

ORIGINAL ARTICLE

AIRWAY DISEASES

European Academy of Allergy and Clinical Immunology task force report on 'dose-response relationship in allergen-specific immunotherapy'

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Abstract

Background: For a century, allergen-specific immunotherapy (SIT) has proven to be an effective treatment for allergic rhinitis, asthma, and insect sting allergy. However, as allergen doses are frequently adapted to the individual patient, there are few data on dose-response relationship in SIT. Allergen products for SIT are being increasingly required to conform to regulatory requirements for human medicines, which include the need to demonstrate dose-dependent effects.

Methods: This report, produced by a Task Force of the EAACI Immunotherapy Interest Group, evaluates the currently available data on dose-response relationships in SIT and aims to provide recommendations for the design of future studies.

Results: Fifteen dose-ranging studies fulfilled the inclusion criteria and twelve reported a dose-response relationship for clinical efficacy. Several studies also reported a dose-response relationship for immunological and safety endpoints. Due to the use of different reference materials and methodologies for the determination of allergen content, variations in study design, and choice of endpoints, no comparisons could be made between studies and, as a consequence, no general dosing recommendations can be made.

Conclusion: Despite recently introduced guidelines on the standardization of allergen preparations and study design, the Task Force identified a need for universally accepted standards for the measurement of allergen content in SIT preparations, dosing protocols, and selection of clinical endpoints to enable dose-response effects to be compared across studies.

Allergen-specific immunotherapy (SIT) is used in the treatment of allergic rhinitis, asthma, and insect sting allergy. The two currently validated administration routes for SIT in adults and children are subcutaneous (SCIT) and sublingual (SLIT). Although the clinical efficacy of SIT has been demonstrated by multiple studies and systematic reviews, a correlation between the allergen dose and the clinical outcome measures has not been clearly established. Presently, SIT dose

regimens used in clinical practice vary widely among countries and clinics, although some suggestions on the optimal dose have been made in a World Health Organization position paper (1). Moreover, no standards for the measurement of allergen content are universally accepted, with manufacturers using in-house reference materials and their own units to express potency (2–6), which may hamper analysis of the few available dose-response studies.

As allergen extracts are usually prepared from natural biological sources, standardization procedures are of crucial importance. The CREATE project (Development of Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification) has recently worked toward the development of certified reference materials of purified natural or recombinant allergens and the validation of enzyme-linked immunosorbent assays (ELISAs) for the quantification of major allergens (7). Additional sources of variability that further hamper a proper dose–response analysis are the large differences in the qualitative composition of the marketed products and the presence of different adjuvants and their quantities. Finally, the efficacy of an allergen extract may also depend on factors that influence bioavailability, for example, the volume in which the allergen is dissolved and for sublingual immunotherapy (SLIT), the form of administration, i.e., drops *vs* tablets. As a result, direct comparisons cannot be made between studies using products from different manufacturers to establish a dose–response relationship for a particular allergen extract.

Studies are available, however, in which several doses of the same extract are compared. Endpoints used in these different SIT dose–response studies vary widely and include clinical efficacy, changes in markers of immune response, number and severity of adverse events (AEs), and surrogate endpoints, such as titrated skin prick tests, nasal and bronchial challenge, and measurements of blood and intranasal cytokines. Recently, the European Medicines Agency (EMA) produced a guideline for standardization of clinical trials with SIT in allergic disease that includes some recommendations for the selection of endpoints (8). However, many SIT studies were designed or published before these recommendations, making it difficult to directly compare findings. Moreover, a change of dose can be accomplished by changing volume or concentration, or both, with or without concomitant change in the adjuvant dose (both in concentration and in volume). In most of the studies, these dosing details were not provided and thus it is not possible to assign any observed variations in effect to a single factor, such as the allergen dose. Thus, even if standardized allergens and validated ELISAs become available, it would be impossible to make cross-comparison between studies.

One of the basic principles in empirical SIT has always been that doses are adapted to the individual patient, with the highest tolerated dose for each individual considered to be the most effective. Dose–response data that could enable the physician to rapidly determine the dose with the most favorable risk-to-benefit ratio are scarce. Allergen products for SIT are being increasingly required to conform to regulatory requirements for human medicines and the recently introduced EMA guidelines on the clinical development of products for SIT (8) state that after establishing a tolerated dose range, studies should be performed to establish a dose–response relationship for clinical efficacy. These new requirements for dose–response data further underline the need to adopt international standards for quantification and investigation of allergen products for SIT, to enable harmonization of clinical trial data. Such a process would require the intro-

duction of standard reference materials and assays for allergen products, validation of clinical endpoints, and the adoption of consistent guidelines on the conduct of clinical studies in SIT by regulatory authorities worldwide. It should be borne in mind, however, that it is intrinsically difficult with SIT to build classical dose–response curves, because the clinical effects of the treatment appear within weeks or months, and no objective marker that could replace the traditional symptom score is available.

A serious attempt at harmonizing potency and characteristics of allergen extracts has already been made in the 1970–1980s. An international team of experts created certified reference material (CRM) of five allergens, for manufacturers to use as a reference for their products (9–13). However, as the use of these CRMs was optional and their quality variable, being extracts of natural allergen sources, their use never became established.

This European Academy of Allergy and Clinical Immunology (EAACI) Immunotherapy Interest Group Task Force Report evaluates the currently available data on dose–response relationship in SIT and is aimed at professionals performing SIT or involved in the design of clinical studies.

Methodology

The Task Force carried out a search of PubMed; the Cochrane Ear, Nose and Throat Disorders Group Trials Register and the Cochrane Central Register of Controlled Trials, which includes EMBASE, CINAHL, IndMed, PakMediNet, CAB Abstracts, Web of Science, BIOSIS Previews, mRCT (Current Controlled Trials), ICTRP (International Clinical Trials Registry Platform); and Google using the keywords: allergen immunotherapy, allergen subcutaneous immunotherapy, allergen SLIT, immunotherapy AND rhinitis, immunotherapy AND asthma, immunotherapy AND venom. The date of the most recent search was May 19, 2011. The searches identified a total of 1739 references. Phase I studies were excluded, and search results were assessed to identify trials of SIT that tested multiple allergen doses. Studies were selected for evaluation if the following information was provided:

- Route of allergen administration (subcutaneous or sublingual);
- allergen used; and
- clinical or surrogate endpoints or safety outcomes.

