Specific Immunotherapy (ITS) with allergenic extracts is an effective treatment of allergic disease and the unique approach able to interfere with the “Allergic March”

The risk of inducing side effects, sometimes of a certain severity, associated to ITS (in particular when administered by subcutaneous route) has stimulated the search of safer therapeutic approach.

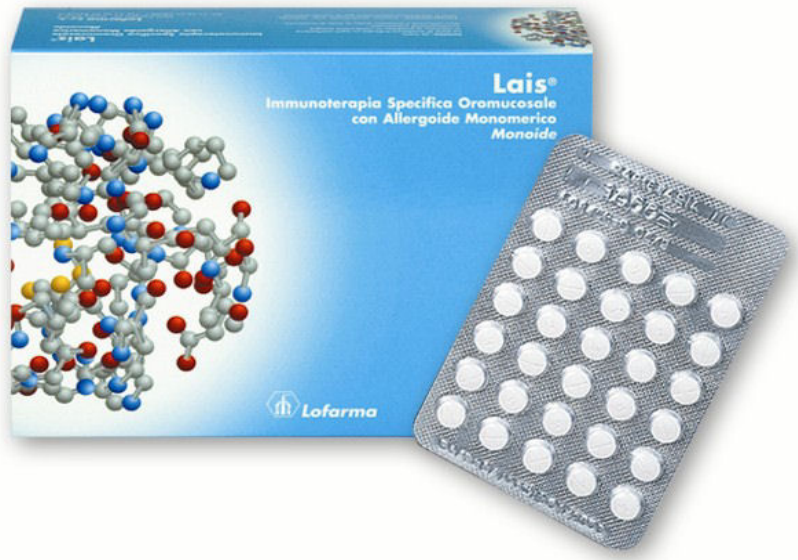
WHAT IS AN ALLERGOID?

- It is an extract obtained by means of a procedure of chemical modification aimed to reduce its allergenic potency while preserving its immunogenic capacity (that is the capacity of inducing Ig response able to also recognize native extract)

LAIS

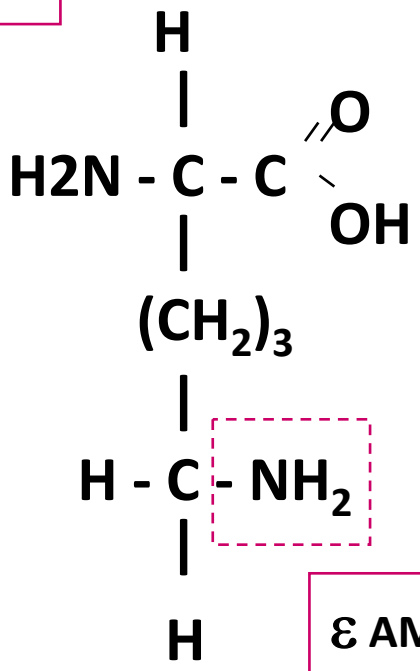
- **Is the first “allergoid” vaccine formulated as tablets to be administered by sublingual route**

It has been developed to overcome one of the limitation of ITS, that is the risk of side effects.



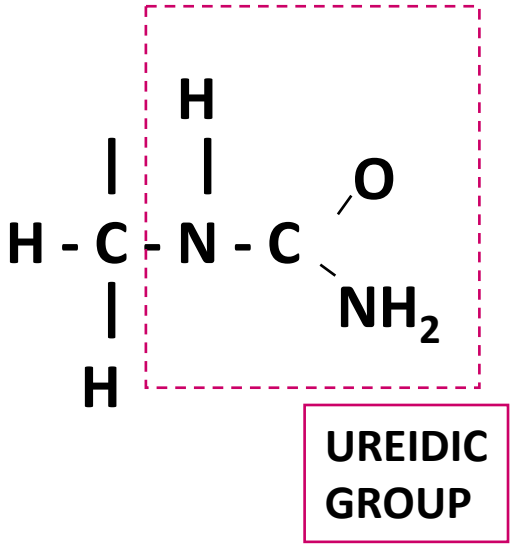
REACTION WITH KCNO

LYSINE:



+ KCNO

HOMOCITRULLINE:

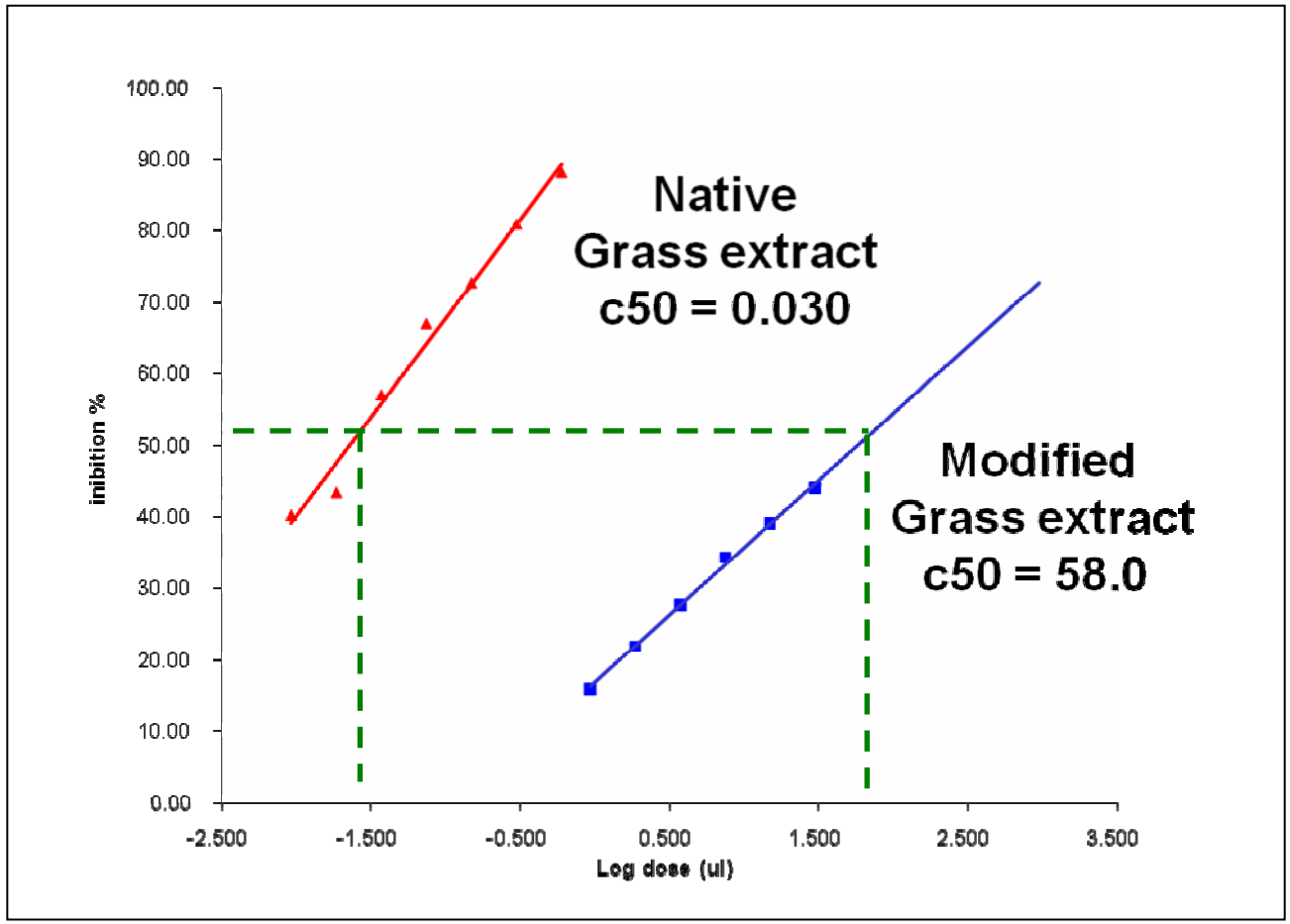


This reaction is known as "**carbamylation**"

