

# Immunotherapy with Fungal Extracts: Efficacy and Practical Issues

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# Learning Objective

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After participating in this session the attendee should understand the problems that are associated with the use of fungal extracts for allergy immunotherapy

# What are the Issues with Using (Fungal) Mold Extracts for AIT?

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# Issues with Immunotherapy with Fungal (Mold) Extracts

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## Determining Exposure:

1. There are hundreds of thousands of species of fungi.
2. Microscopic identification is usually limited to genera or larger classifications (e.g. basidiomycetes, ascomycetes) rather than species.
3. Many spores do not germinate on artificial media (e.g. basidiospores)

# Classification of Fungi

Phylum	Class	Order	Family	Genus
Zygomycota		Mucorales	Mucoraceae	<i>Mucor</i> <i>Rhizopus</i>
Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	<i>Candida</i> <i>Saccharomyces</i>
			Dipodascaceae	<i>Geotrichum</i>
	Dothideomycetes	Capnodiales	Davidiellaceae	<i>Cladosporium / Fulvia / Hormodendron</i>
			Pleosporales	Pleosporaceae
			??	<i>Phoma</i> <i>Epicoccum</i>
		Dothideales	Dothioraceae	<i>Aureobasidium / Pullularia</i>
		Eurotiomycetes	Eurotiales	Trichocomaceae
	Onygenales		Arthrodermataceae	<i>Trichophyton</i> <i>Epidermophyton</i>
	Sordariomycetes	Hypocreales	Nectriaceae	<i>Fusarium</i>
			Hypocreaceae	<i>Trichoderma</i>
			??	<i>Stachybotrys</i>
		Sordariales	Sordariaceae	<i>Chrysonilia (Monilia) / Neurospora</i>
			Chaetomiaceae	<i>Chaetomium</i>
		??	<i>Acremonium / Cephalosporium</i>	
	Leotiomycetes	Helotiales	Sclerotiniaceae	<i>Botrytis</i>
??	??	Plectosphaerellaceae	<i>Verticillium</i>	
Basidiomycota	Microbotryomycetes	Sporidiobolales	??	<i>Rhodotorula</i>
	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	<i>Ustilago</i> <i>Sporisorium / Sphacelotheca</i>

Ascomycota  
>65,000 species

Each genus  
contains  
several  
hundred  
species

Basidiomycota  
> 30,000 species

# Outdoor Fungi Associated with Increased Asthma Disease Activity

Strong Evidence	Moderate Evidence	Weak Evidence
Total spores – 8 studies	Ganoderma – 4 studies	Curvularia- 1study
Ascospores – 7 studies	Coprinus- 4 studies	Drechslera – 1 study
Basidiospores – 7 studies	Epicoccum- 3 studies	Periconia – 1 study
Alternaria – 9 studies	Aspergillus/Penicillium 3 studies	
Cladosporium – 8 studies	Botrytis – 3 studies	

# Issues with Immunotherapy with Fungal (Mold) Extracts

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## Quality of Fungal Extracts:

1. Multiple strains with varying allergens are available and used for each species by different companies.
2. Fungi have high rates of somatic mutation, and allergens change radically with variations in culture medium, physical conditions and duration of culture. These vary by company.
3. Allergens occur in spores, mycelia and substances secreted into the media, the mix used varies by company.
4. Methods of processing of raw material differ by company and effect the final product.

# Quantitation of the Major Fungal Allergens, Alt a 1 and Asp F 1 in Commercial Allergenic Products.

L Vailes, et al. J Allergy Clin Immunol 2001;107:641-6

- Alt a 1 levels in *Alternaria alternata* extracts from 8 companies produced in 1998 and 1999 ranged from  $<.01$  to  $6.09 \mu\text{g/mL}$  (**> 600-fold**) (mean  $1.4 \mu\text{g/mL}$ )
- Asp f 1 levels in *Aspergillus fumigatus* extracts from 8 companies ranged from  $< .01$  to  $64$  (**>6,000-fold**)  $\mu\text{g/mL}$  (mean  $16.3 \mu\text{g/mL}$ )



# Manufacturing and Standardizing Fungal Allergen Products.

RE Esch. J Allergy Clin Immunol 2004;113:210-5

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- Alt a 1 levels in *Alternaria alternata* extracts from 7 companies ranged from .04 to 2.75  $\mu\text{g/mL}$  (46-fold)
- Allergenic potency by ELIZA-inhibition varied 111-fold.
- No two company's *Alternaria alternata* extracts yielded comparable banding patterns by SDS-PAGE

# Randomized Double-Blind, Placebo-Controlled Studies of Immunotherapy with Fungal Extracts

Author	Allergen	Route	Subjects	Dose
Dreborg 1986	Cladosporium	SCIT	30 Children	<10,000 to 100,000 BU
Malling 1986	Cladosporium	SCIT	22 Adults	43,000 BU

# Randomized Double-Blind, Placebo-Controlled Studies of Immunotherapy with Fungal Extracts

Author	Allergen	Route	Subjects	Dose
Horst 1990	Alternaria	SCIT	24 5-56 years	1.6 ug Alt a 1
Tabar 2008	Alternaria	SCIT	23 Mean 13 years	0.1 ug Alt a 1
Kuna 2011	Alternaria	SCIT	45 Children	8.0 ug Alt a 1
Cortellini 2010	Alternaria	SLIT	27 14-42 years	6.0 ug Alt a 1 per month
Tabar 2019	Alt a 1	SCIT	64 12-65 years	0.2 and 0.37 ug Alt a 1

# Diagnosis and Immunotherapy of Mould Allergy. V. Clinical Efficacy and Side Effects of Immunotherapy with *Cladosporium herbarum*.

