EXPERT REVIEWS

The evolution of allergen and non-specific immunotherapy: past achievements, current applications and future outlook

Expert Rev. Clin. Immunol. 11(1), 000–000 (2014)

Giovanni B Pajno*[1], Kari C Nadeau[2], Giovanni Passalacqua[3], Lucia Caminiti[1], Ben Hobson[2], David C Jay[2], Stefania Arasi[1], Fernanda Chiera[1], Giuseppina Salzano[1]

[1]*Department of Pediatrics- Allergy Unit, University of Messina, Via Consolare Valeria-Gazzi, 98124 Messina, Italy*
[2]*Institute of Immunity, Transplantation and Infectious Diseases, Stanford University, California,USA*
[3]*Allergy and Respiratory Diseases, IRCCS San Martino-IST-University of Genova, Genova, Italy*
**Author for correspondence:*
Tel.: +39 0902 213 165
Fax: +39 0902 212 143
Giovanni.pajno@unime.it

Recent epidemiological studies estimated that more than 30% of European suffer from allergic rhinitis or conjunctivitis, while up to 20% suffer from asthma and 15% from allergic skin conditions, while for many other regions the prevalence is increasing. Allergen immunotherapy represents the only available treatment that can modify the allergic disease process, and thus is worth considering as a treatment in affected individuals. A beneficial effect of allergen immunotherapy has been shown in both adults and children affected by allergic rhinitis, allergic conjunctivitis, allergic asthma and hymenoptera venom allergy. The present study represents an overview on allergen immunotherapy, focusing on the principal aspects of the use of immunotherapy in the past, its recent clinical applications and future outlook.

KEYWORDS: allergen-specific immunotherapy • allergic asthma • allergic inflammation • allergic rhinitis • atopy • cytokines • monoclonal antibody • prevention • respiratory allergy • treatment

Allergen immunotherapy (AIT) represents the only available treatment that can modify the evolution of allergic diseases, thus representing a valuable treatment in affected individuals. AIT was found clinically effective, in numerous randomized double-blind placebo-controlled trials, in allergic rhinitis, allergic conjunctivitis, allergic asthma and hymenoptera venom allergy. The beneficial effects were confirmed in both adults [1] and children [2]. This is of relevance, since it is estimated that up to 30% of Europeans suffer from allergic rhinitis/conjunctivitis, up to 20% from asthma and 15% from allergic skin conditions. The prevalence of allergic diseases, asthma excluded, is still increasing [3].

Food allergies are also becoming more frequent and severe [4]. Current lifestyles, including diet, 'westernization', industrialization, exposure to pollutants, congregation, are major triggers of symptoms in allergic patients and they are not expected to change on a global scale in the next years. Therefore, AIT still remains a 'basic' treatment of allergic diseases, both for therapy and prevention, since external factors cannot be modified.

Subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) represent the two routes of administration of allergen vaccines currently available and approved [5]. SCIT and SLIT share many characteristics but somewhere differ (Table 1).

Currently, both routes are used in clinical practice for the treatment of allergic patients in Europe and other countries, whereas in the USA products for SLIT have been approved only recently. In the last decade, a new method of AIT (also called oral desensitization or oral immunotherapy [OIT]) had been extensively investigated for the active treatment of IgE-mediated food allergy. In food OIT, the food is usually given orally starting with low doses and increasing at variable rates to achieve a progressive desensitization or tolerance [6,7].

Moreover, allergen non-specific modalities of treatment for allergy disorders, mostly with omalizumab, are currently used and investigated. Omalizumab is a recombinant humanized monoclonal IgE-blocking antibody used as add-on therapy in adults and children over 6 years of

 10.1586/1744666X.2015.977260 ISSN 1744-666X

Table 1. Comparison between subcutaneous immunotherapy and sublingual immunotherapy: similarities and diversities.

Shared	Effective for both allergic rhinoconjunctivitis and allergic asthma
	Disease modifying – Possible prevention of new sensitivities in patients (mostly children) who are monosensitized to house dust mite – Persistence of benefit for several years after discontinuation – Possible prevention of allergic asthma?
	Immunological mechanisms of action
Differing	Severity of systemic reactions (favors SLIT)
	Effectiveness of multiple allergen extracts (favors SCIT)
	Adherence to therapy (favors SCIT)
	Treatment of young allergic children (favors SLIT)

SCIT: Subcutaneous immunotherap; SLIT: Sublingual immunotherapy.

age, with inadequately controlled severe persistent asthma [8]. Pilot studies were recently performed to assess the safety and efficacy of combining omalizumab treatment with OIT in children with severe cow's milk allergy [9] and in patients with chronic generalized urticaria [10]. The promise of better immunotherapy(ies) appears closer than ever before. In this review, we focus on different types and routes of AIT, exploring recent human clinical trials data and we provide suggestions for future developments.

A historical overview

Allergen-specific immunotherapy, named SIT in the past, and more recently AIT, was introduced in clinical practice by Noon in 1910 [11]. The initial scientific intent was to 'desensitize' the human body, as already done for infectious diseases, against aerodispersed 'toxins'. Noon and Freeman did not know the exact immunological mechanism (IgE were discovered in the 1960s) at the basis of respiratory allergy, but they understood that the administration of progressively increasing doses of an extract of grass pollen could be able to reduce the specific conjunctival reactivity in hay fever. The SCIT approach was therefore increasingly applied, with favorable clinical results, especially in pollen-induced allergy. Thus, the practice of AIT became routine, and rapidly increased when the IgE-induced mechanism was demonstrated [12], and the first specific diagnostic tests were introduced. Concerning the scientific approach, the first double-blind placebo-controlled trials started in the USA during the 1960s [13]. For about 60 years, SCIT remained the only route of administration, often not correctly prescribed and using low-quality extracts. Although SCIT was repeatedly demonstrated to be effective in respiratory allergy, a non-negligible risk of severe or even fatal adverse events remained [14,15]. This was in part attributable to technical/human errors, and could therefore be avoided [16], but a large fraction of the severe adverse events reported, remained unpredictable, even if all precautions were taken. Based on this, alternative routes of administration were repeatedly approached and, following the reports of severe or fatal adverse events [17], attempts were made to achieve desensitization via non-subcutaneous administration of the extracts: oral route (allergen immediately ingested), bronchial route, intranasal route (LNIT) [18]. After some experimental trials, it appeared that the oral route required too high amount of allergens, and that the bronchial route was charged by a high risk of adverse events [19]. LNIT appeared to be safe and effective, but poorly applicable in clinical practice. The oral and bronchial administration routes were totally discontinued for respiratory allergies, whereas LNIT was virtually abandoned due to practical problems [19]. In 1986, for the first time, the use of allergen-specific immunotherapy administered by the sublingual route (SLIT) was described [20] and it immediately appeared as a promising route, despite an initial skepticism due to the low doses used and the inadequate design of the early studies. Indeed, within few years SLIT was officially accepted as the only viable alternative to SCIT for treating respiratory allergies in most international guidelines [21–23]. During the last 25 years, numerous randomized controlled trials and meta-analyses confirmed the clinical efficacy of SLIT (for review see [24,25]), and several post-marketing surveys supported its good safety profile of SLIT [24–27].

Current status: some more questions

Currently, the official indications recommend SCIT for rhinitis/asthma/hymenoptera venom allergy, and SLIT for respiratory allergy only. Of note, SLIT has been recently accepted for clinical use also in the USA [28], limited to grass tablets. Despite the abundant literature, confirming the efficacy of AIT, some aspects are still a matter of debate. For instance, the wide variability in standardization methods, usually done by in-house references, make the published studies difficult to compare each other. Other debated aspects are the use and efficacy of allergen mixtures [29,30], the use and prescription in polysensitized patients and the use of AIT in asthma alone [31]. The clinical practice, prescription and use of extracts still remain profoundly different between Europe and the USA [32], and there is still a large variability in prescription rules [33]. In the USA, until 2014 only SCIT was approved, and allergists prepared at their office a 'personalized' mixture containing all the sensitizing allergens. In Europe and other countries, where SLIT was available since many years, the prescription was done for a maximum of three allergens, usually not mixed together. Whereas for SCIT, the regimens of administration are quite standardized, for SLIT many modalities are available (e.g., no build-up phase, different pharmaceutical preparations, different maintenance regimens). Again, the reporting of side effects is not standardized, although recent recommendations have been provided by WHO for both systemic and local side effects [34,35]. Great efforts are currently made to harmonize and uniform the design of clinical trials and their reporting [36–38]. Especially for SLIT, additional problems emerge, such as the optimal maintenance dose, the best administration regimen and the duration of the treatment.

At present, some other relevant problems on AIT are emerging, and interesting studies have been reported: adherence and how to improve it [39,40], molecular-based diagnosis in appropriate prescription of AIT [41], pharmacoeconomy aspects and preventative effects [42] and mechanisms of action [43,44]. All the above-mentioned problems are part of the present historical framework of AIT.

Over the horizon, the most realistic option is the treatment of IgE-mediated food allergy: oral immunotherapy (OIT), which seems to offer a suitable therapy for patients with food allergy [45]. So far, clinical trials reported overall positive effects, with desensitization achieved in at least 70% patients [46]. However, there are still some unmet needs: allergen dosage of foods and formulation; achievement of a true permanent tolerance in treated patients and safety of OIT. Current status of immunotherapies both specific and non-specific is depicted in Figure 1.