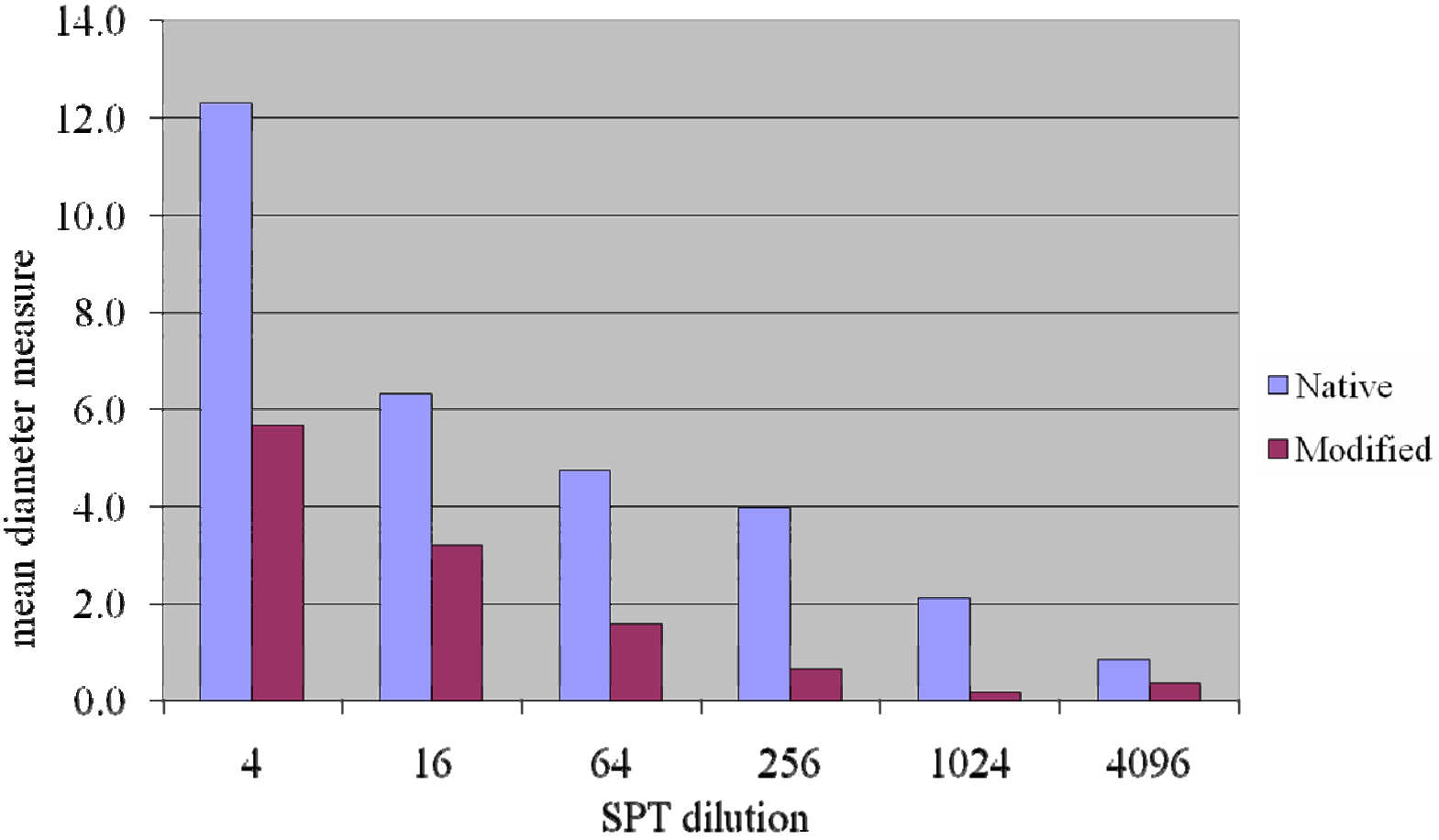
Features of LAIS

- Reduced allergenicity as demonstrated by in vitro and in vivo experiments

In vitro comparison between native and modified grass extract by EAST-inhibition



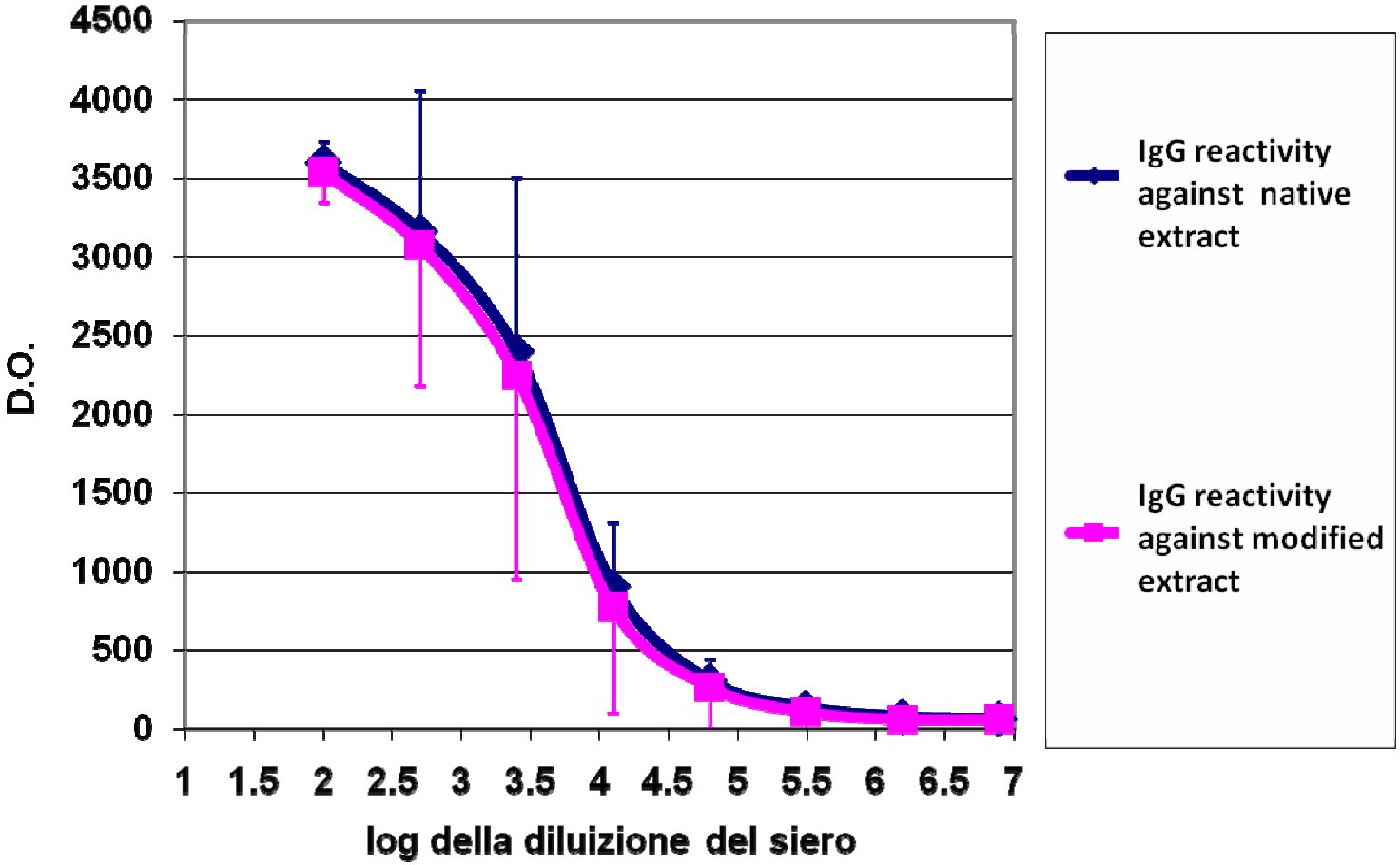
In vivo comparison between native and modified grass extract by SPT



Features of LAIS

- Reduced allergenicity as demonstrated by in vitro and in vivo experiments
- Preserved immunogenicity as demonstrated by ELISA experiments using sera of animals immunized with extract allergoid

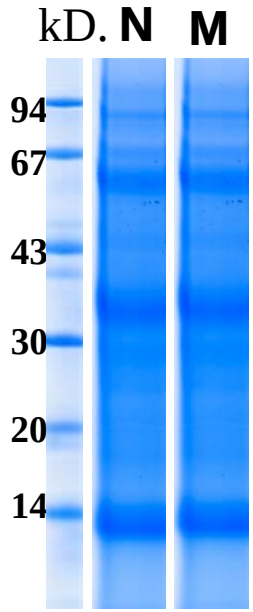
Determination of specific IgG levels in a pool of sera from mice immunized with KCNO-modified grass extract



Features of LAIS

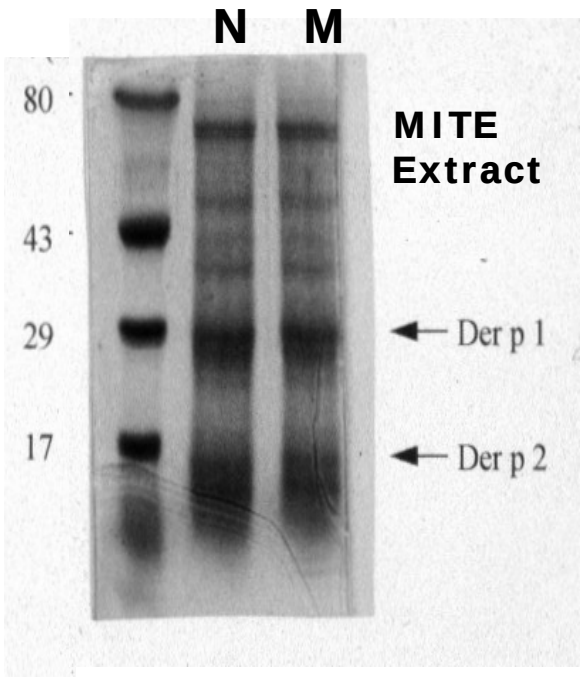
- Reduced allergenicity as demonstrated by in vitro and in vivo experiments
- Preserved immunogenicity as demonstrated by ELISA experiments using sera of animals immunized with extract allergoid
- Preserved molecular dimension of the proteic component as demonstrated bySDS-PAGE

