo ($P = .0257$)	–	104
Grass pollen	B cell epitopes of Phl p 1, Phl p 2, Phl p 5, and Phl p 6 (BM32)	SCIT (3)	II (70)	9 wks	Reduction (~20%-24%) in TNSS for 40-20 μ g dose vs placebo ($P = .003$, 0.03 respectively)	Reduction (~11%-24%) in TOSS for 10- 40 μ g dose vs placebo (No P value) Reduction (~12%-17%) in skin reaction for 20- 40 μ g dose vs placebo (No P value)	25
Grass pollen	B cell epitopes of Phl p 1, Phl p 2, Phl p 5, and Phl p 6 (BM32)	SCIT (7)	IIb (181)	14 mo	Reduction (22%) in combined SMS for pooled active groups vs placebo (But $P = .085$)	Reduction (24%) in SS for pooled active groups vs placebo ($P = .026$)	26

Abbreviations: 8x6Q2W, grass allergen peptides at 6 nmol at 2-wk intervals for a total of 8 doses; CPT, Conjunctival Provocation Test; EPSR, Early Phase Skin Response; LPSR, Late Phase Skin Response; MiniRQLQ, Mini-Rhinoconjunctivitis Quality-of-Life Questionnaire; NNSS, Night-time Nasal Symptom Score; RSMS, Combined daily Rhinoconjunctivitis Symptom and Medication Score; SMS, Symptom Medication Score; SS, Symptom Score; TNNSS, Total Non-Nasal Symptom Score; TOSS, Total Ocular Symptom Score; TRSS, Total Rhinoconjunctivitis Symptom Score. Bold values indicate B cell epitope-based molecular AIT.

TABLE 3 Hypoallergenic derivatives

Target	Molecule	Route (Number of injection/ application)	Phase (Number of patients)	Length of treatment	Primary outcome	Secondary outcome	References
Birch pollen	rBet v1 fragment and rBet v1 trimer	SCIT (8 + unknown number of maximal dose)	II (124)	Unknown	Increase in specific IgG level for active groups vs placebo ($P < .001$) Reduction (no % provided) in cutaneous reaction for active groups vs placebo ($P < .05$) Reduction in pollen season IgE boosting for trimer vs. placebo. ($P < .01$) and fragments vs placebo ($P < .02$)	-	31
Birch pollen	rBet v1 fragments and rBet v1 trimer	SCIT in non-allergic subjects (4)	I (16)	48 wk	Significant increase in Bet v 1-specific IgG in active groups vs placebo	-	33
Birch pollen	rBet v 1-FV (folding variant), pollen extracts (comparator)	SCIT (11-12 for rBet v 1-FV, 14-18 for pollen extract)	II (51)	6 mo in 2 consecutive years	No significant reduction in combined SMS score for active group vs allergen extracts ($P = .330$) after year 1 and year 2 ($P = .812$).	No significant increase in specific IgG1, IgG4 for active group vs allergen extracts (no P value) after year 1 and year 2 (no P value). No significant change in NPT for active group vs allergen extracts (no P value) after year 1 and year 2 (no P value).	34
Peanut	<i>Escherichia coli</i> - encapsulated modified rAra h 1, rAra h 2 and rAra h 3	Rectal administration (4 for healthy, 13 for peanut- allergic patients)	I (15)	4 wk for healthy, 16 wk for peanut- allergic patients	Adverse reactions in 50% of peanut- allergic patients between doses 9-12. Interruption of the protocol	Skin reaction reduced after treatment ($P = .02$) No significant changes for total IgE, peanut IgE, or peanut IgG4.	35
Birch pollen	rBet v 1-FV (folding variant)	SCIT (10)	II (36)	10 wk	2.8- to 3.35-fold higher TSS decrease for all active treatment groups vs placebo (at least $P = .0009$)	Increase in IgG1 level for active groups vs placebo (at least $P = .0419$)	105

Abbreviations: NPT, Nasal Provocation Test; SMS, Symptom Medication Score; TSS, Total Symptom Score

These IgE levels reached the ones measured in the placebo group after seasonal exposure.

Allergen hydrolysates broaden the number of potential T cell epitopes to optimize T cell and B cell responses while maintaining hypoallergenicity. A phase IIb SCIT study assessed the efficacy and the safety of short-course AIT with peptide hydrolysates from *Lolium perenne* (LPP) pollen allergens.²⁰ The treatment induced a modest reduction in the combined symptom and medication score (18% along the entire pollen season). Compared with placebo, treatment with LPP decreased reactivity to conjunctival provocation test and, during the pollen season, rhinoconjunctivitis quality-of-life (QOL) global score. Equivalent moderate improvement was previously reported for short-term grass pollen immunotherapies with adjuvanted extracts.²¹ Around 10% of LPP-treated patients developed mild early systemic reactions in a 30 minutes of period post-administration. Finally, a small phase III trial (N = 21) confirmed the benefits from short-course LPP-based AIT (more than 50% reduction in combined symptom and rescue medication scores throughout the pollen season).²² Immunological mechanisms associated with successful LPP immunotherapy were characterized by the reduction in basophils, seasonal grass pollen-specific IgE as well as IL-4 + Th2 cells, the up-regulation of Foxp3+, follicular regulatory T and IL-10 + Breg cells together with allergen-specific IgG4-blocking antibodies. However, effectiveness of allergen hydrolysates needs firm confirmation as such strategy tested in the context of HDM allergy did not mediate long-term improvement of symptoms and generation of blocking antibodies.²³