H-J Malling et al. Allergy 1986;41:507-19

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- 22 adults with asthma worse in the late summer with no or minimal symptoms outside the *Cladosporium* season.
- Positive PST and bronchial challenge with *Cladosporium* extract.
- 19 had additional positive PSTs.

# Side Effects of Immunotherapy with *Cladosporium herbarum*.

- Symptoms, medication use and PEF recorded during *Cladosporium* season in 1981.
- Placed on standardized *Cladosporium* extract (n=11) or placebo (n=11) in March 1982.
- Projected top dose of 100,000 BU not tolerated, mean maintenance dose 19,000 BU.
- Delayed local reactions > 8 cm occurred in 73% and systemic reactions in 100% of subjects. SRs = 32 episodes of mild asthma, 27 moderate attacks, 3 anaphylaxis episodes.

# Clinical Efficacy of Immunotherapy with *Cladosporium herbarum*.

Overall Score Sx plus Med	Improved	Unchanged	Deteriorated
Placebo	1	2	8
Cladosporium	5	4	2

Since the spore counts in 1982 were 2-3 time those in 1981, unchanged might be considered improved. Using this approach clinical efficacy would be: Placebo 27% and Cladosporium 81% ( $p = .01$ )

SPT ( $p < .01$ ) and bronchial allergen challenge ( $p < .02$ ) improved vs. placebo. H-J Malling et al. *Allergy* 1986;41:507-19

# A Double-Blind, Multicenter Immunotherapy Trial in Children Using a Purified and Standardized *Cladosporium herbarum* Preparation. I Clinical Results.

S Dreborg, et al. Allergy 1986;41:131-40.

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- 30 children ages 5-17 years whose asthma occurred or worsened during the *Cladosporium* season.
- Positive PST or RAST to *Cladosporium*.
- 29 were positive to other allergens, 24 to *Alternaria*.
- Recorded symptoms, medication use and PEFs August to October 1981 (pretreatment) and after 5-7 months of AIT in 1982.

# Immunotherapy Trial in Children Using a Purified and Standardized *Cladosporium herbarum* Preparation.

- Employed a highly purified, biologically standardized extract containing 10 strains of *Cladosporium herbarum*.
- Of 16 children treated with *Cladosporium*, 5 reached the projected maintenance dose of 100,000 BU, 6, intermediate levels, and 5 < 10,000 BU.
- Generalized reactions occurred in 81% of patients and large local reactions (>10 cm) in 25% during 10 months of AIT.



# Immunotherapy Trial in Children Using a Purified and Standardized *Cladosporium herbarum* Preparation.

Parameter	Act vs. Placebo		
	Cladosporium	Placebo	p value
PST	↓62%	↓43%	NS
Conjunctival Challenge	↓ p=.01	NC	NS
Bronchial Challenge	↓ p<.01	NC	p<.05
Med scores	Peak 2 <i>Cladosporium</i> spore weeks		
	↓	↑	p<.01
Symptoms	NC	NC	NS
PEF	NS	NS	NS

