

Recombinant Allergen Immunotherapy: Clinical Evidence of Efficacy—A Review

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Abstract Recombinant allergens for immunotherapy aim to overcome the problems of natural extracts as they can be produced in unlimited amounts with exact physiochemical and immunological properties. These can be modified to have more favourable characteristics including reduced IgE reactivity or enhanced immunogenicity. Different types of recombinant allergens have been evaluated in clinical phase II and III trials whilst others are currently under development. In this review, we identified double-blind, placebo-controlled randomised clinical trials assessing the efficacy and safety of various recombinant allergen preparations. The majority of studies have up to now focused on cat, grass, birch, ragweed and bee venom allergens. Some studies have shown some of these preparations to be effective and well tolerated. However, there are still outstanding issues regarding optimum doses, minimising side effects and long-term effects.

Keywords Allergy · Rhinitis · Asthma · Recombinants · T-cell epitopes · Wild-type recombinants · Modified/hypoallergen allergens · Fusion proteins · Cat · Grass · Birch · Ragweed · Venom allergy · Immunotherapy · Efficacy

Introduction

Allergen immunotherapy (AIT) is the only disease-modifying treatment currently available for allergic conditions. In systematic

reviews and meta-analyses of randomised clinical trials (RCTs), subcutaneous immunotherapy (SCIT) has been found to be an effective treatment for asthma [1], allergic rhinitis [2] and hymenoptera venom allergy [3–5]. The sublingual route (SLIT) has also been shown to be an effective and safe alternative for allergic rhinitis and conjunctivitis [6, 7]. There is also emerging evidence of using other routes such as intra-lymphatic, epicutaneous and oral as well as using AIT in other conditions including atopic dermatitis and food allergy.

Non-polymerised (unmodified) allergen extracts are currently the most widely prescribed regimens [8]. However, one of the main concerns of researchers and clinicians in this field has been the quality of these natural extracts, in particular, the presence of contaminants or undefined components that may promote allergic immune responses, variable amounts of relevant allergens and thus differences in immunogenicity with some individuals responding better than others [9]. Another important issue is the safety profile and risk of adverse reactions on administration, which is currently preventing more widespread use of immunotherapy [10].

Recombinant allergens for immunotherapy aim to overcome the problems of natural extracts as they can be produced in unlimited amounts at highly standardised quality with exact physiochemical and immunological properties. These can be modified to have more favourable characteristics such as reduced IgE reactivity or enhanced immunogenicity [11]. Furthermore, potencies and ratios can be exactly adjusted for each molecule, which may lead to a “patient-tailored” individualised therapy in the future [12].

Different types of recombinant allergens have already been evaluated in clinical phase II and III trials whilst other allergens are currently under development, being tested on animal models or early phase trials.

In this review, we aim to evaluate the clinical efficacy and safety of recombinant AIT preparations based on double-blind, placebo-controlled randomised clinical trials (DB PC RCTs).

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Recombinant allergens for immunotherapy

Different strategies for producing AIT preparations using recombinant DNA technology have been developed [13].

T-cell epitope-containing peptides were designed to modulate allergen-specific T-cell responses without Ig-E mediated activation of effector cells [9]. T-cell epitopes derived from Fel d 1, Der p 1, Cry j 1, Cry j 2 and Api m 1 have been produced. However, trials mainly performed with peptides from the major cat allergen Fel d 1 have shown that the peptides can induce systemic late-phase side effects [14]. Another issue is that complex mixtures of the peptides need to be administered to cover the diverse T-cell epitope repertoire of patients [15].

Recombinant technology has enabled the production of hypoallergenic variants with an altered tertiary structure obtained by making mutations and deletions in allergens around B-cell epitope (IgE-binding) areas. Reduced IgE capacity can also be obtained by fragmentation, oligomerisation or fusion of allergen variants. All these molecules possess most T-cell epitopes of the wild-type allergen, therefore being able to target the broad T-cell repertoire of allergic patients while exhibiting reduced IgE reactivity. It is anticipated that they should not induce IgE-mediated side effects when administered to allergic patients. In addition, they should allow increases of the doses injected compared with wild-type molecules and induction of a Th1 response [14, 15].

