Allergen specific sublingual immunotherapy in children with asthma and allergic rhinitis

Ivana Đurić-Filipović, Marco Caminati, Gordana Kostić, Đorđe Filipović, Zorica Živković
Belgrade, Serbia

Background: **The incidence of asthma and allergic rhinitis (AR) is significantly increased, especially in younger children. Current treatment for children with asthma and allergic rhinitis include allergen avoidance, standard pharmacotherapy, and immunotherapy. Since standard pharmacotherapy is prescribed for symptoms, immunotherapy at present plays an important role in the treatment of allergic diseases. This article presents insights into the up-to-date understanding of immunotherapy in the treatment of children with allergic rhinitis and asthma.**

Data sources: **PubMed articles published from 1990 to 2014 were reviewed using the MeSH terms "asthma", "allergic rhinitis", "children", and "immune therapy". Additional articles were identified by hand searching of the references in the initial search.**

Results: **Numerous studies have shown that sublingual application of allergen specific immunotherapy (SLIT) is an adequate, safe and efficient substitution to subcutaneous route of allergens administration (SCIT) in the treatment of IgE-mediated respiratory tract allergies in children. According to the literature, better clinical efficacy is connected with the duration of treatment and mono sensitized patients.**

Conclusions: **At least 3 years of treatment and stable asthma before the immunotherapy are positive predictors of good clinical efficacy and tolerability of SLIT. SLIT reduces the symptoms of allergic diseases and the use of medicaments, and improves the quality of life of children with the diseases.**

World J Pediatr 2016;12(3):283-290

Key words: **allergic asthma; allergic rhinitis; allergic sensitization; immunotherapy**

Review article

Allergen-specific immunotherapy (AIT) or allergen vaccination is to treat allergic subjects by using increased amounts of allergen(s) (allergenic extract or vaccine) to achieve desensitization that is to reduce the appearance of symptoms during the natural exposure to the allergen.[1] The results of immunotherapy were reported in the beginning of the 19th century,[2] but the interest in the mucosal route was re-examined by a group of German investigators in the 1970s. It is important to point out that many trials with SLIT in the past were small in sample size and/or had an open label design.

According to the literature, sublingual application of allergen specific immunotherapy (SLIT) induces three categories of immunological changes: modulation of allergen-specific antibody responses; reduction in recruitment and activation of pro-inflammatory cells; and changes in the pattern of allergen specific T-cell responses.[3]

During pollen SLIT, allergen-specific IgE increases in weeks although it is not associated with adverse events. The early increase of allergen-specific IgE is followed by blunting of seasonal rises in IgE and an increase in allergen-specific IgE/IgG4. These elevations are both time and allergen-dose dependent[4] and progressive for at least 2 years[5] although the magnitude is lower than that observed during SCIT.[6,7] Studies showed increases in specific IgG4 in the absence of demonstrable efficacy,[8] whereas others showed no difference in IgG levels, likely related to the lower allergen doses employed[9] particularly in relation to mite SLIT.[10,11] Functional assays showed that a serum obtained after grass pollen SLIT was able to inhibit IgE-binding *in vitro*.[5]

Author Affiliations: Faculty of Medical Science Kragujevac, Department of Immunology, Svetozara Markovica 69, 34000 Kragujevac, Serbia (Đurić-Filipović I); Unita di Allergologia Centro Regionale di riferimento per la prevenzione, la diagnosi e la terapia delle malattie allergiche Ospedale Universitario Borgo Trento, Piazzale Stefani 1, Verona, Italy (Caminiati M); Children's Hospital, Clinical Centre Kragujevac, Zmaj Jovina 30, 34000 Kragujevac, Serbia (Kostić G); Institution for Emergency Medical Care, Bulevar Franša Depera 5, 11000 Belgrade, Serbia (Filipović Đ); Children's Hospital for Lung Diseases and Tuberculosis, Medical Center "Dr Dragisa Misovic", Heroja Mihajla Tepića 1, 11000 Belgrade, Serbia (Živković Z)

Corresponding Author: Ivana Đurić-Filipović, MD, Branka Krsmanovića 11/13, Belgrade, Serbia (Tel: +38163512678; Email: drivanica@yahoo.com)

doi: 10.1007/s12519-016-0022-1

©Children's Hospital, Zhejiang University School of Medicine, China and Springer-Verlag Berlin Heidelberg 2016. All rights reserved.

Healthy and allergic subjects exhibit all 3 subsets of Th-lymphocytes although in different proportions. IL-10 secreting Treg cells are the dominant subset against common environmental allergens in healthy subjects, whereas allergen-specific IL-4–secreting T cells (Th2-like) exist in high frequency in allergic subjects. Peripheral tolerance to allergens involves multiple suppressive factors, such as IL-10, TGF-β, cytotoxic T lymphocyte antigen (CTLA-4), and programmed death 1 (PD-1). A change in the dominant subset might lead to either the development of allergy or its reversal.[12] A recent study[13] also showed the novel mechanism for the inhibition of tolerance induction by a Th2-type immune response via GATA3 which directly binds to the FOXP3 promoter region and inhibits its expression. Similar to Th cells, B cells can be classified into subsets according to the cytokines that they produce. One functional B-cell subset, Breg cells, has recently been shown to contribute to the maintenance of the fine equilibrium required for tolerance. Breg cells control excessive inflammatory responses through IL-10, thereby inhibits proinflammatory cytokines and supports Treg cell differentiation.[14]