# Double-Blind, Placebo-Controlled Rush Immunotherapy with a Standardized *Alternaria* Extract.

M Horst, et al. J Allergy Clin Immunol 1990;85:460-72

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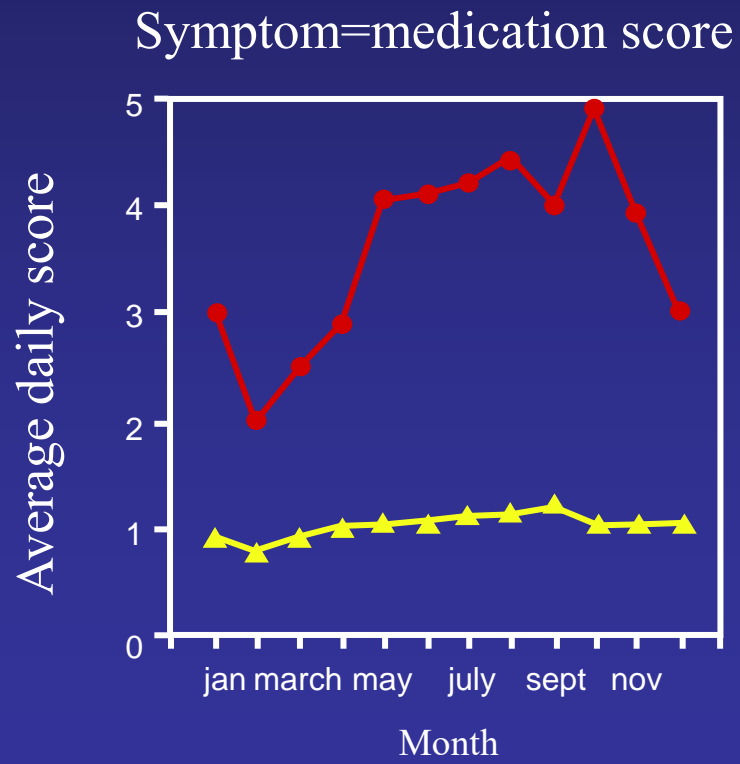
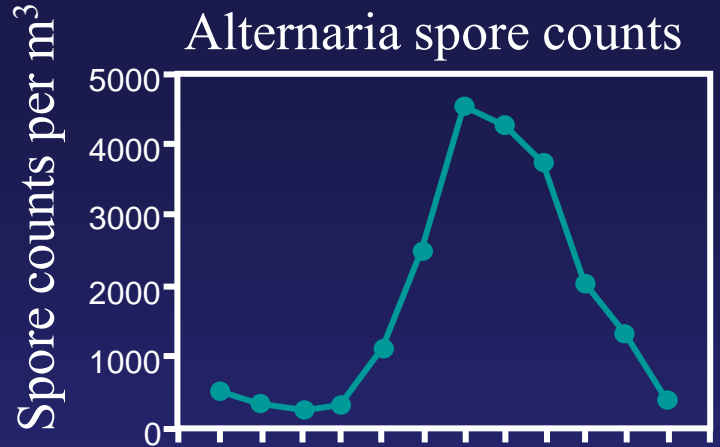
24 patients ages 5 to 56 years:

1. Rhinitis +/- asthma with possible seasonal increase in summer/autumn.
2. PST and RAST to *Alternaria* +/- *Stemphylium*, but no other sensitivities

# Double-Blind, Placebo-Controlled Rush Immunotherapy with a Standardized *Alternaria* Extract.

- Lyophilized/standardized *Alternaria* extract from five different cultures.
- Maintenance dose containing 1.6  $\mu\text{g}$  Alt a 1 a dose selected to be well tolerated.
- Administered by rush over 2 days, then weekly X6, then every other week for one year. All reached projected dose.

# Alternaria Immunotherapy: Pollen/Symptoms



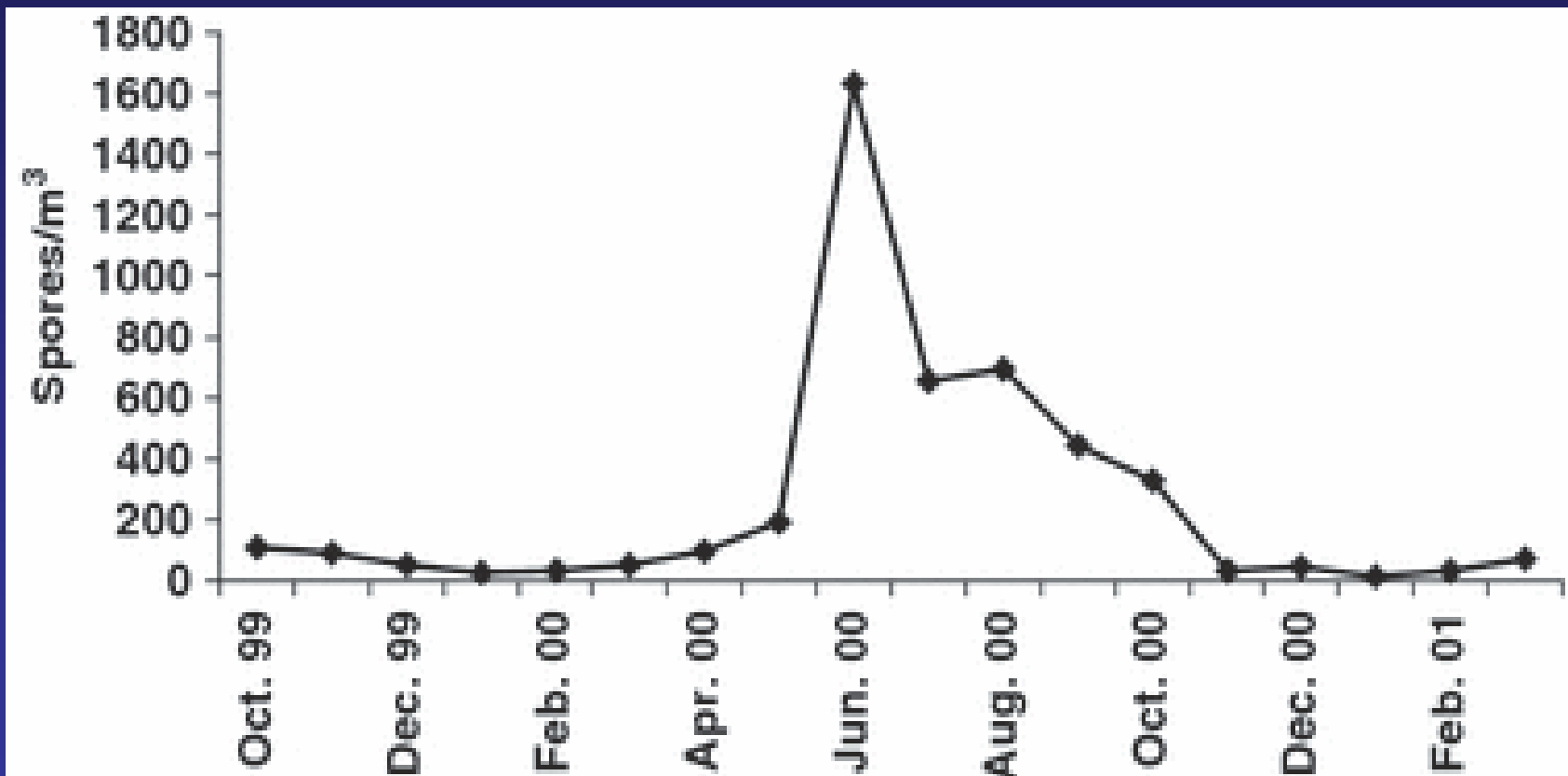
M Horst, et al.  
J Allergy Clin Immunol  
1990;85:460-72

