Allergen Immunotherapy
Vaccine Modification

Peter Socrates Creticos, MD[a,b]

KEYWORDS

• Allergic rhinitis • Allergen immunotherapy • Vaccine modification

KEY POINTS

- New modalities of allergen immunotherapy may allow effective immunization with shorter treatment regimens, improved patient compliance, and the potential of safer agents.
- Toll receptors on specific regulatory cells provide a unique pathway to initiate regulatory pathways capable of down-regulating the untoward allergic diathesis.
- Synthetic peptides offers the ability to immunize allergic subjects with a concise 4-injection intradermal regimen. The smaller peptides are less likely to trigger crosslinking of IgE on mast cells, thereby minimizing the risk of allergic reactions and anaphylaxis.

OVERVIEW

Allergic rhinitis (AR) is a common clinical condition; both its incidence and prevalence seem to be increasing in North America, perhaps reflective of population shifts, climate changes, and genetic susceptibility. Demographic surveys identity up to 20% to 40% of the population as sufferers of AR/conjunctivitis and approximately 8% troubled with asthma.[1–4]

Allergen immunotherapy (AIT) comes to the forefront in our therapeutic approach to immunoglobulin E (IgE)–mediated diseases (allergic rhinoconjunctivitis, allergic asthma, food allergy, venom sensitivity, and possibly even atopic eczema), as it affords a means of redirecting the untoward immune response, reestablishing immunologic tolerance, and accomplishing long-term clinical remission.

Although effective, current immunotherapy regimens are burdened by tedious treatment regimens that not only negatively impact on patient adherence and compliance but also serve as barriers to limit access to this form of disease-modifying therapy. Furthermore, systemic reactions to immunotherapy, although infrequent, can be severe and potentially life threatening.

Thus, there is a recognized need for newer therapeutic agents that improve the safety of AIT, provide an ease of delivery to patients that fosters compliance and

[a] Division of Allergy & Clinical Immunology, Johns Hopkins Medicine, Baltimore, MD 21287, USA; [b] Creticos Research Group, 1300 Saint Paul's Way, Crownsville, MD 21032, USA
E-mail address: psocrates@comcast.net

Immunol Allergy Clin N Am 36 (2016) 103–124
http://dx.doi.org/10.1016/j.iac.2015.08.010 **immunology.theclinics.com**
0889-8561/16/$ – see front matter

allows access to a greater proportion of the allergic population that could benefit from this disease-modifying treatment, and achieves an acceptable therapeutic benefit for most patients committing to the course of treatment.

Through the years, various chemical modifications of allergens have been tried in an attempt to enhance efficacy, improve safety, and foster adherence with AIT. In many cases, these previous approaches have been viewed as unsuccessful, or only partially successful, in that the allergenicity and immunogenicity have either decreased, or increased in tandem, with no resultant efficacy/safety benefit ratio realized. However, recent clinical trials have led to promising results in immunization approaches with modified allergens, including immune-stimulatory adjuvants, recombinant allergens, and T-cell-tolerizing constructs, as well as with alternate routes of delivery, including oral and sublingual, intralymphatic, and epicutaneous methods, as vehicles for immunization in allergic respiratory disease[5–11] (**Box 1**).

MODIFIED ALLERGEN APPROACHES

Background

Through the years, various groups have attempted to improve AIT through a variety of techniques through which the allergen is modified. In the 1970s to 1980s, efforts by Norman and Marsh at Hopkins modified grass and ragweed (RW) allergens by partially denaturing them in formalin; this led to allergens with markedly reduced allergenicity; but unfortunately, the immunogenicity of allergoids, as judged by the IgG antibody response, was also decreased, as was the clinical effectiveness.[12,13] Sehon and Lee attempted to modify and decrease allergenicity by coupling the allergens to a polyethylene glycol backbone. Again, the result was the same: allergenicity and immunogenicity decreased together.[14,15]

Box 1
Modified AIT constructs

Injectable immunotherapy approaches

Alum salts (SQ)

Chemical modifications (SQ)
- Allergoids/polymerized allergens
- Novel adjuvants (SQ; IM)

DNA vaccines
- TLR-9 (CpG oligonucleotides) (SQ)
  - Linked to allergen; cocombined
  - Nanoparticle-based VLPs
- TLR-4 (MPL) (SQ)
- Lysosomal plasmids (IM)

Peptides (T-cell epitopes) (ID)

Recombinant allergens (SQ)

Alternate routes for immunization

Sublingual immunotherapy

Intralymphatic

Epicutaneous

Abbreviations: ID, intradermal; IM, intramuscular; MPL, monophosphoryl lipid A; SQ, subcutaneous; VLPs, viral-like particles.

Then Patterson developed polymerized allergens, wherein a glutaraldehyde-linked polymerization rendered an allergen product that in animal studies was shown to be less reactive on immunization but maintained immunogenicity. Studies of allergic patients with polymerized grass and RW allergens demonstrated efficacy and tolerability in double-blind placebo-controlled clinical trials. However, this project was fraught with regulatory issues related to standardization of the allergen.[16–18]

Subsequently, in the mid-90s, Gefter developed synthetic T-cell tolerizing peptides from cat Fel d 1 and RW Amb a 1. The concept was based on the recognition that specific peptide sequences (epitopes) form the whole allergen and were capable of inducing what was thought to be anergy or, in fact, tolerance.[19,20]

In collaborative studies with cat peptides and RW peptides, Norman and colleagues demonstrated peptide immunization significantly reduced clinical symptoms without an increased IgG antibody response. Although significant therapeutic results were observed, the effects were less than that achieved with unmodified AIT; furthermore, patients experienced late-onset adverse symptoms that mimicked natural allergen exposure.[21–24]

During this same time, groundbreaking work demonstrated that bacterial DNA could have profound effects on the humoral and cellular limbs of the immune system and that this could be used to advantage to manipulate the immune response to proteins and, hence, allergens.[25–38]

Capitalizing on the potential to induce T-cell tolerance with long-term suppression of the allergic diathesis, Raz and colleagues[39–46] demonstrated first, in animal models, and then in human studies that allergen vaccination with immune-stimulatory DNA was capable of redirecting the untoward Th2:Th1 allergic diathesis.

These novel approaches are discussed below.

Immunostimulatory Adjuvants

CpG oligonucleotide conjugated to ragweed Amb a 1

An adjuvant approach, developed by Dynavax Technologies, Inc., in which immune-stimulatory DNA is conjugated to the principal allergic moiety of RW (Amb a 1) (Amb a 1–immunostimulatory complex [AIC]; synonymously termed Toll-Like Receptor 9 [TLR-9] vaccine) has been shown to greatly enhance the Amb a 1–specific Th1 immune response in animals and is capable of diminishing RW-induced pulmonary hyperreactivity in mice. AIC has been shown to be demonstrably less allergenic than unconjugated Amb a 1 or RW extract. This combination of decreased allergenicity and enhanced Amb a 1–specific immune response with AIC was thought to afford an opportunity to use an allergen modification capable of simultaneously providing improved clinical efficacy, an enhanced safety profile, and a more convenient dosing regimen. The basis for enhancement of Th1 response by immune-stimulatory DNA sequences is derived from the recognition that bacterial DNA induces an immune response (through its toll-like receptor ligand on plasmacytoid dendritic cells) that is characterized by a potent interleukin (IL)-12 activation of Th1 cells to secrete interferon (IFN)-gamma and a much lower level of activation of Th2 cells secreting IL-4 and IL-5.

Initial phase 1-2 safety and dose-ranging studies by Creticos and colleagues[47,48] in the early 2000s demonstrated that the vaccine was well tolerated and induced the expected immunologic changes in humans. In 2006, Creticos and colleagues reported the proof-of-concept study for this TLR-9 agonist in a 2-year double-blind placebo-controlled clinical study of 25 RW-allergic subjects immunized with a brief 6-injection regimen (0.06–12.0 mcg AIC; administered before the first RW season only). The mean peak-season rhinitis symptoms, as measured by a standard visual analog scale (VAS), were significantly improved in both RW seasons (year 1: 68% [$P = .006$]; year 2: 72%

[P = .02]) in the TLR-9 vaccine group versus Placebo. Similar findings were observed for the peak-season daily nasal symptom diary scores (year 1: 55% [P = .02]; year 2: 53% [P = .02]). These data provided consistent evidence that the vaccine conferred meaningful long-term clinical efficacy, which extended over the 2 RW seasons under study.[49]

Furthermore, the vaccine blunted the typical seasonal increase in Amb a 1–specific IgE antibody in the first RW season and markedly suppressed the IgE antibody titer in the following RW season, providing evidence for long-term immune tolerance (as defined by suppression of the allergic antibody, IgE). In addition, a reduction in the number of IL-4–positive basophils in AIC-treated patients correlated with lower VAS scores (r = 0.49; P = .03)—an important corollary, as IL-4 is a key cytokine involved in B-cell class switching to IgE antibody.[49]

In a separate DBPC Canadian study (n = 57 RW-AR subjects), a subset (n = 19) consented to nasal provocation and biopsy. Tulic and colleagues[50] reported that the 6-week injection course with the TLR-9 vaccine (administered before the first RW season) modified the nasal inflammatory immune response to allergen challenge after RW season; the study findings showed that treatment significantly reduced the increase in nasal eosinophilia (P = .02) and IL-4 mRNA-positive cells (P = .008), which was paralleled by an increase in number of IFN-gamma mRNA-positive cells (P = .002) as compared with PL-treated subjects. A positive trend in nasal symptom score improvement was observed by the second RW season in the vaccine-treated group.

