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Abstract

The Future of the Allergists and Specific Immunotherapy (FASIT) workshop provides a regular platform for global experts from academia, allergy clinics, regulatory authorities and industry to review developments in the field of allergen immunotherapy (AIT). The most recent meeting, held in February 2017, had two main themes: advances in AIT and hot topics in AIT from the regulatory point of view. The first theme covered opportunities for personalised AIT, advances in adjuvants and delivery systems, and the development of new molecules and future vaccines for AIT. Key topics in the second part of the meeting were the effects of the enactment of European Directive 2001/83 on the availability of allergens for therapy and diagnosis across the EU, the challenges of conducting Phase III studies in the field, the future role of allergen exposure chambers in AIT-studies, and specific considerations in performing AIT-studies in the paediatric population.

Finally, the group highlighted the forthcoming EAACI guidelines and their particular importance for the standardisation of practice in the treatment of allergies. This supplement presents a comprehensive insight into those panel discussions and highlights unmet needs and also possible solutions to them for the future.

Keywords: Allergen immunotherapy (AIT); biomarkers; clinical trials; in vivo diagnostics; precision medicine; legislation.

Abbreviations: AIT, allergen immunotherapy; BF, binding factor; BHR, bronchial hyper-responsiveness; Breg, B regulatory cell; CCR, chemokine receptor; CD, cluster of differentiation; DAO, diamine oxidase; DC, dendritic cell; ECP, eosinophil cationic protein; EMA, European Medicines Agency; FAB, facilitated antigen binding; Id, intradermal test; IFN, interferon; Ig, immunoglobulin; IL, interleukin; In, intranasal test; ISAC, Immuno Solid Phase Allergen Chip Assay; MCP, monocyte chemoattractive protein; PCR, polymerase chain reaction; SCIT, subcutaneous inmmunotherapy; sIg, specific Ig; SLIT, sublingual immunotherapy; SPT, skin prick test; TARC, thymus and activation-regulated chemokine; tIg, total Ig; Treg, T regulatory cell.

Introduction

The Future of the Allergists and Specific Immunotherapy (FASIT) workshop was first held in 2006, and has since been repeated at 2–3-year intervals to review developments in the field of allergen immunotherapy (AIT). Attendees are drawn from academia, allergy clinics, regulatory authorities and industry. The most recent workshop took place in Hamburg in February 2017, and this supplement provides a review of the topics discussed during the meeting and includes recommendations for future activities.

Advances in allergen immunotherapy

Since its introduction, just over a century ago, researchers have endeavoured to provide a progressively deeper understanding of the chemical and physiological processes underpinning AIT. AIT is a highly effective treatment for immunoglobulin E- (IgE-) mediated diseases that has been shown to: provide long-term relief of symptoms of allergic rhinitis and asthma; prevent the progression from allergic rhinitis to asthma in children; and have potential for preventing sensitisation to house dust mite allergy in at-risk infants (1–7).

Looking to the future, we can expect this new understanding to present new opportunities to intervene to prevent allergic responses and to personalise treatment for patients. Biomarkers and improved diagnostic techniques will help to identify the likely best responders to a particular intervention, and new therapeutic antigens and formulations will help to optimise the patient's response.

Opportunities for personalised medicine

Personalised medicine encompasses the interlinking concepts of precision medicine, stratified medicine and P4 medicine (Figure 1).