Additional data were included, when available:

- Presence and/or dilution of an adjuvant;
- major allergen content in manufacturers units;
- treatment period;
- duration of up-dosing period; and
- duration of maintenance period.

Because there are no European or international standards for measurement of allergen content, and considerable heterogeneity between study endpoints, only the outcomes of individual dose–response studies were analyzed and no attempt was made to compare data from different studies. When information was not provided on the assay or standards used

to determine the major allergen content of the extracts, these data were requested from the manufacturer.

The Task Force assessed the quality of the studies and assigned a score according to the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) scoring system (14), Delphi list (15), and Consolidated Standards of Reporting Trials (CONSORT) grading (16).

Results

Study design and quantification of allergen content

Analysis of the search results identified fifteen studies that used different doses of allergen extracts (Table 1). Three were non-randomized and open (17–19), one was randomized and double blind for the dosing groups, including an open control group (20). Two studies were randomized and double blind for the treatment groups, but had no control group (21, 22), and the remaining nine trials were randomized, double-blind, placebo controlled (23–31). Two large trials (23, 31) produced several companion papers describing patient subgroups or immunological data (32–35). The studies including allergen, dose, main endpoints, and results are summarized in Table 1.

Until present, no placebo-controlled prospective dose–response studies have been published in venom immunotherapy (VIT), because of the potentially life-threatening nature of an anaphylactic reaction to a sting in patients treated with placebo. One VIT study reported a dose-dependent increase in venom-specific serum IgG and greater clinical efficacy with a 100 µg monthly venom maintenance dose as compared with a 50 µg maintenance dose. Insufficient protection to a sting challenge, while on 100 µg monthly venom maintenance dose, could be overcome by increasing the maintenance dose to either 150 or 200 µg, especially in patients with elevated baseline tryptase levels or mastocytosis (19).

The dosing data for the selected studies are presented in Table 2. Of the fifteen studies, the two earliest, both published in the 1960s, provided no information on the source of allergens or if the allergen content was quantified (22, 27). For both VIT studies, only the quantity of venom in micrograms is mentioned, as is customary in VIT, without providing information on the content of the major allergen (17, 19). Five studies used investigators' in-house assays to determine the allergen content of commercially obtained allergen preparations (17, 18, 21, 30, 32), with only one study citing a published assay method (18). In the remaining seven studies, the allergen content was determined using the manufacturers' in-house assay (20, 24–26, 28, 29, 31). None of the published reports of these studies provided information on the source of antibodies or reference material used to quantify the major allergens.

For the studies that cited allergen content determined by manufacturers' in-house assays, the Task Force requested detailed information on the type of immunoassay used, sources of antibodies, source of reference material used in the preparation of standard curves, and any published data on the assay used from the manufacturers. Where available, this information is presented in Table 2.

Study evaluation according to GRADE and CONSORT and Delphi list

The Task Force evaluated the quality of evidence of studies using the GRADE scoring system (14) and Delphi list (15). In addition, the group evaluated studies for compliance with eight selected recommendations for reporting of randomized controlled trials (RCTs) outlined in the CONSORT statement (36), see Table 3. The results of these evaluations are presented in Tables 2 and 3.

- Three studies reporting a dose–response relationship for clinical endpoints had a GRADE score of 4, indicating a high quality of evidence (23, 24, 26). Two of these studies also showed a dose–response relationship for immunological endpoints (23, 24) and one for safety endpoints (26).
- One study that did not show a dose–response relationship for any of the main endpoints had a GRADE score of 3, indicating a medium quality of evidence (30).
- The eight RCTs (23–26, 28–31) published after the first CONSORT statement was issued complied with at least three of the eight selected CONSORT recommendations.

Study outcomes

Thirteen studies (out of 15) reported a dose–response relationship for clinical efficacy (17, 19–29, 31), eight of these also reported a dose–response effect for immunological endpoints (17, 21, 23–25, 28, 29, 31) and two reported a dose–response effect for safety and tolerability scores (20, 26). One study reported a dose–response relationship for immunological endpoints and safety scores, but not for clinical endpoints (18). One study reported no significant dose–response relationship for any of the efficacy endpoints, but a dose–response relationship was observed for safety scores (30). In total, nine studies (out of ten) reported a dose–response relationship for immunological endpoints (17, 18, 21, 23–25, 28, 29, 31) and five (of seven) for safety endpoints (18, 20, 24, 26, 31).

A few SCIT studies reported the use of an adjuvant (18, 20, 22); however, information on whether the adjuvant concentration remained constant or whether this changed along with the administration volume and allergen dose was not reported in any publication. As far as data obtained on request from the manufacturers showed, the concentration of the adjuvant was always changed together with the allergen.

Two double-blind placebo-controlled (DBPC) RCTs were performed in pediatric populations, one SCIT study in children with perennial asthma reported a dose–response effect for clinical efficacy and development of new allergies (27), and a SLIT study in children with allergic rhinoconjunctivitis with/without seasonal asthma showed a dose–response relationship for both clinical and immunological endpoints (31).

