The role of allergoids in allergen immunotherapy: from injective to sublingual route

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Summary

Allergen immunotherapy (AIT) is aimed at inducing tolerance to allergens, such as pollens, dust mites or moulds, by administering increasing amounts of the causative allergen through subcutaneous or sublingual route. The evidence of efficacy of AIT is high, but the issue of safety, especially for the subcutaneous route, must be taken into account. The search for safer AIT products aimed at reducing the allergenicity, and thus adverse reactions, while maintaining the immunogenicity, that is essential for effectiveness, gave rise to the introduction of allergoids, which were conceived to fulfill these requirements. In the first allergoids glutaraldehyde or formaldehyde were used as cross-linking agent to polymerize allergens, this resulting in high molecular weight molecules (200,000 to 20,000,000 daltons) which were significantly less allergenic due to a decreased capacity to bridge IgE on its

specific receptor, while maintaining the immunogenicity and thus the therapeutic efficacy. In recent years further agents, acting as adjuvants, such as L-tyrosine, monophosphoryl lipid A, aluminium hydroxide, were added to polymerized extracts. Moreover, a carbamylated monomeric allergoid was developed and, once adsorbed on calcium phosphate matrix, used by subcutaneous route. At the same time, in virtue of its peculiarities, such allergoid revealed particularly suitable for sublingual administration. A lot of clinical evidences show that it is well tolerated, largely safer and effective. Importantly, the higher safety of allergoids allows faster treatment schedules that favor patient compliance and, according to pharmaco-economic studies, they might be more cost-effective than other AIT options.

Key words

Allergen immunotherapy; safety; efficacy; allergoids; monomeric allergoids

Background

Allergen immunotherapy (AIT) was introduced in 1911 by Noon and Freeman, with the provisional name of "desensitizing vaccine"[1]. This treatment was aimed at reducing the reactivity to allergens, namely grass pollen, by subcutaneous administration of increasing amounts of the causative allergen, but remained for decades merely empirical. The discovery of IgE antibodies in the 1960s [2] was crucial for the development of scientific knowledge on the mechanism of allergy, leading to a marked improvement in the diagnosis but also in the quality of allergen extracts for AIT [3). The introduction in the 1980s of immunotherapy products of high biological potency was a further step towards the quality improvement and the consequent reliability of AIT, but the issue of safety came to light. Reports of fatal reactions to subcutaneous immunotherapy from the UK [4] and the USA [5] were published, inducing to reappraise, especially in patients with allergic rhinitis, the feasibility of a treatment burdened by the risk of severe adverse reactions. Such an issue motivated the search for safer AIT products, intending to reduce the allergenicity, and thus adverse reactions while

maintaining the immunogenicity that is essential to induce the immunological modification associated with effective AIT. The first approach to reach this goal was accomplished by introducing the allergoids, conceived to fulfill such the requirements, then followed by a dose reduction in co-administration of the allergen dosage concomitant to adjuvants, and by routes of administration different from the injective route.

The evolution of allergoids for subcutaneous immunotherapy

The first study on allergoids obtained by polymerization of allergens using glutaraldehyde as a cross-linking agent dates back to 1973 [6]. Such chemical treatment resulted in high molecular weight molecules (200,000 to 20,000,000 daltons) which were significantly less allergenic due to a decreased capacity to bridge IgE on its specific receptor while maintaining the immunogenicity and thus the therapeutic efficacy. After 10 years of studies, Grammer et al. concluded that this approach was the most successful in providing a good balance of safety, efficacy and, and immunogenicity in multiple clinical trials [7]. In Europe, the allergoids obtained by the treatment of the partially purified pollen extracts with formaldehyde were evaluated. In 1982 Puttonen et al. showed that the formaldehyde treatment resulted in a change of the net charge of proteins to the more acidic site, in a considerable reduction of the activities of naturally occurring enzymes of native allergen extracts, and the observation of only a trace of activity in the RAST inhibition assay [8]. In the study by Bousquet et al. a lyophilized extract of grass pollen was dissolved in a phosphate buffer, adding formaldehyde to the solution to obtain a 10 mg/ml pollen extract. After incubation, the solution was dialyzed at +4” C to remove formaldehyde and lyophilized. The product was administered by a rush schedule and compared to SCIT with a common standardized grass extract. Both treatments were effective on grass induced rhinitis, more severe reactions were observed with the standardized extract, but also patients treated with the allergoid had SRs [9]. The reduction but not abolition of SRs was also confirmed with other kinds of allergoids, such as the formalinized alum-absorbed allergoid. In a double-blind, placebo-controlled study on patients with grass-pollen allergy high doses of grass allergoid, corresponding to a cumulative pre-seasonal dosage of 46,050 protein nitrogen units (PNU), were