# Double-blind, Placebo-controlled, Study of *Alternaria alternata* Immunotherapy: Clinical Efficacy and Safety

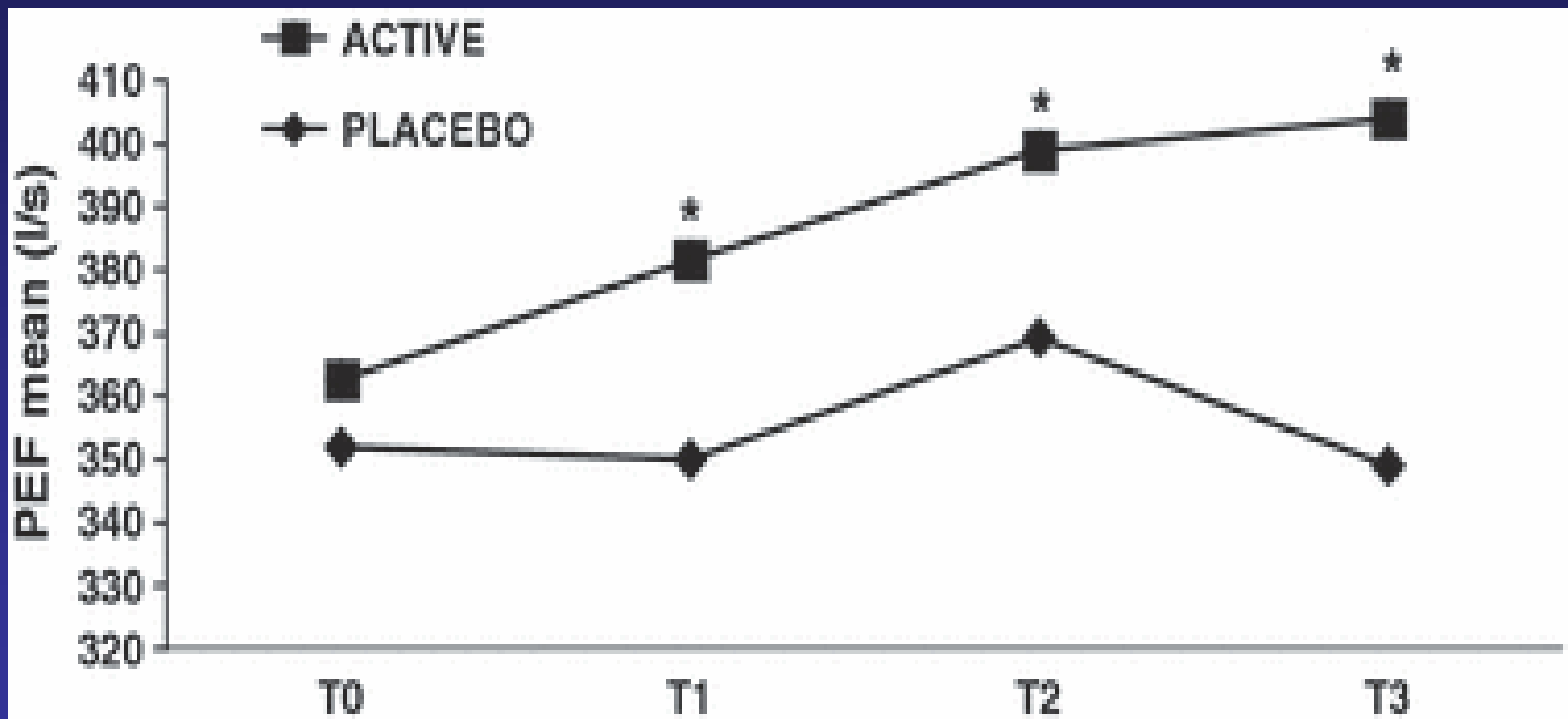
AI Tabar, et al. *Ped Allergy Immunol* 2008;19:67-75

- 23 subjects completed a one-year comparison of *Alternaria* SCIT with 0.1 ug Alt a 1 and placebo.
- SMS scores were significantly better ( $p < .05$ ) in active vs. placebo at 6 months (peak *Alternaria* spores) but not at end of study.
- Physician's evaluation favored active over placebo ( $p < .05$ ). PEFs improved over baseline only in active group.

