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Allergen immunotherapy: 100 years, but it does not look like

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Summary
Allergen immunotherapy (AIT) is the only treatment able to act on the causes and not merely on the symptoms of allergy. AIT was introduced 100 years ago but remained an empirical treatment for more than 40 years, when the first controlled trial in 1954 opened the era of scientific evidence. A major advance was the introduction of venom immunotherapy to prevent anaphylaxis from insect stings in 1978. Concerning inhalant allergens, currently AIT may be administered in two forms, subcutaneous (SCIT), and sublingual immunotherapy (SLIT). A large number of trials, globally analyzed in a number of meta-analyses, gave sound evidence to the efficacy and safety of SCIT and SLIT in allergic rhinitis and asthma. Adverse systemic reactions are still a drawback for SCIT, while safety and tolerability of SLIT are very good, provided recommended doses and schedules of administration are used. A significant advance for SLIT development was the registration in Europe of the standardized quality tablets. New applications, such as food allergy and atopic dermatitis, as well as new routes of administration, are currently under evaluation. After 100 years of use, AIT has a central role in the management of allergy and the ongoing improvement seems able to warrant to AIT an even brighter future.

Introduction

Allergen immunotherapy (AIT) is the only treatment targeting the causes of allergy (1). In fact, drug treatment is clinically efficacious but acts only on symptoms, and allergen avoidance is theoretically able to reduce the sensitivity to the specific allergen, such as the house dust mite or cat epithelium, but a complete avoidance is hardly feasible. By AIT, gradually increasing doses of the specific causative allergen are administered to reduce the clinical reactivity of allergic subjects. AIT was introduced 100 years ago as a treatment for pollen allergy (2), but its effectiveness was scientifically demonstrated in 1954, when the first controlled trial was published (3), paving the way to a high number of trials in the ensuing years. In 1978, the introduction of venom immunotherapy for subjects allergic to Hymenoptera stings was a major achievement for AIT, because of the complete capacity to prevent fatal reactions to stings and the good safety of this treatment (4). Concerning inhalant allergens, until the 1980s the only way to administer AIT was by the injective, subcutaneous route, but the availability in the market of high biological potency allergen extracts raised the issue of safety because of a series of fatal systemic reactions to treatment (5, 6). In 1986 a new AIT method of administration for aeroallergens, by the sublingual route, was introduced (7)

and in the following years it proved as an effective and safe treatment for respiratory allergy and thus as a true option to the injection route (8). To date, a very large number of randomized clinical trials (RCTs) provided a solid evidence of efficacy of both subcutaneous immunotherapy (SCIT), and sublingual immunotherapy (SLIT) that was accurately examined by the tool of meta-analysis.
An important aspect in the RCTs on AIT is the proper assessment of baseline severity of the allergic disease. In many RCTs a run-in season without AIT is lacking. A retrospective symptom score for the evaluation of baseline disease severity is not reliable, as suggested by the significant difference between the retrospectively and prospectively assessed symptom scores in the placebo group observed in the RCTs that evaluated such issue. The lack of a run-in period using the same symptom score as during AIT makes difficult to assess the severity of the allergic disease (in most cases allergic rhinoconjunctivitis) and this biases the randomization of patients and the reliable assessment of long-term outcome of AIT.

Efficacy and safety of SCIT with inhalant allergens

Efficacy of SCIT in allergic asthma

The first meta-analysis on SCIT was performed in 1995, including 20 double-blind, placebo controlled RCTs of immunotherapy on patients with allergic asthma (9). Significant differences in favour of active treatment over placebo were detected for symptomatic improvement (odds ratio 3.2), for reduction in medication (odds ratio 4.2, and reduction in bronchial hyperreactivity, BHR (odds ratio 6.8). The same authors repeated the meta-analysis in 2003, when 75 RCTs were available (10). At that time, the parameter of meta-analysis had become the standardized mean difference (SMD). A significant improvement in asthma symptom scores in actively treated compared to placebo treated (SMD -0.72), a significant reduction of allergen specific BHR, and a reduction in non-specific BHR as well, were found. The overall results of the previous meta-analysis were thus confirmed considering a much higher number of RCTs. The latest meta-analysis update was done in 2010, including 88 RCTs (11). Of them, 42 dealt with dust mite allergy, 27 with pollen allergy, 10 with animal epithelia, 2 with for Cladosporium mould allergy, 2 with latex and 6 with multiple allergens. There was a significant improvement in asthma symptom scores (SMD -0.59) and, using a number needed to treat (NNT) analysis, it would have been necessary to treat 3 patients with SCIT to avoid one deterioration in asthma symptoms and to treat 4 patients to avoid one requiring increased medication. SCIT significantly reduced allergen specific BHR and reduced non-specific BHR.