administered, with only one systemic reaction. All patients were evaluated before and during the treatment by symptom-medication scores, specific nasal and skin reactivity, and immunological (specific IgE, IgG, IgG1 and IgG4 antibodies) parameters. The actively treated patients had significantly lower symptom-medication scores than placebo during the month of May and showed a significant decrease in specific skin and nasal reactivity, and a significant early increase in specific IgE, IgG, IgG1, and IgG4, with a subsequent decrease of IgE and IgG1 [10]. A similar aluminum hydroxide-adsorbed depot allergen preparation produced by allergen modification by formaldehyde and titrated in therapeutic units (TU) was studied in a placebo-controlled trial on children with grass pollen-induced allergic rhinitis. Children in the immunotherapy group received 7 injections of grass pollen allergoid before grass pollen season and remained on maintenance treatment 27 months. Clinical and laboratory parameters were compared between the active and placebo-treated groups. After 1 year of immunotherapy, the rhino-conjunctivitis symptom-medication score was significantly lower in the immunotherapy group, and skin test reactivity and nasal reactivity to grass pollen were significantly decreased. Grass-specific IgG, IgG1 and IgG4 increased significantly already at the end of the s build-up therapy, while the seasonal increase in IgE was blunted by active treatment [11]. A recent double-blind, placebo-controlled trial evaluated the dose-response relationship of the same allergoid preparation comparing a single species (*Phleum pratense*) and a multiple species mixture. Three doses of *P. pratense* allergoid (1800 TU, standard-dose 6000 TU and 18 000 TU) were compared with placebo and the marketed 6-grass pollen allergoid (6000 TU). The primary endpoint was the change in wea size in response to the intra-cutaneous testing before and after treatment, while secondary outcomes were the change in total nasal symptom score measured assessed in the allergen exposure chamber, the changes in *P. pratense*-specific IgG4 and the incidence of adverse events. All three doses of *the P. pratense* and the 6-grass pollen allergoid preparations were significantly superior to placebo for the primary endpoint, while no significant differences in the change in nasal scores were detected. The high-dose of *P. pratense*, when compared to the standard-dose, did not yield any additional significant benefit, but was associated with a slight increase in adverse reactions [12].

Further allergoid preparations include the addition to polymerization (by glutaraldehyde or formaldehyde) of L-tyrosine and monophosphoryl lipid A, aluminum hydroxide.

Henmar et al. performed a direct comparison of three intact allergen extracts and four allergoids using IgE inhibition and basophil activation assays to measure the allergenicity, the human T cell proliferation and specific IgG-titres following mouse immunizations to assess immunogenicity of all products. The results showed important differences in both allergenicity and immunogenicity, that require specific documentation of clinical safety and efficacy for each product [13]. As far as safety is concerned, the Paul-Ehrlich-Institute published a report on adverse drug reactions (ADRs) to injective immunotherapy from 1991 to 2000. ADRs to allergoids classified as serious were evaluated between 0.01% and 0.0005%, corresponding to one serious ADR in 10,000 to 200,000 injections. "Although based only on absolute numbers, the hypothetical assumption regarding better tolerance of the allergoids compared to native allergen preparations was not confirmed, while concerning delayed ADRs 75% of them were related to unmodified semi-depot preparations, and 25% were related to allergoids [14]. In a recent review by Rajakulendran et al. on novel strategies for AIT, which analyzed the data from grass pollen allergoids currently available, the pharmaco-economic aspects were also considered. Based on the available studies, the authors concluded that allergoids, mainly based on their shorter schedules of administration, might be more cost-effective than other AIT options [15].

The development of allergoids for sublingual immunotherapy

A particular allergoid to be administered by sublingual route has been developed. and used for almost 30 years. The product used was a carbamylated monomeric allergoid, which is a chemically modified allergen obtained by substitution of ε-aminogroups of allergen lysine residues, which reduces IgE-binding activity while preserving immunogenicity. Initially this allergoid was used for subcutaneous route [16] once adsorbed into a matrix of calcium phosphate; at the same time the peculiarities (monomericity) of this allergoid made it particularly suitable for sublingual administration. The definition of monomeric derives from the selectivity of carbamylation, which does not concern the

