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Sublingual Immunotherapy in Real-Life: When and Why

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Summary

Sublingual immunotherapy (SLIT) has gained wide acceptance in many European countries and has raised the level of interest in immunotherapy among practicing allergists and primary care physicians. SLIT was firstly accepted as a viable alternative to subcutaneous immunotherapy (SCIT) in the World Health Organization (WHO) Position Paper, published in 1998, and then included in ARIA guidelines. Since 1986, 60 DBPC-RCT trials have been published. There seem to be 2 distinct and perhaps sequential immunologic responses to SLIT; generation of regulatory T- cells (Tregs) secreting interleukin (IL)-10 and transforming growth factor (TGF)-β and immune deviation from Th2 to Th1 responses. The available meta-analyses are in favor of SLIT (rhinitis and asthma in adults and children).SLIT appears to be better tolerated than SCIT: a few case of SLIT-related anaphylaxis have been reported but no fatalities. SLIT may alter the natural history of respiratory allergy by preventing the onset of new skin sensitizations and/or reducing the risk of asthma onset. The clinical effects of SLIT are not immediate, such those of traditional drugs (ie, bronchodilatators or anti-histamines), but the immune modulation is profound and long-lasting (for 5-8 years after discontinuation). Special SLIT indications exist in everyday clinical practice in the following patients: uncontrolled with optimal pharmacotherapy, in whom drugs induces undesirable side effects, affected by moderate to severe rhinitis with moderate to severe nasal eosinophilia, associated with non specific bronchial hyper responsiveness (BHR) and/or bronchial asthma.

Keywords: Sublingual immunotherapy; Atopic march; Rhinitis; Asthma; Real-life

Abbreviations

IT: Immunotherapy; SLIT: Sublingual Immunotherapy; SCIT: Subcutaneous Immunotherapy; FEV1: Forced Expiratory Volume in one Second; PD20: Provocation Dose causing a fall of 20% in FEV1; MCh: Methacholine; SMS: Symptom & Medication Score; BSACI: British Society of Allergy and Clinical Immunology; EAACI: European Academy of Allergy and Clinical Immunology; DBPC-RCTs: Double-Blind, Placebo Controlled, Randomized Controlled Trials

Historical Background

Subcutaneous allergen injection immunotherapy has been the principal immunotherapy approach in the treatment of allergic respiratory airway diseases [1]. Since the earliest attempts, immunotherapy (IT) was administered subcutaneously (SCIT) [2, 3] but during the last century other modalities of administration (gastrointestinal, nasal and bronchial) were anecdotally explored [4, 5, 6, 7]. In 1986, the British Committee for the Safety of Medicine [8] reported 26 deaths caused by SCIT, and raised serious concerns about the safety and the risk/benefit ratio of SCIT. This fact promptly increased the interest in non-injection routes (oral, bronchial, nasal, sublingual) [9] and, since the first published controlled study [10] the sublingual route (SLIT) appeared the most promising alternative to the traditional SCIT. Recently the sublingual administration of allergen extract has become popular in many European countries and has also demonstrated efficacy in both allergic rhinitis [11] and bronchial asthma [12,13]. It is interesting to assess the acceptability of SLIT in guidelines. In 1992 SLIT was not even mentioned in the BSACI Position Paper [14]. In 1993, the EAACI Position Paper on Specific Immunotherapy (SIT) proposed that SLIT might be used as an investigational therapy to prove its efficacy and safety [15]. In 1998 the World Health Organization [1] and the European Academy of Allergy and Clinical Immunology [14] performed an extensive review of the literature, and excluded the "pure" oral and bronchial routes from the clinical practice because of the lack of efficacy. The nasal route was accepted as an effective option, but just in rhinitis and adults and its use is presently declining. In the same documents, it was concluded that SLIT is a viable alternative to the injection route and may be used in adults with allergic rhinitis, but there was insufficient evidence to use it in children. In 2001, the ARIA document [16] confirmed and extended the indication of SLIT also to children, and this was confirmed in the ARIA update in 2008 [17]. In 2008, the World Allergy Organization (WAO) decided that it was important and timely to advise on the current state of the art of SLIT, and to develop practice parameters for SLIT use: in the World Allergy Organization SLIT Position Paper 2009 [18] 60 DBPC-RCTs are considered; their results were also pooled and evaluated in several meta-analyses which concluded that SLIT is significantly efficacious compared with placebo for rhinitis and asthma in adults and children [11,12,13,19]. In the last two years, some adequately powered, well-designed DBPC-RCTs with grass drops [20] or tablets [21,22,23] including hundreds of patients, were published. These studies have confirmed the efficacy of SLIT for these allergens and, more importantly, have demonstrated a dose-effect relationship. In parallel to the clinical trials, post-marketing surveys [24], mechanistic investigations [25,26], prevention studies [27, 28] and pharmaco-economic assessments [29] were also published in the last 10 years, so that several aspects of SLIT were gradually clarified. Concerning safety, all clinical trials and post-marketing surveys have consistently agreed that SLIT is safe, and the majority of side effects are local and mild. In more than 20 years and everyday use, only 6 cases of anaphylaxis with SLIT have been reported, some of which were with mixture of multiple unrelated allergens using non standardized extracts, but 2 patients had

