

REVIEW ARTICLE

One hundred years of allergen immunotherapy European Academy of Allergy and Clinical Immunology celebration: review of unanswered questions

M. Calderón¹, V. Cardona² & P. Demoly³ on behalf of the EAACI 100 Years of Immunotherapy Experts Panel*

¹Imperial College London, National Heart and Lung Institute, London, UK; ²University Hospital of Vall d'Hebron, Barcelona, Spain; ³University Hospital of Montpellier, Montpellier, France

To cite this article: Calderón M, Cardona V, & Demoly P. on behalf of the EAACI 100 Years of Immunotherapy Experts Panel. One hundred years of allergen immunotherapy European Academy of Allergy and Clinical Immunology celebration: review of unanswered questions. *Allergy* 2012; DOI:10.1111/j.1398-9995.2012.02785.x

Keywords

allergen immunotherapy; efficacy; new developments; safety treatment regimens

Correspondence

Moisés Calderón, Imperial College London, National Heart and Lung Institute, London, UK.

Tel.: +44 20 73518024

Fax: +44 20 73497780

E-mail: m.calderon@imperial.ac.uk

*EAACI Experts Panel members: Giovanni Passalacqua, Allergy and Respiratory Diseases, DIMI, Department of Internal Medicine, Genoa, Italy; Jörg Kleine-Tebbe, Allergy & Asthma Center Westend, Berlin, Germany; Erka Valovirta, Suomen Terveystalo Allergy Clinic, Turku, Finland; Stephen R Durham, Imperial College London, National Heart and Lung Institute, London, UK; Ronald Dahl, Aarhus University, Denmark; Hans-Jørgen Malling, Allergy Unit, University Hospital, Copenhagen, Denmark; Antony Frew, University of Southampton; Montserrat Fernandez-Rivas, Hospital Clínico San Carlos, IdISSC, Madrid, Spain; Rudolf Valenta, Christian Doppler Laboratory for Allergy Research, Medical University of Vienna, Austria; Ulrich Wahn, Charité University of Medicine, Berlin, Germany; Albrecht Bufe, Ruhr University, Bochum, Germany.

Abstract

Allergen immunotherapy was introduced by Leonard Noon 100 years ago and is the only disease-modifying treatment for allergic individuals. Improved understanding of immunology has taught us a great deal about the underlying mechanisms involved in allergen immunotherapy; however, despite these developments, a number of important questions remain unanswered. Several of these questions relate to the practice of allergen immunotherapy in the clinic, such as: Is it possible to unify units of allergen potency? Which treatment schedules are best? Is allergen immunotherapy effective in all patient groups? Is there a dose–response relationship for efficacy and safety?, and Is there evidence for long-term effects following allergen immunotherapy? Others are related to new developments, such as new indications, or developments in the production of allergens. On the centenary of Noon's discovery, European experts in the field of immunotherapy met in Geneva under the aegis of the EAACI to discuss these controversial issues. This study presents outcomes and conclusions from these discussions.

Accepted for publication 19 December 2011

DOI:10.1111/j.1398-9995.2012.02785.x

Edited by: Thomas Bieber

In the 100 years since allergen immunotherapy was introduced by Leonard Noon, this treatment has been developed and refined and is now practiced routinely to treat allergic individuals worldwide. The efficacy and safety of allergen

immunotherapy have been demonstrated in a number of clinical studies, and advances in laboratory techniques and our knowledge of immunology have taught us a great deal about the underlying mechanisms involved. However, despite these

Table 1 Outline of the EAACI summit

Questions	Experts	Panel
Is it really possible to unify allergen units?	Giovanni Passalacqua, Jörg Kleine-Tebbe	Stefan Vieths, Roy Gerth van Wijk, Marianne van Hage, Emilio Alvarez Cuesta
Is there a real dose–efficacy and dose–safety relationship?	Erkka Valovirta, Stephen Durham	Walter G Canonica, Carmen Vidal, Manuel Branco-Ferreira, Frederic de Blay
Is a particular schedule better than another?	Ronald Dahl, Hans-Jorgen Malling	Claus Bachert, Carmen Moreno, Glennis Scadding, Beatrice Bilo, Michael Rudenko
Is allergen immunotherapy effective and safe in polysensitized patients?	Moisés Calderón, Antony Frew	Sabina Rak, Nikos Papadopoulos, Pascal Demoly, Oliver Pfaar, Rodrigo Rodrigues Alves
Are the new modalities of allergen immunotherapy realistic?	Montserrat Fernandez-Rivas, Rudolf Valenta	Cezmi Akdis, Marek Jutel, Peter Schmid-Grendelmeier, Gabriella Senti
Is there evidence for long-term efficacy and preventive effect of allergen immunotherapy?	Ulrich Wahn, Albrecht Bufe	Eva Varga, Peter Eng, Alain Didier, Susanne Halken

considerable advances, there are a number of questions that remain unanswered. Several of these relate to recent developments in the practice of allergen immunotherapy, such as efficacy in polysensitized patients, the most effective treatment schedules, new indications and new modes of allergen administration, while other points for discussion relate to the recently introduced European Medicines Agency (EMA) guidelines on the quality of allergen products for human use and the clinical development of products for allergen immunotherapy (1, 2). For many years, allergen immunotherapy has been practised on a named patient basis; however, new requirements for marketing authorization from competent authorities require that clinical studies demonstrate dose–response data for clinical efficacy and safety, and long-term benefits of treatment after discontinuation of immunotherapy, which require the ability to draw comparisons between clinical studies, raising the issue of the need to standardize units of allergen potency.

On the 24th of February 2011, a group of European experts in the field of immunotherapy met in Geneva to discuss these controversial issues, which are outlined in Table 1. Designated experts presented the main arguments for and against each of the topics to the general audience. The audience then split into separate working groups, chaired by the presenters to discuss each of the topics. Arguments for and against each topic and the outcomes of these discussions are presented as follows.

Is it really possible to unify allergen units?

In Europe, each allergen manufacturer uses proprietary units to express the potency of their allergen preparations. These units are established in different ways, and there is considerable variation between extracts from different manufacturers in terms of their protein content and composition. Comparison of the potency of preparations derived from same allergen sources is therefore presently impossible.

Table 2 presents allergen product units used by different European manufacturers and illustrates the considerable heterogeneity in the definitions used to describe potency. Meta-analyses and systematic reviews of clinical studies involving

allergen immunotherapy are hampered by this use of different units. Unification of the units used to describe allergen potency would enable a better understanding of the heterogeneity of results between clinical studies and, in addition, might also help in establishing the optimal maintenance dose for patients who are allergic to a given allergen source.

This problem has been recognized for some time: almost 10 years ago, the CREATE project set out to develop candidate reference materials for important major allergens (single proteins) and to validate these recombinant allergens in immunoassays for the detection of their natural counterparts and isoforms in commercial allergen preparations, which represent complex mixtures (3, 4). However, when the same allergen preparations were tested using different assays (involving different antibodies and/or different standards), different results were obtained. As allergenic proteins exist in several different isoforms, and antibodies can display an intrinsic preference for one isoform over another, it was not possible to identify a standard assay that would work well with allergen preparations from different laboratories or manufacturers. Following on from the CREATE project, the biological standardization programme (BSP090), supported by the European Directorate for the Quality of Medicines (EDQM), the European Pharmacopeia, and the Centre for Biologics Evaluation and Research (CBER), has set out to establish robust assays for two candidate molecules, Bet v 1 and PhI p 5b, and to validate these in different laboratories by vigorous ring trials with a view to becoming part of the European Pharmacopeia (5).