4 | ALLERGEN-DERIVED B CELL EPITOPES

This approach consists of genetic fusion of peptides derived from the IgE binding epitopes of major allergens to a protein carrier. As IgE binding epitopes are mainly conformational, these surface-exposed peptides are hypoallergenic. Moreover, the lack of allergen-specific T cell epitopes would reduce drastically the T cell-mediated late adverse events, whereas the carrier-specific T cells mediate the activation of allergen peptide-specific B cells.

The first recombinant B cell epitope-based molecule tested in human cohorts, BM32, consisted of four proteins carrying hypoallergenic peptides from Phl p 1, Phl p 2, Phl p 5 and Phl p 6, respectively, and fused to hepatitis B preS protein²⁴ (Table 2).

A safety and dose-finding phase II study based on three subcutaneous injections of BM32 formulated with alum demonstrated that the fusion proteins did not elicit early side-effect even at a dose of up to 160 µg although few systemic late-phase reactions were observed.²⁵ BM32 treatment decreased slightly the total nasal symptom scores (TNSS) (20% and 24% reduction for the 40 and 20 µg dose-based treatment, respectively). No significant change in TNSS was observed in the 10µg dose active and placebo groups). Grass pollen allergen-specific IgG1 and IgG4 responses were elicited in vaccinated patients, and these antibodies were able to inhibit allergen-induced basophil degranulation and

T cell proliferation. No boost of allergen-specific IgE responses was observed in the BM32-treated groups. In a subsequent phase IIb efficacy trial, improvement concerning symptom medication, rhinoconjunctivitis quality-of-life questionnaire (RQLQ) and asthma symptom scores was observed in low- or high-dose BM32-treated patients over two seasons.²⁶ However, this positive trend did not reach significance for the primary end-point (daily combined symptom and medication scores). The humoral and cellular immune responses were characterized in detail for five patients from this study (five BM32 injections) and compared with the ones developed following conventional AIT with grass pollen extracts (thirty-five injections).²⁷ Both treatments triggered similar reduction in IL-5 + Th2 cells and transient induction of allergen-specific IL-10 + T cells. Extract-based AIT but not BM32 boosted the allergen-specific IgE response, whereas conventional AIT promoted slightly more allergen-specific IgG antibodies than the recombinant derivative.

5 | HYPOALLERGENIC DERIVATIVES

The disruption of IgE binding epitopes generates hypoallergenic derivatives which display very low IgE reactivity but conserve their capacity to generate IgG antibody and T cell responses. However, the mapping of the IgE binding epitopes, a key prerequisite step for such development, is challenging as they are mainly conformational.²⁸ Alternative method consists of chemical modification of allergen extracts to derive allergoids with reduced allergenicity (their clinical efficacy is discussed in Chapter 9).²⁹

During the last decade, a collection of preclinical studies characterized recombinant hypoallergenic variants derived from multiple allergenic sources including HDM, animal dander, pollen, fungi or food (for a review, see Ref. ³⁰).

Although their hypoallergenicity, characterized notably by the absence of basophil activation, was clearly evidenced together with their ability to induce T cell response and blocking IgG antibodies, the therapeutic potential of these AIT candidates was poorly investigated in corresponding animal models of allergy. Recombinant hypoallergenic allergen derivatives have been evaluated in clinical trials for the treatment of birch pollen and peanut allergy (Table 3).

A first phase II trial assessed the clinical efficacy of preseasonal AIT using hypoallergenic Bet v 1 fragments or trimer in birch pollen-allergic patients.³¹ Interestingly, these two Bet v 1-derived hypoallergens triggered specific blocking IgG antibodies capable to inhibit immediate phase reactions. These vaccinations down-regulated the typical boost of allergen-specific IgE observed during the birch pollen season. Despite these positive effects, the analyses of the combined symptom and medication scores did not show significant differences between the vaccinated groups and placebo.³² It must be pointed out systemic adverse effects appeared several hours following AIT and more frequently with fragments, suggesting that these side reactions are more likely T cell than IgE-mediated.³² Recently, Bet v 1 fragments were assessed as well as

a prophylactic vaccine in a phase I trial using a cohort of non-allergic individuals.³³ The actively treated patients developed large production of Bet v 1 blocking IgG antibodies. Although mild Bet v 1-specific sensitizations were measured, such specific IgE titer was unable to trigger skin reactions to birch pollen, demonstrating that the blocking antibodies are capable to prevent the immediate phase reaction.³³