Efficacy of SCIT in allergic rhinitis

The only meta-analysis thus far available for allergic rhinitis (AR) was performed by Calderon et al. in 2007, who included 51 RCTs (12). Symptom score data from 15 trials were suitable for the analysis and showed an overall reduction in the SCIT group (SMD -0.73). Medication score data were suitable from 13 RCTs and showed an overall reduction in the SCIT group (SMD -0.57). The authors concluded that SCIT in properly selected patients with seasonal AR results in a significant reduction in symptom scores and medication use. A particular aspect is the efficacy of SCIT in patients with multiple sensitization to inhalant allergens; by reviewing studies simultaneously using 2 or more distinct allergen extracts it was found that 11 studies based on the administration of 2 extracts were effective, while in studies using multiple allergens 3 studies showed clear efficacy and in the other 2 studies inadequate doses of extracts or omission of clinically relevant allergens in the treatment regimen could account for the lack of efficacy (13). Thus, it was argued that simultaneous administration of more than 1 allergen extract is clinically effective, and this is relevant considering that most patients candidate to immunotherapy are sensitized to multiple allergens (14).

Safety of SCIT

The two reports in the 1980s of fatal anaphylactic reactions to SCIT (5, 6) made important the issue of safety, that actually was related to a decreasing use of such form of immunotherapy, especially after SLIT was introduced. Safety was also taken into account in the meta-analyses: in the conclusion of the latest meta-analysis on SCIT in asthma the authors stated that the possibility of local or systemic adverse effects (such as anaphylaxis) must be considered (11). In the meta-analysis on rhinitis, SCIT showed a relatively low risk of severe adverse events. Adrenaline was given in 0.13% (19 of 14085 injections) of those on active treatment and in 0.01% (1 of 8278 injections) of the placebo group for treatment of adverse events, and there were no fatalities (12).
Indeed, studies specifically addressed on SCIT safety are also available. In 2000, Mellerup et al. evaluated the safety of 3 different induction regimens of clustered immu-

notherapy (several injections administered during each visit) with both aqueous and alum depot extracts in 657 patients receiving 10369 injections (15). Overall, 454 systemic (immediate and late) reactions were observed in 257 patients corresponding to 4.4% of the injections and 39.1% of the patients. Most of the systemic reactions were not clinically important and less than 1% were anaphylactic reactions. Treatment with cat and mite allergen extracts showed the highest frequency of severe side-effects, probably because these extracts were used predominantly in asthmatic patients.

An observational retrospective study performed in Spain on 1147 patients treated by cluster schedules reported 42 systemic reactions in 39 patients, with a rate of reactions corresponding to 0.6% per injection and 3.4% per patient. No anaphylactic reactions occurred (16).

In a recent multicentric survey conducted in Italy using only standardized depot extracts, 1738 patients received SCIT from 8 different manufacturers, for a total of 2038 courses (300 patients received two extracts) (17). Overall, 95 reactions were observed in 57 patients (3.28%), corresponding to 4.7% of the courses and 1.56/1000 injections. Twenty-five patients had more than one adverse event. There were 34 grade 2, 60 grade 3 and one grade 4 reactions and no fatality. Systemic reactions occurred more frequently in patients with asthma than in patients with only rhinitis (4.1% vs. 1.1%), and were equally distributed between the build-up and the maintenance phase. Ragweed and grass extracts caused significantly more side effects than other allergens.

