

SUBLINGUAL IMMUNOTHERAPY: NO MORE DOUBTS

G. Passalacqua (1), C. Lombardi (2), L. Guerra (1),
E. Compalati (1), F. Fumagalli (1), G.-W. Canonica

GENERAL ASPECTS

Allergen-specific immunotherapy (IT), or allergen vaccination, is the practice of administering to allergic subjects gradually increasing amounts of an allergen (so-called vaccine). The scope of immunotherapy is to achieve, and to maintain, a "hyposensitization" towards the allergen, thus improving symptoms and reducing the need for medications when exposure to the allergen occurs. Since the beginning (1), IT was administered subcutaneously (SCIT), although other modalities of administration (gastrointestinal, nasal and bronchial) were proposed anecdotally (2-5). Those routes of administration are presently termed as *local or non-injection routes*.

In 1986, the British Committee for the Safety of Medicines (6) reported 26 deaths caused by SCIT, and raised serious concerns about the safety and the risk/benefit ratio of SCIT. Although it was subsequently shown (7, 8) that life-threatening events were often due to avoidable errors (wrong dose, improper prescription, incorrect administration), the interest in non-injection routes rapidly increased (9). Since the first report of a controlled study (10) the sublingual route appeared the most promising alternative to the traditional SCIT.

In 1998 a panel of experts of the World Health Organization, based on an extensive review of the literature, concluded that sublingual (SLIT) immunotherapy was a viable alternative to the injection route, and that its use in the clinical practice in adults is justified (11). These conclusions were then confirmed in a position paper by the European Academy of Allergology and Clinical Immunology (EAACI) (12). In 2001, the ARIA document (13) confirmed and extended the indication of SLIT also to children.

Currently, SLIT is widely used in several European Countries, where its selling is progressively reaching that of SCIT. There are several manufacturers com-

mercializing SLIT with different administration schedules and dosages, but all the vaccines commercialized in Europe are standardized, either biologically or immunologically. The extracts can be labelled in Allergen Units (AU), Index of Reactivity (IR), Biological Units (BU), Standard Units (STU) etc, and in some cases also the content in micrograms of the major allergens is available.

The *sublingual-swallow* mode is preferred, although some Authors also used the *sublingual-spit* administration. The amount of allergen given during a course of SLIT is always higher than in an equivalent SCIT, therefore the treatment is often termed *high-dose SLIT*.

EFFICACY

The first randomized trial with SLIT was published in 1986 (10); after 12 years, in 1998, the WHO position paper mentioned 4 studies and the EAACI/ESPACI document published in the same year considered 6 studies. To date, 29 randomized placebo controlled trials (14-42), conducted with adequate methods have been published in peer-reviewed journals. Eleven out of them were conducted in paediatric patients (Table 1).

Only three studies (18, 29, 35) provided negative result (no significant change vs placebo) in rhinitis induced by common allergens (grass, mites, birch and parietaria). There are also sparse positive studies with olive, cypress and mixed-trees extracts. In all the studies, as usual, the primary outcome variable for clinical efficacy was the symptom score, combined with the drug intake score. In some studies, other objective parameters such as the specific nasal or conjunctival reactivity, or immunological changes were assessed. Overall, the magnitude of the clinical efficacy ranged from about 20% to more than 60% reduction of symptoms and/or medication scores. It is true that some studies failed to demonstrate a difference between placebo and SLIT. Torres-Lima (35) et al found no statistically significant clinical effect

(2) Allergy Service, Dept of Internal Medicine, S.Orsola Hospital, Brescia - Italy.

(symptom score and medication intake) in rhinitis due to grass, but in this study the self-evaluation by the patients was largely positive and a systemic immunological effect was also seen. Usually, SLIT performed less well with mite extracts (19, 32), such as happens

with SCIT. In mite allergy, the duration of the treatment is crucial; in fact those treatments lasting at least 2 years gave positive results (23, 25, 30, 36). Moreover, in a recent study by Bufe *et al* (42) showed that the greater benefit with SLIT was obtained in more severe