Another preseasonal phase II study compared the efficacy of a folding variant of Bet v 1 (rBet v 1-FV) with the conventional birch pollen extracts. No statistical difference between AIT using rBet v 1-FV or pollen extracts could be observed in terms of symptom medication score and development of birch pollen-specific IgG1 and IgG4.²⁷

Hypoallergenic rAra h 1, rAra h 2 and rAra h 3 peanut allergens, encapsulated together in heat/phenol-killed *E coli*, were studied in a phase I trial. Rectal administrations induced severe adverse reaction in 50% of the treated peanut-allergic patients, leading the discontinuation of the study. Of note, the formulation vaccine did not influence the level of total IgE, peanut IgE or peanut IgG4.³⁵

6 | DNA/mRNA-BASED PLATFORMS

Preclinical studies showed that DNA/mRNA encoding allergens are promising AIT approaches through the induction of allergen-specific Th1 responses characterized by the suppression of allergen-specific IgE and the development of specific IgG2a and IFN- γ .³⁶ However, DNA-based vaccines are commonly poorly immunogenic in primates and humans and particularly when administered by conventional injection. The current anti-allergy DNA molecules tested in phase I/II were optimized for the MHC-II presentation by the fusion with sequence encoding lysosomal-associated membrane protein (LAMP) (Table 4). Conventional intramuscular administration of cedar pollen allergen Cry j 2-LAMP DNA demonstrated the safety of the treatment in Japanese red cedar/mountain cedar-sensitized patients.³⁷ Conversion of some cedar pollen-specific prick tests from positive to negative was observed, and similar results were also obtained for unrelated allergens, suggesting a possible bystander suppressive effect. Specific IgE titers remained unchanged, and no significant development of specific IgG was observed. However, these results could be explained by the absence in the recombinant DNA plasmid of sequence encoding leader peptide leading to intracellular antigen expression.

Phase I studies testing ARA-LAMP-vax, a single multivalent peanut Ara h 1, h 2, h 3 LAMP DNA, were conducted (NCT03101267 and NCT02851277), but the results were not published.

mRNA encoding allergens could be considered safer than corresponding DNA due to short in vivo lifetime limiting allergen expression and the absence of integration into the host genome. In mice, prophylactic vaccinations with mRNA encoding Phl p 5 remarkably triggered long-term Th1-biased memory responses to prevent grass pollen allergy.³⁸ The development of mRNA-based strategies was mainly impaired by the potent innate immunogenicity of mRNA and

inefficient in vivo delivery. Nucleoside-modified and purified synthetic mRNA drastically reduced the innate immune activation, and its delivery in association with lipid nanoparticles (LNPs) amplified the specific immune response, respectively.³⁹ However, such new mRNA platform remains to be evaluated in the context of allergic diseases.

7 | VLP PLATFORM

Virus-like particles (VLPs) represent a promising platform for AIT. Not only their size (20-200 nm) optimizes the uptake by antigen-presenting cells as well as the lymphatic trafficking⁴⁰ but also antigen display in repetitive structures promotes the cross-linking of B cell receptors for the development of potent antibody responses.⁴¹ Three main VLP-based strategies were designed for the treatment of allergic diseases: VLP packaged with TLR9 ligand CpG motifs and particles displaying Pro-Th2/Th2 cytokines or allergens (full length or B cell epitopes).⁴² The packaging of CpG into VLP not only reduced the side-effects commonly observed with free CpG but also optimized the TLR9 activation in plasmacytoid dendritic cells (pDCs) and B cells leading to the induction of Th1 cytokines and IgG class switching. As arrays of self-antigens on VLPs can trigger strong antibody response to these self-antigens,⁴³ the particulation of Pro-Th2 (IL-33 notably)/Th2 (IL-4, IL-5, IL-13) cytokines could break the self-tolerance to down-regulate the Th2 response.

Remarkably, preclinical studies evidenced that VLP displaying full-length cat allergen Fel d 1 was hypoallergenic through its very weak binding capacity to immobilized IgE and inability to activate basophils but trigger potent IgG responses.⁴⁴ Similar results were recently obtained with VLP displaying the HDM allergen Der p 2. Moreover, VLP-Der p 2 triggered strong allergen-specific blocking IgG and prevented the development of the HDM-allergic response in mice.⁴⁵ Promising preclinical efficacy data were produced as well with VLP displaying peanut allergen Ara h 1 or Ara h 2.⁴⁶ The protective effect of these two molecules was also mediated by the IgG production.

