

Environmental exposure chambers in allergen immunotherapy trials: Current status and clinical validation needs

Karen Rösner-Friese, DVM,^a Susanne Kaul, DVM,^a Stefan Vieths, PhD,^a and Oliver Pfaar, MD^b *Langen, Wiesbaden, and Mannheim, Germany*

As required by the European Medicines Agency and the US Food and Drug Administration for pivotal trials involving allergen immunotherapy (AIT) products, clinical efficacy assessment is currently based on double-blind, placebo-controlled field studies with natural allergen exposure during the allergen season. However, this study design is associated with several drawbacks, such as the high variability of allergen exposure in different trial sites or seasons and the presence of confounding environmental factors. On the contrary, environmental exposure chambers (EECs) aim to operate with a stable and reproducible allergen exposure under highly standardized environmental conditions. Technical validation parameters for different EECs worldwide have been published by several groups. However, full clinical validation of EEC study outcomes is required for their classification as an appropriate alternative to natural allergen exposure for AIT product efficacy assessment. Some clinical validation parameters have already been addressed for EEC units. The reliability of provoked symptoms in repeated EEC sessions is high, but the predictive power of EEC settings for the clinical response on natural exposure and the impact of seasonal priming on test results still have to be validated systematically,

as does the inter-EEC variability. Thus the authors recommend a continued in-depth validation of EECs to exploit the potential of this technology for future AIT product development. (*J Allergy Clin Immunol* 2014;■■■■:■■■■-■■■■.)

Key words: *Environmental exposure chamber, allergen immunotherapy, clinical validation, pivotal studies*

Clinical testing of products for allergen immunotherapy (AIT) is traditionally conducted in clinical trials by using natural allergen exposure in an outpatient setting. However, the sensitivity, reproducibility, and comparability of such clinical trials are affected by the variability of airborne allergen exposure: pollen counts are subject to strong local, seasonal, and diurnal variations and affected by confounding environmental conditions, such as rain, temperature, humidity, and wind speed.¹⁻³ Furthermore, the actual individual pollen exposure is influenced by the respective subject's lifestyle (indoor/outdoor activity) and residence (urban/rural).²⁻⁴ The reliability and reproducibility of such seasonal field studies also depends on the difficulty in defining and capturing the peak pollen season and individual variability in maintaining the study diary.¹⁻⁵ Thus only limited

From ^athe Division of Allergology, Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Langen, and ^bthe Center for Rhinology and Allergology Wiesbaden and the Department of Otorhinolaryngology, Head and Neck Surgery, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim.

Disclosure of potential conflict of interest: K. Rösner-Friese is employed by the Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich-Institut. S. Kaul is employed by Paul-Ehrlich-Institut. S. Vieths has provided consultancy to the Food Allergy Resource and Research Program; has provided expert testimony for the Medical University of Vienna; has received research support from Monsanto Company; has received payment for lectures from Deutsche Dermatologische Gesellschaft, the Spanish Society of Allergy and Clinical Immunology, Westdeutsche Arbeitsgemeinschaft für pädiatrische Pneumologie und Allergologie e.V., Gesellschaft für pädiatrische Allergologie und Umweltmedizin, Ärzteverband Deutscher Allergologen, and the American Academy of Allergy, Asthma & Immunology; has received royalties from Schattauer Allergologie Handbuch and Elsevier Nahrungsmittelallergien und Intoleranzen; and has received travel support from the German Research Foundation, the Federal Institute for Risk Assessment, the Austrian Society for Allergology and Immunology, the European Directorate for the Quality of Medicines and Health Care, the European Academy of Allergy and Clinical Immunology, the World Allergy Organization, Association Monégasque pour le Perfectionnement des Connaissances des Médecins, the Federal Office of Consumer Protection and Food Safety, the German Chemical Society (GDCh), the Austrian Society for Dermatology and Venerology, AKM Allergiekongress, and the Austrian Food Chemical Society. O. Pfaar is current chairman of the Immunotherapy Interest Group (IT IG) of the European Academy of Allergy and Clinical Immunology; is current Secretary of Section ENT of Deutsche Gesellschaft für Allergologie und Klinische Immunologie; has received consultancy fees from Allergopharma, Bencard/Allergy Therapeutics, HAL-Allergy, Novartis/LETI, MEDA, ALK-Abelló, Biotech Tools s.a., GfK Bridgehead, NAVIGANT-consulting, Sanofi, Guidepoint Global Advisors, Thermo Fisher, and Stallergenes; is Scientific Board Member of Mobile Chamber Experts (MCX), a GA²LEN Partner;

is employed by the Department of Otorhinolaryngology, Head and Neck Surgery, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; his institution has received research grants from Allergopharma, ALK-Abelló, Stallergenes, HAL-Allergy, Artu Biologicals, Allergy Therapeutics/Bencard, Hartington, Lofarma, Novartis/Leti, GlaxoSmithKline, Essex-Pharma, Cytos, Curallergic, Roxall, Biomay, Thermo Fisher, Circassia, Biotech Tools s.s., European Union (FP7-HEALTH.2013-Innovation 1), and MEDA-Pharma GmbH. He has received grants for the "Spezifische Immuntherapie"-award 2014 and the "Nachwuchsförderpreis"-award 2010 of the Deutsche Gesellschaft für Allergologie und klinische Immunologie; has received lecture fees from ALK-Abelló, Allergopharma, Stallergenes, HAL-Allergy, Allergy Therapeutics/Bencard, Hartington, Lofarma, Novartis/Leti, GlaxoSmithKline, Roxall, Paul-Ehrlich-Institut, GEKA mbH, Thermo Fisher, and MEDA-Pharma GmbH; is co-editor and an author of the textbook *Allergien bei Kindern und Jugendlichen* (publisher: Schattauer-Verlag, Germany); is author of different chapters of *Allergologie-Handbuch* (publisher: Schattauer-Verlag, Germany); has received payment for development of educational presentations from GlaxoSmithKline, Bencard, and Novartis; and has received travel support from HAL-Allergy and Allergopharma.

Received for publication June 4, 2014; revised September 24, 2014; accepted for publication October 31, 2014.