structural conformation, with no increase of the size of the allergen molecule as occurs with polymerization. The first double-blind, placebo-controlled trial on the efficacy of an allergoid administered by the sublingual route was published into Lancet as a demonstration of its originality. In patients with mite-induced rhinitis, active treatment resulting in significantly lower symptom scores and a significant decrease of the immune-mediated inflammatory response [17]. The second trial evaluated the efficacy of sublingual tablets of monomeric allergoid obtained from grass pollen in children with rhinitis and asthma caused by grass pollen. Children receiving a preseasonal active treatment had a significant reduction of symptoms scores, particularly bronchial symptoms, and a decrease of nasal eosinophil cationic protein, with good tolerance to the allergoid [18]. The safety in children was confirmed in subjects aged less than 5 years treated with either mite of grass pollen monomeric allergoids [19]. A further safety study evaluated 105 patients (28 children and 77 adults) undergoing SLIT with a mite or grass pollen or Parietaria pollen by an ultra-rush schedule reaching the top dose in 20 minutes. Only one patient (0.9%) had an adverse reaction consisting of gastric pyrosis, with spontaneous recovery [20]. Indeed, several other studies on the efficacy and safety of monomeric allergoids are available, which were analyzed in 2010 by Mösges et al., in a systematic review and meta-analysis. The global number of patients with allergic rhinitis included in these studies were 266 for grass pollen and 241 mite allergoid. The average improvement in symptom scores was 34% for grass pollen and 22% for mite allergoid in comparison with the placebo group, and the average improvement in medication scores was 49% and 24% for grass pollen and mite allergoid, respectively. Few side effects, with no systemic reactions, were reported in the trials [21]. The most recent studies investigated the dose-dependence and dose-finding of monomeric allergoids. The first study evaluated the efficacy and safety of the dose of 1000 or 2000 allergy units (AU) in 34 mite allergic patients, using as primary outcome the change of the threshold of allergen concentration inducing a positive nasal provocation test. After 12 weeks all patients treated with 1000 AU and all but one treated with 2000 AU had an increase in the threshold dose inducing positive provocation tests. The rate of adverse reactions, all mild, was comparable with the two doses [22]. In a

randomized, double-blind, phase 2 study on 158 adult patients with grass pollen-induced rhinoconjunctivitis, four different doses, equal to 300, 600, 1000 and 2000 UA/day were administered. The rate of patients with no symptoms to conjunctival provocation test after treatment was 54.3, 47.6, 59.0 and 51.4%, respectively, suggesting 1000 UA/day as the optimal dose. No serious adverse event was reported [23]. However, in a 12-week double-blind, placebo-controlled dose-finding study on 131 patients with mite-induced rhino-conjunctivitis receiving the dose of 300, 1000, 2000. Or 3000 UA/day, the highest rate of treatment response, as assessed by the conjunctival provocation test, was observed with the 2000 UA/day (88.5%). An overall number of 20 treatment-related adverse events (all mild) were recorded [24]. The positive clinical outcomes of the carbamylated monomeric allergoid are supported by immunological investigations, which disclosed that the mechanisms of action are those illustrated for AIT in general. In fact, SLIT with mite monomeric allergoid was shown to down-regulate allergen-specific IgE and to increase interferon-gamma- and interleukin (IL)-10 production, commonly associated with the development of allergen tolerance [25]. The up-regulation of IL-10 was detected also during a short-term course (60 days) of SLIT with grass monomeric allergoid, along with allergen-specific T-cell proliferation and reduction of allergen-specific in vitro proliferation [26]. In a study comparing two induction schedules of SLIT with mite monomeric allergoid of different duration (98 days vs. 16 days) the more rapid induction scheme was associated with a reduction in TNF-alpha and IL-4 at the end of induction [27].

For complete information of the reader, Table 1 summarizes the main results of all the available studies on SLIT with carbamylated monomeric allergoid,

Conclusions

The introduction of allergoids was an actual advance for AIT with inhalant allergens, providing a response to the problem of systemic reactions to injective immunotherapy, which rather commonly hindered the performance of the treatment, being rarely able even to result in fatal events. Abundant literature supports the role of allergoids in AIT, including for injective AIT several types, obtained by different chemical treatments of the natural allergens to reduce allergenicity while maintaining the

immunogenicity and thus the therapeutic efficacy. Also, a product to be used by the sublingual route is available, which consists of the carbamylated monomeric allergoid, which has good evidence of efficacy and safety. Still, there is room for allergoids characterization, taking into account the allergoids require more sophisticated analytical methods than native extracts [28]. In addition, in the current landscape of the regulatory requests governing allergen products, special requirements need to be implemented for control of allergoids [29]. We have identified a total of 24 journal articles reporting 313 participants as total number of active patients and 298 participants as total number of placebo/control group (Lais Mites: 64 active/ 61 placebo-control ; Lais Birch 55 active /82 placebo-control; Lais Grass 114 active/ 95 placebo-control; Lais Parietaria 80 active/ 60 placebo-control).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONFLICT OF INTEREST

CC, MS declare no conflict of interest, financial or otherwise. C Incorvaia is a scientific consultant for Stallergenes Italy. FF, CE, GA, MG are employees of Lofarma SPA.

