



Modified Allergens for Immunotherapy

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Abstract

Purpose of Review During the past few decades, modified allergens have been developed for use in allergen-specific immunotherapy (AIT) with the aim to improve efficacy and reduce adverse effects. This review aims to provide an overview of the different types of modified allergens, their mechanism of action and their potential for improving AIT.

Recent Findings In-depth research in the field of allergen modifications as well as the advance of recombinant DNA technology have paved the way for improved diagnosis and research on human allergic diseases. A wide range of structurally modified allergens has been generated including allergen peptides, chemically altered allergoids, adjuvant-coupled allergens, and nanoparticle-based allergy vaccines. These modified allergens show promise for the development of AIT regimens with improved safety and long-term efficacy. Certain modifications ensure reduced IgE reactivity and retained T cell reactivity, which facilitates induction of immune tolerance to the allergen. To date, multiple clinical trials have been performed using modified allergens. Promising results were obtained for the modified cat, grass and birch pollen, and house dust mite allergens.

Summary The use of modified allergens holds promise for improving AIT efficacy and safety. There is however a need for larger clinical studies to reliably assess the added benefit for the patient of using modified allergens for AIT.

Keywords Allergen-specific immunotherapy · COPs · SPIREs · Nanoparticles · Allergoids · Hypoallergens

Abbreviations

AIT	Allergen immunotherapy
ARC	Allergic rhinoconjunctivitis
BAL	Bronchoalveolar lavage
BP	Birch pollen
Breg	Regulatory B cells
COPs	Contiguous overlapping peptides
CpG ODNs	CpG oligodeoxynucleotide
CTLA-4	Cytotoxic T lymphocyte-associated protein-4
DBPC	Double-blind and placebo-controlled clinical trial
DRF	Dose regimen finding
FcεRI	Fc epsilon receptor 1
FcγRIIb	Fc gamma receptor IIb
H ₂ R	Histamine receptor 2
HDM	House dust mite
IFN-γ	Interferon gamma
ILC	Innate lymphoid cells
ISCOMs	Immunostimulation complex

LPS	Lipopolysaccharides
MHC	Major histocompatibility complex
PAD	Peptide antigen desensitization
PD-1	Programmed death-1
PEG	Polyethylene glycol
SCIT	Subcutaneous immunotherapy
SLIT	Sublingual immunotherapy
SPIREs	Synthetic peptide immuno-regulatory epitopes
TLR	Toll-like receptor
Treg	Regulatory T cells
VLPS	Virus-like particles

Introduction

The prevalence of allergic diseases has increased worldwide during the last century [1–3]. Globally, more than 300 million people suffer from asthma and around 400 million—from allergic rhinitis [4]. Allergic diseases markedly reduce the quality of life and they are associated with fatigue, sleep problems, and decreased productivity [5]. Symptoms of allergy may be partially controlled by allergen avoidance and pharmacotherapy. Nonetheless, many patients suffer from ongoing symptoms despite adherence to treatment. To date, allergen immunotherapy (AIT) remains the only disease-modifying intervention offering

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long-lasting therapeutic effect [6]. However, despite the benefits of AIT for most of the patients, there is a need to establish new therapeutic strategies for non-responding individuals. Novel technologies of allergen modifications offer possibilities for the development of personalized treatment, improved clinical outcome, and reduced side effects of AIT [7, 8]. The usage of biomarkers for patient selection, such as the identification of AIT responder endotype, has also been recently focused [9, 10]. Considerable efforts have been undertaken to improve AIT vaccines and recent guideline documents and meta-analyses demonstrated that there is a huge unmet need to improve in the area [11–16]. Avoidance of development of new sensitizations is also one of the main requirements [17]. In this review, we will focus on the possible allergen modifications and preclinical in vitro and in vivo studies, as well as clinical trials evaluating their safety and efficacy.

Mechanisms of AIT

Successful AIT is characterized by the development of clinical and immunological tolerance and modifies the response to allergen exposure. The following cellular and molecular events take place during allergen-specific immunotherapy: (a) early desensitization effect (within hours after the first injections)—reduced degranulation and activity of basophils and mast cells, (b) induction of allergen-specific IL-10-producing regulatory T cells (Treg) and regulatory B cells (Breg), (c) early and continuous increase in specific IgG4 and—after several months—substantially decreased allergen-specific IgE/IgG4 ratio, (d) reduced number of tissue eosinophils and mast cells [18•, 19•, 20•] (Fig. 1).

It is not clear why basophils and mast cells undergo rapid desensitization even in the presence of specific IgE and become less susceptible to degranulation, which is accompanied by decrease risk of systemic anaphylaxis. It has been

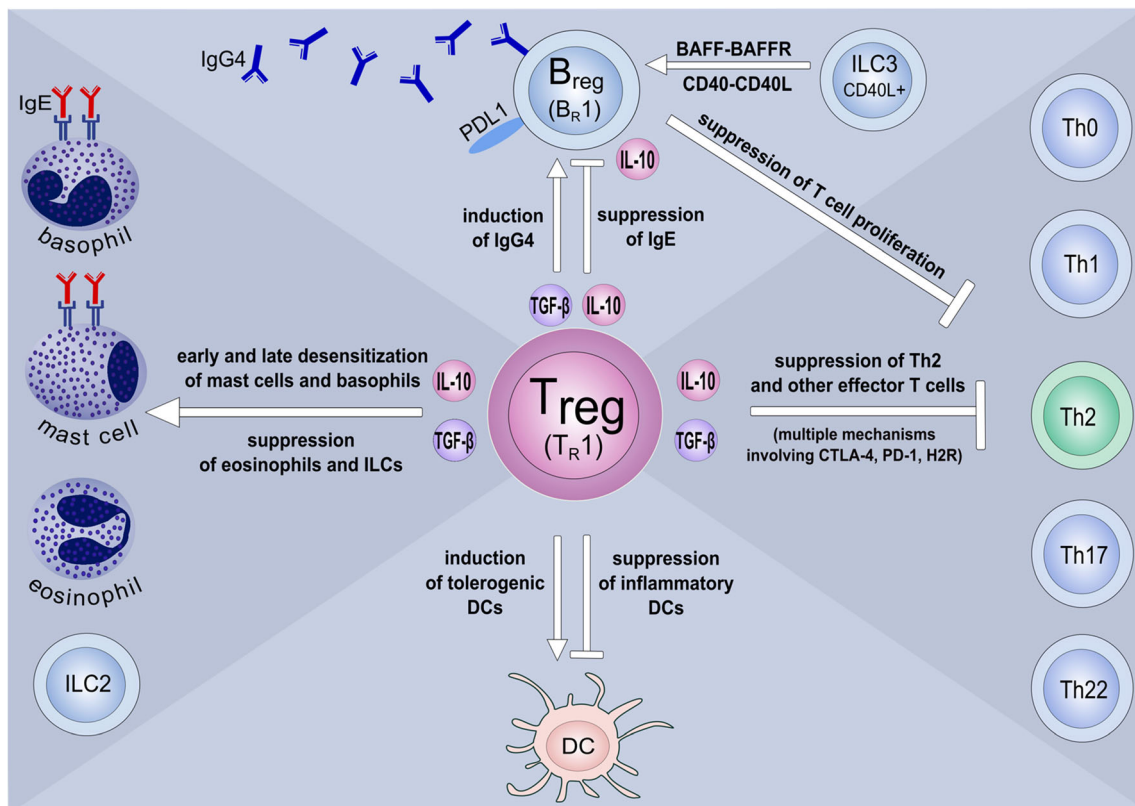


Fig. 1 Mechanisms of allergen-specific immunotherapy. One of the initial events in the development of allergen tolerance is an induction of allergen-specific regulatory T cells (T_R1), which contribute to the control of allergic reactions. Transforming growth factor- β (TGF- β) and interleukin-10 (IL-10) released by Tregs suppress the effector cells of allergic inflammation—mast cells, basophils, and eosinophils—and inhibit seasonal increases of group 2 innate lymphoid cells (ILC2). Additionally, these cytokines limit Th2-type immune responses and induce immunoglobulin class switch to IgG4 and IgA. IgG4 antibodies compete with IgE for allergen binding, decreasing IgE-facilitated antigen presentation and limiting IgE-mediated activation of mast cells and

basophils. Effector T cell responses are suppressed by T_R1 cells through multiple mechanisms involving not only IL-10 and TGF- β , but also cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death-1 (PD-1) and histamine receptor 2 (H2R). T_R1 -mediated suppression of inflammatory dendritic cells (DCs) prevents generation of effector cells and induction of tolerogenic DCs expressing IL-10, TGF- β , IL-27, and C1q take place. An increased frequency of allergen-specific regulatory B cells (B_{R1}) also contributes to allergen tolerance via release of IL-10. Induction of IL-10-producing immature translational Breg cells may be dependent on the help provided by CD40L-expressing ILC3

postulated that a key mechanism underlying this effect is related to exhaustion of effector cells' mediators through a repetitive stimulation and release during the administration of increasing doses of allergen at the early stages of AIT [21]. In addition, early desensitization following allergen challenge may be mediated by upregulation of histamine receptor 2 (H₂R) that inhibits FcεRI-cross-linking-induced activation and mediator release of basophils [22].