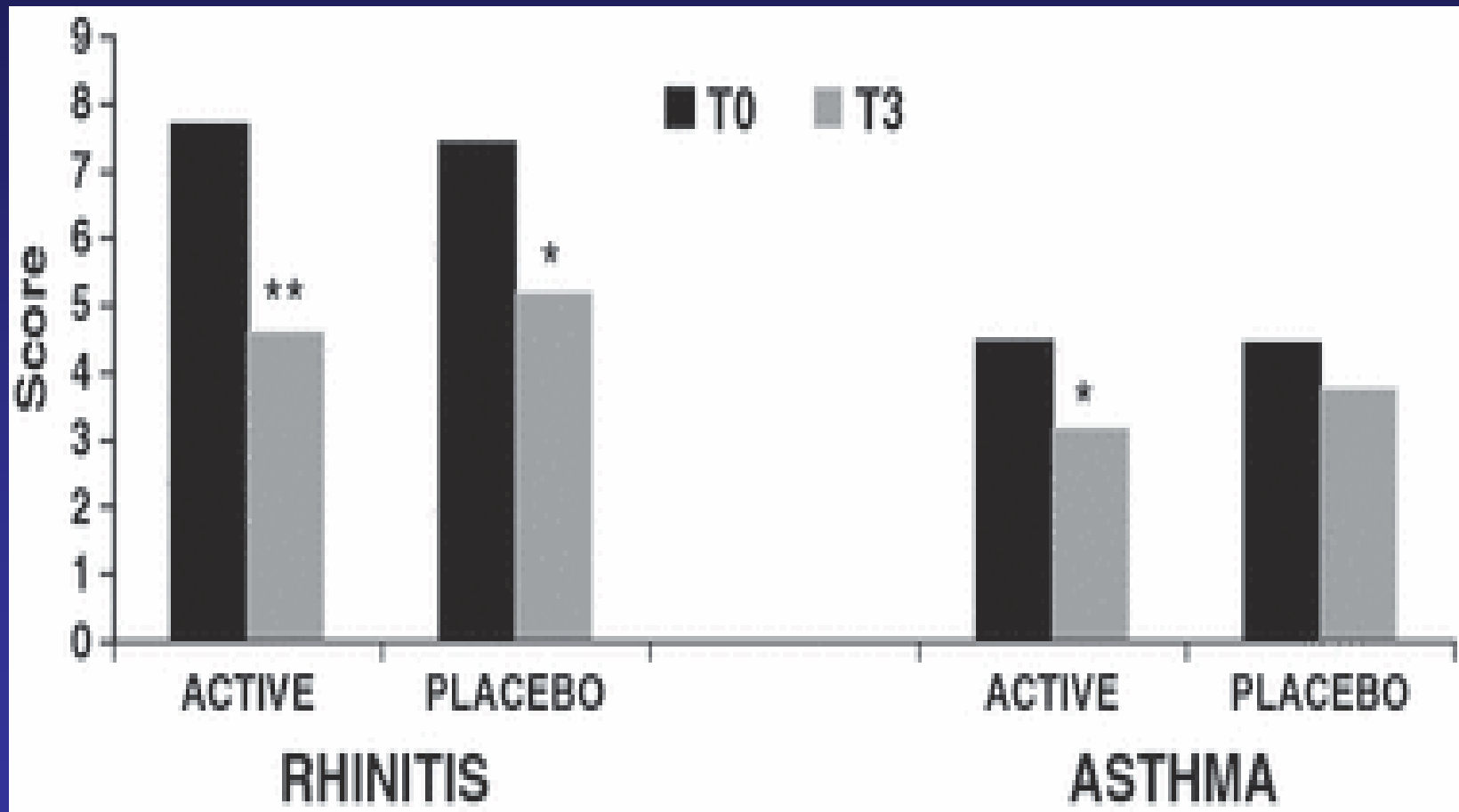
# *Alternaria* Spores during Study Period



# Immunotherapy with *Alternaria alternata*: PEFs



# Immunotherapy with *Alternaria alternata*: Symptom Scores





# Sublingual Immunotherapy for *Alternaria*-induced Allergic Rhinitis: A Randomized, Placebo-controlled Trial.

G Cortellini, et al. Ann Allergy Asthma Immunol 2010;105:382-386

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- 27 adolescents and adults with rhinitis occurring only during the summer-fall, sensitized to *Alternaria* and no perennial or other summer/fall allergens.
- Observed one season, then randomized to 10 months of alternate day SLIT (6 mcg Alt a 1/month or placebo).

# Sublingual Immunotherapy for *Alternaria*-induced Allergic Rhinitis: A Randomized, Placebo-controlled Trial.

- Symptom scores SLIT vs. placebo: 182 vs. 315 ( $p < 0.02$ ).
- Symptom/medication scores SLIT vs. placebo: 231 vs. 414 ( $p = 0.01$ ),
- Symptom/medication scores reduced 38% vs. placebo.
- Well tolerated, one patient reported oral itching.