CRediT AUTHOR STATMENT

IC, CC, CE, FF, MG Conceptualization, Resources. CC, IC Writing - Original Draft. FF, GA, MS Writing - Review & Editing

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Allergen	Study	Study objective	Study design	Scheme -Duration- Dose	No patient	Patology	Results
Lais Mites - Chemically Modified Allergen Extract of house dust mites (*Dermatophagoides pteronyssinus 50%, Dermatophagoides farinae 50%)*	*Pacor ML (1995) [30]*	Efficacy and safety	Open observational Study	Continuative – 2 years- increasing doses 25/100/300/1000 AU alternate days, each dose for 3 times; maintenance dose: 1 tablet of 1000 AU weekly.	14/-	Asthma of light or moderate degree	Before and after the treatment: • Reduction of the number and severity of asthma attacks (p<0.001) • Improving the expiratory peak flow (PEF) (p<0.001). • No side effects were observed and all patients concluded the study
	Passalacqua G (1998) [17]	Efficacy and safety	Randomised, placebo controlled, double-blind, parallel study	Continuative - 24 months- increasing doses 25/ 50/ 100/200/300/600/1000 AU alternate days, each dose for 3 times; maintenance dose: 2 tablets of 1000 AU twice weekly.	10 Active / 9 Placebo	Perennial rhinoconjunctivitis, at least for 2 years	Active vs Placebo: • Neutrophilic infiltration decreased (p=0·002). • Eosinophilic infiltration decreased before challenge (p=0·001). • ICAM-1 expression reduced before challenge (p=0·01) and during and after treatment (p=0·002) • ECP decreased after 12 months of treatment (p=0·04) • The treatment was well tolerated. 1 local (oral itching) side-effects in active group
	Lombardi (2001) [31]	Safety	Observational Study	Continuative – 31.9 months - increasing doses 25/ 50/ 100/200/300/600/1000 for 8 weeks every other day; maintenance dose 2000 AU once a week.	69/	Perennial or seasonal rhinitis and/or mild asthma	• 17 adverse events corresponding to 7.5% of patients and 0.52 per 1000 doses: 7 episodes of rhinitis, 3 of oral itching, and 1 of abdominal pain. Two cases of urticaria and two of abdominal pain/nause were controlled by a temporary dose-adjustment, and one case of urticaria and conjunctivitis required oral antihistamines. • Medical intervention was needed in six patients only during a 3-year period. • No severe systemic side-effect *The events reported as results of Lombardi's study were observed in 198 patients receiving different SLIT treatments (69 patients – Mites ;75 patients – Grasses; 46 – Parietaria; 4 Birch; 1 Olive; 3 Compositae)
	Passalacqua G (2006) [32]	Efficacy and Safety	Randomized, placebo-controlled, double-blind, multicenter	Continuative – 2 years - Increasing doses 25/ 50/ 100/200/300/600/1000 AU on alternate days, each dose for 3 times; maintenance dose: 1 tablet of 1000 AU twice weekly.	34/34	Mild persistent rhinitis with/without mild intermittent asthma, since at least 2 years	Active vs Placebo: • Fifty-six patients completed the study (28 Active/ 28 Placebo) • A significant difference in the clinical score after 1 year of treatment (P = 0.027) • A significant difference for the symptom *nasal obstruction* after 1 year (P=0.05) and 2 years (P=0.033) • A significant global drug intake at the first year of treatment (P = 0.036) • A significant change in SLIT group was seen for the item *change in health status* (P = 0.05) after the second year of treatment. • No relevant side effect was reported (30 *vs* 43 events) • The need for extra visits was lower in the active group (25% *vs* 43%)