Clinical evaluation of VLP-based strategies for the treatment of allergic disease was restricted to VLP encapsulating CpG type A or displaying a Der p 1 B cell epitope (Table 4).⁴⁷⁻⁵⁰ Efficacy trials showed that VLP-CpG are safe and could induce a significant improvement in rhinoconjunctivitis symptoms, quality-of-life scores in patients suffering from allergic rhinitis to HDM.⁴⁹ However, the effectiveness of these nanoparticles was more modest for the control of asthma during steroid withdrawal.⁵⁰ Although clinical evaluations of VLP displaying cytokines or full allergens remain to be fully investigated, two recent animal studies evidenced their high propensity to break self-tolerance leading to potent polyclonal neutralizing responses. Vaccinations of cats with VLPs displaying full Fel d 1 reduced the endogenous allergen level and allergenicity of cat tear samples.⁵¹ The generation of anti-eIL-5 response in horses following administrations of VLP-equine IL-5 (eIL-5) could reduce eosinophil-mediated seasonal insect-bite hypersensitivity (IBH).⁵²

TABLE 4 DNA/mRNA/VLP platforms

Target	Molecule	Route (Number of injection/ application)	Phase (Number of patients)
Japanese red Cedar pollen	DNA encoding fusion rCry j 2-lysosomal-associated membrane protein 1 (Cry j 2-LAMP)	Intramuscular (4 for phase Ia, 5 for phase Ib)	Ia (24) Ib (15)
Japanese red Cedar pollen	DNA encoding fusion Cry j 2-LAMP	Intradermal	II (150)
Peanut	DNA encoding fusion multivalent rAra h 1, h 2, h 3-lysosomal-associated membrane protein (ARA-LAMP-Vax)	Intradermal/ Intramuscular	I (30)
HDM	Linear Der p 1 B cell epitope conjugated to bacteriophage Q β -derived VLP (Q β -Der p 1)	SCIT/Intramuscular (3)	I (24 healthy subjects)
HDM	HDM allergen extracts mixed with Q β packaging A-type CpG motif (CYT003-QbG10)	SCIT (2 without QbG10, 6 with QbG10)	I/IIa (20)
HDM	CYT003-QbG10	SCIT (6)	phase IIb (299)
Persistent allergic asthma	CYT003-QbG10	SCIT (7)	phase II (63)

Abbreviations: ACQS, Asthma Control Questionnaire Score; ASMS, Asthma Symptom and Medication Score; CPT, Conjunctival Allergen Provocation Test; CSMS, Combined Symptom and Medication Score; DNA encoding allergens; MiniRQLQ, Mini-Rhinoconjunctivitis Quality-of-Life Questionnaire; RH, Rhinoconjunctivitis; SPT, Skin Prick Test

Bold values indicate B cell epitope-based molecular AIT.

8 | MAT FUSION OR MHC CLASS-II TARGETING ALLERGENS

To optimize major histocompatibility class-II (MHC-II) antigen presentation, allergens could be fused to the modular antigen translocating (MAT) module. MAT consisted of HIV-1 TAT-derived translocation peptide with the intracellular domain from Ii invariable chain for efficient targeting to endosomal/lysosomal compartments.

MAT module drastically increased cat allergen Fel d 1 allergen uptake by PBMCs. Moreover, restimulation of PBMCs from cat allergic donors with MAT-Fel d 1 induced a shift from Th2 to Th1/Treg in the cytokine profile (high levels of IFN- γ , IL-10 together with a reduction in IL-4, IL-5).⁵³ Intralymphatic administration of MAT-Fel d 1 promoted Th1-biased Fel d 1-specific responses capable to suppress established Fel d 1-induced sensitization.⁵⁴ Intralymphatic immunotherapy (ILIT, see below) with MAT-Fel d 1 was shown to significantly increase allergen tolerance compared with the placebo in titrated nasal provocation and skin prick tests. These positive effects were

associated with stimulation of IL-10 + Treg and increased levels of cat dander-specific IgG4.^{55,56}

9 | NEW ROUTES FOR AIT

One way to improve the patient compliance with AIT is to explore innovative routes of administration which could shorten the duration of treatment. Intralymphatic immunotherapy (ILIT) consists of few ultrasound-guided injections of low allergen doses into inguinal lymph nodes. This route of administration improves the antigen presentation and avoids leakage of allergens in blood vessels and tissues rich in mast cells to minimize local and systemic side-effects. Although few ILIT randomized controlled clinical trials were published to date (reviewed in Ref. ⁵⁷), the clinical data showed that short-course ILIT reduced the risk of systemic adverse effects and improved clinical symptoms with the same efficiency than three to five years of conventional SCIT. These promising efficacy data need to be consolidated through more clinical trials to evaluate ILIT long-term effects.

Length of treatment	Primary outcome	Secondary outcome	References
42 d for phase Ia, 300 d for phase Ib	No systemic side-effects, only local reaction at injection site (no <i>P</i> value) SPT-negative conversion for or JRC/MC/unrelated allergens (no <i>P</i> value)	No significant increase in levels of specific IgG and IgE (no <i>P</i> value for IgG, at least <i>P</i> > .18 for IgE)	37
18 mo	No published results		NCT03101267
2 yr	No published results		NCT02851277
3 mo	No systemic side-effects, only local reaction at injection site (no <i>P</i> value) Potent anti-Der p 1 peptide IgG1/IgG3 responses. No specific IgG4	–	47
9 wk	Improvement in CPT (100-fold median individual increase in allergen tolerance, <i>P</i> < .0001) Reduction in RH and asthma symptoms scores (RH: from 10.5 to 2, <i>P</i> < .0001; asthma from 2 to 0, <i>P</i> < .0001)	–	48
6 wk	Reduction (68%) in CSMS post-treatment for active group vs placebo (<i>P</i> = .04). But no significant differences for pre- to post-treatment comparisons	Reduction in MiniRQLQ (35%-41%) for active groups vs placebo post-treatment (at least <i>P</i> = .0498) Improvement in CPT (10-fold median individual increase in allergen tolerance, <i>P</i> < .007) for active groups vs placebo	49
10 wk	Reduction (17%) in ASMS for active group vs placebo (<i>P</i> = .001) Improvement in ACQS (9%) for active group vs placebo (<i>P</i> = .002)	–	50