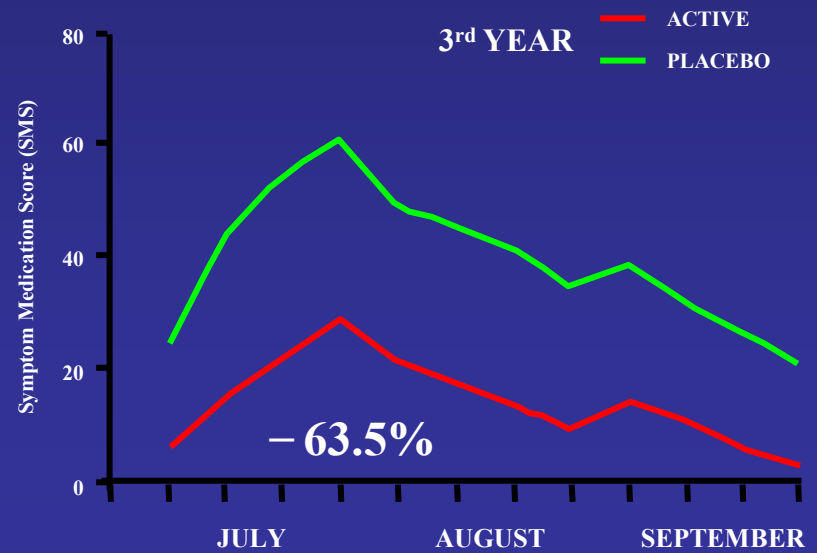
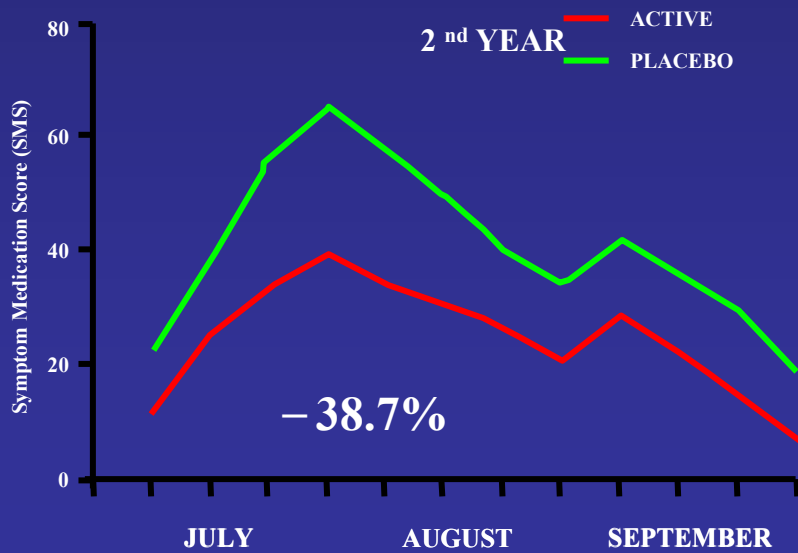
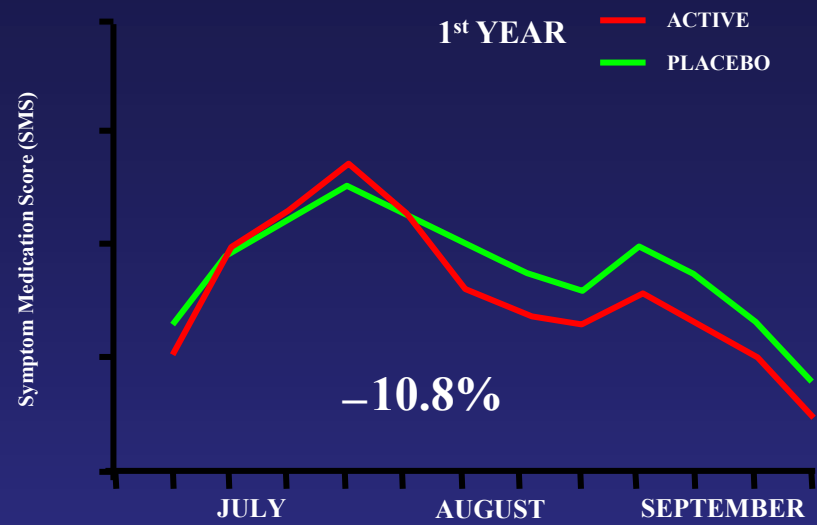
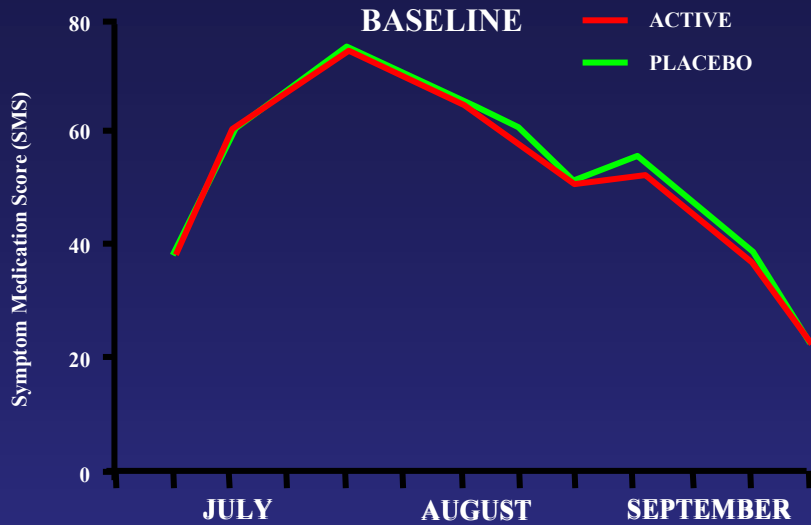
# Efficacy and Safety of Immunotherapy for Allergies to *Alternaria alternata* in Children.