Based on the findings from these clinical studies, Dynavax explored both a higher immunization dose (30 μg) and a booster regimen (30 μg × 2 before the second RW season). The high-dose phase 1 safety study by Vaishnav and Creticos provided the safety data needed for the subsequent performance of the first large-scale multicenter clinical trial. Findings from this study also demonstrated a significant decrease in the late-phase skin test reaction in the AIC-treated group versus PL (P = .008), and a significant increase in both anti-RW and anti-Amb a 1 IgG antibody compared with baseline and PL (P<.05), with no significant increase in IgE allergic antibody levels, findings reaffirming the premise that AIC's mechanism of action involves redirection of the untoward Th2 inflammatory response.[51]

The subsequent phase 2/3 multicenter clinical trial of the TLR-9 vaccine (TOLAMBA) (n = 462 RW-allergic patients), conducted from 2004 to 2006 and encompassing 2 RW seasons, was reported at the 2006 American Academy of Allergy, Asthma, and Immunology (AAAAI) annual meeting by Busse. Active-treated patients reported improvement in the defined efficacy end point total nasal symptom scores (TNSS) reported as change from baseline compared with PL during the peak RW season (treatment effect: year 1: 21.0% [P = .04]; year 2: 28.5% [P = .02]). However, subjects in the booster arm of the study did not achieve significant improvement in their TNSS (treatment effect: 13.5%; P = .28). After discussion with regulatory authorities, Dynavax commenced to undertake a second DBPC multicenter phase 2/3 clinical trial.[52]

In 2007, Dynavax announced that their second large-scale 2-year multicenter trial with the TLR-9 vaccine (n = 716 patients) was being halted after the first year based on their preliminary analyses of the 1-year data, which unfortunately showed that the TNSS in the overall study population provided an insufficient level of symptoms that could be ascribed to ragweed in the enrolled patients, thus making it impossible to demonstrate a meaningful therapeutic effect between the active arms and the PL-treated group, if the study were to continue.[53]

In 2008, the company reported the findings from their Canadian environmental exposure chamber (EEC) study (n = 253 patients) in which, although a measureable clinical effect on TNSS (in the chamber) was observed, the primary end point did

not achieve statistical significance (treatment effect [intention to treat]: 41% vs PL; $P = .09$).[54]

Based on the company's concerns about the subjective nature of assessing efficacy by symptom scores, Dynavax decided to discontinue their clinical development program for the RW allergy vaccine program. However, the company continues to pursue its TLR-9 agonist program in hepatitis and cancer, its collaborative TLR agonist program in asthma and COPD with AstraZeneca, and its collaborative ventures with GlaxoSmithKline in autoimmune diseases.

Monophosphoryl lipid A adjuvant

Another novel toll-like receptor vaccine under development for North America is Allergy Therapeutics' TLR-4 adjuvant (Pollenex Quattro). This compound is a novel therapeutic extract wherein the native allergen (eg, grass; RW) is modified in 3 ways to achieve a safe and effective immune-modifying vaccine: it capitalizes on adsorbing a glutaraldehyde-modified allergoid (chemical modification that reduces the allergen's inherent IgE reactivity but preserves its immunogenicity) onto an L-tyrosine absorbent (to enhance slow-release kinetics), combined with the immunostimulatory adjuvant (monophosphoryl lipid A [MPL]). MPL is derived from detoxified lipopolysaccharide (originating from gram negative bacterium *Salmonella minnesota*), a Th1–inducing adjuvant. The clinical trials have demonstrated clinical efficacy with an *ultrashort* 4-injection preseason regimen.[55–59]

Their major DBPC randomized multicenter grass study conducted in 84 centers in North America, the United Kingdom, and Austria (1028 patients; aged 18–59 years) compared grass modified allergen tyrosine adsorbate–MPL (MATA-MPL) (n = 514) with placebo (n = 514). The vaccine-treated patients reported significant improvement (reduction) in their symptom and medication usage scores as compared with the PL-treated group on analysis of their electronic diary-recorded data over the 4 peak weeks of the 2007 grass pollen season (difference between MATA-MPL versus PL in mean combined symptom medication score: 13.6%; $P = .0038$). Subgroup analysis provided evidence for a greater benefit in patients with more severe symptoms (16.6%; $P = .0023$), sites with higher allergen burden (31%; $P<.0001$), and European subjects (27.4%; $P = .0341$). Serum grass-specific IgG increased 6.5-fold from baseline in the MATA-MPL-treated patients and was significantly elevated versus PL ($P<.001$). Overall, the treatment was well tolerated (Treatment-Related Adverse Events leading to dropout: 2.5% vs 0.4% PL); 92% of subjects completed the study. The most commonly reported adverse events (AEs) were injection site reactions, and there was the occurrence of one severe systemic reaction (generalized erythema in an MATA-MPL subject). However, after treatment, one study subject was reported to have developed transverse myelitis (>4 weeks after the final MATA-MPL injection) (see *clinical hold* notation). Of note, the 95.7% compliance attests to the patients' willingness to adhere to a short (3–4 weeks) 4-injection regimen.[58]

A parallel randomized DBPC multicenter RW clinical study was also initiated in North America in 2007; however, because of a subsequent clinical hold (now lifted), this trial only had 381 of the planned 993 subjects receive all 4 study injections. Even so, a clinical effect was observed in the primary end point for the RW MATA-MPL–treated group versus PL (12%; $P<.05$).[59]

More recently, in 2013, Allergy Therapeutics published their Canadian EEC study with their MPL adjuvant. This allergen preparation contains a glutaraldehyde-modified RW allergoid absorbed onto tyrosine combined with the aforementioned immune-stimulatory adjuvant (MPL) derived from gram-negative bacterium (*Salmonella minnesota*).

RW allergic subjects (n = 228 adults) were randomized in a double-blind placebo-controlled fashion to either active therapy (MATA-MPL, administered in 4 weekly injections) or placebo. Controlled RW pollen exposure (3500 ±500 pollen grains per cubic meter) in the EEC was performed at baseline (before treatment) and again at 3 weeks after the completion of treatment.

Study subjects that received the MPL-adjuvant vaccine showed a relative mean improvement in their total symptom scores (TSS) (48%; $P<.05$). The 4-injection regimen was well tolerated, and no serious AEs (SAEs) occurred in the study; nonetheless, 94.7% of the subjects in the MPL-adjuvant group and 67.7% in the placebo group reported AEs. The difference between the active and placebo groups was significant but mainly due to injection-site reactions of swelling (77% vs 16%), pruritus (80% vs 5%), pain (60% vs 33%), and warmth (27% vs 2%).[60]

The company markets in Europe a dust mite (DM) allergoid product in which purified allergens from *Dermatophagoides pteronyssinus* are modified through treatment with glutaraldehyde and combined with the L-tyrosine absorbent for the treatment of perennial mite allergy. In a clinical trial comparing the safety and efficacy of conventional (administered over 3 weeks) versus clustered dosing of the DM allergoid in 30 adult patients with persistent perennial AR, no AEs were recorded. Improvements in nasal challenge symptom scores and nasal peak inspiratory flow and an increase in IgG4-specific antibody were observed. In a 1-year follow-up study reported at the 2015 European Academy of Allergy and Clinical Immunology (EAACI)–Barcelona meeting, investigators reported a 50% reduction (improvement) in symptom scores on the nasal challenge, a sustained increase in IgG4, and a treatment-induced IL-10, a recognized marker of clinical tolerance.[61,62] Based on these findings, the company is also focused on development of an ultrashort course DM allergoid incorporating the MPL adjuvant.