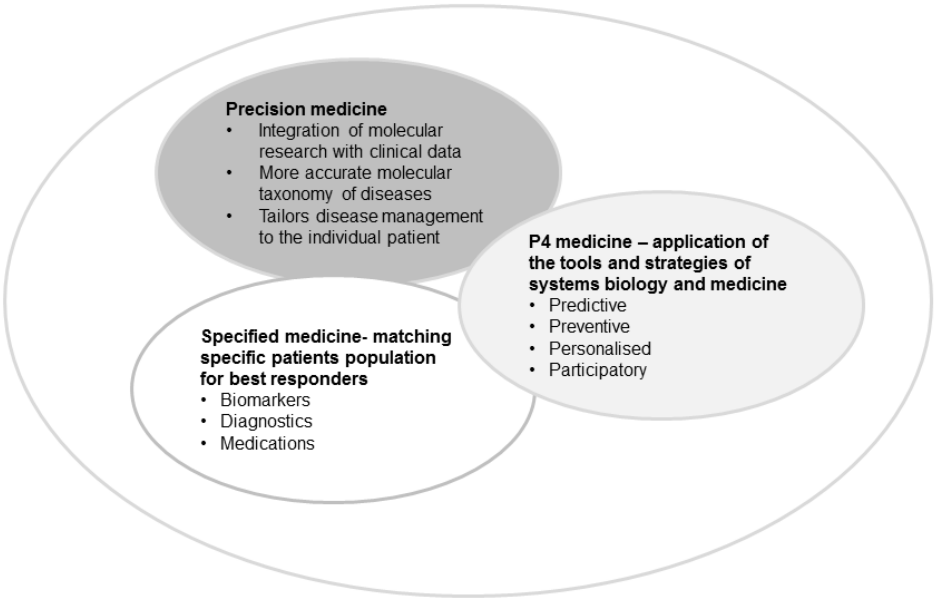

Figure 1. Personalised medicine in practice

The concept of personalised medicine is not new, but ongoing scientific and technological developments in bioinformatics, computational power and medical imaging offer the potential for clinicians to predict which patients will respond to which therapeutics (8–10). While traditionally clinicians have focussed on selecting the right treatment for the patient (stratifying by phenotype), increasingly it will be more important to select the right patient for the treatment (stratifying by endotype or theratype) (see Box 1 for definitions) (8,11,12).

Several inflammatory phenotypes have been identified using biomarkers (11,13,14), but endotype classification remains elusive due to the complexity of these phenotypes; rather than a single endotype associated with a particular disease mechanism, asthma is believed to have a number of subendotypes associated with each mechanism. However, such classification has obvious benefits for optimal treatment design (9,10,13,15).

Box 1. Useful definitions in personalised medicine (10,11)

Definitions

- **Phenotype**: any observable characteristic of a disease without any implication of a mechanism
- **Endotype**: a subtype of a disease condition, which is defined by a distinct pathophysiological mechanism
  - Endotypes should link the key pathogenic mechanisms with clinical phenotype via biomarkers
- **Theratype**: variant classified according to response to a therapeutic
  - A patient with the same phenotype and endotype as another patient, but who does not respond to the same treatment, has a different theratype
- **Biomarker**: measurable indicator used to examine any aspects of health or disease

Biomarkers

Individual steps in the pathway from exposure to allergens to expression of the allergic response are characterised by the production of small molecules and proteins; typically, the most common of these (usually serum samples in allergic diseases) are used as biomarkers (16). This field remains experimental in AIT; no biomarker predictive or indicative of clinical efficacy has so far been identified and validated (16). This paucity of biomarkers to aid the identification of responders to AIT, and for evaluating its efficacy objectively was recognized in a recent international consensus document (17), which classified it as an unmet need.

The European Academy of Allergy and Clinical Immunology (EAACI) recognised this need (18) and established a task force to review all candidate biomarkers used in trials of AIT (16). The aim of the task force was to:

- Collect and review surrogate and predictive immunological and clinical biomarkers, and biomarker data on the effect of AIT on allergenic rhinitis with and without asthma
- Identify surrogate candidate biomarkers that may reflect, or correlate with, the immunological and clinical effects of immunotherapy
- Identify surrogate predictive clinical and immunological candidate biomarkers to monitor the effects of AIT in the target organ and systemically during the early and late allergic responses following immunotherapy
- Identify surrogate cellular, humoral and molecular candidate biomarkers to monitor the effects of AIT during and after discontinuation of treatment
- Confirm (or reject) the candidate biomarkers for monitoring AIT

The resulting report from the extensive literature research and subsequent discussions by the members of the task force consists of recommendations for seven biomarker domains (Table 1) (16). Having reviewed advantages and disadvantages and identified challenges and unmet needs in each domain, the task force recommended that future biomarker studies should have a novel design approach that aims to identify clinical relevance as surrogate or predictive markers of the efficacy of AIT. In particular, it recommended investigating allergen-specific (s)IgG4 as a biomarker for compliance and sIgE : total (t)IgE ratio and IgE-FAB as markers of clinical outcome (16). The design of clinical trials of AIT, in particular the choice of endpoints, should be carefully considered to obtain good quality data on potential biomarkers and to validate biomarkers.