Outcomes of the discussion

- The group agreed that, presently, it is not possible to combine information from potency testing, biological testing and quantitative information on composition into one unit.
- There is inherent variability in the source materials and extraction methods employed by different allergen manufacturers, as well as among patients in terms of exposure, sensitizations, response to skin testing, polyclonal immunoglobulin (Ig) E responses, etc. Although variations in

Table 2 Definitions of allergen preparation units used by some European allergen manufacturers

Allergen manufacturer	Website	Units	Definitions (given by allergen manufacturers)
ALK-Abelló, D	http://www.alk-abello.com	SQ-U	100 000 Standardized Quality – Units is the optimal maintenance dose of allergen extract administered, to the average patient during SCIT.
		SQ-T	75 000 Standardized Quality – Tablet units is the optimal maintenance dose of grass allergen extract administered to the average patient during SLIT.
Allergopharma*, G	http://www.allergopharma.com/	TU	Therapeutic units: demonstration of clinical efficacy and safety in clinical studies determines an appropriate dose of the product, and TU are assigned accordingly. TU reflect the quality and consistency of the product on the basis of a comparison with the IHRP as well as the clinical efficacy and safety.
Allergy Therapeutics*, UK; Bencard, G	http://www.allergytherapeutics.com/	TU	TU is derived from the corresponding ODC prick test strength. ODC = concentration with the lowest rate of false positive and false negative results at a specified cut-off. $CET = \sqrt{\left(\frac{FN}{TNP}\right)^2 + \left(\frac{FP}{TNP}\right)^2}$ see also (62)
		SU	Standardized Units (SU) is the specified reactivity of specific IgG (not IgE) with the allergoid; in the case of grasses, Phl p 1, and in case of birch, Bet v 1.
Bial-Aristegui†, S	http://www.bial.com/es/	DBU/TSU	Diagnostic Biological Units /Treatment Standardized Units (based on SPT using histamine 10 mg/ml as reference).
Diater Laboratorios, S	http://www.diater.com/en.html	HEP/ml (10 000 UB/ml)	Extract provokes a specific skin reaction in the median sensitive patient with a wheal of the same size as a wheal provoked by a positive reference solution consisting of histamines 54.3 mM (for example histamine dihydrochloride 10 mg/ml), when both solutions are administered using the same technique (SPT) on at least 20 individuals who are clinically allergic and cutaneously reactive to the allergen concerned.
HAL*, NL	http://www.hal-allergy.com/	AU*	Erythema size total of two diameters, arithmetical mean of 50 mm = D50.
		AUM	Intradermal test in 15 highly allergic patients, chosen from a pool of patients with no controls
Immunotek, S	http://www.immunotek.com	TU (therapeutic units): 1 TU = 1 BU (same protein content)	HEP-BU are calculated according to the Nordic Guidelines: 'the activity of an allergen extract is 10 000 BU or 10 HEP per ml when the extract provokes a specific skin reaction in the median sensitive patient with a wheal of the same size as a wheal provoked by a positive reference solution consisting of histamine 54.3 mM (histamine dihydrochloride 10 mg/ml), when both solutions are administered using the same technique (SPT) on at least 20 individuals who are clinically allergic and cutaneously reactive to the allergen concerned'.

Table 2 (continued)

Allergen manufacturer	Website	Units	Definitions (given by allergen manufacturers)
LETI [†] , S	http://www.leti.com/eng/Index.asp	HEP	'An allergen extract is defined as having 10 HEP when it causes a specific reaction on the skin consisting of a wheal of the same average size as a positive reference consisting in histamine 54.3 mM (histamine HCl at a concentration of 10 mg/ml), when both solutions are used with the same technique (SPT) in a minimum of 20 individuals who are sensitized and who have a skin reaction to the allergen in question'.
Lofarma [‡] , I	http://www.lofarma.it/en/index.html	AU	Biological unit that is equivalent to 1/40 of the corresponding unmodified allergen challenge dose assessed by nasal challenge test in volunteers suffering from allergic rhinitis.
		BU/ml	BU is equivalent to 1/100 of the concentration of extract, which, before being chemically modified, induces at SPT a mean wheal equivalent to histamine 10 mg/ml.
		10 000 U/ml	Corresponds to 4 µg equivalent/ml of Group 1 major allergens (Der p 1 and Der f 1).
Roxall, S	http://www.roxall.com/	AU	Biological unit that is equivalent to 1/40 of the corresponding unmodified allergen challenge dose assessed by nasal challenge test in volunteers suffering from allergic rhinitis.
		BU/ml	BU is equivalent to 1/100 of the concentration of extract, which, before being chemically modified, induces at SPT testing a mean wheal equivalent to histamine 10 mg/ml.
		10 000 U/ml	Corresponds to 4 µg Equivalent/ml of Group 1 major allergens (Der p 1 and Der f 1).
Stallergenes*, F	http://www.stallergenes.com/	IR	The IR unit has been defined to measure the allergenicity of an allergen extract. The allergen extract contains 100 IR/ml when, on a SPT using a Stallerpoint [®] , it induces a wheal diameter of 7 mm in 30 patients sensitized to this allergen (geometric mean). The cutaneous reactivity of these patients is simultaneously demonstrated by a positive SPT to either 9% codeine phosphate or 10 mg/ml histamine.

BU, biological units; HEP, histamine equivalent prick-testing; IHRP, in-house reference preparation; ODC, optimal diagnostic concentration; SPT, skin prick test; SQ, standardized quality; TU, therapeutic units.

*Full Member.

[†]Associate Member of the European Allergen Manufacturers Group (EAMG; <http://www.eamg.com>).

[‡]information based on (62).

patient response must be accepted, developments in recombinant allergens may reduce variability between allergen preparations.

- In general, it is more important to demonstrate the clinical effect of an individual product than to have one unit to describe potency and insufficient clinical data.
- The inherent variability associated with biological testing, such as skin prick tests (SPT) and intra-dermal tests, as well as between patient cohorts from different geographical regions, highlights the limitations of these methods for comparing the potency of allergen preparations.

- Another limitation of biological tests and potency assays that use ELISA or cell-based assays is that they do not provide information on the composition of an extract.
- Qualitative analysis of active products (e.g. using mass spectrometry) could identify the components of a preparation that are responsible for its activity; quantitative methods based on mass spectrometry technology are currently being developed.
- Even if there was a unified unit for allergen potency (such as US allergy units, which are related to total potency),

the composition of extracts from different companies are different, making it impossible to switch from one company's product to another in the clinic.

- The group concluded that at present it is not possible to unify allergen units from different companies.

Is there a real dose–efficacy and dose–safety relationship?

To date, several clinical studies have been performed to compare different doses of the same allergen source and relate these to efficacy and safety outcomes. Several studies have observed a dose–response relationship. A recent report from the EAACI immunotherapy Task Force reviewed published dose-ranging SCIT and SLIT studies and observed that thirteen of the fifteen identified studies reported a dose–response relationship for clinical efficacy, with eight also reporting a dose–response effect for immunological endpoints and two for safety outcomes (6). Several of the studies reporting a dose–response effect for efficacy had a high quality of evidence as assessed by the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) scoring system (7). However, as clinical endpoints vary widely between studies and there is currently no universally accepted standard for the measurement of allergen content, comparisons between studies or a meta-analysis were not possible.

Nevertheless, despite this evidence for a dose–response relationship, the currently available data have some shortcomings, which relate to the qualitative and quantitative differences in allergen compositions used in the different studies, the use of different adjuvants, different adjuvant/allergen ratios in SCIT studies, the use of sublingual tablets vs drops and the different volumes of solutions administered in SLIT studies (6).

Outcomes of the discussion

- The group agreed that there was evidence from individual SCIT and SLIT studies to support a dose–response effect for efficacy.
- In view of the current regulatory requirements for allergen products to demonstrate a dose–response relationship for efficacy in phase II clinical studies, communication between academic researchers, allergen manufacturers and regulatory authorities is vitally important.
- The logistical and financial implications of gathering dose–response data make it impossible to do this for all currently available allergen sources. Therefore, choices will have to be made – the group identified grass pollen, birch pollen, mite and venom as the principal allergen sources for which dose–response data should be obtained.
- The group agreed that it is worthwhile to measure the allergen content of the aforementioned preparations and to relate this to clinical efficacy. Owing to variations in allergen content and formulation between products from individual manufacturers, preparations should be studied individually.