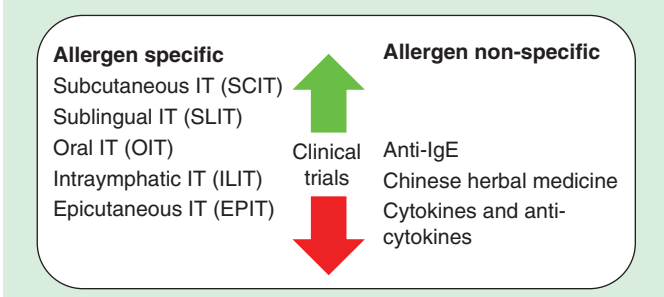

Figure 1. Different routes and forms of immunotherapy. For oral immunotherapy, intralymphatic immunotherapy and epicutaneous administration, more clinical studies are wanted as for non-specific immunotherapy.

The 'near & far' future

An historical narration should look to the past (SCIT), as well as to the future (SLIT and OIT). The future itself is part of the history and, therefore we are considering also the future applicable developments of AIT. The very favorable safety profile of SLIT suggested new possible approaches, especially in conditions other than respiratory allergy, which are characterized by an almost pure IgE-mediated reaction. As mentioned above, oral/sublingual approaches for desensitizing (or inducing temporary tolerance) to food allergens are very promising. The majority of trials were performed with cow's milk desensitzation, with a percentage of positive outcome in 70–80% of children [47]. The results of oral desensitization with peanut or egg white are, so far, convincing and based on relatively large trials [48–50]. Isolated exploratory studies also used SLIT in food allergy with Pru p 3 (lipid transfer protein of peach), or hazelnut extract [51,52]. Other possible developments are related to the engineering of molecules, or to the use of selected allergenic components (purified or recombinant). The birth of molecular allergology was a major advance in the diagnosis of allergy disorders. However, the advance in diagnosis needs to be mirrored in AIT. The improvement of the quality of products for AIT has the potential to increase the credibility of this treatment that, despite its long history, is often underestimated by the medical community. Indeed, due to regulatory and marketing problems, the use of allergen molecules or recombinant proteins seems to belong to a more far future [53]. Finally, novel administration approaches have been recently proposed. First, the intralymphatic immunotherapy [54] and, second the epicutaneous administration [55,56], which represent a reliable perspective.

Allergen immunotherapy

Mechanisms of allergic diseases

Allergic diseases (AD) are defined as a group of immune-mediated disorders [57].

Most AD are IgE-mediated, including allergic rhinoconjunctivitis, bronchial asthma, acute urticaria and angioedema, anaphylaxis, oral allergy syndrome, extrinsic eczema and several food allergies. Less often, AD are IgE-independent like contact dermatitis, food protein-induced enterocolitis and celiac disease, or mixed, IgE and not IgE mediated, such as atopic dermatitis and eosinophilic gastroenteropathies [58].

We will focus on the mechanisms of IgE-mediated immune responses involved with the pathogenesis of AD. The first step is called allergic sensitization, which occurs when an atopic individual is exposed to an allergen for the first time. During sensitization, both microbes and irritants can damage and activate the epithelium, facilitating the entry of allergens into the body through skin, airways or gut. In some cases, the epithelial barrier may already be altered due to genetic or environmental factors, such as mutations in filaggrin associated with the atopic dermatitis [59]. Activated epithelial cell and immune cells in the environment secrete GM-CSF and several chemoattractants that guide dendritic cells (DCs) from the bone marrow to the epithelium and underlying mucosa, where they differentiate into mature and competent antigen-presenting cells (APCs) [60,61]. Activated APCs or DCs process the allergen, migrate to draining lymph nodes or to the site of local mucosal lymphoid tissue. There, they present allergen-derived peptides to naïve T cells and promote their activation and differentiation into Th2 cells. Triggering of bronchial epithelial cell by allergen exposure leads to activation to production of chemokines, cytokines and endogenous danger signal that recruit and activate DCs (Figure 2).

Th2 cells are characterized by production of type 2 cytokines IL-4, IL-5, IL-10 and IL-13. Innate ILC2 cells can be activated by epithelial-derived IL-33, IL- [25] and thymic stromal lymphopoietin and Th2 cytokines and assist Th2 cells in secreting type 2 cytokines [62]. Th2 and ILC2 cells play an integral role in orchestrating type 2 cytokine production during allergic inflammation [63]. However, regulatory mechanisms exist to control Th2-mediated inflammation. Treg can directly and indirectly dampen Th2 induction and cytokine secretion, as well as induce and maintain tolerance to allergens [64]. A defective Treg function is believed to contribute to allergic response. Further, Th2 polarization is also under epigenetic regulation, and reduction in levels of certain miRNAs can diminish Th2 responses and polarize toward the Th1 responses [65,66].

Along with Th2 cytokine production, IgE secretion is an integral component of type 2 immunity. Th2 cells interact with cognate B cells in lymphoid tissues and, via MHC class II

recognition and CD40 ligation, and IL-4 and IL-13 secretion, induce B cells to undergo class-switching to IgE and differentiation, leading to secretion of IgE [67]. These high-affinity, allergen-specific IgE are distributed locally at the affected anatomical sites and systemically via circulation. After reaching the interstitial fluid, allergen–IgE complexes bind to and activate the high-affinity IgE receptor FcεRI on tissue-resident mast cells, sensitizing them to release immune mediators in an allergen-specific manner. Subsequent allergen exposure leads to crosslinking, stabilization and aggregation of IgE–FcεRI complexes on mast cells and basophils, causing their immediate degranulation of preformed mediators, including histamines, serglycin proteoglycans and serine proteases [68]. This early-phase reaction, via the h1 histamine receptor, causes vasodilation, bronchoconstriction, nerve end stimulation, mucus hypersecretion, which clinically appear as rhinitis, asthma, cough and urticaria [57]. Mast cells and basophils continue to contribute to late-phase reactions, releasing newly synthesized cytokines, grow factors and chemokines that sustain inflammation and recruitment of other cells, such as T cells, neutrophils, eosinophils, basophils and monocytes, which also contribute to the inflammatory response [69].

Persistent allergen exposure results, therefore, in chronic allergic inflammation and recruitment of innate and adaptive immune cells. The persistent inflammation leads to interactions among immune and structural cells, including vascular endothelial cells, epithelial cells, smooth muscle cells, fibroblasts and nerve cells, which result in the complex organ dysfunction. For example, chronic asthma can result in airway remodeling involving all layers of the airway wall, characterized by goblet cell hyperplasia and increased mucus production, smooth muscle hyperplasia, subepithelial fibrosis, increased vascularity and impaired epithelial barrier function [68–70]. Many of the characteristics of airway remodeling are driven in part by Th2-mediated inflammatory stimuli, involving eosinophils, mast cells and Th2 cells. Repeated epithelial injury and repair responses result in interactions between epithelial cells and mesenchymal cells form an epithelial–mesenchymal trophic unit that sustains Th2 responses and sensitization to additional allergens [71]. These events worsen allergic symptoms, such as mucus secretion, bronchoconstriction, cough, rhinorrhea and itching [57]. Although airway remodeling is just one example, the underlying mechanisms may be broadly applicable to other chronic AD. However, studying chronic AD in humans is problematic and much of our current knowledge is based on mouse models that do not always correlate with clinical findings.

Advances in understanding the immunopathogenic mechanisms of AD constantly emphasized the direct and indirect role of immune cells in mediating pathogenesis. Indeed, much is missing in determining the exact mechanisms, particularly regarding allergic sensitization and the temporary or permanent effects of chronic AD.

Allergen-specific immunotherapy: how does it work?

AIT consists in the administration of increasing doses of the sensitizing allergen, to reduce symptoms and medication use in patients with allergic respiratory disorders or hymenoptera venom allergy. AIT induces a tolerance by acting on immunological mechanisms of allergic inflammation. The efficacy is well documented in patients with venom hymenoptera allergy, asthma and rhinoconjuntivitis to inhalant allergens [72]. Immunotherapy has been also experimentally used in the treatment of food allergy with promising results [6].

The choice of the allergen for immunotherapy is made according to clinical history and symptoms, and increased specific IgE. The allergen products available may be either crude extracts or vaccines adapted chemically and/or by absorption on different carriers: aqueous, depot and modified vaccines, mixtures of allergen vaccines. Where possible, standardized vaccines of known potency and preservation should be used. Standardization allows definition of the 'potency' of allergenic extracts and warrants that the batches of vaccine produced from different lots of raw material have comparable activities [73–75].

Moreover, to enhance the Treg activity, new formulations are available, including allergoids, adjuvants (monophosphoryl lipid A, toll-like receptor [TLR] ligands, living or heat-killed bacteria, small molecules such as the active 1,25-dihydroxy vitamin D3) and more recently vector systems (liposomes, virosomes, D, L-lactic-co-glycolic acid or immunostimulating complexes) [76]. To further improve tolerability and low-volume application via subcutaneous route, a short course with allergoid immunotherapy seems promising [77].