The important risk factors for severe anaphylactic reactions are: failure to observe patients for an appropriate time period, errors in dosage, failure to reduce the dosage after a longer than scheduled interval or during the pollen season, failure to postpone injection due of concomitant infection or asthma exacerbation, uses of multiple allergen extracts (1, 15). Globally, SCIT is well tolerated in most patients and anaphylactic reactions are rare, however the risk of a fatal reaction, although very low, may be considered unacceptable in treating patients with respiratory allergy, and this was a major impulse for the development of SLIT.

Efficacy and safety of SLIT

Efficacy of SLIT in allergic asthma

When the first meta-analysis on efficacy of SLIT was performed, there was an insufficient number of RCTs on asthmatic patients to allow a separate analysis (18). However, in the meta-analysis by Olaguibel et al. (19) including 7 RCTs conducted on children aged up to 14 years SLIT was significantly effective on asthma symptoms (SMD -1.42). The significantly positive effect in children was later confirmed by a meta-analysis of 9 studies, including a total number of 441 subjects, 232 actively treated and 209 placebo-treated patients [20]: a significant reduction was found in both symptoms scores (SMD -1.14; p = 0.02) and drug use (SMD -1.63) (20). By contrast, in a meta-analysis including 25 studies on subjects of any age, with a global number of 1706 patients, the SMD of the reduction of asthmatic symptoms did not reach the statistical significance (21). However, using the intention-to-treat method for outcome measures, significant decreases of asthma symptoms and drug consumption and significant increases of lung function and BHR were detected. The NNT to avoid leaving 1 patient with the same symptoms or worse was 3.7, that is, comparable to that reported for SCIT in asthmatic patients (11).

Efficacy of SLIT in rhinitis

The first meta-analysis was performed in 2005, when 22 RCTs were available (18), and showed a significantly higher efficacy of SLIT versus placebo, with an SMD corresponding to -0.42 for symptom scores and to -0.43 for medication scores. Differences concerning subgroups, such as the patients age and the kind of allergen, were not detected because of the relatively low numbers, but this aspect was assessed in subsequent analyses. In 2006 a meta-analysis on the efficacy of SLIT in children with rhinitis included 10 RCTs with an overall number of 484 patients (245 actively and 239 placebo-treated). A significant reduction of both symptoms (SMD -0.56) and medication (SMD -0.76) was observed (22). A notable aspect was highlighted from the sub-analysis addressing the length of treatment and the kind of allergen administered, that demonstrated a higher efficacy for durations longer than 18 months and for pollen allergens compared to house dust mites. In 2010, the global meta-analysis on rhinitis was updated, including 49 RCTs, 2333 patients treated with SLIT and 2256 treated with placebo (23). Significant reductions were confirmed for symptoms (SMD -0.49) and medication scores (SMD -0.32) in favour of the active treatment. Indeed, further recent meta-analyses addressed the kind of allergen. The meta-analysis limited to studies on grass pollen allergy found that SLIT

significantly reduces both symptoms (SMD -0.32) and medication use (SMD -0.33) compared with placebo, is more efficacious in adults than in children, and prolonging the duration of preseasonal treatment for more than 12 weeks improves the treatment efficacy (24). Concerning house dust mites, a meta-analysis on SLIT with mite extracts, showed "promising evidence of efficacy" but suggesting "more data, derived from large population-based high quality studies" (25).

If one considers that meta-analysis is not the perfect method, because of the heterogeneity of the included studies (that when statistically analyzed is generally highly significant), another approach is to evaluate single studies conducted on high numbers of patients that allow adequate statistical power. The recent preparations for SLIT in tablets of grass pollen extract were evaluated on large populations, including 855 adults treated by a timothy grass extract (26), 628 adults treated using a 5-grass pollen extract (27), and 278 children treated using the same 5-grass preparation (28). These studies, known as "big trials" showed a highly significant improvement in symptoms and rescue-medications scores in actively treated compared with placebo-treated patients during the grass pollen season. In addition, the big trials provided important observations concerning the dose dependence of clinical efficacy: only high doses, corresponding to 75.000 Standard Quality (SQ) in the trial with the timothy grass pollen (26), and to 300 index of reactivity (IR) in the trial with the 5-grass extract (27), were effective. Calculating the monthly cumulative dose in mcg of major allergen, the World Allergy Organization Position Paper on SLIT suggested as optimal the dose of 600 mcg of the grass pollen major allergen Phl p 1 (29).