Allergen	Study	Study objective	Study design	Scheme -Duration- Dose	No patient	Patology	Results
	Cosmi L (2006) [25]	Efficacy	Open, randomized, two arm parallel group: one treated with SLIT, one untreated (UT) and receiving only rescue symptomatic drugs	Continuative - 1 year and half – increasing doses (25/ 50/ 100/200/300/600/1000 AU for 8 weeks every other day; the maintenance dose of 1000 AU once a week.	12 SLIT-treated/ 13 untreated (UT)	Perennial rhinitis and/or rhinitis plus mild asthma	Active vs Control: • Twenty patients (80%) completed the study (11 T and 9 UT). • A significant reduction of symptom medication scores after 12 and 18 months of treatment (P<0.05) • Reduction of Dp-specific IgE after 12 and 18 months (P<0.05 and P<0.005 respectively) of therapy • The serum levels of CXCL10 (an IFN-g-driven chemokine) after 12 and 18, but not after 6 months, of treatment were significantly higher (P<0.05) • IL-10 were significantly increased (P<0.05) in culture supernatants of PBMC from 6 month-treated patients in comparison with those detected at the beginning of therapy
	Giordano T (2006) [33]	Efficacy and safety	Open observational study	Continuative – 1 year- Four-day build-up :1st day 500 AU, 2nd day 1.000 AU, 3rd day 1500 AU, 4th day 2000 AU . Maintenance: 5-365 day 1000 AU twice weekly	27	moderate/ severe rhinitis, with or not moderate asthma, perennial or seasonal	• Improvement of the VAS scores was observed. • Decrease of the drug consumption {p<0.01). • No side effects: Only two mild adverse reactions: somnolence and tiredness *The study observed 39 patients house-dust mite (n. 27), grass pollen (n. 7), olive pollen (n. 3), cat dander (n. 1) and Parietaria pollen (n. 1).
	D'Anneo RW (2010) [34]	Efficacy and Safety	Prospective, open-label, randomized study included two parallel groups one treated with SLIT, one treated with standard pharmaco -therapy (control group)	Continuative - 12 months – 300 AU tablet each day for 4 day and the 12-month; maintenance dose 2000 AU/week	15/15	Intermittent or persistent rhinitis or rhino conjunctivitis and/or intermittent, mild-persistent or persistent moderate-severity allergic asthma	SLIT group vs Control: • All patients very well tolerated both the four-day build-up phase and the 12-month maintenance phase • Visual Analogue Scale rises significantly, about 45%, in both groups (p=0.001). • Reduction in the global symptom score SLIT group vs control group, about 52% (p=0.0004). • Smaller rescue drug consumption SLIT group vs control group, about 9%. • The difference between before SLIT (T0) and after 12 months (Tl) was highly significant in skin reactivity (p=0.000003). The control group had a small increase in skin-reactivity (2.6±15.7%) with significance between T0 and Tl (p=0.5226).
Lais Betulle-Chemically modified	*Burastero SE (2009) [35]*	Efficacy and Safety	Open observational, parallel grouped: active and placebo	Continuative - 6 months – 1.000 AU every day	11/11	Seasonal allergic rhino conjunctivitis with or not mild asthma	• Two patients had transient itching in their mouth, spontaneously disappeared. • During the pollen season symptoms/drug usage scores improved of 30% and 40% respectively in actively treated and control patients (p<0.0001); well-days (days without intake of rescue medications and symptoms score less than 2) were in 33% and 23% of patients respectively (p=0.0024).

Allergen	Study	Study objective	Study design	Scheme -Duration- Dose	No patient	Patology	Results
allergen extract of trees pollens (Betula pendula 50%, Alnus incana 50%)	*L. Bommarito (2009) [36]*	Efficacy	Open, randomized, parallel group: three active groups	Continuative -18 months- pre-coseasonal regimen (T1) (6,000 AU/week for 10 weeks/year for two years); perennial regimen (T2) (2,000 AU/week for the entire study period)	8 T1+ 8 T2 /5 T3 (Drug Therapy alone)	Allergic rhinoconjunctivitis with/without mild intermittent asthma	• T1 vs T2: significant improvement of both nasal obstruction ($p<0.01$) and other symptoms ($p<0.01$). • Significant reduction of antihistamine consumption as well as rescue medication score in T1 vs T3 patients ($p<0.05$). • T2 vs T3 patients reported less nasal congestion and ocular symptoms in 2008 season ($p< 0.01$). • No significant AR have been observed.
	Passali D (2010) [37]	Efficacy and Safety	Prospective, open, randomized study, with three parallel groups and control group	Continuative -6 months - 1,000 AU (Group A); 500/1,000/1500 AU up-dosing in 4-day (Group B); 300/600/900/1200 AU (Group C) up-dosing in 4-day; Maintenance: 1,000 AU 5-7 times a week	4 (Group A) / 3 (Group B) / 3 (Group C) / 3 (control)	Rhinitis and oculo-rhinitis	Treated VS Control • All patients tolerated all the three dosage very well, no patient interrupted • A statistically significant ($p < 0.02$) reduction of SMSs vs control group • Significant ($p < 0.01$) decrease in nasal reactivity the three SLI T-treated groups, while the untreated controls remained unchanged • A significant increase in VAS values has been observed in all 3 study groups, in comparison to the controls ($p < 0.001$). • During up-dosing 4 slight side-effects in 4 patients, 1 somnolence and 1 tiredness, and 2 oral itching. No side-effects were recorded during the maintenance treatment.
	Marogna M (2013) [38]	Efficacy and Safety	Open randomized parallel 4 groups study: Group 1: BUD 400 mcg/day + anti Lt/s Group 2: BUD 800 mcg/day Group 3: BUD 1600 mcg/day Group 4 : BUD 400 mcg/day + SLIT	Discontinuos - 3 seasons of treatment (February to April) – four-day build-up phase followed by a maintenance phase of three years (1000 Allergic Unit once a day for five days/week)	Group 1 (n=21) / Group 2 (n=21) / Group 3 (n=21) / Group 4 (n=21)	Seasonal mild and persistent asthma and normal lung function associated with AR	• A significantly performance associated with the use of SLIT; only patients of group 4, achieved an appreciable control (mean 24; SEM 0.242). • A significant improvement in allergy symptoms-medications scores (SMS), in patients of group 4 (decrease of 87%) than in all other groups ($p < 0.01$). • The FEV1 increase and the albuterol intake in group 4 was significantly lower after three years ($p < 0.001$), • Reduction of nasal eosinophils and nasal corticosteroids in group 4 • Significant difference in the PD_{20} was detected at baseline between the controls and the 1,000 AU and between the 1,000 and 2,000 AU groups • During the three years of SLIT course, two patients reported one episode of occurred during the maintenance phase and self-resolved without any therapy in less than two hours.
Lais Grasses-Chemically modified allergen	*Bordignon V (1994) [39]*	Efficacy	Randomised, placebo-controlled, double-blind parallel study	Discontinuos – 3 seasons of treatment (February to April) – 25/100/300 and 1,000 AU every other day (3 times a week)	30/30	Perennial rhino conjunctivitis and/or asthma at least for 2 years	Active vs Placebo: • A statistically significant reduction of nasal and bronchial symptoms particularly after the second and the third years of treatments ($p < 0.01$). • Significant reduction of drugs consumption ($p < 0.01$)