The main routes of administration routinely used are SCIT, SLIT and OIT. SCIT is currently used for the treatment of insect venom allergy, allergic asthma and allergic rhinitis; SLIT is used for respiratory allergy caused by inhalants and recently for food allergy and OIT provides a new therapeutic approach in the treatment of food allergy.

The routes of administration may be chosen according to the allergen involved (hymenoptera venoms treatment is available only with SCIT), vaccine(s) approval by regulatory agencies or ethics committees, age of patient, cost/benefit, handling routes, severity of reported side effects and patient's preference. The ideal candidate to AIT should be carefully selected, assessing compliance and ability to communicate with the physician, considering symptoms score, response to avoidance measures, medication use and side effects of drug treatments. In addition, patients with poorly controlled asthma should not receive immunotherapy before achieving an improvement in lung function with pharmacological treatment [71].

AIT promotes the development of specific immune tolerance modifying both humoral and cellular responses to the allergen. The whole process takes place in two phases: early desensitization and T-cell tolerance induction.

Early desensitization is characterized by decrease in mast cell and basophil activity, degranulation and systemic anaphylaxis. These early events involve APCs such as DCs and B cells and can be due to the action of adjuvants or 3D structure intact allergens.

In fact, APCs among which DCs are recruited in the first contact between immune system and allergens, modulate, in

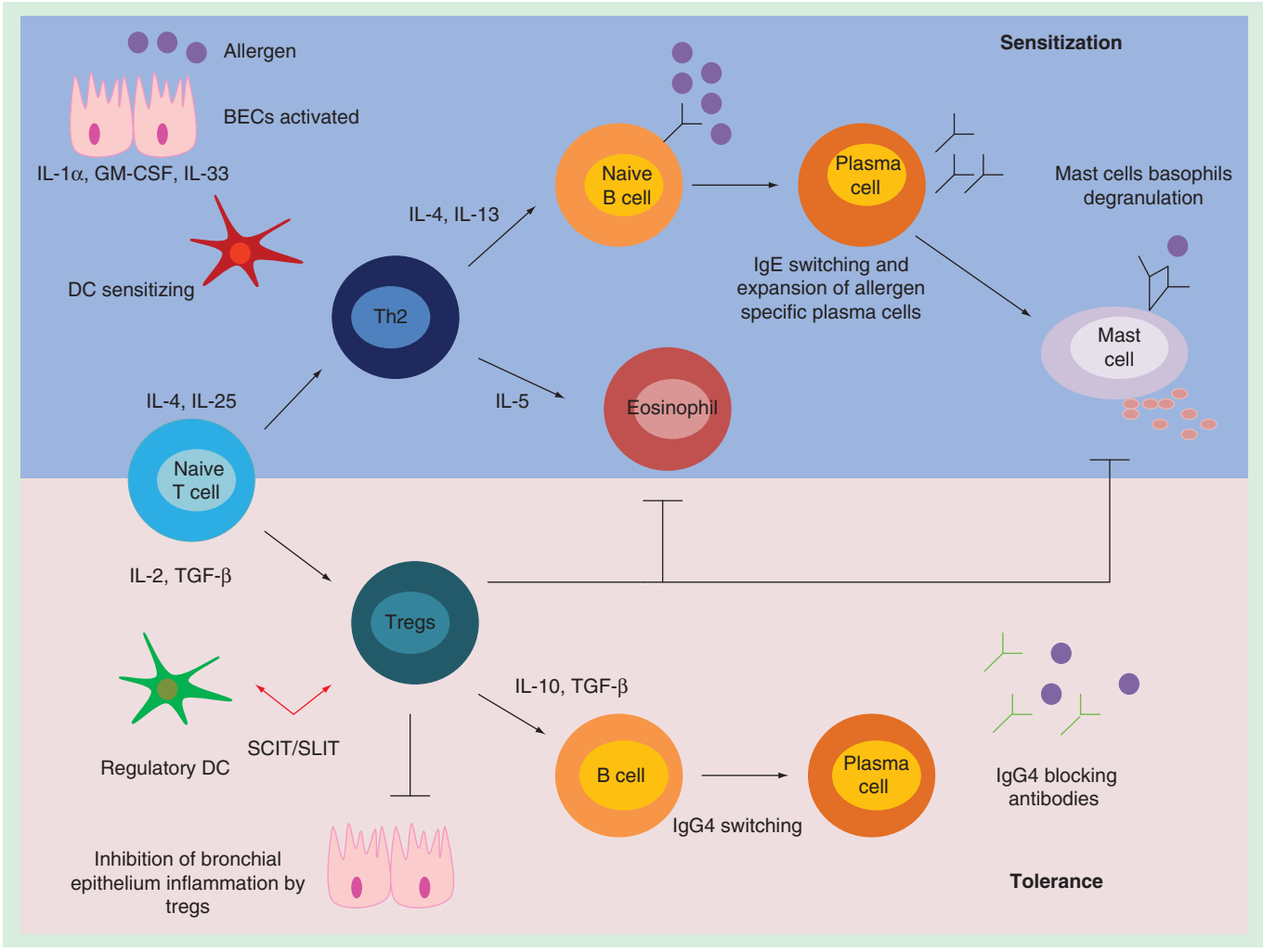

Figure 2. Immunologic mechanisms underlying sensitization and tolerance.
B cells, that is, plasma cells are stimulated by allergen(s) to produce specific IgE antibodies either directly or by allergen(s) processed by antigen-presenting cells: dendritic cells and bronchial epithelial cells .These antibodies bind to receptors on mast cells and basophils following the release of pharmacologically vasoactive amines and eosinophils activation.
Proposed mechanisms of action of immunotherapy for both subcutaneous immunotherapy and sublingual immunotherapy: induction of Treg; production of immunosuppressive IL-10 and TGF-β cytokines to downregulate antigen processing by dendritic cells. Immunomodulation of target cells, B cells, mast cells/basophils and modulation of immunoglobulin isotypes, with downregulation of IgE production by the production of IgG4, which are 'blocking antibodies'.

the healthy, the development of Th1, Th2 and Tregs and favor a state of immunologic tolerance. AIT seems to prevent the onset of new allergic disease by acting on DCs function and expression of costimulatory molecules on their surface [76]. Histamine receptors also play a key role on immune regulator system: HR type 2 and type 4 are involved in eosinophils, basophils and mast cells recruitment in early/late desensitization phases and enhancement of Treg expression [78].

After a short interval (days/weeks) from starting immunotherapy, a second phase takes place: increase in $CD4^+CD25^+$ Treg secreting IL-10 and TGF-β (Figure 2).

In literature, five subsets of Treg are described: naïve $CD4^+CD25^+Foxp3^+$ (nTregs), induced $CD4^+Foxp3^+$ (iTregs), type 1 IL-10 secreting Tregs (Tr1), which includes Th3 secreting TGF-β and IL-1, $CD8^+$ Treg and IL-17-producing $Foxp3^+$ Treg. But other cellular clusters are involved in tolerance induction such as: DCs, IL-10 secreting B cells (Bregs), NK cells and resident tissue cells.

Treg perform their immunomodulatory function inhibiting DCs, effector T cells (Th1, Th2 and Th17), basophils, mast cells, eosinophils, IgE production and promoting IgG and/or IgA secretion. In particular, nTregs seem to be already present in the thymus of the newborn and their function develop over time with the increasing thymic Foxp3 mRNA expression in nonatopic subjects, instead atopic children show a delay in Treg maturation [79,80]. Moreover in the periphery, foreign antigens enhance iTregs to express Foxp3 and their presence at an early age seems to prevent asthma. AIT induces a reduction in allergen-stimulated proliferation of peripheral blood mononuclear cell as it has been shown in a study where Treg secreting

IL-10 and TGF-β increase after 70 days of SCIT [81]. The secretion of IL-10 produces suppression of proliferation and release of proinflammatory cytokines by T lymphocytes, also inhibiting IgE and promoting IgG4 production while TGF-β enhances switching to IgA [71].

In addition to Treg, B lymphocytes also have a subset with regulatory phenotype (Bregs), which secrete inhibitory cytokine and express Foxp3. The Breg subtypes include Br1 and Br3 secreting, respectively IL-10 and TGF-β and exert their function in early stage by inducing Treg recruitment.

AIT also affects specific humoral responses, inducing a shift in allergen-specific IgE production to specific IgG4. However, during the early desensitization phase there is an increase of both allergen-specific IgE and allergen-specific IgG4 and IgG1 followed by gradual decrease of the ratio-specific IgE/specific IgG4 since 6 months to 3 years after beginning of SIT [82].

Allergen-specific IgG4 seem to inhibit the allergen binding and crosslinking of IgE on the surface FcεRI receptors of mast cells and basophils, thus preventing activation and release of mediators. However, allergen-specific IgG4 activity is not strictly related with serum concentration. So, it is important to define the blocking activity of allergen-specific IgG4 rather than its serum level [83]. As previously mentioned, specific IgA also increases as demonstrated in several studies showing a correlation between IgA rise and TGF-β secretion and between IgG4 increase and IL-10 secretion in peripheral mucosal response to allergens both in healthy conditions and during AIT [84,85]. The key feature of allergen-specific IgA is related to the induction of IL-10 secretion by monocytes [71].