- Rigorous standards must be applied to these studies, and the same measurement techniques should be used in each. Endpoints, such as combined symptom and rescue medication scores, must be clearly defined and standardized across studies.
- Despite only 2 of the 15 studies in the EAACI Task Force Report reporting a dose–response relationship for safety, the group was convinced that a dose–response relationship for safety exists and is evident during SCIT up-dosing in clinical practice. The panel concluded that the lack of a dose–response relationship for safety in clinical studies reflects the need for standardized reporting of adverse events; this should apply not only in the context of clinical trials, but also in routine clinical practice.
- Guidelines are available for assessing systemic adverse events during SCIT. Similar guidelines are needed for the reporting of adverse events in SLIT, especially local reactions, which occur most frequently.
- The group concluded that surrogate antigen challenges in the eye and nose would be useful to perform in parallel with phase II dose–response studies, as there is presently little data to correlate these tests with actual symptoms during the allergen season. Their relationship to clinical efficacy could be evaluated.
- It is presently not possible to identify a marker that is predictive of a clinical response to allergen immunotherapy. Such a marker should be serum- or plasma-based, as it is not feasible to perform complex T cell-based assays in the context of multicenter clinical trials. Candidate markers are as follows: sIgE, IgG1, IgG4, facilitated allergen binding (FAB) inhibition, ratio of sIgE to total IgE, ratio of sIgG4 to sIgE, and basophil sensitivity.
- At present, the utility of pollen chambers in dose–response studies has not been adequately studied.

Is a particular schedule better than another?

Induction and maintenance schedules for SCIT and SLIT vary widely in dosing interval, treatment duration and whether treatments are administered pre-seasonally, pre-co-seasonally or perennially. Published randomized controlled trials (RCTs) of allergen immunotherapy have employed many different treatment schedules, and no direct comparisons have been made. Some schedules may be preferable to others regarding the following: efficacy, including long-term efficacy; safety and risk reduction; patient convenience; cost; adherence; and the allergen extracts that are available.

The induction regimen is of minor importance to the long-term clinical efficacy of allergen immunotherapy and represents a titration to the dose essential for an immunological response (8). Conventional dose-increase schedules for SCIT imply one, or rarely two, weekly injections until the maintenance dose is reached. A slow induction of immune tolerance results in a lower frequency of adverse effects compared with more aggressive regimens (9). An alternative to conventional dose-increase regimens is rush immunotherapy; this regimen

may save time, as the maintenance dose can normally be reached in 3–5 days (10), with some schedules reaching a maintenance dose within 2.5 h. However, the risk of inducing severe adverse effects is high, and this regimen should be limited to hospitalized patients (11). A compromise between these two extremes is cluster immunotherapy, which involves administration of two to four injections spaced at 30-minute intervals in weekly sequences. The advantage is a reduction in the time needed to reach the maintenance dose without jeopardizing patient safety, at the expense of a slightly increased risk of inducing adverse effects compared with conventional immunotherapy (11, 12). A pharmacoeconomic analysis concluded that a cluster regimen resulted in a global saving of USD 244.95 per patient compared with a conventional protocol (13). The situation is slightly different for SLIT, where very short build-up phases or the omission of the build-up do not result in an increased occurrence of adverse effects (14).

The length of the maintenance phase is an important issue and mainly depends on the specific allergen and the clinical allergic reaction encountered (15). Traditionally, a treatment duration of 3 years is recommended; however, scientific data to support this are scarce (16). The optimal duration has not been investigated in clinical studies, and no international guidelines exist. It has been shown that 3 years of SLIT gives a beneficial effect that lasts for a further 2 years after stopping treatment (17). In addition, results from a study with a 300IR 5-grass pollen SLIT tablet demonstrated a significant sustained efficacy after three seasons of 2- and 4-month pre- and co-seasonal treatment (18), as well as during the first treatment-free year after three seasons (19).

Outcomes of the discussion

- The group concluded that it was impossible to compare between schedules. The optimal method to identify the best schedule for allergen immunotherapy was to discern studies where treatment was safe and effective.
- Safety should be the first consideration in the choice of schedule, followed by efficacy, convenience to the patient, cost and long-term benefit of treatment.
- The use of allergoids necessitates fewer injections, but shows no advantage in terms of efficacy and safety.
- The initial phase of the schedule should consider primarily the safety of, and convenience to the patient. At this point, it is not necessary to consider efficacy, which is related to the maintenance phase and duration of treatment.
- For SCIT, there is evidence that pre-medication with antihistamines can reduce the severity of adverse reactions and allow a higher maintenance dose to be reached, which is decisive for the efficacy of the treatment.
- For venom SCIT, treatment duration should be at least 3 years to lifelong. In aeroallergen SCIT, treatment duration should probably be at least 3 years. It is not yet clear whether longer treatment improves efficacy.
- In SLIT, up-dosing is not necessary for all currently approved preparations, but might improve tolerability. Preseasonal treatment should last at least 8 weeks. It is

unclear whether efficacy is improved with up to 16 weeks of pre-seasonal treatment. The effect of pre-medication on adverse effects of SLIT has not been investigated.

Is allergen immunotherapy effective and safe in polysensitized patients?

Epidemiological and clinical trial data show that 51–81% of allergic patients are polysensitized (according to SPT and/or IgE assay results) (20–22). Among allergists, there are a variety of strongly held opinions on the best way to perform allergen immunotherapy in polysensitized patients. In the United States, allergists tend to treat for all sensitivities identified as individually important by skin testing, using mixtures of extracts prepared from bulk vials, whereas in Europe, patients, even those with multiple sensitivities, are normally only treated with one or few single-allergen sources, deemed to be the most clinically relevant, which are supplied direct from the manufacturer. Mixed allergen extracts are, however, available and are used in some parts of Europe as individual prescriptions (named patient products) or as custom mixes from manufacturers.

The prevailing view in Europe is that (i) a polysensitized subject is not necessarily polyallergic and (ii) multiple allergies do not always constitute a clinical problem (15, 23); the most troublesome allergy is treated with a single-allergen source preparation. The opposing view (which predominates in North America) is that, as long as multi-allergen therapy is effective and does not induce new sensitizations, there is an advantage in treating as many of the patient's actual or potential allergies as possible.

In considering whether allergen immunotherapy is effective and safe in polysensitized patients, two quite separate questions arise:

- 1 Is monovalent allergen immunotherapy efficacious and safe in patients who are polysensitized?
- 2 Is polyvalent allergen immunotherapy efficacious?

Most recent clinical trials of allergen immunotherapy have been designed to demonstrate the efficacy of monovalent products. To maximize the power of the study, it is usual to exclude patients who are sensitized to other allergens, on the basis of skin or blood testing. Other trials exclude anyone who has clinical symptoms on exposure to allergens other than those in the test allergen extract, but allow those whose dominant symptoms are to the test allergen to remain in the trial. One recent study of a timothy grass preparation provided a useful insight, showing that patients with multiple allergic sensitization, as judged by skin tests, responded at least as well as those who were monosensitized to grass pollen (24). In another study using SLIT with 5-grass pollen tablets, a subgroup analysis of 628 adults with different clinical profiles of allergic rhinoconjunctivitis enrolled in a study of the efficacy and safety reported that the average rhinoconjunctivitis total symptom score was identical for mono- and polysensitized patients (25).

Regarding the use of polyvalent immunotherapy, a recent review by Nelson et al. (26) highlighted the lack of evidence for the efficacy and safety of this approach, with only 13

trials published between 1961 and 2007, of which very few were well designed and adequately powered.

Outcomes of the discussion

- The key question for practitioners faced with a polysensitized patient is how the physician should decide on appropriate treatment?
- The group identified the need to clearly define polysensitization; patients with grass pollen allergy may be sensitized to multiple components of grass pollen but are referred to as monosensitized. Patients who cross-react to several allergens should not be considered polysensitized
- Before starting treatment, it is necessary to determine the importance of a demonstrated sensitization in causing clinical symptoms.
- The group questioned the suitability of SPT to identify clinically relevant sensitizations and concluded that in rare cases, when clinical history is strong with negative SPT or/and sIgE, the use of specific allergen challenge test may help to identify the responsible allergen for local allergy.
- Regarding monovalent allergen immunotherapy, a *post hoc* analysis of clinical trials identified no data that enabled comparison of the efficacy of allergen immunotherapy in polyallergic vs monoallergic patients and concluded that, because of the need for very large and long

studies, there was limited scope for large-scale clinical trials to address this question as a primary outcome.