Table 1 Summary of the selected SCIT and SLIT dose-ranging studies

Authors	Allergen	RDB PC	Age range	Duration	Maintenance doses and enrolled patients per group*	Clinical outcomes	Surrogate endpoints (clinical and immunological)	Main results
SCIT studies in respiratory allergies								
Johnstone et al. (27)	Multiple mixed	Yes	2–15 years	4 years	Controls (<i>n</i> = 42) 1:10 000 000 w/v (<i>n</i> = 49) 1:5000 w/v (<i>n</i> = 39) 1:250 w/v (<i>n</i> = 43)	Days with no wheezing No asthma in the last year New allergies Exercise-induced asthma Severity of symptoms†	No	Days with no wheezing on exertion and during upper respiratory illness and patients without asthma attacks significantly ↑ in the two higher dose groups. No patient of the two higher dose groups developed new allergies
Franklin et al. (22)	Ragweed plus mix of other allergens	No	NR	Open dose-escalation 1 year+ Trial 4 months	1:1000 w/v (<i>n</i> = 13) 1:50 w/v (<i>n</i> = 12)		No	Higher dose significantly better than lower dose
Creticos et al. (21)	Ragweed	No	Adults	18 months	13 pts progressively on 0.6, 12.4, 24.8 µg Amb a 1	Rhinitis symptoms- medications during seasons	Nasal-specific challenge at each dose† Specific IgG	The nasal reactivity (mediators) decreased at the two higher doses of 12.4 and 24.8 µg as compared with pretreatment challenge Progressive increase in specific IgG ↓ symptom and medication score in all patients vs baseline season ↓ of allergen-specific bronchial reactivity and conjunctival reactivity vs controls with no difference among the doses at 1 and 2 years Significant increase in AEs (local and systemic) with increasing maintenance doses ↓ of medication and PEF score in all three active groups compared to pretreatment, no change in control group No difference between doses in all parameters except for specific IgG, IgG1 and IgG4 and skin reactivity (in favor of the high dose) Increased frequency of systemic AEs after administration of higher dose Progressive ↓ in skin reactivity across doses. Significant overall dose-dependent effect for increase in sIgG4 and decrease in CD4 + /IL-4 + peripheral blood mononuclear cells (PBMCs)
Haugaard et al. (20)	HDM	No	10–64 years	2 years	Control (<i>n</i> = 19) 0.7 µg Der p 1 (<i>n</i> = 19) 7 µg Der p 1 (<i>n</i> = 20) 21 µg Der p 1 (<i>n</i> = 16)	Peak expiratory flow (PEF) and medication score AEs	Bronchial-specific reactivity† Conjunctival-specific reactivity	
Olaguibel et al. (18)	HDM	No	6–50 years	18 months	Max tolerated dose: <4 µg Der p 1 (<i>n</i> = 22) 4–16 µg Der p 1 (<i>n</i> = 15)	Asthma symptoms and medications† AEs	Eye-specific reactivity Skin prick tests Specific IgE, IgG, IgG1, IgG4	
Ewbank et al. (25)	Cat	Yes	Adults	5 weeks	Placebo (<i>n</i> = 7) 0.6 µg Fel d 1 (<i>n</i> = 7) 3.0 µg Fel d 1 (<i>n</i> = 7) 15 µg Fel d 1 (<i>n</i> = 7)	No	Titrated skin prick tests Specific IgE, IgG4 Cytokines (flow cytometry)	

Table 1 (Continued)

Authors	Allergen	RDB PC	Age range	Duration	Maintenance doses and enrolled patients per group*	Clinical outcomes	Surrogate endpoints (clinical and immunological)	Main results
Nanda et al. (29)	Cat	Yes	Adults	1 year	Placebo (<i>n</i> = 7) 0.6 µg Fel d 1 (<i>n</i> = 6) 3.0 µg Fel d 1 (<i>n</i> = 6) 15 µg Fel d 1 (<i>n</i> = 7)	No	Titrated-specific nasal challenge and skin prick tests† Specific IgE, IgG4 Cytokines at 5 wks and 1 year	Significant ↓ in nasal and skin reactivity at 5 wks and 1 year with the highest dose Significant dose effect across doses Specific IgG4 significantly ↑ at 5 wks and 1 year in the medium- and high-dose group No change in cytokines Overall dose-dependent ↓ in skin prick test and late cutaneous response vs baseline No change in nasal reactivity Specific IgG4 significantly ↑ in low and high dose Dose-dependent ↑ in TGFβ and ↓ in TNFα No change in specific IgE Symptom and medication scores: no difference between doses in whole season, significant difference in the peak season
Lent et al. (28)	Dog	Yes	Adults	5 weeks	Placebo (<i>n</i> = 7) 0.6 µg Can f 1 (<i>n</i> = 6) 3 µg Can f 1 (<i>n</i> = 7) 15 µg Can f 1 (<i>n</i> = 7)	No	Titrated skin prick test Nasal challenge Specific IgE, IgG4 Lymphocyte proliferation, TGFβ and TNFα	Overall more AEs in the high-dose group
Frew et al. (26)	Grass	Yes	18–60 years	6.4 months (3–8 months)	Placebo (<i>n</i> = 103) 2 µg Phl p 5 (<i>n</i> = 104) 20 µg Phl p 5 (<i>n</i> = 203)	Symptom/medication scores whole season† and peak season AEs	Quality of life (QoL) Bad days (VAS)	QoL: significant difference between doses Bad days (VAS): no difference between doses
SLIT studies in respiratory allergies								
Durham et al. (24)	Grass	Yes	18–65 years	18 weeks	Placebo (<i>n</i> = 286) 0.5 µg Phl p 5/d (<i>n</i> = 136) 5 µg Phl p 5/d (<i>n</i> = 139) 15 µg Phl p 5/d (<i>n</i> = 294)	Rhinoconjunctivitis SS and medication use† QoL % well days AEs	Specific IgE, IgG4	Symptom score ↓ in the 15 µg dose, significant ↓ of medication use in the 15 µg group QoL and % of well days significantly increased in the 15 µg dose group compared to placebo, no difference between the two lower doses and placebo Dose- and time-dependent increase in specific IgG4 Increased frequency of mild AEs between 0.5 and 5.0 µg dose group but not between 5.0 and 15 µg

Table 1 (Continued)