Nevertheless, the long-term efficacy of AIT is not fully related to IgG4 concentration, since some studies reported an 80% IgG4 reduction 2 years after stopping the treatment with persistence of the clinical effects [86]. In addition, hypoallergenic Derp1/Dpr2 fragments combination vaccines for immunotherapies may induce allergen-specific IgG increase in animal models [87]. These variations of humoral response during AIT are reached both with subcutaneous and sublingual routes, even if SLIT seems to induce less systemic changes than SCIT, but there are additional local mechanisms in the oral mucosa and/or regional lymph nodes equally important [25]. In fact, the oral mucosa provides high permeability and an environment with a distinct set of APCs and a low number of inflammatory cells [88]. Moreover, AIT efficacy is associated with reduction of the immediate response to allergen exposure and the late phase reaction in respiratory tract and skin. In this context, the immunodeviation with proliferation of Th1-type T lymphocytes and increasing mRNA for Th1-type cytokines was reported with grass-pollen immunotherapy [89,90]. Although clinical desensitization and immune modulation have been demonstrated with OIT, the strength of the current evidence from clinical trials is insufficient about the induction of tolerance [91]. Recently, an increase of antigen-induced Treg was claimed during peanut OIT [92].

Concerning allergic respiratory disorders, a meta-analysis of 20 published prospective studies showed that AIT is effective in the treatment of asthma both in adults and children [93]. Moreover, a meta-analysis of 16 studies involving 759 patients highlighted that SCIT is highly effective in the treatment of allergic rhinitis [94]. The persistence of benefit for years after discontinuation [95] and the possibility that AIT might reduce the progression to asthma [96] and prevent new sensitization [97] should broaden the indications for immunotherapy in the near future. These observations highlight the possibility of an early intervention in allergic children. Therefore, both long-lasting effects and preventive effects of AIT demonstrate its different 'therapeutic profile' in comparison with pharmacotherapy of allergic diseases.

In the last two decades, SLIT has emerged as a good alternative to SCIT with a more favorable safety profile, allowing the at-home administration of the vaccine(s). SLIT has been extensively used in Europe, due to a lower risk of severe systemic adverse reaction [98]. Several meta-analyses on SLIT, including four in allergic rhinitis and three in allergic asthma showed its efficacy in both diseases [99–101], while some open studies documented that SLIT can modify the natural history of allergic diseases and the progression from allergic rhinitis to asthma, when used in pediatric age [102]. In a study using grass pollen SLIT with a co-seasonal schedule of 3 years, the authors reported that children with rhinoconjunctivitis have a risk of asthma development 3.8-times greater in control than in treated group [103]. Another study, performed on 30 children with allergic rhinitis with or without intermittent asthma, randomized to pharmaceutical treatment only or plus SLIT for 3 years, showed that the incidence of mild asthma was significantly lower in the SLIT-treated arm with a decrease of methacholine reactivity [104]. However, there is a growing need to improve the quality of the studies because a CONSORT analysis on SCIT and SLIT trials highlighted that the published manuscripts available are often suboptimal [105]. The clinical efficacy of immunotherapy in patients with asthma and/or allergic rhinitis is usually evaluated by a simple and arbitrary symptom/medication score. These parameters are not univocal and largely variable. Markers or biomarkers are needed, in order to choose the proper immunotherapy treatment and for follow-up. At present, *in vivo* (early and late skin reaction) and *in vitro* (IgE, IgG subclasses, IgA mucosal lymphocyte subpopulations, cytokines and systemic inflammatory markers) have been proposed as potential markers [106].

Allergen-non-specific approaches

Anti-IgE

The pathogenesis of allergic diseases involves complex mechanisms that elicit unwanted immune responses. This section of the review focuses on allergen-non-specific immunotherapies being used to treat allergic diseases. Emphasis will be placed on the function of therapeutic agents for which there are clinical efficacy and safety data, with little discussion of animal studies.

Anti-IgE (omalizumab; Xolair) is a humanized monoclonal anti-IgE approved by the EMA and FDA for the treatment of severe asthma induced by perennial allergens. Its safety profile and efficacy in reducing IgE-mediated pathogenic responses has generated wide interest, and numerous Phase I–IV trials are

currently available for a wide variety of allergic conditions: allergic asthma, IgE-mediated food allergy, chronic urticaria. Omalizumab binds to soluble IgE, reduces their concentration and downregulates the high-affinity IgE receptor (FcεRI) on the surface of basophils, mast cells and DCs, attenuating antigen-specific IgE-mediated cellular responses and diminishing the release of several potent inflammatory mediators upon allergen encounter [107–109]. Thus, omalizumab acts to non-specifically dampen a wide variety of responses mediated by IgE-FcεRI signaling, leading to a reduction in Th2 cytokine production and related allergic inflammation [107,110,111].

For non-approved indications, early studies showed that omalizumab can effectively reduce the clinical symptoms in patients with moderate-to-severe asthma and seasonal allergic rhinitis [107,112]. These findings demonstrated how anti-IgE treatment can reduce allergic disease exacerbations and hospitalizations, increasing quality of life. Importantly, these findings promoted interest in using anti-IgE treatment in addition to AIT to decrease immunotherapy-related side effects and complications. An early trial focusing on the effectiveness of using omalizumab in addition to SCIT found that in patients with multiple allergic symptoms, a combination of omalizumab with SCIT improved their clinical symptoms, largely independent of whether the allergen was covered by SCIT [113]. These findings showed that anti-IgE treatment can markedly improve the safety profile of immunotherapy. Compellingly, anti-IgE therapy alone increased the threshold sensitivity of patients with peanut allergy and decreased the risk of anaphylaxis following accidental ingestion. While the study did not assess the role of anti-IgE therapy on actually desensitizing the patients to peanuts, it did display the ability of anti-IgE treatment to be effective for a range of allergies, and to possibly allow for rapid and higher threshold doses of allergen during immunotherapy [114].

In a Phase I study assessing the safety and clinical efficacy of using omalizumab with rush milk OIT, the advantages of combining anti-IgE treatment with OIT for the rapid and sustained desensitization of allergic subject were clearly demonstrated. Allergic subjects received omalizumab treatment prior to the rush phase, where increasing incremental doses of cow's milk protein were ingested every 30 min for 6 h, followed by 7–11 weeks of daily doses with weekly increases. Nine out of eleven patients successfully reached the maximal daily dose of milk with few reported adverse reactions, and by the end of the study they were able to tolerate large doses of milk in their diet [9]. This study showed that combining anti-IgE therapy with allergen-specific OIT can allow for rapid allergen desensitization without increased risk of allergic reactions. Further, a Phase I study has demonstrated that combined anti-IgE treatment and OIT to multiple food allergens simultaneously can rapidly, safely and effectively desensitize patients with multiple food allergies, an important finding as 30% of children with food allergies are reactive to more than one food allergen [115]. Combining anti-IgE treatment with OIT induces multiple immunological changes that promote desensitization, including reductions in allergen-specific Th2 cells and decreased basophil activation [116,117]. However, these immunological changes will require further study.

Cytokine & anti-cytokine therapy

Many cytokines produced by immune and structural cells contribute to the pathogenesis of allergic diseases, thus promoting studies in allergic diseases. IL-5 specifically regulates growth, differentiation, survival and influx of eosinophils. Thus, inhibition via anti-IL-5 is an attractive therapeutic agent in eosinophilic allergic diseases, including asthma and atopic dermatitis. Anti-IL-5 antibody therapy using mepolizimab, reslizumab and benralizumab have been used in several trials to improve the clinical symptoms of asthma and other eosinophilic diseases [118–126] with very favorable results.

IL-4 is produced by Th2 cells, eosinophils and mast cells, where it maintains the inflammatory response to allergens in several allergic diseases. Therapies inhibiting IL-4 alone have been problematic since both IL-4 and IL-13 signal through a receptor complex sharing IL-4Rα, leading to approaches blocking both IL-4 and IL-13 [127]. IL-13 is produced by activated Th2 cells, mast cells and DCs, and is similarly involved in the pathogenesis of several allergic diseases. Blockade of IL-4Rα with the anti-IL-4Rα antibody dupilumab or mutant IL-4 pitrakinra block both IL-4 and IL-13 signaling, successfully improving lung function and clinical symptoms of asthmatic subjects, though other therapies blocking both IL-4 and IL-13 have been ineffective [127–130]. IL-13 blocking treatments (lebrikizumab and tralokinumab) improved lung function and clinical symptoms of asthmatic subjects, but other anti-IL-13 therapies resulted ineffective [131–133].

Anti-IL-4 & IL-13

Modulation of Th2-mediated inflammatory cytokine signaling, specifically IL-4 and IL-13, is currently being explored as a therapy for allergic asthma. Efforts to treat asthma by targeting IL-4 began in the early 2001 with pascolizumab, a humanized anti-IL-4 monoclonal antibody that appeared promising in preclinical studies with cynomolgus monkeys [127,131–133]. However, Phase I and Phase II trials in mild-to-moderate asthmatic patients showed little clinical efficacy, and development was discontinued [108,109]. Preclinical studies demonstrated beneficial effects of monoclonal anti-IL-13 therapy, CAT-354 [127,131–133], leading to the development of multiple anti-IL-13 monoclonal antibodies: lebrikizumab, IMA-638 and tralokinumab [127,131–133]. Numerous Phase II trials have been conducted using these anti-IL-13 biologicals to treat various forms of asthma. Lebrikizumab interacted with serum periostin levels to improve lung function in patients with asthma uncontrolled by glucocorticoids [127,131–133], while tralokinumab improved lung function and reduced rescue β2-agonist use in patients with uncontrollable moderate-to-severe asthma, IMA-638 reduced IL-13 fluid responses induced by nasal allergen challenge, but had no significant effect on allergen-induced airway hyperresponsiveness or sputum eosinophils in patients with mild atopic asthma [127,131–133].