Immunotherapy is to reorient allergen specific immune response in the course of IL-10 secreting cells such as Treg and Breg cell subsets.[15] The immunologic mechanisms of SLIT are less well-established than those of subcutaneous immunotherapy. In children or adults with seasonal allergic rhinoconjunctivitis to grass pollen, no significant effect of SLIT on T-cell functions (i.e. cytokine production and proliferation) was observed in several studies.[9,16-18] SLIT did not induce any detectable changes in dendritic cells (DCs) nor T-lymphocytes in the epithelium or lamina propria of the oral mucosa. Immunization through the sublingual route was nevertheless shown in other studies to decrease the production of the Th2 cytokine IL-13 and the proliferation of peripheral blood mononuclear cells (PBMCs) from patients allergic to house dust mite.[19,20]

Suárez-Fueyo A et al[21] reported that grass tablet SLIT leads to increased (specific IgE and sIgG4) and Th2 responses during the first 4 weeks of therapy. They also found that production of blocking antibody and sIgG4 correlates with a decrease in sIgE synthesis and IL-4$^+$ Th2 responses. A reduction in IL-4 cell frequency correlates with increased frequency of T cells with a regulatory phenotype. By the second year of treatment, allergen desensitization is evident.

There is a growing evidence supporting the role of regulatory T cells in controlling the development of asthma and allergic disease in a variety of models, although it is not clear whether the subsets of regulatory T cells are the most important.[22] A revised hygiene hypothesis proposes that limited exposure to infectious pathogens during infancy, particularly mycobacteria and parasites, may prevent the establishment of both Th1 and T regulatory repertoire, explaining in part the increased prevalence of allergies in developed countries.[23] Regulatory T cells can control or regulate all effectors mechanisms activated during allergy and Th2 responses through the production of IL-10/TGF-β and/or cell to cell contact. IL-10 is a potent suppressor of total and allergen-specific IgE, whereas it induces an antibody isotype switch towards IgG4. TGF-β also decreases IgE production and induces immunoglobulin isotype switch towards IgA.[24] TGF-β contributes to the generation of both Th17 and Treg cells. TGF-β directs the peripheral conversion of effector T cells into Fox p3 Treg cells,[25] where as in the presence of IL-6, TGF-β promotes the generation of Th17 from naive T cells.[26]

There is an association between atopy and a defect in T reg functions. For example, children born with a dysfunctional Fox p3 gene presented with a deficit in CD4$^+$CD25$^+$ regulatory T cells develop severe autoimmune diseases often associated with eczema, elevated IgE levels, eosinophilia and food allergy [polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX) syndrome].[27]

A study[28] revealed that DCs from children with allergic rhinitis can be impaired in their capacity to

Table 1. Clinical efficacy in DBPC–RTC–part I

Author (ref)	Age range	Patients A/P	Allergen	Duration	Disease
Tari, 1990[32]	5-12	30/28	HDM	18 mon	AR
Hirsch, 1997[33]	6-16	13/14	HDM	12 mon	AR
Vourdas, 1998[34]	7-17	34/32	Olive	2 s	AR
La Rosa, 1999[35]	6-14	20/21	Parietaria	6 mon, 2 s	AR
Pajno, 2000[10]	8-15	12/12	HDM	2 y	A
Caffarelli, 2000[36]	4-14	24/20	Grass	3 mon	AR
Yuksel, 1999[37]	5-15	21/18	Grass	4 mon	AR
Bahceciler, 2001[38]	7-15	8/7	HDM	6 mon	AR
Ippoliti, 2003[19]	5-12	47/39	HDM	6 mon	AR
Pajno, 2003[39]	8-14	15/15	Parietaria	13 mon	RAS
Wuthrich, 2003[40]	6-13	10/12	Grass	2 y	AR
Bufe, 2004[41]	6-13	68/74	Grass	1+2 y	AR
Rolinck-Werninhause, 2004[9]	3-14	39/38	Grass	3 y	AR
Niu, 2006[42]	6-12	56/54	HDM	6 mon	A
Valovirta, 2006[43]	6-14	65/33	Hazelnut, birch, elm	18 mon	RC
Lue, 2006[8]	6-12	10/10	HDM	8 mon	A
Pham-Ti, 2007[44]	5-11	55/56	HDM	18 mon	A
Roder, 2007[45]	6-18	108/96	grass	2 y	RC
Wahn, 2008[46]	4-17	139/139	Grass	8 mon	RC
Bufe, 2009[47]	5-16	126/127	Grass		RC
Stelmach, 2009[48]	6-17	25/25	Grass	6 mon, 2 s	A

A/P: active *vs.* placebo group; HDM: house dust mite; A: asthma; AR: rhinitis allergica; RC: rhino conjunctivitis; s: season.

produce IL-10. Interestingly, allergen-specific IL-10-secreting Tr1 cells are highly represented in healthy individuals in comparison with allergen-specific IL-4-secreting Th2 cells, suggesting that regulatory T cells are predominant during natural immune responses to environmental allergens in nonatopic donors.[29,30] Regulatory T lymphocytes can control an established allergic response via distinct mechanisms: IL-10 and TGF-β decrease IgE production and enhance IgG4 and IgA production, respectively.