Authors	Allergen	RDB PC	Age range	Duration	Maintenance doses and enrolled patients per group*	Clinical outcomes	Surrogate endpoints (clinical and immunological)	Main results
Valovirta et al. (31)	Pollen mix: Birch, alder, hazel	Yes	5–15 years	18 months	Placebo (<i>n</i> = 33) 3.6 µg Bet v 1/wk (<i>n</i> = 33)	Symptom and medication score†	Conjunctival provocation test	↓ symptoms with both doses
Savolainen et al. (35)					30 µg Bet v 1/wk (<i>n</i> = 32)		Methacholine bronchial provocation test	Medications ↓ with higher dose, no overall difference between the two doses
Nieminen et al. (33)							Late phase skin reaction	No significant change in the other parameters
							IL-5, IL-10, IL-17, IL-18 mRNA, FOXP3 mRNA	↑ in IL-18 and FOXP3 mRNA expression in high-dose group only
Didier et al. (23)	Grass	Yes	18–45 years	4–6 months	Placebo (<i>n</i> = 156) 100 IR/d (<i>n</i> = 157) 300 IR/d (<i>n</i> = 155) 500 IR/d (<i>n</i> = 160)	Rhinoconjunctivitis SS† % days with rescue medication	Specific IgE, IgG4	Significant reduction in symptom scores vs placebo only with the 300 and 500IR
Didier et al. (32)						% of symptom free days QoL AEs		Significant reduction of days without symptoms, days without medications
								Significant improvement of QoL
								Dose-dependent increase in specific IgG4
								No dose-dependent differences in frequency of AEs
Skoner et al. (30)	Ragweed	Yes	18–50 years	18 weeks	Placebo (<i>n</i> = 40) 4.8 µg Amb a 1/d (<i>n</i> = 39) 48 µg Amb a 1/d (<i>n</i> = 36)	Average daily SS† Medication score NPT AEs	Specific IgE, IgG4	No significant reduction in symptom and medication scores compared to placebo in both dose groups
								In an analysis of covariance correcting for preseasonal symptoms, both symptom and medication scores significantly ↓ in the high-dose group only vs placebo
								↑ in IgG4 with both doses
								Equal safety
SCIT studies in insect sting anaphylaxis								
Golden et al. (17)	Bee Yellow jacket Hornet	No	NR	20 weeks	50 µg (<i>n</i> = 23) raw venom 100 µg-1978 (<i>n</i> = 22) 100 µg-1976 (<i>n</i> = 20)	Sting challenge†	Specific IgG	Better protection at challenge with the 100 µg dose
Rueff et al. (19)	Bee Yellow jacket	No	19–72 years	NR	Increase from 100 to 150, 200 or even 250 µg	Sting challenge†	No	Higher concentrations of specific IgG with the 100 µg dose
								8 of 10 patients still reacting at challenge when treated with 100 µg were fully protected when treated with 150 µg maintenance dose and 28 of 30 patients were fully protected at 200 µg maintenance dose and 1 patients at 250 µg

RDB PC, randomized double-blind placebo controlled; NR, not reported; AEs, adverse events; NPT, nasal provocation test; PEF, peak expiratory flow; QoL, quality of life; SS, symptom score; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; ↑, increase; ↓, decrease.

Clinical endpoints include global evaluations, SS, QoL assessments, medication scores. Clinical surrogate endpoints include SPT, skin LPR, CPT, bronchial and nasal challenge tests.

*Monthly maintenance dose if not otherwise specified.

†Indicates the primary outcome, when stated.

Table 2 Dosing data of the evaluated individual studies

SCIT studies in respiratory allergies

Johnston 1961, DBPC RCT

Antigen mixture (=all inhalant allergens patients were allergic to, no details)

Low dose: 10^{-7} = 1 : 10 000 000 wt/v

High dose: 1 : 5000 wt/v

Highest tolerated dose: maximum 0.5 ml of 1 : 250 wt/v

Manufacturer: not specified

Volume: 0.5 ml all groups

Adjuvant: none

Up-dosing: 1 : 10 000 000 w/v group, 5 weeks; 1 : 5000 w/v group, 9 weeks; 1 : 250 w/v group, NA

Frequency at maintenance: 28 days

Duration: 4 years

GRADE scoring: 2, low quality of evidence

Delphi score*: 6.5

Franklin 1967, double-blind randomized continuation of already installed high-dose ragweed pollen immunotherapy

Antigen mixture (no details) with aqueous ragweed pollen extract in two doses

Low dose: mean 0.3 ml of a 'concentration' of 1 : 1000

High dose: mean 0.3 ml of a 'concentration' of 1 : 50

Manufacturer: not specified

Volume: variable as tolerated: low-dose range 0.1–0.4 ml (mean 0.3 ml); high-dose range 0.07–0.5 ml (mean 0.3 ml)

Adjuvant: none

Frequency at maintenance: every 2–3 weeks

Duration: duration trial immunotherapy 3 months, but before that approximately 6-month dose-escalation immunotherapy with several pollens, including ragweed pollen

Duration: 4 years

GRADE scoring: 2, low quality of evidence

Delphi score: 8.5

Creticos 1989, randomized double blind with no control group

Ragweed concentrations: dose escalation. Evaluation of the patients when 0.6 µg, 12.4 mg and 24.8 µg Amb a 1 dose was reached. (description µg)

Major allergen content determined by radial immunodiffusion

Volume: NA

Adjuvant: none

Frequency at maintenance: 4 weeks

Duration: 0.6 µg was reached at 4–5 months, 12.4 µg at 6–10 months, 24.8 µg at 12–18 months

GRADE scoring: 2, low

Delphi score: 5

Haugaard 1993, randomized, double blind in active group with open control group (controls received no injections)

D pteronyssinus 10 000, 100 000 and 300 000 SQ-U and open control group

100 000 SQ-U contained 23 000 UI Der p 1 (7 µg/ml) and 34,500 UI Der p 2; quantified by