Another approach aiming to eliminate IgE- as well as T-cell-mediated side effects is the use of recombinant fusion proteins. These consist of allergen-derived peptides typically between 20 and 35 amino acids in length, from the conformational IgE-binding sites but lacking IgE reactivity. They can either be coupled chemically to a carrier protein or produced as recombinant fusion proteins with a carrier protein [16]. Viral carrier proteins have been used for this purpose [17, 18]. Upon immunisation, these preparations focus IgG antibodies towards the IgE-binding sites on allergens but do not activate allergen-specific T cells because the T-cell help is derived from T-cell epitopes of the carrier protein [13].

Recombinant AIT: current clinical data and future developments

Both phase II and phase III clinical trials have been performed within the last decade [15]. The following gives an overview on different approaches as outlined above.

1) T-cell epitope peptides

Targeting of allergen-specific T cells has been achieved through the use of synthetic peptides containing T cell epitopes [19]. Early attempts to investigate T cell-directed peptide immunotherapy in clinical trials used two 27 amino acid polypeptides (IPC-1/IPC-2; AllervaxCat©) from the major cat allergen Fel d 1

administered subcutaneously in individuals with cat-allergic rhinitis and/or asthma. Four clinical trials evaluating safety and efficacy generated mixed results, with three studies [20–22] identifying some modest clinical benefit (Table 1). The other study [23] found that SCIT did not reduce immediate- or late-phase skin reactivity to cat extract containing Fel d 1 at 2, 6 and 24 weeks after treatment. In addition, there was no significant modification of cat antigen-specific cytokine production. Treatment with peptides was associated with a high incidence of adverse events including some acute allergic reactions and more frequent delayed symptoms of rhinitis, asthma and pruritus. Delayed symptoms of asthma were later characterised as IgE-independent, late asthmatic reactions caused by activation of airway allergen-specific T cells in asthmatic subjects [8, 24].

A number of small clinical studies were then performed using mixtures of shorter peptides from Fel d 1 administered intra-dermally. A single intradermal injection of a 12-peptide mixture was found to reduce late-phase skin reactions (LPSR) to challenge with whole allergen [25]. However, a DBPC, multi-dose (cumulative dose 90 µg of each peptide) trial of the same peptide mixture showed significant reductions in early- and late-phase skin response to intradermal challenge with allergen extract [26]. Smith et al. [27], when they compared cells from blood taken before with that after peptide immunotherapy, found a significant reduction in both proliferation and IL-13 production by allergen-stimulated CD4+ T cells. CD4(+) CD25(+) T cells suppressed both proliferation and IL-13 production by CD4(+) CD25(−) T cells before and after therapy but peptide therapy was not associated with any change in suppressive activity of these cells. Alexander et al. (2005) found that in the actively treated group, but not placebo, there were significant decreases in the late asthmatic reaction (LAR) to whole cat dander ($p=0.03$) at 3–4 months' follow-up. There were also significant improvements in asthma quality of life scores: rhinitis-sleep, non-nose/non-eye symptoms and nasal problems.

A 7-peptide formulation (Cat-PAD; Toleromune Cat©), based on the previously tested 12-peptide mixtures described, was assessed in a phase IIa safety and tolerability single-centre study [28]. A single injection (in 5 separate dose cohorts) was administered either intradermally or subcutaneously to subjects with cat allergen-induced allergic rhinoconjunctivitis. A dose of 3 nmol administered intradermally resulted in a reduction in the LPSR of approximately 40 % (placebo, 10 %). The adverse events profile was indistinguishable from that of placebo.

The same peptide formulation was evaluated in a DB PC, parallel-group trial, where 202 subjects attended an

Table 1 Summary of recombinant allergen double-blind, placebo-controlled, randomised clinical trials