Safety of SLIT

All meta-analyses on efficacy also evaluated the safety and found that the most common adverse events are local reactions in the mouth and in the gastrointestinal tract once the extract is swallowed, and that systemic reactions such as asthma, rhinitis, or urticaria are rare (18-23). In the meta-analysis by Calamita et al was also calculated the number needed to harm, that is, the number of patients to treat to have 1 adverse reaction, which corresponded to 14.3 (21). Most local reactions are self-limiting and easy to manage. Such good safety was confirmed by a systematic review, in which no difference in the rate of systemic reactions was found comparing trials using low allergen doses and high allergen doses, respectively (30). Anaphylactic reactions are extremely rare; an analysis of a series of case reports showed that in most cases the reaction is caused by a mistake, such as the assumption of very high doses or the use of inappropriate allergen mix (31). However, an increased risk is apparent in subjects in whom SLIT was indicated because of previous systemic reactions to SCIT (32, 33), especially when no updosing regimens are used (33), and this suggests to reconsider the occurrence of systemic reactions to SCIT as an admission criterion to SLIT (34).

Efficacy and safety of venom immunotherapy

The optimal treatment of allergic reactions to Hymenoptera stings is venom immunotherapy (VIT). This valuable therapy was introduced in the late 70s by two controlled studies using vespid venom (4) and honeybee venom (35), respectively. More recently, another double-blind placebo-controlled study demonstrated the efficacy of VIT with ant venom (36). For ethical reason, following the single demonstrations by double-blind trials, subsequent studies were conducted in an open fashion. A meta-analysis performed in 2000 on the available studies confirmed the clinical efficacy of VIT (37). Remarkably, to date no fatal reaction to stings was reported during the treatment, this attributing to VIT a complete capacity to prevent mortality. Concerning overall efficacy, VIT prevents any kind of reaction in more than 90% of treated patients, though a lower rate has been reported for honeybee venom (38). Most residual reactions are slight-moderate, though also severe reactions were reported. In case of incomplete protection, doubling the maintenance dose to 200 mcg is recommended, but in some patients even higher doses may be needed (39).

VIT has a good safety profile and noteworthy no fatal reaction to treatment, differently from immunotherapy with inhalant allergens, was reported. However, as emerged in a number of studies, that were analyzed in a recent systematic review, VIT with honeybee venom has a lower safety compared with vespid venom. In fact, the rate of side-effects is significantly higher with honeybee venom (25%) than with vespid venom (5.8%) (40). Thus, to improve the safety of bee venom is a need to meet for VIT. A number of approaches have been tried: 1) using venom allergoids, that is, monomethoxy-polyetilen glycol-modified venom, resulted in better safety but lower efficacy; 2) using retard, alum-adsorbed preparations gave a significantly lower rate of large local reactions but not of systemic reactions; 3) similar results on significant reductions

only of large local reactions were obtained using purified vs. nonpurified venoms; 4) the preventive medication with antihistamines reduced both local and systemic reactions; 5) the treatment with the anti-IgE antibody omalizumab prevented severe systemic reactions allowing to reach the maintenance dose (40, 41).

A recent study reported that baseline serum tryptase levels may be useful as predictors of side effects during the build-up phase of VIT, however a significant association was detected for wasp venom but not for bee venom VIT (42). Interestingly, another study showed that tryptase levels decline during long-term VIT (median time 4.2 years, range 2-12 years), as measured by a 2.5% decrease per year; the authors suggested that such decrease could be induced by a dampened mast cell function or a decline in mast cell burden during VIT (43).

These observations highlight the issue of mastocytosis in HVA. Such condition is less rare than previously believed and recent studies demonstrated that VIT is effective and relatively safe (though the incidence of side-effects is increased, especially with vespid venom) in venom-allergic patients with mastocytosis (44).