Corresponding author: Susanne Kaul, DVM, Division of Allergology, Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich-Strasse 51-59, D-63225 Langen, Germany. E-mail: Susanne.Kaul@pei.de. Or: Oliver Pfaar, MD, Center for Rhinology and Allergology Wiesbaden, An den Quellen 10, D-65189 Wiesbaden, Germany. E-mail: oliver.pfaar@allergiezentrum.org.

0091-6749/\$36.00

© 2014 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2014.10.045>

Abbreviations used

AIT:	Allergen immunotherapy
AR:	Allergic rhinitis
ARTSS:	Average rhinoconjunctivitis total symptom score
EEC:	Environmental exposure chamber
EMA:	European Medicines Agency
SCIT:	Subcutaneous immunotherapy
SLIT:	Sublingual immunotherapy
TNSS:	Total nasal symptom score

information regarding the onset and duration of action of an investigational compound can be drawn from such natural exposure trials.^{1,4,6}

The use of environmental exposure chambers (EECs) can overcome these drawbacks by means of defined, stable, and reproducible allergen exposure under highly standardized environmental conditions.^{1,3,6} Furthermore, in allergic rhinitis (AR) trials EEC assessments exclude bias because of the use of rescue medication and enable controlled symptom scoring.^{1,3,5,7,8}

EECs have been used for efficacy assessment and outcome evaluation in AR trials since the 1980s: Horak and Jäger⁹ first reported the Vienna Challenge Chamber in 1987. At the same time, the Environmental Exposure Unit in Kingston, Canada, was established for AR research.^{3,10} During the following decades, the EEC model was continually evolved, and additional facilities were established: the Fraunhofer Environmental Challenge Chamber in Hannover/Germany and smaller or primarily commercially operated EEC units in Denmark (Copenhagen), the United States (Atlanta), and Japan (Wakayama, Osaka, and Tokyo). The EEC setting has since been extensively used to evaluate the efficacy and safety of antiallergic medication (antihistamines/corticoids), with special emphasis on the onset and duration of action of these drugs.^{11–23} These trials are reviewed by Day et al.³

Immunotherapy trials using the EEC system are first mentioned in the 1990s by Horak et al.²⁴ and Donovan et al.²⁵ More recently, the effects of novel allergen products for both sublingual immunotherapy (SLIT) and subcutaneous immunotherapy (SCIT) were evaluated by means of EEC challenge.^{26–28} However, to date, the use of EEC settings in AIT trials is relatively low and generally limited to phase II trials. This can be attributed to the fact that until now the European Medicines Agency (EMA) and the US Food and Drug Administration do not recognize EEC trials without evaluation of efficacy during natural exposure as the primary end point as pivotal (phase III) studies.^{29,30} However, the current EMA guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases²⁹ accepts the EEC model for efficacy analysis in dose-finding and pharmacodynamic studies.

Existing challenge chambers are technically validated and reported to operate within their specifications in terms of environmental conditions (eg, temperature, humidity, and air pressure) and allergen distribution. Moreover, the use of EECs as a promising outcome measure adjunct to natural pollen exposure in phase III trials is discussed in the recently published European Academy of Allergy and Clinical Immunology position paper on clinical outcomes used in allergen immunotherapy trials.³¹ However, with respect to clinical validation of EECs, there are still basic questions to be answered, as pointed out by the EMA in 2008²⁹: Is the treatment effect biased in out-of-season EEC sessions because

of the lack of seasonal priming? How well does the EEC setting reproduce the real-life situation? In other words, how does the controlled allergen challenge compare with natural exposure?

This review article aims at summarizing and discussing published data on the validation status of EEC methods with respect to technical aspects and an emphasis on clinical validation. A focus will be on the unmet needs for validation from a clinical and regulatory point of view, especially regarding treatment outcome measurements in the EEC system.

TECHNICAL VALIDATION OF EXISTING EECs

Currently used pollen chambers have undergone systematic technical validations. The main cornerstones of technical EEC validation are homogenous spatial allergen distribution, temporal stability of allergen levels (intrasession and intersession), and stable, defined, and reproducible environmental conditions throughout an exposure session. Temperature, humidity, and volumetric air flow rate have to be monitored and kept within a narrow range of target values during the entire challenge.^{2,3,7,10,32}

There are 2 standard methods for controlling and documenting spatial homogeneity and temporal stability of pollen levels: (1) Rotorod samplers positioned throughout the subject's seating area for offline measurement (calculating the pollen dispersal levels in the EEC retrospectively after completion of the exposure session)^{3,32} and (2) laser particle counters for real-time determination (determining the actual pollen dispersal levels during the EEC session).^{3,32}

Krug et al.³² found a strong correlation between the 2 methods in the Fraunhofer unit. Target pollen concentrations during a 4-hour exposure and from day to day showed only slight variations (day-to-day variation, 3.7% to 5.5%), with spatial distribution of pollen within the chamber varying by $\pm 10\%$. Constant ragweed pollen levels over an 8-hour challenge period and reproducible pollen levels in 5 subsequent sessions have been shown also for the Kingston Environmental Exposure Unit¹⁰: the average daily ragweed pollen concentrations varied from the predefined level by less than 5%.

Ito et al.³³ demonstrated that the pollen distribution in the EEC in Osaka varied from 80% to 110% of target dispersal values, and the rate of pollen level variations throughout an exposure session did not exceed 15%.

Published technical validation data of existing EECs are compiled in Table I.^{2,3,10,15,23,32–37} The various technical setups and specifications of existing EEC facilities in Europe, Canada, and the United States have been reviewed by Day et al.³

In summary, existing EEC facilities have been reported as technically validated and proved to function within the predefined specifications. Thus it can be concluded that controlled and reproducible inhalation exposure to routine airborne allergens (eg, grass, tree, ragweed, mountain cedar, Japanese cedar pollen, and house dust mite) can be ensured. Furthermore, technical validation is in progress for new EEC setups, such as mobile EEC systems.

However, comparison of the results obtained in distinct units remains difficult because of the differences between physical setup and engineering features of worldwide existing facilities.