A large number of Phase II trials investigating these and other anti-IL-13 biologicals have been completed recently, and

published results of these studies are on the horizon. While many studies have investigated the effects of monoclonal blocking antibodies to either IL-4 or IL-13, recent efforts have focused on simultaneously targeting both IL-4 and IL-13 receptors through the common IL-4Rα subunit [127,131–133]. The first evidence that dual modulation of IL-4 and IL-13 receptor signaling could lead to clinical benefits in asthmatics came through altrakincept, a formulation of human soluble, recombinant IL-4Rα subunit [127,131–133]. Since then, a variety of strategies to inhibit IL-4/IL-13 signaling through blockade of the IL-4Rα subunit have been developed, including small molecule antagonists (AIR-645), mutated recombinant IL-4 (pitrakinra) and monoclonal antibodies (AMG 317 and dupilumab) [116–120]. AIR-645, an inhaled oligonucleotide antagonist, decreased sputum eosinophils and serum IgE in a small cohort of mild asthmatic patients [127,131–133]. In a large, 300-patient study, none of the three doses of humanized monoclonal antibody AMG-317 tested resulted in a significant reduction in asthma control questionnaire score [117]. Two parallel clinical studies demonstrated that subcutaneous administration of pitrakinra, a soluble, mutated variant of IL-4, improved lung function and decreased events requiring β2-agonist rescue in atopic asthmatics [118]. Weekly administration of the anti-IL-4Rα monoclonal antibody dupilumab led to significant improvements in asthma control and lung function while decreasing biomarkers of Th2-inflammation in moderate-to-severe asthmatic patients [127,131–133]. Although none of these therapies is currently FDA approved, these results are exciting and suggest that modulation of IL-4/IL-13 signaling may be clinically effective in a wide range of conditions associated with Th2-driven inflammation.

Thymic stromal lymphopoietin (TSLP) is secreted by epithelial cells and functions early in allergic responses by promoting Th2-mediated inflammation through signaling in both hematopoietic and non-hematopoietic cell lineages. Research work by many laboratories, including Ziegler's laboratory at the Benaroya Institute, has laid the groundwork for important clinical applications. Inhibition of TSLP with anti-TSLP antibody, AMG-157, is a promising therapy that improved lung function and decreased eosinophil influx in subjects with atopic asthma [134].

CRTH2 antagonists

The chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) recognizes prostaglandin D_2 and is expressed on Th2 cells, where its activation plays a significant role in the pathogenesis of Th2-mediated allergic responses by directing Th2 and eosinophils to sites of allergic inflammation. Treatment with small molecules CRTH2 antagonists like OC000459 and ACT-129968 could improve the clinical symptoms of eosinophilic esophagitis, mild asthma and rhinoconjunctivitis [135–138]. However, some CRTH2 were ineffective, and others are still in clinical trials with no published results of efficacy [139,140].

Herbal medicine & immunomodulants

Chinese herbal medicine

Food allergy herbal formula-2 (FAHF-2) is a combination of herbs used in Chinese traditional medicine for treatment of atopic asthma, rhinitis, dermatitis and food allergy. FAHF-2 treatment inhibited peanut anaphylaxis in mice and Phase I clinical trials have shown some association with beneficial immunomodulatory effects. The mechanisms of FAHF-2 are unclear, but it may suppress Th2 responses and promote Th1 responses and decrease basophil activation [141,142]. Phase II clinical trials are currently ongoing to investigate the efficacy of this product [142].

Toll-like receptor agonists & natural polymers

Stimulation of TLR9 with unmethylated CpG sequences, TLR7 with single-stranded RNA or TLR4 with lipopolysaccharides derivatives promotes a Th1-polarizing environment, which has been shown to suppress Th2 activity in allergic disease [143,144]. SCIT with a CpG-containing DNA sequence conjugated to ragweed allergen significantly reduced allergic responses in subjects [145]. Treatment of subjects with allergic asthma or rhinoconjunctivitis with TLR9 agonist QbG10, a virus-like particle containing a CpG motif, improved their clinical symptoms and lung function [146,147]. Agonists of TLR7 have also shown promise and are being prepared for clinical trials for treatment of asthma [148–150]. Using TLR4 agonist monophosphoryl lipid A, a lipopolysaccharide derivative, as an adjuvant immunotherapy has successfully improved the clinical symptoms of allergic rhinoconjunctivitis in subjects undergoing immunotherapy for grass pollen and ragweed [148,151–153].

In conclusion, future research for allergen-non-specific immunotherapies will continue to focus of increasing their clinical efficacy and safety. Especially for safety, more trials focused on side effects are awaited. Anti-IgE, anti-cytokine treatments, TLR agonists, chitin, CRTH2 agonists and Chinese herbal medicine could allow for safer and more effective immunotherapy. Our hope is that the ideal allergen-non-specific immunotherapy will not only be cost-effective and clinically efficacious, but also induce sustained tolerance in patients afflicted with allergic diseases. Finally, the more and more detailed characterization of some diseases, asthma in particular, are introducing in parallel to the concept of phenotype (clinical, biological, cytological), the more profound will be the aspect of endotype. This virtually encompasses all clinical, cytological, until genic aspects and would be the premise for an endotype-oriented therapy [154].

Expert commentary & five-year view

AIT started as a simple 'vaccination' against generic toxins. When the mechanisms of IgE-mediated reactions became more and more clear, new perspectives emerged, mainly in the recent years. Initially, AIT was used on an empirical basis only for seasonal allergies, then its therapeutic potential was also recognized and extended to hymenoptera venom allergy. Afterward, the immunological mechanisms of allergy were detailed, so that the use of AIT could be expanded. The earliest controlled and randomized trials achieved a scientifically based context. During the mid-1980s, SLIT was introduced in clinical practice. Since then, new achievements were made on: mechanisms of action, identification of the appropriate doses to be used, subsequent amelioration of the extracts and administration and, finally the

attempts to develop new formulations. It remains a mystery why such a well-ascertained treatment still remains 'on the leash' of randomized controlled trials. The new efforts should be made on OIT in order to overcome several issues: severity and type of food allergy responsive to specific immunotherapy, degree of protection, shared schedules for desensitization in research settings and well-established risk:benefit ratio.

Allergen-non-specific immunotherapy, mostly with omalizumab, was effective for the treatment of patients with refractory asthma, severe IgE-mediated food allergy and chronic urticaria. Omalizumab in combination with SCIT, or SLIT, or OIT, may increase either the efficacy or the safety of immunotherapy alone in patients with severe allergic disorders.

Altogether, anti-IgE, anti-cytokine treatments, Chinese herbal medicine and other allergen-non-specific therapies could offer new approaches for the treatment of allergic diseases: data are encouraging, however, further studies are needed concerning both efficacy and safety.

Currently, AIT in different routes (SCIT or SLIT) represent an effective therapeutic approach in patients with IgE-mediated respiratory disorders. Moreover, in allergic disorders other than respiratory, evidence is emerging that these diseases also could respond to AIT (Figure 3). Another relevant question is the impact of the early intervention in allergic children. A careful selection of children to whom prescribing AIT still remains an aspect to be faced, with the awareness that early use of AIT can alter the natural history of the allergic diseases by suppressing airway inflammation at a time when a child is only intermittently symptomatic. Relief of allergic symptoms and long-lasting efficacy are two goals that can be attained in allergic patients treated with AIT. These effects are of particular relevance in childhood when bronchial asthma is largely not severe, and children often show one or few sensitizations. Allergy immunotherapy or allergy vaccination is a time-honored treatment, which currently expands its role to the wide spectrum of IgE-mediated disorders. Also, it must be considered that molecular diagnosis is now a reality [41], and there is room for a more detailed diagnosis and prescription of AIT. Nonetheless, due to regulatory and technical problems, the use of purified/recombinant molecules for AIT remains a 'distant' prospective [53].

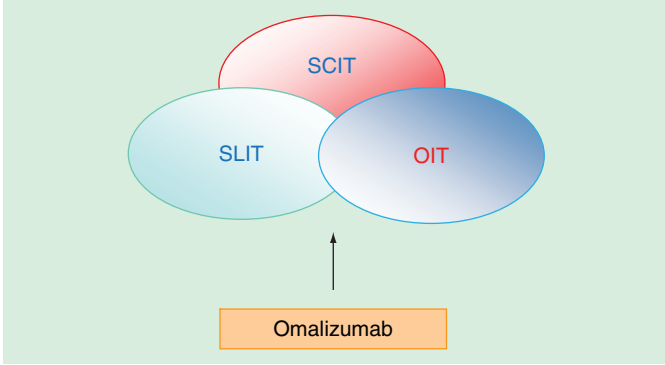

Figure 3. The main routes and forms of immunotherapy.
Due to the complexity of IgE-mediated disorders, the triad could be considered as complementary therapy. Thus, SCIT, SLIT and OIT could be used in various combinations and timing in order to optimize both adherence to treatment and therapeutic effects. Omalizumab in combination with SCIT, SLIT or OIT may increase either the efficacy or safety of immunotherapy in patients with severe allergic disorders.
OIT: Oral immunotherapy for the active treatment of IgE-mediated food allergy; SCIT: Subcutaneous immunotherapy for the treatment of allergic asthma and allergic rhinitis; SLIT: Sublingual immunotherapy for the treatment respiratory allergies and IgE-mediated food allergies.