Table 1. Recommendations for classifying biomarkers for allergen immunotherapy (adapted from Shamji et al, 2017 (16)

Domain and examples	Advantages	Disadvantages
1. IgE		
tIgE; sIgE; sIgE : tIgE	Serum based biomarker; current gold standard for selection of patients for AIT; can reflect immunogenicity and allergen exposure; elevated sIgE : tIgE is potential positive predictive marker for AIT	No clinical and functional relevance to rise in sIgE in early phase treatment; sIgE : tIgE has not been validated; equivalence between tIgE units and sIgE units only demonstrated for one singleplex IgE assay platform
2. IgG subclasses		
sIgG1, sIgG4 (including sIgE : sIgG4)	Serum-based biomarker; consistent results in SCIT and SLIT studies; indicative of allergen exposure; can be informative when used in conjunction with functional study; ISAC can be used to determine IgG4 blocking activity; data on local antibody levels are available	No firm relationship between sIgG4 antibody levels and clinical efficacy has been established
3. Serum inhibitory activity for IgE		
IgE-FAB; IgE-BF	Studies demonstrate association between symptom scores, rescue medication scores and IgE-BF; IgE-FAB is a highly-reproducible serum-based assay; some studies have shown association between IgE-FAB and symptom and rescue medication scores;	Equipment for measuring IgE-BF is no longer available; no published data on IgE-FAB in responders vs non-responders; only limited data on correlation of IgE-FAB with clinical response to AIT
4. Basophil activation		
CD63; CD203c; BHR; DAO	Serum-based test; ex vivo basophil activation with the sensitising allergen reflects FcεRI-mediated in vivo response	Limited number of studies, with inhibition of basophil activation demonstrated in only some, not all; this type of assay technically more challenging than others; dose-response curves are required for accurate interpretation of data; 5–10% of population show no basophil response to IgE cross-linking
5. Cytokines and chemokines		

CCR3; ECP; eotaxin; IFN-g; IL-2; IL-2R; IL-4/5/6; IL-8/9/10; IL-13/18; MCP-1; TARC; transthyretin	These assays explore mechanisms of AIT and may help provide proof of concept at early stages of drug development	Poor availability of allergen-specific T cells dilutes the cytokine signal among all cytokines secreted from T cells with other specificities; currently no potentially predictive cytokines or chemokines have been identified; study results are inconsistent
6. Cellular biomarkers		
DCs; Bregs; Tregs	Tregs may play a key role in skewing the Th2 to Th1 immune response; change in allergen-specific B cells towards Bregs may be significant in the course of AIT; serum markers of DC polarisation, which could represent an early indication in the innate immune system of the orientation of adaptive immune responses, have been identified and can be measured by quantitative PCR; DCs may be a more persistent serum marker of transition from Th2 to regulatory immune response during AIT	No specific marker exists for Tregs and there are insufficient data to link appearance or function of Tregs with clinical efficacy, moreover their early appearance means they have limited predictive utility; low frequency of allergen-specific T and B cells makes these assays technically challenging – currently these are impossible for routine clinical practice; characterisation of Bregs is technically challenging and cannot be achieved in routine practice; changes in levels of monocytes and monocyte-derived DCs during AIT, but no information available on impact of AIT on myeloid and plasmacytoid DCs; candidate DC-associated markers of efficacy identified in a single study, but results need to be corroborated; some DC-associated markers also expressed by other leukocyte subsets
7. **In vivo *biomarkers***		
SPT; Id; In; chamber studies	Provocation tests may indicate a change in responsiveness or sensitivity to allergen following AIT and have been used as surrogate markers of clinical response to AIT; chambers permit greater control of temperature, humidity, pollen exposure and improve the accuracy and cost-effectiveness of time-course and dose-response studies; provocation tests are recommended for understanding mechanisms and permit biomarker discovery both at local level and in peripheral blood and are accepted by the EMA as primary endpoints in early trials of AIT	EMA does not accept results of provocation tests in pivotal Phase 3 studies because they may not replicate natural allergen exposures; little standardisation of tests methods; absence of standardised/ harmonised scoring methods; allergens required for tests may not be approved in all countries and so not available for international standardisation studies; chamber studies can be expensive and standardisation and confirmation of reproducibility within/between sites not always established; intradermal tests do not necessarily correlate with improvement of symptoms

AIT, allergen immunotherapy; BF, binding factor; BHR, bronchial hyper-responsiveness; Breg, B regulatory cell; CCR, chemokine receptor; CD, cluster of differentiation; DAO, diamine oxidase; DC, dendritic cell; ECP, eosinophil cationic protein; EMA, European Medicines Agency; FAB, facilitated antigen binding; Id, intradermal test; IFN, interferon; Ig, immunoglobulin; IL, interleukin; In, intranasal test; ISAC, Immuno Solid Phase Allergen Chip Assay; MCP, monocyte chemoattractive protein; PCR, polymerase chain reaction; SCIT, subcutaneous inmmunotherapy; sIg, specific Ig; SLIT, sublingual immunotherapy; SPT, skin prick test; TARC, thymus and activation-regulated chemokine; tIg, total Ig; Treg, T regulatory cell.