Nanoparticle-based immunomodulation with viral-like particles

Cytos Biotechnology Ltd has focused on a nanoparticle-based immunomodulator. Their construct uses viral-like particles (VLPs) to deliver synthetic cytosine deoxynucleotide phosphorylated to guanine deoxynucleotide (CpG) oligonucleotide (G10) contained in a bacteriophage Qb capsid VLP admixed to allergen (eg, house dust mite [HDM]) or administered independent of allergen (TLR-9 agonist alone).[63]

In an initial safety and dose-ranging study, Kundig and colleagues[64] studied a VLP construct in which allergen (a synthetic 16 amino acid sequence of *Der p 1*) was incorporated into a VLP derived from bacteriophage, Qbeta (QB) (*QB-Der p 1*). This study assessed 24 healthy volunteers who were randomly assigned to varying dosing regimens (10 vs 50 mcg) and routes of immunization (intramuscular [IM] vs subcutaneous [SQ]). The *QB-Der p 1* vaccine was well tolerated in this single-injection study design, and no SAEs or systemic reactions were observed. The 50-mcg single dose, regardless of route of administration, was more efficient at generating an IgG antibody titer (1:2000); this robust B-cell response in humans is consistent with the observations made by the group in mice. The study demonstrated that a VLP could be used as a carrier to efficiently deliver allergen to antigen-presenting cells (APCs), inducing high titers of antibody without requiring the use of an adjuvant.[64]

This study was followed by an open-label study by Senti and colleagues[65] in which 20 HDM-allergic adult volunteers were immunized with *QB-Der p 1* to which the CpG oligonucleotide (*G10*) was admixed. The treatment regimen used a dose-escalating cluster schedule that led to a *fixed* 6 SQ injection regimen administered at 1- to 2-week intervals. The QbG10 adjuvant was well tolerated, abrogated the response to allergen

challenge in the CPT, significantly attenuated both rhinoconjunctivitis and asthma symptoms, and demonstrated expected shifts in IgG antibody and skin test reactivity.[65]

In 2011, Klimek and colleagues[66] reported the findings from a phase 2b clinical trial of the TLR-9 agonist construct in which 299 patients with perennial HDM allergy were randomized in a double-blind placebo-controlled fashion to 2 different dosing regimens of the QbG10 adjuvant (6 weekly SQ injections of either 0.5 or 1.0 mg CYT003-QbG10) or placebo.

The clinical study findings demonstrated that the higher-dose vaccine regimen improved rhinoconjunctivitis symptom medication scores (0.31 vs 0.52; $P = .04$ vs placebo), positively shifted conjunctival provocation (10-fold increase in allergen tolerance), and was generally well tolerated. However, although no drug-related SAEs were reported, 16 patients discontinued the study (8 because of AEs, of which 6 were defined as treatment-related AEs).[66]

Based on subsequent data that demonstrated that QbG10 acted through an allergen-independent mechanism of action and that combination with an allergen did not result in any benefit over allergen alone, Cytos halted work in AR and instead focused on pursuing persistent allergic asthma requiring long-term treatment with inhaled corticosteroids (ICS).

In 2013, Cytos reported the midstage results with their CYT003-QbG10 compound in a small phase 2a asthma study (n = 63). They reported that treatment resulted in maintained asthma control and pulmonary function in patients with Global Initiative for Asthma (GINA)-defined persistent asthma even with the reduction in ICS in the step-down phase of the study.[67]

However, in April 2014, the company reported that their larger phase 2b asthma study (n = 360 patients with moderate to severe persistent allergic asthma) did not meet its primary end point. Specifically, the study was not able to show *clinically relevant improvement* in asthmatic patients treated with CYT003-QbG10 versus PL for the primary end point of the study (improvement in the Asthma Control Questionnaire score at week 12 of therapy). Of note, a confounding observation was that a clinical improvement was observed in PL and all dose levels of the investigational drug. Furthermore, additional secondary end points, including pulmonary function, likewise failed to show a significant difference versus PL.[68]

Following this setback, Cytos announced that they had terminated the 1-year study and have halted further development of the program.

Lysosomal-associated membrane protein

Immunomic (ITI) has focused on developing a novel vaccine that acts through the innate immune system to achieve immune modulation. The concept takes advantage of lysosomal processing of antigens with major histocompatibility complex (MHC) class II presentation thereby promoting an efficient means of upregulating the innate immune system through a glycoprotein found on the lysosomal membrane (lysosomal-associated membrane protein [LAMP]).

Animal studies provide evidence that in the presence of modified allergen (eg, bacterial DNA encoded into the lysosome), natural killer cells are primed to secrete IFN-gamma, which acts on DCs to secrete IL-12 and IFN-gamma through a T-box transcription factor (T-bet)–mediated process that favorably orients the immune response to Th1 differentiation with attenuation of the Th2 inflammatory profile.

Initial studies in Japanese red cedar (JRC)–allergic subjects have demonstrated that a 4 IM injection regimen with the LAMP construct (CrJ2 nucleotide sequence from JRC inserted into LAMP) was well tolerated and resulted in induction of allergen-specific IgG to JRC; suppression and subsequent elimination of skin test reactivity to JRC,

mountain cedar, and Cry 12; and conversion of skin test–positive subjects to nonreactors (skin test negative).[69–71]

Further phase 2 studies are underway.

Synthetic Peptides

T-cell–tolerizing peptides

As noted, initial studies carried out by Gefter and colleagues in the mid-1990s provided evidence that synthetic T-cell-tolerizing peptides could be used to suppress IgE-mediated allergic diseases, such as AR and asthma, through induction of immune tolerance. Their laboratory developed both cat (two 27 amino acid peptides derived from Fel d 1) and RW (short amino acid sequences derived from Amb a 1) T-cell–tolerizing peptides.[20]

Subsequent collaborative studies with Norman and colleagues at Johns Hopkins and Mass General used a cat-room challenge model to demonstrate that varying doses of SQ-injected cat peptides could improve clinical symptoms on natural cat-room exposures.[21,22] Creticos and colleagues undertook 2 large multicenter trials with varying doses and dosing regimens with the RW peptide vaccine, which provided evidence for modest clinical improvement but reduced the need for rescue medication to relieve breakthrough nasal symptoms in the RW season.[23,24]

However, these first-generation peptides did not achieve the expected degree of clinical benefit and, perhaps because the peptides were too large or given in high SQ doses, were observed to induce delayed-onset AEs, such as chest tightness. This developmental program was terminated in the late 1990s.

Synthetic peptide immunoregulatory epitopes

A new class of synthetic peptides, termed synthetic peptide immunoregulatory epitopes (SPIREs) emanated from the laboratories of Barry Kay and Mark Larché at Imperial College in London.

These synthetic T-cell–tolerizing peptides were designed as smaller peptide units (eg, cat: 7 peptides, 13–17 amino acids in length), are assembled from different T-cell epitopes, are administered in much smaller quantities than the Gefter's laboratory's molecules (75 μg vs 750 μg), and are administered intradermally, thereby more efficiently interacting with resident antigen-presenting cells. Following acquisition of the technology from Imperial College, Circassia Ltd has further refined the formulation of these peptides to prevent dimer formation and, hence, preserve bioactivity of the peptides.

These novel immunoregulatory peptides are designed to induce immunologic tolerance by binding to MHC class II molecules on antigen-presenting cells, thereby inducing the upregulation of regulatory T cells. The smaller size of these synthetic peptides is an inherent advantage, as the peptides should be of insufficient length to trigger cross-linking of IgE on mast cells and basophils, thus significantly reducing the risk of IgE-mediated allergic reactions and anaphylaxis.[72–80]

CAT–SYNTHETIC PEPTIDE IMMUNOREGULATORY EPITOPES

The lead compound for Circassia is their Cat-SPIRE construct. It consists of 7 separate small peptides (13–17 AA in length) derived from Fel d 1, the major cat allergenic moiety. After reconstitution, it is administered as a series of 4 intradermal (ID) injections into the dermis.

The selection of peptides in Cat-SPIRE was defined based on MHC class II binding studies in conjunction with T-cell proliferation and histamine release assays performed

on ex vivo blood samples from cat allergic volunteers. The T-cell proliferative responses to an *internal standard* allergen extract of cat dander and Cat-SPIRE were closely correlated and provided confirmatory evidence that most of the T-cell reactivity to cat dander could be ascribed to the 7 synthetic peptide epitopes selected for Cat-SPIRE. As further evidence for an optimal peptide mix, the peptides induced in vitro IL-10 release from peripheral blood mononuclear cells in greater than 90% of cat-allergic subjects.

The peptide construct's IgE-binding activity was evaluated through performance of basophil histamine release assays on whole blood from cat-allergic individuals. These assays affirmed that Cat-SPIRE had significantly less capability than whole allergen to cross-link IgE and induce histamine release.[72–80]

The antiinflammatory properties ascribed to the cat peptides are most well defined in a study of transgenic mice by Campbell and colleagues[76] in which treatment with a single peptide in Cat-SPIRE resulted in reductions in bronchoalveolar lavage total cells and eosinophils, reductions in pulmonary and systemic TH2 inflammatory cytokines, reduced recruitment of TH2 inflammatory cells to the lungs, and reduced proliferative responses to cat Fel d 1.