CLINICAL VALIDATION OF EXISTING EECs

Systematic clinical validation of EECs existing worldwide has not been conducted thus far. Only a few parameters

TABLE I. Published technical validation data of EECs

Facility	Antigen dispersed	Validation parameters	References
Kingston EEU, Ontario, Canada	Ragweed pollen	T, H Spatial distribution and temporal stability of allergen	Day et al ³ ; Day and Briscoe ¹⁰ ; Day et al ³⁵
Vienna Challenge Chamber, Vienna, Austria	Grass pollen	T, H Spatial distribution and temporal stability of allergen	Day et al ³ ; Horak et al ¹⁵
Fraunhofer ECC, Hannover, Germany	Grass pollen	T, H, airflow rate Spatial distribution and temporal stability of allergen Correlation offline and real-time pollen counter methods	Krug et al ³²
Biogenic Research Chamber, San Antonio, Tex	Mountain cedar pollen	T, H, airflow rate/characteristics Spatial distribution of allergen Sensitivity/correlation offline and real-time pollen counter methods*	Jacobs et al ²
ACC Osaka, EEU Wakayama, OHIO chamber Tokyo, Japan	Japanese cedar pollen	T, H Spatial distribution and temporal stability† of allergen Correlation of 2 pollen counter methods‡	Ito et al ³³ ; Enomoto et al ³⁶ ; Hashiguchi et al ³⁴
Chamber at National University Copenhagen, Denmark	House dust mite aerosol	Aerodynamic properties of the unit Effect of human activity Reproducibility of airflow kinetics	Rønborg et al ³⁷
AEU Atlanta, Ga	Ragweed pollen	Temporal stability of allergen	Berkowitz et al ²³

ACC, Allergen challenge chamber; AEU, allergen exposure unit; ECC, environmental challenge chamber; EEU, environmental exposure unit; H, humidity; T, temperature.

*Evaluated first in the unoccupied fully equipped chamber and then in the occupied unit.

†Only determined by Ito et al³³ and Enomoto et al.³⁶

‡Only determined by Ito et al.³³

considered crucial for the acceptance of efficacy analysis in an EEC as the primary end point in pivotal AIT trials have already been addressed. Table II^{2,5,6,32,34,37-39} provides an overview of validation parameters required from a clinical and regulatory point of view and their validation status. Table II differentiates between AR symptoms evoked in the EEC and the AIT treatment outcome obtained with the EEC setting, if applicable. The subsequent sections will follow this principle.

Sensitivity/specificity of symptom induction by EEC challenge

Sensitivity of the pollen chamber system is mainly driven by the concentration of target pollen or the respective antigen dispersed in the chamber. Systematic validation studies focused on the identification of reasonable dispersal levels inducing adequate symptoms but no severe side effects in the majority of allergic subjects without affecting healthy volunteers (Table II). Studies including several different pollen concentrations (eg, 1000, 2000, 4000, and 8000 grass pollen grains/m³)^{3,32} showed a significant pollen concentration effect on symptom severity.^{2,32,34}

Pollen concentrations used in the EEC system are typically much higher compared with mean pollen counts measured during the natural pollination period. Higher antigen concentrations are chosen to ensure elicitation of the full range of symptoms occurring during the peak pollen season.^{3,10} Ito et al³³ also noted that pollen counts at 1.6 m in height (at which human subjects breathe) might be higher than rooftop

recordings from public pollen measuring stations. Jacobs et al² addressed the relevance of priming exposure sessions on the elicitation of symptoms in all sensitized subjects, including low responders. However, depending on preselection of the study population, priming exposure sessions might not be necessary, as shown by Hashiguchi et al.³⁴ Typical pollen and antigen loads used in various EEC facilities worldwide are compiled in Table III.^{5,6,10,24,36,40-43}

The specificity of individual exposure units was investigated by means of provocation of healthy subjects, which was performed by Jacobs et al² and Rønborg et al.⁴⁰ Both studies confirmed a distinct allergic reaction in sensitized subjects only. Masked sham runs were included in most of the validation studies listed in Table II, revealing a minimum placebo response in sensitized subjects.

Reproducibility of AR symptoms evoked in the EEC

Reproducibility of clinical findings captured in the EEC system is not only crucial in regard to repetitive EEC sessions but also for comparison between different EEC facilities.

Focus on repetitive EEC sessions. Several well-designed studies investigated whether the degree of symptom induction in different EEC sessions is reproducible (Table II). Hohlfeld et al⁶ compared the diagnostic value (specificity, sensitivity, and reproducibility) of several outcome measures (nasal and ocular symptom scores, nasal flow rate, and exhaled nasal nitric oxide) in 60 patients subjected to grass pollen exposure on 2 measurements out of the pollen season with an interval of 2 weeks. The total nasal symptom score

TABLE II. Overview of clinical EEC validation parameters and their validation status with respect to AR symptoms and AIT treatment outcomes

Validation parameter	Already addressed	Facility	Antigen dispersed	References
Sensitivity/specificity of AR symptom induction by EEC challenge	Yes	Fraunhofer ECC, Germany OHIO Chamber, Japan Biogenic Research Chamber, Texas Chamber at National University, Copenhagen, Denmark	Grass pollen Japanese cedar pollen Mountain cedar pollen House dust mite	Krug et al ³² Hashiguchi et al ³⁴ Jacobs et al ² Rønborg et al ³⁷
Reproducibility of				
I. AR symptoms				
A. on different EEC sessions (retest reliability)	Yes	Fraunhofer ECC, Germany OHIO Chamber, Japan	Grass pollen Japanese cedar pollen	Hohlfeld et al ⁶ ; Krug et al ³² Hashiguchi et al ³⁴
B. between different EEC facilities (inter-EEC variability)	No			No data
II. AIT treatment outcomes	No			No data
Correlation EEC vs natural exposure				
I. With reference to AR symptoms	Yes	Biogenic Research Chamber, Texas Fraunhofer ECC, Germany	Ragweed pollen Grass pollen	Jacobs et al ⁵ Hohlfeld et al ⁶
II. With reference to AIT treatment outcome	No			No data
Seasonal priming				
I. Correlation of AR symptoms in-season EEC/out-of-season EEC	Yes	ACC Osaka, Japan Fraunhofer ECC, Germany	Japanese cedar pollen Grass pollen	Yuki et al ^{38*} Badorrek et al ³⁹
II. Correlation of AIT treatment outcome in-season EEC/out-of-season EEC	No			No data

ACC, Allergen challenge chamber; ECC, environmental challenge chamber; EEU, environmental exposure unit.