Concerning the optimal duration of VIT, 3-5 years are currently recommended, based on the observations from several studies showing long-lasting protection from stings after 3-5 years of treatment (45-47). In the most recent study, of 181 patients who underwent VIT for more than 3 years, 100 (55.2%) were stung after discontinuing the treatment. At the time of the first sting after stopping VIT, 8 patients had a systemic reaction. Of 40 patients who were stung more than once after stopping VIT, 7 (17.5%) had more severe reactions with the subsequent stings. All the patients reported that their reactions after ending VIT were milder than before treatment (47). However, in patients with systemic reactions to VIT or not completely protected from stings longer durations are recommended (41). In patients with mastocytosis, because of reports of fatal reactions to stings following discontinuation of VIT (48), a life-long duration of the treatment is recommended (44).

The history of AIT through the guidelines on rhinitis and asthma

In their brilliant article on the centenary year of AIT, Durham and Leung reviewed the recommendations from the guidelines of the World Health Organization and the US practice parameters for immunotherapy (49). To review the development of the opinion on AIT through the position papers on allergic rhinitis (Allergic Rhinitis and its Impact on Asthma – ARIA) and asthma (Global Initiative for Asthma – GINA) it is also interesting. The first ARIA document stated that "There is good evidence that immunotherapy using inhalant allergens to treat seasonal or perennial rhinitis and asthma is clinically effective" (50). In the 2008 update the statement was modified to "There is sound evidence that immunotherapy using inhalant allergens is clinically effective in the treatment of allergic rhinitis and asthma" (51).

Concerning the GINA guidelines, in the original document in 1995 it was stated "Specific immunotherapy, directed at treating an underlying allergy to grass and other pollen, domestic mites, animal dander, or Alternaria, may be considered when avoiding allergens is not possible or appropriate medications fail to control asthma symptoms" (52). No significant changes were done in the subsequent updates until 2005, when it was affirmed "Several studies have demonstrated that specific immunotherapy using extracts of common aeroallergens may have some benefit in patients with allergic asthma, but several large, well-conducted studies have not demonstrated such a benefit" (53). In the 2010 update the available evidence from meta-analysis lead to acknowledge that "A Cochrane review that examined 75 randomized controlled trials of specific immunotherapy compared to placebo confirmed the efficacy of this therapy in asthma in reducing symptom scores and medication requirements, and improving allergen-specific and non-specific airway hyperresponsiveness" (53). This seems to substantially mirror the evolution of AIT.

Possible development of AIT

A major advance for the modern AIT is the ongoing process of pharmaceutical registration of the products. Future perspectives for AIT include the introduction of new materials, new applications in fields other than respiratory allergy and insect venom allergy, and new routes of allergen administration (Tab. 1).

New materials for AIT

The recently introduced preparations in standardized tablets for grass pollen allergy, that were developed to fulfil the requirements of the regulatory agencies and made possible to assess allergen extracts with the same methods used for drugs, currently represent the optimal materials to be used for SLIT and are registered in Europe with the indi-

Table 1 - Future perspectives for AIT

New materials	Recombinant allergens
	Modified allergens
	Hypoallergenic isoforms
	Tolerogenic peptides
	Adjuvants
New applications	Food allergy
	Latex allergy
	Atopic dermatitis
New routes of administration	Intralymphatic
	Epicutaneous

cation for treatment of grass pollen allergy in adults and children. The availability of such material also for other allergens is needed and is currently under evaluation.
The new proposed materials for AIT concern active components, such as recombinant allergens (54), modified allergens, hypoallergenic isoforms and tolerogenic peptides, as well as adjuvants able to enhance the efficacy, such as monophosphoryl lipid A (55) or bacterial DNA, the latter inducing tolerance to allergens through the stimulation of the Toll-like receptors, that control innate immunity. These materials are aimed at improving the safety of SCIT, as with hypoallergenic isoforms and tolerogenic peptides, or the clinical efficacy, especially concerning asthma (56, 57).