In addition, regulatory T cells exhibit a direct inhibitory effect on Th1 and Th2 T cells, through cell to cell contact, or by decreasing the antigen presenting function of DCs. Regulatory T cells producing IL-10 and/or TGF-β are induced not only in atopic patients by successful immunotherapy, but also during natural allergen exposure in healthy people. As per the hygiene hypothesis, limited exposure to bacteria and parasites in developed countries may result in a poor establishment of a Treg repertoire during childhood, thereby contributing to an increase in the frequency of allergies.[24,30]

Akdis et al[31] described that SLIT can induce multiple mechanisms and receptors such as IL-10, TGF-β, CTLA-4, PD-1, and histamine receptor 2 (HR2) which can play an important role in reorienting the immune response in allergic patients and correlate with the positive effects on clinical course of the disease.

Efficacy of immunotherapy on asthma and allergic rhinitis (AR)

Clinical efficacy of SLIT

The evaluation of clinical efficacy of SLIT relies on the assessment of symptom severity and rescue medication (Table 1).

Most clinical trials used the assessment of traditional symptom scores (graded from 0 to 3) plus recording of doses of rescue medications. In some trials, other evaluation parameters were applied, including visual analogue scale (VAS), combined score, symptom-free days and medication-free days. Most trials showed positive results for one or more parametrs (Table 2); one study presented negative results[42] and two studies reported partial or clinical efficacy.[33,42]

Tari et al[32] found clinical efficacy of SLIT in 58 children aged 5-12 sensitized to house dust mite (HDM) diagnosed with asthma and AR. After 18 months of continuous use of SLIT, they found a significant decrease of allergic symptoms and reduction of the use of recue medicines. Valovirta et al[43] first reported the effect of SLIT in 18 months at 2 different doses for tree/pollen allergy in 88 children with seasonal allergic rhinitis. They proved that the 18 months of SLIT can provide dose dependent benefits in terms of significant reduction of symptoms and medication.[43]

These studies revealed the efficacy of SLIT in

Table 2. Clinical efficacy in DBPC–RTC–part II

Author	Main positive results	No changes
Tari, 1990[32]	Symptom and drug score	
Hirsch, 1997[33]	For asthma	Medication score, rhinitis score, self-assessment
Vourdas, 1998[34]	Dyspnea score, conjunctivitis	Medication score, rhinitis score and global assessment
La Rosa, 1999[35]	Rhinitis score after 2 y	Medication score, R score after 1 y
Pajno, 2000[10]	Asthma s score after 2 y, nights, medication score 1st and 2nd year	Asthma score 1st y, VAS
Caffarelli, 2000[36]	TSS, asthma score, symptom-medicine score for high pollen count	Medication score and ocular score
Yuksel, 1999[37]	Antihistamine, rhinitis score, overall efficacy by doctor	Beta2 use, asthma score, PEF
Bahceciler, 2001[38]	Asthma score, beta2, PEF, exacerbation	Nasal symptom score
Ippoliti, 2003[19]	Asthma score, rhinitis score, FEV1	Drugs
Pajno, 2003[39]	Ocular score, VAS	Bronchial and nasal
Wuthrich, 2003[40]	Drug score, the 2nd y	Drug score 1st, symptom score
Bufe, 2004[41]	Symptom drug score only the 3rd y	Symptom and drug score
Rolinck-Werninhause, 2004[9]	Drug score, symptom drug score	Ocular, nasal, bronchial symptom score
Niu, 2006[42]	Nighttime, daytime, total asthma score, FEV1, FVC, global assessment	Oral steroids, PEF, FEV1 and FVC between groups
Valovirta, 2006[43]	Total symptom score, nose, ling, eye symptom during birch season	Total drug score, methacholine, skin test
Lue, 2006[8]	Night symptoms, day symptoms, FEV1, drugs *vs.* base line	Days, drugs , FEV1, PEF *vs.* placebo
Pham-Ti, 2007[44]	QOL (quality of life questionnaire)	Asthma symptoms, asthma medication, asthma free days
Roder, 2007[45]		Main daily score, symptom-free days, medication free days, QoL
Wahn, 2008[46]	Rhinitis score, meds, meds free day	
Bufe, 2009[47]	RC score, asthma score, meds, well day	
Stelmach, 2009[48]	Asthma score, asthma med	Eye symptoms

PEF: peak expiratory flow; FEV1: forced expiratory volume; FVC: forced vital capacity; VAS: visual analogue scale; QoL: quality of life.

reducing symptom scores during pollen season in children with rhinitis. Furthermore, there was also a significant reduction in the administration of medication. A large trial on SLIT assessed the effect of grass tablets on asthma in children aged 5 to 16 years.[41] Asthma symptoms (coughing, wheezing, shortness of breath, and exercise-induced symptoms) reduced significantly, whereas rescue medication reduced but not significantly. The allergens that were used in this study included *Phleum pratense*, 5-grass mix, *Parietaria* and *Betulaceae* pollens, and HDM.