SDS-PAGE Profile of native and modified extracts or purified allergens

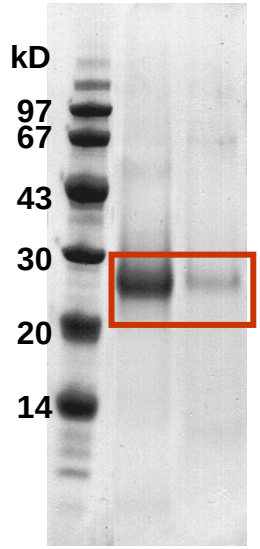


Grass Extract

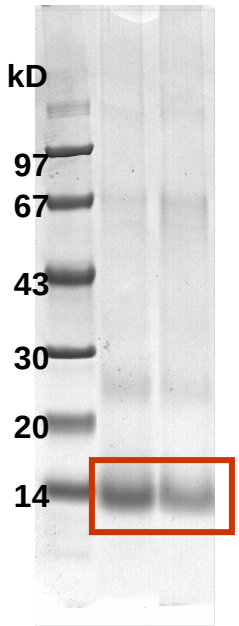
Phl p 1
Phl p 5



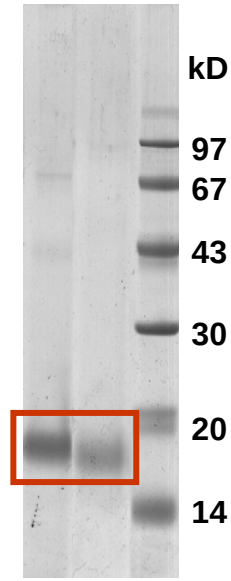
Der p 1



Par j 1



Der p 2



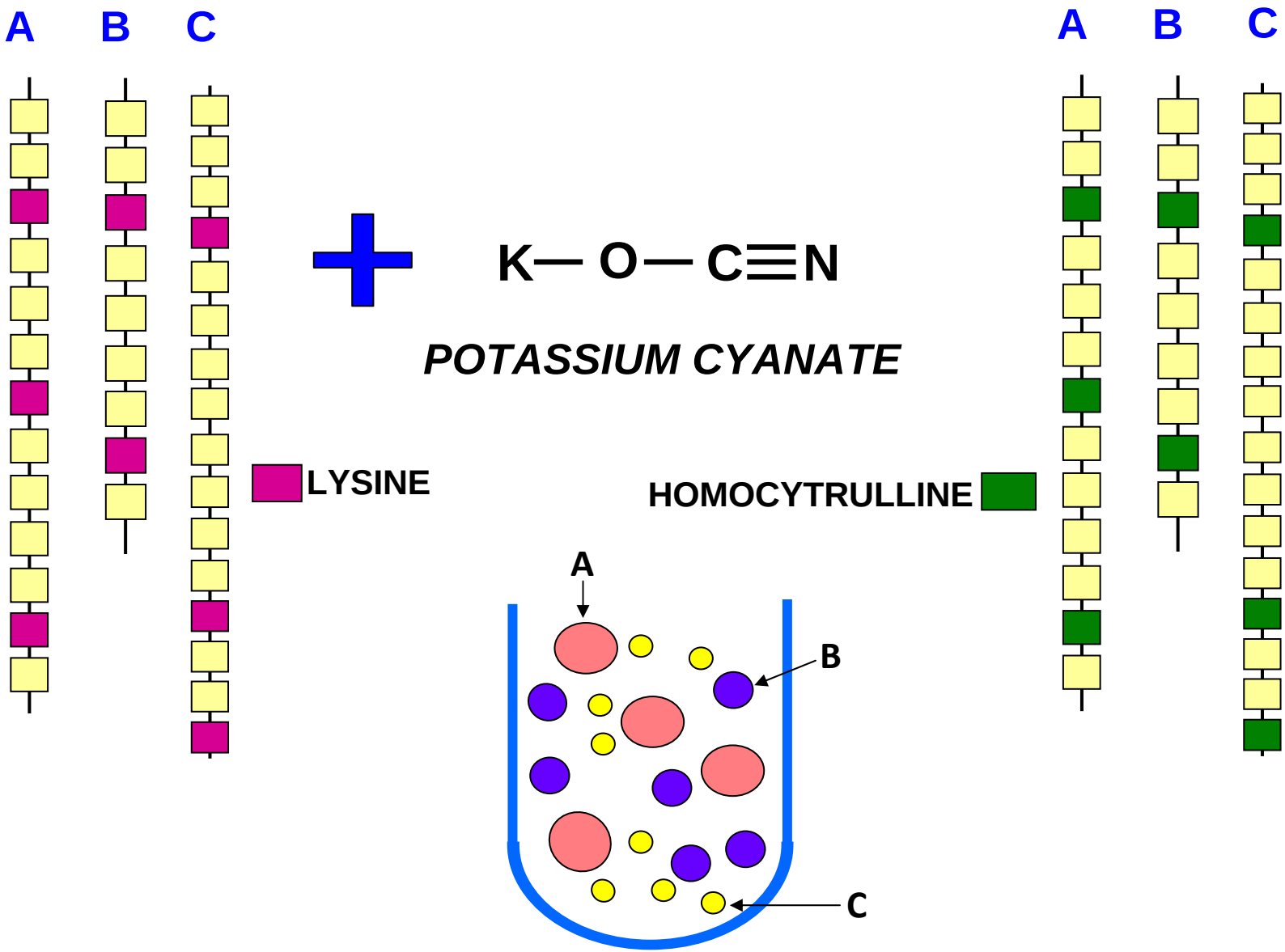
The last feature (monomericity, that is the preservation of the molecular dimension) makes LAIS particularly suitable for sublingual administration.

In this case the capability to pass through by mucosal tissue is an important element in the expression of the therapeutic effect of LAIS

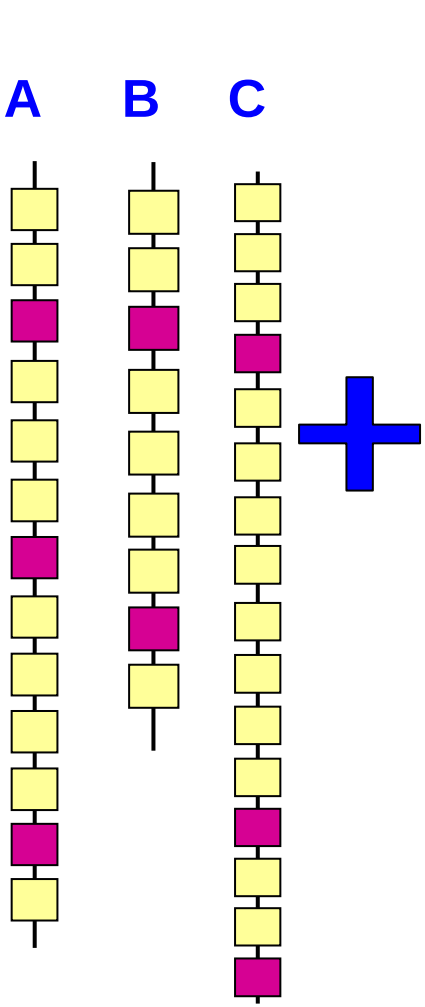
For this reason no any polymeric extract can be administered by sublingual route.

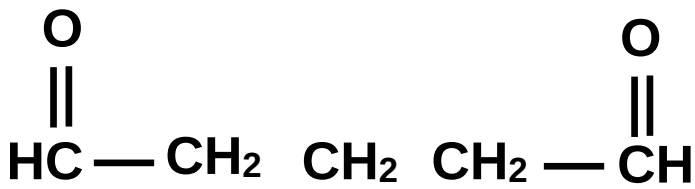
The large molecular dimension (>1000 kD) of their components make it unable to pass through mucosal tissue and therefore it could not be effective.