Immunoelectrophoresis; whole mite culture

Volume: 1 ml all groups

Adjuvant: aluminum hydroxide, same dose all 3 dosing groups

Frequency at maintenance: 6–8 weeks

Duration: 24 months

GRADE scoring: 2, low quality of evidence

Delphi score: 8

Olaguibel 1997, open dose titration trial, nonrandomized

D pteronyssinus: maximum tolerated dose

Pangramin® (ALK-Abelló)

(100 BU = 40 µg Der p 1, 20 µg Der p 2. Der p 1/2 quantified by monoclonal antibody enzyme immunoassays ELISA in solid phase, whole mite culture dialyzed to remove particles smaller than 5 kDa (55))

Volume: depending on dose 0.4–0.8 ml (lower dose had a higher volume as it was taken from the diluted vial)

Adjuvant: aluminum hydroxide (no data on concentration)

Frequency at maintenance: 4 weeks

Table 2 (Continued)

Duration build-up: 3–4 months
 Total duration: approximately 15 months
 GRADE scoring: 0, very low quality of evidence
 Delphi score: 7

Ewbank 2003, DBPC RCT

Cat hair and dander extract (ALK-Abelló, Wallingford, CT, USA) high dose 2344 BAU, medium 469 BAU, low 94 BAU, placebo
 (10 000 BAU = 64 µg Fel d 1. Groups 15, 3, 0.6 µg Fel d 1)
 Assay: in-house assay ALK. Placebo caramelized sugar + histamine
 Volume: 0.5 ml
 Adjuvant: none
 Frequency at maintenance: weekly (only 1 dose given)
 Duration up-dosing: 4 weeks (cluster)
 Duration: 5 weeks (4 run-in, 1 maintenance week)
 GRADE scoring: 2, low quality of evidence
 Delphi score: 10

Nanda 2004, DBPC RCT

Cat hair and dander extract (ALK Austin, TX, USA) high dose 2344 BAU, medium 469 BAU, low 94 BAU, placebo
 (10 000 BAU = 64 µg Fel d 1. Groups 15, 3, 0.6 µg Fel d 1)
 Assay: in-house assay ALK. Placebo caramelized sugar + histamine
 Volume: 0.5 ml
 Adjuvant: none
 Frequency at maintenance: monthly
 Duration up-dosing: 4 weeks (cluster)
 Duration: 12–13 months (4 weeks run-in, 1 year maintenance)
 GRADE scoring: 3, medium quality of evidence
 Delphi score: 9

Lent 2006, DBPC RCT

AP dog extract (Hollister Stier Laboratories) high dose calculated to deliver 15 µg, intermediate 3 µg, low 0.6 µg Can f 1 and placebo
 (1 : 100 wt/vol extract of AP dog (Hollister-Stier Laboratories, Spokane, WA, USA), contained approximately 161 µg/ml
 Can f 1 (information provided by the extract manufacturer)
 Assay: in-house assay Hollister-Stier. Placebo caramelized sugar + histamine
 Volume: 0.5 ml
 Adjuvant: none
 Frequency at maintenance: monthly
 Duration up-dosing: 4 weeks (cluster)
 Duration: 5 weeks (4 weeks run-in, 1 dose maintenance)
 GRADE scoring: 2, low quality of evidence
 Delphi score: 9

Frew 2006, DBPC RCT

Phleum pratense: High dose 100 000 SQ-U, low dose 10 000 SQ-U, placebo
 Alutard® (ALK-Abelló, Hørsholm, Denmark)
 (100 000 SQ-U = 20 µg Phl p 5. Quantification method not mentioned; probably rocket immunoelectrophoresis or radio immunodiffusion (56))
 Volume: 100 000 SQ-U/ml
 Adjuvant: aluminum hydroxide (<100 000 SQ doses used were diluted with regard to allergen as well as ALOH)
 Frequency at maintenance: 6 weeks (±2 weeks)
 Duration build-up: 8 weeks
 Total duration: up-dosing 8 weeks, average maintenance 6.4 months (range 3–8 months)
 GRADE scoring: 4, high quality of evidence
 Delphi score: 9.5

SLIT studies in respiratory allergies

Durham 2006, DBPC RCT

Phleum pratense pollen: high dose 75 000 SQ-T, Median dose 25 000 SQ-T, low dose 2500 SQ-T, placebo
 Grazax® (ALK-Abelló, A/S, Denmark)
 (75 000 SQ-T = 15 µg Phl p 5. Quantification method not mentioned; probably rocket immunoelectrophoresis or radio immunodiffusion (56))

Table 2 (Continued)