Pajno et al[39] followed up 21 children with asthma symptoms (8-15 years old) who were sensitized to HDM for two years. In the first year, they did not find clinical improvement of the symptoms. Children in the experimental group used less medicaments comparing with those on standard pharmacotherapy.[39] In the second year symptom scores and the use of recue medicaments significantly decreased in the children on SLIT. A study from Taiwan proved the efficacy of SLIT on children with asthma sensitized to HDM concerning day and night symptoms scores and parameters of respiratory function.[42]

Vourdas et al[34] reported the improvement of dyspnea scores in children with asthma sensitive to olive pollen, but not the medication score. The main problem of meta-analysis is a large heterogeneity of the included trials, often without a proper sample size calculation. Thus, meta-analyses provide only suggestive evidence.

Beside DBPC-RCTs, there are also other clinical studies like open controlled trials and studies that compared two routes of administrations of allergens (most common SCIT and SLIT). Up to now, there is only one open controlled trial in pediatric population. In the mentioned study, Marcucci compared two different doses of SLIT. The results showed better efficacy in the terms of symptom and medication score in the group that received a higher dose.[49] Larenas-Linnemann et al[50] also reported positive effects of SLIT in children. Further studies[51,52] described long-term effects of SCIT and only one study showed possible long-term effects of HDM SLIT.[53] A study[54] reported steroid sparing effects of HDM allergen specific immunotherapy (ASIT).

Safety and tolerability of SLIT in allergic children

The overall safety of SLIT has been widely proven and accepted.[55] Though the safety profile should be demonstrated for the single extract of each brand,[56] life-threatening and non-life-threatening severe systemic adverse events (SAEs) related to SLIT are very rare. Among double blind-placebo controlled-randomized clinical trials (DB-PC-RCTs) for allergic asthma, allergic rhinitis or allergic rhino-conjunctivitis[55,57-69] involving children, one reports the use of epinephrine.[59] In one patient, enrolled in the placebo group, epinephrine was given at the investigational site because of wheezing, probably related to previous exposure to a grassy field according to the investigators. Within the active group, an inappropriate administration of epinephrine occurred in the emergency department where the patient was later diagnosed with viral pharyngitis. Another patient experienced a SLIT-related non life-threatening systemic reaction after the first dose of grass AIT (tablet). Epinephrine was administered but the investigator graded the severity of the event as moderate. In the real-life setting, five cases of SLIT-related SAEs described as anaphylaxis have been published (Table 3).[60-63] In two of them, epinephrine was administered.

Though not always clearly reported and uniformly classified, non life-threatening SAEs account for a minority of SLIT-related side effects. In DB-PC-RCT,[55,57-59] the prevalence of systemic adverse events was lower than 20%, and severe reactions were rated between 1% and 2% of total recorded events. In the real-life setting, most of the systemic reactions reported by post-marketing surveys were mild and resolved spontaneously without any treatment (Table 4).[39,60,64-70] Potential risk factors for systemic adverse reactions are still a matter of debate. Suboptimal administration conditions (use of non-standardized extracts, administration of products containing a mixture of many allergens, overdosing) have been reported as a potential trigger of SAEs.[69] Patient-related non-specific risk factors, though not clearly defined, include cardiovascular diseases and long-term therapy with non-cardioselective beta-blockers.[70] However, they are not usually considered as an absolute contra-indication to SLIT assumption. Uncontrolled asthma, oral lesions or infections and previously recurrent SAEs occurred with SCIT may represent specific risk factors.[70,71]

SLIT-related reactions mostly consist of local adverse events (LAEs). According to MeDRA classifi-cation,[72] they include signs and symptoms involving oropharyngeal and gastrointestinal reactions. It is quite difficult to accurately estimate the prevalence of LAEs as they are not always included in the safety analysis, nor deeply discussed in DB-PC-RCTs, which are not usually designed to evaluate treatment tolerability.[55,57-59] Furthermore, LAEs do not cause alteration of objective parameters so that recording them and grading their severity is not easy. It may account for a potential underestimation and the great variability of their prevalence, rated between 50% and 85%.[73,74] Many post-marketing surveys considering the pediatric population reported a large number of local reactions (Table 2). Most of them involved the oral mucosa but several cases of abdominal pain were also recorded.[39,60,64-68]

Table 3. Published cases of SLIT-induced systemic reactions described as anaphylaxis

References	Patient sex (age, y)	Allergen (manufacturer if stated)	Phase	Onset timing	Clinical presentation	Use of epinephrine
Aydogan, 2008[65]	F (11)	HDM, grass pollen mix (Stallergenes)	Maintenance	3 min	Abdominal pain, chest pain, fever, nausea	Not specified
Blazowski, 2008[69]	F (16)	HDM (stallergenes)	Maintenance (overdose)	10 min	Hypotension collapse, flushing, urticaria	Yes
Rodriguez-Perez et al, 2008[67]	F (7)	HDM, pecar tree	Maintenance	30 min	Wheezing, dyspnea, anxiety, flushing, dizziness	Yes
De Groot and Bijl, 2009[70]	M (13)	Grass (Grazax, ALK-Abello)	First dose	15 min	Generalized urticaria, swelling of tongue	No

HDM: house dust mite; SLIT: sublingual application of allergen specific immunotherapy.