Study	Allergen No. of peptides and length	Population Disease	AIT Route Duration	Results of main outcomes
T-cell epitope peptides				
Norman 1996 [21]	Cat Fel d 1 peptide 2×27	95 adults AR and AA	SCIT 7.5, 75 and 750 mcg (4 weeks)	Six weeks after treatment, the 750-mcg group showed a significant reduction in nasal and lung symptoms compared with placebo
Simons 1996 [23]	Cat Fel d 1 peptide 2×27	42 adults AR and AA	SCIT 250 mcg (4 weeks)	SCIT did not reduce immediate- or late-phase skin reactivity to cat extract containing Fel d 1 at 2, 6 and 24 weeks after treatment. No significant modification of cat antigen-specific cytokine production
Pene 1998 [22]	Cat Fel d 1 peptide 2×27	31 adults AA	SCIT 7.5, 75 and 750 mcg (6 weeks)	Post-treatment bronchial provocation test (BPT) was not significantly different between groups. In the medium- and high-dose groups there was a significant improvement between baseline and post-treatment days. No correlation between BPT and the decreased in IL-4 production
Maguire 1999 [20]	Cat Fel d 1 peptide 2×27	133 adults AR and AA	SCIT 75 and 750 mcg (8 injections)	750 mcg improved pulmonary function (FEV1) in patients with reduced baseline FEV1 and global evaluation of the subjects' ability to tolerate cats
Haselden 2001 [24]	Cat Fel d 1 peptide	34 adults AR and AA	ID 80 ug of Fel d 1 chain 1-derived peptides	Immunostaining of bronchial biopsy specimens showed no changes in the numbers of eosinophils, neutrophils, basophils, mast cells, CD3(+), CD4(+) or CD8(+) T cells, CD25(+) cells or macrophages, or cells mRNA(+) for IL-4, IL-5 or IL-13 when the FC1P day was compared with the diluent control day. There were no significant differences in eosinophil numbers in either broncho-alveolar lavage fluids or in peripheral blood after FC1P challenge
Oldfield 2002 [26]	Cat Fel d 1 peptide	16 adults AA	ID 5, 10, 25 and 50 mcg (total dose 90 mcg) peptide at 3–4-day intervals over 2 weeks and observed for 6 h after each injection	Well tolerated. Late-phase cutaneous reactions to whole cat dander were significantly reduced in the peptide group compared to placebo between baseline and both follow-ups, but the difference between groups was not significant. Decrease in IFN-γ, IL-4 and IL-13, increase in IL10
Fellrath 2003 [30]	Bee venom Api m 1 peptide 3: 11, 12, 18	16 adults	SC 3 LSPs with cumulative dose of 251.1 ug of each LSP within 3 h	Increased allergen-specific IgG4, increased IFN-γ and IL-10 in PBMC
Smith 2004 [27]	Cat Fel d 1 peptide	16 adults AR and AR	ID 12 visits (30 weeks)	Significant reduction in both proliferation and IL-13 production by allergen-stimulated CD4+ T cells
Worm 2011 [28] DB PC RCT escalating single dose	Cat Fel d 1 peptide	88 adults AR and AA	ID / SC	Cat allergen extract, but not peptides, induced histamine release in blood basophils. The dose of vaccine resulting in the greatest inhibition of the late-phase skin response to intradermal whole allergen challenge was 3 nmol

Table 1 (continued)

Study	Allergen No. of peptides and length	Population Disease	AIT Route Duration	Results of main outcomes
Patel 2013 [29]	Cat Cat-PAD mixture of 7 peptides	202 adults AR	ID Cat-PAD is an equimolar mixture of 7 peptides at 50 or 100 nmol/ml or placebo 18 – 22-week 1-year follow-up study	Cat-PAD improved the ocular and nasal components of rhinoconjunctivitis symptoms in subjects with cat allergy. Greater efficacy with 4 administrations of a 6-nmol dose 4 weeks apart than with 8 administrations of a 3-nmol dose 2 weeks apart. The effect of 6 nmol persisted 1 year after the start of treatment and was significantly different from that of 3 nmol and placebo. The treatment effect persisted 1 year after the start of treatment
Recombinant hypoallergens				
Niederberger 2004 [33]	Birch rBet v 1 fragments/trimer	124 adults AR and AA	SC 1, 2, 4, 8, 10, 20, 40 and 80 mcg of protein (12 months)	In the active group: (1) induction of protective IgG Abs that inhibited allergen-induced release of inflammatory mediators; (2) reduction of cutaneous sensitivity; (3) improvement of symptoms.
Purohit 2008 [34]	Birch Bet v 1 trimer/fragments	84 adults AR and AA	SC maximum dose was 80 mcg (12–14 months)	No significant improvement of combined symptom medication score. Trends towards improvement in the subjects' well-being and clinical symptoms (nasal scores). Treatment induced strong IgG1 and IgG4 allergen-specific antibody responses. Systemic reactions were more frequently by fragments. A large proportion of adverse side effects appeared hours following injections
Rak 2009 [36]	Birch rBet v 1	483 adults AR and AA	SLIT 12.5, 25 and 50 mcg	First clinical trial that demonstrated clinically relevant efficacy of a recombinant allergen SLIT tablet in birch-related rhinoconjunctivitis
Meyer 2013 [37]	rBet v 1-FV folding variant	36 adults AR	SC weekly injections (10 weeks)	The total symptom score significantly decreased in all active groups compared with placebo
Recombinant wild-type allergens				
Jutel 2005 [31]	Grass Phl p1, 2, 5a/b, 6	62 adults AR and AR	SC 40 mcg total protein; 10 mcg Phl p 1; 5 mcg Phl p 2&6; 10 mcg Phl p 5a&b; (18 months)	Significant improvement in symptom medication in subjects receiving recombinant allergens compared to placebo, with reductions in both symptoms and medication usage. RQOQ showed clinically relevant significant improvements in overall assessment and in 5 of 7 separate domains. Conjunctival provocation showed a clear trend in favour of active treatment. All treated subjects developed strong allergen-specific IgG1 and IgG4 antibody responses
Pauli 2008 [32]	Birch Bet v 1 recombinant birch pollen allergen vaccine (rBet v 1a), licenced birch pollen extract, natural purified birch pollen allergen (nBet v 1)	134 adults AR and AA	SC 0.5, 5, 50 mcg/ml Bet v 1 (24 months)	Significant reductions in AR symptoms, rescue and skin sensitivities were observed in the 3 actively treated groups compared with placebo during 2 consecutive