Monomeric Allergoid

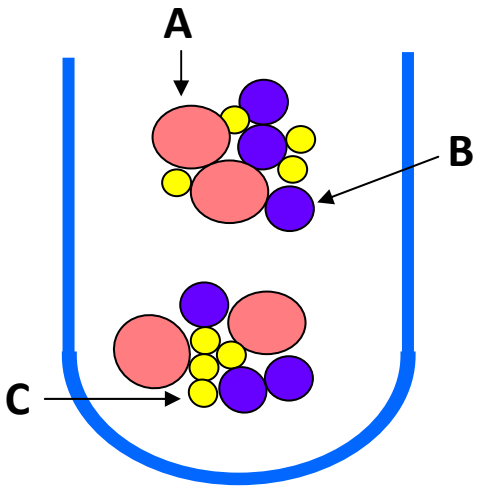


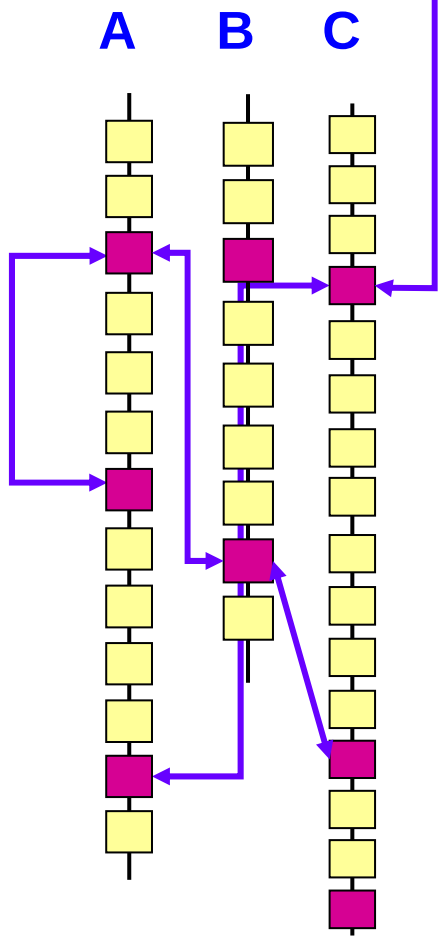
Polymeric Allergoid





GLUTARALDHEYDE





Toxicological study

- To demonstrate the safety profile of LAIS acute (using a single subcutaneous administration of active principle, at dose higher of that one used in humans) or chronic (after repeatet subcutaneous administration of active principle) toxicity studies have been performed
- No sign of toxicity at level of the main organs have been evidenced

Other important observations derive from

pharmacokinetic studies

in humans

Int Arch Allergy Immunol. 2005 Nov;138(3):197-202. Epub 2005 Oct 3.

Pharmacokinetics of Der p 2 allergen and derived monomeric allergoid in allergic volunteers.

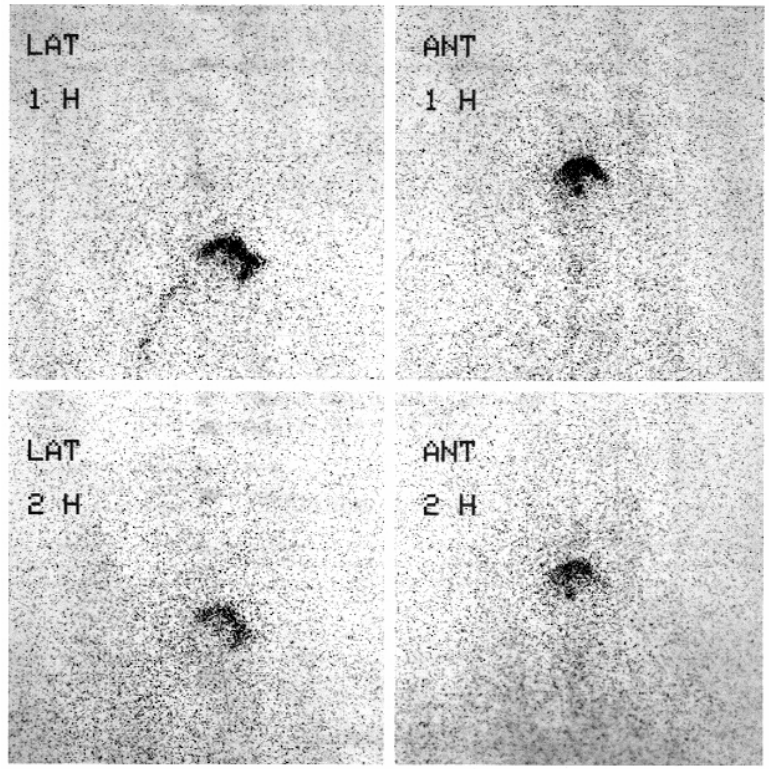
Bagnasco M, Altrinetti V, Pesce G, Caputo M, Mistrello G, Falagiani P, Canonica GW, Passalacqua G.

Medical and Radiometabolic Therapy, University of Genoa, Genoa, Italy.

Protocol:

- Purified Der p 2 or modified Der p 2 (major allergen of mites were radiolabelled with I123 and administered sublingually to allergic volunteers.

- The subject were allowed to swallow 6 min after administration and submitted to scintigraphic analysis at different times (30 min, 1, 2, 3 and 20 hrs)



Persistence of radioactivity in the mouth

Serum samples were obtained at different time and radioactivity present was measured

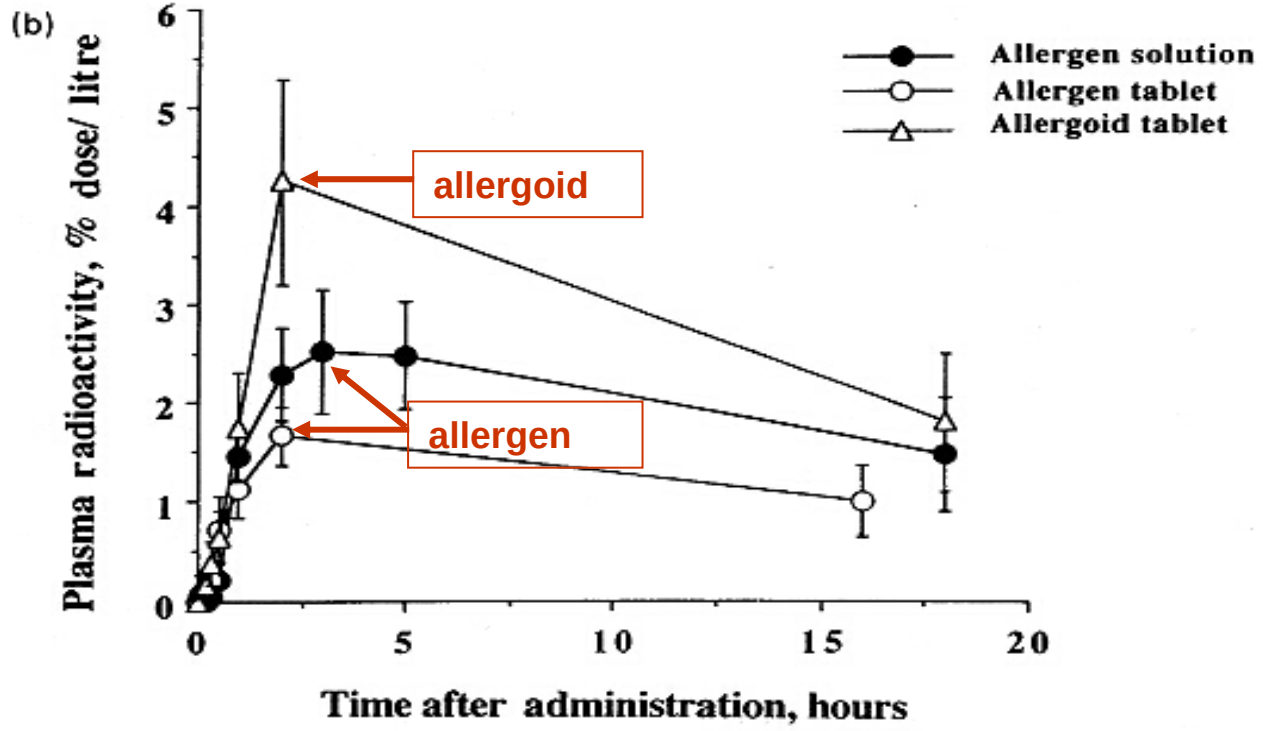
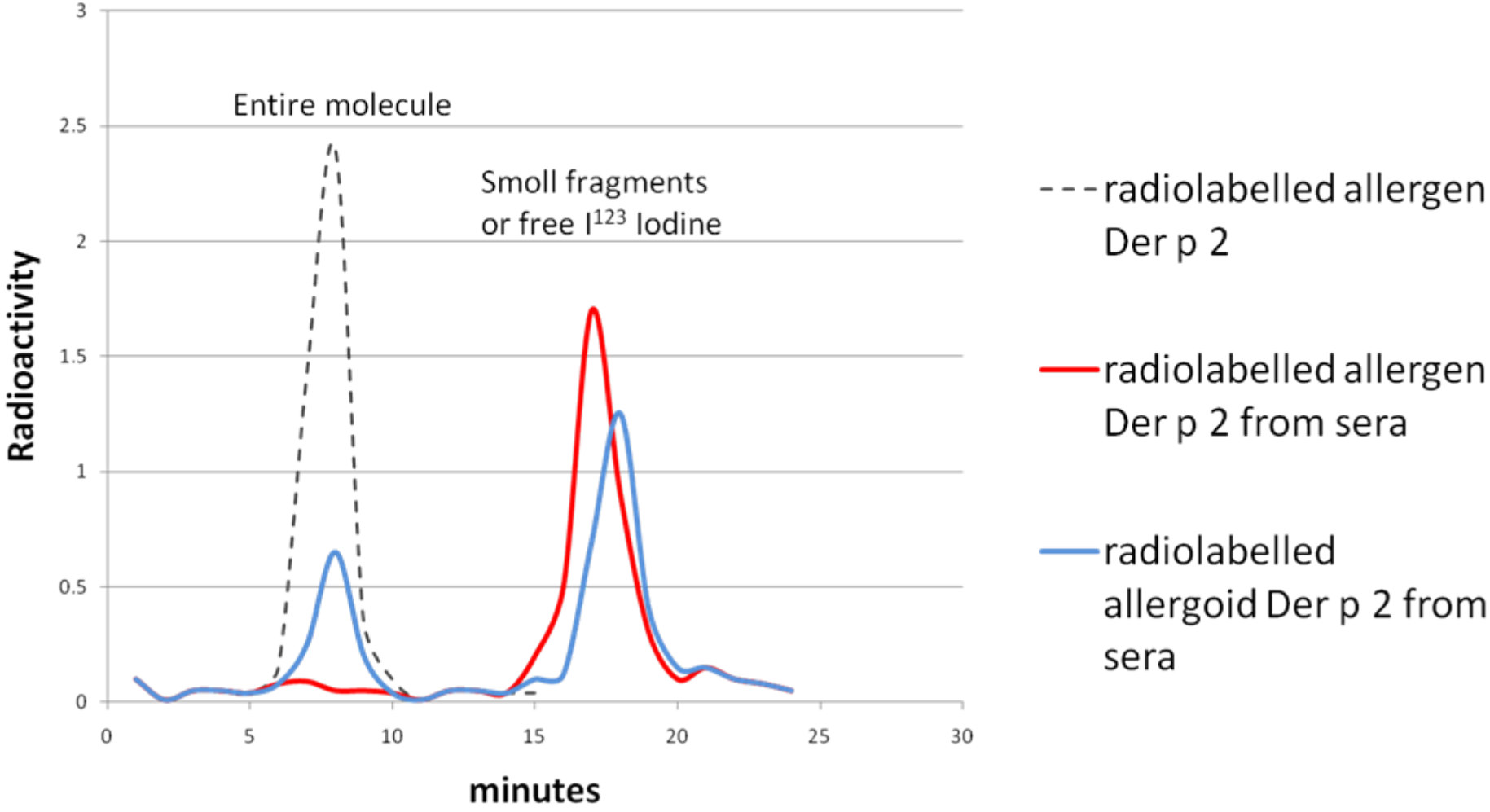