Table 4. SLIT-induced adverse events reported in post-marketing surveys

References	Study population	Number of patients	Follow-up (mon)	Local reactions	Systemic reactions	Use of epinephrine
Di Rienzo et al, 1999[71]	Pediatric	268	3-84	1	7	0
Pajno et al, 2003[50]	Pediatric	354	36	6	11	0
Drachenberg et al, 2004[72]	Adult and Pediatric	43	12	15	19	0
Agostinis et al, 2005[73]	Pediatric	36	12-36	2	0	0
Di Rienzo et al, 2005[74]	Pediatric	126	24	9	0	0
Fiocchi et al, 2005[75]	Pediatric	65	12	7	6	0
Agostinis et al, 2008[76]	Pediatric	433	6-24	161	17	0
Rodriguez-Perez et al, 2008[67]	Adult and Pediatric	43	12	21	7	2
De Castro et al, 2013[77]	Pediatric	70	12	6	2	0

SLIT: sublingual application of allergen specific immunotherapy.

Tolerability does not threaten patient's safety but has a great effect on clinical outcomes.[75-77] In fact, in clinical trials and even more in the real life setting severity, persistence or simply poor awareness of local reactions may increase the risk of treatment discontinuation despite its efficacy.[77] A correlation between allergen/dose/treatment schedule and local AEs has not been clearly demonstrated even if they frequently occur after the administration of the first doses. Oral lesions or infections and previous AEs are considered patient-related specific risk factors.[71] However, it is crucial for treatment adherence that doctors recognize and grade local reactions and that patients know that they may occur without any risk for their safety. The World Allergy Organization has recently proposed a grading system for SLIT-induced local adverse events[78] that will certainly help in achieving the goal.

Quality of life studies

Quality of life (QOL) is an important issue in allergic rhinitis and has been evaluated in a number of studies that have shown how it is impaired in untreated patients and improved by effective treatment. However, there is only a few data concerning QOL after sublingual immunotherapy (SLIT). QOL assessment was based on the Rhinoconjunctivitis Quality of Life Questionnaire, which consists of 28 items distributed in 7 domains: sleep problems (3 items), general problems (7 items), practical problems (3 items), nasal problems (4 items), eye symptoms (4 items), activities (3 items), and emotions (4 items). Responses are scored on a 7-point Likert scale, whereas domains and overall scores are scored on a 0-to-6 scale, with lower scores indicating better QOL. When evaluating the effects of SLIT on QOL, a reduction of at least 1 point after treatment is considered clinically relevant.[79]

A recent study[80] showed the improvement of QOL in polisensitized patients with AR and/or asthma on SLIT. Bousquet et al[81] compared QOL in patients with asthma receiving SLIT and placebo, and the changes from baseline to end point showed significant differences in favour of the SLIT group compared with the placebo group.

However, Khinchi et al[82] observed no statistical significant difference in in QOL scores among three groups, i.e. SLIT, SCIT and placebo, using a 36-item short-form health survey (SF-36) questionnaire.

Future perspectives

AIT therapy is evolving rapidly, but some fundamental information potentially affecting therapeutic results is still missing. First, a better understanding of the mechanisms of immunomodulation is necessary to optimize their therapeutic effects. In order to reduce

the frequency and severity of adverse reactions and the risk of anaphylaxis, many novel approaches to allergen immunotherapy have been patented in the last decade. These modifications involved changes of the allergen to reduce IgE binding while maintaining T cell reactivity or immunogenicity. Such approaches include allergoids, recombinant allergen mutants or complexes, peptides, DNA sequences from allergens, and adjuvances.[83] Adjuvants are defined as chemical or bacterial products that enhance or alter the immune response to a defined antigen. In Europe (but not the USA), allergen extracts for subcutaneous immunotherapy were generally adsorbed to aluminum hydroxide (alum). The second widely used adjuvant is monophosphoryl lipid A (MPL), used in combination with tyrosine-adsorbed and glutaraldehyde-treated allergen (allergoid).[84]

Conclusions

Immunotherapy with its immune modulator effects is very important part in the treatment of children with asthma and allergic rhinitis. Current data have shown clinical efficacy of SLIT in children with AR, but the data on clinical efficacy of SLIT in asthma are controversial. Symptoms and rescue medicaments intake are a reasonable outcome measure, but objective parameters like FEV1 and PEF should be included as co primary endpoints.

Funding: None.
Ethical approval: Not needed.
Competing interest: None.
Contributors: All authors contribute to the data collection. Ivana Đurić-Filipović collected data on SLIT clinical efficacy and immunological effects. Marco Caminati collected data on safety and tolerability. Gordana Kostić collected data about quality of life, Đorđe Filipović reviewed and revised the manuscript, Zorica Živković proof-read the manuscript and gave critical input and discussion on the manuscript.