- In patients with both seasonal and perennial sensitizations, the group concluded that it is worthwhile to treat the most severe and clinically relevant allergy, which is likely to be the seasonal component of seasonal allergic rhinitis.
- It is impossible to extrapolate findings from one allergen to another.
- Regarding polyvalent allergen immunotherapy, the simultaneous delivery of multiple unrelated allergens can be clinically effective when an allergic response to those allergens has been shown; however, well-designed and adequately powered clinical trials are needed to validate this as a treatment option.
- Multi-allergen immunotherapy in polysensitized patients needs more supporting data to validate it as a treatment option.

Are the new modalities of allergen immunotherapy realistic?

New modalities of allergen immunotherapy include new clinical indications (Table 3), such as food or nickel allergy, urticaria and atopic dermatitis; new routes of allergen administration (Table 4), including oral, epicutaneous and intralymphatic routes, which aim to improve the efficacy and

Table 3 New indications for allergen immunotherapy

	State of the art	References
Food allergy	Two controlled trials of SCIT in peanut allergy have shown a significant increase in the peanut threshold and reduction in skin reactivity in nine actively treated patients. Systemic reactions occurred in 23% of the rush build-up doses and 39% of the maintenance doses. Mucosal routes of administration have been investigated in an attempt to improve the safety profile. SLIT with hazelnut and peach in double-blind, placebo-controlled (DBPC) trials have shown an increase in the food threshold and a good safety profile with mild systemic reactions in <0.5% of doses. Local oral reactions were reported in 7% and 87% of patients treated with hazelnut and peach, respectively. Controlled and noncontrolled trials of specific oral tolerance induction (SOTI) with milk, egg and peanut have shown that 50–100% of patients are able to tolerate a normal serving or a substantial amount that protects them from accidental exposures. Systemic reactions were frequent, mostly in the build-up phases in the hospital, but also during the maintenance phase at home.	27–33, 35–40, 63–65
Nickel allergy	Open, noncontrolled, observational studies have reported positive results for efficacy (reduction in symptoms, need for medication, reduction in epicutaneous or intra-dermal test with nickel, increase in orally tolerated nickel, tolerance of nickel containing foods). Two trials failed to demonstrate efficacy. DBPC studies are needed to establish the usefulness of allergen immunotherapy in nickel allergy.	66–72
Urticaria	There are some open, non-controlled studies and case reports on the use of house dust mite (HDM) allergen immunotherapy in chronic urticaria, although a causal relationship between HDM sensitization and chronic urticaria has not been established. DBPC clinical trials are needed to evaluate this therapeutic approach.	73–75
Atopic dermatitis	Analysis of seven combined observational studies and five combined placebo-controlled trials involving SLIT and SCIT showed a significant improvement of atopic dermatitis. SOTI failed to demonstrate efficacy.	76–78

Table 4 New routes of allergen administration

	State of the art	References
SLIT in Venom IT	A DBPC trial of SLIT in bee venom allergy reported a significant reduction in sting challenge large local reactions, a significant increase in specific IgG and no changes in specific IgE compared with baseline. No adverse effects were reported. Another study in 21 patients with previous systemic reactions after vesperula stings reported mild adverse reactions in the SLIT (9.5%) and SCIT (15%) groups. Over the two-year treatment period, four patients in the SLIT group were field stung and only one reacted. In the SCIT group, nine patients were field stung and only one reported a systemic reaction.	79, 80
Intralymphatic IT (ILIT)	An open-label trial of ILIT in 165 patients with rhinoconjunctivitis caused by grass pollen allergy reported an improvement of allergen symptoms and reduced consumption of rescue medication that was long lasting and comparable to a three-year course of SCIT. Reduction in SPT reactivity and specific IgE was comparable between ILIT and SCIT. ILIT caused fewer and milder adverse effects compared with SCIT and patients showed better treatment compliance	81, 82
Epicutaneous immunotherapy (EPIT)	A DBPC clinical trial of EPIT in 37 patients with grass pollen rhinoconjunctivitis reported significantly decreased scores in the nasal provocation test in the first year. Only subjects who received active treatment had significantly reduced scores compared with baseline at the end of the second year. Another randomized DBPC trial of EPIT with grass pollen in 30 children reported significantly reduced symptom scores and antihistamine intake in actively treated patients. No change in SPT was observed after treatment, and no systemic or local reactions were observed.	83, 84

safety of allergen immunotherapy; and the development of new recombinant or chemically modified allergens (Table 5), which is driven by recent advances in protein engineering and is aimed at improving the specificity and efficacy/risk profile of allergen immunotherapy.

Food allergy is probably the most widespread new indication for allergen immunotherapy. Current standard management consists in avoidance of the offending food/s and, in the case of accidental reactions, rescue medication that may include self-injectable adrenaline for those patients at risk of anaphylaxis. However, as many food allergies are persistent, complete avoidance is difficult to achieve and severe accidental reactions are frequent. There is therefore a need for an active therapy to induce tolerance. Some SCIT studies have demonstrated efficacy in allergen immunotherapy of peanut allergy, but with a poor safety profile (27, 28). Alternative routes of allergen administration, such as SLIT, specific oral tolerance induction (SOTI) (29–40) and epicutaneous administration, are being investigated in an attempt to improve the safety profile. Clinical trials of SLIT and SOTI are promising and have shown a disease-modifying effect, although further studies are needed to establish the balance between efficacy and safety, the optimal dosing and duration, whether permanent tolerance is developed and the immunological mechanisms involved.

Some clinical studies of allergen immunotherapy in atopic dermatitis have shown an acceptable balance of efficacy and safety, but it is still a matter of debate whether atopic dermatitis alone is an indication for allergen immunotherapy. Nickel and house dust mite (HDM) allergen immunotherapies have been investigated in nickel allergy and chronic urticaria, respectively in open noncontrolled studies, which have so far not provided evidence of clinical efficacy (Table 3).

Research into new routes of allergen administration has mainly been aimed at improving convenience as well as the risk/benefit profile of allergen immunotherapy. Although several of the studies involving new routes, summarized in Table 4, have reported fewer adverse effects compared with traditional SCIT, further studies are needed to confirm these findings and to establish if efficacy is comparable.

Another important area of development is the use of new technological approaches to improve the efficacy/risk profile of the allergen/antigen preparation itself. One approach is through the use of recombinant allergen molecules and hypoallergenic allergen derivatives. Several of these new modalities have shown promising results in clinical trials (Table 5).

Outcomes of the discussion

- The group agreed that basic research has produced several products that have shown promising results in proof-of-concept and phase I studies. Successful phase III trials have so far only been conducted with recombinant hypoallergenic Bet v 1.
- Development of new allergen products for allergen immunotherapy should begin with a sound scientific basis; there is a need to identify and define the clinically relevant allergen molecules and efficacy markers for use in clinical studies.
- There is a need for health economic studies to illustrate to political authorities that treatment of allergic diseases with allergen immunotherapy is economically favourable compared with symptomatic treatment, with the aim of increasing research funding for studies on new modalities of immunotherapy.