A total of 3 recent studies have led to the identification of a set of biomarkers that may prove useful for monitoring treatment efficacy. None of the potential biomarkers has yet been validated, and larger studies are required to achieve this.

A pilot study suggests that measuring levels of sIgE, sIgG4 and functional antibodies in nasal fluid, rather than in peripheral blood, can detect differences in IgE and IgG4, and in a range of components of IgG4, in treated and untreated patients with allergies. Moreover, data from nasal fluid correlate more closely with symptom scores (unpublished data, personal communication M.Shamji). In addition to validation of these findings, more research is required to investigate the role of other immunoglobulins, especially IgA, in target organ samples from patients undergoing AIT.

Stimulation with allergens is known to activate basophils, which can be assessed by measuring surface activation markers, such as CD63, CD203c and CD107a, or by measuring histamine release. Histamine release is most easily measured in assays based on binding histamine to intracellular DAO. An early method achieved this by using an enzyme-affinity-gold method (19), but a recent study evaluated a simpler and more immediately-available method based on detecting fluorochrome-labelled DAO using flow cytometry in patients with allergic rhinitis (20). Following *in vitro* grass pollen allergen stimulation, the proportion of fluorochrome labelled-DAO negative basophils was significantly higher in those who received SCIT and SLIT compared with non-treated atopic controls (all. $P<0.001$). Similarly, significantly lower ($P<0.01$) proportions of CRTh2+ basophils expressed activated CD63, CD203c, and CD107a and significantly fewer ($P<0.001$) patients had symptoms of rhinitis (20). Taken together, these findings indicate that histamine bound to labelled DAO may be useful for monitoring treatment for allergic rhinitis.

Another study published in 2016 investigated gene expression on dendritic cells (DC) driving differentiation of Th2 cells or Tregs in patients receiving SLIT for grass pollen allergy (21). The investigators used quantitative PCR to investigate peripheral blood mononuclear cells derived directly from blood samples and identified three markers that were differentially expressed in DC2 cells (CD141, GATA3 and RIPK4) and two markers differentially expressed in DC10 cells (C1QA and FcgRIIIA). Expression of CD141, GATA3 and RIPK4 was downregulated in responders compared to the non-responders. C1QA and FcgRIIIa were upregulated in active responders and downregulated in the active non-responders. There was also a correlation between these five biomarkers and efficacy, which was seen as early as two months, after initiation of AIT (21).

It should be noted that, often, the choice of objectives and endpoints in the design of AIT studies results in the generation of heterogeneous data, which are not helpful for the identification of potential biomarkers. A better understanding of how such noise is generated, and how it can be eliminated, will help elevate the discovery and validation of biomarkers with clinical relevance in AIT.

Precision medicine

Figure 2 summarises the precision medicine approach to disease management. Starting with the whole patient pool, the disease phenotype is used to select the right patient and the right treatment. If the patients fail to respond to the chosen treatment, the endotype must be assessed. There are several difficulties to be addressed here. First, how is 'response/non-response' determined (immune response or clinical outcome?) and then how should the immunological testing to assess the endotype be performed (provocation tests, allergen exposure chambers (AECs)). Non-responders who have the same phenotype and endotype as responders clearly have a different theratype. This should prompt investigations to establish if the correct endotype and phenotype have been assigned – it is possible that the patient has a previously undefined endotype, because research to identify the mechanisms of allergies and hypersensitivities is not complete.