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a severe reaction after the first dose of a grass tablet. It has also been reported that use of multiple allergens for SLIT does not increase the rate of side effects in children [30]. Furthermore, it has been suggested [31] that the safety profile of SLIT does not differ in children below the age of 5 years (a relative contraindication to SCIT).

Why: impact of sublingual immunotherapy on the atopic march

During the last 20 years it has become clear that asthma and rhinitis are two clinical aspects of a single disorder involving the whole respiratory tract [32] and it is also true that, in atopic subjects, there is a clinical progression of the disease, usually starting with atopic dermatitis and food allergy in the infancy and evolving through rhinitis and asthma. This progression is usually termed "allergic march" [33]. Also in adults the clinical features of allergic disease may change over the time. For instance, it has been shown that allergic patients tend to develop new skin sensitizations [34] and that an evolution from rhinitis to asthma may occur [35,36].Interventions that can alter the natural history of atopic march may reduce the risk of developing asthma or prevent the onset of new allergen sensitizations. Presently, none of the currently available medications, including H1-antihistamines and inhaled steroids, display such properties [37,38,39,40]. Conversely, allergen immunotherapy is capable of modifying the immunological response to the offending allergens at the earliest stages, probably acting via T regulatory cells [41,42]. More than 40 years ago, in an observational study, Johnston [43] observed that a significantly smaller proportion of children treated with SCIT developed asthma, versus children receiving medications only, over a period of 14 years. Subsequently, the Preventing Allergy Treatment (PAT) Study [44] suggested the preventive effect of SCIT on the development of asthma in children with rhinitis, and this effect was shown to persist 7 years after discontinuation [45]. In parallel, it was consistently shown that SCIT was able to reduce the onset of new sensitizations in both adults and children restoring the imbalance between Th1 and Th2 lymphocyte subsets [34, 46, 47]. The long-lasting persistence of the clinical effects of SCIT after discontinuation is an additional indirect confirmation of the effect on the natural history [48, 49]. In the recent years the sublingual administration of immunotherapy (SLIT) has gained more and more credibility, and it is now included in guidelines and used in many European countries. The clinical effects of SLIT are not immediate such as with traditional drugs (i.e. bronchodilators or antihistamines), but the immunomodulation is profound and long-lasting. The main advantage of SLIT is the self-administration at home and the faovurable safety profile. It has been repeated show that SLIT, such as SCIT, can downregulate the inflammatory phenomena at the target organs during the exposure to allergens[50,51,52]and can reduce the degree of bronchial hyperresponsiveness [53,54], which is indirectly related to bronchial inflammation. There are two randomized open controlled studies suggesting that SLIT reduces the risk of asthma onset in children with rhinitis: the first study showing that SLIT may prevent the onset of asthma in children with rhinitis was published in 2004 [27]. This randomized, open, controlled study involved 113 children suffering from seasonal rhinitis because of grass pollen at enrolment. Of these children, 54 were randomly allocated to drug treatment plus SLIT and 59 to standard pharmacotherapy alone. After 3 years, 99 children were re-evaluated: development of asthma was 3.8 times more frequent in the control group. The second open controlled trial [28] involved 216 children (age 5-17 years) suffering from allergic rhinitis with or without intermittent asthma. They were randomly allocated 2:1 to drugs plus SLIT or drugs only, and followed for 3 years. Symptoms and medication scores were recorded yearly during the period of exposure, whereas the presence of persistent asthma was assessed at 3 year. There was a significant reduction of symptom-medication scores only in the SLIT group throughout the study. There were 196 patients evaluated at 3 years, and the occurrence of persistent asthma was 2/130 (1.5%) in the SLIT group and 19/66 (30%) in the control group, with a number to treat of four. Overall, the rate of prevention of the onset of asthma in children, as reported in the aforementioned trials, is quite similar to that described for SCIT in the PAT study. There are two open randomized studies showing that SLIT reduces the onset of new allergen sensitizations. In the study mentioned earlier, conducted in children [28], at the third year of follow-up, the rate of onset of new sensitizations was 4/130 (3%) in the SLIT group and 23/66 (35%) in the control group. Another randomized open controlled trial [55] assessed the onset of new allergy skin test sensitizations after 3 years in 511 patients, randomly allocated to SLIT (319 subjects) or drug alone (192 subjects). At the end of the study, new sensitizations, compared with baseline, appeared in 64/170 (38%) of controls and 16/271 (5.9%) of SLIT patients ($p<0.001$). Few studies have investigated the long-term effect of SLIT. Di Rienzo et al [56] followed in a prospective open controlled trial 60 children with asthma/rhinitis due to house dust mite for 10 years. They were subdivided into 2 matched groups with 35 subjects undergoing 4-5 years of SLIT and 25 subjects receiving drug therapy alone. The patients were evaluated at baseline, at the end of SLIT and 4 to 5 years after SLIT discontinuation. In the SLIT group there was a significant difference compared with baseline for the presence of asthma ($p<0.001$), whereas no difference was observed in the control group. This difference was also seen 5 years after SLIT discontinuation. A 15-year follow-up of mite-allergic patients treated with SLIT for 3, 4 or 5 years has suggested that a 4-year course represents the best combination of clinical efficacy and long term effect [57]. Again, a retrospective study on 59 patients allergic to house dust mite [58] suggested that 4 years of SLIT achieved long-lasting effects of 7-8 years, whereas this effect was lost with shorter courses of treatment.