AUTHOR, YEAR	Age range	Patients A/P *	Allergen	Durat.	Cumulative Dose	VS SCIT	Dise - ase **
Tari, 1990	5-12	30/28	Mites	18 mths	365 STU	5	R/A
Sabbah, 1994	13-51	19/29	Grasses	17 wks	4.500 IR	50	R
Feliziani, 1995	14-48	18/16	Grasses	3.5 mths	25 BU	6	R
Troise, 1995	17-60	15/16	Parietaria	10 mths	105 BU	20	R
Hirsch, 1997	6-16	15/15	Mites	1 yrs	570 mcg Der p1	5	R/A
Passalacqua, 1998 (monoid)	15-46	10/9	Mites				
	2 yrs	10.000 AU	20	R			
Vourdas, 1998	7-17	33/31	Olive	2 yrs	4 mg Ole e 1	300	R/A
Clavel, 1998	8-55	62/28	Grasses	6 mths	28 mcg Phl p 5	100	R/A
Horak, 1998	16-48	18/16	Birch	4 mths	250 STU	NS	R
Hordijk 1998	18-45	30/27	Grasses	6 mths	300.000 BU	NS	R/A
Bousquet, 1999	15-37	15/15	Mites	2 yrs	104,000 IR 4.2 mg Der p 1	200	A
Passalacqua, 1999	15-42	15/15	Parietaria	8 mths	265 BU 16 mcg Par j 1	7	R/A
Pradalier, 1999	6-25	59/61	Grasses	4 mths	11,000 IR 935 mg Phl p5	150	R/A
La Rosa, 1999	6-14	20/21	Parietaria	6 mths	75,000 IR 52.5 mg Par j1	375	R/A
Purello, 1999	14-50	14/16	Parietaria	8 mths	200 BU 12 mcg Par j 1	3	R/A
Pajno, 2000	8-15	12/12	Mites	2 yrs	360 mcg Der p1	4	A
Guez, 2000	6-51	24/18	Mites	2 yrs	90,000 IR 2,2 mg Der p 1	2	R
Caffarelli, 2000	4-14	24/20	Grasses (monoid)	3 mths	37.000 AU	5	R/A
Ariano, 2001	19-50	10/10	Cypress	8 mths	250.000 RU	5	R/A
Bahceciiler, 2001	7-15	8/7	Mites	6 mths	7.000 IR 0.56 mg Der p1	NS	R/A
Voltolini, 2001	15-52	24/13	Trees	24 mths	250,000 RU	5	R
Lima, 2002	16-48	24/22	Grasses	18 mths	0.9 mg Phl p 5		
per month	50	R					
Mortemousque 2003	6-60	26/19	Mites	24 mths	90,000 IR 2.2 mg Der p 1	NS	C
Andre 2003	6-55	48/51	Ragweed	7 mths	Variable	NS	R
Ippoliti 2003	5-12	47/39	Mites	6 mths	57 mcg Der p1	5	R/A
Pajno 2003	8-14	15/15	Parietaria	13 mths	23 mcg Par j1	NS	R/A/C
Wutrich 2003	6-13	11/11	Grasses	2 yrs	NA	NS	R/A
Tonnel 2004	7-45	15/17	Mites	2 yrs	1.28 mg Der p1	NS	R
Bufe 2004	6-13	68/74	Grasses	3 yrs	2,650,000 AU 9.6 mg Phl p5	10	R/A

Table 1: summary of the randomized placebo controlled studies with SLIT

* A=Active; P= Placebo. ** A= Asthma; R= Rhinitis; C= Conjunctivitis

patients. There is also a meta-analysis study (43) of twenty-two randomized placebo-controlled trials involving 979 patients. This meta-analysis concluded that SLIT significantly differs from placebo in reducing symptoms and need for medications in allergic rhinitis, and that the safety is overall satisfactory.

SLIT has been demonstrated effective also on asthma symptoms. Some studies (25-27) documented a reduction of the days with asthma, of the use of β_2 agonists, of the intake of systemic steroids and of the clinical symptoms. André *et al* (37) evaluated the efficacy of SLIT with a ragweed extract given to 99 patients for 6 months, and found a lower occurrence of asthma in the active group. Ippoliti *et al* (38) evaluated the effects of SLIT in 86 children with asthma and rhinoconjunctivitis due to mites and found, after 6 months of treatment, a significant improvement also of asthma. As an indirect evidence of the effects of SLIT on bronchial inflammation, several studies have documented a significant reduction in bronchial hyperreactivity after SLIT (44-46). Of note, one of these studies was conducted in children in double blind fashion (46). One single study evaluated the effects of SLIT on allergic conjunctivitis (36): the Authors studied 60 patients with perennial allergic conjunctivitis for 24 months and found a significant increase in the threshold dose for conjunctival provocation and a significant clinical efficacy over placebo. Finally, the possible use of SLIT in latex allergy has been recently envisaged in an open label study and one controlled study (47, 48).