References

1 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper J Allergy Clin Immunol 1998;102:558-622.
2 Black JH. The oral administration of pollen: clinical report. J Lab Clin Med 1928;13:709-713.
3 Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. Allergy 2006;61:151-165.
4 Didier A, Malling HJ, Worm M, Horak F, Jager S, Montagut A, 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;120:1338-1345.
5 Dahl R, Kapp A, Colombo G, de Monchy JGR, Rak S, Emminger W, et al. Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. J Allergy Clin Immunol 2008;121:512-518.
6 Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004;172:3252-3259.
7 UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;117:319-325.
8 Lue KH, Lin YH, Sun HL, Lu KH, Hsieh JC, Chou MC. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. Pediatr Allergy Immunol 2006;17:408-415.
9 Rolinck-Werninghaus C, Kopp M, Liebke C, Lange J, Wahn U, Niggemann B. Lack of detectable alterations in immune responses during sublingual immunotherapy in children seasonal allergic rhinoconjunctivitis to grass pollen. Int Arch Allergy Immunol 2005;136:134-141.
10 Pajno GB, Morabito L, Barberio G, Parmiani S. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. Allergy 2000;55:842-849.
11 Bahceciler NN, Arikan C, Taylor A, Akdis M, Blaser K, Barlan IB, et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. Int Arch Allergy Immunol 2005;136:287-294.
12 Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic Individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004;199:1567-1575.
13 Mantel PY, Kuipers H, Boyman O, Rhyner C, Ouaked N, Ruckert B, et al. GATA3-driven Th2 responses inhibit TGF-beta1-induced FOXP3 expression and the formation of regulatory T cells. PLoS Biol 2007;5:e329.
14 Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol 2012;30:221-224
15 Laaksonen K, Junikka M, Lahesmaa R, Terho EO, Savolainen J. *In vitro* allergen-induced mRNA expression of signaling lymphocytic activation molecule by PBMC of patients with allergic rhinitis is increased during specific pollen immunotherapy. J Allergy Clin Immunol 2003;112:1171-1177.
16 Gabrielsson S, Soderlund A, Paulie S, van der Pouw Kraan TC, Troye- Blomberg M, Rak S. Specific immunotherapy prevents increased levels of allergen-specific IL-4 and IL-13-producing cells during pollen season. Allergy 2001;56:293-300.
17 Faith A, Richards DF, Verhoef A, Lamb JR, Lee TH, Hawrylowicz CM. Impaired secretion of interleukin-4 and interleukin-13 by allergen-specific T cells correlates with defective nuclear expression of NF-AT2 and jun B: relevance to immunotherapy. Clin Exp Allergy 2003;33:1209-1215.
18 Oldfield WL, Larché M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002;360:47-53.
19 Ippoliti F, De Santis W, Volterrani A, Lenti L, Canitano N, Lucarelli S, et al. Immunomodulation during sublingual therapy in allergic children. Pediatr Allergy Immunol 2003;14:216-221.