New applications for AIT

The consensus documents and guidelines on AIT currently consider only respiratory allergy and insect venom allergy, but there are other important fields of possibly useful application. Food allergy, especially in the clinical form of anaphylactic reactions, that may occur also to inadvertent assumption of small amounts of the culprit food, would be an important application for AIT, with a significance comparable to that for insect venom allergy. However, the first attempts to desensitize patients allergic to peanut by SCIT were burdened by frequent and severe adverse reactions (58), and thus the injective route was abandoned. Positive results in terms of both efficacy and safety were obtained by SLIT using an hazelnut extract (59) and a peach extract quantified in major allergen Pru p 3 (60). A number of studies on AIT with food extracts are presently ongoing and it is likely they will make AIT a treatment option for an allergy that currently can be managed only by avoidance of the culprit food and by drug treatment of the reactions (61).
Immunotherapy for latex allergy has similar features. In fact, the first studies on SCIT with standardized latex extracts reported an high rate of side effects (62), while subsequent trials using SLIT showed good efficacy and safety (63). Hypoallergenic preparations for SCIT are also proposed (63). Currently, latex extracts for SLIT are commercially available and this new indication is likely to be included in next consensus documents.
In addition, a number of studies showed that AIT, in both subcutaneous and sublingual routes, is feasible for use in atopic dermatitis, which has long been considered a possible trigger to worsen the disease severity and thus a contra-indication. In fact, especially when atopic dermatitis is correlated to hypersensitivity to house dust mites, good clinical results were achieved in most studies (64).

New routes of administration

New routes of administration were recently proposed, based on intralymphatic and epicutaneous administration, both showing encouraging results in first studies (65-67). In particular, in a trial on grass pollen allergy 165 patients were randomized to receive either 54 injections with grass pollen extract over 3 years or 3 intralymphatic injections over 2 months; despite the great difference in cumulative allergen doses, the intralymphatic administration was as effective as standard SCIT, and was significantly better tolerated (65). In an animal model, also Hymenoptera venom was effective and safe by intralymphatic injection (66).
The study on epicutaneous immunotherapy concerned a small population, including 37 patients with grass pollen allergy, 21 of them being actively treated and 16 being placebo treated; active treatment achieved significantly lower scores to specific nasal challenge and was well tolerated (67). Further investigations are needed to consider these new routes of AIT for possible use in current practice.

Conclusions

AIT has a 100 year-long history but the continuous advances in the scientific knowledge of the immunologic mechanisms underlying hypersensitivity or tolerance to allergens have lead to a significant evolution of this treatment. The refinement of the materials to be used in AIT

offers to patients allergic to inhalant allergens and to insect venom a valuable treatment acting on the causes of allergy. The ongoing process concerning new applications and new routes of administration seems able to warrant to AIT an even brighter future.