Epicutaneous route of administration prevents allergen absorption into the systemic circulation and maximize antigen presentation as epidermal layer of human skin is rich in antigen-presenting Langerhans cells (LCs).⁵⁸

Epicutaneous immunotherapy (EPIT) at low dose was shown to be particularly interesting for the treatment of food allergy as the daily exposure of food allergens at high doses in SCIT or oral immunotherapy (OIT) often generated severe and mild systemic side-effects, respectively.⁵⁹

Several placebo-controlled, double-blind trials evaluating EPIT for the treatment of cow's milk, peanut or grass pollen allergy evidenced the high safety and tolerability profiles of these approaches (reviewed in Ref. ^{60,61}). Notably and contrary to OIT, EPIT administrations of food allergens prevented persistent GI symptoms or eosinophilic esophagitis. However, it must be pointed out that intradermal EPIT can induce mild symptoms localized to the patch sites. In a phase IIb trial, a one-year-long low-dose peanut EPIT delivered through occlusive patch induced a modest but statistically significant treatment effect compared with the placebo (defined as a 10-fold or higher increase in successfully consumed dose along oral food challenge from

baseline to week 52), the highest positive changes being mainly observed in 4- to 11-year-old children cohorts.⁶² A second phase 2b randomized trial confirmed that 250 µg peanut patch reached primary efficacy end-point vs placebo patch following one-year EPIT.⁶³

However, EPIT induced lower positive effects than reported high-dose OIT following one year of therapy.^{60,61} Extension of EPIT treatment to two years could increase the level of desensitization, although the absence of placebo arm in this open-label extension trial prevented firm interpretation.⁶³

EPIT-associated immunologic changes, similarly observed in OIT or SLIT, included increases in peanut-specific IgG4⁶⁴ and reduction in T cell, allergen-specific IgE and basophil responses.

A novel EPIT based on dissolvable microneedle array (PLD-MNA) coated with powdered peanut allergens mixed with 1,25-dihydroxyvitamin D3 and CpG adjuvants (See Chapter 9) was recently evaluated for the treatment of peanut allergy in preclinical studies.⁶⁵ Compared with intradermal EPIT, PLD-MNA EPIT did not provoke any local skin inflammation. Moreover, six patch applications could induce immune tolerance with comparable efficacy as 12 intradermal EPIT using five times more peanut allergens.