Fig. 3. Plasma radioactivity (% dose/Litre ± SEM) at different times after the administration of the allergen and allergoid in tablets from 0-time to 3 hours (a) and from 0-time to 18 h (b). The kinetics of the allergen administered as aqueous solution (historical datum [13]), is also plotted for comparison. ● = allergen solution; ○ = allergen tablet; △ = allergoid.

Gel chromatography of radiolabelled Der p 2 allergen (----) or serum from patients treated with radiolabelled Der p 2 allergen (__)or allergoid (__)



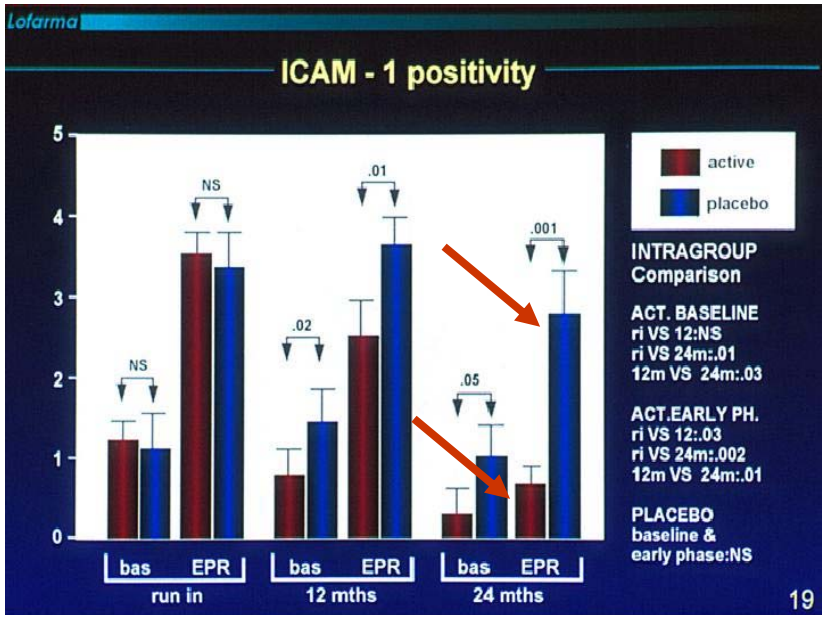
Observations derived from pharmacokinetic studies

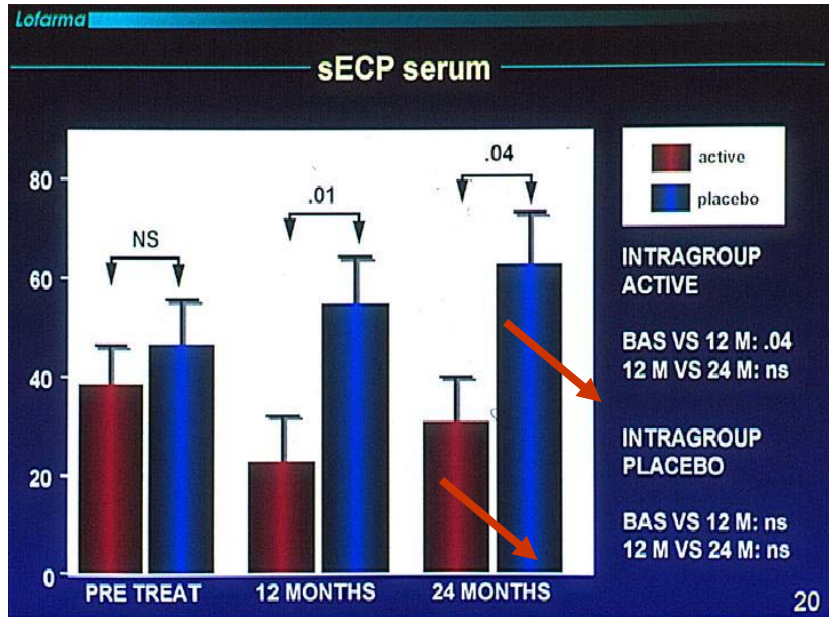
- Allergoid and native allergens show the same pattern at buccal level where they remain for many hours
- Allergoid is able to reach systemic circulation in intact form unlike native allergen
- Allergoid is less sensitive to proteolytic degradation at gastro-intestinal level

Allergoid extract seems therefore act through two concomitant effects: one involving local and the other systemic mechanisms strongly suggesting the active principle present into the LAIS tablets is made all bio-available

What about mechanisms of action of LAIS?

- **LAIS reduces the expression of inflammation markers**





- **Lais reduces the specific IgE levels**

doi: 10.1111/j.1365-2222.2006.02429.x
Clinical and Experimental Allergy, 36, 261–272