Therefore, the future is represented by the attempt to encourage the development of new therapeutic strategies in the quest for disease-modifying treatment option for IgE-mediated allergies, with the goal to implement new evidence practice guidelines. In our opinion, after 104 immunotherapy's history it seems to meet the patients' expectation for long-lasting relief by allergic symptoms.

Financial & competing interests disclosure

GB Pajno is a Board Member of the Paediatric Section of the European Academy of Allergy and Clinical Immunology, and received fees for this. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Both subcutaneous immunotherapy and sublingual immunotherapy are the standard delivery methods in allergen-specific immunotherapy.
- Oral immunotherapy represents the new active treatment for IgE-mediated food allergy. It could be performed in selected medical centers and under strict medical supervision.
- Treg perform their immunomodulatory function inhibiting dendritic cells, effector T cells (Th1, Th2, Th17), basophils, mast cells, eosinophils. Treg play a pivotal role in mechanism(s) of action of specific immunotherapy.
- Non-specific immunotherapy, mostly with anti-IgE antibody, may change in the future, the therapeutic approach for severe allergic disorders.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Burks AW, Calderon MA, Casale T, et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. J Allergy Clin Immunol 2013;131(5):1288-96
2. Calderon MA, Gerth van Wijk R, Eichler I, et al. Perspectives on allergen –specific immunotherapy in childhood: an EAACI position statement. Pediatr Allergy Immunol 2012;23(4):300-6
3. Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis. and eczema in childhood :ISAAC Phases One and Three repeat multicountry cross-sectional surveys. ISAAC Phase Three Study Group. Lancet 2006;368(9537):733-43
4. Muraro A, Werfel T, Hoffmann Sommergruber K, et al. EAACI Food. Allergy and Anaphylaxis Guidelines: diagnosis and management of food Allergy. 2014;69(8):1008-25
5. Calderon MA, Demoly P, Gerth van Wijk R, et al. EAACI: a European Declaration on Immunotherapy. Designing the future of allergen Specific immunotherapy. Clin Trans. Allergy 2012; 2(1):20
6. Pajno GB. Oral desensitization for milk allergy in children: state of the art. Curr. Opin. Allergy Clin. Immunol 2011;11(6): 560-4
7. Pajno GB, Cox L, Caminiti L. Ramistella V, Crisafulli G. Oral Immunotherapy for Treatment of Immunoglobulin E-Mediated Food Allergy: the Transition to Clinical Practice. Pediatr. Allergy Immunol. Pulmonol 2014;27(2):42-50

• **A comprehensive review of the current possibilities on the transition of oral immunotherapy (OIT) into clinical practice.**

8. Normansell R, Walker S, Milan SJ, et al. Omalizumab for asthma in adults and children. Cochrane Database Syst Rev 2014;1:CD003559
9. Nadeau KC, Schneider LC, Hoyte L, et al. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. J Allergy Clin Immunol 2011;127(6):1622-4

• **The study demonstrates that anti-IgE antibody therapy could be used in combination with OIT for active treatment of IgE-mediated food allergy.**

10. Kaplan AP. Biologic agents in the treatment of urticaria. Curr Allergy Asthma Rep 2012; 12(4):288-91
11. Noon L. Prophylactic inoculation against hay fever. Lancet 1911;1:1572
12. Ogawa M, Kochwa S, Smith C, et al. Clinical aspects of IgE myeloma. N Engl J Med 1969;281(22):1217-20
13. Lowell FC, Franklin WA. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. N Engl J Med. 1965;273(13):675-9
14. Bukantz SC, Bagg AS, Lockey RF. Adverse effects and fatalities associated with subcutaneous allergen immunotherapy. Clin Allergy Immunol 2008;21:455-68
15. Windom HH, Lockey RF. An update on the safety of specific immunotherapy. Curr Opin Allergy Clin Immunol 2008;8(6): 571-6
16. Aaronson DW, Gandhi TK. Incorrect allergy injections: allergists' experiences and recommendations for prevention. J Allergy Clin Immunol 2004;113(6):1117-21
17. Committee on the safety of Medicine: desensitizing of vaccine. BMJ 1989;293:948
18. Passalacqua G, Canonica GW. Local nasal specific immunotherapy for allergic rhinitis. Allergy Asthma Clin Immunol 2006; 15:2(3):117-23
19. Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. J Allergy Clin Immunol 2003;111(3):437-48
20. Scadding K, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to dust mite. Clin Allergy 1986;16:483-91
21. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy : therapeutical vaccines for allergic diseases. A WHO position paper Allergy 1998;102(4pt1):558-62
22. Bousquet J, Van Cauwenberge P, Khaltaev N. Aria Workshop Group; World Health Organization. Allergic Rhinitis and its Impact on Asthma. J Allergy Clin Immunol 2001;108(5 suppl):S146-50
23. 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
24. Canonica GW, Bousquet J, Casale T, et al. World Allergy Organization position paper on sublingual immunotherapy. Allergy 2009;64(Suppl 91):1-59

• **The first position paper focused on sublingual immunotherapy.**

25. Canonica GW, Cox L, Pawankar R, et al. Sublingual immunotherapy: world Allergy Organization position paper 2013 update. World Allergy Organ 2014;7(1):6
26. Cox LS, Linnemann DL, Nolte H, et al. Sublingual immunotherapy: a comprehensive review. J Allergy Clin Immunol 2006;117(5):1021-35
27. Cox L, Aaronson D, Casale TB, et al. Allergy Immunotherapy Safety :location Matters! J Allergy Clin Immunol Pract. 2013;1(5):455-7
28. Nelson HS. Sublingual immunotherapy: the U.S. experience. Curr Opin Allergy Clin Immunol 2013;13(6):663-8
29. Nelson HS. Specific immunotherapy with allergen mixes: what is the evidence? Curr Opin Allergy Clin Immunol 2009;9(6): 549-53
30. Passalacqua G. The use of single versus multiple antigens in specific allergen immunotherapy for allergic rhinitis: review of the evidence. Curr Opin Allergy Clin Immunol 2014;14(1):20-4
31. Passalacqua G. Specific immunotherapy in asthma: a comprehensive review. J Asthma 2014;51(1):29-33
32. Cox L, Jacobsen L. Comparison of allergen immunotherapy practice patterns in the United States and Europe. Ann Allergy Asthma Immunol. 2009;103(6):451-9
33. Baena Cagnani CE, Larenas Linemann D, Sisul C, et al. Allergy training and immunotherapy in Latin American: results of a regional overview. Ann Allergy Asthma Immunol 2013;111(5):415-19
34. Passalacqua G, Baena Cagnani C, Bousquet J, et al. Grading local side effects of sublingual immunotherapy for respiratory allergy: speaking the same language. J Allergy Clin Immunol 2013;132(1):93-8
35. Larenas Linneman D, Lockey RF, Passalacqua G. Speaking a common language in immunotherapy. WAO grading of systemic reactions. J Allergy Clin Immunol 2010;125(3):569-74
36. Casale T, Canonica GW, Bousquet J, et al. Recommendations for Appropriate Sublingual Immunotherapy (SLIT) Clinical Trials. J Allergy Clin Immunol 2009; 124(4):665-70
37. Canonica GW, Baena Cagnani C, Bousquet J, et al. Recommendations for

standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. Allergy 2007;62(3):317-24

38. Pfaar O, Demoly P, Gerth van Wijk R, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. Allergy 2014;69(7):854-67
39. Passalacqua G, Baiardini I, Senna G, Canonica GW. Adherence to pharmacological treatment and specific immunotherapy in allergic rhinitis. Clin Exp Allergy 2013;43(1):22-8
40. Pajno GB, Caminiti L, Passalacqua G. Changing the route of immunotherapy administration: an 18-year survey in pediatric patients with allergic rhinitis and asthma. Allergy Asthma Proc 2013;34(6): 523-6
41. Canonica GW, Ansotegui IJ, Pawankar R, et al. A WAO-ARIA-GA2LEN consensus document on molecular-based Allergy diagnostics. World Allergy Organ J 2013; 6(1):17
42. Canonica GW, Passalacqua G. Disease modifying effect and economic implications of Sublingual immunotherapy. J Allergy Clin Immunol 2011;127(1):44-5
43. Soyer OU, Akdis M, Ring J, et al. Mechanisms of peripheral tolerance to allergens. Allergy 2013;68(2):161-70
44. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol 2014;133(3):621-31

- **This review depicted the immune mechanisms of tolerance with regard to allergen immunotherapy.**