AIT is associated with induction of peripheral T cell tolerance characterized by generation of IL-10- and TGF-β-producing allergen-specific regulatory T cells (T_R1) that have a potential to inhibit Th2-type responses and suppress other effector T cells through multiple mechanisms engaging cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death-1 (PD-1) and histamine receptor 2 (H₂R) [23, 24]. Moreover, IL-10 and TGF-β produced by T_R1 cells are responsible for class-switching towards IgG4 and IgA [5, 25, 26]. Non-inflammatory IgG4 antibodies protect from allergic reactions, capturing the allergen to prevent IgE-mediated activation of mast cells and basophils. Additionally, IgG antibodies interrupt allergen presentation to Th₂ cells [27–29]. It has been demonstrated that IgG4 production induced upon AIT is confined to IL-10-producing B regulatory cells (B_R1) that play an essential role in peripheral tolerance by suppressing effector T cells, inhibiting proinflammatory cytokines and promoting differentiation of regulatory T cells [30, 31]. Recently, Komlosi et al. have shown that immunosuppressive IL-10-producing Bregs may be induced through receiving help from CD40L-expressing group 3 innate lymphoid cells (ILC3) [32].

After several months of AIT, decreased numbers of eosinophils and mast cells and limited release of their mediators can be observed. Successful AIT has a disease-modifying effect, improves quality of life in allergic patients, as well as reduces symptoms and medication requirement [19••].

In conclusion, mechanisms of AIT research have now a new era with the studies on multiple omics and big data analyses. Single cell methods and focusing on individual allergen-specific cells will bring a huge knowledge as AIT represents the only human allergen-specific in vivo intervention. In addition, knowledge on immune tolerance development coming from other disciplines adds significantly to our understanding in AIT [33].

Structure of Allergens

Although an allergen is defined as a protein that induces the production of IgE antibodies and elicits allergic reactions in sensitized patients, the question why some proteins act as allergens and others do not remains largely unanswered [34]. There are however certain associations between allergenic and non-allergenic proteins, their structure and related-biological functions [35••]. For example, the major allergen from house

dust mite (HDM), *Dermatophagoides pteronyssinus* 1 (Der p 1), is a cysteine protease, which directly penetrates the tissue barrier (i.e. airway epithelial barriers, skin epithelium, and mucosal barriers), whereas the other major HDM allergen, Der p 2, has an immunoglobulin-like fold with two major β-sheets and transports lipids or lipopolysaccharides (LPS) [34]. Two terms that are used to describe a protein that acts as an allergen are allergenicity and immunogenicity. Allergenicity is determined by the interaction of the epitope with the Fab region of its cognate antibody that mediates allergic reactions [36]. The immunogenicity is the ability of an allergen to induce a humoral and/or cell-mediated immune response. The ideal AIT regimen facilitates induction of immune tolerance by reducing allergenicity while maintaining the immunogenicity.

Modified Allergens

The first report of the use of AIT dates back to 1911, when Leonard Noon injected grass pollen extract into an allergic patient, achieving clinical improvement for almost 1 year [37]. Since then, significant progress has been made in the field of allergen characterization and understanding of the mechanisms driving immune tolerance. Natural allergen extracts often vary greatly in their potency and stability, which makes them difficult to standardize. Moreover, since extracts consist of a mixture of allergens, containing also non-allergenic and/or toxic proteins and other molecules, sensitization to additional allergenic components has been reported in patients receiving birch pollen [38] and grass extracts [39]. Concerns regarding patient safety and treatment efficacy are the main reasons for the search for novel approaches for AIT. New vaccines based on modified allergens give hope for highly effective and convenient forms of AIT with reduced risk of side effects. Through genetic engineering of allergens, decreased IgE reactivity and retained T cell reactivity may be obtained. Here, we provide an overview of the modifications of allergens, their mode of action and efficacy in clinical trials based on the recent findings in this area.

T Cell Epitope Peptide-Based Vaccines

The development of T cell epitope-based peptides aimed to weaken the binding of IgE to immune-dominant T cell epitopes and reduce the allergic inflammation. The study of Hoyne et al. has indicated inhibition of T cell responses and reduction of allergic symptoms in a murine model using the major T cell epitope of house dust mite allergen—Der p 1 [40]. T cell epitope peptide-based vaccines may contain either allergen-derived contiguous overlapping peptides (COPs) or synthetic peptide immuno-regulatory epitopes (SPIREs). The advantages of these therapies include a long-lasting sustained efficacy after only a short course of AIT [41••].

COPs have emerged as a promising tool for the use in AIT [42]. They are designed to encompass the complete allergen sequence with overlaps. Such overlapping peptides recognize potential T cell epitopes of allergens and decrease the binding to IgE. Pellaton et al. demonstrated extremely limited IgE binding to COPs derived from the sequence of the major allergen of birch pollen (Bet v 1) in both murine and human model in phase I clinical trial [43]. Another phase I/IIa clinical trial indicated the promising treatment with a mixture of three COPs termed “AllerT” which derive from the whole Bet v 1 sequence. The subcutaneous administration of AllerT elicits a strong immune response as compared to the 3-years AIT using purified allergens [44]. The efficacy of Bet v 1 COPs has also been confirmed in phase IIb clinical trial, showing significant improvement in the quality of life [45]. These findings indicated that even short-period AIT using COPs might provide a long-term immunologic memory.

SPIREs were first developed by Larché [46]. These synthetic T cell tolerating peptides contain 13–17 amino acids derived from major cat allergen Fel d 1, and have the ability to induce a population of CD4⁺ T cells with suppressor/regulatory activity in cat-allergic and asthmatic patients. A study by Campbell et al. using a murine model demonstrated that treatment with a Cat-SPIRE reduced eosinophil and total cell number in bronchoalveolar lavage (BAL), recruitment of Th2 cells to the lungs, pulmonary and systemic Th2 inflammatory cytokines, and proliferative responses to Fel d 1 [47]. Phase IIa clinical trial with Fel d 1 SPIREs has demonstrated safety and caused a great reduction in the late-phase skin response [48]. However, the recent international phase III clinical trial with Cat-PAD, a mixture of seven SPIREs from Fel d 1, revealed marked placebo effect. Although the treatment was well-tolerated, showed highly favorable safety profile and the study’s endpoint outcomes differed between placebo and active group, they did not achieve their primary endpoint and the use of Cat-PAD needs to be investigated further upon detailed analysis of this clinical trial data (<http://www.circassia.com/media/press-releases/circassia-announces-top-line-results-from-cat-allergy-phase-iii-study/>).

In another study, Couroux et al. reported the evidence of a long-term therapeutic effect in rhinoconjunctivitis patients using Cat-PAD intradermally [49]. The efficacy of the SPIREs has also been assessed in the treatment of HDM and grass pollen allergy. Another recent clinical trial using intradermally administered SPIREs derived from *Cynodon dactylon* 1 (Cyn d 1), *Lolium perenne* 5 (Lol p 5), *Dactylis glomerata* 5 (Dac g 5), *Holcus lanatus* (Hol l 5), and *Phleum pratense* (Phl p 5), which are five different allergens from Bermuda grass, Ray grass, Cat grass, Perennial grass, and Timothy grass, respectively. The results demonstrated an acceptable safety profile and potential to limit ARC symptoms after the allergen challenge [50]. Furthermore, substantial reduction of the symptoms and persistent effect were achieved

in the treatment with HDM-SPIREs in an exposure chamber model [51]. Also, intradermal HDM-peptide antigen desensitization (HDM-PAD) has been shown to be safe and well tolerated [52], and has been approved for phase IIb clinical trial.

Recombinant Allergen Derivative-Based Vaccines

Recombinant allergens are purified proteins made from allergen-encoded DNA, designed for the development of in vitro diagnostic tests (IgE reactivity) and in vivo allergen sensitivity testing [53]. Compared to natural allergens, recombinant allergens are very specific for direct sensitization and easy for the detection of cross-reactivity. However, they are more expensive to produce and it is more difficult to obtain a market authorization [54]. Recombinant allergens are produced in wild-type, chemically modified and genetically modified forms.