ORIGINAL PAPER

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L. Cosmi[1]*, V. Santarlasci[1]*, R. Angeli*, F. Liotta*, L. Maggi*, F. Frosali*, O. Rossi*, P. Falagiani†, G. Riva†, S. Romagnani*, F. Annunziato* and E. Maggi*
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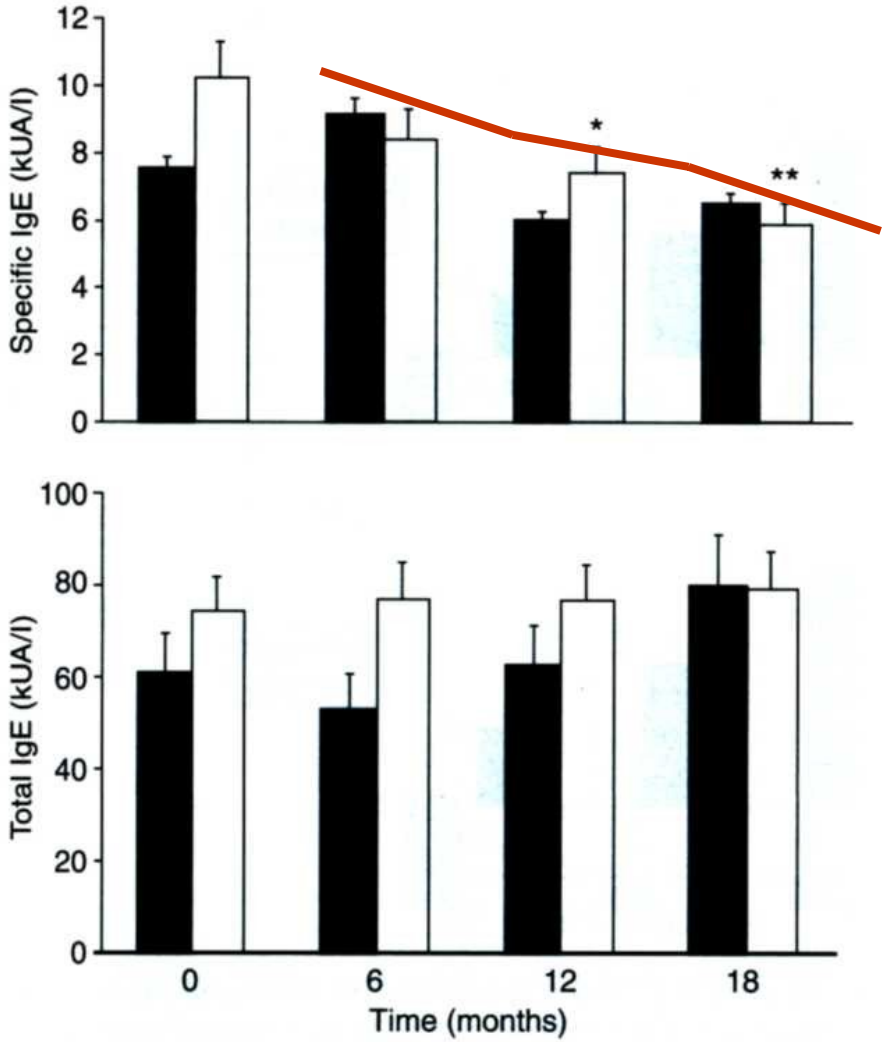

Untreated controls

Allergoid SLIT treated patiens

LAIS reduces lymphocytes proliferative capacity after specific stimulation

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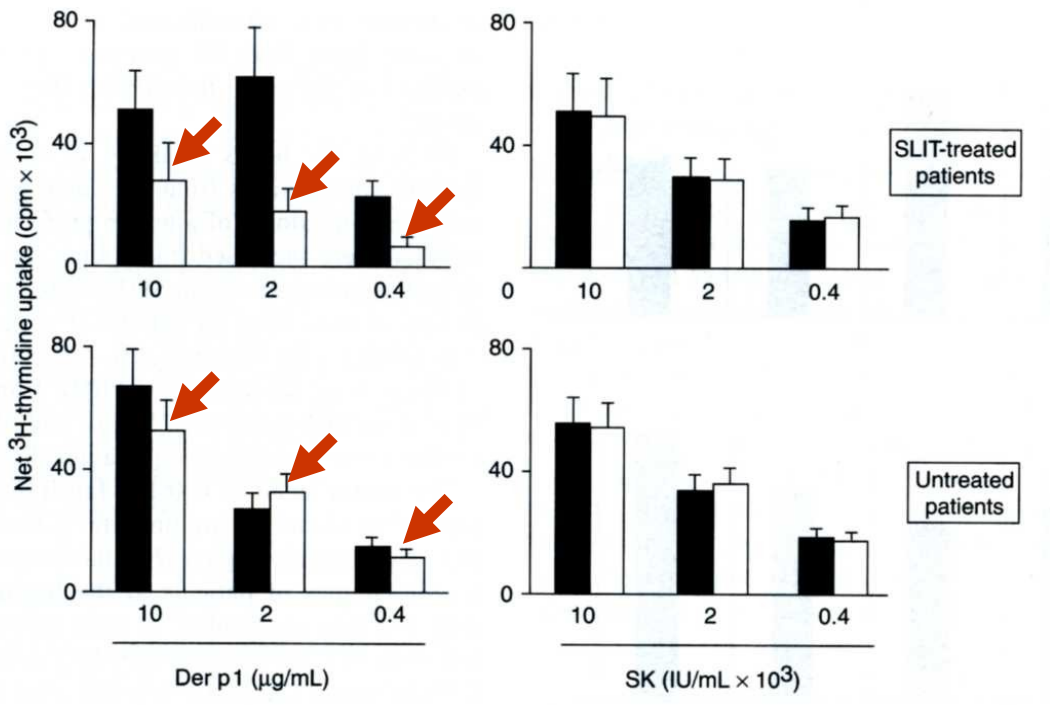
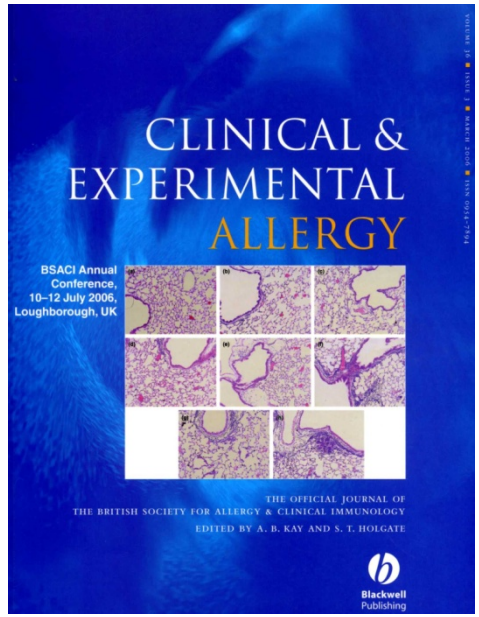
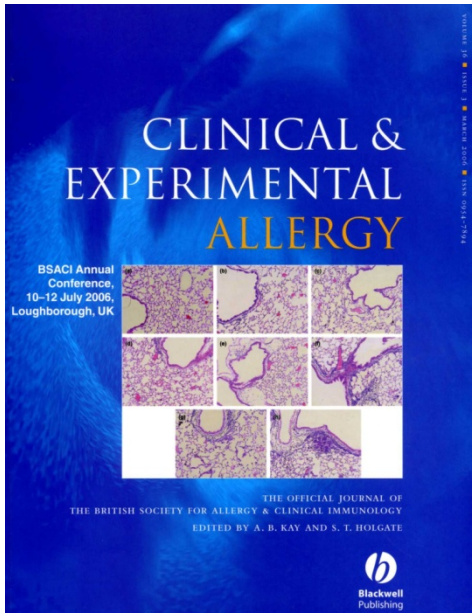