One of the most important aspect of the efficacy of SLIT is the comparison with SCIT. To date there are two double blind double dummy studies published as full paper is available (49, 50). The first was conducted in grass pollen allergic patients, and showed that the clinical efficacy of the two routes was comparable. Indeed, in this study, a placebo control group was not included. The other comparison study (50) was more rigorous in design, and involved also a placebo group and a baseline evaluation. This study showed that SLIT and SCIT were equally effective, although the safety and tolerability were superior with the non-injection route.

SAFETY

The primary rationale of SLIT is to minimize or ideally abolish the risk of adverse events. With SCIT, the rate of systemic reactions is quite variable, and depends on the administration schedule, the allergen and the survey method. In previous studies, the occurrence of systemic reactions was reported to

range between 0.5% and 2.9% of patients (51, 52), up to 5.6% in children (53). Indeed, in about 20% of the published clinical trials with SCIT no information is provided about the side effects, and in the remaining studies side effects are reported to occur on average in 14% of patients. The occurrence of systemic reactions is in general about 0.05-0.6% of doses administered. Near fatal events are rare, but the risk exists, especially in asthma patients (54, 55).

On the contrary, with SLIT no life-threatening adverse event or fatality has even been described in the literature and in the clinical practice. The most frequently reported side effect is oral/sublingual itching after taking the dose, followed by stomachache and nausea. These events are invariably described as mild and self-resolving, and they can be easily managed by a temporary reduction of the dose given. A noticeably high rate of gastrointestinal adverse events seems to occur only when dosing exceeding 300 times the SCIT are used (28). Other systemic side effects, as headache, rhinitis, asthma or urticaria were described only sporadically in the randomized trials, and their incidence did not differ from the placebo groups.

Andre' *et al.* reviewed the safety of SLIT in the trials performed with vaccines from a single manufacturer (56), involving 690 subjects (347 active + 343 placebo), with 218 children (103 active + 115 placebo). The large majority of events were mild and their occurrence did not differ in active and placebo groups. The only difference, again, was in the oral and gastrointestinal side effects that were more frequent in SLIT patients, although mild. The occurrence of side effects and dropouts was similar in adults and children.

Post-marketing surveillance studies, better reflect the everyday clinical practice and provide therefore valuable information on the safety. Di Rienzo *et al* (57) followed-up 268 children aged between 2 and 15 years and receiving SLIT up to 3 years. The overall incidence of systemic side effects was 3% of the patients and 1/12.000 doses, and no discontinuation of the SLIT was needed. Of 8 side effects described, only one (urticaria) was judged of moderate severity and required a single dose of oral antihistamine. A pharmacosurveillance study performed in adults (58), with 198 patients observed for about 3 years, provided similar figures. Also one large post marketing survey performed by Pajno *et al* (59), confirmed, in children, that the occurrence of side effects was very low. In this survey, both the sublingual-swallow and the sublingual-spit modalities were used. The occurrence of side effects was 6% of patients and 0.15 per 1,000 doses. Agostinis *et al* studied the safety in a small group of 36 children below 5 years of age side effects were reported to occur in 5%

of patients and at a rate of 0.071 per 1,000 doses (60). Drachenberg *et al* (61) reported in a mixed population of 159 subjects an occurrence of adverse events in 6.3% of patients, but detailed data on side effects are not provided in the article. We recently performed a survey involving 128 children aged between 3 and 5 years, and found side effects in 5.6% of patients (0.2 per 1,000 doses); two side effects were local and mild and the remaining (nausea, diarrhea) were moderate. A summary of the results of the post marketing surveys is shown in table 2. Finally, no increased risk of side effects has been shown in those patients suffering from oral allergy syndrome (62), and the use of an ultra rush build-up phase did not increase the risk of side effects (63). In a study specifically addressing this issue, it has been shown that a detailed education of the patient with written instructions allow to easily manage side effects also at patient's home without discontinuing therapy (64).