Table 1 (continued)

Study	Allergen No. of peptides and length	Population Disease	AIT Route	Duration	Results of main outcomes
Coupled					pollen seasons. Clinical improvement was accompanied by marked increases in Bet v 1-specific IgG levels, which were higher in the rBet v 1-treated group than in the birch and nBet v 1-treated groups. No severe systemic adverse events were observed in the rBet v 1-treated group
Tulic 2004 [38]	Ragweed Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate (AIC)	57 adults AR	SC	6 escalating doses of AIC 0.06–12 mcg (6 weeks)	AIC was safe and well tolerated. Short-course immunotherapy with AIC can modify the response of nasal mucosa to allergen challenge by increasing Th1 cytokine production and decreasing Th2 cytokine production and eosinophilia. This modification was not immediate but was observed 4 to 5 months after completion of immunotherapy and seasonal ragweed pollen exposure
Creticos 2006 [39]	Ragweed Amb a 1, a ragweed pollen antigen, conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA (AIC)	25 adults AR	SC	0.06, 0.3, 1.2, 3, 6, 12 mcg of AIC (6 weeks)	A 6-week regimen of the AIC vaccine appeared to offer long-term clinical efficacy in the treatment of ragweed allergic rhinitis. AIC did not alter the vascular permeability response (measured by the albumin level in nasal-lavage fluid) to nasal provocation. AIC group had better peak-season rhinitis scores on the VAS, peak-season daily nasal symptom diary scores and midseason overall quality-of-life scores than the placebo group. AIC induced a transient increase in Amb a 1-specific IgG antibody. Clinical benefits of AIC were again observed in the subsequent ragweed season, with improvements over placebo in peak-season rhinitis VAS and peak-season daily nasal symptom diary scores. The season-specific IgE antibody response was suppressed

DBPC RCT, double-blind, placebo-controlled, randomised clinical trial; A, allergic rhinitis; A, allergic asthma; SC, subcutaneous; SLIT, sublingual; ID, intradermal

environmental exposure chamber in which they were exposed to cat allergen before and after treatment with two different regimens of Cat-PAD over a 3-month period [29]. Clinical efficacy was assessed as a change in total rhinoconjunctivitis symptom scores 18 to 22 weeks and 50 to 54 weeks after the start of treatment. Treatment with Cat-PAD showed greater efficacy with four administrations of a 6-nmol dose 4 weeks apart than with eight administrations of a 3-nmol dose 2 weeks apart. Interestingly, the treatment effect of

6 nmol persisted 1 year after the start of treatment and was significantly different from that of 3 nmol and placebo. The treatment effect was apparent on both nasal and ocular symptoms at 1 year. Importantly, the treatment was safe and well tolerated with an adverse events profile similar to that of placebo.

Other than cat allergy, bee venom allergy has been another area targeted for treatment with T-cell peptides. Bee venom-allergic subjects were treated with three long synthetic peptides encompassing the entire Api m

1 sequence in a rush desensitisation protocol to a maintenance dose of 100 µg of each peptide repeated at days 4, 7, 14, 42 and 70 [30]. Increased T cell proliferation due to the treatment peptides was observed, along with increases in IFN-gamma and IL-10 levels, but not Th2 cytokines. Allergen-specific IgG4, but not IgE, levels increased during the study. No significant changes in skin reactions to intradermal allergen extract challenge were seen. No severe adverse events were reported.