The study by Jutel et al. investigated the use of a mixture of the wild-type form of five recombinant timothy grass allergens (Phl p 1, 2, 5a, 5b, 6) in allergic rhinitis patients. The data indicated that every patient developed strong allergen-specific IgG1 and IgG4 antibody responses and a 36% reduction in symptoms and medication usage [55]. Another study on birch pollen (BP) allergy compared the safety and efficacy of recombinant Bet v 1a, purified natural Bet v 1, and standard BP extract with placebo in allergic patients for 2 years. The results showed a 50% reduction of rhinoconjunctivitis symptoms in three different groups compared to placebo [56]. The rBet v 1-based vaccine was safe and effective for BP allergy and promoted a highly specific immune response. The study from Nony et al. included rhinoconjunctivitis patients with BP allergy who were treated with the sublingual tablet made of pharmaceutical-grade recombinant Bet v 1. The clinical data demonstrated a decrease in symptom scores in patients receiving sublingual rBet v 1 tablets compared to placebo [57]. Both rBet v 1-based vaccine and rBet v 1 tablets were safe and effective for BP-allergic patients. Since anti-Bet v 1 IgE antibodies recognize the homologous structure of the major soya bean allergen, Gly m 4, Bet V 1 sensitization may be associated with food allergy. A recent study by Treudler et al. investigated the effect of AIT with cross-reactive rBet v 1 folding variant and demonstrated a promising immunogenic effect on specific IgE and IgG4 antibodies against Bet v 1 and homologous soya allergen; however, the clinical efficacy requires further investigation [58].

The recombinant DNA technology and the combination of allergen sequences and structures allow the production of genetically engineered recombinant allergens in different forms such as oligomers, fragments, mutants, mosaic molecules, chimeric or allergen hybrids, and allergen-derived B cell hypoallergens [59].

Allergen oligomers were developed by Vrtala et al. using the three copies of the major BP allergen, Bet v 1, covalently linked to short oligonucleotide [60]. These recombinant Bet v 1 oligomers displayed conserved structures of B cell and T cell epitopes of wild-type allergen and formed high molecular weight aggregates, which changes the presentation of IgE epitopes and reduces the cell surface-bound-IgE cross-linking. Campana et al. indicated that rBet v 1 oligomers displayed a significantly reduced allergenic activity compared with rBet v 1 wild-type and also induced a stronger release of Th1 cytokines from BP-allergic patients [61]. The allergen fragmentations were first established in major BP allergen (Bet v 1) expressed in *Escherichia coli* (*E. coli*) [62]. The 3-dimensional structure of the Bet v 1 was separated into two parts of the Bet v 1 cDNA representing amino acids (aa) 1-74 and 75-160 and represented no allergenicity with a random coil conformation. Immunization of mice and rabbits with rBet v 1 fragments induced IgG antibodies, which cross-reacted with complete Bet v 1 and Bet v 1-related allergens and strongly inhibited binding of IgE to these allergens [63]. Another study performed in atopic children using the hypoallergenic fragment 1-55 and 52-102 of *Parietaria Judaica* 2 (Par j 2) [64]. This result indicates that hypoallergenic 1-55 fragment increases the release of interferon gamma (IFN- γ). Chen et al. designed two recombinant fragments of a major HDM allergen (Der p 2) with aa 1-53 and aa 54-129 and a hybrid molecule (aa 54-129+1-53) by genetic engineering. rDer p 2 derivatives (fragments and hybrid) showed reduced IgE reactivity compared to the Der p 2 wild-type allergen. The allergenic activity of the Der p 2 derivatives was reduced more than 10-fold in vitro in basophil activation assays and in vivo in skin prick testing in HDM-allergic patients [65].

Allergen mutations are aimed at replacing the original amino acids with a designed amino acid sequence in order to change its physicochemical characteristics. A study on the Bet v 1 allergen compared amino acid substitutions in several Bet v 1 isoallergens and homologs with an algorithm that was developed to predict residues importance in IgE binding. A Bet v 1 6-point mutant was first developed by site-directed mutagenesis. It exhibited drastically reduced reactivity with serum IgE from BP-allergic patients [66]. The site-directed mutagenesis method offers the opportunity to study the function of different allergens [67]. A preclinical study using recombinant hypoallergenic carp parvalbumin, mutant Cyp c 1, which is a major fish allergen, warranted first-in-man immunotherapy studies to evaluate the safety of this innovative vaccine [68]. The result showed a reduction in allergenic activity in vitro and a preservation of immunogenicity in vivo.

Mosaic allergens are constructed by reassembling the peptides derived from the wild-type allergen. The aim is also to produce allergen molecules that lack IgE binding. The study from Campana et al. generated the three recombinant mosaic proteins derived from Bet v 1 by reassembly of codon-

optimized Bet v 1 fragments, which contain the major T cell epitopes and some peptides that displayed allergen-specific blocking IgG antibody. Immunization with this recombinant hypoallergenic Bet v 1 could induce IgG antibodies and inhibit IgE reactivity in patients compared to wild-type allergen [69]. Seven recombinant mosaic proteins were generated by reassembly of non-IgE-reactive peptides of Fel d 1. Immunization of rabbits with three different mosaic allergens which constructs of hypoallergenic peptides, Fel d 1, induced high levels of IgG antibodies that inhibited IgE reactivity of cat-allergic patients compared to wild-type allergen [70].

Chimeric or allergen hybrids are constructed by connecting the genetic information of at least two different proteins. Two-hybrid molecules derived from the two HDM major allergens, Der p 1 and Der p 2, were generated and purified in *E. coli*. The IgE reactivity of both hybrid proteins was significantly reduced in vitro. Furthermore, in vivo skin prick test (SPTs) in HDM-allergic patients confirmed that the hybrid proteins had a significantly lower potency to induce cutaneous reactions than the individual components. Hybrid molecules also induced higher T cell proliferation responses than the wild-type allergens. Immunization in mice with these hybrid proteins induced Der p 1- and Der p 2-specific IgG and inhibited the binding of IgE in patients [71]. Other study using two hybrid proteins from Der p 1 and Der p 2 fragments with and without cysteines also showed the similar result [72]. A hypoallergenic Phl p 2, was generated by fragmentation of the major pollen allergen (Phl p 2) sequence and reassembled with a truncated Phl p 6 allergen. These allergen hybrids retained the reduction of IgE reactivity in ELISA and allergenic activity in basophil activation assays. Immunization with the hybrid molecule demonstrated the increased immunogenicity, which leads to higher levels of allergen-specific IgG antibodies compared to the wild-type allergen [73]. The other allergen hybrids studies showed the similar result in venom allergy and Japanese cedar pollen allergy [74–77].

The allergen-derived B cell epitope-based vaccine represents a novel development of recombinant hypoallergenic allergen derivatives that have been engineered to reduce IgE reactivity and thus the allergenic activity of allergens to be used for AIT with the goal to improve their safety [78•]. The recombinant B cell epitope-based vaccine (BM32), a recombinant hypoallergenic B cell epitope to grass pollen allergy was constructed and evaluated in several clinical trials [79, 80]. A hypoallergenic cat vaccine based on Fel d 1–derived peptides fused to hepatitis B PreS domain was generated and exhibited a strongly reduced IgE-mediated allergenic activity [81]. In a recent study in grass pollen allergy, the vaccine was generated using the same strategy. The result indicated that the recombinant hypoallergenic grass pollen allergy vaccine BM32 induces hepatitis B-specific immune responses and protect against hepatitis B virus infection in vitro [82].

Allergoid-Based Vaccines

Allergoids are chemically modified allergens, which preserve the linear epitopes recognized by the major histocompatibility complex (MHC) and TLR. The recognition of allergoids by MHC and TLR retain the immunogenicity and reduce the allergenicity. The chemicals used for production of allergoids include: formaldehyde and aluminum hydroxide (vaccines such as Allergovit and Acaroid), glutaraldehyde and tyrosine (Pollinex-R as tyrosine-adsorbate), glutaraldehyde and tyrosine and monophosphoryl lipid A (MPL) (Pollinex Quattro), and glutaraldehyde and aluminum hydroxide (Purethal, Depigoid or depigmented allergoid) [83]. MPL strongly induces a Th1-type immune response, characterized by increased allergen-specific IgG antibody levels and decreased IgE response during allergen exposure [84]. By using a mixed grass pollen allergoid vaccine (an aluminum hydroxide-adsorbed allergoid preparation of 6 grass pollen allergens), grass pollen allergoid SCIT and standard SLIT were compared and showed that allergoid SCIT was found better and cost-effective [85]. The improvement of allergic symptoms was observed in two studies with HDM-allergoid treated patients [86, 87]. The efficacy, tolerability, and safety were confirmed in a cohort of adults and children receiving subcutaneous AIT with another alum-based allergoid for 2 years [88]. Allekotte et al. indicated that the treatment with mite monomeric allergoids is well-tolerated and can be considered a safe treatment for patients suffering from HDM-induced ARC [89]. A dose study with 2000 UA/day sublingual monomeric allergoid tablets in HDM-allergic patients was well tolerated and decreased the conjunctival provocation tests reaction [90]. Moreover, it has been demonstrated that grass pollen allergoid dosage can be accelerated with safety and tolerability [91]. The newest chemically modified allergen is the mannan-coated allergoids, which upregulate human DCs to generate functional FOXP³ (+) Treg cells through programmed death ligand 1. Thus allergoids conjugated to nonoxidized mannan may be suitable for the use in AIT [92].