When: A Clinical practice improvement (Cpi) program for sublingual immunotherapy

The disease-modifying effects of SLIT have only been apparent in the past 10 years because the previous clinical trials were aimed at demonstrating the clinical efficacy and the safety of treatment. Furthermore, studies assessing long-term and preventive effects require several years of follow-up of the patients. As with SCIT, the preventive and long-term effects of SLIT can be reliably evaluated in long-term trials involving many patients. The less restrictive open randomized trials can provide supportive results in this sense. The need to work on an obserbational basis arises from the fact that randomized blind controlled trials often do not adapt well to real-life, since experimental schemes are not easily transferred into clinical practice [59,60]. Setting up a CPI program is a novel approach in this field, but fulfils the following basic needs:

a) Standardization of the methods for selecting and treating patients in real-life;

b) Uniformity of methods for data collection and analysis of key clinical endpoints;

c) Observational studies on large case list with correction- as necessary- of diagnostic and therapeutic methods in everyday clinical practice;

The fundamental steps of this decisional tree are:

1) At the first visit (admission): skin prick test, full spirometry with

body pletismography, methacholine (MCh) challenge, assays of specific IgE for the main pneumoallergens, eosinophil count in nasal secretions;

2) During the first year: treatment with drugs and monitoring based on clinical diaries of the symptoms and consumed drugs;

3) During the next five years patients who had not responded to standard treatment with drugs after the first year were asked for informed consent and were given SLIT, usually for moderate to severe rhinitis and rhinitis with asthma;

4) Re-evaluation of the immunoallergic profile after three and five years of SLIT.