*Correlation of symptoms in end-of-season EECs/out-of-season EECs evaluated in 2 study populations.

TABLE III. Published data on typical pollen and antigen loads used in various EECs worldwide

Facility	Antigen source	Target antigen concentration	References
Fraunhofer ECC, Germany	Grass pollen	4000 ± 300 grains/m ³	Hohlfeld et al ⁶
Kingston EEU, Canada	Ragweed pollen	3500 ± 500 grains/m ³	Day and Briscoe ¹⁰ ; Day et al ⁴¹
Vienna Challenge Chamber, Austria	Birch pollen	1000 grains/m ³	Horak et al ²⁴
	Der p 1	40 ng/m ³	Horak et al ⁴²
	Grass pollen	1500 ± 120 grains/m ³	Horak et al ⁴³
Chamber at National University Copenhagen, Denmark	Der p 1	50 or 100 ng/m ³	Rønborg et al ⁴⁰
EEU Wakayama, Japan	Japanese cedar pollen	2000 grains/m ³	Enomoto et al ³⁶
Biogenics Research Chamber, Texas	Ragweed pollen	3500 ± 500 grains/m ³	Jacobs et al ⁵

ECC, Environmental challenge chamber; EEU, environmental exposure unit.

(TNSS) proved to have the best reproducibility and sensitivity/specificity.

Reproducible symptoms and lung function parameters on 5 consecutive grass pollen exposures (3-6 days apart) outside the natural pollination period in response to a predefined pollen concentration were previously demonstrated by the same group.³² No significant difference in the TNSS, nasal flow rate, nasal secretion, or FEV₁ were detected from day to day in 24 volunteers with seasonal AR ($P > .05$).

The above studies convincingly demonstrated the reproducibility of symptoms on repetitive EEC sessions as long as a sufficient time gap (a minimum of 3 and preferably 7 days) between subsequent EEC challenges is ensured. This is of particular importance in view of experimental priming effects, which would lead to exacerbated symptoms from one exposure to the next, as seen from study data generated in the Canadian and Japanese challenge units,^{5,33,38} where EEC sessions were performed on 2 to 4 consecutive days.

Focus on differences between EEC facilities. The comparability of individual EEC facilities in regard to symptom induction has not been demonstrated thus far (see the section on unmet needs for validation).

Reproducibility of treatment outcome measured by using an EEC

The reproducibility of AIT treatment outcomes measured on subsequent EEC sessions (retest reliability) or by different EEC facilities (inter-EEC variability), respectively, has not been investigated thus far to the authors' knowledge (see the section on unmet needs for validation).

Notably, Day et al^{35,41} investigated whether repetitive EEC studies using an identical study protocol yielded the same efficacy results for antiallergic drugs. The authors were able to demonstrate similar treatment effects (onset of action and symptom relief) of cetirizine and loratadine in comparison with

TABLE IV. Unmet needs for clinical validation of the EEC approach in AIT trials

Unmet needs	Essential parameters*	Additional parameters
Correlation EEC vs natural exposure	Treatment effect	Symptoms
Impact of seasonal priming	Treatment effect	Symptoms
Reproducibility	I. Treatment outcome on different EEC sessions (retest reliability) II. Treatment outcome between different EEC facilities (inter-EEC variability)	Symptoms between different EEC facilities (inter-EEC variability)

*In view of the general acceptance of the EEC model in pivotal AIT trials.

placebo in 2 EEC studies conducted out of the respective pollen season approximately 4 years apart by using 2 different study populations.

Correlation EEC versus natural exposure with respect to AR symptoms

A perceived limitation of experimental allergen exposure models is that the controlled indoor setting does not resemble natural exposure conditions. During seasonal exposure multiple cofactors are relevant, such as different weather conditions, pollutants, and a wide variety of different allergen sources. Moreover, the absence of seasonal priming, short exposure time compared with real-time exposure, the trial context (artificial environment, reduced normal life activity of participants, and proximity to other volunteers), and subjects' demographics limited to the EEC area (especially in trials with multiple EEC sessions) are also often reported as limitations of the EEC approach.^{1-3,5,8} In view of these drawbacks, a correlation of clinical symptoms evoked under experimental conditions in comparison with natural exposure is frequently requested to confirm the validity of data obtained by pollen chamber exposure.^{2,4,5,7,8}

To date, 2 studies have been published that performed a comparative analysis of symptoms induced by seasonal pollination versus out-of-season challenge in the pollen chamber.

Jacobs et al⁵ recently demonstrated a strong correlation among symptom scores (nasal and ocular) recorded during the natural ragweed season and in response to off-season ragweed challenge with the same set of patients. In the EEC setting the study population could be divided into slow/low and rapid/high responders in terms of symptom induction and severity. Logistic regression analysis revealed that symptom scores elicited in the EEC are strong predictors of symptom levels reached in the natural allergy season. In contrast, the severity of symptom development in the EEC and during natural pollen exposure was not related to the size of the corresponding skin test response, which is consistent with previous studies.^{44,45}

Contrary to earlier observations,⁵ Hohlfeld et al⁶ reported a lack of correlation between the TNSS at the end of a 4-hour grass pollen challenge outside the season and the TNSS recorded from the same patient panel during the natural grass pollen season ($r = 0.25$). However the TNSS assessed 24 hours after EEC challenge significantly correlated with the in-season TNSS ($r = 0.41$). The lack of a positive correlation after 4 hours was attributed to (1) different pollen concentrations during the natural season versus chamber atmosphere and (2) early-phase reactions in the EEC setting versus late-phase reactions under natural conditions. Hohlfeld et al⁶ hypothesized that symptoms occurring 24 hours after challenge instead reflect late-phase reactions and therefore would better correlate with symptoms during field exposure.

The authors further postulated that in-season EEC measurements might better reflect ongoing chronic inflammation and therefore might correlate better with natural exposure.