Adjuvant-Coupled Molecule-Based Vaccines

Aluminum Hydroxide was the first chemical molecule to be used as an adjuvant [93]. It induces a strong Th2 response, stimulates activation of antigen presenting cells (APCs), independent of Toll-like receptor (TLR) signaling, but dependent on the NLRP3 inflammasome. It is still considered the most common and safe adjuvant for injection immunotherapy in humans. A randomized phase II study on hypoallergenic recombinant aluminum hydroxide-adsorbed birch pollen allergen, rBet v 1-FV, can be administered in higher doses than the native protein without increase in adverse effects [94]. The first DBPC SCIT-DRF with a mixture of recombinant Phleum allergens (Phl p 1, 2, 5a, 5b, 6) in patients with

ARC/asthma revealed that eight of 50 patients had systemic reactions grade 1 or 2, but showed no grade 3 or 4 reaction in very high doses either [95].

Interestingly, *in vivo* lymph node targeting of CpG oligodeoxynucleotide (CpG) ODNs has confirmed that CpG ODNs could be used as vaccine adjuvants [96]. A ragweed-pollen antigen (Amb a 1) conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA, administered six weekly injections in rhinitis patients, induced a long-term clinical efficacy in a DBPC phase II trial by Creticos et al. [97].

Nanoparticle-Based Vaccines

Nanoparticles (NP) for AIT are composed of virus-like particles, polymers, liposomes (VLP), and immunostimulation complex (ISCOMs). VLPs have been developed as the drug delivery system. In a study of HDM-allergic patients employing VLPs consisting of bacteriophage Q β coated with a synthetic 16-amino-acid sequence of the allergen Der p 1 (Q β -Der p 1) associated with CpG ODN, the authors demonstrated improvement in rhinoconjunctivitis symptoms and better quality-of-life score [98]. The phase I/IIa study demonstrated that subcutaneous application of HDM allergen, together with A-type CpG ODN packaged into virus-like particles, was safe. All patients achieved practically complete alleviation of allergy symptoms after 10 weeks of immunotherapy [99]. Another recent study examined the effect of Fel d 1 displayed on VLPs *in vitro* and demonstrated that such allergen repetition on VLPs increased the immunogenicity while decreased allergic symptoms by avoiding IgE-mediated activation of mast cells. The data suggest that allergens displayed on VLPs might lead to an effective AIT vaccine [100].

Polymers have been developed in recent years and particularly non-biodegradable polymers that are found ubiquitous in daily life such as bottles and plastic packaging material. The most prominent synthetic water-soluble polymer is polyethylene glycol (PEG), an aliphatic polyether that is widely used in pharmaceutical applications. PEG is often described as the “gold standard” for biomedical applications and obtained this status because of its unique properties [101]. As PEG is a non-biodegradable polymer, cleavage sites need to be introduced for controlled delivery of proteins by PEG-based NP in AIT. To this end, acid-labile PEG-macromonomers that degrade at pH 5 (physiological pH inside the endolysosome) were synthesized by Pohlitz et al. [102]. Encapsulation of allergens into these NPs resulted in targeted delivery and induction of T cell proliferation concurrently with allergen shielding from recognition by IgE antibodies in human *in vitro* experiments.

Liposomes are synthetic spheres made of lipid bilayers that can encapsulate hydrophilic antigens and act as adjuvants [103]. A DBPC study in which asthmatic patients sensitized

Table 1 Clinical trials using allergen-specific immunotherapy with modified allergens

Type of vaccine modification	Allergen source	Molecule	Intervention	Clinical trial	Ref.
T cell epitope peptide-based	Allergen-derived contiguous overlapping peptides	Bet v 1	Subcutaneous injection	Phase I/IIa/Phase IIb	[44]/[45]
	Synthetic peptide-regulatory epitopes	Fel d 1	Intradermal	Phase IIa/follow-up study/phase III	[48]/[49]/ http://www.circassia.com/media/press-releases/circassia-announces-top-line-results-from-cat-allergy-phase-iii-study/ [50]
Recombinant allergen derivative-based	Grass pollen	Cyn d 1, Lol p 5, Dac g 5, Hol l 5, Phl p 5	Intradermal	Phase II	
		Not specified	Intradermal	Phase IIb	[51]
		Not specified	Intradermal	Phase IIb	[52]
	House dust mite	rBet v 1	Sublingual	Phase I	[57]
	House dust mite	Phl p 1, Phl p 2, Phl p 5, Phl p 6	Subcutaneous injection	Phase II	[79]
	Birch pollen	DBPC	Subcutaneous injection	Phase I	[88]
	Grass pollen	Carbamylated monomeric allergoids	Sublingual	Phase II	[89]
Allergoid-based vaccines	House dust mite	Not specified	Subcutaneous injection	Phase II	[90]
	House dust mite	Depegoid	Subcutaneous injection	Phase II	[91]
Adjuvant-coupled/nanoparticle-based	Birch pollen	rBet v 1-FV (recombinant folding variant)	Subcutaneous injection	Phase II	[94]
		rPhl p 1, rPhl p 2, rPhl p 5a, rPhl p 5b, rPhl p 6,	Subcutaneous injection	Phase II	[95]
	Grass pollen	Der p, Der f, Der p 1	Subcutaneous injection	Phase I/IIa	[99]
	House dust mite	Der p 1	Subcutaneous injection	Phase I	[98]
	House dust mite	Der p 1	Subcutaneous injection	Phase I	[104]
	House dust mite	Der p 1	Subcutaneous injection	Phase I	[111]
	Cat, dog, house dust mite	Fel d 1, Can f 1, Der f 1, Der p 1	Subcutaneous injection	Phase I	
	Cow's milk, egg, peanut, nuts, grain, sesame seed	Not specified	Oral	Phase I	[112]
	Peanut	Not specified	Oral	Phase II	[113]

to mites were randomly assigned for vaccination with Der p extract encapsulated in liposomes or empty liposomes for 12 months demonstrated that nearly half (45.8%) of the patients revealed reduction in their allergic symptoms. Vaccination was well tolerated, and no subcutaneous nodules appeared [104].

ISCOMs are spherical complexes about 40 nm in size, composed of saponin adjuvant [105]. ISCOMs are able to trap the antigen by apolar interactions and to elicit strong humoral, cellular, and mucosal Th1 responses after subcutaneous or intranasal immunization [106]. Although ISCOMs have been proposed as potential vaccine vectors in vaccination with influenza or human immunodeficiency virus, no study has yet evaluated the effect of ISCOMs as adjuvants in allergen immunotherapy.

Combined Treatment with Immunomodifiers

FcγRIIb is an immune tyrosine-based inhibitory motif containing receptor 65. The coaggregation of FcεRI and FcγRIIb inhibits FcγRI signaling. One approach that has been tested is the fusion of FcγRIIb to allergens to down-regulate downstream allergen-specific immune responses. In a similar approach, the fusion of allergens to human Fcγ suppressed allergen-induced degranulation of basophils and mast cells by cross-linking Fcγ and FcεRI receptors [107, 108]. Fel d 1 was cloned and expressed together with HIV protein, TAT-derived membrane translocation domain and a truncated peptide of the invariant chain (modular antigen translocation-Fel d 1) in order to intensify allergen internalization and presentation to T cells by APCs. This compound was tested in a recombinant cat allergen DBPC clinical trial using intralymphatic allergen application. The administration of only three increasing doses (1, 3, and 10 mg) at 4-week intervals demonstrated good clinical efficacy assessed by nasal provocation challenge, very high safety profile as well strong immune response measured by increased concentrations of specific IgG4 in serum [109, 110]. The combination AIT and anti-IgE (omalizumab) treatment have been investigated in several trials. A good safety profile, a significant decline in the risk of anaphylaxis and improved rescue medication scores were reported [111, 112]. Recent clinical trials using allergen-specific immunotherapy with modified allergens are summarized in Table 1.

Novel Routes of Vaccine Administration

Recently, the intralymphnode and epicutaneous applications of vaccines were examined. Epicutaneous immunotherapy is administered directly to human skin. Senti et al. indicated that DBPC trial in patients with grass pollen-induced rhinoconjunctivitis is safe and efficacious in a dose-dependent manner [114]. A recent study demonstrated that epicutaneous

allergen application boosts specific T cell responses in BP sensitized patients [115].

Intralymphatic immunotherapy (ILIT) is administered by direct injection of an allergen into the lymph nodes. This treatment requires lower doses of antigen than other treatment because it could be delivered to the specific immune cells. Patients with pollen-induced rhinoconjunctivitis were randomized to receive three intralymphatic inguinal injections of active allergen and the results showed a significant reduction in allergic symptoms at the end of pollen season [116]. The tolerable doses of ILIT for grass pollen allergy were examined and the results demonstrated 3 pre-seasonal injections during 8 weeks are a viable option for treating allergies [117]. In the pilot study of seven patients with a history of symptomatic allergic rhinoconjunctivitis due to grass pollen, three ILIT injections of allergen were performed with monthly intervals. ILIT induced allergen-specific plasmablasts and objective measures of clinical improvement were reported [118].