Volume: oro-dispensable, fast-dissolving tablet
Adjuvant: none
Frequency at maintenance: daily
Total monthly dose: high-dose group 2 250 000; medium 750 000; low 75 000 SQ-T
Duration build-up: none
Total duration: 18 weeks (preco-seasonal, starting approximately 2 months preseasonal)
GRADE scoring: 4, high quality of evidence
Delphi score: 11
Valovirta 2006, DBPC RCT
Tree pollen extract <i>B. verrucosa</i> (birch), <i>C. avellana</i> (hazel) and <i>A. glutinosa</i> (alder):
High dose 40 000 SQ-U, low dose 4800 SQ-U, placebo
1 000 000 SQ-U contains 150 µg (Bet v 1/Aln g 1/Cor a 1) (ALK-Abelló, A/S, Denmark)
Quantification method not mentioned; probably rocket immunoelectrophoresis or radio immunodiffusion (56)
Volume: 400 µl. For high and low doses same volume
Adjuvant: none
Frequency at maintenance: 5 days a week
Total monthly dose: not mentioned (weekly cumulative dose: 3.6 µg, 30 µg SLIT 5 times per week)
Duration build-up: 5 weeks
Total duration: 18 months
GRADE scoring: 3, medium quality of evidence
Delphi score: 11
Didier 2007, DBPC RCT
5-grass pollen tablet Oralair® (orchard, meadow, perennial rye, sweet vernal, and timothy grasses) (Stallergènes SA, Antony, France)
High dose 500 IR, medium dose 300 IR, low dose 100 IR, placebo
300 IR contains 25 µg group 5 major grass allergen (Stallergènes)
Quantification method: ELISA sandwich using two in-house monoclonal antibodies with rPhl p 1 and 5 Biomay (Vienna, Austria) reference material (57)
Volume: tablet
Adjuvant: none
Frequency at maintenance: daily
Total monthly dose: high 15 000 IR, medium 9000 IR, low 3000 IR
Duration build-up: 5 days
Total duration: 6 months (preco-seasonal, starting 4 months preseasonal)
GRADE scoring: 4, high quality of evidence
Delphi score: 8
Skoner 2010, DBPC RCT
Glycerinated short ragweed pollen extract (Greer Laboratories, Inc, Lenoir, NC, USA)
High dose 48 µg Amb a 1 or maximum tolerated dose (mean: 30.5 µg), medium dose 4.8 µg Amb a 1 or maximum tolerated dose (mean: 3.21 µg), placebo
Quantification method: radial immunodiffusion assay with FDA references. (Manufacturer's information)
Volume: 50–140 µl
Adjuvant: none
Frequency at maintenance: daily
Total monthly dose: (mean) low: 96.3 µg, high: 915 µg Amb a 1
Duration build-up: 4 doses on 1 day
Total duration: 17 ± 3 weeks (preco-seasonal, starting 8–10 weeks preseasonal)
GRADE scoring: 3, medium quality of evidence
Delphi score: 10
SCIT studies in insect sting anaphylaxis
Golden 1981, Open study, historic controls
Yellow jacket, yellow hornet, white-faced hornet, honeybee or mixed vespid venom (Pharmacia Diagnostics, Piscataway, NY, USA)
High dose 100 µg, low dose 50 µg venom
Quantification method: not stated
Volume: 1 ml (high dose), 0.5 ml (low dose)
Adjuvant: none
Frequency at maintenance: monthly

Table 2 (Continued)

Total monthly dose: 50 or 100 µg
 Duration build-up: 6 weeks
 Total duration: 16 weeks (historic controls 20 weeks), before sting challenge
 GRADE scoring: 3, medium quality of evidence
 Delphi score: 4

Rueff 2001, Open study, retrospective

Lyophilized honeybee venom (*A. mellifera*) or *Vespula* species (Reless; ALK-SCHERAX, Hamburg, Germany)
 Maintenance 100 µg every 4 weeks, but dose increased to 150, 200, and 250 after nontolerated sting challenge
 Quantification method: not stated
 Volume: not stated
 Adjuvant: none
 Frequency at maintenance: monthly
 Total monthly dose: 100, 150, 200, 250 or 400 µg venom
 Duration build-up: normal protocol 18 weeks (conventional)
 Total duration: 10–28 months before sting challenge
 GRADE scoring: 3, medium quality of evidence
 Delphi score: 3

DBPC, double-blind placebo-controlled; GRADE, Grading of Recommendations, Assessment, Development, and Evaluations.

*Delphi score was calculated from 11 criteria from the Delphi list for assessing the quality of randomized controlled trials (15). A higher score indicates a higher quality of study.

Discussion

Positive outcomes establish a dose–response relationship

- Several studies demonstrated a clear dose–response relationship in SIT, showing that the effect increases with the dose.
- A dose–response relationship in SIT efficacy was shown for both clinical (13 of 15 studies) and immunological (9 of 10 studies) endpoints.
- The concept of dose-dependent SIT effects is valid for subcutaneous and sublingual routes of administration. Three of the four SLIT studies and 10 of the 11 SCIT studies showed dose-dependent effects for clinical efficacy.
- In SLIT studies that reported AEs as a clinical endpoint, a dose-dependent increase in efficacy was not associated with an increase in AEs, possibly reflecting the different sites of action within the immune system. In contrast, SCIT studies reporting AEs showed increased frequencies of AEs at higher allergen doses.

Potential sources of bias to consider

Heterogeneity of effects and lack of clinical data

- The considerable heterogeneity in dose-dependent effects between the reviewed studies, reflects variations in study design, selection of patients and endpoints, allergen sources, product standardization, units, and other parameters. Consequently, comparisons between dose–response studies are problematic, and for this reason, studies are presented individually. Results of dose–response studies are clearly linked to a particular product and protocol, route of application, subject age, disease manifestation, and severity.
- At present, no dose–response data exist that enable comparison between SIT protocols. Therefore, it remains

unclear how the mode of dose escalation and maintenance (co-seasonal *vs* perennial *vs* preseasonal) affects the dose dependency.

- The studies identified were mostly carried out before publication of the World Allergy Organization (WAO) and EMA guidelines for standardization of clinical trials with SIT (8, 37).

Methodological issues

- Not all studies were DBPC (9/15).
- Many studies were carried out in small populations.
- Many of the studies did not class endpoints as primary and secondary; therefore, we evaluated all measurements to determine whether any were dose related.
- Not all studies had high GRADE or high CONSORT scores. Of interest, 1/11 SCIT studies and 4/4 SLIT studies report whether a power analysis was performed.
- Published SIT studies that demonstrate dose-dependent effects of SIT compared with placebo, use superiority testing. Significant differences between doses would clearly confirm dose-dependent effects. However, this would necessitate large sample sizes and introduce multiplicity-testing issues. Therefore, differences between a limited number of doses, rather than based on predefined dose–response models, should be sufficient to demonstrate a dose–response effect in SIT (38). An alternative approach would be to use a regression framework to describe a dose–response relationship; however, although this would necessitate fewer patients per dose, many doses would be needed.