Correlation EEC: Natural exposure with respect to AIT treatment outcome

To the authors' knowledge, up to now, studies comparing AIT efficacy results (including placebo effects) measured in the challenge unit with corresponding treatment outcomes of field exposure trials have not been performed (see the section on unmet needs for validation).

Impact of seasonal priming on AR symptom induction in the EEC system

During natural pollen exposure, the continuous antigen trigger results in allergic reactions, with chronic inflammatory processes and late-phase responses being prominent. This so-called seasonal priming possibly results in a different and stronger allergic reaction and altered response to antiallergic treatments.^{7,39,46,47} It is frequently argued that the EEC model lacks the impact of natural priming, and therefore transferability of EEC data to the real-life situation is limited.^{3,7,38,39} Two published studies^{38,39} address the effect of seasonal priming on EEC assessments.

Yuki et al³⁸ found in 32 patients with Japanese cedar pollinosis that symptoms (nasal and ocular) are more quickly induced and are stronger when volunteers are challenged in the EEC just after the end of the natural pollen season compared with EEC exposures out of the natural season. In fact, on the first day of 3 consecutive challenges during the nonpollen season, no significant symptoms could be elicited, but symptoms increased proportionally with the number of exposure days, suggesting an experimental priming effect. After the end of the pollen season, significant symptoms were already induced 15 minutes after the first initiation of exposure. Yuki et al concluded that without priming from natural pollination, medium- to long-term continuous challenge is necessary to induce symptoms reflecting chronic allergic inflammation during natural pollination. However, the power of this study is limited because of the fact that 2 different study populations were used (only 2 patients participated in both parts of the trial) and because stratification was not conducted.

Badorrek et al³⁹ investigated in a well-designed study whether the treatment effect of an established antiallergic treatment (combination of cetirizine and pseudoephedrine) is the same when obtained by in-season and out-of-season EEC challenge. Therefore 70 patients with seasonal AR underwent 4 grass pollen challenges, 2 in and 2 out of the season. Antiallergic treatment (cetirizine plus pseudoephedrine) was administered 2 hours after

challenge initiation, and the effect on the TNSS was assessed as the primary objective. Symptoms provoked in the EEC were slightly increased during the season compared with those outside the season. A decrease in FEV₁ within 12 hours after challenge was more frequently detected after in-season sessions also in patients with no history of asthma. Therefore the authors concluded that seasonal priming during natural pollination increases the risk for pulmonary side effects.

Both studies confirm an increased reactivity of allergic subjects when challenged during or just after the natural pollen season, which is most likely attributed to seasonal priming.

Impact of seasonal priming on treatment outcome measured in the EEC

To date, no controlled study data are available on the impact of seasonal priming effects on AIT efficacy results obtained in EEC trials (see the section on unmet needs for validation). However, regarding the treatment effect of symptomatic AR treatment, Badorrek et al³⁹ detected no significant difference in treatment outcome between in- and off-season EEC exposures (intention-to-treat population: $P > .05$). Regardless of the time point of EEC challenge, treatment with cetirizine plus pseudoephedrine significantly reduced the TNSS compared with that seen after placebo.

USE OF EEC MEASUREMENTS IN AIT TRIALS

Although EECs have been extensively used to evaluate the effects of pharmacotherapy^{13,16,35,48-50} (reviewed by Day et al³), use in AIT trials has been limited to a few studies²⁴⁻²⁸ (reviewed by Devillier et al¹ and Pfaar et al⁵¹).

In an early trial a total of 32 adults with ragweed allergy (16 patients with an anamnesis of minimally 2 years of AIT and 16 patients without AIT) and 11 nonallergic control subjects were exposed to levels of 2500 to 3000 ragweed grains/m³ over a single challenge of 3 hours outside the ragweed pollen season.²⁵ The authors concluded that ragweed immunotherapy significantly reduces nasal symptoms in patients with ragweed allergy compared with the control group without immunotherapy treatment ($P = .025$). They emphasized that the EEC provides “opportunities for additional studies on immunotherapy for allergic respiratory conditions.”²⁵ A subsequent early trial on 41 patients with birch pollen allergy evaluated the effects of a 4-month course of SLIT as assessed in a 2-hour challenge in the Vienna Challenge Chamber.²⁴ The authors found that in addition to other outcome measures, actively treated patients produced less than half of the nasal secretions at the second challenge (posttreatment) in the active group compared with the placebo group. However, this difference did not reach statistical significance. Rhinomanometric measurements revealed significantly improved nasal airflow compared with that of placebo-treated patients at the second challenge ($P = .028$). Of note, at the beginning of the second challenge, nasal airflow levels were much lower in both the active and placebo groups compared with baseline values (possibly caused by the beginning of the natural pollen flight in the course of the second challenge in March), indicating these findings might be biased by priming.

In a later double-blind, placebo-controlled trial by the same group of investigators, the extent and onset of clinical effects of a 5-grass-pollen tablet for SLIT were analyzed in 89 adults with

grass pollen allergy.²⁸ SLIT was performed outside the grass pollen season, and all patients underwent a standardized challenge at baseline and again after 1, 4, 8, and 16 weeks of treatment (outside the grass pollen season). The analysis revealed a significant superiority of active treatment versus placebo based on the average rhinoconjunctivitis total symptom score (ARTSS) as soon as after 4 weeks of treatment. The ARTSS of the actively treated patients further decreased at each of the following challenges.^{28,52} After 4 months of treatment, the relative mean improvement in the ARTSS compared with baseline was 41.8% in the active and 18.5% in the placebo arms, resulting in a significant difference ($P = .003$).²⁸ However, in a subsequent analysis a correlation of changes in patterns of peripheral CD4⁺ T cells, antibody responses, or basophil activation with the clinical effects in the same population of study patients could not be demonstrated.⁵²⁻⁵⁴ Nevertheless, increased expression of C1Q and STAB1 as biomarkers indicating activity of regulatory dendritic cells was found in PBMCs from clinical SLIT responders. In contrast, these immunologic changes were not found in nonresponders or placebo-treated patients.⁵⁵