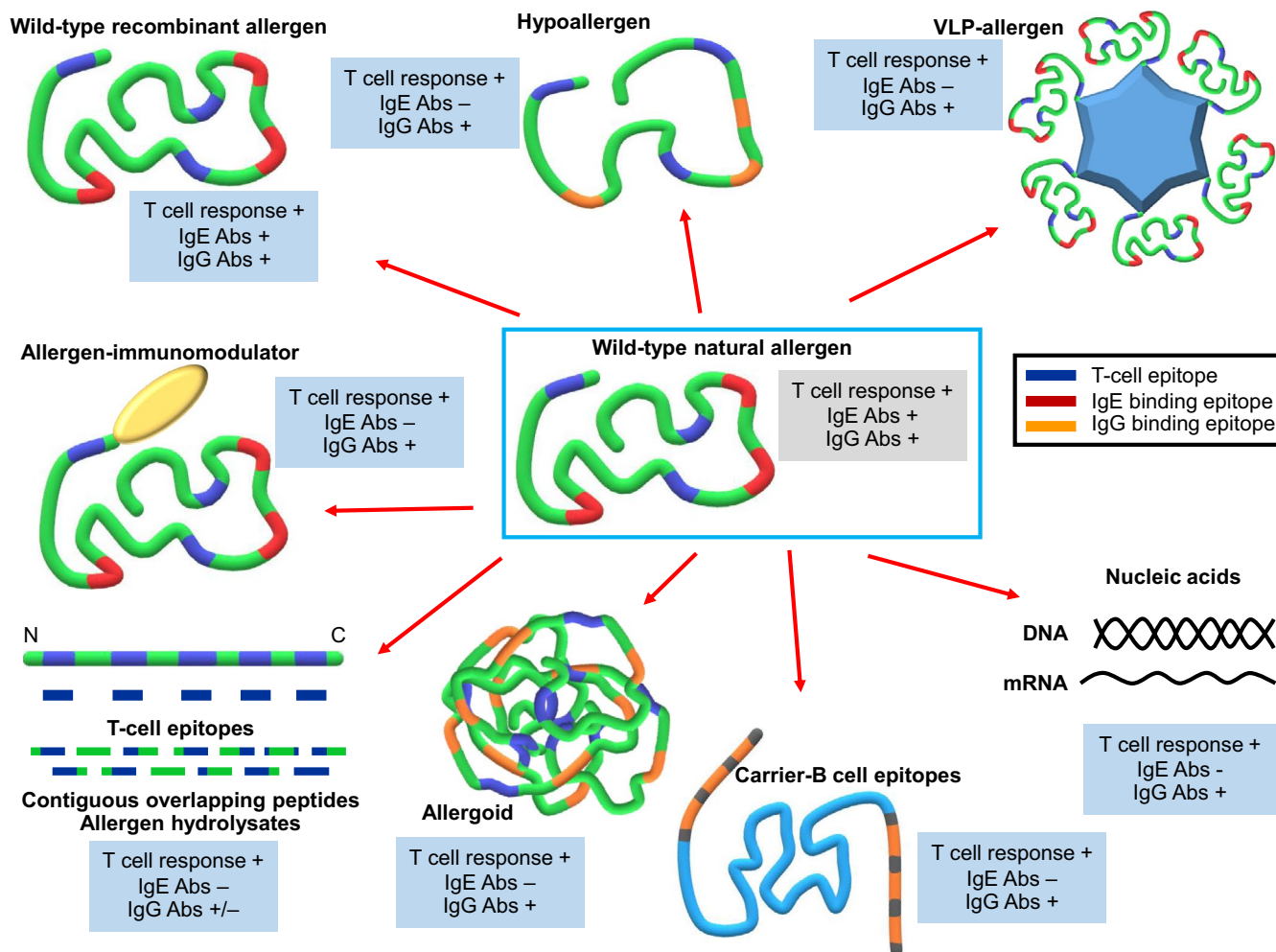


FIGURE 2 Evolution of molecular AIT approaches. The molecular cloning of clinically relevant allergens together with the mapping of their T cell and IgE binding epitopes enabled the development of innovative strategies for safer and more efficient AIT. Most of these molecular platforms promote in vivo potent IgG blocking antibody responses (IgG Abs +, with the exception of peptides carrying T cell epitopes). Remarkably, de novo synthesis of specific IgE (IgE Abs-) is prevented by their hypoallergenicity, the combination of pro-Th1 adjuvants/immunomodulators or allergen-encoding nucleic acids

10 | ADJUVANT

Formulations with adjuvant could enhance the protective allergen-specific responses in AIT but also reduce the antigen dose and the frequency of antigen administration, minimizing side-effects.⁶⁶

Alum, the most commonly used adjuvant in human vaccines, is licensed for SCIT in Europe but not in the United States. No adjuvant is currently used in SLIT protocols world-wide. Although alum was considered as a Th2 adjuvant in mouse models of allergen sensitizations, this immunostimulatory molecule was capable in sensitized patients to revert established Th2-biased allergic response to Th1.⁶⁷ Its use in AIT for years evidenced that alum is a safe adjuvant with few established side-effects. However, the duration of SCIT (minimum 2-3 years) results inevitably in long-life time of aluminium exposure which potentially produce chronic toxic side-effects. Local effects are more commonly observed at the injection sites.⁶⁸

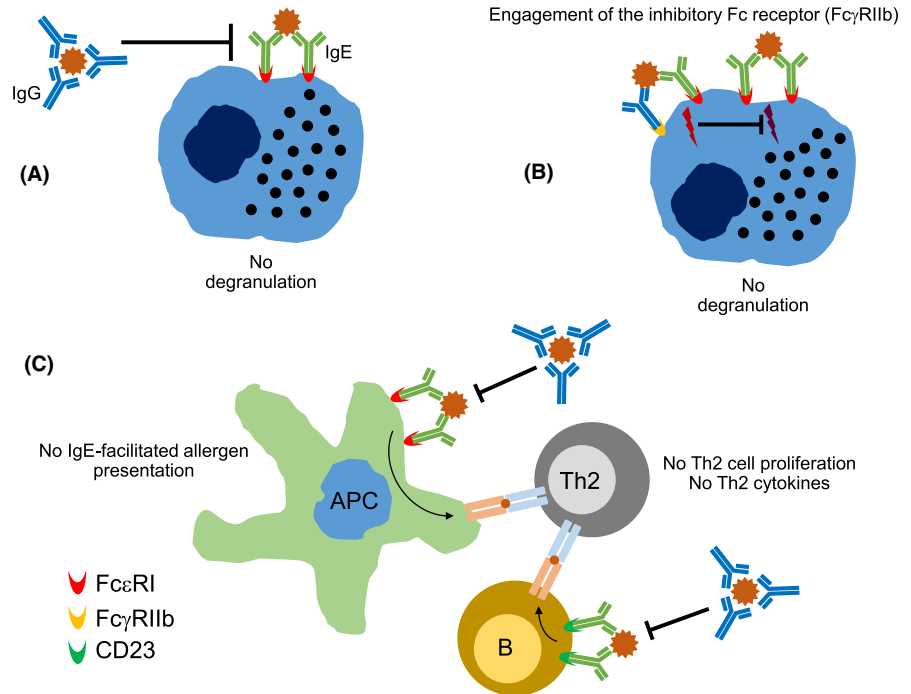
Microcrystalline tyrosine (MCT) represents an alternative depot formulation to optimize the slow release of adsorbed or

co-precipitated antigens. Moreover, the rapid MCT biodegradation avoids toxicity risk by prolonged accumulation. One clinical four injection-based SCIT trial evaluating HDM (allergoids) and formulated with MCT showed that this adjuvant is safe and promotes the development of allergen-specific IgG4 Ab production.⁶⁹ Nasal challenge test led to a significant decrease in symptom scores and mean nasal peak inspiratory flow.