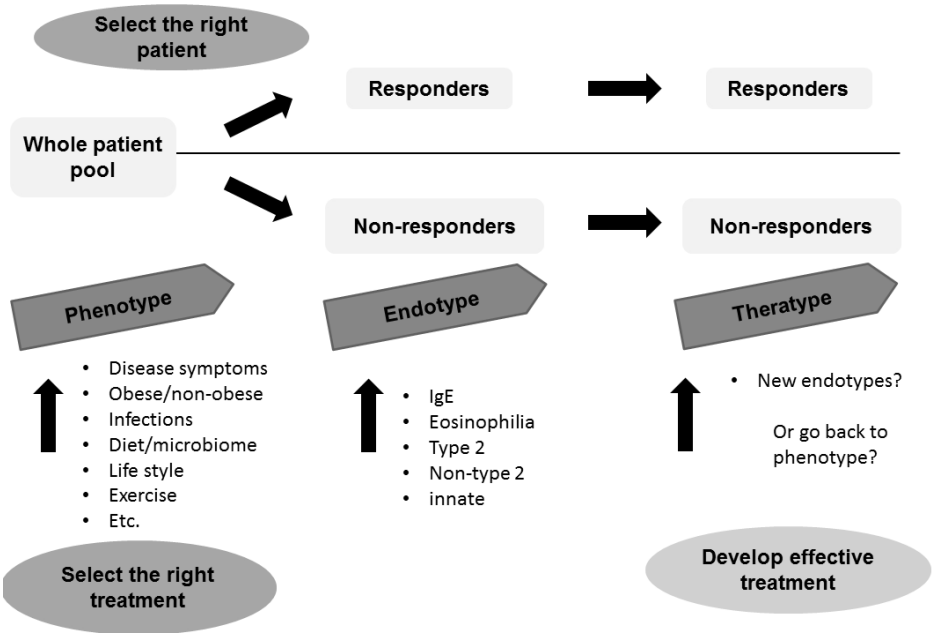

Figure 2. Precision medicine in practice. IgE, immunoglobulin E

This approach is complicated. A consideration of the interplay between anatomical, biochemical, immunological, genetic, metabolic, microbiologic, and psychological factors with the exposome (allergens, irritants, pollutants) that influence the nature of an allergic disease endotype highlights their complexity and explains why allergic asthma, for example, is now considered to have several 'subendotypes' (9,11). The understanding of disease endotypes based on pathophysiological principles, and their validation across clinically meaningful outcomes in allergic diseases, is essential if precision medicine is to be successful – and there is a broad consensus between academics, manufacturers and regulators that the potential of precision medicine to identify treatment needs (including unmet needs), improve clinical trial design (especially in Phase 3), to avoid unsuccessful treatment (and hence cost), and to increase patients' quality of life means that it is essential that this work continues (9,15).

In the last 2 years, several studies and reviews have sought to link allergic disease phenotypes and endotypes with potential targets for treatment in a precision medicine approach (13,14,22–26). In January 2017, the PRACTALL group published a summary on the potential for precision medicine in allergic disease, which concluded that while some progress has been made in defining endotypes for allergic disease and identifying biomarkers to guide a precision medicine approach to treatment, further validation and quantification of these biomarkers are needed to allow their translation into practice in the clinical management of allergic disease (18).

The process of using endotypes and biomarkers to manage patients with allergies may seem abstract to some clinicians; however, it has been shown that factors such as obesity, diet, microbiome, lifestyle and exercise can be used to link phenotypes and endotypes when recruiting patients and stratifying them for treatment. It is known that Th1 cells are upregulated by histamine through the H1 receptor (which is predominantly expressed in Th1 cells), that Th2 cells are suppressed by histamine through H2 receptors (predominantly expressed in Th2 cells) and that regulatory cells are upregulated by histamine through the H2 receptor (27–30). It has recently been shown that levels of histamine-secreting gut bacteria are increased in patients with asthma (31). Non-obese patients with asthma produce more histamine-secreting bacteria than obese patients with asthma, providing a physical marker for stratifying patients at risk for severe asthma (31). Similarly, the composition of the diet – relative proportions of protein, fat and carbohydrate – is known to affect the composition of the gut microbiome (32). A more recent study has shown that the composition of the microbiome (available from samples of faeces) can be used to predict obesity and severe asthma [personal communication M. Jutel]. The investigators used these findings to hypothesise that regional differences in diet could account for differences in predisposition to disease and even responses to AIT. This type of testing is relatively inexpensive and accessible; so if its utility can be validated, there is great promise here for patient stratification.

Therapeutic developments

Vaccines

Where vaccines and AIT have been seen as different areas historically, AIT are now classified as therapeutic vaccines leading to an immune modulation, with the aim of preventing and relieving allergic symptoms (33). Traditionally, AIT products are allergens isolated from biological sources (such as pollen and mites), used unmodified or treated with aldehydes and then formulated (with or without an adjuvant such as aluminium salts) for administration, with little or no ‘design’ of the active ingredients.

Modern methods of vaccine discovery rely on an ‘identify–design–formulate–administer’ model that utilises systems biology methodologies rather than the old ‘isolate–inactivate–inject model’, and AIT can also benefit from this approach. A number of the next generation of AIT possibly rely on recombinant or synthetic proteins or DNA rather than biological allergen sources for their active ingredients and use techniques such as reverse vaccinology (Table 2) to move from active principle to finished vaccine (34,35). Reverse vaccinology is a ‘top down’ approach that begins with identification of a large number of potentially interesting genome sequences, selected in silico using attributes such as transmembrane domains, leader peptides, homology to known surface proteins, lipoprotein signatures, and presence of outer membrane anchoring motifs and host cell binding motifs (such as the arginine-glycine-aspartic acid (RGD) motif) (35). This group of candidates is narrowed down using a series of computational and experimental (mainly based on mass

spectroscopy) methods. The resulting candidates are then tested extensively by *in vitro* assays and selected animal models: In order to progress and qualify for development, the candidates must elicit a specific and correct polarised immune response, be representative of the natural source, conserved and have a good safety profile (35).

