Abstract: PURPOSE OF REVIEW: Allergen-specific immunotherapy is the only curative treatment for allergic diseases. In spite of the great progress in both vaccine development and the methods of allergen immunotherapy (AIT) in recent years, several key problems related to limited efficacy, side-effects, low patient adherence and the relatively high costs due to the long duration (3-5 years) remain to be solved. The current approaches aiming at optimization of AIT are reviewed, including both conceptual studies in experimental models and proof-of-concept - as well as large, multicenter clinical studies. RECENT FINDINGS: The most promising approaches to improve efficacy and safety of vaccine-based AIT include bypassing IgE binding and targeting allergen-specific T cells using hypoallergenic recombinant allergen derivatives and immunogenic peptides, the use of new adjuvants and stimulators of the innate immune response, the fusion of allergens to immune modifiers and peptide carrier proteins and new routes of vaccine administration. SUMMARY: The cloning of allergen proteins and genetic engineering enabled the production of vaccines that have well defined molecular, immunologic and biologic characteristics as well as modified molecular structure. These new compounds along with new immunization protocols can bring us closer to the ultimate goal of AIT, that is, complete cure of a large number of allergic patients.

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Allergen-specific immunotherapy is the only curative treatment for allergic diseases. In spite of the great progress in both vaccine development and the methods of allergen immunotherapy (AIT) in recent years, several key problems related to limited efficacy, side-effects, low patient adherence and the relatively high costs due to the long duration (3–5 years) remain to be solved. The current approaches aiming at optimization of AIT are reviewed, including both conceptual studies in experimental models and proof-of-concept – as well as large, multicenter clinical studies.

Recent findings
The most promising approaches to improve efficacy and safety of vaccine-based AIT include bypassing IgE binding and targeting allergen-specific T cells using hypoallergenic recombinant allergen derivatives and immunogenic peptides, the use of new adjuvants and stimulators of the innate immune response, the fusion of allergens to immune modifiers and peptide carrier proteins and new routes of vaccine administration.

Summary
The cloning of allergen proteins and genetic engineering enabled the production of vaccines that have well defined molecular, immunologic and biologic characteristics as well as modified molecular structure. These new compounds along with new immunization protocols can bring us closer to the ultimate goal of AIT, that is, complete cure of a large number of allergic patients.

Keywords
allergen immunotherapy, allergen immunotherapy administration routes, novel vaccines

INTRODUCTION
The advances in immunology and bioengineering provide a rationale for improvement of the current allergen immunotherapy (AIT) methods in the fields of both new vaccines and novel methods, including new routes of application. These new approaches should contribute to the solving of the major problems of AIT, which include poor selection and monitoring criteria, as well as relatively high costs and the burden for the patients related to the multiple visits and duration of 3–5 years.

Major current approaches to develop more efficacious and safer AIT vaccines are shown in Table 1 [1–20,21**,22–27,28**,29,30].

BYPASSING IGE BINDING AND TARGETING ALLERGEN-SPECIFIC T CELLS
Allergen fragments, fusions, hybrids and chimeras [1,3–5,31,32] are used to avoid recognition by conformation-dependent B-cell epitopes and utilize linear amino acid sequence of T-cell epitopes. These approaches provide the possibility to enhance the tolerogenic T-cell-dependent signal due to the administration of higher doses of preparation with the low risk of anaphylaxis [11,32].

Especially, the application of peptides representing linear T-cell epitope peptides [33,34*,35*,36–39] has been recently investigated in clinical trials showing promising results. An interesting approach relates to overlapping peptides of Bet v 1. A large multicenter study [39] showed good clinical efficacy in birch pollen-allergic patients with rhinitis.

THE USE OF RECOMBINANT ALLERGENS AND THEIR MIXTURES
The first study [13] using a vaccine that contained a mixture of five recombinant native grass pollen

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KEY POINTS

- Improvements of SIT are key to the progress in allergy prevention and treatment.
- Research has been focused on new vaccine formulations, which provide better efficacy and safety mainly by bypassing IgE responses and targeting T cells.
- Novel adjuvants, carrier proteins and new routes also show promising insights.
- The application of the advanced vaccines is limited by the high costs of clinical development.