Furthermore, administration of an anti–IL-10 monoclonal antibody (immediately after treatment with the peptide) blocked these effects, thereby emphasizing the potential critical regulatory interaction between the T-cell–tolerizing peptide and IL-10, a critical cytokine in the underlying mechanism of action ascribed to SPIREs for reestablishment of immune tolerance.[76]

Cat–Synthetic Peptide Immunoregulatory Epitopes Clinical Studies

Phase 1/2a safety and efficacy trial

In 2011, Worm and colleagues[78] published their initial safety and efficacy findings on the cat Fel d 1 peptide. Eighty-eight volunteers were randomized to (single-dose) escalating ID injections or to SQ injections of the peptide. The primary end point for the study was safety and tolerability. The Cat-SPIRE construct was well tolerated in doses up to 12 nmol (ID) and 20 nmol (SQ) (the ID dose was equivalent to 150 mcg of Fel d 1). No SAEs were observed, and no subject withdrew from the study because of an AE. The study also assessed the effect of the drug on the late-phase skin test response, a recognized surrogate clinical end point for efficacy of immunotherapy; although not statistically significant, the 3-nmol ID peptide dose resulted in a 40% reduction in the late phase skin response (vs 10% for placebo). This initial study demonstrated that the cat-peptide could be administered at an effective dose that might obviate the buildup phase required for Subcutaneous immunotherapy.

Phase 2b environmental chamber study

The phase 2 dose-ranging studies with Cat-SPIRE were performed using an EEC methodology, as this model provides a controlled setting in which to evaluate AIT. As such, it avoids many of the pitfalls that can plague natural field trials (eg, weather change, outdoor pollution, exposure to confounding allergens). Furthermore, in a chamber setting, it is possible to expose patients to predefined allergen levels that are known to cause symptoms of sufficient severity to allow assessment of *drug effect*.

In the study design, subjects had a baseline EEC challenge that consisted of 4 consecutive days on which patients were exposed for 3 hours each day in the chamber. The subjects returned at 18 to 22 weeks after the start of treatment and underwent a repeat 4-day chamber challenge. The primary end point for the study was the Total Rhinitis Symptom Score (TRSS) (defined as the difference in TRSS at each time point on each day between baseline and posttreatment challenge).

One hundred twenty-one (121) subjects who met a qualifying *threshold* symptom score were randomized to one of 4 treatment arms or placebo: (1) 3 nmol × 4 ID injections, 2 weeks apart; (2) 6 nmol × 4 ID injections, 2 weeks apart; (3) 3 nmol × 4 ID injections, 4 weeks apart; (4) 3 nmol × 8 ID injections, 2 weeks apart; (5) placebo × 8 ID injections.

Dosing over 12 to 14 weeks, as opposed to 6 weeks, demonstrated a greater shift in the TRSS. Subgroup analysis showed that those patients who received 8 ID injections of 3 nmol of peptide 2 weeks apart had the greatest reduction in TRSS (reduction of symptoms versus baseline: −4.8 points versus PL: 3.1 points [$P<.05$]). Although not significant, the 6-nmol dose showed a magnitude of improvement that was superior to the 3-nmol dose. An interesting corollary was that improvement in clinical scores was greater on later challenge days, whereas scores in the placebo group remained largely unchanged; this would be consistent with the findings from the late phase skin response in the Worm study.[78]

No SAEs were observed with any of the 4 treatment regimens, and respiratory system treatment-emergent AEs (TEAEs) occurred at a low frequency in all groups, with no difference between active groups or placebo.[81,82]

This EEC study provided preliminary evidence that a sustained treatment effect could be achieved with Cat-SPIRE. The positive effect observed in the EEC at 18 to 22 weeks is consistent with the cat-room exposure study by Norman and colleagues,[21] which showed a stronger effect at approximately 26 weeks than at any earlier time point.

Phase 2b Cat–synthetic peptide immunoregulatory epitopes environmental exposure chamber trial with 1-year follow-up

The focus of the study by Patel and colleagues[83] was to further explore whether a persistent treatment effect could be achieved with Cat-SPIRE in patients with cat-induced AR.

This randomized DBPC trial used the same baseline challenge methodology as the earlier cited study and had patients undergo the cat-allergen challenge in the EEC at 18 to 22 weeks and at 50 to 54 weeks after the start of treatment. The primary end point was defined as the change in TRSS (posttreatment vs baseline EEC challenges) at 1 to 3 hours on days 2 to 4 in the chamber.

A total of 202 cat-allergic patients were randomize to (1) 4 ID doses of 6-nmol cat peptide, 4 weeks apart (n = 66); (2) 8 ID doses of 3-nmol cat peptide, 2 weeks apart (n = 67); or (3) placebo (n = 69).

At the 18- to 22-week chamber challenge, a distinct treatment effect was observed on all chamber exposure challenge days in the nonasthmatic (per protocol) population with the 6 nmol × 4 ID injections, 4-weeks-apart regimen of Cat-SPIRE versus placebo (median change: −5.77 versus −3.67; mean change: −5.56 versus −3.52; Least square means change: −5.52 versus −3.56; [*P* value (analysis of covariance) = .05]). The treatment was well tolerated.

In phase 2 of this clinical trial, patients were reconsented to continue in the study and undergo a repeat chamber exposure to cat at 1-year (50–54 weeks). Treatment with Cat-SPIRE was shown to provide a treatment effect that persisted at 1 year after the start of treatment compared with placebo (6 nmol × 4 ID injections, 4 weeks apart versus 3 nmol × 8 ID injections, 2 weeks apart [$P = .0342$]; 6 nmol × 4 ID injections, 4 weeks apart versus placebo [$P = .0104$]). Furthermore, the effect on the TRSS at the 1-year challenge was heightened in comparison with the changes observed at the phase 1 (18–22 week) challenge (**Fig. 1**).

A distinct treatment effect was observed at all time points after 1 hour on days 2 to 4 in EEC at the 50- to 54-week challenge in the nonasthmatic population for the 6 nmol × 4

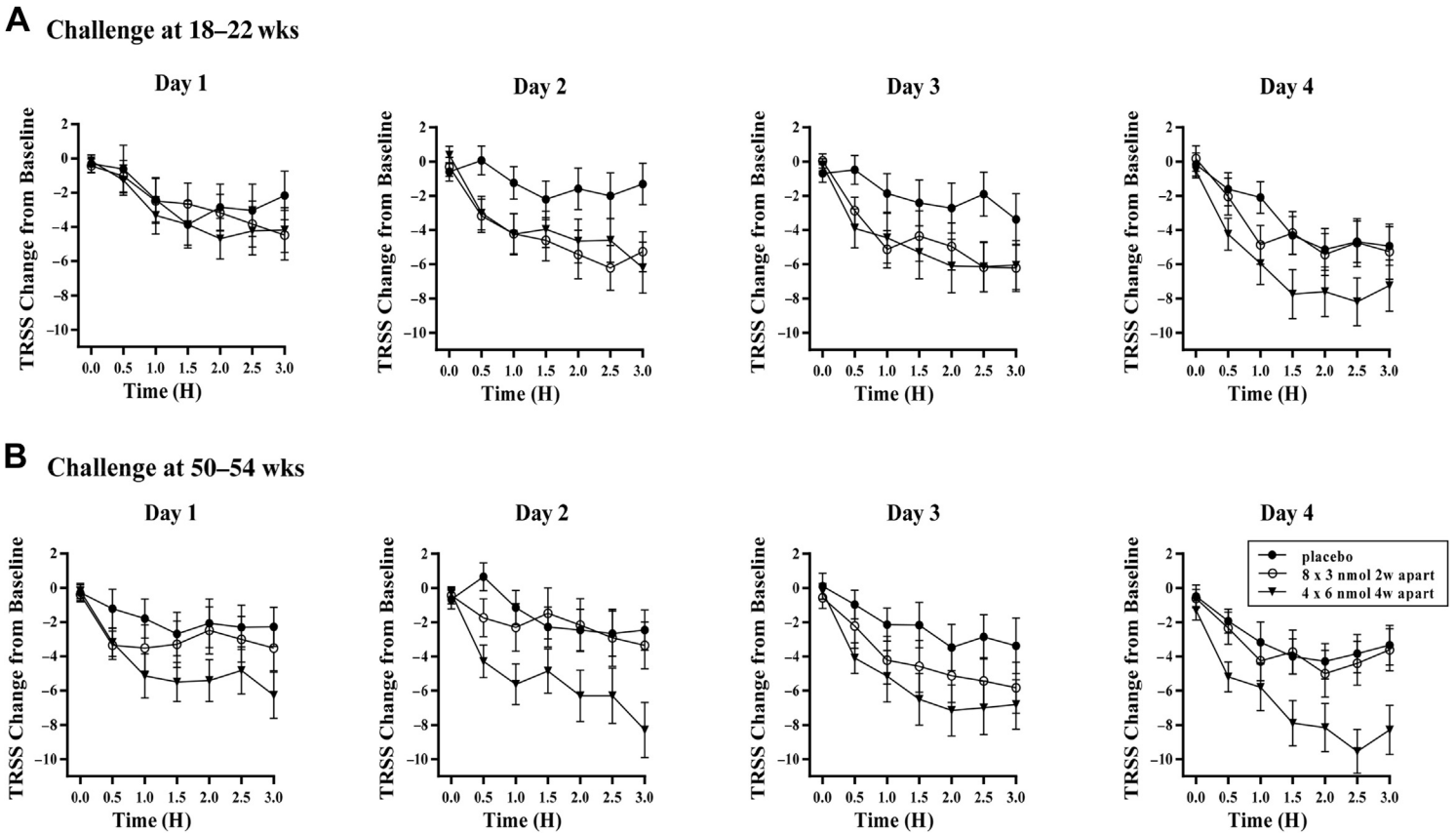

Fig. 1. Delta changes in total rhinoconjunctivitis symptom scores for the treatment effect observed with cat-SPIRE in the environmental exposure chamber. (*From* Creticos PS. Advances in synthetic peptide immuno-regulatory epitopes. World allergy Organ J 2014;7:30.)

dose regimen versus placebo. Furthermore, the clinical effects were similar when assessing all patients (including patients with asthma) with the 6 nmol × 4 dose regimen versus placebo.