2) Wild-type recombinants

Two DBPC RCTs using SCIT have been performed with recombinant allergens delivered exactly as their natural counterparts, one for grass pollen and one for birch pollen allergy.

A clinical phase II trial by Jutel et al. evaluating the efficacy of a mixture of five recombinant timothy grass pollen allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6) administered subcutaneously found significant improvements in combined symptom and medication scores and quality of life after 18 months in the AIT group compared to placebo in 57 grass pollen-allergic patients [31]. Active treatment induced increases in both IgG1 and IgG4 *Phleum pratense*-specific antibody concentrations. IgG1 concentrations increased approximately 60-fold, peaking during the first 12 months of the study. IgG4 concentrations showed a continuing upward trend, achieving an approximately 4,000-fold increase by the end of treatment. Interestingly, some subjects in the active group had no Phl p5a/b-specific IgE at the beginning of the study and failed to develop IgE antibodies despite repeated injections of the allergen, but nevertheless, they developed IgG1 and particularly IgG4 responses. This finding suggests that this recombinant grass pollen “cocktail” can induce immunogenic tolerance without causing new sensitisations.

However, systemic side reactions were observed in 24 % of actively treated patients, suggesting that recombinant wild-type allergens are as allergenic as the corresponding natural allergens and may induce IgE-mediated side effects during immunotherapy. The high frequency of systemic side effects may also have been related to the rather high dose of allergens injected (40 µg rPhl p).

A subsequent phase II DBPC study by Pauli et al. compared three subcutaneous preparations (recombinant birch pollen allergen rBet v 1a, licenced birch pollen extract, natural purified birch pollen allergen nBet v 1) and placebo in 134 birch pollen-allergic adults [32]. Significant reductions in rhinoconjunctivitis symptoms, rescue medication and skin sensitivities were revealed in the three actively treated groups compared with placebo during two consecutive pollen seasons. Combining symptom and medication scores

showed a 59 % reduction in disease severity in patients treated with birch pollen extract, 57 % for rBet v 1 and 60.5 % for nBet v 1 compared with placebo. Furthermore, Bet v 1-specific IgG1, IgG2 and IgG4 increased in all groups. No serious or systemic adverse events related to rBet v 1 treatment were observed. However, local reactions at the injection site occurred more frequently in the rBet v 1 group. Of note, new IgE specificities were induced in 3 of 29 patients treated with birch pollen extract, but in none of the rBet v 1-treated or nBet v 1-treated patients. This finding on recombinant Betv1 vaccination was in line with the results of Jutel et al. with a recombinant five-grass pollen “cocktail”, as outlined above. Overall, this study confirmed the proof of concept of “non-inferiority” of recombinant “wild-type”-Bet v1 vaccines compared to natural birch pollen-extract sources in the clinical efficacy of “wild type” as a means of clinical efficacy and immunological responses. The clinical results were obtained with a rather modest maintenance dose of Bet v 1 (15 µg), which may explain the low frequency of severe systemic reactions.

3) Modified/hypoallergen allergens

As a next step in recombinant technologies in allergen vaccines, hypoallergenic derivatives of the major isoform Bet v 1a have been developed either by expressing three copies of the gene in sequence to produce a trimer or by cleaving the gene in order to create two recombinant peptides (fragments) that together represent the whole sequence of Bet v 1.

Niederberger et al. [33] performed a multi-centre, DBPC, parallel-group, randomised trial over a 12-month period with one pre-seasonal treatment course. One hundred twenty-four birch pollen-allergic patients were treated with eight subcutaneous injections containing increasing doses (1, 2, 4, 8, 10, 20, 40 and 80 µg of protein) of the trial preparations (rBet v 1 fragments, 30 %; rBet v 1 trimer, 30 %) or placebo (40 %) in 1- to 2-weekly intervals. Active treatment induced protective IgG antibodies that inhibited allergen-induced release of inflammatory mediators. They also observed a reduction of cutaneous sensitivity as well as an improvement of symptoms in actively treated patients. The antibodies inhibited allergen-induced basophil histamine release in vitro, and IgG1 antibody titres correlated with improvement in clinical symptoms, as judged based on a 10-point interval scale and reduction in skin test reactivity to Bet v 1. Specific IgE responses showed a three-fold increase in the placebo group as a consequence of seasonal pollen exposure, whereas those in the two treatment groups were blunted.