Table 5 New developments in allergens

	Advantages/disadvantages	State of the art	References
Peptide immunotherapy targeting allergen-specific T cells	Advantages: lack IgE reactivity and therefore cannot induce IgE-mediated adverse effects. Disadvantages/problems: 1) T-cell epitope-containing peptides can activate allergen-specific T cells, leading to T cell-mediated late-phase adverse effects, 2) Owing to major histocompatibility complex diversity among patients, it is impossible to perform treatment with one or few peptides, 3) Treatment seems to reduce T-cell activation, but the effects on IgE-mediated symptoms are unclear.	Trials of allergen-derived peptides containing T-cell epitopes without IgE reactivity showed no relevant clinical improvement and patients experienced considerable late-phase adverse effects. Further studies were performed to optimize the treatment, and trials, performed by Circassia, are ongoing.	85, 86
Recombinant wild-type allergens for SCIT, SLIT	Advantages: allow the formulation of well-defined products containing specified amounts of each allergen. Can be easily produced under defined conditions that should satisfy regulators and health authorities. Disadvantages/Problems: induce the same adverse effects as natural allergens.	Clinical efficacy has been demonstrated for recombinant wild-type allergen-based products in birch and grass pollen allergy using SCIT. Trials are ongoing to prepare tablets based on recombinant major birch pollen allergen, Bet v 1 for SLIT and a mix of recombinant grass pollen allergens for SCIT.	87, 88
Recombinant hypoallergenic allergen derivatives for SCIT	Advantages: can be manufactured as defined molecules under defined conditions and thus fulfil requirements of modern allergen products. Do not induce IgE-mediated adverse effects and therefore can be given in higher doses than natural allergens. Disadvantages/problems: can induce T cell-dependent adverse effects.	Clinical efficacy has been demonstrated for SCIT with recombinant hypoallergenic Bet v 1 derivatives up to phase III.	89–93
CpG-adjuvanted allergens	Advantages: the approach is applicable to every purified allergen and may reduce IgE reactivity and enhance immunogenicity. Disadvantages/problems: chemical coupling of CpGs is difficult to control, and it is not clear whether comparable results can be obtained for different allergens. Enhancement of immunogenicity and Th1 bias was modest in humans.	A SCIT trial performed with CpG-conjugated Amb a 1 showed that the allergen preparation induced allergen-specific blocking IgG antibodies, and, similar to effects observed in the first SCIT trial with recombinant hypoallergenic derivatives of the major birch pollen allergen Bet v 1, reduced seasonal boosts of IgE production.	94
Virus-like particles	Advantages: allergens or allergen-derived peptides that induce allergen-specific IgG. Disadvantages/problems: difficult to manufacture.	Peptides from the house dust mite allergen Der p 1 coupled to the viral carrier protein induced allergen-specific IgG antibodies in non-allergic persons.	95, 96

Table 5 (continued)

	Advantages/disadvantages	State of the art	References
Recombinant fusion proteins containing allergen-derived peptides	Advantages: allergen products should lack IgE- as well as T cell-mediated adverse effects, but induce robust allergen-specific blocking IgG antibodies similar to wild-type allergens or recombinant hypoallergenic allergen derivatives. The concept is applicable to all allergens with known sequence. They may be useful also for prophylactic allergen immunotherapy Disadvantages/problems: results only available from safety skin testing of patients, but not yet from clinical trials.	Safety has been demonstrated in a skin test study for grass pollen allergen immunotherapy. The first immunotherapy trials for a grass pollen product based on this principle is scheduled for end of 2011.	97–99
Genetic immunization	Advantages: induces Th1-biased immune responses, which, at least in prophylactic murine models, seem to prevent allergic sensitization and may also affect established allergy. Production of proteins for allergen immunotherapy is not necessary. Disadvantages/problems: production and release of allergen in the host cannot be controlled and may cause severe and unpredictable adverse effects.	At present, protocols are being developed in murine models using nucleic acids coding for hypoallergenic molecules or which reduce the dose and duration of allergen expression in the patient.	100–102

- Using recombinant technology, allergen/antigen preparations can be produced under carefully controlled and reproducible conditions that fulfil manufacturing requirements set out by regulators.
- Recombinant technology also brings the possibility to modify allergens or to produce peptide or allergen derivatives, with a view to minimize IgE- and T cell-mediated adverse effects and to increase immunogenicity.
- There is a need for new modified allergen molecules to enter clinical studies. It is hoped that promising results obtained with a recombinant hypoallergenic rBet v 1 derivative, which has reached phase III clinical trials, will pave the way.

Is there evidence for long-term efficacy and preventive effect of allergen immunotherapy?

In addition to alleviating allergic symptoms, one of the goals of allergen immunotherapy is to induce long-term tolerance that persists after discontinuation of treatment.

The EMA defines long-term efficacy in allergen immunotherapy as the documented capacity to significantly reduce symptoms and medication use compared with placebo for at least two years after termination of treatment in a RCT. Some controlled trials have suggested that the effect of SCIT may last for several years after discontinuation of treatment (41–44); however, only one of these studies is randomized

and controlled. Moreover, the aforementioned studies do not include markers that correlate with a long-term effect on symptoms and medication. Two recent SLIT studies with grass pollen tablets have shown sustained efficacy after discontinuation of treatment (17, 19).

Several clinical studies have reported that allergen immunotherapy prevents new sensitizations (45–50), although the design of these studies offers differing strengths of evidence. Several studies have also reported prevention of the progression of allergic rhinitis to asthma (25, 42, 44, 51–55). An observational study of children aged 6–12 years with seasonal allergic rhinoconjunctivitis induced by both birch and grass pollen who were followed for 10 years after termination of treatment found that the incidence of seasonal asthma was significantly reduced by early SCIT intervention (44). Similar results were obtained in another open controlled trial in children, where 3 years of SLIT significantly reduced the occurrence of persistent asthma (56).

Candidates for a preventive approach are the following:

- Infants or toddlers with a positive family history of allergy, atopic dermatitis or food allergy in infancy (secondary prevention).
- Young infants with a positive family history before any manifestation of atopy (primary prevention).
- School children with allergic manifestations of the upper airways without asthma (secondary prevention).

A double-blind, placebo-controlled study on asthma prevention in high-risk infants, funded by the NIH, has treated a pilot cohort of children with a liquid extract of grass pollen, dust mite and cat allergens (57). The ongoing pan-European GAP trial aims to investigate the prevention of asthma in grass rhinitic children (58). The outcomes of these preventive intervention studies will provide evidence as to whether or not allergen immunotherapy can play a role in the primary or secondary prevention of atopic diseases.

If a clear preventive effect of allergen immunotherapy is observed, the question of when to start treatment to achieve maximum efficacy arises. A minimal age limitation of 5 years is still a usual recommendation in SCIT guidelines, although this is based on a single study with a rush induction protocol (59). Trials are currently underway to address this question (57). An additional question is whether to restart treatment in case of relapse; however, it is first necessary to determine when a sufficient level of efficacy has been achieved (60).

Outcomes of the discussion

- Data from older studies provide a moderate quality of evidence for the long-term efficacy of allergen immunotherapy. These studies, however, provide useful information for the design of new studies.
- To date, there is more evidence of a long-term preventive effect with SCIT compared with SLIT.
- Long-term efficacy data for SCIT and SLIT are only available in adults.
- Demonstration of long-term efficacy is required for the mandatory paediatric investigation plan (PIP) that must accompany applications for marketing authorization submitted to the EMA.
- For environmental allergens, the most urgent task is to focus on the prevention of asthma. There is an opportunity for studies of secondary prevention in high-risk populations that have expressed clinical symptoms or patterns of sensitization. These patients should be given high priority, as there are currently no drugs that are effective in prevention.
- The priority given to the development of studies on primary prevention using allergen immunotherapy should be reconsidered.

Conclusion

It has taken allergen immunotherapy some time before reaching its current level of robustness. Several appropriately designed clinical trials have nevertheless proved its effectiveness in allergic rhinitis, asthma and venom allergy (61). As illustrated here, although some questions remain unanswered, allergen immunotherapy should now be regarded as a new therapeutic class.

Acknowledgments

We would like to thank the invited observers and other participants for their valuable contribution to the discus-

sions (in alphabetical order): Jerónimo Carnés, Marie David, Luis Delgado, Irmgard Eichler, Cecile Hilaire, Henrik Jacobi, Lars Jacobsen, Véronique Janet, Simon A. Lawton, Lise Lemonier, Kaare Lund, Jan Lötvall, Alberto Martínez, Antonella Muraro, Eva Perea, Bruno Robin and Marianella Salapatas. We also thank Dr Ron Hogg of OmniScience SA for assistance in preparing the manuscript. Finally, we thank ALK-Abelló, Bial-Aristegui, LETI and Stallergens for their unrestricted funding and partnering during the EAACI 100 years of Immunotherapy in Allergy campaign.