References

1. Bousquet J, Lockey R, Malling HJ (eds). Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO Position Paper. J Allergy Clin Immunol 1998; 102:558-62.
2. Noon L, Cantab BC, Eng FR. Prophylactic inoculation against hay fever. Lancet 1911;177;1572-3.
3. Frankland A, Augustin R. Prophylaxis of summer hay fever and asthma: a controlled trial comparing crude grass pollen extract with the isolated main protein components. Lancet 1954;266;1055-8.
4. Hunt KJ, Valentine MD, Sobotka AK, et al. A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med 1978;299:157-61.
5. Committee on Safety in Medicine: CSM update: desensitizing vaccines. BMJ 1986; 293;948.
6. Lockey RF, Benedikt LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987;79; 660-77.
7. Scadding GK, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. Clin Allergy 1986;6:483-91.
8. Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. J Allergy Clin Immunol 2003;111:437-48.
9. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials Am J Respir Crit Care Med 1995;151:969-74.
10. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. Cochrane Database Syst Rev 2003;4: CD001186.
11. Abramson MJ, Puy RM, Weiner JM. Injection immunotherapy for asthma. Cochrane Database Syst Rev 2010;8: CD001186.
12. Calderon MA. Alves B, Jacobson M, et al. Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 2007;1: CD001936.
13. Nelson H. Multiallergen immunotherapy for allergic rhinitis and asthma. J Allergy Clin Immunol 2009;123:763-9.
14. Ciprandi G, Alesina R, Ariano R, et al. Characteristics of patients with allergic polysensitization: the POLISMAIL study. Eur Ann Allergy Clin Immunol 2008;40: 77-83.
15. Mellerup MT, Hahn GW, Poulsen LK, Malling H. Safety of allergen-specific immunotherapy. Relation between dosage regimen, allergen extract, disease and and systemic side-effects during induction treatment. Clin Exp Allergy 2000;30: 1423-29.
16. Serrano P, Justicia JL, Sanchez C, et al. Systemic tolerability of specific subcutaneous immunotherapy with index-of-reactivity-standardized allergen extracts administred using clustered regimens: a retrospective, observational, mutlicenter study. Ann Allergy Asthma Immunol 2009;102:247-52.
17. Schiappoli M, Ridolo E, Senna G, et al. A prospective Italian survey on the safety of subcutaneous immunotherapy for respiratory allergy. Clin Exp Allergy 2009;39: 1569-74.
18. Wilson DR, Torres-Lima M, Durham S: Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;60: 4-12.
19. Olaguibel JM, Alvarez Puebla MJ. Efficacy of sublingual allergen vaccination for respiratory allergy in children. Conclusions from one meta-analysis. J Investig Allergol Clin Immunol 2005;15:9-16.
20. Penagos M, Passalacqua G, Compalati E, et al. Meta-analysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. Chest 2008;133: 599-609.
21. Calamita Z, Saconato H, Pela AB, Atallah AN: Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. Allergy 2006;61:1162-72.
22. Penagos M, Compalati E, Tarantini F, et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3-18 years of age: a meta analysis of randomized placebo-controlled, double blind trials. Ann Allergy Asthma Immunol 2006;97:141-8.
23. Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. Cochrane Database Syst Rev 2010;12: CD002893.
24. Di Bona D, Plaia A, Scafidi V, Leto-Barone MS, Di Lorenzo G: Efficacy of sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a systematic review and meta-analysis. J Allergy Clin Immunol 2010;26: 558-66.
25. Compalati E, Passalacqua G, Bonini M, Canonica GW. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. Allergy 2009;64:1570-79.
26. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once daily grass allergen tablet: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;117:802-9.
27. Didier A, Malling HJ, Worm M, et al. Optimal dose, efficacy, and safety of once daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol 2007;120:338-45.
28. Wahn U, Tabar A, Kuna P, et al. SLIT Study Group. Efficacy and safety of 5-grass pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. J Allergy Clin Immunol 2009;123:160-6.
29. Canonica GW, Bousquet J, Casale T, et al (eds). Sublingual immunotherapy: World Allergy Organization Position Paper 2009. Allergy 2009;64 (suppl 91):1-59.
30. Gidaro GB, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. Clin Exp Allergy 2005;35:565-71.
31. André C, Fadel R: Anaphylaxis caused by allergen sublingual immunotherapy? Allergy 2007;62 :1220-1.
32. de Groot H, Bijl A. Anaphylactic reaction after the first dose of sublingual immunotherapy with grass pollen tablet. Allergy 2009;64:963-4.
33. Cochard MM, Eigenmann PA. Sublingual immunotherapy is not always a safe alternative to subcutaneous immunotherapy. J Allergy Clin Immunol 2009;124:378-79.
34. Incorvaia C, Mauro M. Do indications to sublingual immunotherapy need to be revised? J Allergy Clin Immunol 2010;125:277-8.