Another three-arm DBPC study was performed in birch pollen-allergic individuals to assess the clinical

efficacy of a single course of pre-seasonal injection immunotherapy with either a recombinant Bet v 1 trimer, an equimolar mixture of two recombinant Bet v 1 fragments or placebo [34]. This study failed to demonstrate significant differences for the primary end point (symptom scores) between active- and placebo-treated patients in the first birch pollen season after a pre-seasonal short-time therapy course of eight injections in weekly intervals (maximum dose 80 µg Bet v 1). The authors concluded that this could be explained by a relatively modest cumulative dose of the vaccines as well as a low pollen exposure in the respective regions. Furthermore, no significant difference in skin sensitivity and nasal challenge test was observed among the three groups 12 months after initiating immunotherapy. However, both regimens (fragments and trimer) induced strong IgG1 and IgG4 responses against the Bet v 1 allergen in all treated subjects, with levels peaking at the end of treatment and remaining substantially reduced 7 months after discontinuation of injections. Local injection-site reactions were most frequent in the trimer group, affecting 59.5 % of patients as opposed to 37.8 % and 30.6 % in the fragment and placebo groups, respectively. Systemic reactions, the majority being grade 1 but also some grade 2, were observed in both actively treated groups but were more frequently elicited by fragments. Generalised urticaria was observed in 21 % of the fragment-treated patients and 16 % of the trimer group, with no episodes in the placebo group. The majority of these reactions occurred with the 80-µg maximum dose.

An rBet v 1 vaccine, formulated as a dissolving tablet, has also been developed for SLIT application [35]. A multi-centre, randomised, DB PC phase II trial was performed to evaluate the efficacy and safety of SLIT with three rBet v 1 dose levels. A total of 483 adults with birch pollen-related allergic rhinoconjunctivitis were randomised into 50-µg, 25-µg, 12.5-µg or placebo treatment groups. The patients were treated daily during 4 months pre-season and throughout the birch pollen season. As primary end point the Average Adjusted Symptom Score (AASS), a symptom score adjusted for rescue medication intake, and as secondary end point the Average Rescue Medication Score (ARMS) were recorded during the pollen season. All three actively treated groups differed significantly from the placebo group in AASS and there was no difference in efficacy among the three doses. The relative difference compared to placebo was about 20 % (mean). Rescue medication intake was reported to be high in the study but still the ARMS was approximately 33 % (mean) lower for the actively treated groups, with no significant difference among the three doses

compared to placebo. The safety profile was good with generally mild to moderate and most commonly local side effects. As there was no difference in efficacy among the three active treatment doses, the lowest dose (12.5 µg) showed the best efficacy/safety profile [36].

Recently, a new hypoallergenic folding variant of recombinant Bet v 1 (rBet v 1-FV) has been evaluated in a DB PC dose-ranging study [37]. Before AIT, 37 adult patients were exposed to birch pollen for 8 h in an environmental exposure chamber (EEC), then randomised to four maintenance dose groups of rBet v 1-FV (20 µg, 80 µg, 160 µg, 320 µg) and placebo. Patients were treated for 10 weeks with weekly subcutaneous injections and then re-exposed in the EEC. The total symptom score significantly decreased in all active groups compared with placebo (−18.8 % for placebo patients; −71.9 %, $p=0.0022$ for 20 µg; −75.6 %, $p=0.0007$ for 80 µg; −81.8 %, $p=0.0009$ for 160 µg; −78.3 %, $p=0.0003$ for 320 µg). IgG1 increased significantly in all active groups compared to placebo. All four active doses were well tolerated, and no serious adverse event occurred. It was concluded that a maintenance dose of 80 µg of rBet v 1-FV appears to be the ideal dose for AIT.

4) Recombinant vaccines coupled with immunostimulatory DNA sequences

The first clinical trial evaluating Amb, a 1-immunosstimulatory phosphorothioate oligonucleotide conjugate (AIC), as a novel immunotherapeutic compound in humans suffering from ragweed allergic rhinitis was performed by Tulic et al. [38]. Six escalating doses of AIC (0.06–12 µg, $n=28$) or placebo ($n=29$) were given at weekly intervals before the ragweed season. No difference between treatment groups was observed in symptom scores or medication use during the first ragweed season. During the second ragweed season, however, there was a significant decrease in chest symptoms and a trend toward reduced nasal symptoms in the AIC-treated group. AIC was safe and well tolerated by all patients. On re-challenge and repeat nasal biopsy after the end of the first ragweed season, the AIC-treated patients had a significantly reduced increase in eosinophils and IL-4 mRNA-positive cells and an increased number of IFN-γ mRNA-positive cells compared with placebo-treated patients.