Author (ref)	N pats	Age range	Follow-up	AE % of patients	AE/1,000 doses
Di Rienzo (56)	268	2-15 years	3 years	3 %	0.1/1,000
Lombardi (57)	198	> 14 years	3 years	7.5 %	0.5/1,000
Pajno (58)	354	5-15 years	36 months	6 %	0.15/1,000
Agostinis (59)	36	3-5 years	2 years	5 %	0.07/1,000
Di Rienzo	128	3-5 years	2 years	5.6%	0.2/1,000

Table 2: Adverse events (AE) reported in the post marketing surveys.

NEW ACQUISITIONS

■ **Long-lasting efficacy.** One of the most relevant clinical characteristics of SCIT is its long-lasting effect after discontinuation (65). So far it was claimed that SCIT is superior to SLIT in view of this effect. Recently, a prospective parallel groups, carried out in 60 children suffering from allergic asthma/rhinitis due to mite was published (66). Thirty-five children underwent a 4-5 year course of SLIT with a standardized extract and twenty-five received only drug therapy. The patients were evaluated at baseline, at the end of SLIT and 4-5 years later. In the SLIT group a significant difference versus baseline for the presence of asthma ($p < 0.001$) was documented even 5 years after discontinuation, whereas no difference was observed in the control group.

■ **Preventive effect.** It has been proven that SCIT is able of preventing the onset of asthma in rhinitis subjects (67). Such demonstration has been recently provided also for SLIT (68): in a study in 130 children with rhinitis, the onset of asthma after 3 years was 3.8 times more frequent in the control group than in the

SLIT group. is still lacking for SLIT. Also, SCIT is capable of preventing the onset of new sensitization (69, 70). We have recently studied this aspect in a large population of patients with asthma and/or rhinoconjunctivitis receiving SLIT in a real-life setting (45). The study involved 319 active treatments plus 192 controls receiving only drug therapy. After the third year, new skin sensitization appeared in 5.8% of the SLIT patients and 38% of the controls ($p < 0.001$). This was the first demonstration that SLIT exerts a preventive effect on new sensitizations.

■ **Adherence.** Similarly to drug therapy, also for immunotherapy the adherence is critical for the successful outcome of the treatment. In the case of SLIT, the adherence has been always regarded as a weak point, because the treatment is self-administered. In the case of SCIT, the adherence is believed to be optimal since a physician or a nurse gives injections. Indeed, the few studies available for SCIT, show that the adherence is quite poor (71, 72). We attempted to systematically evaluate the adherence to SLIT in a real-life setting (73). Consecutive patients receiving SLIT in tablets for mites or pollens underwent random telephonic interviews and were asked to count the remaining tablets at that moment. By means of a computerized program, it was possible to establish exactly the number of taken tablets versus the expected at a given time. After one year of observation, the data from 35 patients (mean age 26.5) were available. The adherence was 95% for pollen immunotherapy and 97% for mite immunotherapy.

■ **Mechanisms of action** The knowledge on the mechanisms of action of SCIT is rapidly evolving, and it is now well ascertained that it can modulate the Th1/Th2 balance and increase the number of T regulatory cells (74). On the contrary, SLIT has been studied so far mainly from a clinical point of view in order to assess its efficacy and safety, and only few systematic observations on the mechanisms of action have been made. Nevertheless, some of the more recent studies began to investigate different immunological aspects of SLIT. For instance, it is well ascertained that SLIT down-regulates the expression of adhesion molecules on nasal and conjunctival epithelia and the concomitant influx of inflammatory cells (23, 24). Very importantly, in the study by Ippoliti *et al* (38), no change in CD40+ peripheral lymphocytes was detectable, but a decrease of serum ECP and IL-13 was clearly shown in the active treated group. In particular, the decrease of IL-13 accounts for a reduced Th2 activity. This latter fact is in agreement with the hypothesis of an effect on the Th1/Th2 balance. A recent study by Marcucci *et al*, conducted in double blind on 24 children with mite allergy, showed that

SLIT prevents the increase of nasal tryptase after specific allergen challenge, although no change in nasal ECP could be detected (75).

It seems that the contact of the vaccine with the oral mucosa is critical to the effect of SLIT. The recent pharmacokinetics studies performed in vivo in humans with the radiolabelled ¹²⁵I-Par j 1 allergen have shown that the allergen does not cross the oral mucosa, but it persists adsorbed for hours after its administration (76, 77). Overall, the clinical effects of SLIT are identical to SCIT, and the data presently available suggest that the mechanisms of action should not differ between the two routes.