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extract of grass pollens (Holcus lanatus 33%, Phleum pratense 33%, Poa pratensis 33%)	*Pacor M.L. (1996) [40]*	Efficacy	Open non comparative	Discontinuos – 6 pre-seasonal months for 2 years- increasing doses 25/100/300 up to 1,000 AU every other day (3 times a week)	34	Seasonal rhino conjunctivitis	• After 1 year reduction of symptoms: sneezing (p<0.001), nasal itching (p<0.001) and ocular symptoms (p<0.001) and improvement at the second year • Significant reduction of antihistamine consumption (p<0.001) • Treatment well tolerated and no side effects
	Caffarelli C. (2000) [18]	Efficacy and safety	Randomised, double-blind, placebo-controlled study	Continuative- 3 months before pollen season- increasing doses 25/50/100/200/300/600 and 1,000 AU every other day (3 times a week)	24 active / 24 placebo	Seasonal rhinitis and/ or rhino-conjunctivitis and/or bronchial asthma	Active vs Placebo: • 44 out of 48 patients (91.6%), all 24 in the active treatment group and 20 of 24 given placebo, completed the study: three because they moved away, and one because of a mild side-effect (abdominal pain) • Significant reduction of total symptoms (P<0.05) during the pollen season • Treatment well tolerated and compliance was good • EG2/EGl increased significantly only in the placebo group during natural allergen exposure (P< 0.01)
	Lombardi C (2001) [41]	Efficacy and safety	Open, controlled study	Discontinuos – 3 months of pre-seasonal treatment for 3 years (1995-1997) - cumulative dosage, 36,000 AU	26 (pharmaco-therapy + SLIT) / 25 (pharmaco-therapy only)	Seasonal rhinoconjunctivitis and/or asthma (mild intermittent or mild persistent)	Active vs Control: • Significant increase (p=.0.01) of PD20 at the methacholine • Significant clinical improvement both for rhinitis (p = 0.001) and asthma (p=0.001) • Reduction of drug intake (p= 0.001) • Improvement of rhinitis symptom without modification of drug intake • Treatment well tolerated and no relevant side effects during the 3 years.
	Lombardi C (2001) [31]	Safety	Observational Study	Continuative – 9.2 months - increasing doses 25, 50, 100, 200, 300, 600, 1000 for 8 weeks every other day; maintenance dose 2,000 AU once a week	75/-	Perennial or seasonal rhinitis and/or mild asthma	• 17 adverse events corresponding to 7.5% of patients and 0.52 per 1000 doses: 7 episodes of rhinitis, 3 of oral itching, and 1 of abdominal pain. Two cases of urticaria and two of abdominal pain/nause were controlled by a temporary dose-adjustment, and one case of urticaria and conjunctivitis required oral antihistamines. • Medical intervention was needed in six patients only during a 3-year period. • No severe systemic side-effect *The events reported as results of Lombardi's study were observed in 198 patients receiving different SLIT treatments (69 patients – Mites ;75 patients – Grasses; 46 – Parietaria; 4 Birch; 1 Olive; 3 Compositae)