In this way actually we consider for SLIT administration patients with clinical profile of moderate to severe rhinitis and mild/moderate asthma, with positive MCh challenge for PD20FEV1 < 400µ gr, moderate to severe nasal eosinophilia (>10%), RAST/CAP for house dust mite, cat and pollens class II^ or more, poor responders to treatment with drugs alone . During the last 10 years we have modified our decisional tree for immunotherapy excluding from the protocol:

a) Use of local nasal immunotherapy(LNIT), because this type of IT displayed no preventive effects on the onset of asthma [61,62];

b) Use of subcutaneous immunotherapy (SCIT), because SLIT has showed well demonstrated clinical efficacy (as SCIT), but main advantage as the self-administration and more safety profile.

Conclusions

SLIT represents an advance in immunotherapy, mainly because of its safety, as testified by several controlled trials and several post-marketing surveillance studies. The recent data from the literature have shown that SLIT resembles the traditional SCIT in the main characteristics, such as the long-lasting effect and the prevention of the onset of new sensitizations. It is true that there are only few data about the mechanisms of action of SLIT, but the clinical observations make it conceivable that the mechanisms of action of SLIT do not greatly differ from those of SCIT. For instance, since a clinical effect on both asthma and rhinitis has been confirmed, the existence of a systemic immunological effect should be postulated. Despite SLIT is self-administered, adherence [55] and costs [63] seem not represent a problem, although a careful instruction and a strict follow-up of the patients are required. At present, the indication of SLIT are the same of SCIT, but the good safety profile makes SLIT, in principle, an optimal choice especially for pediatric patients.

References

1. Bousquet J, Lockey RF, Malling HJ (1998) WHO Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. J Allergy Clin Immunol 102: 558-562.
2. Noon L (1953) Prophylactic inoculation against hay fever. Int Archs Allergy appl Immun 4: 285-288.
3. Freeman J (1960) Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. Ann Allergy. 18: 427-434.
4. Black JH (1927) The oral administration of pollen. J Lab Clin Med 12: 1156.
5. Herxeimer H (1951) Bronchial hypersensitization and hyposensitization in man. Int Arch Allergy Appl Immunol 2: 40-59.
6. Taylor G, Shivalkar PR (1972) Local nasal desensitization in allergic rhinitis. Clin Allergy 2: 125-136.
7. Taudorf E, Weeke B (1983) Orally administered grass pollen. Allergy 38: 561-564.
8. Committee on the safety of medicines. CSM update. Desensitizing vaccines. (1986) Br Med J 293:348.
9. Valovirta E, Passalacqua G, Canonica GW (2004) Non-injection routes for immunotherapy of allergic diseases. Clin Allergy Immunol 18: 607-623.
10. Scadding K, Brostoff J(1986) Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. Clin Allergy 16: 483-491.
11. Wilson DR, Lima MT, Durham SR (2005) Sublingual immunotherapy for allergic rhinitis:systematic review and meta-analysis. Allergy 60: 4-12.
12. Penagos M, Passalacqua G, Compalati E, Baena-Cagnani CE, Orozco S, et al. (2008) Meta-analysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. Chest 133: 599-609.
13. Calamita Z, Saconato H, Pela' AB, Atallah AN (2006) Efficacy of sublingual immunotherapy in asthma: Systematic review of randomized-clinical trials using the Cochrane Collaboration method. Allergy 61: 1162-1172.
14. Position Paper on allergen immunotherapy. Report of a BSACI working party. (1992) Clin Exp Allergy 23: 1- 44.
15. EAACI Position Paper on local immunotherapy. (1998) Malling HJ edt Allergy 53: 933-944.
16. Bousquet J and Van Cauwenberge P edts (2001) Allergic Rhinitis and Its Impact on Asthma. J Allergy Clin Immunol 108: 146-150.
17. Bousquet J, Kaltaev N, Cruz AA, Denburg J, Fokkens WJ, et al. (2008)Allergic Rhinitis and Its Impact on Asthma (ARIA) 2008 update (in collaboration with World Health Organization, GA(2)LEN and AllerGen). Allergy 63: 8-160.
18. Sublingual immunotherapy: World Allergy Organization Position Paper (2009). Allergy 64: 1-59.
19. Penagos M, Compalati E, Tarantini F, Baena-Cagnani R, Huerta J, et al. (2006) Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in children. Meta-analysis of randomized controlled trials. Ann Allergy Asthma Immunol 97: 141-148.
20. Pfaar O, Klimek L (2008) Efficacy and safety of specific immunotherapy with a high-dose sublingual grass pollen preparation: a double-blind, placebo-controlled trial. Ann Allergy Asthma Immunol 100: 256-263.
21. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S (2006) Sublingual immunotherapy with once-daily grass-allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 117: 802-809.
22. Dahal R, Kapp A, Colombo G, De Moncky JG, Rak S, et al. (2006) Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 118: 434-440.
23. Didier A, Malling HJ, Worm M, Horak F, Jager S, et al. (2007) Optimal dose, efficacy and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol 120: 1338-1345.
24. Passalacqua G, Guerra L, Fumagalli F, Canonica GW (2006) Safety profile of sublingual immunotherapy. Treat Respir Med 5: 225-234.
25. Cosmi L, Santarlasci V, Angeli R, Liotta F, Maggi L, et al.(2006) Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma and interleukin-10-production. Clin Exp Allergy 36: 261-272.
26. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-schmid B, Ebner (2007) Sublingual immunotherapy induces IL-10- producing T regulatory cells, allergen-specific tolerance, and immune deviation. J Allergy Clin Immunol 120: 707-713.
27. Novembre E, Galli E, Landi F, Caffarelli C, Pifferi M, et al. (2004) Coseasonal sublingual immunotherapy reduces the developement of asthma in children with allergic rhinoconjunctivitis. J Allergy Clin Immunol 114: 851-857.
28. Marogna M, Tomassetti D, Bernasconi A, Colombo F, Massolo A, et al (2008) Preventive effects of sublingual immunotherapy in childhood: an open randomized controlled study. Ann Allergy Asthma Immunol 101: 206-210.
29. Berto P, Frati F, Incorvaia C (2008) Economic studies of immunotherapy: a review. Curr Opin Allergy Clin Immunol 8: 585-590.
30. Agostinis F, Foglia C, Landi M, Cottini M, Lombardi C, et al. (2008) The safety of sublingual immunotherapy with one or multiple pollen allergens in children. Allergy 63: 1637-1639.