Allergens showed efficacy in reducing symptom-medication scores in patients with grass pollen-induced allergic rhinitis. In addition, a strong immune response to grass pollen allergens was demonstrated by induction of high allergen-specific IgG1 and IgG4 antibody specific to grass extract. The treatment also showed a very good safety profile. Clinical trials with recombinant allergen preparation mainly for grass pollen, birch pollen and house dust mites showed good clinical efficacy compared with placebo.

The potential of hypoallergenic derivatives with the most reduced IgE reactivity has been evaluated in a mouse model for cat allergy and by skin tests on cat-allergic patients [40]. A hypoallergenic folding variant of the major birch pollen allergen Bet v 1 (Bet v1-FV) was investigated in a dose-finding study [41**]. The optimal dose for specific immunotherapy (SIT) was assessed using environmental exposure chamber and the maintenance dose of 80 µg of rBet v 1-FV was found to be the most effective and well tolerated.

However, it has to be noted that the indirect comparison does not show significantly higher efficacy of the currently tested recombinant preparation as compared with the extracts. Thus, it is still difficult to justify the high costs of vaccine development and licensing until new studies appear [6].

THE FUSION OF ALLERGENS TO IMMUNE MODIFIERS AND PEPTIDE CARRIER FUSION PROTEINS

Major cat allergen 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) [45] in order to intensify allergen internalization and presentation to T cells by antigen-presenting cells. This compound was tested in a first recombinant cat allergen double-blind, placebo-controlled clinical trial using the intranasal route. The administration of only three increasing doses (1 µg, 3 µg and 10 µg) at 4-week intervals showed 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 [27,28**].

1,25-DihydrovitaminD3 has been shown to increase regulatory T-cell responses by affecting dendritic cells for their tolerogenic properties. In a mouse model for cat allergy, vaccine containing rFel d 1 covalently coupled to VitD3 was tested [46], showing superiority to rFel d 1 only.

Another approach represents allergen covalently coupled to carbohydrate-based particles for targeting of dendritic cells and enhanced adjuvanticity in SIT [47]. Promising data have been provided in studies in mouse model of cat allergy [48].

FcγRIIb is an immune tyrosine-based inhibitory motif containing receptor [55]. The coaggregation of FcγRI and FcγRIIb inhibits FcγRI signaling, so one
Table 1. Vaccine development for allergen-specific immunotherapy

<table>
<thead>
<tr>
<th>Type of vaccine or approach</th>
<th>Developmental status</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bypassing IgE and targeting T cells</strong></td>
<td></td>
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<tr>
<td>Hypoallergenic hybrid molecules</td>
<td>Effects in human cell cultures and mouse models</td>
<td>Derived from the Der p 1 and Der p 2 allergens, the hybrid proteins with reduced IgE reactivity induce more T-cell proliferation</td>
<td>[1,2]</td>
</tr>
<tr>
<td><strong>Fusion of major allergens and chimeric allergens</strong></td>
<td>Effects in human cell cultures and mouse models</td>
<td>Major allergens or their fragments are fused and expressed as a single recombinant protein. T-cell reactivity is preserved and IgE binding is attenuated. Prevention of IgE production has been demonstrated in mice</td>
<td>[3,4]</td>
</tr>
<tr>
<td><strong>Fragments of major allergens</strong></td>
<td>A multicenter clinical trial was reported in 2004. The approach has not been pursued further</td>
<td>Use of fragments of a major allergen (Bet v 1) that do not bind IgE. IgE binding is attenuated and T-cell reactivity is preserved</td>
<td>[5]</td>
</tr>
<tr>
<td><strong>Peptide immunotherapy</strong></td>
<td>Several clinical studies have been performed. Long peptides (27 amino acids in length) have been associated with side-effects. Short peptides have been shown to be safe and tolerable in people allergic to cats in a dose-ranging phase Ila clinical trial (NCT00867906). Overlapping peptides investigated in a large multicenter study</td>
<td>T-cell epitope peptides (Fel d 1, Api m 1) that do not bind IgE and induce T-cell tolerance have been used in cat and bee venom allergy</td>
<td>[6–10]</td>
</tr>
<tr>
<td>Unrefolded and folding variants of recombinant allergens</td>
<td>Several ongoing clinical trials have reported promising results</td>
<td>Major recombinant allergens (Api m 1, Bet v 1) are not properly refolded and lack their native conformation. IgE binding is abolished and T-cell reactivity is protected</td>
<td>[11,12]</td>
</tr>
<tr>
<td>Polymers of major allergens</td>
<td>A multicenter clinical trial was finalized several years ago and this approach has not been pursued further</td>
<td>Major allergen (Bet v 1) is trimerized. Mast cell and basophil degranulation is attenuated, and T-cell reactivity is preserved in vitro</td>
<td>[5]</td>
</tr>
<tr>
<td><strong>Cocktails of recombinant natural allergens</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mixtures of several major recombinant allergens</td>
<td>One clinical trial reported promising results. A double-blind placebo-controlled dose-response study is under evaluation</td>
<td>Phi p 1, Phi p 2, Phi p 5a, Phi p 5b and Phi p 6 were used in combination as a mixture of five recombinant grass pollen allergens. A reduction in symptoms and the need for symptomatic medication was observed in people allergic to grass pollen</td>
<td>[13]</td>
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<tr>
<td><strong>Adjuvants and peptide carrier proteins</strong></td>
<td></td>
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<tr>
<td>GpG oligonucleotide-conjugated allergens</td>
<td>A large multicenter clinical trial did not reach its endpoints</td>
<td>A toll-like receptor 9-triggering CpG oligonucleotide is fused to major ragweed allergen Amb a 1</td>
<td>[14,15]</td>
</tr>
<tr>
<td>Allergens coupled to virus-like particles</td>
<td>A rapid induction of high IgG antibody titers was observed in healthy human volunteers</td>
<td>Highly repetitive virus capsid-like recombinant particles are coupled to house dust mite major allergen Der p 1</td>
<td>[16]</td>
</tr>
<tr>
<td>Carbohydrate-based particles</td>
<td>Effects shown in mouse models</td>
<td>Carbohydrate-based particles bound to the allergen rPhl p 5b induced a stronger antibody and cytokine responses</td>
<td>[17]</td>
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</tbody>
</table>