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	Quercia O (2001) [42]	Efficacy and safety	Prospective, randomized, open controlled trial with three parallel groups.	Continuative for 16 days: 25/100/300/1000 AU. After for 2 years: Continuative Group 1 1,000 AU/week - Pre-seasonal Group 2: 5,000 AU/week for 10 weeks/year, on demand drug therapy alone (Group 3) for 2 years	Group 1 (n=10), Group 2 (n=11) and Group 3 (n=11).	Rhino-conjunctivitis with/without mild intermittent asthma	• Significant VAS improvement in both SLIT groups, after the first and second pollen season, compared to baseline and to Group 3($p<0.05$). • Less symptoms and need for medications resulted during the second season ($p<0.05$). • Lower drug assumption was significantly in both SLIT groups during the second season ($p<0.05$) • Lower global symptoms score in comparison Group 1 and Group 2 vs Group 3 during the second pollen season ($p<0.05$) • Treatment well tolerated, only 2 patients reported local or mild adverse events and one of this has interrupted the study (Group 1 - originally 11).
	A.G. Palma Carlos (2006) [43]	Efficacy and safety	Monocentric randomised, double-blind, placebo controlled	Discontinuos - pre-seasonal months for 2 years - 25, 100, 300 and 1,000 AU every other day (3 times a week) for 14 weeks 1,000 AU 2 times a week till May.	17 Active / 16 Placebo	Seasonal rhinoconjunctivitis with or not intermittentor mild persistent asthmas since at least two years	Active vs Placebo: • 20 patients out of the 33 enrolled (60.6%) completed the study (13 Active/ 7 Placebo) • Statistically significant decrease of symptom scores (conjunctivitis $p<0.02$, rhinorrea $p<0.03$ and sneezing $p< 0.03$) • Statistically significant decrease of nasal reactivity at the second year of treatment ($p<0.03$) • Lower consumption of inhaled steroids, mean monthly scores ($P < 0.02$) • Treatment well tolerated; 2 mild local adverse events occurred without interruption of therapy
	Burastero, S.E (2008) [26]	Efficacy	Open, observational pilot study	Continuative – 60 days - dose of 2,000 AU once a day	1[illegible]	Rhinoconjunctivitis with or not mild asthma for at least 2 years	• Decrease in Allergen-Specific Proliferation to the rPhl p 1 and to the raw grass extract after 2 Months of SLIT ($P= .002$ and $.04$) • Increase in Transcription of IL-10 ($P < .001$) and TGF-β ($P = .06$), at rPhl p1–Stimulated Lymphocytes • Correlation indexes of pre-treatment and post-treatment changes in IL-10 vs TGF-β expression were 0.17 ($P .47$) and 0.16 ($P .70$), respective
Lais Parietaria-Chemically modified allergen extract of parietaria pollens	*Ariano R (1998) [44]*	Efficacy and safety	Randomised, placebo controlled, double-blind parallel study.	Continuative – 38 week treatment – 25, 100, 300 and 1,000 AU, maximum three times a week. The dosage of 1,000 AU once a week till the end of the study	15/15	Allergic rhinitis with or without asthma	Active vs Placebo: • Improvement of score symptoms and drug consumption with a statistically significant difference at the end of the treatment ($p<0.01$) • Comparison of the areas of the skin tests and RAST before and after treatment showed no statistically significant difference in the two groups. • Comparison of nasal or bronchial provocation test before and after treatment with statistically significant difference ($p<0.05$) No side effect observed: one patient of active group discontinued the treatment owing to digestive troubles (Active Group – 14 out of 15 completed the study)