31. Di Rienzo V, Minelli M, Musarra A, Sambugaro R, Pecora S, et al.(2005) Post-marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. Clin Exp Allergy 35: 560-564.

32. Passalacqua G, Ciprandi G, Guerra L, Pasquali M, Canonica GW (2004). An update on the asthma-rhinitis link. Curr Opin Allergy Clin Immunol 4: 177-183.

33. Spergel JM, Paller AS (2003) Atopic dermatitis and the atopic march. J Allergy Clin Immunol 112: s118-s127.

34. Paino GB, Barberio G, De Luca F, Morabito L, Parmiani S(2001) Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 31: 1392-1397.

35. Spergel JM (2005) Atopic march:link to upper airways. Curr Opin Allergy Clin Immunol 5: 17-21.

36. Lombardi C, Passalacqua G, Gargioni S, Ciprandi G, Senna GE, Scordamaglia A, et al. (2001) The natural history of respiratory allergy: a follow-up study of 99 patients up to 10 years. Respir Med 95: 9-12.

37. Ulrich Wahn (1998) ETAC Study Group. Allergic factors associated with the development of asthma and the influence of cetirizine in a double-blind, randomized, placebo-controlled trial: first results of ETAC- Early Treatment of the Atopic Child. Pediatr Allergy Immunol 9: 116-124.

38. Simons FE. (2007) Early Prevention of Asthma in Atopic Children (EPAAC) Study Group. Safety of cetirizine treatment in young atopic children: an 18-month study. Pediatr Allergy Immunol 18: 535-542.

39. Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F, et al. (2006) Intermittent inhaled corticosteroids in infants with episodic wheezing. N Engl J Med 354: 1998-2005.

40. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, et al. (2006) Long-term inhaled corticosteroids in preschool children at high risk for asthma. An important study of inhaled corticosteroids in childhood asthma showing no preventive potential after cessation of treatment. N Eng J Med 354: 1985-1997.