■ **The problem of the ideal dose.** Based on the data of literature, the effective dose of allergen to be administered ranges between 3 and 375 times the doses of SCIT, but this is the only available information. This dose interval of efficacy is wide, and there is no proof that one particular dosage performs better than another. This point is controversial since in some cases, both positive and borderline results have been obtained either with very high doses (>100 times the corresponding SCIT) and lower doses (3-5 times the corresponding SCIT). So far there is no clear evidence that the efficacy is proportional to the dose administered, within the mentioned range, although the recent study by André *et al.* has suggested that the clinical efficacy improves as the dose of allergen given increases (37). On the other hand, it is ascertained that increasing the dose of allergen increases the occurrence of gastrointestinal side effects.

CONCLUSIONS

The treatment of respiratory allergy is based on: allergen avoidance, pharmacological treatment and immunotherapy. IT is a unique allergen-oriented immunomodulator which affects the immune response to allergens and whose action develops over long times (months). The SLIT represents a significant advance in immunotherapy, mainly because of its safety, as testified by numerous controlled trials and several post-marketing surveillance studies. The more recent data from the literature have shown that SLIT is quite similar to the traditional SCIT, since it exerts a long-lasting effect and can prevent the onset of new sensitizations. Moreover, since a clinical effect on both asthma and rhinitis has been demonstrated, the existence of a systemic immunological effect should be postulated.

Several points need further developments (78): first the definition of an optimal maintenance dose and

second the formal demonstration of a preventive effect on asthma. Moreover, The good safety profile makes SLIT, in principle, an optimal choice for paediatric patients, but more studies are needed in this field to establish and define the indications and limits (79). Finally a detailed cost/effectiveness analysis is still lacking.

SLIT is self-administered, and therefore it requires a careful instruction and a strict follow-up of the patients. As for SCIT, the prescription must be made only by specialists, after a detailed diagnosis has been established and the expected benefit/cost ratio has been carefully evaluated.

ACKNOWLEDGEMENTS :

This work has been partially supported by the Foundation ARMIA (Associazione Ricerca Malattie Immunologiche e Allergiche)

References

- 1) Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;i:1572-1573.
- 2) Black JH. The oral administration of pollen. *J Lab Clin Med* 1927; 12: 1156
- 3) Herxheimer H. Bronchial hypersensitization and hyposensitization in man. *Int Arch Allergy Appl Immunol* 1951; 40: 40-57
- 4) Taylor G, Shivalkar PR. Local nasal desensitization in allergic rhinitis. *Clin Allergy* 1972; 2: 125-126
- 5) Taudorf E, Weeke B. Orally administered grass pollen. *Allergy* 1983; 38: 561-564
- 6) Committee on the safety of medicines. CSM update. Desensitizing vaccines. *Br Med J* 1986; 293: 948
- 7) Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy and skin testing. *J Allergy Clin Immunol* 1987; 79: 660-677
- 8) Reid MJ, Lockey RF, Turkeltaub PC, Platt-Mills TAE. Survey of fatalities from skin testing and immunotherapy. *J Allergy Clin Immunol* 1993; 92: 6-15
- 9) EAACI Immunotherapy position paper. Malling HJ & Weeke B eds, *Allergy* 1993; 48.
- 10) Scadding K, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to dust mite. *Clin Allergy* 1986; 16: 483-491
- 11) World Health Organization Position Paper. Allergen immunotherapy : therapeutical vaccines for allergic diseases. Bousquet J, Lockey R, Malling HJ eds. *Allergy* 1998 : 53 suppl.
- 12) EAACI Position Paper on local immunotherapy. Malling HJ ed. *Allergy* 1998; 53: 933.
- 13) Allergic Rhinitis and its Impact on Asthma. Bousquet J and Van Cauwenberge P eds. *J Allergy Clin Immunol* 2001; 108(5 suppl) : S146-S150
- 14) Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double blind study. *Allergol Immunopathol* 1990; 18: 277-284.
- 15) Sabbah A, Hassoun S, Le Sellin J, Andre C, Sicard H. A double blind placebo controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. *Allergy* 1994; 49: 309-313.
- 16) Feliziani V, Lattuada G, Parmiani S, Dall'Aglio PP. Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts. A double blind study. *Allergol Immunopathol* 1995; 23: 173-178