Before SLIT

After 6 months

LAIS increases IL-10 cytokine (marker of immume tolerance



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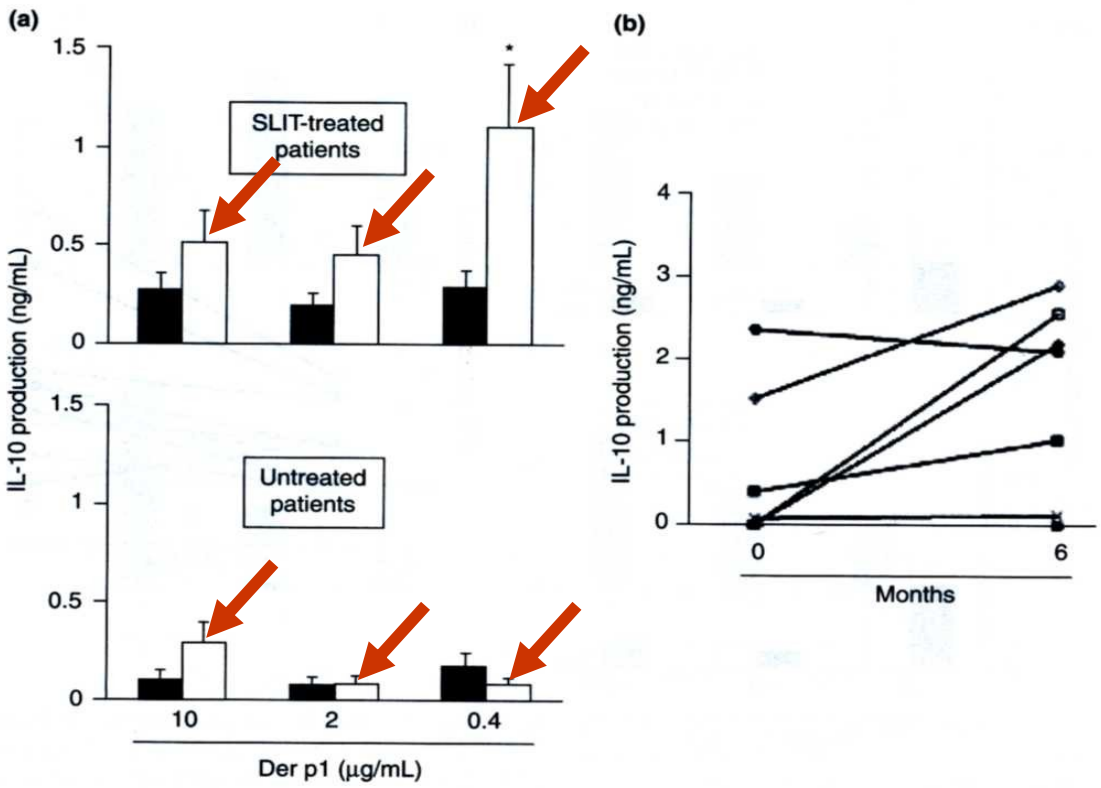
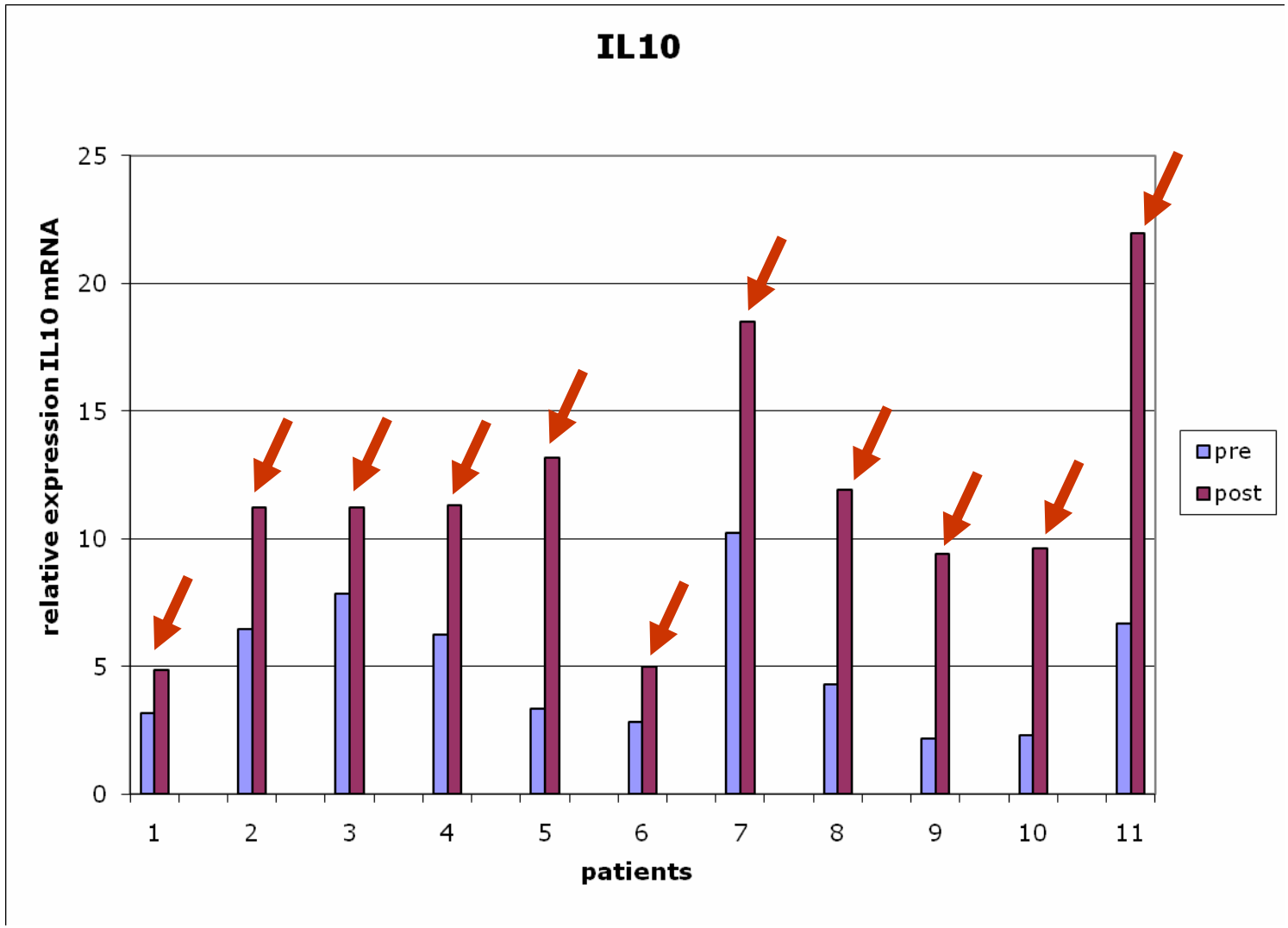


Before SLIT

After 6 months

Sublingual immunotherapy with grass monomeric allergoid down-regulates allergen-specific T-cell proliferation and increases IL-10 production

Burastero et al. Ann Allergy, Asthma Immunol 2008;100:343-50



Sublingual monomeric allergoid Lais Lofarma

LAIS-Tablet – Up-dosing (initial induction phase)
1st day:1 tablet 300 UA =300 AU
2nd day:2 tablets 300 UA =600 AU
3rd day:3 tablets 300 UA =900 AU
4th day:4 tablets 300 UA =1,200 AU

LAIS-Tablet - Maintenance Therapy
From 2 to 5 tablets 1,000 AU (= 2-5,000 AU) per week, in different days.

Definition of AU (Allergenic Unit)

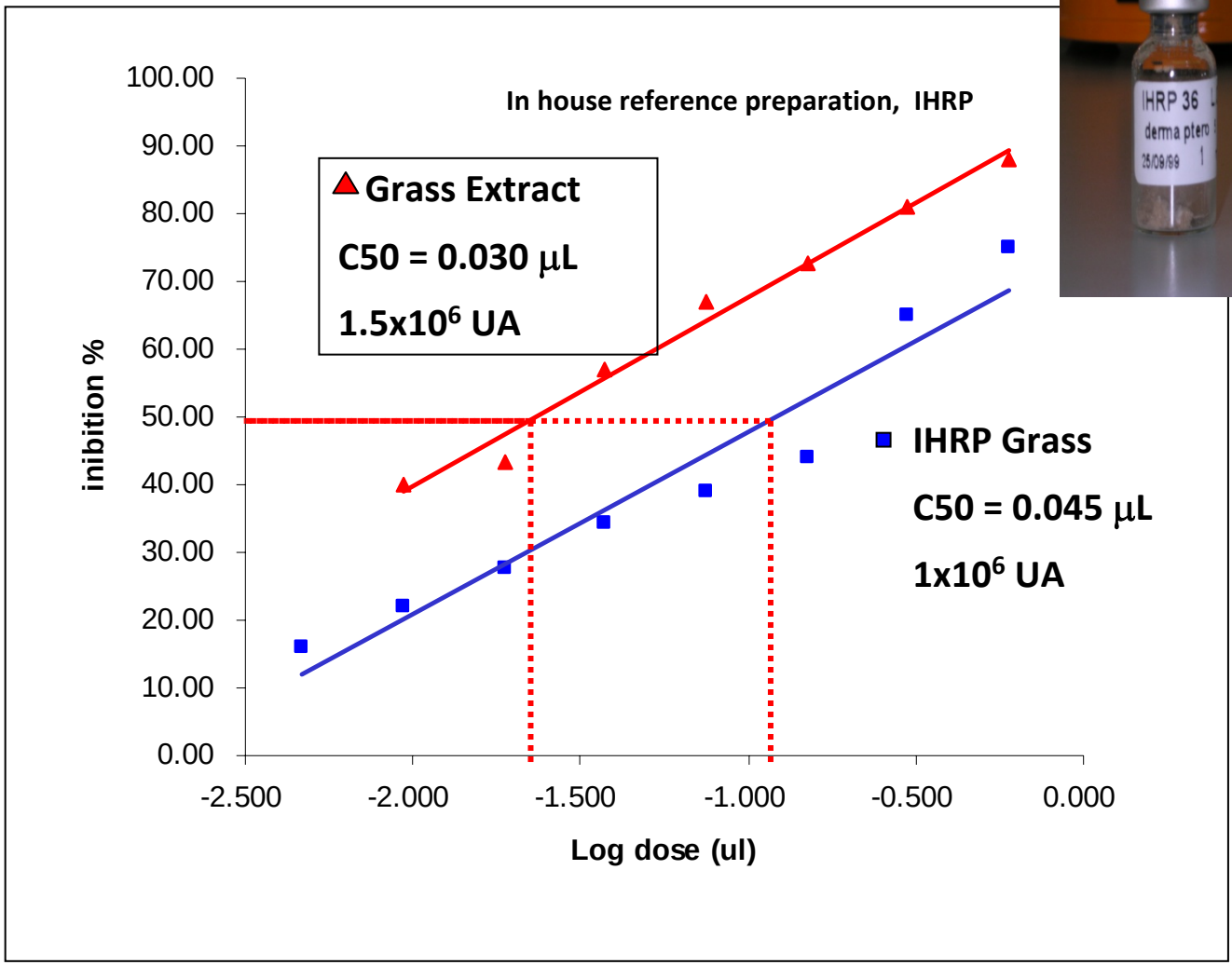
1 AU = 1/40 of the dose of native allergen (IHR, in house reference standard) able to provoke symptoms during nasal provocation test.

In this way it is attributed to IHR preparation its total AU

The total AU of a new batch is determined comparing its allergenic activity with that one expressed by IHR.