Unresolved biological issues

- Until present, the units used to quantify allergen preparations in Europe were based on in-house reference materials and protocols for extract characterization and standardization. Moreover, measurements of major

Table 3 Methodological characteristics of the evaluated studies

Author	CONSORT flow chart	Drop outs placebo/active/active (%)	ITT OR per protocol		Adequate blinding	Method of randomization reported	Flaw in method	Power analysis	Delphi score*	GRADE score
			Specified	Used						
SCIT studies in respiratory allergies										
Johnstone et al. (27)	NA ¹								6.5	2
Franklin et al. (22)	NA ¹								8.5	2
Creticos et al. (21)	NA ¹								5	2
Haugaard et al. (20)	NA ¹								8	2
Olaguibel et al. (18)	NA ¹								7	0
Ewbank et al. (25)	No, but no dropouts	0/0/0		ITT, no dropouts	Yes	No	Only challenge test and immunology	No	10	2
Nanda et al. (29)	No	14/0/0/14		PP	Yes	No	Only challenge test and immunology	No	9	3
Lent et al. (28)	Yes, in words	0/14/0/0		Almost ITT (only 1 drop-out)	Yes	No	Only challenge test and immunology	No	9	2
Frew et al. (26)	Yes	12/16/17	No	PP	Yes	Incomplete		Yes	9.5	4
SLIT studies in respiratory allergies										
Durham et al. (24)	Yes	13/10/10/15	ITT	All randomized patients	Yes	Yes	No calculation of differences between active groups	Yes	11	4
Valovirta et al. (31)	Yes	18/3/22	ITT	Patients excluded with evaluable clinical data	Yes	No	Post hoc sign level changed from 1.5 to >5%	Yes	11	3
Didier et al. (23)	Yes	6/11/14/12	ITT	ITT analysis excluding drop outs with no efficacy data and LOCF	Yes	Incomplete		Yes	8	4
Skoner et al. (30)	No	10/13/8	No	PP Missing data excluded	Yes	Yes	Below calculated number of patients to treat and†	Yes	10	3
SCIT studies in insect sting anaphylaxis										
Golden et al. (17)	NA ²	NA	NA		NA	NA			4	3
Rueff et al. (19)	NA ²							No	3	3

A, active group; ITT, intention to treat; PP, per protocol; LOCF, last observation carried forward; NA¹, not applicable since the first CONSORT statement was issued in 1996 (16); NA², not applicable because the study was not randomized; P, placebo; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; CONSORT, Consolidated Standards of Reporting Trials; GRADE, Grading of Recommendations, Assessment, Development, and Evaluations.

*Delphi score was calculated from 11 criteria from the Delphi list for assessing the quality of randomized controlled trials (15). A higher score indicates a higher quality of study.

†Post hoc analysis of co-variance: correct for differences of baseline symptoms.

allergen content are obtained by in-house assays, and limited information is publicly available on details, such as assay type, reference materials, antibodies, and protocols. Therefore, values from different laboratories or allergen manufacturers cannot be compared.

- A consortium of independent research laboratories, allergen manufacturers and the Paul-Ehrlich-Institute (6, 39) has carefully selected reference materials and searched for robust assays to detect major allergens in complex allergen preparations. Recombinant major birch pollen (rBet v 1a) and major timothy grass pollen allergen (rPhl p 5a) are promising candidates for potential reference materials, which will lead to improved standardization of birch and *Phleum pratense* pollen extracts.
- Immunoassays for the detection of allergen-specific IgG4, which are commonly used to follow SIT-induced immune responses during dose-finding studies, are not standardized. These immunoassays are based on in-house references, and their values cannot be compared between studies. Future assays should employ single (major) allergens, calibrated to validated reference materials.

Unresolved dosing problems

- The relationship between allergen dose and adjuvant concentration is a subject of investigation and debate. In multi-dose SCIT studies, information about the amount of adjuvant, i.e., alum, in relation to allergen dose, should be considered. An extra group might be added, in which the adjuvant concentration is varied, but in human studies the practicalities of realizing this option are limited. According to the data obtained from the manufacturers on request, the adjuvant was diluted down when lower doses were produced for the trials. Recently, animal studies have suggested that the alum dose in relation to the allergen dose is relevant for promoting a protective immune response during SCIT (40–42). Allergen-specific IgG4 measurements after short-term SCIT suggest an important role of adjuvant/allergen ratio also in humans (43, 44).
- Animal studies during preclinical development may be useful for investigating the relationship between allergen dose and adjuvant concentration. This relationship might vary depending on the allergen source as well as on the nature of the adjuvant. It does not seem feasible to test several allergen/adjuvant ratios in human studies.
- The relationship between frequency of administration and peak dose of sublingual allergen application and their contribution to the immunological effect of SLIT has sparked many discussions. Preclinical animal studies indicate that the frequency of administration, even with constant weekly cumulative dose, is of significant importance for the resulting effect, favoring a daily regimen for SLIT (45).
- The dose–response relationship and, consequently, the optimal dose have been established with a robust methodology only for grass allergens in SLIT tablets (23, 24). Dose-ranging studies with adequate methodology are

lacking for the remaining relevant allergens (e.g., mite, birch, parietaria, and ragweed).

- For studies investigating the dose dependency of safety, an universal grading system is needed.

General methodological and regulatory issues

Potential role of surrogate endpoints

- Significant differences between SIT doses measured using surrogate endpoints are not necessarily clinically relevant. Therefore, the choice of endpoint should enable estimation of the clinical benefit of any dose–response effect. Combined symptom and rescue medication scores, proposed in the EMA guidelines (8), would enable such an estimation. Because of the difficulties in designing SIT dose-finding studies of sufficient power, clinical surrogate parameters could be a substitute for clinical endpoints; however, these require validation.
- Clinical surrogate endpoints for SIT dose-finding studies include challenge models (conjunctival, nasal, or bronchial provocation tests), allergen exposure (chamber) models, and patient-reported outcomes (e.g., quality of life). SIT dose-finding studies should, nevertheless, include clinical endpoints, such as symptom severity after allergen challenge.
- Laboratory parameters, such as allergen-specific IgG or IgG4 levels, T-cell reactivity, or cytokine production are not necessarily correlated with the clinical outcome. The *in vitro* parameter that best correlates with clinical efficacy in respiratory allergen SIT is FAB analysis (Facilitated Antigen binding) (46, 47). Even so, although laboratory tests can provide supportive information for dose-finding studies, they cannot be used to determine a suitable therapeutic dose.