Both epicutaneous and intralymphatic route of administration showed efficacy similar to subcutaneous immunotherapy in grass pollen allergy, but fewer application and lower total doses of allergen were required using these routes.

Conclusions

There are still some major unmet needs in AIT which are open for improvement. From the economic point of view, allergen immunotherapy may be beneficial for healthcare system, offering improved clinical outcome at reduced costs compared to standard pharmaceutical treatment and compensation of the costs of illness [119, 120]. Development of novel vaccines with less injections and curative effect will also open a new avenue in the health economics of allergy treatment [121]. AIT products are also biological products for medicine some of which are used in *in vivo* diagnostic tests, including skin prick tests, provocation tests, intradermal tests, and epicutaneous tests. The regulatory approaches for AIT products differ globally. Novel vaccines have to pass all the stages of regulation for marketing authorizations that show several challenges that require further consideration [119].

The studies on modified allergens reported here show the way for promoting the safety, specificity, and high effectiveness of allergen vaccines. The possible utility of novel allergens has been intensively investigated through *in vitro*, *in vivo*, and human clinical trials. Most of the studies reveal positive impact in the treatment of allergic subjects. This potential evidence is being applied in the development of an ideal AIT, which aims to substantially improve quality of life have possible usage in prevention and end up with cure in allergic individuals [9, 122].

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Platts-Mills TA. The allergy epidemics: 1870–2010. *J Allergy Clin Immunol*. 2015;136(1):3–13. <https://doi.org/10.1016/j.jaci.2015.03.048>.
2. Backman H, Raisanen P, Hedman L, Stridsman C, Andersson M, Lindberg A, et al. Increased prevalence of allergic asthma from 1996 to 2006 and further to 2016—results from three population surveys. *Clin Exp Allergy*. 2017;47(11):1426–35. <https://doi.org/10.1111/cea.12963>.
3. Loftus PA, Wise SK. Epidemiology of asthma. *Curr Opin Otolaryngol Head Neck Surg*. 2016;24(3):245–9. <https://doi.org/10.1097/MOO.0000000000000262>.
4. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ J*. 2014;7(1):12–3. <https://doi.org/10.1186/1939-4551-7-12>.
5. Larsen JN, Broge L, Jacobi H. Allergy immunotherapy: the future of allergy treatment. *Drug Discov Today*. 2016;21(1):26–37. <https://doi.org/10.1016/j.drudis.2015.07.010>.
6. Patel HD, Chambliss JM, Gupta MR. Utility and comparative efficacy of recombinant allergens versus allergen extract. *Curr Allergy Asthma Rep*. 2017;17(9):63. <https://doi.org/10.1007/s11882-017-0727-9>.
7. Valenta R, Ferreira F, Focke-Tejkl M, Linhart B, Niederberger V, Swoboda I, et al. From allergen genes to allergy vaccines. *Annu Rev Immunol*. 2010;28(1):211–41. <https://doi.org/10.1146/annurev-immunol-030409-101218>.
8. Campana R, Huang HJ, Freidl R, Linhart B, Vrtala S, Wekerle T, et al. Recombinant allergen and peptide-based approaches for allergy prevention by oral tolerance. *Semin Immunol*. 2017a;30:67–80. <https://doi.org/10.1016/j.smim.2017.08.017>.
9. Shamji MH, Kappen JH, Akdis M, Jensen-Jarolim E, Knol EF, Kleine-Tebbe J, et al. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI position paper. *Allergy*. 2017;72(8):1156–73. <https://doi.org/10.1111/all.13138>.
10. Hoffmann HJ, Valovirta E, Pfaar O, Moingeon P, Schmid JM, Skaarup SH, et al. Novel approaches and perspectives in allergen immunotherapy. *Allergy*. 2017;72(7):1022–34. <https://doi.org/10.1111/all.13135>.
11. Dhami S, Kakourou A, Asamoah F, Agache I, Lau S, Jutel M, et al. Allergen immunotherapy for allergic asthma: a systematic review and meta-analysis. *Allergy*. 2017a;72(12):1825–48. <https://doi.org/10.1111/all.13208>.
12. Nurmatov U, Dhami S, Arasi S, Pajno GB, Fernandez-Rivas M, Muraro A, et al. Allergen immunotherapy for IgE-mediated food allergy: a systematic review and meta-analysis. *Allergy*. 2017;72(8):1133–47. <https://doi.org/10.1111/all.13124>.
13. Dhami S, Nurmatov U, Arasi S, Khan T, Asaria M, Zaman H, et al. Allergen immunotherapy for allergic rhinoconjunctivitis: a systematic review and meta-analysis. *Allergy*. 2017b;72(11):1597–631. <https://doi.org/10.1111/all.13201>.
14. Larenas-Linnemann DES, Antolin-Amerigo D, Parisi C, Nakonechna A, Luna-Pech JA, Wedi B, et al. National clinical practice guidelines for allergen immunotherapy: an international assessment applying AGREE-II. *Allergy*. 2017; <https://doi.org/10.1111/all.13316>.
15. Roberts G, Pfaar O, Akdis CA, Ansotegui IJ, Durham SR, Gerth van Wijk R, et al. EAACI guidelines on allergen immunotherapy: allergic rhinoconjunctivitis. *Allergy*. 2017; <https://doi.org/10.1111/all.13317>.
16. Ryan D, Gerth van Wijk R, Angier E, Kristiansen M, Zaman H, Sheikh A, et al. Challenges in the implementation of the EAACI AIT guidelines: a situational analysis of current provision of allergen immunotherapy. *Allergy*. 2017; <https://doi.org/10.1111/all.13264>.
17. Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Macchia L, Di Lorenzo G. Efficacy of allergen immunotherapy in reducing the likelihood of developing new allergen sensitizations: a systematic review. *Allergy*. 2017;72(5):691–704. <https://doi.org/10.1111/all.13104>.
18. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J*. 2015;8(1):17. <https://doi.org/10.1186/s40413-015-0063-2>. **An excellent review on the mechanism of AIT and immune tolerance to allergens.**
19. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*. 2014;133(3):621–31. <https://doi.org/10.1016/j.jaci.2013.12.1088>. **An excellent review on the mechanisms of AIT.**
20. van de Veen W, Wirz OF, Globinska A, Akdis M. Novel mechanisms in immune tolerance to allergens during natural allergen exposure and allergen-specific immunotherapy. *Curr Opin Immunol*. 2017;48:74–81. <https://doi.org/10.1016/j.coi.2017.08.012>. **An update in the mechanisms in immune tolerance to allergens.**
21. Maintz L, Bussmann C, Bieber T, Novak N. Contribution of histamine metabolism to tachyphylaxis during the buildup phase of rush immunotherapy. *J Allergy Clin Immunol*. 2009;123(3):701–3. <https://doi.org/10.1016/j.jaci.2008.11.009>.
22. Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. *J Allergy Clin Immunol*. 2012;130(5):1153–8 e2. <https://doi.org/10.1016/j.jaci.2012.04.039>.
23. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med*. 2004;199(11):1567–75. <https://doi.org/10.1084/jem.20032058>.
24. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med*. 2008a;205(12):2887–98. <https://doi.org/10.1084/jem.20080193>.
25. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells

- and toll-like receptors. *Allergy*. 2008b;63(11):1455–63. <https://doi.org/10.1111/j.1398-9995.2008.01774.x>.
26. Fujita H, Soyka MB, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *Clin Transl Allergy*. 2012;2(1):2. <https://doi.org/10.1186/2045-7022-2-2>.
 27. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol*. 2004;172(5):3252–9. <https://doi.org/10.4049/jimmunol.172.5.3252>.
 28. van Neerven RJ, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arnved J, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol*. 1999;163(5):2944–52.
 29. James LK, Till SJ. Potential mechanisms for IgG4 inhibition of immediate hypersensitivity reactions. *Curr Allergy Asthma Rep*. 2016;16(3):23. <https://doi.org/10.1007/s11882-016-0600-2>.
 30. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol*. 2013;131(4):1204–12. <https://doi.org/10.1016/j.jaci.2013.01.014>.
 31. Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol*. 2012;30(1):221–41. <https://doi.org/10.1146/annurev-immunol-020711-074934>.
 32. Komlasi ZI, Kovacs N, van de Veen W, Kirsch A, Fahmer HB, Wawrzyniak M, et al. Human CD40L-expressing type 3 innate lymphoid cells induce IL-10-producing immature transitional regulatory B cells. *J Allergy Clin Immunol*. 2017; <https://doi.org/10.1016/j.jaci.2017.07.046>.
 33. Jensen-Jarolim E, Bax HJ, Bianchini R, Crescioli S, Daniels-Wells TR, Dombrowicz D, et al. AllergoOncology: opposite outcomes of immune tolerance in allergy and cancer. *Allergy*. 2017;73(2):328–40. <https://doi.org/10.1111/all.13311>.
 34. Scheurer S, Toda M, Vieths S. What makes an allergen? *Clin Exp Allergy*. 2015;45(7):1150–61. <https://doi.org/10.1111/cea.12571>.
 35. Pomes A. Allergen structures and biologic functions: the cutting edge of allergy research. *Curr Allergy Asthma Rep*. 2008;8(5):425–32. **An excellent review on the allergen structures and biologic functions.**
 36. Aalberse RC. Structural biology of allergens. *J Allergy Clin Immunol*. 2000;106(2):228–38. <https://doi.org/10.1067/mai.2000.108434>.
 37. Noon L. Prophylactic inoculation against hay fever. *Lancet*. 1911;1:1572–3.
 38. Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP system. *Allergy*. 2002;57(5):423–30.
 39. Marcucci F, Sensi L, Incorvaia C, Dell'Albani I, Di Cara G, Frati F. Specific IgE response to different grass pollen allergen components in children undergoing sublingual immunotherapy. *Clin Mol Allergy*. 2012;10(1):7. <https://doi.org/10.1186/1476-7961-10-7>.
 40. Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med*. 1993;178(5):1783–8. <https://doi.org/10.1084/jem.178.5.1783>.
 41. Prickett SR, Rolland JM, O'Hehir RE. Immunoregulatory T cell epitope peptides: the new frontier in allergy therapy. *Clin Exp Allergy*. 2015;45(6):1015–26. <https://doi.org/10.1111/cea.12554>. **An excellent review on the T cell epitope peptides.**
 42. O'Hehir RE, Prickett SR, Rolland JM. T cell epitope peptide therapy for allergic diseases. *Curr Allergy Asthma Rep*. 2016;16(2):14. <https://doi.org/10.1007/s11882-015-0587-0>.
 43. Pellaton C, Perrin Y, Boudousquie C, Barbier N, Wassenberg J, Corradin G, et al. Novel birch pollen specific immunotherapy formulation based on contiguous overlapping peptides. *Clin Transl Allergy*. 2013;3(1):17. <https://doi.org/10.1186/2045-7022-3-17>.
 44. Spertini F, Perrin Y, Audran R, Pellaton C, Boudousquie C, Barbier N, et al. Safety and immunogenicity of immunotherapy with Bet v 1-derived contiguous overlapping peptides. *J Allergy Clin Immunol*. 2014;134(1):239–40 e13. <https://doi.org/10.1016/j.jaci.2014.04.001>.
 45. Spertini F, DellaCorte G, Kettner A, de Blay F, Jacobsen L, Jutel M, et al. Efficacy of 2 months of allergen-specific immunotherapy with Bet v 1-derived contiguous overlapping peptides in patients with allergic rhinoconjunctivitis: results of a phase IIb study. *J Allergy Clin Immunol*. 2016;138(1):162–8. <https://doi.org/10.1016/j.jaci.2016.02.044>.
 46. Larche M. Immunotherapy with allergen peptides. *Allergy Asthma Clin Immunol*. 2007;3(2):53–9. <https://doi.org/10.1186/1710-1492-3-2-53>.
 47. Campbell JD, Buckland KF, McMillan SJ, Kearley J, Oldfield WL, Stern LJ, et al. Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. *J Exp Med*. 2009;206(7):1535–47. <https://doi.org/10.1084/jem.20082901>.
 48. Worm M, Lee HH, Kleine-Tebbe J, Hafner RP, Laidler P, Healey D, et al. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy. *J Allergy Clin Immunol*. 2011;127(1):89–97, e1-14. <https://doi.org/10.1016/j.jaci.2010.11.029>.
 49. Couroux P, Patel D, Armstrong K, Larche M, Hafner RP. Fel d 1-derived synthetic peptide immuno-regulatory epitopes show a long-term treatment effect in cat allergic subjects. *Clin Exp Allergy*. 2015;45(5):974–81. <https://doi.org/10.1111/cea.12488>.
 50. Ellis AK, Frankish CW, O'Hehir RE, Armstrong K, Steacy L, Larche M, et al. Treatment with grass allergen peptides improves symptoms of grass pollen-induced allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2017;140(2):486–96. <https://doi.org/10.1016/j.jaci.2016.11.043>.
 51. Hafner R, Couroux P, Armstrong K, Salapatek A, Patel D, Larche M. Persistent Treatment effect achieved at one year after four doses of Der p derived synthetic peptide immuno-regulatory epitopes in an exposure chamber model of house dust mite allergy. *J Allergy Clin Immunol*. 2014;133(2):Ab289-Ab.
 52. Larche M, Hickey P, Hebert J, Hafner R. Safety and tolerability of escalating doses of house dust mite-peptide antigen desensitization (HDM-PAD). *J Allergy Clin Immunol*. 2013;131(2):Ab37-Ab.
 53. Larche M. Peptide and recombinant immunotherapy. *Immunol Allergy Clin N Am*. 2011;31(2):377–89, xi. <https://doi.org/10.1016/j.iac.2011.03.008>.
 54. Niederberger V, Eckl-Dorna J, Pauli G. Recombinant allergen-based provocation testing. *Methods*. 2014;66(1):96–105. <https://doi.org/10.1016/j.jymeth.2013.07.037>.
 55. Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol*. 2005;116(3):608–13. <https://doi.org/10.1016/j.jaci.2005.06.004>. **A successful study of AIT using the mixture of grass pollen allergens.**
 56. Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2008;122(5):951–60. <https://doi.org/10.1016/j.jaci.2008.09.017>.
 57. Nony E, Bouley J, Le Mignon M, Lemoine P, Jain K, Horiot S, et al. Development and evaluation of a sublingual tablet based on