- 17) Troise C, Voltolini S, Canessa A, Pecora S, Negrini AC. Sublingual immunotherapy in parietaria pollen induced rhinitis: a double blind study. *J Invest Allergol Clin Immunol* 1995; 5: 25-30
- 18) Hirsch T, Sahn M, Leupold W. Double blind placebo controlled study of sublingual immunotherapy with house dust mite extracts in children. *Pediatr Allergy Immunol* 1997; 8: 21-27
- 19) Clavel R, Bousquet J, Andre' C. Clinical efficacy of sublingual swallow immunotherapy: a double blind placebo controlled trial of a standardized five grass pollen extract in rhinitis. *Allergy* 1998; 53: 493-498
- 20) Horak F, Stubner UE, Berger U, Marks B, Toth J, Jager S. Immunotherapy with sublingual birch pollen extract: a short term double blind study. *J Invest Allergol Clin Immunol* 1998; 8: 165-171.
- 21) Vourdas D, Syrigou E, Potamianou P *et al.* Double blind placebo controlled evaluation of sublingual immunotherapy with a standardized olive tree pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive tree pollen sensitization. *Allergy* 1998; 53: 662-671.
- 22) Hordijk GJ, Antwelink JB, Luwema RA. Sublingual immunotherapy with a standardized grass pollen extract: a double blind placebo controlled study. *Allergol Immunopathol* 1998; 26: 234-240
- 23) Passalacqua G, Albano M, Fregonese L *et al.* Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite induced rhinoconjunctivitis. *Lancet* 1998; 351: 629-632.
- 24) Passalacqua G, Albano M, Riccio AM *et al.* Clinical and immunological effects of a rush sublingual immunotherapy to parietaria species: a double blind placebo controlled trial. *J Allergy Clin Immunol* 1999; 104: 964-968
- 25) Bousquet J, Scheinmann P, Guinneeapain MT *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
- 26) Purello D'Ambrosio F, Gangemi S, Isola S *et al.* Sublingual immunotherapy: a double blind placebo controlled trial with Parietaria judaica extract standardized in mass units in patients with rhinoconjunctivitis, asthma or both. *Allergy* 1999; 54: 968-973
- 27) Pradalier A, Basset D, Claudel A *et al.* Sublingual swallow immunotherapy (SLIT) with a standardized five grass pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. *Allergy* 1999; 54: 819-828
- 28) La Rosa M, Ranno C, Andre' C *et al.* 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
- 29) Guez S, Vatrinet C, Fadel R, Andre' C. House dust mite sublingual swallow immunotherapy in perennial rhinitis: a double blind placebo controlled study. *Allergy* 2000; 55: 369-375
- 30) Pajno GB, Morabito L, Barberio G, Parmiani S. Clinical and immunological effects of longterm sublingual immunotherapy in asthmatic children sensitized to mite: a double blind study. *Allergy* 2000; 55: 842-849.
- 31) 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.
- 32) Ariano R, Spadolini I, Panzani RC. Efficacy of sublingual specific immunotherapy in Cupressaceae allergy using an extract of Cupressus arizonica. A double blind study. *Allergol Immunopathol (Madr)*. 2001;29: 238-244.
- 33) Bahcecililer NN, Isik U, Barlan IB, Basaran N. Efficacy of sublingual immunotherapy in children with asthma and rhinitis: a double-blind, placebo-controlled study. *Pediatr Pulmonol*. 2001; 32: 49-55.
- 34) Voltolini S, Modena P, Minala P *et al.* Sublingual immunotherapy in tree pollen allergy. Double blind, placebo controlled study with a biologically standardized extract of tree pollen (alder, birch and hazel) administered by a rush schedule. *Allergol Immunopathol* 2001; 29: 103-110