Pollinex Quattro, composed of specific pollen allergoids formulated with monophosphoryl lipid A (MPLA) and adsorbed to MCT, is the only ultra-short (three weeks) four injection-based SCIT recommended by EAACI guidelines for the treatment of pollen allergy.⁷⁰ Phase III trials showed that preseasonal treatments with Pollinex Quattro were well tolerated and clinically effective in different cohorts of grass and tree pollen-allergic patients.⁷¹ The therapeutic efficacy was notably characterized by the reduction in symptom and/or medication scores.

However, a pivotal phase III for the treatment of birch pollen allergy with Pollinex Quattro Plus 1.0 mL birch 100% (PQBirch) failed

FIGURE 3 Key roles of allergen-specific IgG blocking antibodies in successful AIT. The down-regulation of the allergic response by allergen-specific IgG antibodies can be mediated by at least three mechanisms: (A) direct allergen neutralization; (B) engagement of the inhibitory Fc receptor (FcγRIIb), both preventing allergen-induced activation of mast cells and basophils; and (C) prevention of IgE-facilitated antigen presentation by B cells or APCs leading to reduction in T cell activation



as there was not statistically significant difference between active and placebo arms in the combined symptom and medication score over peak birch pollen season.⁷² Nevertheless, specific IgG and IgG4 titres showed highly statistically significant differences between active and placebo.

MPLA, a potent detoxified TLR4 ligand, is an approved adjuvant for human vaccines. By its capacity to promote potent Th1-biased immune response, MPLA-based AIT could be more effective. With the exception of one phase I/IIa clinical study testing the effect of MPLA when combined with grass pollen SLIT,⁷³ MPLA was exclusively used in ultra-short SCIT and formulated with grass, birch and ragweed allergoid preparations and MCT (as explained above,^{70,71,74}).

MPLA-adjuvanted SLIT was shown to be safe and was more efficient than unadjuvanted SLIT for the reduction in allergic rhinitis symptoms. Moreover, MPL induced higher and smaller increases in specific IgG and IgE levels, respectively, compared with other formulations. The efficacy of new AIT based on single MPLA formulation needs to be more investigated in larger studies.

Numerous preclinical studies evidenced the potential of probiotics as well as active metabolites of vitamins A and D (retinoic acid and 1,25-dihydroxyvitamin D3, respectively) for the control of the allergic response.^{75,76} However, the evaluation of their adjuvant properties in AIT protocols remained poorly investigated in clinical trials. A first study showed that combination of *L rhamnosus* and peanut OIT induced possible sustained unresponsiveness in 82% of the treated peanut-allergic children (compared to 3% in the placebo group).⁷⁷ However, the exact contribution of the probiotic needs to be delineated due to the lack of peanut OIT-only treatment group. A follow-up study showed that combined probiotic and peanut oral immunotherapy can induce long-lasting clinical benefit (up to 4 years) compared with placebo. More than 50% of the treated

patients tolerated regularly moderate-to-large amounts of peanut, compared with only 4% of the placebo group.⁷⁸ In grass pollen-sensitized children developing allergic rhinitis, vitamin D supplementation improved the effectiveness of grass-specific SLIT in terms of reduction in nasal symptoms ($P = .04$), asthma symptoms ($P = .001$) and combined symptom and medication score ($P = .001$).⁷⁹ Another trial compared *L rhamnosus* GG and vitamin D as adjuncts for grass pollen SLIT.⁸⁰ Whereas similar reduction in the symptom and medication score and improvement in lung function was observed in all SLIT-treated groups, the supplementation of *L rhamnosus* GG triggered more CD4(+)CD25(+)Foxp3(+) than SLIT alone.