20 Fenoglio D, Puppo F, Cirillo I, Vizzaccaro A, Ferrera A, Tosca MA et al. Sublingual specific immunotherapy reduces PBMC proliferations. Eur Ann Allergy Clin Immunol 2005;37:147-151.
21 Suárez-Fueyo A, Ramos T, Galán A, Jimeno L, Wurtzen PA, Marin A, et al. Grass tablet sublingual immunotherapy down regulates the TH2 cytokine response followed by regulatory T-cell generation. J Allergy Clin Immunol 2014;133:130-138.
22 Hawrylowicz CM, O'Garra A. Potential role of interleukin-10 secreting regulatory T cells in allergy and asthma. Nature Rev Immunol 2005;5:271-283.
23 Taylor A, Verhagen J, Akdis CA, Akdis M. T regulatory cells in allergy and health: a question of allergen specificity and balance. Int Arch Allergy Immunol 2004;135:73-82.
24 Yazdanbakhsh M, Kremsner PG, Van Ree R. Allergy, parasites and the hygiene hypothesis. Science 2002;296:490-494.
25 Zhang L, Zhao Y. The regulation of Foxp3 expression in regulatory CD4(1) CD25(1)T cells: multiple pathways on the road. J Cell Physiol 2007;211:590-597.
26 Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 2006;441:231-234.
27 Gambineri E, Torgerson T, Ochs H. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of Foxp3, a critical regulator of T cell homeostasis. Curr Opin Rhumatol 2003;15:430-435.
28 Gentile D, Schreiber R, Howe-Adams J, Trecki J, Patel A, Angelini B, et al. Diminished dendritic cell interleukin 10 production in atopic children. Ann Allergy Asthma Immunol 2004;92:538-544.
29 Ling EM. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 2004;363:608-615.
30 Akdis C, Joss A, Akdis M, Blaser K. Mechanisms of IL10 induced T cell inactivation in allergic inflammation and normal response to allergens. Int Arch Allergy Immunol 2001;124:180-182.
31 Akdis M, Akdis C. Mechanisms of allergen-specific immunotherapy: Multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol 2014;133:621-631.
32 Tari G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A doubleblind study. Allergol Immunopathol (Madr) 1990;18:277-284.
33 Hirsch T, Sahn M, Leupold W. Double-blind placebo-controlled study of sublingual immunotherapy with house dust mite extract (D.pt.) in children. Pediatr Allergy Immunol 1997;8:21-27.
34 Vourdas D, Syrigou E, Potamianou P, Carat F, Batard T, André C, et al. Double blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. Allergy 1998;53:662-672.
35 La Rosa M, Ranno C, Andre´ C, Carat F, Tosca MA, Canonica GW. Double-blind placebo-controlled evaluation of sublingual swallow immunotherapy with standardized Parietaria judaica extract in children with allergic rhinoconjunctivitis. J Allergy Clin Immunol 1999;104:425-432.
36 Caffarelli C, Sensi LG, Marcucci F, Cavagni C. Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial. Allergy 2000;55:1142-1147.
37 Yuksel H, Tanac R, Gousseinov A, Demir E. Sublingual immunotherapy and influence on urinary leukotrienes in seasonal pediatric allergy. J Investig Allergol Clin Immunol 1999;9:305-313.
38 Bahceciler NN, Isik U, Barlan IB, Basaran MM. Efficacy of sublingual immunotherapy in children with asthma and rhinitis: a double-blind, placebo-controlled study. Pediatr Pulmonol 2001;32:49-55.
39 Pajno GB, Vita D, Parmiani S, Caminiti L, La Grutta S, Barberio G. Impact of sublingual immunotherapy on seasonal asthma and skin reactivity in children allergic to Parietaria pollen treated with inhaled fluticasone propionate. Clin Exp Allergy 2003;33:1641-1647.
40 Wuthrich B, Bucher Ch, Jorg W, Bircher A, Eng P, Schneider Y, et al. Double-blind, placebo-controlled study with sublingual immunotherapy in children with seasonal allergic rhinitis to grass pollen. J Investig Allergol Clin Immunol 2003;13:145-148.
41 Bufe A, Ziegler-Kirbach E, Stoeckmann E, Heidemann P, Gehlhar K, Klimek L, et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. Allergy 2004;59:498-504.
42 Niu CK, Chen WY, Huang JL, Lue KH, Wang JY. Efficacy of sublingual immunotherapy with high-dose mite extracts in asthma: a multicenter, double-blind, randomized, and placebo-controlled study in Taiwan. Respir Med 2006;100:1374-1383.
43 Valovirta E, Jacobsen L, Ljorring C, Koivikko A, Savolainen J. Clinical efficacy and safety of sublingual immunotherapy with tree pollen extract in children. Allergy 2006;61:1177-1183.
44 Pham-Thi N, Scheinmann P, Fadel R, Combebias A, Andre C. Assessment of sublingual immunotherapy efficacy in children with house dust miteinduced allergic asthma optimally controlled by pharmacologic treatment and mite-avoidance measures. Pediatr Allergy Immunol 2007;18:47-57.
45 Roder E, Berger MY, Hop WC, Bernsen RM, de Groot H, Gerth van Wijk R. Sublingual immunotherapy with grass pollen is not effective in symptomatic youngsters in primary care. J Allergy Clin Immunol 2007;119:892-898.
46 Wahn U, Tabar A, Kuna P, Halken S, Montagut A, De Beaumont O. Efficacy and safety of 5 grass pollen subllingualimmunotherapy in pediatric allergic rhinoconjunctivitis. J Allergy Clin Immunol 2009;123:160-166.
47 Bufe A, Eberle P, Franke-Beckmann E, Funck J, Kimmig M, Klimek L, et al. Safety anefficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. J Allergy Clin Immunol 2009;123:167-173.
48 Stelmach I, Kaczmarek-Woz´niak J, Majak P, Olszowiec-Chlebna M, Jerzynska J. Efficacy and safety of high-doses sublingual immunotherapy in ultra-rush scheme in children allergic to grass pollen. Clin Exp Allergy 2009;39:401-408.
49 Marcucci F, Sensi L, Di Cara G, Incorvaia C, Frati F. Dose dependence of immunological response to sublingual immunotherapy Allergy 2005;60:952-956.
50 Larenas-Linnemann D, Blaiss M, Van Bever HP, Compalati E, Baena-Cagnani CE Pediatric sublingual immunotherapy efficacy: evidence analysis, 2009-2012. Ann Allergy Asthma Immunol 2013;110:402-415.
51 Hedlin G, van HM. The role of immuntherapy in the managment of childhood asthma. Ther Adv Respir Dis 2012;127:30-38.
52 Tabar AI, Arroabarren E, Echechipía S, García 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.