Another recently published clinical trial investigated the effects of AIT with rBet v 1 produced as a folding variant in 37 patients with birch pollen allergy exposed in a Canadian EEC.²⁶ In this dose-finding phase II trial, patients received a preseasonal short-term course of AIT over 10 weeks (randomized into 4 maintenance groups and one placebo group) and were challenged twice under standardized exposure of 8 hours at baseline and after therapy (before the season). The analysis of the total symptom score revealed a mean reduction in the posttreatment total symptom score of 18.8% for placebo-treated patients, 71.9% for those receiving 20 µg of maintenance AIT, 75.6% for those receiving 80 µg of maintenance AIT, 81.8% for those receiving 160 µg of maintenance AIT, and 78.3% for 320 µg of maintenance AIT compared with baseline scores. This improvement was significant versus placebo for all treatment arms investigated ($P = .0003-.0022$). The authors concluded that considering efficacy, immunologic effects, and tolerability, a maintenance dose of 80 µg of rBet v 1 would be preferable for SCIT in adults with birch pollen allergy.²⁶ However, this has to be confirmed in further trials with this recombinant allergen product.⁵⁶

The same EEC was used in a randomized, double-blind, placebo-controlled phase IIb study on the effects of a short course of monophosphoryl lipid A–adjuvanted AIT in adults with ragweed pollen allergy.²⁷ The pollen exposure at baseline and after treatment was conducted outside the natural ragweed pollen season and was standardized to an average airborne ragweed pollen concentration of 3500 ± 500 grains/m³. The analysis of total symptom scores before (baseline) and after short-course AIT revealed a relative mean improvement in active- versus placebo-treated patients of 48% ($P < .05$).²⁷

UNMET NEEDS FOR VALIDATION FROM A CLINICAL AND REGULATORY VIEW

As summarized above, the first steps toward a systematic clinical validation of the EEC model have been taken, and considerable efforts have been made to increase the body of evidence for the validity and reliability of the EEC approach in AIT trials. Nevertheless, several unmet needs require attention and evaluation before the EEC system can be accepted as

equivalent to traditional field trials for the proof of efficacy of AIT products.³¹

Table IV compiles the unmet needs (highest relevance first) that are considered crucial in view of a systematic clinical validation of the EEC model in AIT trials.

The proof has yet to be delivered that the treatment outcome obtained in the EEC setting correlates with effects found after natural exposure.^{1,7,8,29,32} Correlation is not expected for symptom severity, which is mainly driven by the amount and allergenicity of pollen/antigen exposure. Both are highly variable during the natural allergy season, whereas the allergen challenge system tries to control these variables within a tight range.

Thus a lack of correlation in terms of symptom levels appears comprehensible, as shown by Hohlfeld et al.⁶ Future validation studies should rather focus on the clinical efficacy of AIT products after field versus EEC exposures. Comparative analyses should be performed in the same study population and, if feasible, in a multicenter approach. In particular, evidence needs to be provided that the EEC model is capable of predicting the performance of investigational products for AIT during the natural allergy season.³² This is considered crucial for accepting that the EEC setting is of equal value as the traditional field design in pivotal AIT trials.

Another important issue is to address the impact of seasonal priming on the treatment outcome captured by the EEC challenge. It remains to be elucidated whether the magnitude of the treatment effect is biased by the time point of allergen challenge (in-season vs out of season) and which exposure paradigm correlates best with natural exposure. The EMA guideline²⁹ states that “the influence of measurement within or without the pollen season (influence of inflammation) has to be evaluated before such tests could be used as primary endpoints.” Given an impact of seasonal priming on the treatment outcome, further work is required on experimental priming effects mimicking natural priming by means of subsequent EEC sessions. In this regard, when in-season EEC challenges are performed, one should be aware of the enhanced allergic responsiveness during the natural pollen period and the increased risk of allergic side effects. This is why lung function monitoring should be routinely performed during in-season challenges.

Finally, efforts should be made to confirm reproducible treatment outcomes on repetitive EEC sessions and between different EEC facilities applying the same study population and protocol.^{7,8} However, comparability studies of individual EEC facilities face major challenges, such as great geographic distances between individual pollen chambers and the specific technical features of each EEC facility. This makes comparative analysis extremely demanding. In fact, it will be almost impossible to achieve a numeric correlation of treatment outcomes between individual EEC facilities. Instead, comparative analysis should focus on the correlation of treatment outcomes in terms of clinical relevance and statistical significance.

CONCLUSION

The EEC approach represents a promising tool to facilitate AIT trials and enhance the reliability and comparability of clinical trial data by controlling multiple confounding variables inherent to traditional field-based AR trials.

Over the last decades, great efforts have been undertaken to validate the EEC system itself in terms of technical features.

Recent work focused on clinical aspects of EEC validation, such as the correlation of EEC-captured data with field-based trial data. However, the predictive power of challenge settings for the clinical response on natural exposure, the role of seasonal priming, and the reproducibility of clinical trial data generated in controlled inhalation systems requires further systematic investigations. There is still a way to go before the body of evidence will be sufficient for using EECs for efficacy testing in pivotal AIT trials. In view of the potentially added scientific and economic value of the EEC technology on AIT product development, it is worth taking up this challenge in the future.