The direct conjugation of adjuvant molecules to allergens was also considered as a strategy to improve AIT protocols. The first allergen/adjuvant conjugate consisted of chemical coupling of CpG motifs to the major ragweed pollen Amb a 1 allergen.⁸¹ Preclinical studies showed that Amb a 1-CpG was capable to suppress the established Amb a 1-specific Th2 response through induction of IFN- γ . In contrast, the mixture of CpG with the ragweed allergen did not exhibit such suppressive activities. The efficacy of AIT using Amb a 1 conjugated to type B CpG was evidenced in a phase II clinical trial.⁸² The conjugate was well tolerated and was capable to reduce the peak season rhinitis score and improved the quality of life of the treated ragweed-allergic subjects. However, these positive clinical outcomes were not similarly reproduced in a phase III study.⁸³

Listeria monocytogenes-derived flagellin A, a strong TLR5 ligand, was genetically fused to ovalbumin or pollen Art v 1 and Bet v 1 allergens for the efficient antigen targeting to TLR5 positive APCs.⁸⁴⁻⁸⁶ Flagellin-allergen fusion proteins were shown to generate in vitro IL-10-producing tolerogenic DCs capable to suppress Th1 and Th2 cytokine responses. Prophylactic vaccinations with FlaA-WT or

hypoallergenic Art v 1 were able to prevent the development of Art v 1 sensitization. Although Art v 1-specific IgG1 and IgG2a antibodies were detected, their blocking capacity was not evaluated in this study. Nevertheless, these promising preclinical data encourage future assessment in clinical trials.

The conjugation of non-oxidized mannan to allergoids optimized the antigen uptake by mannose receptor and DC-SIGN expressed on DCs.⁸⁷ Subsequent DC activation, characterized by IL-6, IL-10 secretion and expression of programmed death ligand 1, promoted the development of allergen-specific Th1- and IL-10+/Foxp3 + Treg subsets for the suppression of the allergic-specific Th2 responses and production of blocking specific IgG antibodies in mice.

11 | DISCUSSION

Molecular forms of AIT are continuously emerging and preclinical studies as well as phase I/II trials evidenced their potential to replace the conventional allergen extract-based treatment in order to minimize the side-effects and increase efficacy of AIT (Figure 2). However, the few phase III studies testing the efficacy of these new allergen derivatives were quite disappointing, either by the failure to meet the pre-determined end-points, the modest long-term evidenced efficacy or by the absence of clear advantages over the use of allergen extracts, leading to the prevention of their market authorizations. Larger trials will be needed to evidence the clinical impact of these different molecular AIT and particularly long-term benefits. The recent implementation of regulations forcing the pharmaceutical companies to produce GMP-grade allergen extracts and to evaluate their efficacy in clinical trials following good clinical practice (GCP) standards could slow down the development of these new immunotherapeutic molecules.⁸⁸ Indeed, these pharmaceutical companies would prefer to prioritize the consolidation of their efficacy data to allow the registration of their allergen extract-based AIT. Moreover, shortening of conventional AIT by the optimization of antigen formulations and route of administrations would reduce considerably the need in allergens and therefore would negatively impact their profit margins.

Actually, it is impossible to replace allergen extracts with a cocktail of the corresponding recombinant allergens in terms of GMP production costs and clinical testing. Consequently, the design of molecular AIT is based on the selection of clinically relevant allergens from any allergenic source. We cannot also exclude that AIT based on single molecules could trigger the activation of bystander Tregs, conferring protection against other allergens, as previously shown.^{89,90}

An important question remains to be addressed for the appropriate molecular strategy selection: is it preferred to favour the induction of allergen-specific T cells with a regulatory phenotype or to focus strictly on the development of IgG blocking antibodies for successful AIT? To our knowledge, we must note there is no head-to-head comparison of these different approaches. Although there

are no clearly identified surrogate markers to monitor the benefits of AIT,⁹¹ there is now a growing consensus that blocking activities of allergen-specific IgG4 antibodies correlates with the improvement of the clinical symptoms^{92,93} (Figure 3).

Therefore, we hypothesize that the most promising molecular AIT is the ones capable to elicit potent blocking IgG response as B cell epitope- or VLP-allergen-based platforms. Of note, single passive immunization with anti-Fel d 1 monoclonal IgG4 antibodies improved clinical symptoms with the same efficacy than conventional SCIT for two years, confirming the importance of blocking IgG4 response for successful AIT.⁹⁴

The natural evolution of IgE responses to allergens throughout childhood evidenced the molecular spreading of the sensitization profiles.^{95,96} The first specific IgE response is triggered against one or few initiator allergenic molecules (as Phl p 1 for *Phleum pratense* pollen or Der p 1, Der p 2 and Der p 23 for HDM). Early allergen-specific immunoprophylaxis, tailored on the initiator molecules from any source, could represent an elegant strategy to prevent the development of allergic disease.⁹⁷ Such early interventions could be recommended as primary prevention for the not yet sensitized but at-risk children or as a secondary prevention for healthy children sensitized to initiator allergens to counter onset of allergic symptoms. In this context, maternal allergen-specific IgG was shown to prevent the development of allergic sensitization in the offspring,⁹⁸ suggesting that allergen-specific vaccinations in pregnant women could be critical for the prevention of allergy. All together, these findings support the hypothesis that immunoprophylaxis can be recommended against allergies, through development of such specific IgG.

In conclusion, the described innovative molecular AIT needs more extensive clinical evaluation to determine the most effective strategies able to replace the conventional allergen extracts. Combined with the development of accurate molecular diagnosis and the identification of biomarkers for the monitoring of AIT, these new approaches give hope to allergic patients for an allergy-free future.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

PP and AJ equally contributed to and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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