- 35) Lima MT, Wilson D, Pitkin L *et al.* Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis : a randomized controlled trial. *Clin Exp Allergy* 2002; 32: 507-514
- 36) Mortemousque B, Bertel F, De Casamayor J, Verin P, Colin J. House-dust mite sublingual-swallow immunotherapy in perennial conjunctivitis: a double-blind, placebo-controlled study. *Clin Exp Allergy*. 2003;33: 464-469.
- 37) André C, Perrin-Fayolle M, Grosclaude M *et al.* A double blind placebo controlled evaluation of SLIT with a standardized ragweed extract in patients with seasonal rhinitis. *Int Arch Allergy Immunol* 2003; 131:111-118
- 38) Ippoliti F, De Sanctis W, Volterrani A *et al.* Immunomodulation during sublingual therapy in allergic children. *Pediatr Allergy Immunol* 2003; 14: 216-221.
- 39) Pajno G, Vita D, Caminiti D, Parmiani 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 Dec;33(12):1641-7.
- 40) Wuthrich B, Bucher Ch, Jorg W, Bircher A, Eng P, Schneider Y, Schnyder F, Eigenmann P, Senti G. Double-blind, placebo-controlled study with sublingual immunotherapy in children with seasonal allergic rhinitis to grass pollen. *J Invest Allergol Clin Immunol*. 2003;13(3):145-8.
- 41) Tonnel AB, Scherpereel A, Douay B, Mellin B, Leprince D, Goldstein N, Delecluse P, Andre C. Allergic rhinitis due to house dust mites: evaluation of the efficacy of specific sublingual immunotherapy. *Allergy*. 2004 May;59(5):491-7.
- 42) Bufer A, Ziegler-Kirbach E, Stoeckmann E, Heidemann P, Gehlhar K, Holland-Letz T, Braun W. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. *Allergy*. 2004 May;59(5):498-504.
- 43) Wilson DR, Torres LI, Durham SR. Sublingual immunotherapy for allergic rhinitis (Cochrane review). *Cochrane Database Syst Rev*. 2003 ;(2) : CD002893
- 44) Lombardi C, Gargioni S, Venturi S, Zoccali P, Canonica GW, Passalacqua G. Controlled study of preseasonal immunotherapy with grass pollen extract in tablets: effect on bronchial hyperreactivity. *J Invest Allergol Clin Immunol* 2001; 11: 41-45.
- 45) Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Randomized controlled open study with SLIT in real life: clinical efficacy and more. *Allergy* 2004, in press.
- 46) Pajno GB, Passalacqua G, Vita D, Parmiani S, Barberio G. Sublingual immunotherapy abrogates seasonal bronchial hyperresponsiveness in children with parietaria-induced respiratory allergy: a randomized controlled trial. *Allergy* 2004; 59: 883-7
- 47) Patriarca G, Nucera E, Pollastrini E, Roncallo C, Buonomo A, Bartolozzi F, De Pasquale T, Gasbarrini G, Schiavino D. Sublingual desensitization: a new approach to latex allergy problem. *Anesth Analg*. 2002; 95: 956-960
- 48) Cistero Bahima A, Sastre J, Enrique E, Fernandez M, Alonso R, Quirce S, Gandarias B, Parmiani S, Rico P. Tolerance and effects on skin reactivity to latex of sublingual rush immunotherapy with a latex extract. *J Invest Allergol Clin Immunol*. 2004;14: 17-25.
- 49) Quirino T, Iemoli E, Siciliani E, Parmiani S. Sublingual vs injective immunotherapy in grass pollen allergic patients: a double blind double dummy study. *Clin Exp Allergy* 1996; 26 : 1253-1261
- 50) Khinchi MS, Poulsen LK, Carat F, André C, Hansen AB, Malling H-J. 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
- 51) Tinkelman DG, Cole WQ, Tunno J. Immunotherapy: a one-year prospective study to evaluate risk factors of systemic reactions. *J Allergy Clin Immunol*. 1995; 95: 8-14.
- 52) Lin MS, Tanner E, Lynn J, Friday GA. Nonfatal systemic reactions induced by skin testing and immunotherapy. *Ann Allergy* 1993; 71: 557-562