REFERENCES

- Devillier P, Le Gall M, Horak F. The allergen challenge chamber: a valuable tool for optimizing the clinical development of pollen immunotherapy. *Allergy* 2011; 66:163-9.
- Jacobs RL, Ramirez DA, Andrews CP. Validation of the biogenics research chamber for *Juniperus ashei* (mountain cedar) pollen. *Ann Allergy Asthma Immunol* 2011;107:133-8.
- Day JH, Horak F, Briscoe MP, Canonica GW, Fineman SM, Krug N, et al. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. *Clin Exp Allergy Rev* 2006;6:31-59.
- Day JH, Ellis AK, Rafeiro E, Ratz JD, Briscoe MP. Experimental models for the evaluation of treatment of allergic rhinitis. *Ann Allergy Asthma Immunol* 2006; 96:263-77.
- Jacobs RL, Harper N, He W, Andrews CP, Rather CG, Ramirez DA, et al. Responses to ragweed pollen in a pollen challenge chamber versus seasonal exposure identify allergic rhinoconjunctivitis endotypes. *J Allergy Clin Immunol* 2012;130:122-7.
- Hohlfeld JM, Holland-Letz T, Larbig M, Lavae-Mokhtari M, Wierenga E, Kapsenberg M, et al. Diagnostic value of outcome measures following allergen exposure in an environmental challenge chamber compared with natural conditions. *Clin Exp Allergy* 2010;40:998-1006.
- Zieglmayer PU. Are results of environmental exposure units transferable to real-life exposure? *Curr Opin Allergy Clin Immunol* 2013;13:244-8.
- Bernstein JA. Correlation between a pollen challenge chamber and a natural allergen exposure study design for eliciting ocular and nasal symptoms: early evidence supporting a paradigm shift in drug investigation? *J Allergy Clin Immunol* 2012;130:128-9.
- Horak F, Jager S. Die Wiener Provokations-Kammer (Vienna Challenge Chamber). Eine neue Methode des Allergenexpositionstests. *Wien Klin Wochenschr* 1987;99:509-10.
- Day JH, Briscoe MP. Environmental exposure unit: a system to test anti-allergic treatment. *Ann Allergy Asthma Immunol* 1999;83:83-9.
- Day JH, Buckeridge DL, Clark RH, Briscoe MP, Phillips R. A randomized, double-blind, placebo-controlled, controlled antigen delivery study of the onset of action of aerosolized triamcinolone acetonide nasal spray in subjects with ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1996;97:1050-7.
- Day JH, Briscoe MP, Clark RH, Ellis AK, Gervais P. Onset of action and efficacy of terfenadine, astemizole, cetirizine, and loratadine for the relief of symptoms of allergic rhinitis. *Ann Allergy Asthma Immunol* 1997;79:163-72.
- Day JH, Briscoe MP, Rafeiro E, Ellis AK, Pettersson E, Akerlund A. Onset of action of intranasal budesonide (Rhinocort aqua) in seasonal allergic rhinitis studied in a controlled exposure model. *J Allergy Clin Immunol* 2000;105:489-94.
- Day JH, Briscoe MP, Rafeiro E, Hewlett D, Chapman D, Kramer B. Randomized double-blind comparison of cetirizine and fexofenadine after pollen challenge in the Environmental Exposure Unit: duration of effect in subjects with seasonal allergic rhinitis. *Allergy Asthma Proc* 2004;25:59-68.
- Horak F, Zieglmayer P, Zieglmayer R, Lemell P. Onset of action of loratadine/montelukast in seasonal allergic rhinitis patients exposed to grass pollen. *Arzneimittelforschung* 2010;60:249-55.
- Horak F, Zieglmayer P, Zieglmayer R, Lemell P. The effects of bilastine compared with cetirizine, fexofenadine, and placebo on allergen-induced nasal and ocular symptoms in patients exposed to aeroallergen in the Vienna Challenge Chamber. *Inflamm Res* 2010;59:391-8.
- Horak F, Stübner P, Zieglmayer R, Kawina A, Moser M, Lanz R. Onset and duration of action of ketotifen 0.025% and emedastine 0.05% in seasonal allergic conjunctivitis. Efficacy after repeated pollen challenges in the Vienna challenge chamber. *Clin Drug Investig* 2003;23:329-37.