An important corollary from this work is that the mean level of airborne Fel d 1 allergen content in the EEC (48 ng/m^3) is within the range reported in homes with cats (10–200 ng/m^3); hence, the EEC unit provides a real-world assessment of the disease. Furthermore, the improvement in TRSS with Cat-SPIRE in the EEC (~4 units change) compares favorably with allergen chamber studies of sublingual immunotherapy cat allergy drops (1.6 units change) and a chamber study of the antihistamine fexofenadine, 180 mg (1.3 units change).[79]

Another important outcome from this study was the finding that no significant changes in cat-specific IgE levels were observed after the 1-year follow-up visit for any of the treatment regimens when compared with the baseline EEC visit. This finding is reassuring based on the premise that the peptides should be of insufficient size to cross-link IgE on mast cells and basophils.

In these trials, Cat-SPIRE was reasonably well tolerated. Of note, there were no asthma-related SAEs in the asthmatic subset of patients. Most of the TEAEs were mild in severity, and no TEAEs were rated as severe on patient diaries. Six subjects dropped out of the study because of a TEAE; except for one patient who experienced a hypersensitivity reaction to the drug, none of the other patient withdrawals were assesses as study drug related.

With respect to respiratory TEAEs, there were no reductions in forced expiratory volume in the first second of expiration greater than 30% (the prospectively defined cutoff in the study). Three study patients who received (6 nmol) peptide experienced an episode of dyspnea, bronchospasm, or asthma, whereas 14 subjects who received 3 nmol and 11 subjects who received placebo reported similar symptoms.[83]

These data from the 1-year follow-up study demonstrate a persistence of effect with the initial 4-injection peptide treatment regimen. The findings from this study extended the observations made in the earlier EEC clinical trial and provide evidence that a long-lasting effect on symptoms can be achieved through immunization with Cat-SPIRE in cat-allergic individuals.

Phase 2b Cat–synthetic peptide immunoregulatory epitopes clinical trial: 2-year environmental exposure chamber follow-up

Of the 86 patients who completed all visits at the 1-year follow-up trial with Cat-SPIRE, 51 agreed to reconsent, remain blinded, and enroll in the 2-year follow-up study. No further treatment was administered to study patients.

Study patients underwent repeat chamber challenge at the 2-year time point (102–106 weeks) with the same protocol challenge methodology (4 consecutive days of 3-hour allergen exposures in the EEC).

A sustained improvement in mean TRSS (from baseline) was observed for the patient group who received 6 nmol (−5.87) versus the 3-nmol group (−3.05) versus the placebo group (−2.02) at the 2-year (102–106 weeks) posttreatment challenge. Although this positive trend did not reach significance for the primary end point ($P = .13$), a statistically significant difference was observed for the prespecified secondary end point that assessed effect at the time point when the cumulative allergen challenge was greatest (day 4, third hour) ($P = .02$).[84]

The third-year posttreatment data presented at the EAACI 2015 meeting provided evidence for persistence of the treatment effect in the subgroup of patients with higher baseline symptoms and in which the 6-nmol dose had been administered.[85]

The encouraging findings from these EEC studies provided the basis for undertaking the first large-scale clinical trial with Cat-SPIRE in patients who live with cats in their home environment. This global study is currently ongoing, and clinical trial results should be available within 1 to 2 years.

Other Peptide Constructs

Circassia also has ongoing clinical trials with peptide constructs of grass, RW, and house DM in various stages of clinical development. The initial trials have again focused on the environmental chamber model to ascertain efficacy and safety in a controlled setting.[80,86–88]

Summary

The work by Durham and colleagues[89] with SCIT in which a 3-year course of immunotherapy with a modified standardized grass extract can induce sustained benefit, serves as the benchmark for new immunotherapy constructs. The potential for synthetic peptide immune-regulatory epitopes to achieve long-lasting clinical benefit, safely and with a concise treatment regimen, is certainly a desired goal in AIT.

OVERLAPPING PEPTIDES

Anergis uses its technology of contiguous overlapping peptides (COP) for the development of allergy vaccines. Products based on COP reproduce the complete amino acid sequence of the allergen in separate synthetic long peptides, designed to provide the complete allergen sequence covering all T-cell epitopes, but to not cross-react with IgE, and thereby avoid the early phase response. The objective is ultrafast allergy desensitization after only 2 months of treatment.[90,91]

In 2014, Spertini reported data from a phase 2b study for AllerT, a birch pollen allergy vaccine candidate. In this placebo-controlled, double-blind, randomized multicenter trial, a total of 239 subjects were divided into 3 groups (placebo, AllerT 50 μg, and AllerT 100 μg, respectively). From November 2012 to March 2013, the subjects received 5 injections over a period of 2 months as a preseasonal treatment. During the subsequent 2013 birch pollen season, compared with the placebo group, the combined Rhinoconjunctivitis Symptom and Medication Score (RSMS, primary end point) was reduced by 30% ($P = .024$, statistically significant) with AllerT 50 μg and by 19% ($P = .190$; *NS*) with AllerT 100 μg. Both AllerT doses were associated with similar statistically significant improvements in the total score of the Rhinoconjunctivitis Quality of Life Questionnaire (Mini-RQLQ) and in the rhinoconjunctivitis symptom score throughout the birch pollen season.[92]

Previous data showed that both AllerT doses induced highly significant increases in anti-Bet v 1 IgG4 antibody (~20-fold; $P<.0001$) versus PL in season 1. In the 2014 trial, the sustained efficacy of AllerT was assessed in the same group of birch-allergic study patients during a second follow-up season without additional treatment. Study results showed persistence of the anti-Bet v 1 IgG4 antibody biomarker, which was accompanied by sustained improvement in clinical parameters of efficacy (RSMS; Mini-RQLQ; nighttime nasal symptom score). These immunologic data provide the first observation that antibody responses to a peptide epitope could be distinguished from natural allergen. As a corollary, no significant difference was observed in Bet v 1–specific IgE levels between active-treated and PL patients. AllerT was safe and well tolerated throughout the 2-month preseasonal treatment.[93,94]

Intralymphatic Immunotherapy

Another intriguing approach to immunization of allergic patients is that of intralymphatic injection (ILIT) of the allergen, which was being developed by Imvision Therapeutics. The premise was based on animal and human investigations which have demonstrated that RNA, DNA, oligopeptides, proteins, and adjuvants can be delivered directly to the regional lymph nodes where they are capable of strongly enhancing immune responses.[11,95–98]

With respect to allergic conditions, ILIT has been investigated in studies of seasonal allergic conditions (birch and grass) and cat allergy. Senti and colleagues[95] performed an open-label, single-center study of ILIT versus SCIT in 165 patients with grass pollen-induced rhinoconjunctivitis in which subjects were randomized to SCIT (54 injections over 3 years; cumulative standardized quality units (SQ-U) dose: 4,031,540 units) or 3 ILITs (at 4-week intervals; cumulative dose: 3000 SQ-U). ILIT resulted in attenuate of hay fever symptoms, inhibition of skin test reactivity to grass pollen, a decrease in serum-specific IgE, and a positive shift in nasal responsivity on nasal provocation. ILIT was well tolerated and resulted in fewer AEs as contrasted with SCIT. Most relevant, the nasal provocation procedures, performed at 4 months, 12 months, and 3 years, demonstrated attenuation to grass challenge within 4 months.[95]

The cat study reported by Senti and colleagues[97] evaluated 20 cat-allergic subjects who were randomized to either placebo or active therapy with a modified recombinant allergen rFel d 1 (recombinant of major cat allergen) fused to a translocator peptide trans-activating transcription factor (TAT) and to part of the human invariant chain (Ii), to generate a modular antigen transporter (MAT) vaccine (MAT-Fel d 1). The product was administered via injection using a conventional needle and syringe directly into lymph nodes in the groin.[97]

This initial study demonstrated that the active vaccine was well tolerated, with no AEs related to the 3-injection procedure other than reports of swelling and itching in the lymph node in the groin used for injection. Furthermore, the MAT-Fel d 1 vaccine increased cat-specific IgG4 6-fold ($P = .003$); the IgG4 response positively correlated with IL-10 production ($P = .026$); and the threshold for nasal provocation to cat was significantly shifted (74-fold vs <3-fold; $P<.001$).[97]

Hylander and colleagues performed a hybrid open-label (followed by DBPC phase) study in birch-allergic patients. Seven birch-allergic patients received birch SCIT (Alutard; ALK) over 3 years (14-week buildup phase to achieve 100,000 SQ-U administered every 6–8 weeks during the 3-year maintenance phase of the study) and served as open-label controls. Twenty-one patients (6 on open-label ILIT; 15 in a cohort randomized to receive ILIT [n = 7] or PL [n = 8]) received 3 ultrasound-guided IL injections of either birch or grass ILIT (1000 SQ-U) or PL. Patients on ILIT were shown to have an improvement on nasal provocation, which was associated with a decrease in inflammatory cells in nasal lavage fluids. Albeit a small number of patients were undergoing treatment, ILIT was well tolerated; patients reported improvement in their seasonal allergic symptoms (scored on a 10-point VAS: 0 [unchanged] to 10 [total symptom relief]) (SCIT: 6.1 [$P = .03$]; ILIT: 5.5 [$P = .05$] versus baseline, respectively).