- recombinant Bet v 1 in birch pollen-allergic patients. *Allergy*. 2015;70(7):795–804. <https://doi.org/10.1111/all.12622>.
58. Treudler R, Franke A, Schmiedeknecht A, Ballmer-Weber B, Worm M, Werfel T, et al. BASALIT trial: double-blind placebo-controlled allergen immunotherapy with rBet v 1-FV in birch-related soya allergy. *Allergy*. 2017;72(8):1243–53. <https://doi.org/10.1111/all.13112>.
 59. Tscheppe A, Breiteneder H. Recombinant allergens in structural biology, diagnosis, and immunotherapy. *Int Arch Allergy Immunol*. 2017;172(4):187–202. <https://doi.org/10.1159/000464104>.
 60. Vrtala S, Hirtenlehner K, Susani M, Akdis M, Kussebi F, Akdis CA, et al. Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. *FASEB J*. 2001;15(11):2045–7. <https://doi.org/10.1096/fj.00-0767fje>.
 61. Campana R, Vrtala S, Maderegger B, Dall'Antonia Y, Zafred D, Blatt K, et al. Altered IgE epitope presentation: a model for hypoallergenic activity revealed for Bet v 1 trimer. *Mol Immunol*. 2011;48(4):431–41. <https://doi.org/10.1016/j.molimm.2010.09.016>.
 62. Vrtala S, Hirtenlehner K, Vangelista L, Pastore A, Eichler HG, Sperr WR, et al. Conversion of the major birch pollen allergen, Bet v 1, into two nonanaphylactic T cell epitope-containing fragments: candidates for a novel form of specific immunotherapy. *J Clin Invest*. 1997;99(7):1673–81. <https://doi.org/10.1172/JCI119330>.
 63. Vrtala S, Fohr M, Campana R, Baumgartner C, Valent P, Valenta R. Genetic engineering of trimers of hypoallergenic fragments of the major birch pollen allergen, Bet v 1, for allergy vaccination. *Vaccine*. 2011;29(11):2140–8. <https://doi.org/10.1016/j.vaccine.2010.12.080>.
 64. Pace E, Duro G, La Grutta S, Ferraro M, Bruno A, Bousquet J, et al. Hypoallergenic fragment of Par j 2 increases functional expression of toll-like receptors in atopic children. *Allergy*. 2006;61(12):1459–66. <https://doi.org/10.1111/j.1398-9995.2006.01234.x>.
 65. Chen KW, Fuchs G, Sonneck K, Gieras A, Swoboda I, Douladiris N, et al. Reduction of the in vivo allergenicity of Der p 2, the major house-dust mite allergen, by genetic engineering. *Mol Immunol*. 2008;45(9):2486–98. <https://doi.org/10.1016/j.molimm.2008.01.006>.
 66. Ferreira F, Ebner C, Kramer B, Casari G, Briza P, Kungl AJ, et al. Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J*. 1998;12(2):231–42.
 67. Swoboda I, De Weerd N, Bhalla PL, Niederberger V, Sperr WR, Valent P, et al. Mutants of the major ryegrass pollen allergen, Lol p 5, with reduced IgE-binding capacity: candidates for grass pollen-specific immunotherapy. *Eur J Immunol*. 2002;32(1):270–80. [https://doi.org/10.1002/1521-4141\(200201\)32:1<270::Aid-Immu270>3.0.Co;2-X](https://doi.org/10.1002/1521-4141(200201)32:1<270::Aid-Immu270>3.0.Co;2-X).
 68. Zuidmeer-Jongejan L, Huber H, Swoboda I, Rigby N, Versteeg SA, Jensen BM, et al. Development of a hypoallergenic recombinant parvalbumin for first-in-man subcutaneous immunotherapy of fish allergy. *Int Arch Allergy Immun*. 2015;166(1):41–51. <https://doi.org/10.1159/000371657>.
 69. Campana R, Vrtala S, Maderegger B, Jertschin P, Stegellner G, Swoboda I, et al. Hypoallergenic derivatives of the major birch pollen allergen Bet v 1 obtained by rational sequence reassembly. *J Allergy Clin Immunol*. 2010;126(5):1024–31, 31 e1–8. <https://doi.org/10.1016/j.jaci.2010.05.023>.
 70. Curin M, Weber M, Thalhammer T, Swoboda I, Focke-Tejkl M, Blatt K, et al. Hypoallergenic derivatives of Fel d 1 obtained by rational reassembly for allergy vaccination and tolerance induction. *Clin Exp Allergy*. 2014;44(6):882–94. <https://doi.org/10.1111/cea.12294>.
 71. Asturias JA, Ibarrola I, Arilla MC, Vidal C, Ferrer A, Gamboa PM, et al. Engineering of major house dust mite allergens Der p 1 and Der p 2 for allergen-specific immunotherapy. *Clin Exp Allergy*. 2009;39(7):1088–98. <https://doi.org/10.1111/j.1365-2222.2009.03264.x>.
 72. Chen KW, Blatt K, Thomas WR, Swoboda I, Valent P, Valenta R, et al. Hypoallergenic Der p 1/Der p 2 combination vaccines for immunotherapy of house dust mite allergy. *J Allergy Clin Immunol*. 2012;130(2):435. <https://doi.org/10.1016/j.jaci.2012.05.035>.
 73. Linhart B, Mothes-Luksch N, Vrtala S, Kneidinger M, Valent P, Valenta R. A hypoallergenic hybrid molecule with increased immunogenicity consisting of derivatives of the major grass pollen allergens, Phl p 2 and Phl p 6, for allergy vaccination. *Wien Klin Wochenschr*. 2008;120:114.
 74. King TP, Jim SY, Monsalve RI, Kagey-Sobotka A, Lichtenstein LM, Spangfort MD. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellow jacket and paper wasp venom allergen antigen 5s. *J Immunol*. 2001;166(10):6057–65. <https://doi.org/10.4049/jimmunol.166.10.6057>.
 75. Fujimura T, Fujinami K, Ishikawa R, Tateno M, Tahara Y, Okumura Y, et al. Recombinant fusion allergens, Cry j 1 and Cry j 2 from Japanese cedar pollen, conjugated with polyethylene glycol potentiate the attenuation of Cry j 1-specific IgE production in Cry j 1-sensitized mice and Japanese cedar pollen allergen-sensitized monkeys. *Int Arch Allergy Immun*. 2015;168(1):32–43. <https://doi.org/10.1159/000441141>.
 76. Karamloo F, Schmid-Grendelmeier P, Kussebi F, Akdis M, Salagianni M, von Beust BR, et al. Prevention of allergy by a recombinant multi-allergen vaccine with reduced IgE binding and preserved T cell epitopes. *Eur J Immunol*. 2005;35(11):3268–76. <https://doi.org/10.1002/eji.200425522>.
 77. Kussebi F, Karamloo F, Rhyner C, Schmid-Grendelmeier P, Salagianni M, Mannhart C, et al. A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2005;115(2):323–9. <https://doi.org/10.1016/j.jaci.2004.11.041>.
 78. Valenta R, Campana R, Niederberger V. Recombinant allergy vaccines based on allergen-derived B cell epitopes. *Immunol Lett*. 2017;189:19–26. <https://doi.org/10.1016/j.imlet.2017.04.015>. **An excellent review on allergen-derived B cell epitopes.**
 79. Ziegelmayer P, Focke-Tejkl M, Schmutz R, Lemell P, Ziegelmayer R, Weber M, et al. Mechanisms, safety and efficacy of a B cell epitope-based vaccine for immunotherapy of grass pollen allergy. *Ebiomedicine*. 2016;11:43–57. <https://doi.org/10.1016/j.ebiom.2016.08.022>.
 80. Weber M, Niespodziana K, Linhart B, Neubauer A, Huber H, Henning R, et al. Comparison of the immunogenicity of BM32, a recombinant hypoallergenic B cell epitope-based grass pollen allergy vaccine with allergen extract-based vaccines. *J Allergy Clin Immunol*. 2017;140(5):1433–1436.e6. <https://doi.org/10.1016/j.jaci.2017.03.048>.
 81. Niespodziana K, Focke-Tejkl M, Linhart B, Civaj V, Blatt K, Valent P, et al. A hypoallergenic cat vaccine based on Fel d 1-derived peptides fused to hepatitis B PreS. *J Allergy Clin Immunol*. 2011;127(6):1562–70 e6. <https://doi.org/10.1016/j.jaci.2011.02.004>.
 82. Cornelius C, Schoneweis K, Georgi F, Weber M, Niederberger V, Ziegelmayer P, et al. Immunotherapy with the PreS-based grass pollen allergy vaccine BM32 induces antibody responses protecting against hepatitis B infection. *Ebiomedicine*. 2016;11:58–67. <https://doi.org/10.1016/j.ebiom.2016.07.023>.
 83. Nguyen NT, Raskopf E, Shah-Hosseini K, Zadayan G, Mosges R. A review of allergoid immunotherapy: is cat allergy a suitable target? *Immunotherapy*. 2016;8(3):331–49. <https://doi.org/10.2217/imt.15.121>.