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<th>Type of vaccine or approach</th>
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<tbody>
<tr>
<td>Monophosphoryl lipid A (MPL) formulated with allergoid</td>
<td>Clinical trials have reported safety and efficacy. A phase III study has been completed (NCT00414141)</td>
<td>Th1-inducing adjuvant MPL-A facilitates short-term specific immunotherapy (SIT) together with a grass pollen allergoid</td>
<td>[18]</td>
</tr>
<tr>
<td>Hypoallergenic vaccine based on allergen-derived peptides fused to peptide carrier protein (hepatitis B Pre-S)</td>
<td>Effects shown in mouse models. Multicenter clinical trial going on</td>
<td>Recombinant fusion proteins show reduced allergenic activity with lowered basophil activation and there is no IgE reactivity to the fusion protein</td>
<td>[19,20,21]</td>
</tr>
<tr>
<td>New routes of administration</td>
<td></td>
<td></td>
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<tr>
<td>Intralymphatic vaccination</td>
<td>A clinical trial has reported safety and efficacy. A phase III study is ongoing (NCT011166269)</td>
<td>Allergen-SIT vaccines are administered directly into inguinal lymph nodes with the aim of delivering high amounts of allergens into secondary lymphatic organs</td>
<td>[22,23]</td>
</tr>
<tr>
<td>Epicutaneous vaccination</td>
<td>A clinical trial in grass pollen-induced rhinoconjunctivitis demonstrated safety and efficacy. A phase II study is ongoing in children with a peanut allergy (NCT01197053)</td>
<td>High numbers of antigen presenting cells (LCs) are delivered to a nonvascularized area. The method is well tolerated, needle-free and has the potential for self-administration</td>
<td>[24]</td>
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<tr>
<td>Fusion with immune response modifiers</td>
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<tr>
<td>Targeting FcγRII</td>
<td>Effects have been shown in human cell cultures and mouse models</td>
<td>Fusion of allergens to human Fcγ has been reported to inhibit allergen-induced basophil and mast cell degranulation by cross-linking FcγRI and FcγRII receptors</td>
<td>[25,26]</td>
</tr>
<tr>
<td>Modular antigen translocation vaccines</td>
<td>A clinical trial has been finalized and demonstrated safety and efficacy with evidence of immune regulation</td>
<td>The coexpression of major recombinant allergens together with the transactivator of transcription peptide and truncated invariant chain peptide can target antigens to the MHC II molecules in the trans-Golgi compartment</td>
<td>[27,28]</td>
</tr>
<tr>
<td>Combined treatment with immune response modifiers</td>
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<tr>
<td>Pretreatment with anti-IgE mAb before SIT</td>
<td>Several investigator-initiated clinical trials are ongoing to reduce SIT-induced side-effects to enable relatively rapid dose increase and to use relatively high doses</td>
<td>Significantly fewer systemic allergic reactions were observed, more patients were able to reach the target maintenance immunotherapy dose</td>
<td>[29,30]</td>
</tr>
</tbody>
</table>
strategy 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 FcγRI receptors [25,26].