P Kuna, et al. J Allergy Clin Immunol 2011;127:502-8

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- 50 subjects, 5-18 years of age, with allergic rhinitis and/or asthma only in the fall, positive SPT, sIgE, and nasal allergen challenge to *Alternaria* and Negative to mugwort, the only overlapping exposure.
- Observational year plus 3 years on immunotherapy
- Maintenance SCIT contained 8 mcg Alt a 1.
- Few local and one repeated mild SR.

# Symptom/medication Scores

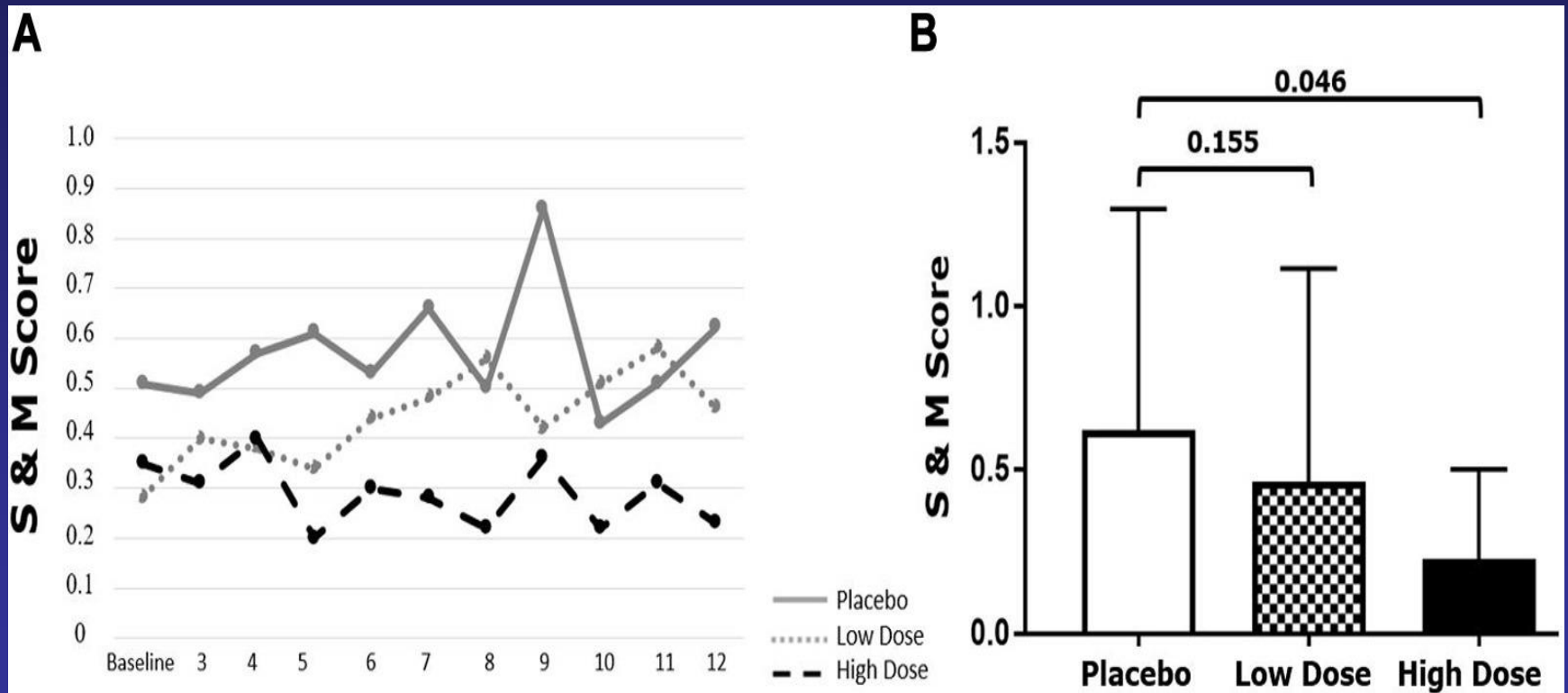


# Double-blind, Randomised, Placebo-controlled Trial of Allergen-specific Immunotherapy with the Major Allergen Alt a 1

AI Tabar, et al. J Allergy Clin Immunol 2019;144:216-23

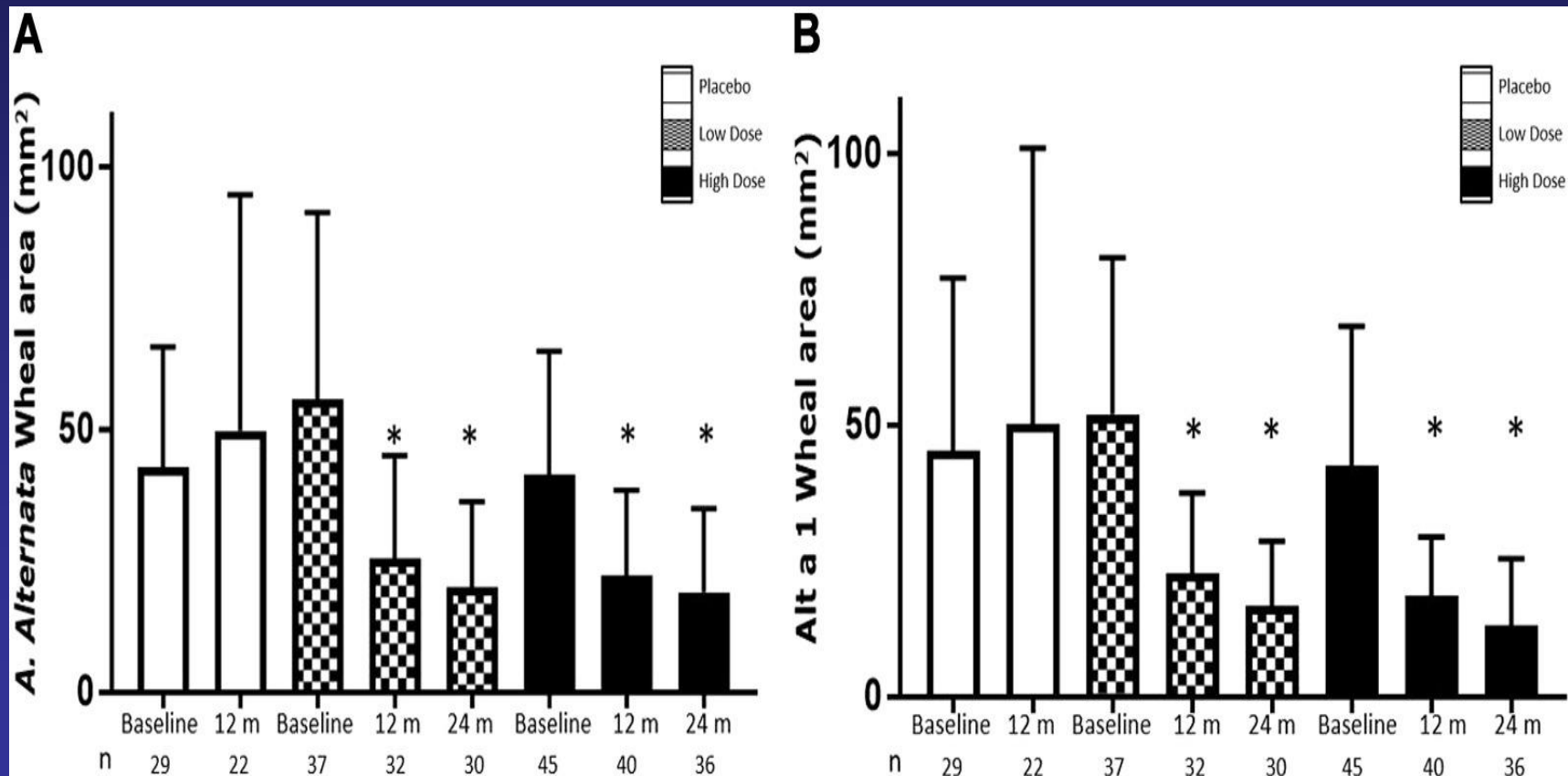
- One year trial of placebo and purified Alt a 1 0.2 ug and 0.37 ug subcutaneously.
- Subject age 12-44 years, with rhinoconjunctivitis with or without asthma, allergic to *Alternaria* and not to danders or mites.
- 113 enrolled, 64 (57%) available for evaluation after 12 months.

# Immunotherapy with the Major Allergen Alt a 1: Symptom/Medication Scores



Al Tabar, et al. J Allergy Clin Immunol 2019;144:216-23

# Immunotherapy with the Major Allergen Alt a 1: Mean Wheal SPTs to Alternaria and Alt a 1



# Fungal Immunotherapy for ABPA and Allergic Fungal Sinusitis

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- The studies are not randomized or placebo controlled.
- In the majority of studies the fungi for AIT are selected by reactions to a fungal skin test panel, not the organism causing AFS in the patient.
- In the majority, AIT dosing is based on end-point titration, which has been shown to be ineffective for ragweed AR.



# The Approach to the Mold Allergic Patient

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- Mold immunotherapy should largely be limited to *Alternaria* and perhaps *Cladosporium*
- Under conditions of demonstrated exposure and with apparently potent extracts, other fungi to which the patient is sensitive may be considered for immunotherapy.
- Mold mixes should not be used.