18. Horak F, Stübner P, Ziegelmayer R, Harris AG. Comparison of the effects of desloratadine 5-mg daily and placebo on nasal airflow and seasonal allergic rhinitis symptoms induced by grass pollen exposure. *Allergy* 2003;58:481-5.
19. Horak F, Stübner P, Ziegelmayer R, Kavina A, De Vos C, Burtin B, et al. Controlled comparison of the efficacy and safety of cetirizine 10 mg o.d. and fexofenadine 120 mg o.d. in reducing symptoms of seasonal allergic rhinitis. *Int Arch Allergy Immunol* 2001;125:73-9.
20. Stübner P, Horak F, Ziegelmayer R, Arnaiz E, Leuratti C, Perez I, et al. Effects of rupatadine vs placebo on allergen-induced symptoms in patients exposed to aeroallergens in the Vienna Challenge Chamber. *Ann Allergy Asthma Immunol* 2006;96:37-44.
21. Stübner P, Ziegelmayer R, Horak F. A direct comparison of the efficacy of antihistamines in SAR and PAR: randomised, placebo-controlled studies with levocetirizine and loratadine using an environmental exposure unit—the Vienna Challenge Chamber (VCC). *Curr Med Res Opin* 2004;20:891-902.
22. Krug N, Hohlfeld JM, Geldmacher H, Larbig M, Heermann R, Lavallee N, et al. Effect of lometoprednol etabonate nasal spray suspension on seasonal allergic rhinitis assessed by allergen challenge in an environmental exposure unit. *Allergy* 2005;60:354-9.
23. Berkowitz RB, Woodworth GG, Lutz C, Weiler K, Weiler J, Moss M, et al. Onset of action, efficacy, and safety of fexofenadine 60 mg/pseudoephedrine 120 mg versus placebo in the Atlanta allergen exposure unit. *Ann Allergy Asthma Immunol* 2002;89:38-45.
24. Horak F, Stübner P, Berger UE, Marks B, Toth J, Jager S. Immunotherapy with sublingual birch pollen extract. A short-term double-blind placebo study. *J Investig Allergol Clin Immunol* 1998;8:165-71.
25. Donovan JP, Buckeridge DL, Briscoe MP, Clark RH, Day JH. Efficacy of immunotherapy to ragweed antigen tested by controlled antigen exposure. *Ann Allergy Asthma Immunol* 1996;77:74-80.
26. Meyer W, Narkus A, Salapatek AM, Hafner D. Double-blind, placebo-controlled, dose-ranging study of new recombinant hypoallergenic Bet v 1 in an environmental exposure chamber. *Allergy* 2013;68:724-31.
27. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. *J Allergy Clin Immunol* 2014;133:121-9, e1-2.
28. Horak F, Ziegelmayer P, Ziegelmayer R, Lemell P, Devillier P, Montagut A, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol* 2009;124:471-7.
29. Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency (EMA). Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases. London: European Medicines Agency; 2008. CHMP/EWP/18504/2006.
30. US Food and Drug Administration—Center for Drug Evaluation and Research. Allergic rhinitis: clinical development programs for drug products. Draft Guidance. Silver Spring (MD): US Food and Drug Administration; 2000.
31. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy* 2014;69:854-67.
32. Krug N, Hohlfeld JM, Larbig M, Buckendahl A, Badorrek P, Geldmacher H, et al. Validation of an environmental exposure unit for controlled human inhalation studies with grass pollen in patients with seasonal allergic rhinitis. *Clin Exp Allergy* 2003;33:1667-74.
33. Ito K, Terada T, Yuki A, Ichihara T, Hyo S, Kawata R, et al. Preliminary study of a challenge test to the patients with Japanese cedar pollinosis using an environmental exposure unit. *Auris Nasus Larynx* 2010;37:694-9.
34. Hashiguchi K, Tang H, Fujita T, Suematsu K, Tsubaki S, Nagakura H, et al. Validation study of the OHIO Chamber in patients with Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2009;149:141-9.
35. Day JH, Briscoe M, Widlitz MD. Cetirizine, loratadine, or placebo in subjects with seasonal allergic rhinitis: effects after controlled ragweed pollen challenge in an environmental exposure unit. *J Allergy Clin Immunol* 1998;101:638-45.
36. Enomoto T, Ide T, Ogino S. Construction of an environmental exposure unit and investigation of the effects of cetirizine hydrochloride on symptoms of cedar pollinosis in Japan. *J Investig Allergol Clin Immunol* 2007;17:173-81.
37. Rønberg SM, Mosbech H, Johnsen CR, Poulsen LK. Exposure chamber for allergen challenge. The development and validation of a new concept. *Allergy* 1996;51:82-8.
38. Yuki A, Terada T, Ichihara T, Fujii K, Hyo S, Kawata R, et al. Evaluating the effects of testing period on pollinosis symptoms using an allergen challenge chamber. *Allergol Int* 2011;60:533-9.
39. Badorrek P, Dick M, Hecker H, Schaumann F, Sousa AR, Murdoch R, et al. Anti-allergic drug testing in an environmental challenge chamber is suitable both in and out of the relevant pollen season. *Ann Allergy Asthma Immunol* 2011;106:336-41.
40. Rønberg SM, Mosbech H, Poulsen LK. Exposure chamber for allergen challenge. A placebo-controlled, double-blind trial in house-dust-mite asthma. *Allergy* 1997;52:821-8.
41. Day JH, Briscoe M, Rafeiro E, Chapman D, Kramer B. Comparative onset of action and symptom relief with cetirizine, loratadine, or placebo in an environmental exposure unit in subjects with seasonal allergic rhinitis: confirmation of a test system. *Ann Allergy Asthma Immunol* 2001;87:474-81.
42. Horak F, Jager S, Berger U, Toth J, Nimberger G, Kyrein HJ, et al. Controlled exposure to mite allergen for a dose-finding of dimethindene maleate (DMM). *Agents Actions* 1994;41, Spec No:C124.
43. Horak F, Stübner P, Ziegelmayer R, Harris AG. Effect of desloratadine versus placebo on nasal airflow and subjective measures of nasal obstruction in subjects with grass pollen-induced allergic rhinitis in an allergen-exposure unit. *J Allergy Clin Immunol* 2002;109:956-61.
44. Ellis AK, Ratz JD, Day AG, Day JH. Factors that affect the allergic rhinitis response to ragweed allergen exposure. *Ann Allergy Asthma Immunol* 2010;104:293-8.
45. Radcliffe MJ, Lewth GT, Prescott P, Church MK, Holgate ST. Do skin prick and conjunctival provocation tests predict symptom severity in seasonal allergic rhinoconjunctivitis? *Clin Exp Allergy* 2006;36:1488-93.
46. Toth J, Schultze-Werninghaus C, Marks B, Temmel AF, Stubner P, Jager S, et al. Environmental priming influences allergen-specific nasal reactivity. *Allergy* 1998;53:1172-7.
47. Connell JT. Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. *J Allergy* 1969;43:33-44.
48. Patel P, D'Andrea C, Sacks HJ. Onset of action of azelastine nasal spray compared with mometasone nasal spray and placebo in subjects with seasonal allergic rhinitis evaluated in an environmental exposure chamber. *Am J Rhinol* 2007;21:499-503.
49. Horak F, Jäger S, Toth J, Berger U. Efficacy and tolerability of astemizole-D and Loratadine-D during prolonged, controlled allergen challenge in the Vienna Challenge Chamber. *Arzneimittelforschung* 1996;46:1077-81.
50. Barchuk WT, Salapatek AM, Ge T, D'Angelo P, Liu X. A proof-of-concept study of the effect of a novel H3-receptor antagonist in allergen-induced nasal congestion. *J Allergy Clin Immunol* 2013;132:838-46, e1-6.
51. Pfaar O, Kleine-Tebbe J, Hörmann K, Klimek L. Allergen-specific immunotherapy: which outcome measures are useful in monitoring clinical trials? *Immunol Allergy Clin North Am* 2011;31:289-309.
52. Van Overtvelt L, Baron-Bodo V, Horiot S, Moussu H, Ricarte C, Horak F, et al. Changes in basophil activation during grass-pollen sublingual immunotherapy do not correlate with clinical efficacy. *Allergy* 2011;66:1530-7.
53. Bonvalet M, Moussu H, Wambre E, Ricarte C, Horiot S, Rimaniol AC, et al. Allergen-specific CD4+ T cell responses in peripheral blood do not predict the early onset of clinical efficacy during grass pollen sublingual immunotherapy. *Clin Exp Allergy* 2012;42:1745-55.
54. Baron-Bodo V, Horiot S, Lautrette A, Chabre H, Drucbert AS, Danzé PM, et al. Heterogeneity of antibody responses among clinical responders during grass pollen sublingual immunotherapy. *Clin Exp Allergy* 2013;43:1362-73.
55. Zimmer A, Bouley J, Le Mignon M, Pliquet E, Horiot S, Turfkruyer M, et al. A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. *J Allergy Clin Immunol* 2012;129:1020-30.
56. Makatsori M, Pfaar O, Leonart R, Calderon MA. Recombinant allergen immunotherapy: clinical evidence of efficacy—a review. *Curr Allergy Asthma Rep* 2013;13:371-80.