84. Rosewich M, Lee D, Zielen S. Pollinex Quattro an innovative four injections immunotherapy in allergic rhinitis. *Hum Vacc Immunother*. 2013;9(7):1523–31. <https://doi.org/10.4161/hv.24631>.
85. Reinhold T, Bruggenjurgen B. Cost-effectiveness of grass pollen SCIT compared with SLIT and symptomatic treatment. *Allergo J Int*. 2017;26(1):7–15. <https://doi.org/10.1007/s40629-016-0002-y>.
86. El-Qutob D, Moreno F, Subtil-Rodriguez A. Specific immunotherapy for rhinitis and asthma with a subcutaneous hypoallergenic high-dose house dust mite extract: results of a 9-month therapy. *Immunotherapy*. 2016;8(8):867–76. <https://doi.org/10.2217/imt-2015-0017>.
87. Roger Reig A, Ibero Iborra M, Carrillo Diaz T, Lopez Abad R, Sanchez Moreno V, Alvarez Nieto J, et al. Perceived efficacy and satisfaction of patients with subcutaneous hypoallergenic high-dose house dust mite extract. *Eur Ann Allergy Clin Immunol*. 2017;49(3):100–5.
88. Mahler V, Klein C, Sager A, Zimmermann J. House dust mite-specific immunotherapy with two licensed vaccines: outcome under clinical routine conditions. *Immun Inflamm Dis*. 2017;5(2):132–40. <https://doi.org/10.1002/iid3.141>.
89. Allekotte S, Huser C, Dieterich P, Singh J, Compalati E, Mosges R. A randomized Dbpc dose-finding trial of slit allergoids tablets in house dust mites (HDM) allergic patients. *J Allergy Clin Immun*. 2015;135(2):Ab388-Ab.
90. Huser C, Dieterich P, Singh J, Shah-Hosseini K, Allekotte S, Lehmacher W, et al. A 12-week DBPC dose-finding study with sublingual monomeric allergoid tablets in house dust mite-allergic patients. *Allergy*. 2017;72(1):77–84. <https://doi.org/10.1111/all.12913>.
91. Chaker AM, Al-Kadah B, Luther U, Neumann U, Wagenmann M. An accelerated dose escalation with a grass pollen allergoid is safe and well-tolerated: a randomized open label phase II trial. *Clin Transl Allergy*. 2016;6:4. <https://doi.org/10.1186/s13601-016-0093-z>.
92. Sirvent S, Soria I, Cirauqui C, Cases B, Manzano AI, Diez-Rivero CM, et al. Novel vaccines targeting dendritic cells by coupling allergoids to nonoxidized mannan enhance allergen uptake and induce functional regulatory T cells through programmed death ligand 1. *J Allergy Clin Immunol*. 2016;138(2):558–67 e11. <https://doi.org/10.1016/j.jaci.2016.02.029>.
93. De Gregorio E, Tritto E, Rappuoli R. Alum adjuvanticity: unraveling a century old mystery. *Eur J Immunol*. 2008;38(8):2068–71. <https://doi.org/10.1002/eji.200838648>.
94. Klimek L, Bachert C, Lukat KF, Pfaar O, Meyer H, Narkus A. Allergy immunotherapy with a hypoallergenic recombinant birch pollen allergen rBet v 1-FV in a randomized controlled trial. *Clin Transl Allergy*. 2015;5:28. <https://doi.org/10.1186/s13601-015-0071-x>.
95. Klimek L, Schendzielorz P, Pinol R, Pfaar O. Specific subcutaneous immunotherapy with recombinant grass pollen allergens: first randomized dose-ranging safety study. *Clin Exp Allergy*. 2012;42(6):936–45. <https://doi.org/10.1111/j.1365-2222.2012.03971.x>.
96. Yu CS, An M, Li M, Liu HP. Immunostimulatory properties of lipid modified CpG oligonucleotides. *Mol Pharm*. 2017;14(8):2815–23. <https://doi.org/10.1021/acs.molpharmaceut.7b00335>.
97. Creticos PS, Schroeder JT, Hamilton RG, Balcer-Whaley SL, Khattignavong AP, Lindblad R, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med*. 2006;355(14):1445–55. <https://doi.org/10.1056/NEJMoa052916>.
98. Kundig TM, Senti G, Schnetzler G, Wolf C, Vavricka BMP, Fulurija A, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J Allergy Clin Immun*. 2006;117(6):1470–6. <https://doi.org/10.1016/j.jaci.2006.01.040>.
99. Senti G, Johansen P, Haug S, Bull C, Gottschaller C, Müller P, et al. Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial. *Clin Exp Allergy*. 2009;39(4):562–70. <https://doi.org/10.1111/j.1365-2222.2008.03191.x>.
100. Engeroff P, Caviezel F, Stomi F, Thoms F, Vogel M, Bachmann MF. Allergens displayed on virus-like particles are highly immunogenic but fail to activate human mast cells. *Allergy*. 2017;73(2):341–9. <https://doi.org/10.1111/all.13268>.
101. Herzberger J, Niederer K, Pohlitz H, Seiwert J, Worm M, Wurm FR, et al. Polymerization of ethylene oxide, propylene oxide, and other alkylene oxides: synthesis, novel polymer architectures, and bioconjugation. *Chem Rev*. 2016;116(4):2170–243. <https://doi.org/10.1021/acs.chemrev.5b00441>.
102. Pohlitz H, Bellinghausen I, Schomer M, Heydenreich B, Saloga J, Frey H. Biodegradable pH-sensitive poly(ethylene glycol) nanocarriers for allergen encapsulation and controlled release. *Biomacromolecules*. 2015;16(10):3103–11. <https://doi.org/10.1021/acs.biomac.5b00458>.
103. Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. *Immunol Cell Biol*. 2004;82(5):488–96. <https://doi.org/10.1111/j.1440-1711.2004.01272.x>.
104. Basomba A, Tabar AI, de Rojas DHF, Garcia BE, Alamar R, Olaguibel JM, et al. Allergen vaccination with a liposome-encapsulated extract of dermatophagoides pteronyssinus: a randomized, double-blind, placebo-controlled trial in asthmatic patients. *J Allergy Clin Immun*. 2002;109(6):943–8. <https://doi.org/10.1067/mai.2002.124465>.
105. Sanders MT, Brown LE, Deliyannis G, Pearse MJ. ISCOM-based vaccines: the second decade. *Immunol Cell Biol*. 2005;83(2):119–28. <https://doi.org/10.1111/j.1440-1711.2005.01319.x>.
106. Lenarczyk A, Le TT, Drane D, Malliaros J, Pearse M, Hamilton R, et al. ISCOM based vaccines for cancer immunotherapy. *Vaccine*. 2004;22(8):963–74. <https://doi.org/10.1016/j.vaccine.2003.09.014>.
107. Zhu D, Kepley CL, Zhang M, Zhang K, Saxon A. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits fc epsilon RI-mediated degranulation. *Nat Med*. 2002;8(5):518–21. <https://doi.org/10.1038/nm0502-518>.
108. Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A. A chimeric human-cat fusion protein blocks cat-induced allergy. *Nat Med*. 2005;11(4):446–9. <https://doi.org/10.1038/nm1219>.
109. Senti G, Cramer R, Kuster D, Johansen P, Martinez-Gomez JM, Graf N, et al. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J Allergy Clin Immunol*. 2012a;129(5):1290–6. <https://doi.org/10.1016/j.jaci.2012.02.026>.
110. Zaleska A, Eiwegger T, Soyer O, van de Veen W, Rhyner C, Soyka MB, et al. Immune regulation by intralymphatic immunotherapy with modular allergen translocation MAT vaccine. *Allergy*. 2014;69(9):1162–70. <https://doi.org/10.1111/all.12461>.
111. Massanari M, Nelson H, Casale T, Busse W, Kianifard F, Geba GP, et al. Effect of pretreatment with omalizumab on the tolerability of specific immunotherapy in allergic asthma. *J Allergy Clin Immunol*. 2010;125(2):383–9. <https://doi.org/10.1016/j.jaci.2009.11.022>.
112. Begin P, Dominguez T, Wilson SP, Bacal L, Mehrotra A, Kausch B, et al. Phase 1 results of safety and tolerability in a rush oral immunotherapy protocol to multiple foods using omalizumab. *Allergy Asthma Clin Immunol*. 2014;10(1):7. <https://doi.org/10.1186/1710-1492-10-7>.
113. MacGinnitie AJ, Rachid R, Gragg H, Little SV, Lakin P, Cianferoni A, et al. Omalizumab facilitates rapid oral desensitization for peanut allergy. *J Allergy Clin Immunol*. 2017;139(3):873–81 e8. <https://doi.org/10.1016/j.jaci.2016.08.010>.
114. Senti G, von Moos S, Tay F, Graf N, Sonderegger T, Johansen P, et al. Epicutaneous allergen-specific immunotherapy ameliorates

- grass pollen-induced rhinoconjunctivitis: a double-blind, placebo-controlled dose escalation study. *J Allergy Clin Immunol*. 2012b;129(1):128–35. <https://doi.org/10.1016/j.jaci.2011.08.036>.
115. Campana R, Moritz K, Neubauer A, Huber H, Henning R, Brodie TM, et al. Epicutaneous allergen application preferentially boosts specific T cell responses in sensitized patients. *Sci Rep*. 2017b;7(1):11657. <https://doi.org/10.1038/s41598-017-10278-1>.
 116. Hylander T, Larsson O, Petersson-Westin U, Eriksson M, Kumlien Georen S, Winqvist O, et al. Intralymphatic immunotherapy of pollen-induced rhinoconjunctivitis: a double-blind placebo-controlled trial. *Respir Res*. 2016;17(1):10. <https://doi.org/10.1186/s12931-016-0324-9>.
 117. Patterson AM, Bonny AE, Shiels WE 2nd, Erwin EA. Three-injection intralymphatic immunotherapy in adolescents and young adults with grass pollen rhinoconjunctivitis. *Ann Allergy Asthma Immunol*. 2016;116(2):168–70. <https://doi.org/10.1016/j.anai.2015.11.010>.
 118. Schmid JM, Nezam H, Madsen HH, Schmitz A, Hoffmann HJ. Intralymphatic immunotherapy induces allergen specific plasmablasts and increases tolerance to skin prick testing in a pilot study. *Clin Transl Allergy*. 2016;6(1):19. <https://doi.org/10.1186/s13601-016-0107-x>.
 119. Bonertz A, Roberts GC, Hoefnagel M, Timon M, Slater JE, Rabin RL, et al. Challenges in the implementation of EAACI guidelines on allergen immunotherapy: a global perspective on the regulation of allergen products. *Allergy*. 2017;73(1):64–76. <https://doi.org/10.1111/all.13266>.
 120. Asaria M, Dhami S, van Ree R, Gerth van Wijk R, Muraro A, Roberts G, et al. Health economic analysis of allergen immunotherapy for the management of allergic rhinitis, asthma, food allergy and venom allergy: a systematic overview. *Allergy*. 2017;73(2):269–83. <https://doi.org/10.1111/all.13254>.
 121. Pfaar O, Lang S, Pieper-Furst U, Astvatsatourov A, Gerich F, Klimek L, et al. Ultra-short-course booster is effective in recurrent grass pollen-induced allergic rhinoconjunctivitis. *Allergy*. 2017;73(1):187–95. <https://doi.org/10.1111/all.13240>.
 122. Lee MF, Song PP, Lin TM, Chiu YT, Chen YH. For t 2 DNA vaccine prevents *Forcipomyia taiwana* (biting midge) allergy in a mouse model. *Allergy*. 2016;71(4):522–31. <https://doi.org/10.1111/all.12832>.