Another recent technique, systems vaccinology (Table 2) has the potential to identify molecular immunological signatures and modes of action of successful AIT, which can be used to develop novel and improved therapies; to improve inclusion criteria and predict vaccine efficacy in clinical trials; and to rapidly identify non-responders.

Integrated reverse vaccinology combines all the techniques listed in the table to look at whole systems (e.g. B cells and epigenetic modulation), to identify molecular and immunological signatures of protection enabling identification or T and B cell epitopes that form the basis of the vaccine (36).

Table 2. Methods in modern vaccinology. Modified from Poland et al., 2013 (37) **with permission from Elsevier.**

Theory/model	Description	Tools
Reverse vaccinology	Combination of genomic data and informatics tools to **identify functional antigens**	Genomics, transcriptomics, proteomics, epitope prediction and immune monitoring
Immune network theory	**Prediction of immunity** based on action or interaction of genes and pathways	Transcriptomics, proteomics and pathway analytics
Vaccinomics	Studies of vaccine immune responses in order to develop **rationally designed vaccines**	Transcriptomics, proteomics, epigenomics, immunogenetics, computer modelling and immune monitoring
Systems vaccinology	Systems biology approach to understand and **predict immune responses** to vaccines	Transcriptomics, proteomics, epigenomics and computer modelling
Structural vaccinology	Structural biology approach to **identify optimal epitopes** for functional antibodies	Proteomics, X-ray and nuclear magnetic resonance data, and immune monitoring
Vaccine informatics	Use of bioinformatics to **facilitate vaccine development**	Computer modelling, epitope and human leukocyte antigen binding prediction, data mining, immune response models.
Integrated reverse vaccinology	A combination of informatics and combined systems biology data linking **signatures of protection** with antigens	All of the above

Use of systems biology approaches may potentially lead to individualised vaccines taking into account the innate and environmental factors – such as gender, age (due to changes in the immune system with age), size (determining the size of the dose given), season (types of allergen co-present during therapy) and location (can affect size and general health of population) – that can influence

treatment success (34,35,38,39). Currently, the integrated vaccinology approach is at an early stage, with vast amounts of data available, but little correlation with real-life situations.

The development of enhanced antigens, as well as vastly expanding knowledge of the interactions between the innate and the adaptive immune system, has stimulated a search for new adjuvant and delivery systems. The new possibilities include manipulation of the relationship between antigen uptake and activation of immunity, enhancing the safety and efficacy of the vaccine and improving compliance by reducing the numbers of vaccinations that patients require (40–42). The development of new adjuvants will be discussed in the next section.

Adjuvants and delivery systems

Adjuvants act as enhancers or potentiators of AIT, mostly by activating CD40, CD80 and CD86 – although there are many other proteins that could be targeted (43,44). Current adjuvants include aluminium salts (alum), ligands for toll-like receptors (TLRs), derivatives of chitin and complement fractions (Figure 3) (45). Different adjuvants stimulate different immune response pathways: stimulation of Tregs, Bregs and IgG4 are associated with tolerance, while stimulation of Th17 and IgG upregulates lytic immunity. An effective adjuvant provides a balance between these two effects.

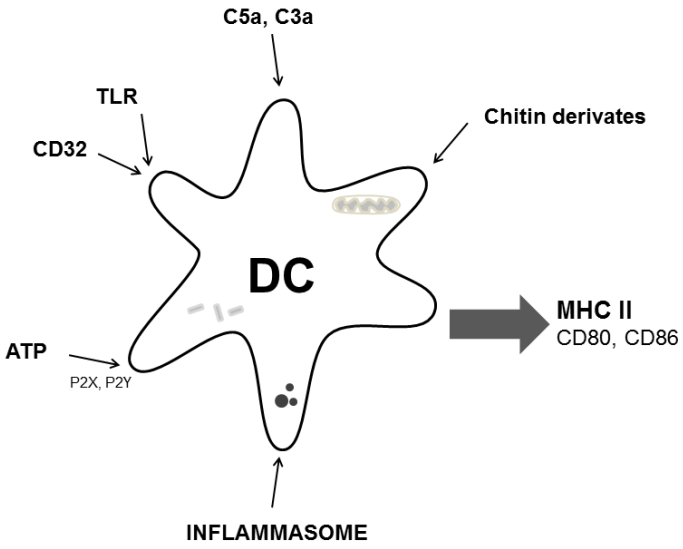

Figure 3. Current adjuvants. TLR, toll like receptor; DC, dendritic cell; CD, cluster of differentiation; MHC, major histocompatibility complex.