Allergen	Study	Study objective	Study design	Scheme -Duration- Dose	No patient	Patology	Results
(Parietaria judaica 50%, Parietaria officinalis 50%)	*Lombardi C (2001) [31]*	Safety	Observational Study	Continuative – 16.3 months - increasing doses 25, 50, 100, 200, 300, 600, 1000 for 8 weeks every other day; maintenance dose 2,000 AU once a week.	46/-	Perennial or seasonal rhinitis and/or mild asthma	• 17 adverse events corresponding to 7.5% of patients and 0.52 per 1000 doses: 7 episodes of rhinitis, 3 of oral itching, and 1 of abdominal pain. Two cases of urticaria and two of abdominal pain/nause were controlled by a temporary dose-adjustment, and one case of urticaria and conjunctivitis required oral antihistamines. • Medical intervention was needed in six patients only during a 3-year period. • No severe systemic side-effect • The events reported as results of Lombardi's study were observed in 198 patients receiving different SLIT treatments (69 patients – Mites ;75 patients – Grasses; 46 – Parietaria; 4 Birch; 1 Olive; 3 Compositae)
	Arena A (2003) [45]	Efficacy and tolerability	Prospectic Observational Study	Continuative - Three Years - increasing doses 25/50/100/300 AU and 1000 AU for 3 alternate days. Maintenance phase most patients received 2000 AU twice weekly	24 SLIT / 11 SIT / 9 pharmacological therapy	Rhinitis and/or mild intermittent or persistent asthma or conjunctivitis	• 8 patients interrupted the immunotherapy during the study period: 3 SLIT group and 5 SIT group • The physician's opinion on efficacy, by symptoms and drug consumption reduction, was statistically better in the SLIT group than in the other two groups ($p< 0.0001$). • The difference between the patient's degree of satisfaction of treatments was statistically significant in favour of SLIT treatments ($p< 0.0001$). * The events reported as results of a study observed in 110 patients receiving different treatments (Parietaria, Graminacea, Olea, Dermathopaghoides)
	Lombardi C (2004) [46]	Safety	Multicenter observational Study	Continuative – 18 ± 2 weeks- 1000 AU tablets - count: 3952/4050 tablet	18	Allergic rhinitis and/or asthma at least 2 years	• 11 mild side effects were reported in 6 (7%*) patients: 6 oral itching, 2 rhinitis, 2 nausea, and 1 generalized itching • Omitted dose was documented in 11 patients. *on a total of 86 patients: 41 received SLIT to mite and 45 to pollens (24 grasses, 18 Parietaria, 3 Ragweed).
	Gammeri E (2005) [20]	Safety and the tolerability	Open sequential Non controlled	Continuative – 20 minutes – every 5 minutes, of increasing doses of SLIT 100 AU, 300 AU, 600 AU, 1000 AU, 2000 AU	24	intermittent/persistent rhinitis or intermittent/ mild persistent asthma	Only 1 patient out of 105* (0.9 %) had a mild local symptom (gastric pyrosis) that occurred 30 minutes after the last initial dose and spontaneously disappeared as the treatment was continued. *The study observed 105 patients [Dust (n = 56), Parietaria (n = 34) and Timothy-grass (n =15)]
	La Grutta S (2007) [47]	Efficacy	Prospective, open-controlled randomised	Continuative – 1 year- 16 days build-up 25/100/300/1000 AU Maintenance 1000 AU, 2 times a week for 1 year	33 SLIT / 23 Control *56 pt allergic to House Dust mite with (n-36) or without Parietaria	mild persistent asthma with or not moderate intermittent moderate rhinitis	Active vs Control • All patients completed the study • Greater reduction daily of the mean symptom score ($p<0.01$) and drug consumption ($p<0.001$) in the SLIT than in the control group. • MCh PD20 increased only in the SLIT group($p<0.0005$) • The reduction of nasal eosinophils was statistically greater ($P<0.05$) only in the SLIT group.

Allergen	Study	Study objective	Study design	Scheme -Duration- Dose	No patient	Patology	Results
	D'Anneo RW (2008) [48]	Efficacy and safety	Prospective, randomized, With three parallel Groups receiving either two different dosages of SLIT or the standard chronic	Continuative – 6 months - 1,000 AU/week - 3,000 AU/week	24 (SLIT 1,000 AU/week) / 21 (SLIT 3,000 AU/week) / 21 (drug therapy)	Seasonal rhinoconjunctivitis and/or asthma (mild intermittent or mild persistent)	• VAS: at the 3rd month: $p < 0.05$ improvement in group of higher dose vs control; after 6 months VAS in the SLIT groups is statistically better than control ($p < 0.05$) • Reduction in rescue medication consumption between 3 and 6 months ($p < 0.05$) in all 3 groups. • Reduction bronchial reactivity in the SLIT groups ($p < 0.001$). • Significant increase of MCh PD20 at the end of the study, in both the patients treated with 1,000 AU ($p < 0.05$) and in those treated with 3,000 AU ($p < 0.001$) • No adverse events were observed, no patient interrupted the study
	Passali D (2010) [37]	Safety and efficacy	Prospective, open, randomized study, with three parallel groups and control group	Continuative – 6 months - 1,000 AU (Group A) – 4-day up-dosing 500/1000/1500 AU (Group B) - 4-day up-dosing 300/600/900 /1200 AU (Group C) Maintenance: 1,000 AU 5-7 times a week	4 (Group A) /3 (Group B) / 2 (Group C) / 2 (Control)	Rhinitis and oculo-rhinitis	Treated VS Control • All patients tolerated all the three dosage very well, no patient interrupted • A statistically significant ($p < 0.02$) reduction of SMSs vs control group • Significant ($p < 0.01$) decrease in nasal reactivity the three SLI T-treated groups, while the untreated controls remained unchanged • A significant increase in VAS values has been observed in all 3 study groups, in comparison to the controls ($p < 0.001$). • During up-dosing 4 slight side-effects in 4 patients, 1 somnolence and 1 tiredness, and 2 oral itching. No side-effects were recorded during the maintenance treatment.

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