A carrier protein, the Pre-S domain of hepatitis B virus, has been used to fuse to two nonallergenic peptides of Fel d 1 or Bet v 1 [19,20,21]. This approach eliminated both IgE-mediated and T-cell-mediated side-effects. The Pre-S domain of hepatitis B virus contains antigenic sites for both B and T cells and provides T-cell help. Peptides derived from the IgE-reactive areas of Bet v 1 were fused to a hepatitis B surface protein. This fusion peptide reduced T-cell activation in vitro and induced allergen-specific IgG in a rabbit model.

Similar results were provided by Chen et al. [49,50] (using carrier-bound Der p 2 peptides or rDer p 2/1 mosaic proteins).

**COMBINATION WITH IMMUNE RESPONSE MODIFIERS**
The combination AIT and anti-IgE (omalizumab) treatment has been investigated [36] in several trials. A good safety profile, significant decrease in the risk of anaphylaxis and improved rescue medication scores were reported [29,30,51].

**ROUTES OF VACCINE ADMINISTRATION**
The efficacy of sublingual immunotherapy (SLIT) for the treatment of allergic rhinitis and asthma has been well established; however, the direct head-to-head studies comparing SLIT and subcutaneous immunotherapy (SCIT) are lacking [52,53]. In addition, sustained disease-modifying effects of SLIT have been demonstrated in randomized, double-blind, placebo-controlled trials in adults and in children [54,55]. The immunologic mechanisms of SLIT are similar to SCIT. They involve increased blocking IgG4, IgA antibodies, activation of Treg cells, increased IL-10 production and increased numbers of sublingual FOXP3-expressing T cells [56–58]. However, the magnitude of the changes, especially increase in specific IgG4 response, is rather modest. Future studies should address this issue by using novel adjuvants and increasing the effective doses of allergen. Recently, sugar-modified antigens have been shown to effectively induce oral tolerance. A C-type lectin receptor, SIGNR-1 (CD209b), is capable of conditioning of dendritic cells to induce tolerance in gastrointestinal lamina propria in a model of food-induced anaphylaxis [59]. rBet v 1a formulated in amylopectin-based microparticles has also been investigated in a mouse model [60].

Work is going on to use intralymphnode and epicutaneous routes of vaccine application. The demonstrated advantage of these approaches involves fewer doses, that is, patients’ visits at the office and lower total dose of allergen required to achieve clinical efficacy [22–24]. However, both indirect efficacy comparisons show similar efficacy to SCIT in the treatment of grass pollen allergy. Interestingly, intralymphatic vaccines are capable of inducing strong T-cell responses associated with cytotoxic activity and IFN-γ production, which are important in long-term protection against viral infections.

**CONCLUSION**
In spite of the progress in improving the efficacy and safety of AIT, there is still the great potential for further modifications, which are hoped to broaden the pool of candidates for AIT, aiming at much better treatment and prevention of allergic diseases as well as other diseases related to immune dysregulation. However, the major barrier for the clinical application of these new technologies is the capacity to perform a large number of phase 3 confirmatory double-blind, placebo-controlled clinical trials.

**Acknowledgements**

**Conflicts of interest**
Marek Jutel is Investigator in clinical trials of Allergopharma GmbH, Anergis AG, Stallergens, Leti GmbH, Hal Allergy, is Steering Committee member – EU 7th Framework Programme 601763–1, BM4SIT and received lecture fees from Allergopharma GmbH and Stallergens.

**REFERENCES AND RECOMMENDED READING**
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Immunotherapy and new treatments