Aluminium salts are widely-used but imperfect adjuvants. The ways in which chitin and IFNɣ exert their adjuvant effects are fairly well understood, but this is not true for all adjuvants.

Hydroxyapatite has also been investigated as a novel adjuvant and provided greater increases in IgE when bound with ovalbumin compared with soluble ovalbumin (46), but other exciting prospects come from work to develop advanced antibody vaccines for infectious diseases and cancer, which

has stimulated the development of new adjuvants or delivery systems. Some of these may be relevant for AIT. For example, a number of teams have investigated nanoparticle delivery systems based on liposomes or polyethylene glycol (PEG) hydrogels that incorporate adjuvants with the drug (47,48).

There are some issues that need to be taken into account when attempting to transfer this technology to AIT. Vaccination-driven antibody responses include TLR-5 dependent responses (interestingly, TLR5-mediated sensing of gut microbiota plays an essential role in the efficacy of vaccines) (49). Similarly, these nanoparticles have been shown to enhance IL10, IgG1 and CD8 responses, and increase Th2 immunity, whereas allergists would be looking for enhanced IgG4 and reduced Th2 immunity. Although liposomes and hydrogel formulation of drugs for inflammatory skin disease have been approved for use, to date, uses in AIT have only been investigated in mice (46,48,50). This means there are no safety data for this nanoparticle plus allergen combination. From a safety perspective, it will be necessary to choose the right adjuvant (or nanoparticle) for the right allergen; for example, allergens with anaphylactic potential will initially be the secondary choice.

To really capitalise on the potential of these new adjuvant approaches, a rational approach is required to design a system that will deliver the required IgG4 enhancement and reduced Th2 immunity. Systems biology approaches will play a key role in streamlining this process, and will help to hone the list of possible adjuvants down to those that will be of most use to allergists.

New molecules for AIT

Advances in new molecules for AIT are following trends seen in many other areas of medicine. A number of companies are pursuing hypoallergenic recombinant allergens. Early examples of these include the Bet v 1 and the Bet v 1 folding variant (51), which were investigated against birch pollen allergy (52,53).

More recently, AllerT has provided good preclinical evidence of activity against birch pollen allergy. AllerT is synthesised from three contiguous overlapping peptides derived from Bet v 1 – the major allergen of birch pollen – which have been combined in a way that reduces IgE binding (54). This is under development by Anergis S. A. This molecule demonstrated a five-log reduction in the ability of AllerT to compete for Bet v 1 specific IgE in *in vitro* studies, and between a 10-fold and 100-fold reduction in skin test sensitivity in a Phase 1 skin prick test feasibility study comparing AllerT with birch pollen extract and also recombinant Bet v 1. Recently, the results of a Phase 2 field trial have been published that compared AllerT 50µg against AllerT 100µg and placebo (55). Treatment led to a robust increase in allergen-specific IgG4 and no change in allergen-specific IgE in the treatment groups. Interestingly, the lower dose seems more efficacious – it may be that there is a fairly thin line between tolerance and activation, and if the dose is just too high, collateral inflammation can be induced that neutralises some of the tolerogenic effects of the therapy. This effect can also be seen as an increased incidence of rhinitis and also other respiratory symptoms. A few mild and reversible delayed systemic reactions-occurred but the treatment was generally well tolerated. Some patients experienced administration site adverse events. By contrast, Anergis have recently announced top line results of their latest Phase 2b field-based clinical trial, investigating AllerT for patients with birch pollen allergy (56). The study did achieve its primary endpoint, however with only marginal efficacy. The future of the approach remains uncertain.

BM32 is a hypoallergenic, recombinant B cell epitope-based grass pollen allergy vaccine under development by Biomay (57,58). It contains recombinant fusion proteins consisting of allergen-derived Phl p 1, Phl p 2, Phl p 5, and Phl p 6 – the major allergens of Timothy grass pollen – peptides and the hepatitis B surface protein domain/ preS, as a hapten-type l carrier. In a dose-finding study with 10, 20 and 40µg of vaccine, there were reductions in total nasal symptom scores (TNSS; $P<0.03$) and total ocular symptom scores (TOSS) from baseline to 360 minutes in all groups during a challenge in an AEC. In the 20 and 40µg groups for TNSS, a significant difference was identified from baseline. For TOSS, the greatest reduction was in the 20µg group and for TRSS it was in the 40µg group (58). Some patients experienced allergic reactions – rhinitis (seven patients), conjunctivitis (one), palm pruritus/erythema (one), urticaria (three). This molecule is now in Phase 3 clinical development.