- 53) Ostergaard PA, Kaad PH, Kristensen T. A prospective study on the safety of immunotherapy in children with severe asthma. *Allergy*. 1986; 41: 588-593.
- 54) Stewart GE, Lockey RF. Systemic reactions from allergen immunotherapy. *J Allergy Clin Immunol*. 1992; 90: 567-578.
- 55) Lockey RF, Nikoara-Kasti GL, Theodoropoulos DS, Bukantz SC. Systemic reactions and fatalities associated with allergen immunotherapy. *Ann Allergy Asthma Immunol* 2001; 87 (1 supp): S46-S55
- 56) Andre' C, Vatrinet C, Galvain S, Carat F, Sicard H. Safety of sublingual swallow immunotherapy in children and adults. *Int Arch Allergy Immunol* 2000; 121: 229-234
- 57) Di Rienzo V, Pagani A, Parmiani S, Passalacqua G, Canonica GW. Post-marketing surveillance study on the safety of sublingual immunotherapy in children *Allergy* 1999; 54: 1110-1113
- 58) Lombardi C, Gargioni S, Melchiorre A *et al*. Safety of sublingual immunotherapy in adults: a post marketing surveillance study. *Allergy* 2001; 56: 889-892
- 59) Pajno GB, Peroni DG, Vita D, Pietrobelli A, Parmiani S, Boner AL. Safety of Sublingual immunotherapy in children with asthma. *Paediatr Drugs*. 2003; 5: 777-81
- 60) Agostinis F, Tellarini L, Falagiani P, Canonica GW, Passalacqua G. Safety of SLIT in very young children. *Allergy* 2004 in press
- 61) Drachenberg KJ, Urban E, Prohl S, Woroniecki SR. Sublingual specific immunotherapy for adults and children: a post-marketing surveillance study. *Allergol Immunopathol (Madr)*. 2004; 32: 76-81.
- 62) Lombardi C, Canonica GW, Passalacqua G. Sublingual immunotherapy is clinically safe in patients with oral allergy syndrome. *Allergy* 2000; 55: 677-678
- 63) Grosclaude M, Bouillot P, Alt R *et al*. Safety of various dosage regimens during induction of sublingual immunotherapy. *Int Arch Allergy Immunol* 2002; 129: 248-253
- 64) Frati F, Sensi L, Di Rienzo V, Senna GE, Incorvaia C, Marcucci F. A model for management of sublingual immunotherapy. *Allerg Immunol (Paris)*. 2003 Feb;35(2):56-60
- 65) Passalacqua G, Canonica GW. Long lasting efficacy of specific immunotherapy. *Allergy* 2002; 57: 275-276.
- 66) Di Rienzo V, Marcucci F, Puccinelli P *et al*. Long lasting effect of sublingual immunotherapy in children with asthma due to house dust mites: a ten-year prospective study. *Clin Exp Allergy* 2003; 33: 206-210
- 67) Moller C, Dreborg S, Ferdousi HA *et al*. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; 109: 251-256.
- 68) Novembre E, Galli E, Landi F, Caffarelli C, Pifferi M *et al*. Co seasonal SLIT reduces the development of asthma in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* in press.
- 69) Pajno G, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy* 2001;31: 1392-1397.
- 70) Purello D'Ambrosio F, Gangemi S, Merendino RA *et al*. Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. *Clin Exp Allergy* 2001; 31: 1295-1302.
- 71) Tinkelman D, Smith F, Cole WQ 3rd, Slik HJ. Compliance with an allergen immunotherapy regimen. *Ann Allergy Asthma Immunol* 1995; 74(3): 241-246.
- 72) Lower T, Hensy J, Mandik L, Janosky J, Friday GA Jr. Compliance with allergen immunotherapy. *Ann Allergy*. 1993;70: 480-482.
- 73) Lombardi C, Gani F, Landi M, Falagiani P, Bruno M, Canonica GW, Passalacqua G. Quantitative assessment of the adherence to sublingual immunotherapy. *J Allergy Clin Immunol* 2004; 113: 1219-20
- 74) Till SJ, Francio JN, Durham SR. Mechanisms of immunotherapy. *J Allergy Clin Immunol* 2004; 113: 1025-1034
- 75) Marcucci F, Frati F, Sensi L *et al*. Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites. *Allergy*. 2003; 58:657-662.
- 76) Bagnasco M, Mariani G, Passalacqua G *et al*. Absorption and distribution kinetics of the mayor *Parietaria* allergen administered by noninjectable routes to healthy human beings. *J Allergy Clin Immunol* 1997; 100: 121-129
- 77) Bagnasco M, Passalacqua G, Villa G *et al*. Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001; 31: 54-60
- 78) Nelson HS. Advances in upper airway disease and allergen immunotherapy. *J Allergy Clin Immunol* 2003; 111: S793-798
- 79) Sopo SM, Macchiaiolo M, Zorzi G, Tripodi S. Sublingual immunotherapy in asthma and rhinoconjunctivitis; systematic review of paediatric literature. *Arch Dis Chil* 2004; 89: 620-4.