53 Ozdemir C, Yazi D, Gocmen I, Yesil O, Aydogan M, Semic-Jusufagic A, et al. Efficacy of long-term sublingual immunotherapy as an adjunct to pharmacotherapy in house dust mite-allergic children with asthma. Pediatr Allergy Immunol 2007;18:508-515.
54 Zielen S, Kardos P, Madonini E. Steroid-sparing effects with allergen specific immunotherapy in childrem with asthma: a randomized controled trial. J Allergy Clin Immunol 2010;126:942-956.
55 Lin SY, Erekosima N, Kim JM, Ramanathan M, Suarez-Cuervo C, Chelladurai Y, et al. Sublingual immunotherapy for the treatment of allergic rhinoconjuntivits and asthma. JAMA 2013;309:1278-1288.
56 Bachert C, Canonica GW, Bufe A. SIT: efficacy depends on product, not on route of application. Pediatr Allergy Immunol 2012;23:401.
57 Yoselin A, Kendirli SG, Yilmaz M, Altintas DU, Karakoc GB. et al. Effect of one-year subcutaneous and sublingual immunotherapy on clinical and laboratory parameters in children with rhinitis and asthma: a randomized, placebo-controlled, double-blind, double-dummy study. Int Arch Allergy Immunol 2012;157:288-298.
58 Aydogan M, Eifan AO, Keles S, Akkoc T, Nursoy MA, Bahceciler NN, et al. Sublingual immunotherapy in children with allergic rhinoconjunctivitis mono-sensitized to house-dust-mites: a double-blind-placebo-controlled randomised trial. Respir Med 2013;107:1322-1329.
59 Blaiss M, Maloney J, Nolte H, Gawchik S, Yao R, Skoner DP. Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. J Allergy Clin Immunol 2011;127:64-71, 71.e1-4.
60 Rodriguez-Perez N, Ambriz-Moreno AJ, Canonica GW, Penagos M. Frequency of acute systemic reactions in patients with allergic rhinitis and asthma treated with sublingual immunotherapy. Ann Allergy Asthma Immunol 2008;101:304-310.
61 Antico A, Pagani M, Crema A. Anaphylaxis by latex sublingual immunotherapy. Allergy 2006;61:1236-1237.
62 Blazowski L. Anaphylactic shock because of sublingual immunotherapy overdose during third year of maintenance dose. Allergy 2008;63:374.
63 de Groot H, Bijl A. Anaphylactic reaction after the first dose of sublingual immuno-therapy with grass pollen tablet. Allergy 2009;64:963-964.
64 Di Rienzo V, Pagani A, Parmiani S, Passalacqua G, Canonica GW. Postmarketing surveillance study on the safety of sublingual immunotherapy in children. Allergy 1999;54:1110-1113.
65 Drachenberg KJ, Urban E, Proll S, Woroniecki SR. Sublingual specific immunotherapy for adults and children: a post marketing survey. Allergol Immunopathol 2004;32:76-81.
66 Agostinis F, Tellarini L, Canonica GW, Falagiani P, Passalacqua G. Safety of sublingual immunotherapy with a monomeric allergoid in very young children. Allergy 2005;60:133.
67 Rienzo VD, Minelli M, Musarra A, Sambugaro R, Pecora S, Canonica WG, et al. Post marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. Clin Exp Allergy 2005;35:560-564.
68 Fiocchi A, Pajno G, La Grutta S, Pezzuto F, Incorvaia C, Sensi L, et al. Safety of SLIT in children aged 3 to 7 years. Ann Allergy Asthma Immunol 2005;95:254-258.
69 Agostinis F, Foglia C, Landi M, Cottini M, Lombardi C, Canonica GW, et al. The safety of sublingual immunotherapy with one or multiple pollen allergens in children. Allergy 2008;63:1637-1639.
70 De Castro G, Zicari AM, Indinnimeo L, Tancredi G, di Coste A, Occasi F, et al. Efficacy of sublilngual immunotherapy on allergic asthma in children's real life. Eur Rev Med Pharmacol Sci 2013;17:2225-2231.
71 Caminati M, Dama A, Schiappoli M, Senna G. Balancing efficacy against safety in sublingual immunotherapy with inhalant allergens: what is the best approach? Expert Rev Clin Immunol 2013;9:937-947.
72 Calderón MA, Simons FE, Malling HJ, Lockey RF, Moingeon P, Demoly P. Sublingual allergen immunotherapy: mode of action and its relationship with the safety profile. Allergy 2012;67:302-311.
73 Passalacqua G, Canonica GW. Specific immunotherapy in asthma: efficacy and safety. Clin Exp Allergy 2011;41:1247-1255.
74 Medical Dictionary for Regulatory Activities (MedDRA). Maintenance support services and organization. http://www.meddra.com (Accessed October 1, 2014).
75 Senna G, Ridolo E, Calderon M, Lombardi C, Canonica GW, Passalacqua G. Evidence of adherence to immunotherapy. Curr Opin Allergy Clin Immunol 2009;9:544-548.
76 Pajno GB, Caminiti L, Crisafulli G, Barberi S, Landi M, Aversa T, et al. Adherence to sublingual immunotherapy in preschool children. Pediatr Allergy Immunol 2012;23:688-689.
77 Senna G, Caminati M, Canonica GW. Safety and tolerability of sublingual immunotherapy in clinical trials and real life. Curr Opin Allergy Clin Immunol 2013;13:656-662.
78 Passalacqua G, Baena-Cagnani CE, Bousquet J, Canonica GW, Casale TB, Cox L, et al. Grading local side effects of sublingual immunotherapy for respiratory allergy: speaking the same language. J Allergy Clin Immunol 2013;132:93-98.
79 Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trias in rhinoconjunctivitis. Clin Exp Allergy 1991;21:77-83.
80 Ciprandi G, Cadario G, Valle C, Ridolo E, Verini M, Di Gioacchino M, et al. Sublingual immunotherapy in polysensitized patients: effect on quality of life. J Investig Allergol Clin Immunol 2010;20:274-279.
81 Bousquet J, Scheinmann P, Guinnepain MT, Perrin-Fayolle M, Sauvaget J, Tonnel AB, et al. Sublingual swallow immunotherapy (SLIT) in patients with asthma due to house dust mites; a double blind placebo controlled study. Allergy 1999;54:249-260.
82 Khinchi MS, Poulsen LK, Carat F, André C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. Allergy 2004;59:45-53.
83 Kahlert H, Grage-Griebenow E, Stüwe HT, Cromwell O, Fiebig H. T cell reactivity with allergoids: influence of the type of APC. J Immunol 2000;165:1807-1815.
84 Ulrich JT1, Myers KR.Monophosphoryl lipid A as an adjuvant. Past experiences and new directions. Pharm Biotechnol 1995;6:495-524.

Received March 26, 2014
Accepted after revision January 2, 2015