Creticos et al. [39] performed a DB PC randomised phase 2 clinical trial of AIC in patients with ragweed-induced seasonal allergic rhinitis. A total of six injections of AIC (0.06, 0.3, 1.2, 3, 6 and 12 µg) or placebo was administered at weekly intervals before the onset of the first ragweed season and the patients ($n=25$) were followed through the subsequent ragweed season without further vaccine administration. Even though the study did not demonstrate that AIC affected the primary end point (nasal vascular permeability as assessed by nasal-lavage

albumin), it demonstrated that a once-weekly, six-injection regimen of AIC substantially reduced allergic rhinitis symptoms during the ragweed season. The suppressed rise in ragweed-specific IgE during the subsequent allergy season indicated immunomodulatory effects associated with a clinical benefit lasting through two ragweed seasons. AIC decreased immediate intradermal skin-test reactivity [mean decrease in wheal size after AIC=8 mm, placebo=3 mm ($p=0.04$ for the difference from baseline)]. Better peak-season rhinitis scores on the visual analogue scale, peak-season daily nasal symptom diary scores and midseason overall quality-of-life scores were observed in the AIC group than in the placebo group. No serious adverse events occurred. However, the limitations of this study were the small number of patients and the unknown long-term safety of the treatment. In a subsequent phase-II/III-trial on 462 ragweed-allergic adult patients, only a modest non-significant improvement of the primary clinical outcome (TNSS) could be demonstrated, which resulted in cessation of further clinical investigation of these CpG motives [40].

5) Fusion proteins: the future of recombinants?

Recombinant fusion proteins have also been developed. A non-allergenic birch pollen allergy vaccine consisting of hepatitis PreS-fused Bet v 1 peptides was found to focus blocking IgG toward IgE epitopes and shift immune responses to a tolerogenic and Th1 phenotype [18]. A hypoallergenic cat vaccine based on Fel d 1-derived peptides also fused to hepatitis B PreS showed no relevant IgE reactivity and exhibited more than 1,000-fold reduced allergenic activity in basophil activation tests. On immunisation of mice and rabbits, the fusion proteins induced Fel d 1-specific IgG antibodies that inhibited the binding of allergic patients' IgE to the allergen without allergic sensitisation to Fel d 1 [41]. It was concluded that this could represent a potentially safe vaccine for the treatment of cat allergy in the future.

Discussion

Recombinant DNA technology has consistently evolved over the last decades, enhancing our knowledge on the sequences and the three-dimensional structures of specific allergens. This information has allowed clinical and research investigators to understand even further the mechanisms of the allergic inflammation and to produce vaccines that can be used for the treatment of allergic diseases.

Recombinant AIT aims to provide a defined ultrapure component vaccine with a better, well-defined and measurable allergen content, which provides absolute standardisation; this

represents a major advantage compared to natural allergen extract vaccines (Table 2).

Considering that recombinant AIT could be used in the future as an alternative for allergen extract-based immunotherapy, in this review, we tried to focus on the published evidence of the clinical efficacy and the safety profile of recombinant AIT in humans with allergic disease. We identified various DBPC RCTs focussing mainly on four inhalant allergens (cat, grass, birch and ragweed) and on insect venom allergy (bee allergen). All these studies vary in the clinical outcomes used, the criteria for selection of patients, the dosages used and the duration of treatment.

Studies evaluating the efficacy, safety and/or the effects on the immune response of Fed d 1 T-cell peptides administered subcutaneously or intradermally have generated mixed clinical results. A large number of adverse events have been associated with the use of these preparations. At this stage, these results are encouraging, but more well-powered studies are needed to consolidate the clinical efficacy of peptide AIT for cat allergy. The advantages of peptide AIT compared to natural allergen AIT for cat allergy need to be thoroughly evaluated. Considering the asthmatic status of patients with cat allergy, the safety profile of this treatment modality also needs to be reviewed.

The efficacy of a mixture of five recombinant timothy grass pollen allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6) administered subcutaneously was demonstrated by significant improvements in combined symptom and medication scores and quality of life in patients with grass pollen allergy [31]. However, these encouraging findings need to be balanced with the high percentage of systemic adverse reactions observed. An optimal risk/benefit dose needs to be properly evaluated.