Concepts of SIT dosing

- In general, pharmacokinetic and pharmacodynamic principles cannot simply be adapted to SIT dose-finding studies (48). SIT is only administered to allergic subjects and not to healthy volunteers, and still very little is known about bioavailability after subcutaneous or sublingual application of allergen preparations (49). Numerous variables potentially influence the efficacy of SCIT (volume of injection, allergen dose, modification of the allergen preparation, nature and concentration of the adjuvant) as well as SLIT dosing (aqueous or solid state, volume of solution, potential adjuvant, peak dose, contact time with the mucosa).
- Dose-finding studies can be designed to define a recommended maximum tolerated dose in allergic individuals. Recent safety studies of SLIT have indicated a logarithmic distribution, which is linked to a continuous increase in both frequency and severity of AEs (50). The concept of maximum tolerated dose requires guidelines on how the frequency and severity of local and systemic AEs should be taken into account (51).
- Unlike IgE response, which has a logarithmic curve and broad dose–response curve (e.g., titrated skin tests,

challenge tests, basophil studies), the dose–response relationship in SIT might follow different rules – some studies report a rather steep relationship between the allergen dose and the clinical effects in SIT. Comparisons of immunological and clinical dose–response curves obtained in parallel would be helpful to investigate the relationship between these endpoints during dose–response studies.

- At present, definitions such as ‘low dose’ or ‘high dose’ are meaningless, unless these are defined relative to a common standard. Designation of lower and higher doses is only relevant for identical allergen preparations used within a given study.

Framework of present regulations on allergen products in Europe

If a medicinal product (i.e., allergen preparation for SIT) is to be developed for two or more European countries, an application for marketing authorization must be submitted via the Mutual Recognition Procedure or the Decentralized Procedure. A marketing authorization granted by the Centralized Procedure is valid in the whole European Community and is mandatory for recombinant or biotechnologically generated products. Three guidelines of the EMA have a potential to shape the future development of allergen preparations for SIT (8, 52–54). Parallel to the European initiatives, the Paul Ehrlich-Institute has recently targeted ‘Named-Patient products’ used for SIT in Europe. So far, no dose–response studies have been requested for these already marketed allergen preparations. New products, however, will be required to show a tolerated dose range and follow the recently introduced EMA guidelines for further development and registration (8).

Future implications

- New clinical trials in children and adults will be necessary for registration of new and currently available products for SIT. These will include dose-finding studies for safety as well as for efficacy endpoints, according to the EMA guidelines outlined above.
- Novel SIT preparations and possibly currently available products that lack dose–response data will be tested for safety and efficacy in dose-escalation schemes. This will produce a large pool of data that will help to define the dose–response relationship in SIT and mark a major advance in the understanding of the treatment of allergic conditions. The Task Force strongly supports this for the most prevalent allergens.
- Improved characterization and standardization of allergen products for SIT is to be expected. As a small-scale follow-up to the CREATE project, validation of allergen reference materials in Europe will continue through the Biological Standardization Program (BSP090) of the European Directorate for the Quality of Medicines & HealthCare in conjunction with the European Pharmacopoeia.
- Vigorous testing by reference laboratories and a ring test trial should prove the suitability of the recombinant

birch and grass pollen allergens rBet v 1 and rPhl p 5a and the robustness of the appropriate antibody-based immunoassays for their detection. The same approach is foreseeable for other recombinant major allergens (i.e., from house dust mite). After validation, these materials could become biological reference preparations and establish the basis for future in-house reference preparations for allergen manufactures and regulatory authorities. Only then would labeling of allergen products, including their major allergen content in mass units, become a possibility.

- The recombinant venom allergens Api m1, Ves v5 and Ves v1 are already available for use in diagnosis. Future use of recombinant venom allergens for VIT will also necessitate that these conform to guidelines for standardization of allergen preparations.

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Conflict of interest

MC has acted as a consultant for ALK-Abelló and a speaker for ALK-Abelló, Allergy Therapeutics (UK) Ltd, MSD and GSK. He has appeared on advisory boards for Allergy Therapeutics (UK) Ltd and ActoGeniX, received honoraria from ALK-Abelló, Allergy Therapeutics (UK) Ltd, Schering-Plough, and MSD, and research grants from ALK-Abelló and GSK. DL has received lecture fees from ALK, Schering Plough, MSD and FDA Allergenic (Brasil) and travel grants from ALK and Stallergenes. JKT has received lecture fees from Allergopharma, ALK-Abelló, Bencard, HAL Allergy, Leti, Novartis, Roxall Medizin/Dr. Beckmann Pharma, and Stallergenes, and consultancy fees from ALK-Abelló, Bencard, HAL Allergy, and Novartis, and research and safety board honoraria from Allergopharma, ALK-Abelló, HAL Allergy, Leti and Merck/Schering-Plough. LJ has acted as consultant for the European Allergen Manufacturers Group. GP has received lecture fees from ALK Abelló, Abbott, Almirall, AstraZeneca, GSK, Lofarma, Menarini, MSD, Phadia, Schering Plough, and Stallergenes. PAE has received lecture fees from ALK, Allergopharma, Astra, Glaxo, MSD, Stallergenes, and Phadia, and consultancy fees from ALK, Allergopharma, MSD and Novartis. EMV declares no conflict of interest. EV has received lecture fees from ALK, GSK, MSD, Schering-Plough, AllergoPharma and Nycomed, and consultancy fees from ALK, Nycomed, Schering-Plough and MSD. CM declares no conflict of interest. HJM has received research support from, and served on the advisory boards of, ALK-Abelló and Stallergenes, and

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