45. Passalacqua G, Landi M, Pajno GB. Oral immunotherapy for cow's milk allergy. Curr Opin Allergy Clin Immunol 2012;12(3): 271-7
46. Pajno GB, Caminiti L, Crisafulli G, Salzano G. Recent advances in immunotherapy: the active treatment of food allergy on the horizon. Expert Rev Clin Immunol 2012;9(10):891-3
47. Kostadinova AI, Willemsen LE, Knippels LM, Garssen J. Immunotherapy-risk/benefit in food allergy. Pediatr Allergy Immunol 2013;24(7):633-44
48. Mansfield LE. Oral immunotherapy for peanut allergy in clinical practice is ready. Allergy Asthma Proc 2013;34:3-205-9
49. Sun J, Hui X, Ying W, et al. Efficacy of allergen-specific immunotherapy for peanut allergy: a meta-analysis of randomized controlled trials. Allergy Asthma Proc 2014; 35(2):171-7
50. Kulis M, Burks WA. A Oral immunotherapy for food allergy: clinical and preclinical studies. Adv Drug Deliv Rev 2013;65(6):774-81
51. Fernández-Rivas M, Garrido Fernández S, Nadal JA, et al. Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p 3 quantified peach extract. Allergy 2009;64((6)):876-83
52. Enrique E, Malek T, Pineda F, et al. Sublingual immunotherapy for hazelnut food allergy: a follow-up study. Ann Allergy Asthma Immunol 2008;100(3):283-4
53. Cromwell O, Häfner D, Nandy A. Recombinant allergens for specific immunotherapy. J Allergy Clin Immunol 2011;127(4):865-72
54. 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(5):1290-6
55. von Moos S, Kündig TM, Senti G. Novel administration routes for allergen-specific immunotherapy: a review of intralymphatic and epicutaneous allergen-specific immunotherapy. Immunol Allergy Clin North Am 2011;31(2):391-406
56. 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(1):128-35
57. Kumar Y, Bhatia A. Immunopathogenesis of Allergic Disorders: current Concepts Disclosures. Expert Rev Clin Immunol 2013;9(3):211-16
58. Wang J, Sampson HA. Food allergy: recent advances in pathophysiology and treatment. Allergy Asthma Immunol Res 2009;1(1): 19-29
59. Bieber T. Atopic dermatitis2.0: from the clinical phenotype to the molecular taxonomy and stratified medicine. Allergy 2012;67(12):1475-82
60. Holgate ST. Innate and adaptive immune responses in asthma. Nat Med 2012;18(5): 673-83
61. Lambrecht B, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. J Allergy Clin Immunol 2014;134:499-507
62. Licona-Limon P, Kim LK, Palm NW, Flavell RA. TH2, allergy and group 2 innate lymphoid cells. Nat Immunol 2013;14(6):536-42
63. Walker JA, Mckenzie AN. Development and function of group 2 innate lymphoid Cells. Curr Opin Immunol 2013;25(2): 148-55
64. Pellerin L, Jenks JA, Bégin P, et al. Regulatory T cells and their roles in immune dysregulation and allergy. Immunol Res 2014;58(2-3):358-68
65. Lu TX, Hartner J, Lim EJ, et al. MicroRNA-21 limits in vivo immune response-mediated activation of the IL-12/ IFN-γ pathway, Th1 polarization, and the severity of delayed-type hypersensitivity. J Immunol 2011;187(6):3362-73
66. Mattes J, Collison A, Plank M, et al. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. Proc Natl Acad Sci 2009;106(44):18704-9
67. Dullaers M, De Bruyne R, Ramadani F, et al. The who, where, and when of IgE in allergic airway disease. J Allergy Clin Immunol 2012;129(3):635-45
68. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. Nature 2008;454(7203):445-54
69. Deckers J, Branco Madeira F, Hammad H. Innate immune cells in asthma. Trends Immunol 2013;34(11):540-7
70. Shifren A, Witt C, Christie C, Castro M. Mechanisms of Remodeling in Asthmatic Airways. Journal of Allergy 2012;2012:316049
71. Holgate ST. Epithelium dysfunction in asthma. J Allergy Clin Immunol 2007; 120(6):1233-44
72. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. J Allergy Clin Immunol 2011; 127(1 suppl):S1-55
73. Bousquet J, Lockey R, Malling HJ. Allergen Immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol 1998;102(4 pt 1): 558-62
74. The use of standardized allergen extracts. American Academy of Allergy, Asthma and Immunology (AAAAI). J Allergy Clin Immunol 1997;99:583-6
75. Nelson HS. The use of standardized extracts in allergen immunotherapy. J Allergy Clin Immunol 2000;106(1pt 1):41-5

76. Moingeon P. Adjuvants for allergy vaccines. Hum Vaccin Immunother 2012;8(10): 1492-8

77. Rosewich M, Lee D, Zielen S. Pollinex Quattro An innovative four injections immunotherapy in allergic rhinitis. Human Vacc Immunother 2013;9(7):1523-31

78. Ito T, Yang M, Wang YH, et al. Plasmocytoid dendritic cells prime IL-producing T regulatory cells by inducible costimulator ligand. J Exp Med 2007; 204(1):105-15

79. Ozdemir C, Kucuksezer UC, Akdis M, Akdis CA. Specific immunotherapy and turning off the T cell: how does it work? Ann Allergy Asthma Immunol 2011;107(5): 381-92

80. Zhang H, Kong H, Zeng X, et al. Subsets of regulatory T cells and their roles in allergy. J Transl Med 2014;12:125

81. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33(5):1205-14

82. Horak F, Zieglmayer P, Zieglmayer R, et al. Early onset of action of a 5-grass pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. J Allergy Clin Immunol 2009;124(3):471-7

83. Akkoc T, Akdis M, Akdis CA. Update in the mechanisms of allergen-specific immunotheraphy. Allergy Asthma Immunol Res 2011;3(1):11-20

84. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33(5):1205-14

85. Meiler F, Zumkehr J, Klunker S, et al. In vivo switch to IL- 10-secreting T regulatory cells in high dose allergen exposure. J Exp Med 2008;205(12):2887-98

86. Bahceciler NN, Arikan C, Taylor A, et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. Int Arch Allergy Immunol 2005;136(3):287-94

87. Chen KW, Blatt K, Thomas WR, et al. Hypollergenic Der p1/Der p2 combination vaccines for immunotherapy of house dust mite allergy. J Allergy Clin Immunol 2012; 130(2):453-43

88. Marcucci F, Incorvaia C, Sensi L, et al. Lack of inflammatory cells in the oral mucosa of subjects undergoing sublingual immunotherapy. Int J Immunopathol Pharmacol 2008;21(3):609-13

89. Varney VA, Hamid QA, Gaga M, et al. Influence of grass Pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. J Clin Invest 1993;92(2):644-51

90. Hamid QA, Schotman E, Jacobson MR, et al. Increases in IL-12 messenger RNA +cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. J Allergy Clin Immunol 1997;99(2):254-60

91. Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. J Allergy Clin Immunol 2011;127(3):576-86

92. Syed A, Garcia MA, Lyu SC, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). J Allergy Clin Immunol 2014;133(2):500-10

93. Abramson MJ, Puv RM, Weiner JM. Is Allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. Am J Respir Crit Care Med 1995;151(4):969-74

94. Ross RN, Nelson HS, Finegold I. Effectiveness of specific immunotherapy in the treatment of allergic rhinitis: an analysis of randomized, prospective, single- or double- blind, placebo-controlled studies. Clin Ther 2000;22(3):342-50

95. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999; 341(7):468-75

- **This important study provides the evidence that allergen immunotherapy maintains efficacy after discontinuation of treatment.**

96. Jacobsen L, Niggemann B, Dreborg S, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma :10-year follow-up on the PAT study. Allergy 2007;62(8):943-8

97. Pajno GB, Barberio G, De Luca F, et al. Prevention of new sensitization in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 2001; 31(9):1392-7

98. Walker SM, Durham SR, Till SJ, et al. Immunotherapy for allergic rhinitis. Clin Exp Allergy 2011;41(9):1177-200

99. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;60(1):4-12

100. Radulovic S, Wilson D, Calderon M, Durham S. Systematic reviews of sublingual immunotherapy (SLIT). Allergy 2011;66(6): 740-52

101. Calderon MA, Penagos A, Sheikh A, et al. Sublingual immunotherapy for allergic conjunctivitis: Cochrane systematic review and meta-analysis. Clin Exp Allerg 2011; 41(9):1263-72

102. Marogna M, Tomassetti D, Bernasconi A, et al. Preventive effects of sublingual immunotherapy in childhood: an open randomized controlled study. Ann Allergy Asthma Immunol 2008;101(2):206-11

103. Novembre E, Galli E, Landi F, et al. Co-seasonal sublingual immunotherapy reduces the development of asthma in children with rhinoconjunctivitis. J Allergy Clin Immunol 2004;114(4):851-7

104. Pajno GB, Passalacqua G, Vita D, et al. Sublingual immunotherapy abrogates seasonal bronchial hyperresponsiveness in children with Parietaria-induced respiratory allergy: a randomized controlled trial. Allergy 2004;59(8):883-7

105. Bousquet PJ, Calderon MA, Demoly P, et al. The Consolidated Standards of Reporting Trials (CONSORT) Statement applied to allergen-specific immunotherapy with inhalant allergens: a Global Allergy and Asthma European Network (GA(2)LEN) article. J Allergy Clin Immunol 2011; 127(1):49-56