The major birch pollen allergen, Bet v 1, which is recognised by more than 90 % of birch pollen allergic patients [42], was selected for the production of hypoallergenic derivatives. Studies [32–34] have shown a significant improvement in symptom scores, medication scores, QoL parameters and allergen-specific immunological responses

Table 2 List of advantages and disadvantages of recombinant AIT

Advantages	Disadvantages
• Better molecular design • Consistent and reproducible product quality • Doses are standardised (mass units) • Optimal doses • Provides a precise monitoring of clinical and laboratory outcomes • Better safety profile (reduction of adverse reactions)	• Rigorous production requirements • Mandatory selection of isoforms • High development costs

compared to placebo. These findings are associated with a good safety profile. The optimal dose needs to be properly established.

The use of short-course 6-week AIT with immunostimulatory phosphorothioate oligonucleotide conjugates (AIC) has mainly been focussed on ragweed allergy. Two studies on allergic rhinitis [38, 39], initially showed that ragweed AIC is safe and well tolerated; however, subsequently a phase-II/III-trial found only a modest non-significant improvement in the primary clinical outcome (TNSS) and resulted in cessation of further clinical investigation of these CpG motives [40].

Recombinant AIT for venom allergy has been evaluated by different groups [30, 43–45] using mainly peptide immunotherapy, which seems to be safe and well tolerated, although they have shown mixed clinical results.

Considering the urgent need to standardise AIT products and to reduce allergic adverse reactions during therapy, a transition to recombinant/synthetic hypoallergenic approaches is expected. Emerging evidence suggests that this effective therapy can be delivered using a limited panel of allergens or even epitopes; this should review the argument that all allergens must be present for optimal efficacy. More importantly, the use of standardised products will allow direct comparisons between studies, allowing a better assessment of the treatment effect. A comparison in the clinical effect size and safety profile of the use of natural and recombinant allergens in AIT for inhalant and venom allergy is necessary. New recombinant AIT studies should (1) be statistically well powered, (2) use the same clinical outcomes and (3) identify the optimal maintenance dose and duration of treatment.

In the further development of recombinant allergens for AIT, there is an urgent need to focus on some other complex allergens, such as house dust mites, which have a well-established causal role in persistent allergic respiratory diseases globally. Some other allergic conditions such as food allergy or atopic dermatitis with poor response to current treatment should be considered in the future development of recombinant AIT vaccines.

The careful selection of isoforms for each allergen and the rigorous production requirements of these products represent a fundamental issue in the development of recombinant AIT. These factors are likely to lead to high-cost development programmes and high final product costs, which may lead to a limit in the market potential. These issues might explain in part why the new vaccines using recombinant allergens are less likely to become available for routine practice in the very near future.

Conclusions

Recombinant DNA technology for producing high-quality vaccines with well-defined molecular, immunological and biological characteristics is currently well established.

Genetic engineering applied to allergy enables modifications of the allergen molecular structure that can reduce allergenic activity and/or increase immunogenicity.

Based on the current evidence published on recombinant AIT from DBPC RCTs, we can conclude that this modality of treatment may represent a very good alternative for the future treatment of inhalant allergy. However, more studies are needed with enough statistical power to detect differences amongst the study groups. These new studies should be designed using standardised clinical outcomes and selecting the patients with the same harmonised clinical criteria.

Compliance with Ethics Guidelines

Conflict of Interest Oliver Pfaar has received research grants from ALK-Abello, Denmark; Allergopharma, Germany; Stallergenes, France; HAL, The Netherlands; Artu Biologicals, The Netherlands; Allergy-Therapeutics/Bencard, UK/Germany; Hartington, Spain; Lofarma, Italy; Novartis/Leti, Germany/Spain; GlaxoSmithKline, UK/Germany; Essex-Pharma, Germany; Cytos, Switzerland; Curalogic, Denmark; Roxall, Germany; Biomay, Austria; Thermo-Fisher, Germany; MEDA-Pharma GmbH, Germany; and/or has served as an adviser and on the speakers' bureaus for some of the aforementioned pharmaceutical companies. Furthermore, he has received travel grants from HAL Allergy, The Netherlands/Germany, and Allergopharma, Germany, and is a consultant for HAL-Allergy, The Netherlands.

Melina Makatsori declares that she has no conflict of interest.

Ramon Leonart declares that he has no conflict of interest.

Moises A. Calderon declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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