35. Müller U, Thurnheer U, Patrizzi R, Spiess J, Hoigné R. Immunotherapy in bee sting hypersensitivity. Bee venom versus whole body extract. Allergy 1979;34:369-78.
36. Brown SG, Wiese MD, Blackman KE, Heddle RJ. Ant venom immunotherapy: a double-blind, placebo-controlled, crossover trial. Lancet 2003;361:1001-6.
37. Ross RN, Nelson HS, Finegold I. Effectiveness of specific immunotherapy in the treatment of hymenoptera venom hypersensitivity: a meta-analysis. Clin Ther 2000;22:351-8.
38. Müller U, Helbling A, Berchtold E. Imunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol 1992;89:529-35.
39. Rueff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving conventional Hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy Clin Immunol 2001;108:1027-32.
40. Incorvaia C, Frati F, Dell'Albani I, et al. Safety of hymenoptera venom immunotherapy: a systematic review. Exp Opin Pharmacother 2011; Epub Ahead of print.
41. Bilò BM, Bonifazi F. Hymenoptera venom immunotherapy. Immunotherapy 2011;3:229-46.
42. Ruëff F, Przybilla B, Bilò MB, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of basseline serum tryptase. J Allergy Clin Immunol 2010;126:105-11.
43. Dugas-Breit S, Przybilla B, Dugas M, et al. Serum concentration of baseline mast cell tryptase: evidence for a decline during long-term immunotherapy for Hymenoptera venom allergy. Clin Exp Allergy 2010;40:643-649.
44. Bonadonna P, Zanotti R, Müller U. Mastocytosis and insect venom allergy. Curr Opin Allergy Clin Immunol 2010;10:347-53.
45. Lerch E, Müller U. Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. J Allergy Clin Immunol 1998;101:606-12.
46. Golden DBK. Discontinuing venom immunotherapy. Curr Opin Allergy Clin Immunol 2001;1:353-6.
47. Hafner T, DuBuske L, Kosnik M. Long term efficacy of venom immunotherapy. Ann Allergy Asthma Immunol 2008;100:162-5.
48. Oude Elberink JN, De Monchy JGR, Kors JW, et al. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. J Allergy Clin Immunol 1997; 99:153-4.
49. Durham SR, Leung DYM. One hundred years of allergen immunotherapy: time to ring the changes. J Allergy Clin Immunol 2011;127:3-7.
50. 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):S147-334.
51. Bousquet J, Khaltaev N, Cruz AA (Eds). Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA2LEN and AllerGen). Allergy, 2008, 63(Suppl. 86):8-160.
52. NHLBI/WHO Workshop report. Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma. January 1995, NIH Publication No. 02-3659.
53. http://www.ginasthma.com
54. Pauli G, Larsen TH, Rak S, et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. J Allergy Clin Immunol 2008;122:951-60.
55. Drachenberg HJ, Wheeler H. 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; 56:498-505.
56. Passalacqua G, Lombardi C, Troise C, Canonica GW. Sublingual immunotherapy: certainties, unmet needs and future directions. Eur Ann Allergy Clin Immunol 2009; 41:163-70.
57. Nagata M, Nakagome K. Allergen immunotherapy in asthma: current status and future perspectives. Allergol Int 2010;59:15-9.
58. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. J Allergy Clin Immunol 1992; 90:256-62.
59. Enrique E, Pineda F, Malek T, 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;116:1073-9.
60. Fernandez-Rivas M, Garrido FS, Nadal JA, et al. Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p3 quantified peach extract. Allergy 2009;64:876-83.
61. Kurihara K. Immunotherapy for food allergy. Allergol Int 2010;59:9-14.
62. Leynadier F, Herman D, Vervloet D, Andrè C. Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. J Allergy Clin Immunol 2000;106:585-90.
63. Rolland JM, Drew AC, O'Hehir RE. Advances in development of hypoallergenic latex immunotherapy. Curr Opin Allergy Clin Immunol 2005;5:544-51.
64. Novak N. Allergen specific immunotherapy for atopic dermatitis. Curr Opin Allergy Clin Immunol, 2007;7:542-6.
65. Senti G, Prinz Vavricka BM, Erdmann I, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. Proc Natl Acad Sci USA 2008;105:17908-12.
66. Martinez-Gomez JM, Johansen P, Erdman I, et al. Intralymphatic injections as a new administration route for allergen-specific immunotherapy. Int Arch Allergy Immunol 2009;50:59-65.
67. Senti G, Graf N, Haug S, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. J Allergy Clin Immunol 2009;124:997-1002.