As opposed to SCIT, ILIT did not result in an increase in IgG4. However, peripheral T-cell activation was evaluated with flow cytometry that showed upregulated expression of CD69 ($P = .02$) and CD98 ($P = .04$) on CD4+ lymphocytes with ILIT but not with PL. However, this cellular activation of peripheral CD4+ T cells was not accompanied by IL-10 secretion.[98]

At the 2015 AAAAI annual meeting, Patterson and colleagues[99] presented the first trial with the 3-injection (50/100/250 protein nitrogen units; $\geq$4 weeks apart)

ultrasound-guided ILIT with a commercially available US grass extract (*Phleum pratense*) in 15 young adults with grass pollen–induced allergic rhinoconjunctivitis with or without intermittent asthma. The ILIT was well tolerated (100% completed all injections), with the total safety score (TSS) showing no difference between active treatment and PL ($P = .8$).[99]

The ability to deliver allergen directly to antigen-processing cells in the lymphoid tissue residing in a regional lymph node (eg, inguinal node) provides an attractive approach: it allows immunization to be accomplished with a smaller dose; it achieves long-lasting benefit with a minimal number of injections; and it seems to be well tolerated.

Epicutaneous Immunotherapy

Another approach that attempts to capitalize on ease of access to antigen-presenting cells is that of epicutaneous delivery of allergen. Studies in the early allergy literature attempted to introduce allergen through ID inoculation or scarification techniques (cutaneous quadrille ruling) in an attempt to circumvent the problem of anaphylaxis associated with subcutaneous injections of allergens. Interestingly, some of this early work demonstrated a positive benefit; however, patient discomfort and imprecise allergen delivery led to discontinued interest in this approach. However, with the development of modern transcutaneous delivery systems, this method of immunization has again generated increased interest as a means of possibly effectively administering allergens.

Senti and colleagues[100–103] have studied allergen patches prepared with grass allergen extract derived from respective pollen species (*Phleum*, *Dactylis*, *Lolium*, *Poa*, *Festuca*, *Holcus*).

Recombinant Vaccines

Natural recombinant vaccines (wild type) have been studied in DBPC clinical trials in grass- and birch-allergic patients.[104,105] A study by Jutel and colleagues[106] with a recombinant timothy grass pollen extract demonstrated significant improvement in the combined symptom medication score and quality of life; however, allergen-specific IgE was not suppressed. Pauli and colleagues[106,107] compared recombinant Bet v 1a, purified Bet v 1, and standard birch pollen extract and showed similar improvements with all 3 treatments in symptom medication diary scores; IgG1/IgG2/IgG4 antibody increases were observed in all 3 treatment groups. Again, no significant shift was observed in the safety profile, and injection site swelling was more frequently observed in the group that received the recombinant birch vaccine.

Subsequent interest has focused on hypoallergenic recombinant allergens wherein conformational changes are inserted onto the native allergen's IgE epitopes to reduce the allergen's inherent allergenicity.

An environmental chamber study (n = 36) by Meyer and colleagues[108] with a novel hypoallergenic recombinant birch extract, administered as 10 weekly injections, demonstrated significant improvement in patient-reported EEC symptoms (pretreatment vs posttreatment score) and was accompanied by the expected increase in allergen-specific IgG1. The investigators, however, reported that grade II AEs were more frequently observed in patients receiving the 2 higher dosing regimens.[108]

Valenta and colleagues[104,105] have focused on studies with recombinant fragments and in a series of studies have shown that, as opposed to intact allergen, the fragments did not induce allergen-specific IgE, did increase protective IgG and IgA antibody, inhibited facilitated antigen binding, and attenuated nasal sensitivity on nasal allergen provocation. However, subsequent DBPC trials could not demonstrate

significant improvement in clinical outcomes, including symptom diary scores and relief medication usage.

Valenta in association with Biomay have recently focused their research and development on a recombinant B-cell epitope vaccine (BM32). This vaccine is a peptide carrier fusion vaccine that contains linear peptides that are part of B-cell epitopes; these peptides are fused to an immunogenic carrier element and, using recombinant technology, are expressed as fusion proteins. A peptide carrier fusion vaccine is capable of inducing allergen-specific IgG that is directed against parts of the IgE epitope and blocks the binding of IgE.[105,109,110]

In a 2-year European phase 2b DBPC trial of the fusion vaccine (BM32), 181 patients were randomized to receive either of 2 doses of BM32 or the matching placebo. The treatment phase consisted of 3 subcutaneous injections administered over the 2 months before the 2013 grass pollen season, followed by a fall boost injection and 3 additional doses before the 2014 season. (The Data Monitoring Committee reviewed the safety data after the completion of the first year of treatment and recommended that all actively treated patients should receive the lower vaccine dose [20 μg of each of the 4 protein components]).[109,110]

Several of the study's secondary clinical outcomes provided statistically significant evidence for therapeutic efficacy with the vaccine, including a 25% difference between active drug and PL in the Rhinoconjunctivitis Symptom Score during the peak pollen season in the second treatment year ($P = .042$) and improvement on the VAS ($P = .014$) and the Rhinoconjunctivitis Quality of Life Questionnaire ($P<.005$). A measureable positive trend was observed for the primary end point (Combined Symptom and Medication Score) versus placebo (22% difference; $P = .085$).[109,110]

The positive clinical findings were paralleled by a sustained induction of allergen-specific IgG antibody, and the vaccine did not boost IgE antibody. The treatment was well tolerated; most side effects were mild to moderate and short-lived (resolving in a short period after drug administration).[109,110]

The company also has a strategic focus for the development of birch, RW, house DM, and cat peptide-carrier fusion proteins.

SUMMARY

There is a recognized need for newer therapeutic agents that improve the safety of AIT, provide an ease of delivery to patients that fosters compliance and allows access to a greater proportion of the allergic population that could benefit from this disease-modifying treatment, and achieve an acceptable therapeutic benefit for most patients committing to the course of treatment.

The recent advances in sublingual AIT are most encouraging, as this now offers patients a noninjectable form of treatment of inhalant allergies.[111]

Furthermore, the continued research and development of the novel therapeutic constructs discussed in this article holds the promise of accomplishing the aforementioned goals in the not-so-distant future.

REFERENCES

1. Arbes SJ Jr, Gergen PJ, Elliott L, et al. Prevalence of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005;116:377–83.

2. Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, health use, and mortality: U.S., 2005-2009. National Health Statistics Report; no 32, 32. Washington, DC: National Center for Health statistics; 2011. p. 1–14.
3. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and Its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008;63(Suppl 86):8–160.
4. Wallace D, Dykewicz M, Bernstein D, et al. The diagnosis and management of rhinitis: an updated practice parameter. J Allergy Clin Immunol 2008;122:S1–84.
5. Broide DH. Immunomodulation of allergic disease. Annu Rev Med 2009;60: 279–91.
6. Valenta R, Ferreira F, Focke-Tejkl M, et al. From allergen genes to allergy vaccines. Annu Rev Immunol 2010;28:211–41.
7. Focke M, Swoboda I, Marth K, et al. Developments in allergen-specific immunotherapy: from allergen extracts to allergy vaccines bypassing allergen-specific immunoglobulin E and T cell reactivity. Clin Exp Allergy 2010;40:385–97.
8. Canonica GW, Bousquet J, Casale T, et al. Sublingual immunotherapy: World Allergy Organization position paper 2009. Allergy 2009;64(Suppl 91):1–59.
9. Akdis M, Akdis C. Therapeutic manipulation of immune tolerance in allergic disease. Nat Rev Drug Discov 2009;8:640–60.
10. Creticos PS. Sublingual and oral immunotherapy for allergic rhinitis. Waltham (MA): UpToDate; 2015.
11. Cox L, Compalati E, Kundig T, et al. New directions in immunotherapy. Curr Allergy Asthma Rep 2013;13:178–95.
12. Marsh DG, Belin L, Bruce CA, et al. Rapidly released allergens from short ragweed pollen. I. Kinetics of release of known allergen in relation to biological activity. J Allergy Clin Immunol 1981;67:206–16.
13. Norman P, Lichtenstein L, Marsh D. Studies on allergoids from naturally occurring allergens. IV. Efficacy and safety of long-term allergoid treatment of ragweed hay fever. J Allergy Clin Immunol 1981;59:314–9.
14. Wie SI, Wie CW, Lee WY, et al. Suppression of reaginic antibodies with modified allergens. III. Preparation of tolerogenic conjugates of common allergens with monomethoxypolyethylene glycols of different molecular weights by the mixed anhydride method. Int Arch Allergy Appl Immunol 1981;64:84–99.
15. Sehon AH, Wy Lee. Suppression of IgE antibodies with modified allergens. J Allergy Clin Immunol 1979;64(4):242–50.
16. Patterson R, Suszko IM, Pruzansky JJ, et al. Polymerization of mixtures of grass allergens. J Allergy Clin Immunol 1977;59:314–9.
17. Grammer LC, Zeiss CR, Suszko IM, et al. A double-blind placebo-controlled trial of polymerized whole ragweed for immunotherapy of ragweed allergy. J Allergy Clin Immunol 1982;69(6):494–9.
18. Grammer LC, Shaughnessy MA, Suszko IM, et al. A double-blind histamine placebo-controlled trial of polymerized whole grass for immunotherapy of grass allergy. J Allergy Clin Immunol 1983;72(5 Pt 1):448–53.
19. Briner TJ, Kuo MC, Keating KM, et al. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. Proc Natl Acad Sci U S A 1993;90:7608–12.
20. Wallner BP, Gefter ML. Immunotherapy with T-cell-reactive peptides derived from allergens. Allergy 1994;49:302–8.
21. Norman PS, Ohman JL Jr, Long AA, et al. Treatment of cat allergy with T cell epitope containing peptides. Am J Respir Crit Care Med 1996;154:1623–8.