Author contributions

MC, VC and PD had the original idea, chose the subject matter, organized the meeting and co-ordinated the writing of the manuscript. MC, VC, PD, GP, JK-T, EV, SRD, RD, H-JM, AF, MF-R, RV, UW and AB participated in discussions, wrote the text and reviewed successive drafts of the manuscript.

Conflicts of interest

MAC has received consulting fees, honoraria for lectures and/or research funding from ALK-Abelló, Stallergenes, Allergopharma and Allergy Therapeutics. VC has received honoraria as a speaker or advisor for ALK-Abelló, LETI and Stallergenes. PD is a consultant and a speaker for Stallergenes, ALK and Therabel and was a speaker for Schering-Plough, MSD, AstraZeneca and GlaxoSmithKline in 2009–2011. GP has received speaking fees from Anallergo, ALK-Abelló, Almirall, AstraZeneca, GSK, MSD, Menarini, Lofarma, Stallergenes, and Schering-Plough. JK-T has received lecture fees from Allergopharma, ALK-Abelló, AstraZeneca, Bencard, Boehringer Ingelheim, Essex, HAL Allergy, Leti, Lofarma, Novartis, Thermo Fisher Phadia, Roxall and Stallergenes; research grants from Allergopharma, ALK-Abelló and HAL Allergy; and has been a consultant for ALK-Abelló, Bencard, HAL Allergy, Novartis and Paul-Ehrlich-Institut (EMA). EV declares no conflict of interest. SRD has received lecture and consultancy fees and research funding from ALK-Abelló Denmark, consultancy fees and research funding from Novartis and consulting fees from Merck, Circassia and Boehringer Ingelheim. RD has served on an advisory board or given lectures for Boehringer Ingelheim, Pfizer, GlaxoSmithKline, ALK-Abelló, Airsonett, Merck, Novartis, Vectura, Elevation Pharma, Roche and Norpharma; His institution has received funding for clinical trials from ALK-Abelló, Stallergenes, Pfizer, Boehringer Ingelheim, AstraZeneca, Novartis, Airsonett, GSK, Centocor, Behring and Chiesi. H-JM has received fees for board membership and expert testimony. AF was an advisor and consultant to ALK-Abelló, Allergopharma, Sterna, Allergy Therapeutics and Stallergenes and has given lectures for ALK-Abelló, Allergopharma, MEDA, Allergy Therapeutics and Stallergenes. AF has performed advisory and consultancy work for ALK-Abelló, Allergopharma, Sterna, Allergy Therapeutics and Stallergenes; and lectures and meetings for ALK-Abelló,

Allergopharma, MEDA, Allergy Therapeutics and Stallergenes. MF-R declares no conflict of interest. RV is a consultant and has received research funding from Phadia (now Thermofisher) and Biomay. UW has received research grants from DFG (German Research Foundation), EU, BMBF (German Ministry of Education and Research), BMG (German Ministry of Health), BMELV (German Ministry of Nutrition, Agriculture and Consumer Protection), ALK-Abelló, Allergopharma, Stallergenes, Phadia, UCB, MSD,

FAES, Novartis, Sanofi-Aventis, Pfrimmer-Nutritia, GSK, Hipp and Symbiopharm; consultancy fees from Allergopharma, Stallergenes, Merck, Novartis, Hitachi, Schering-Plough, Danone, FAES, AstraZeneca, GSK and Medimmune; and lecture fees from Sanofi-Aventis, Stallergenes, Allergopharma, Phadia, Schering-Plough, Danone, Novartis, MSD, GSK and Abbott. AB has received consulting fees from ALK-Abelló, Denmark, Bitop AG, Germany and NETSTAP Forschungs GmbH, Germany.

References

- Committee for Medicinal Products for Human Use (CHMP) and Efficacy Working Party (EWP). Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases; EMEA/CHMP/EWP/18504/2006, 2008.
- Committee for Medicinal Products for Human Use (CHMP). Guideline on allergen products. Production and quality issues; CPMP/EMA/BWP/304831/2007, 2008.
- van Ree R. The CREATE project: a new beginning of allergen standardization based on mass units of major allergens. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M* 2003;**94**:70–73. discussion 74–5.
- van Ree R, Chapman MD, Ferreira F, Vieths S, Bryan D, Cromwell O et al. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. *Allergy* 2008;**63**:310–326.
- Neske F, Schorner C, Buchheit KH, Costanzo A, Hanschmann KM, Himly M et al. BSP090—the follow-up to CREATE. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M* 2009;**96**:12–19. discussion 19–20.
- Calderon MA, Larenas D, Kleine-Tebbe J, Jacobsen L, Passalacqua G, Eng PA et al. European Academy of Allergy and Clinical Immunology task force report on 'dose-response relationship in allergen-specific immunotherapy'. *Allergy* 2011;**66**:1345–1369.
- Brozek JL, Akl EA, Alonso-Coello P, Lang D, Jaeschke R, Williams JW et al. Grading quality of evidence and strength of recommendations in clinical practice guidelines. Part 1 of 3. An overview of the GRADE approach and grading quality of evidence about interventions. *Allergy* 2009;**64**:669–677.
- Malling HJ. Immunotherapy in Europe. *Clin Exp Allergy* 1994;**24**:515–521.
- Cohn JR, Pizzi A. Determinants of patient compliance with allergen immunotherapy. *J Allergy Clin Immunol* 1993;**91**:734–737.
- Hejjaoui A, Ferrando R, Dhivert H, Michel FB, Bousquet J. Systemic reactions occurring during immunotherapy with standardized pollen extracts. *J Allergy Clin Immunol* 1992;**89**:925–933.
- Malling HJ, Weeke B. Immunotherapy. EAACI Position Paper. *Allergy* 1993;**48**(Suppl 48):9–35.
- Pfaar O, Leitzbach S, Hormann K, Klimek L. Cluster protocols in SCIT: enough evidence for practical use? *Curr Opin Allergy Clin Immunol* 2010;**10**:188–193.
- Mauro M, Russello M, Alesina R, Sillano V, Alessandrini A, Dama A et al. Safety and pharmacoeconomics of a cluster administration of mite immunotherapy compared to the traditional one. *Eur Ann Allergy Clin Immunol* 2006;**38**:31–34.
- Lombardi C, Incorvaia C, Braga M, Senna G, Canonica GW, Passalacqua G. Administration regimens for sublingual immunotherapy to pollen allergens: what do we know? *Allergy* 2009;**64**:849–854.
- Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. *Allergy* 2006;**61**(Suppl 82):1–20.
- Tabar AI, Arroabarren E, Echechipia S, Garcia BE, Martin S, Alvarez-Puebla MJ. Three years of specific immunotherapy may be sufficient in house dust mite respiratory allergy. *J Allergy Clin Immunol* 2011;**127**:57–63.
- Durham SR, Emminger W, Kapp A, Colombo G, de Monchy JG, Rak S et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010;**125**:131–138 e1–7.
- Didier A, Worm M, Horak F, Sussman G, de Beaumont O, Le Gall M et al. Sustained 3-year efficacy of pre- and coseasonal 5-grass-pollen sublingual immunotherapy tablets in patients with grass pollen-induced rhinoconjunctivitis. *J Allergy Clin Immunol* 2011;**128**:559–566.
- Didier A, Horak F, Worm M, Melac M, de Beaumont O, Montagut A et al. Long-term clinical efficacy of a 5-grass pollen sublingual immunotherapy tablet in adults with grass pollen-induced allergic rhinoconjunctivitis. Presented at the 30th Congress of the European Academy of Allergy and Clinical Immunology, Istanbul, Turkey 11–15 June 2011.
- Baatenburg de Jong A, Dikkeschei LD, Brand PL. Sensitization patterns to food and inhalant allergens in childhood: a comparison of non-sensitized, monosensitized, and polysensitized children. *Pediatr Allergy Immunol* 2011;**22**:166–171.
- Bousquet PJ, Castelli C, Daures JP, Heinrich J, Hooper R, Sunyer J et al. Assessment of allergen sensitization in a general population-based survey (European Community Respiratory Health Survey I). *Ann Epidemiol* 2010;**20**:797–803.
- Didier A, Chartier A, Demonet G. Specific sublingual immunotherapy: for which profiles of patients in practice? Midterm analysis of ODISSEE (observatory of the indication and management of respiratory allergies [rhinitis and / or conjunctivitis and / or allergic asthma] by specific sublingual immunotherapy). *Revue française d'allergologie* 2010;**50**:426–433.
- Calderon MA, Cox L, Casale TB, Moin-geon P, Demoly P. Multiple-allergen and single-allergen immunotherapy strategies in polysensitized patients: looking at the published evidence. *J Allergy Clin Immunol* 2012; in press.
- Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006;**117**:319–325.
- Malling HJ, Montagut A, Melac M, Patriarca G, Panzner P, Seberova E et al. Efficacy and safety of 5-grass pollen sublingual immunotherapy tablets in patients with different clinical profiles of allergic rhinoconjunctivitis. *Clin Exp Allergy* 2009;**39**:387–393.
- Nelson HS. Multiallergen immunotherapy for allergic rhinitis and asthma. *J Allergy Clin Immunol* 2009;**123**:763–769.

27. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997;**99**(6 Pt 1):744–751.
28. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol* 1992;**90**:256–262.
29. Blumchen K, Ulbricht H, Staden U, Dobberstein K, Beschoner J, de Oliveira LC et al. Oral peanut immunotherapy in children with peanut anaphylaxis. *J Allergy Clin Immunol* 2010;**126**:83–91 e1.
30. Hofmann AM, Scurlock AM, Jones SM, Palmer KP, Lokhnygina Y, Steele PH et al. Safety of a peanut oral immunotherapy protocol in children with peanut allergy. *J Allergy Clin Immunol* 2009;**124**:286–291. 291 e1–6.
31. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;**124**:292–300. 300 e1–97.
32. Longo G, Barbi E, Berti I, Meneghetti R, Pittalis A, Ronfani L et al. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. *J Allergy Clin Immunol* 2008;**121**:343–347.
33. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. *Allergy* 2004;**59**:980–987.
34. Meglio P, Giampietro PG, Gianni S, Galli E. Oral desensitization in children with immunoglobulin E-mediated cow's milk allergy—follow-up at 4 yr and 8 months. *Pediatr Allergy Immunol* 2008;**19**:412–419.
35. Morisset M, Moneret-Vautrin DA, Guegnard L, Cuny JM, Frentz P, Hatahet R et al. Oral desensitization in children with milk and egg allergies obtains recovery in a significant proportion of cases. A randomized study in 60 children with cow's milk allergy and 90 children with egg allergy. *Eur Ann Allergy Clin Immunol* 2007;**39**:12–19.
36. Pajno GB, Caminiti L, Ruggeri P, De Luca R, Vita D, La Rosa M et al. Oral immunotherapy for cow's milk allergy with a weekly up-dosing regimen: a randomized single-blind controlled study. *Ann Allergy Asthma Immunol* 2010;**105**:376–381.
37. Patriarca G, Nucera E, Roncallo C, Pollastrini E, Bartolozzi F, De Pasquale T et al. Oral desensitizing treatment in food allergy: clinical and immunological results. *Aliment Pharmacol Ther* 2003;**17**:459–465.
38. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008;**122**:1154–1160.
39. Staden U, Rolinck-Werninghaus C, Brewster F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. *Allergy* 2007;**62**:1261–1269.
40. Zapatero L, Alonso E, Fuentes V, Martinez MI. Oral desensitization in children with cow's milk allergy. *J Investig Allergol Clin Immunol* 2008;**18**:389–396.
41. Cools M, Van Bever HP, Weyler JJ, Stevens WJ. Long-term effects of specific immunotherapy, administered during childhood, in asthmatic patients allergic to either house-dust mite or to both house-dust mite and grass pollen. *Allergy* 2000;**55**:69–73.
42. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;**341**:468–475.
43. Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP. Twelve-year follow-up after discontinuation of pre-seasonal grass pollen immunotherapy in childhood. *Allergy* 2006;**61**:198–201.
44. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A 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**:943–948.
45. Des Roches A, Paradis L, Menardo JL, Bouges S, Dures JP, Bousquet J. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol* 1997;**99**:450–453.
46. Inal A, Altintas DU, Yilmaz M, Karakoc GB, Kendirli SG, Sertdemir Y. Prevention of new sensitizations by specific immunotherapy in children with rhinitis and/or asthma monosensitized to house dust mite. *J Investig Allergol Clin Immunol* 2007;**17**:85–91.
47. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy* 2001;**31**:1392–1397.
48. Purello-D'Ambrosio F, Gangemi S, Merendino RA, Isola S, Puccinelli P, Parmiani S et al. Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. *Clin Exp Allergy* 2001;**31**:1295–1302.
49. Reha CM, Ebru A. Specific immunotherapy is effective in the prevention of new sensitivities. *Allergol Immunopathol (Madr)* 2007;**35**:44–51.
50. Tella R, Bartra J, San Miguel M, Olona M, Bosque M, Gaig P et al. Effects of specific immunotherapy on the development of new sensitizations in monosensitized patients. *Allergol Immunopathol (Madr)* 2003;**31**:221–225.
51. Crimi N, Li Gotti F, Mangano G, Paolino G, Mastruzzo C, Vancheri C et al. A randomized, controlled study of specific immunotherapy in monosensitized subjects with seasonal rhinitis: effect on bronchial hyperresponsiveness, sputum inflammatory markers and development of asthma symptoms. *Ann Ital Med Int* 2004;**19**:98–108.
52. Keskin O, Tuncer A, Adalioğlu G, Sekerel BE, Sackesen C, Kalayci O. The effects of grass pollen allergoid immunotherapy on clinical and immunological parameters in children with allergic rhinitis. *Pediatr Allergy Immunol* 2006;**17**:396–407.
53. Passalacqua G, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol* 2007;**119**:881–891.
54. Polosa R, Al-Delaimy WK, Russo C, Piccillo G, Sarva M. Greater risk of incident asthma cases in adults with allergic rhinitis and effect of allergen immunotherapy: a retrospective cohort study. *Respir Res* 2005;**6**:153.
55. Polosa R, Li Gotti F, Mangano G, Paolino G, Mastruzzo C, Vancheri C et al. Effect of immunotherapy on asthma progression, BHR and sputum eosinophils in allergic rhinitis. *Allergy* 2004;**59**:1224–1228.
56. Marogna M, Tomassetti D, Bernasconi A, Colombo F, Massolo A, Businco AD et al. Preventive effects of sublingual immunotherapy in childhood: an open randomized controlled study. *Ann Allergy Asthma Immunol* 2008;**101**:206–211.
57. Promoting Tolerance to Common Allergens in High-Risk Children: Global Prevention of Asthma in Children (GPAC) Study. <http://clinicaltrials.gov/ct2/show/NCT00346398?term=Promoting+Tolerance+to+Common+Allergens+in+High-Risk+Children%3A+Global+Prevention+of+Asthma+in+Children+%28GPAC%29+Study&rank=1>.
58. Grazax Asthma Prevention (GAP) Study. <http://clinicaltrials.gov/ct2/show/NCT01061203?term=Grazax+Asthma+Prevention+%28GAP%29+trial&rank=1>.
59. Hejjoui A, Dhivert H, Michel FB, Bousquet J. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. IV. Systemic reactions according to