106. Senna G, Calderon M, Makatsori M, et al. An evidence-based appraisal of the surrogate markers of efficacy of allergen immunotherapy. Curr Opin Allergy Clin Immunol 2011;11(4):375-80

107. Holgate S, Casale T, Wenzel S, et al. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. J Allergy Clin Immunol 2005;115(3):459-65

108. Lin H, Boesel KM, Griffith DT, et al. Omalizumab rapidly decreases nasal allergic response and FcepsilonRI on basophils. J Allergy Clin Immunol 2004;113(2):297-302

109. MacGlashan DW, Bochner BS, Adelman DC, et al. Down-regulation of Fc (epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. The Journal of Immunology 1997;158(3):1438-45

110. Holgate S, Smith N, Massanari M, Jimenez P. Effects of omalizumab on

markers of inflammation in patients with allergic asthma. Allergy 2009;64(12): 1728-36

111. Khoriaty E, Umetsu DT. Oral immunotherapy for food allergy: towards a new horizon. Allergy Asthma Immunol Res 2013;5(1):3-15
112. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. J Allergy Clin Immunol 2001;108(2):184-90
113. Kuehr J, Brauburger J, Zielen S, et al. Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. J Allergy Clin Immunol 2002;109(2):274-80
114. Leung DYM, Sampson HA, Yunginger JW, et al. Effect of Anti-IgE Therapy in Patients with Peanut Allergy. N Engl J Med 2003; 348(11):986-93

•• **This multicenter study reports the evidence that non-specific immunotherapy could be a therapeutic option for the treatment of IgE-mediated food allergy.**

115. Begin P, Dominguez T, Wilson S, et al. Phase 1 results of safety and tolerability in a rush oral immunotherapy protocol to multiple foods using Omalizumab. Allergy Asthma Clin Immunol 2014;10(1):7
116. Bedoret D, Singh AK, Shaw V, et al. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. Mucosal Immunol 2012;5(3): 267-76
117. Gernez Y, Tirouvanziam R, Yu G, et al. Basophil CD203c Levels Are Increased at Baseline and can Be Used to Monitor Omalizumab Treatment in Subjects with Nut Allergy. Int Arch Allergy Immunol 2011;154(4):318-27
118. Corren J. Anti-interleukin-5 antibody therapy in asthma and allergies. Curr Opin Allergy Clin Immunol 2011;11(6):565-70
119. Leckie MJ, Brinke At, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. The Lancet 2007;356(9248): 2144-8
120. Hart TK, Cook RM, Zia-Amirhosseini P, et al. Preclinical efficacy and safety of mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgus monkeys. J Allergy Clin Immunol 2001;108(2):250-7
121. Rothenberg ME, Klion AD, Roufosse FE, et al. Treatment of Patients with the Hypereosinophilic Syndrome with Mepolizumab. N Engl J Med 2008; 358(12):1215-28
122. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM):a multicentre, double-blind, placebo-controlled trial. The Lancet 2012; 380(9842):651-9
123. Spergel JM, Rothenberg ME, Collins MH, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. J Allergy Clin Immunol 2012;129(2):456-63.e453
124. Castro M, Mathur S, Hargreave F, et al. Reslizumab for Poorly Controlled, Eosinophilic Asthma. Am J Respir Crit Care Med 2011;184(10):1125-32
125. Laviolette M, Gossage DL, Gauvreau G, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. J Allergy Clin Immunol 2013;132(5):1086-96.e1085
126. Oldhoff JM, Darsow U, Werfel T, et al. Anti-IL-5 recombinant humanized monoclonal antibody (Mepolizumab) for the treatment of atopic dermatitis. Allergy 2005;60(5):693-6
127. Wenzel S, Wilbraham D, Fuller R, et al. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. The Lancet 2007; 370(9596):1422-31
128. Wenzel S, Ford L, Pearlman D, et al. Dupilumab in Persistent Asthma with Elevated Eosinophil Levels. N Engl J Med 2013;368(26):2455-66
129. Borish LC, Nelson HS, Lanz MJ, et al. Interleukin-4 Receptor in Moderate Atopic Asthma. Am J Respir Crit Care Med 1999; 160(6):1816-23
130. Corren J, Busse W, Meltzer EO, et al. A randomized, controlled, phase 2 study of amg 317, an il-4rα antagonist, in patients with asthma. Am J Respir Crit Care Med 2010;181(8):788-96
131. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab Treatment in Adults with Asthma. N Engl J Med 2011;365(12): 1088-98
132. De Boever EH, Ashman C, Cahn AP, et al. Efficacy and safety of an anti–IL-13 mAb in patients with severe asthma: a randomized trial. J Allergy Clin Immunol 2014;133(4): 989-96.e984
133. Piper E, Brightling C, Niven R, et al. A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. European Respiratory Journal 2013;41(2): 330-8
134. Gauvreau GM, O'Byrne PM, Boulet L-P, et al. Effects of an Anti-tslp antibody on allergen- induced asthmatic responses. N Engl J Med 2014;370(22):2102-10
135. Horak F, Zieglmayer P, Zieglmayer R, et al. The CRTH2 antagonist OC000459 reduces nasal and ocular symptoms in allergic subjects exposed to grass pollen, a randomised, placebo- controlled, double-blind trial. Allergy 2012;67(12): 1572-9
136. Straumann A, Hoesli S, Bussmann C, et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. Allergy 2013;68(3): 375-85
137. Pettipher R, Hunter MG, Perkins CM, et al. Heightened response of eosinophilic asthmatic patients to the CRTH2 antagonist OC000459. Allergy 2014;69(9):1223-32
138. Diamant Z, Sidharta PN, Singh D, et al. Setipiprant, a selective CRTH2 antagonist, reduces allergen-induced airway responses in allergic asthmatics. Clin Exp Allergy 2014; 44(8):1044-52
139. Busse WW, Wenzel SE, Meltzer EO, et al. Safety and efficacy of the prostaglandin D2 receptor antagonist AMG 853 in asthmatic patients. J Allergy Clini Immunol 2013;131(2):339-45
140. Snell N, Foster M, Vestbo J. Efficacy and safety of AZD1981, a CRTH2 receptor antagonist, in patients with moderate to severe COPD. Respir Med 2013;107(11): 1722-30
141. Qu C, Srivastava K, Ko J, et al. Induction of tolerance after establishment of peanut allergy by the food allergy herbal formula-2 is associated with up- regulation of interferon-γ. Clin Exp Allergy 2007; 37(6):846-55
142. Wang J, Patil SP, Yang N, et al. Safety, tolerability, and immunologic effects of a food allergy herbal formula in food allergic individuals: a randomized, double-blinded, placebo-controlled, dose escalation, phase 1 study. Ann Allergy Asthma Immunol 2010;105(1):75-84.e71
143. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18(5):716-25
144. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov 2006;5(6):471-84

145. 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(14):1445-55

146. Beeh K-M, Kanniess F, Wagner F, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. J Allergy Clin Immunol 2013;131(3): 866-74

147. Klimek L, Willers J, Hammann-Haenni A, et al. Assessment of clinical efficacy of CYT003- QbG10 in patients with allergic rhinoconjunctivitis: a phase IIb study. Clin Exp Allergy 2011;41(9):1305-12

148. Kaufman EH, Fryer AD, Jacoby DB. Toll-like receptor 7 agonists are potent and rapid bronchodilators in guinea pigs. J Allergy Clin Immunol 2011;127(2):462-9

149. Matthew G, Drake EHK, Allison D. Fryer and David B. Jacoby. The Therapeutic Potential of Toll-Like Receptor 7 Stimulation in Asthma. Inflamm Allergy Drug Targets 2012;11(6):484-91

150. Biffen M, Matsui H, Edwards S, et al. Biological characterization of a novel class of toll-like receptor 7 agonists designed to have reduced systemic activity. Br J Pharmacol 2012;166(2):573-86

151. DuBuske LM, Frew AJ, Horak F, et al. Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. Allergy Asthma Proc 2011;32(3):239-47

152. Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. Allergy 2001;56(6): 498-505

153. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. J Allergy Clin Immunol 2014; 133(1):121-9.e122

154. Agache IO. From phenotypes to endotypes to asthma treatment. Curr Opin Allergy Clin Immunol 2013;13(3):249-56

AUTHOR QUERY SHEET

Author(s) : Pajno, Nadeau, Passalacqua et al.

Article title : The evolution of allergen and non-specific immunotherapy: past achievements, current applications and future outlook

Article no : 977260

Enclosures : 1) Query sheet
2) Article proofs

Dear Author,

Please check these proofs carefully. It is the responsibility of the corresponding author to check against the original manuscript and approve or amend these proofs. A second proof is not normally provided. Informa Healthcare cannot be held responsible for uncorrected errors, even if introduced during the composition process. The journal reserves the right to charge for excessive author alterations, or for changes requested after the proofing stage has concluded.

The following queries have arisen during the editing of your manuscript and are marked in the margins of the proofs. Unless advised otherwise, submit all corrections using the CATS online correction form. Once you have added all your corrections, please ensure you press the "Submit All Corrections" button.

AQ1	Ref. no. 21 does not match with 'Noon and Freeman'. Please check.