22. Norman PS, Nicodemus CF, Creticos PS, et al. Clinical and immunologic effects of component peptides in Allervax® Cat. Int Arch Allergy Immunol 1997;113:1–3.
23. Creticos PS, Hebert J, Philip G, Allervax® Ragweed Study Group. Efficacy of Allervax® ragweed in the treatment of ragweed-induced allergy. J Allergy Clin Immunol 1997;99(No. 1, Part 2):S401(1631).
24. Creticos PS. Peptide downregulation of the immune response. Chapter 30. In: Marone G, Austen KF, Holgate ST, et al, editors. Asthma and allergic diseases. San Diego: Academic Press; 1998. p. 407–15.
25. Bird AP. DNA methylation and the frequency of CpG in animal DNA. Nucleic Acids Res 1980;8(7):81499–504.
26. Tokunaga T, Yamamoto H, Shimada S, et al. Antitumor activity of deoxyribonucleic acid fraction from Mycobacterium bovis BCG: I: isolation, physiochemical characterization, and antitumor activity. J Natl Cancer Inst 1981;72:955–62.
27. Yamamoto S, Yamamoto T, Shimada S, et al. DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. Microbiol Immunol 1992;36:983–97.
28. Yamamoto T, Yamamoto S, Kataoka T, et al. Synthetic oligonucleotides with certain palindromes stimulate interferon production of human peripheral blood lymphocytes in vitro. Jpn J Cancer Res 1994;85:775–9.
29. Pisetsky DS. Immune activation by bacterial DNA: a new genetic code. Immunity 1996;5:303–10.
30. Krieg AM, Yi AK, Matson S, et al. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 1995;374:546–9.
31. Wagner H. Toll meets bacterial CpG-DNA. Immunity 2001;14(5):499–502.
32. Bauer S, Kirschning CJ, Häcker H, et al. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc Natl Acad Sci U S A 2001;98(16):9237–42.
33. Klinman DM, Yi AK, Beaucage SL, et al. CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin6-, interleukin 12, and interferon gamma. Proc Natl Acad Sci U S A 1996;93:2879–83, 124.
34. Klinman DM, Yamshchikov G, Ishigatsubo Y. Contributions of CpG motis to the immunogenicity of DNA vaccines. J Immunol 1980;158:3635–9.
35. Messina JP, Gilkeson GS, Pisetsky DS. Stimulation of in vitro murine lymphocyte proliferation by bacterial DNA. J Immunol 1991;147:1759–64.
36. Sato Y, Roman M, Tighe H, et al. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. Science 1996;273:352–4.
37. Cowdery JS, Chace JH, Yi AK, et al. Bacterial DNA induces NK cells to produce IFN-gamma in vivo and increases the toxicity of lipopolysaccharides. J Immunol 1996;156:4570–5.
38. Halpern MD, Kurlander RJ, Pisetsky DS. Bacterial DNA induces murine interferon-gamma production by stimulation of interleukin-12 and tumor necrosis factor-alpha. Cell Immunol 1996;167:72–77.8.
39. Raz E, Carson DA, Parker SE, et al. Intradermal gene immunization: the possible role of DNA uptake in the induction of cellular immunity to viruses. Proc Natl Acad Sci U S A 1994;91:9519–23.
40. Raz E, Tighe H, Sato Y, et al. Preferential induction of a T_H1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. Proc Natl Acad Sci U S A 1996;93:5141–5.
41. Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. J Allergy Clin Immunol 2000;105:124–34.

42. Van Nest G, Eiden JJ, Tuck SF, et al. Conjugation of immunostimulatory DNA (ISS) to the major short ragweed allergen, Amb a 1, enhances immunogenicity and reduces allergenicity. J Allergy Clin Immunol 2000;105:S70.
43. Horner AA, Van Uden JH, Zubeldia JM, et al. DNA-based immunotherapeutics for the treatment of allergic disease. Immunol Rev 2001;179:102–18.
44. Broide D, Schwarze J, Tighe H, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. J Immunol 1998;161(12):7054–62.
45. Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. J Allergy Clin Immunol 2000;106(1 Pt 1):124–34.
46. Marshall JD, Abrahi S, Eiden JJ, et al. Immunostimulatory sequences (ISS) DNA linked to Amb a 1 allergen promotes Th1 cytokine expression while downregulating Th2 cytokine expression in PBMC's from ragweed-allergic humans. J Allergy Clin Immunol 2001;108:191–7.
47. Creticos PS, Eiden JJ, Balcer SL, et al. Immunostimulatory oligonucleotides conjugated to Amb a 1: safety, skin test reactivity, and basophil histamine release. J Allergy Clin Immunol 2000;105:S70.
48. Creticos PS, Balcer SL, Schroeder JT, et al. Initial immunotherapy trial to explore the safety, tolerability, and immunogenicity of subcutaneous injections of an Amb a 1 immunostimulatory oligonucleotide conjugate in ragweed-allergic adults. J Allergy Clin Immunol 2001;107:S216.
49. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006;355:1445–55.
50. Tulic M, Fiset P, Christodoulopoulos P, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. J Allergy Clin Immunol 2004;113:235–41.
51. Vaishnav AR, Whaley SB, Hamilton R, et al. Evaluation of safety and clinical efficacy of higher dose immunotherapy with AIC (immunostimulatory DNA conjugated with Amba 1) in patients with ragweed-induced seasonal allergic rhinitis (SAR). J Allergy Clin Immunol 2005;115(No.2):S65–260.
52. Busse W, Gross G, Korenblat P, et al. Phase 2/3 study of the novel vaccine Amb a 1 immunostimulatory oligodeoxyribonucleotide conjugate AIC in ragweed allergic adults. J Allergy Clin Immunol 2006;117(No.2):S88–9.
53. Bernstein DI, Segall N, Nayak A, et al. Safety and efficacy of the novel vaccine TOLAMBA™ in ragweed allergic adults, a dose finding study. J Allergy Clin Immunol 2007;119(No 1):S78–9.
54. Dynavax press release. May 16, 2008: Tolamba TM chamber study misses primary endpoint.
55. Drachenberg KJ, Wheeler AW, Stuebner P, et al. A well-tolerated grass pollen specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A (MPL), reduces allergic symptoms after only four preseasonal injections. Allergy 2001;56:498–505.
56. Drachenberg KJ, Heinzkill M, Urban E, et al. Efficacy and tolerability of short-term specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A (MPL) for children and adolescents. Allergol Immunopathol (Madr) 2003;31(5):270–7.
57. Mothes N, Heinzkill M, Drachenberg KJ, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally

boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. Clin Exp Allergy 2003;33: 1198–208.
58. DuBuske L, Frew A, Horak F, et al. Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. Allergy Asthma Proc 2011;32:239–47.
59. Baldrick P, Richardson D, Woronjecki SR, et al. Pollinex Quattro® ragweed: safety evaluation of a new allergy vaccine adjuvanted with monophosphoryl lipid A (MPL®) for the treatment of ragweed pollen allergy. J Appl Toxicol 2007;27(4): 399–409.
60. Patel P, Holdich T, Weikersthal-Drachenberg K, et al. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. J Allergy Clin Immunol 2014;133:121–9.
61. Roger A, Depreux M, Jurgens Y, et al. A novel and well tolerated mite allergoid subcutaneous immunotherapy: evidence of clinical and immunologic efficacy. Immun Inflamm Dis 2014;2(2):92–8.
62. Roger A, Depreux M, Jurgens Y, et al. An observational post authorisation 1 year follow-up study of the safety, tolerability, satisfaction and effectiveness of a modified mite-allergoid subcutaneous immunotherapy. Barcelona (Spain): EAACI Abstract; 2015.
63. Storni T, Ruedl C, Schwarz K, et al. Nonmethylated CpG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects. J Immunol 2004;172(3):1777–85.
64. Kundig TM, Senti G, Schnetzler G, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. J Allergy Clin Immunol 2006; 117:1470–6.
65. Senti G, Johansen P, Haug S, et al. Use of A-type CpG oligonucleotides as an adjuvant in allergen- specific immunotherapy in humans: a phase 1/2 clinical trial. Clin Exp Allergy 2009;39:62–70.
66. Klimek L, Willers J, Hammann-Haenni A, et al. Assessment of clinical efficacy of CYT003-QbG10 in patients with allergic rhinoconjunctivitis: a phase 2b study. Clin Exp Allergy 2011;41:1305–12.
67. Beeh K, Kanniess F, Wagner F, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent asthma. J Allergy Clin Immunol 2013;131:866–74.
68. Casale T, Cole J, Beck E, et al. CYT003, a TLR9 agonist, in persistent asthma – a randomized placebo-controlled phase 2b study. Allergy 2015;22. http://dx.doi.org/10.1111/all.12663.
69. Arruda LB, Sim D, Priya R, et al. Dendritic cell-lysosomal associated membrane protein (DC-LAMP0- and LAMP-1-Gag chimeras have distinct cellular trafficking pathways and prime T- and B-cell responses to a diverse repertoire of epitopes. J Immunol 2006;177:2265–75.
70. Weiner L, Heiland T, Mackler B, et al. Phase 1 study of JRC-LAMP-Vax, a DNA immunotherapy vaccine to treat Japanese red cedar allergy. Ann Allergy Asthma Immunol 2013;111:A8–9.
71. Weiner L, Mackler B, Heery B, et al. A DNA vaccine immunotherapy for Japanese red cedar. World Allergy Organ J 2014;7(Suppl):23.
72. Oldfield W, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002;360:47–53.
73. Smith T, Alexander C, Kay AB, et al. Cat allergen peptide immunotherapy reduces CD4 T cell responses to cat allergen but does not alter suppression by

CD4 CD25 T cells: a double-blind placebo-controlled study. Allergy 2004;59:1097–101.
74. Verhoef A, Alexander C, Kay AB, et al. T cell epitope immunotherapy induces a CD4(+) T cell population with regulatory activity. PLoS Med 2005;2:e78.
75. Larche M. Immunotherapy with allergen peptides. Allergy Asthma and Cx Immunol 2007;3(2):53–9.
76. Campbell J, Buckland K, McMillan S, et al. Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. J Exp Med 2009;206:1535–47.
77. Larche M, Moldavar D. Immunotherapy with Peptides. Allergy 2011;66:784–91.
78. Worm M, Lee HH, Kleine-Tebbe J, et al. Development and preliminary evaluation of a peptide immunotherapy vaccine for cat allergy. J Allergy Clin Immunol 2011;127:89–97.
79. Worm M, Patel D, Creticos PS. Cat peptide antigen desensitisation for treating Cat allergic rhinoconjunctivitis. Expert Opin Investig Drugs 2013;22(10):1347–57.
80. Creticos PS. Advances in synthetic peptide immune-regulatory epitopes. World Allergy Organ J 2014;7:30.
81. Larche M, Patel D, Patel P, et al. Safety and efficacy of Fel d 1-derived peptide immunotherapy in a DBPC environmental exposure chamber study [ECACI Abstract]. Allergy 2012;67(Suppl 96).
82. Hafner R, Patel P, Salapatek A, et al. Fel d 1 peptide antigen desensitization safety and efficacy in a DBPC EEC study [WAO Abstract]. World Allergy Organ J 2013;6(Supp):150.
83. Patel D, Couroux P, Hickey P, et al. Fel d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: a randomized, placebo-controlled study. J Allergy Clin Immunol 2013;131:103–9.
84. Hafner R, Couroux P, Armstrong K, et al. Two year persistent treatment effect achieved after 4 doses of cat-peptide antigen desensitization in an EEC model of cat allergy. J Allergy Clin Immunol 2013;131(2):AB147.
85. Couroux P, Patel D, Armstrong K, et al. Fel d 1-derived synthetic peptide immune-regulatory epitopes show a long-term treatment effect in cat allergic subjects. Clin Exp Allergy 2015;45:974–81.
86. Hafner R, Salapatek AM, Patel D, et al. Validation of peptide immunotherapy as a new approach in the treatment of allergic rhinoconjunctivitis: the clinical benefits of treatment with Amb a 1 derived T cell epitopes. J Allergy Clin Immunol 2012;129(2):AB368.
87. Hafner R, Salapatek AM, Larche M, et al. Comparison of the treatment effect of house dust mite synthetic peptide immune-regulatory epitopes in the environmental exposure chamber and field setting two years after a short course of treatment. EAACI-2015 abstract, in press.
88. Gliddon DR, Kim YW, Shannon CP, et al. Whole blood immune transcription profiling reveals systemic pathways associated with the mechanism of action of cat-synthetic peptide immune-regulatory epitopes. EAACI-2015 abstract, in press.
89. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999;341:468–75.
90. Pellaton C, Perrin Y, Boudousquie C, et al. Novel birch pollen specific immunotherapy formulation based on contiguous overlapping peptides. Clin Transl Allergy 2013;3:17.
91. Fallrath JM, Kettner A, Dufour N, et al. Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase 1 trial. J Allergy Clin Immunol 2003;111(4):854–6.

92. Spertini F, de Blay F, Jacobsen L, et al. Ultra-fast hypoallergenic birch pollen allergy vaccine AllerT is efficient and safe: results of a phase 2b study. J Allergy Clin Immunol 2014;133(2 Suppl):AB290.
93. Spertini F, Jutel M, Jacobsen L, et al. Sustained efficacy of AllerT allergy vaccine after a second birch pollen season: a phase 2b. J Allergy Clin Immunol 2015; 135(2 Suppl):AB143.
94. Reymond C, Kettner A, Boand V, et al. Persistence of anti-Bet 1 v IgG4 prior and during a second pollen season after AllerT ultra-fast immunotherapy (phase 2b follow up). EAACI-2015 abstract, in press.
95. Senti G, Prinz Vavricka BM, Erdmann I, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. Proc Natl Acad Sci U S A 2008;105:17908–12.
96. Senti G, Johansen P, Kundig TM. Intralymphatic immunotherapy. Curr Opin Allergy Clin Immunol 2009;9:537–43.
97. Senti G, Crameri R, Kuster D, et al. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. J Allergy Clin Immunol 2012;129: 1290–6.
98. Hylander T, Latif L, Petersson U, et al. Intralymphatic allergen-specific immunotherapy: an effective and safe alternative treatment route for pollen-induced allergic rhinitis. J Allergy Clin Immunol 2013;131:412–20.
99. Patterson AM, Bonny AE, Shiels WE, et al. Safety of 3-injection immunotherapy protocol for treating grass pollen-induced rhinoconjunctivitis in adolescents/ young adults. J Allergy Clin Immunol 2015;135(2 Suppl):AB388.
100. Senti G, Graf N, Haug S, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. J Allergy Clin Immunol 2009;124: 997–1002.
101. Agostinis F, Forti S, Di Berardino F. Grass transcutaneous immunotherapy in children with seasonal allergic rhinitis. Allergy 2010;65:410.
102. Senti G, von Moos S, Kündig T. Epicutaneous allergen administration: is this the future of allergen specific immunotherapy? Allergy 2011;66(6):798–809.
103. Senti G, von Moos S, Tay F, et al. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: a double-blind, placebo-controlled dose escalation study. J Allergy Clin Immunol 2012;129:128.
104. Valenta R, Niederberger V. Recombinant allergens for immunotherapy. J Allergy Clin Immunol 2007;119:826–30.
105. Valenta R, Linhart B, Swoboda I, et al. Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens. Allergy 2011;66:775–83.
106. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005;116:608–13.
107. Pauli G, Larsen TH, Rak S, et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. J Allergy Clin Immunol 2008; 122:951–60.
108. Meyer W, Narkus A, Salapatek AM, et al. Double-blind, placebo-controlled, dose-ranging study of new recombinant hypoallergenic Bet v 1 in an environmental exposure chamber. Allergy 2013;68:724–31.
109. Biomay Press Release. (28 January 2015). Clinicaltrials.govidentifier NCT1538979.
110. Valenta R. Clinical efficacy of a recombinant B-cell epitope based grass pollen allergy vaccine - a phase 2b proof of concept study. EAACI-2015 Abstract, in press.
111. Nelson HS, Makatsori M, Calderon MA. SCIT and SLIT: comparative efficacy, current and potential indications-and warnings-US vs. Europe.