On the basis of the difference of allergenic activity its easy to calculate the total AU to be attributed to new batch of extract

Allergenic potency evaluated by EAST-inhibition



To calculate the total AU of allergoid we must take into consideration the protein content too, using the simple proportion

PROTEIN (native allergen) : PROTEIN (allergoid) =
AU (native allergen) : AU (allergoid)

Appendix 3. Published trials of the treatment of dust mite–induced allergic rhinoconjunctivitis.

Author	Method	Study participants		Duration	Intervention	Outcomes	
		Allergoid tablets	Placebo, control			Relative improvement in symptom score	Relative improvement in medications score
L Cosmi (2006)	Open, parallel group design	11	9	2 yrs	SLIT vs. control	2.44% *($p < 0.05$)*	39.51% *($p < 0.05$)*
M La Rosa (1996)	Randomized trial, parallel group design	30	21 SCIT	19 mos	SLIT vs. SCIT	n.s.	n.s.
M Marogna (2007)	Retrospective trial	53	12	1–4 yrs	SLIT vs. control	*$p < 0.001$*	*$p < 0.001$*
ML Pacor (1995)	Open observational study	14		2 yrs	SLIT	n.s.	n.s.
G Passalacqua (1998)	Double-blind, placebo-controlled trial	10	9	23 mos	SLIT vs. placebo	48.4% *($p < 0.0002$)*	n.s.
G Passalacqua (2006)	Double-blind, placebo-controlled trial	28	28	3 yrs	SLIT vs. placebo	13.9% *($p < 0.05$)*	7.83% *($p = 0.036$)*

Note: *n.s. = not specified*

LAIS DERMATOPHAGOIDES: incidence of adverse events irrespectively of relationship to trial drug pooled by study and by organ system

Adverse events	Total N° of AE	Passalacqua		Pacor		La Rosa		Lombardi	Passalacqua (2006)		Cosmi	
	N= 369	Lais Derm (n=10)	Placebo (n=9)	Lais Derm (n=14)		Lais Derm (n=30)	Traditional subcutaneous immunotherapy (n=27)	SLIT therapy (n=198)	Lais Derm (n=28)	Placebo (n=28)	Lais Derm. (n=12)	Untreated (n=13)
Body as whole												
Anaphylaxis	-	-	-	-	-	-	-	-	-	-	-	-
Edema of lips	**6 (1.6%)**	-	-	-	-	-	-	**3**	**3**	-	-	-
Angioedema		-	-	-	-	-	-	-	-	-	-	-
Conjunctivitis	**1 (0.3 %)**	-	-	-	-	-	-	**1**	-	-	-	-
Oral itching	**2 (0.5%)**	**1**	**1**	-	-	-	-	-	-	-	-	-
Flu-like syndrome	**17 (4.6%)**	-	-	-	-	-	-	-	**5**	**12**	-	-
Traumatic fracture	**3 (0.8%)**	-	-	-	-	-	-	-	**2**	**1**	-	-
Digestive system												
Gastrointestinal complaints	**3 (0.8%)**	-	-	-	-	-	-	**3**	-	-	-	-
Respiratory System												
Otitis	**7 (1.9%)**	-	-	-	-	-	-	-	**3**	**4**	-	-
Asthma	**22 (5.9%)**	-	-	-	-	-	-	-	**10**	**12**	-	-
Rhinitis	**16 (4.3%)**	-	**1**	-	-	-	-	**7**	**5**	**3**	-	-
Cough	**7 (1.9%)**	-	-	-	-	-	-	-	**2**	**5**	-	-
Skin and appendages												
Urticaria	**3 (0.8%)**	-	-	-	-	-	-	**3**	-	-	-	-
TOTAL	**84 (22.8%)**	**1 (1.2%)**	**2 (2.4%)**	**0**	**0**	**0**	**0**	**17 (20.2%)**	**27 (32.1%)**	**37 (44.0%)**	**0**	**0**

Appendix 4. Published trials of the treatment of grass pollen–induced allergic rhinoconjunctivitis.

Author	Method	Study participants		Duration	Intervention	Outcomes	
		Allergoid tablets	Placebo, control			Relative improvement in symptom score	Relative improvement in medications score
V Bordignon (1994)	Double-blind, placebo-controlled trial	30	30	3 yrs	SLIT vs. placebo	38.5% *($p < 0.05$)*	74.60% *($p < 0.001$)*
C Caffarelli (2000)	Double-blind, placebo-controlled trial	24	20	1 yr	SLIT vs. placebo	31.66% *($p < 0.01$)*	n.s.
G Cavagni (1996)	Double-blind, placebo-controlled trial	24	20	2 yrs	SLIT vs. placebo	30.45% *($p < 0.01$)*	22.63% *($p<0.05$)*
C Lombardi (2001)	Open controlled trial	26	25	3 yrs	SLIT vs. control	Rhinitis: 17.27% *($p=0.01$)* Asthma: 60.47% *($p=0.01$)*	Rhinitis: 55.55% *($p = 0.01$)* Asthma: 68.43% *($p = 0.01$)*
ML Pacor (1996)	Observational study	34		2 yrs	SLIT	*$p<0.001$*	n.s.
AG Palma-Carlos (2006)	Double-blind, placebo-controlled trial	17	16	2 yrs	SLIT vs. Placebo	*$p<0.03$*	*$p<0.02$*

Note: *n.s. = not specified*

LAIS GRASS: incidence of adverse events irrespectively of relationship to trial drug pooled by study and by organ system

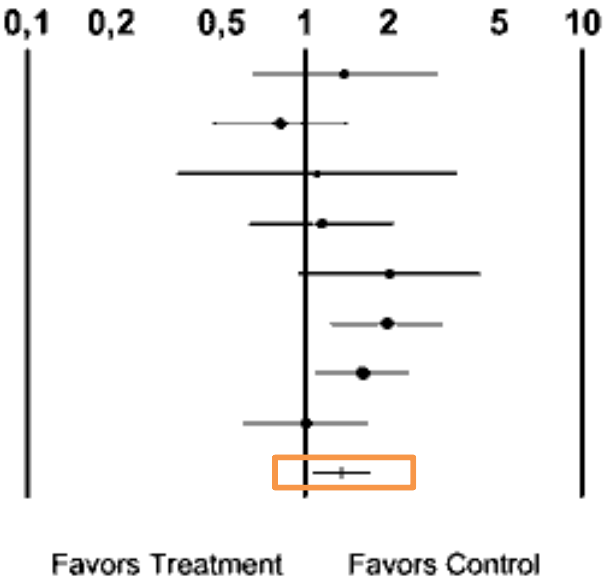
Adverse events	Total N° of AE	Bordignon		Lombardi	Caffarelli		Pacor	Palma Carlos		Lombardi Allergy 2001
Total number of pts evaluated for safety	n=420	Lais Grass (n=30)	Placebo (n=30)	Lais Grass (n=51)	Lais Grass (n=24)	Placebo (n=20)	Lais Grass (n=34)	Lais Grass (n=17)	Placebo (n=16)	Lais Grass (n=198)
Gastrointestinal disorders	3 (0.71%)	-	-	-	-	-	-	-	-	3(1.5%)
Nervous system disorders	1(0.24%)	-	-	1(1.9%)	-	-	-	-	-	
Respiratory, thoracic and mediastinal disorders	7 (1.66%)	-	-	-	-	-	-	-	-	7(3.5%)
Skin and subcutaneous tissue disorders	9(2,14%)	-	-	1(1.9%)	-	-	-	2(11.7%)	-	6(3.03%)
Eye disorders	1(0.24%)									1(0.50%)
TOTAL	21(5.0%)	0	0	2 (3.9%)	0	0	0	2(11.7%)	0	17(8.6%)

META-ANALYSIS

It is a mathematic-statistic method by which is possible to integrate the results of different clinical trials allowing to draw conclusions stronger of that ones we can draw by each single study

It increases the potency of the study and particularly useful when the included clinical studies have been performed on small number of patients.

TreatClass	Treatment	Control	Lower	Effect	Upper	PValue
Beta	125 / 138	118 / 135	,645	1,385	2,976	,402
Beta	39 / 105	42 / 100	,466	,816	1,430	,477
Ca	16 / 26	13 / 22	,347	1,108	3,535	,863
Ca	41 / 81	41 / 87	,628	1,150	2,108	,651
Beta	58 / 73	44 / 67	,946	2,021	4,319	,067
Arb	76 / 147	53 / 151	1,243	1,979	3,151	,004
Ca	114 / 211	88 / 209	1,099	1,616	2,376	,014
Beta	70 / 119	71 / 121	,602	1,006	1,682	,982
Combined (8)	**539 / 900**	**470 / 892**	**1,069**	**1,354**	**1,716**	**,012**



META -ANALYSIS OF THE EFFICACY OF LAIS DERMATOPHAGOIDES (EVALUATION OF SYMPTOMS SCORE)