- the immunotherapy schedule. *J Allergy Clin Immunol* 1990;**85**:473–479.
60. Cox L, Cohn JR. Duration of allergen immunotherapy in respiratory allergy: when is enough, enough? *Ann Allergy Asthma Immunol* 2007;**98**:416–426.
 61. European Declaration on Immunotherapy: Combating Allergy Beyond Symptoms. <http://eaaci.net/eaacimedia/1663-eaaci-presents-the-european-declaration-on-allergen-immunotherapy>.
 62. Larenas-Linnemann D, Cox LS. European allergen extract units and potency: review of available information. *Ann Allergy Asthma Immunol* 2008;**100**:137–145.
 63. Enrique E, Pineda F, Malek T, Bartra J, Basagana M, Tella R et al. Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-controlled study with a standardized hazelnut extract. *J Allergy Clin Immunol* 2005;**116**:1073–1079.
 64. Fernandez-Rivas M, Garrido Fernandez S, Nadal JA, Diaz de Durana MD, Garcia BE, Gonzalez-Mancebo E et al. Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p 3 quantified peach extract. *Allergy* 2009;**64**:876–883.
 65. Kim EH, Bird JA, Kulis M, Laubach S, Pons L, Shreffler W et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol* 2011;**127**:640–646 e1.
 66. Sjoval P, Christensen OB, Moller H. Oral hyposensitization in nickel allergy. *J Am Acad Dermatol* 1987;**17**(5 Pt 1):774–778.
 67. Tammaro A, De Marco G, Persechino S, Narcisi A, Camplone G. Allergy to nickel: first results on patients administered with an oral hyposensitization therapy. *Int J Immunopathol Pharmacol* 2009;**22**:837–840.
 68. Schiavino D, Nucera E, Alonzi C, Buonomo A, Pollastrini E, Roncallo C et al. A clinical trial of oral hyposensitization in systemic allergy to nickel. *Int J Immunopathol Pharmacol* 2006;**19**:593–600.
 69. Bagot M, Terki N, Bacha S, Moysse D, Suck C, Revuz J. [Per os desensitization in nickel contact eczema: a double-blind placebo-controlled clinico-biological study]. *Ann Dermatol Venerol* 1999;**126**:502–504.
 70. Troost RJ, Kozel MM, van Helden-Meeuwse CG, van Joost T, Mulder PG, Benner R et al. Hyposensitization in nickel allergic contact dermatitis: clinical and immunologic monitoring. *J Am Acad Dermatol* 1995;**32**:576–583.
 71. Morris DL. Intradermal testing and sublingual desensitization for nickel. *Cutis* 1998;**61**:129–132.
 72. Panzani RC, Schiavino D, Nucera E, Pellegriano S, Fais G, Schinco G et al. Oral hyposensitization to nickel allergy: preliminary clinical results. *Int Arch Allergy Immunol* 1995;**107**:251–254.
 73. Caliskaner Z, Ozturk S, Turan M, Karayavaz M. Skin test positivity to aeroallergens in the patients with chronic urticaria without allergic respiratory disease. *J Investig Allergol Clin Immunol* 2004;**14**:50–54.
 74. Kulthanan K, Wachirakaphan C. Prevalence and clinical characteristics of chronic urticaria and positive skin prick testing to mites. *Acta Derm Venereol* 2008;**88**:584–588.
 75. Mahesh PA, Kushalappa PA, Holla AD, Vedanthan PK. House dust mite sensitivity is a factor in chronic urticaria. *Indian J Dermatol Venereol Leprol* 2005;**71**:99–101.
 76. Mastrandrea F. The potential role of allergen-specific sublingual immunotherapy in atopic dermatitis. *Am J Clin Dermatol* 2004;**5**:281–294.
 77. Bussmann C, Bockenhoff A, Henke H, Werfel T, Novak N. Does allergen-specific immunotherapy represent a therapeutic option for patients with atopic dermatitis? *J Allergy Clin Immunol* 2006;**118**:1292–1298.
 78. Pajno GB, Caminiti L, Vita D, Barberio G, Salzano G, Lombardo F et al. Sublingual immunotherapy in mite-sensitized children with atopic dermatitis: a randomized, double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2007;**120**:164–170.
 79. Severino MG, Cortellini G, Bonadonna P, Francescato E, Panzini I, Macchia D et al. Sublingual immunotherapy for large local reactions caused by honeybee sting: a double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2008;**122**:44–48.
 80. Patriarca G, Nucera E, Roncallo C, Aruanno A, Lombardo C, Decinti M et al. Sublingual desensitization in patients with wasp venom allergy: preliminary results. *Int J Immunopathol Pharmacol* 2008;**21**:669–677.
 81. Senti G, Johansen P, Kundig TM. Intralymphatic immunotherapy. *Curr Opin Allergy Clin Immunol* 2009;**9**:537–543.
 82. Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci U S A* 2008;**105**:17908–17912.
 83. Senti G, Graf N, Haug S, Ruedi N, von Moos S, Sonderegger T et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2009;**124**:997–1002.
 84. Agostinis F, Forti S, Di Berardino F. Grass transcutaneous immunotherapy in children with seasonal rhinoconjunctivitis. *Allergy* 2010;**65**:410–411.
 85. Norman PS, Ohman JL Jr, Long AA, Creticos PS, Geftner MA, Shaked Z et al. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996;**154**(6 Pt 1):1623–1628.
 86. Simons FE, Imada M, Li Y, Watson WT, HayGlass KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int Immunol* 1996;**8**:1937–1945.
 87. Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005;**116**:608–613.
 88. Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2008;**122**:951–960.
 89. Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci U S A* 2004;**101**(Suppl 2):14677–14682.
 90. Purohit A, Niederberger V, Kronqvist M, Horak F, Gronneberg R, Suck R et al. Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v 1 derivatives. *Clin Exp Allergy* 2008;**38**:1514–1525.
 91. Kahlert H, Suck R, Weber B, Nandy A, Wald M, Keller W et al. Characterization of a hypoallergenic recombinant Bet v 1 variant as a candidate for allergen-specific immunotherapy. *Int Arch Allergy Immunol* 2008;**145**:193–206.
 92. Valenta R, Ferreira F, Focke-Tejkl M, Linhart B, Niederberger V, Swoboda I et al. From allergen genes to allergy vaccines. *Annu Rev Immunol* 2010;**28**:211–241.
 93. Valenta R, Niespodziana K, Focke-Tejkl M, Marth K, Huber H, Neubauer A et al. Recombinant allergens: what does the future hold? *J Allergy Clin Immunol* 2011;**127**:860–864.
 94. Creticos PS, Schroeder JT, Hamilton RG, Balcer-Whaley SL, Khattignavong AP, Lindblad R et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006;**355**:1445–1455.
 95. Kundig TM, Senti G, Schnetzler G, Wolf C, Prinz Vavricka BM, Fulurija A et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy

- adults. *J Allergy Clin Immunol* 2006;**117**:1470–1476.
96. Schmitz N, Dietmeier K, Bauer M, Maudrich M, Utzinger S, Muntwiler S et al. Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. *J Exp Med* 2009;**206**:1941–1955.
 97. Focke M, Swoboda I, Marth K, Valenta R. 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**:385–397.
 98. Edlmayr J, Niespodziana K, Linhart B, Focke-Tejkl M, Westritschnig K, Scheiblhofer S et al. A combination vaccine for allergy and rhinovirus infections based on rhinovirus-derived surface protein VP1 and a nonallergenic peptide of the major timothy grass pollen allergen Phl p 1. *J Immunol* 2009;**182**:6298–6306.
 99. Niespodziana K, Focke-Tejkl M, Linhart B, Civaj V, Blatt K, Valent P et al. A hypoallergenic cat vaccine based on Fel d 1-derived peptides fused to hepatitis B PreS. *J Allergy Clin Immunol* 2011;**127**:1562–1570 e6.
 100. Slater JE, Paupore E, Zhang YT, Colberg-Poley AM. The latex allergen Hev b 5 transcript is widely distributed after subcutaneous injection in BALB/c mice of its DNA vaccine. *J Allergy Clin Immunol* 1998;**102**:469–475.
 101. Hochreiter R, Stepanoska T, Ferreira F, Valenta R, Vrtala S, Thalhamer J et al. Prevention of allergen-specific IgE production and suppression of an established Th2-type response by immunization with DNA encoding hypoallergenic allergen derivatives of Bet v 1, the major birch-pollen allergen. *Eur J Immunol* 2003;**33**:1667–1676.
 102. Roesler E, Weiss R, Weinberger EE, Fruehwirth A, Stoecklinger A, Mostböck S et al. Immunize and disappear-safety-optimized mRNA vaccination with a panel of 29 allergens. *J Allergy Clin Immunol* 2009;**124**:1070–1077 e1–11.