41. Till SJ, Francis JN, Nouri Aria K, Durham SR (2004) Mechanisms of immunotherapy. J Allergy Clin Immunol 113: 1025-1034.

42. Romagnani S.(2006) Regulatory T cells:wich role in the pathogenesis and treatment of allergic disorders? Allergy 61: 3-14.

43. Johnstone DE, Dutton DA (1968) The value of hyposensitization therapy for bronchial asthma in children –a 14-year study. Pediatrics 42: 793-802.

44. Moller C, Dreborg S, Ferdousi HA, Halken S, Høst A, et al.(2002) Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 109: 251-256.

45. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, et al. (2007) (the PAT investigator group). Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 62: 943-948.

46. Purello-D'Ambrosio F, Gangemi S, Merendino RA, Isola S, Puccinelli P, et al. (2001) Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. Clin Exp Allergy 31: 1295-1302.

47. Akdis M, Akdis C. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2007;119(4):780-791.

48. Eng PA, Reinhold M, Gnehm HP (2002) Long-term efficacy of preseasonal grass pollen immunotherapy in children. Allergy 57: 306-312.

49. Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP (2006) Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy 61: 198-201.

50. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, et al. (1999) Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 341: 468-475.

51. Ippoliti F, De santis W, Volterrani A, Lenti L, Canitano N, et al. (2003) Immunomodulation during sublingual therapy in allergic children. Pediatr Allergy Immunol 14: 216-221.

52. Ciprandi G, Fenoglio D, Cirillo I, Vizzaccaro A, Ferrera A, et al. (2005) Induction of interleukin-10 by sublingual immunotherapy for house dust mites: a preliminar report. Ann Allergy Asthma Immunol 95: 38-44.

53. Grembiale RD, Camporota R, Djukanovic R, Marsico S. (2000) Effects of specific immunotherapy in allergic riniti individuals with bronchial hyperresponsiveness. Am J Respir Crit Care Med 162: 2048-2052.

54. Pajno GB, Passalacqua G, Vita D, Caminiti L, Parmiani S, Barberio G (2004) Sublingual immunotherapy abrogates seasonal bronchial hyperresponsiveness in children with Parietaria-induced respiratory allergy: a randomized controlled trial. Allergy 59: 883-887.

55. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. (2004) Randomized controller open study of sublingual immunotherapy for respiratory allergy in real-life: clinical efficacy and more. Allergy 59: 1205-1210.

56. Di Rienzo V, Marcucci F, Puccinelli P, Parmiani S, Frati F, et al. (2003)Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. Clin Exp Allergy 33: 206-210.

57. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G (2010) Long lasting effects of sublingual immunotherapy according to its duration: a 15-year prospective study. J Allergy Clin Immunol 126 : 969-975.

58. Marogna M, Bruno M, Massolo A, Falagiani P (2007) Long-lasting effects of sublingual immunotherapy for house dust mites in allergic riniti with bronchial hyperreactivity: a long term (13-year) retrospective study in real-life. Int Arch Allergy Immunol 142: 70-78.

59. Concato J, Shah N, Horwitz RI (2000) Randomized, controlled trials, observational studies, and the hierarchy of research design. N Eng J Med 342: 1887.

60. Benson K, Hartz AJ (2000) A comparison of observational studies and randomized, controlled trials. Am J Ophthalmol 130: 688.

61. Marogna M, Tiri A, Riva G (2001) Clinical practice improvement program for immunotherapy of respiratory allergic diseases. Int J Immunopath Pharm 14: 93-101.

62. Marogna M, Massolo A (2003) Sublingual immunotherapy in the contest of a clinical practice improvement program in the allergological setting: results of a long term observattional study. Eur Ann Allergy Clin Immunol 35: 133-140.

63. Berto P, Passalacqua G, Crimi N, Frati F, Ortolani C, et al. (2006) Economic evaluation of sublingual immunotherapy vs symptomatic treatment in adults with pollen-induced respiratory allergy: the Sublingual Immunotherapy Pollen Allergy Italy (SPAI) study. Ann Allergy Astma Immunol 97: 615-621.