Circassia's CAT-SPIRE (synthetic peptide immuno-regulatory epitopes) for cat allergy has been evaluated in a long-term, Phase 3, double-blind placebo-controlled field study (CATALYST) (59). The study followed a successful Phase 2, AEC-based study (60). Patients were randomised to receive either four or eight doses of CAT-SPIRE 6nmol every 4 weeks or placebo. At the 1-year follow-up, approximately 60% of patients in each group achieved a response. The company has not provided any analysis of these results but possible reasons include the tendency for owners to tolerise to their cats, or that cat owners with persistent allergy are a treatment resistant subset. If either or both of these was responsible for the high placebo response, they would not be trivial matters to address. The first would require IgG4 levels to be measured throughout any future study, and the second would require careful patient selection to ensure that patients remain symptomatic throughout the course of the study.

The small molecule JAK kinase inhibitor, tofacitinib, has been developed for treating psoriasis and rheumatoid arthritis. Normal administration would not be advisable for allergy, because the drug would be too toxic. However, Schmidt-Weber and colleagues have demonstrated that intraperitoneal administration of tofacitinib permits ovalbumin-specific immunosuppression in mouse models (61). Further work is required to demonstrate efficacy and safety in humans.

A consistent problem with moving new AIT products through to registration is the difficulty in replicating the results of Phase 2 studies in Phase 3. A number of differences in study design and patient selection for Phase 2 and Phase 3 studies might contribute to this problem (Table 3), but so far none of these hypotheses have been tested.

Table 3. Possible reasons for failure of phase 3 trials after successful phase 2 trials

Category	Possible reasons
Endpoints and outcome measures	• Soft, subjective, or pseudo-objective outcome measures compared to e.g. type 1 diabetes mellitus • Use of different endpoints in Phase 2 and Phase 3
Patient selection	• Not based on predictive biomarkers • Change from highly selected patients in Phase 2 to more 'realistic' patient populations in Phase 3 • Inclusion of patients based on symptom score diary, with patients overrating symptoms at trial entry on purpose to meet inclusion criteria
Study type	• Change from single center studies (Phase 2) to multicenter studies with different confounding factors (e.g. different populations with different microbiomes, different environmental exposure) • Regulatory requirement for field studies in Phase 3, with exposure to natural variability in the environment (e.g. unpredictable pollen seasons) • Often no run-in phase to ensure proper randomization of patients and calculation of placebo effect
Choice of dose	• Potential for incorrect conclusions regarding optimal dose to be drawn from Phase 2 studies
High placebo effect	• Possibility that patients involved in successful Phase 2 studies are super-motivated patients • Potential for inflated baseline symptoms that regress to the mean due to overly strict inclusion criteria • Potential for cat owners to develop natural tolerance to their cat's fur or for cat owners with persistent allergy to become a treatment-resistant subset (59)

Summary

New developments in technology offer many opportunities to improve diagnosis and treatment for patients with allergies. It is important to define how these technologies – and especially their products – can best be used to deliver clinically meaningful improvements in therapy for patients.

Hot topics in the regulation of allergen immunotherapy

Meeting the needs of polysensitised patients

The enactment of European Directive 2001/83 EC has changed – and will continue to change – the environment for allergy treatment by defining products for AIT as medicines, which therefore means that they require individual marketing authorisations (MAs), which are granted on the basis of successful clinical studies with single agents for individual allergies. Notwithstanding this, the Directive does allow for allergens (singly and in mixtures) to continue to be prescribed on a named patient basis – that is, without the need for a MA – at the prescriber's discretion. This is an important concession for patients with rare or infrequent allergies, and for patients who have, or appear to have, more than one allergy (62).

In 2016, a survey was conducted among >1000 physicians prescribing AIT in 15 countries (not including the USA) to assess how physicians used AIT and the effect of changing regulations on their practice. This is the largest survey of its type ever conducted. The survey found that (63,64):

- The majority of physicians supported adherence to guidelines that aimed to standardise AIT and wanted high quality, documented allergen products.
- The majority of patients presenting for AIT were polysensitised/polyallergic, but the majority of prescriptions were for single allergies. Respondents preferred to use only single, characterised allergens; they were less enthusiastic for mixtures.
- Most polyallergic patients are treated one allergy at a time, receive more than one single allergen, or in a minority of cases receive a named patient product (NPP) consisting of a mixture of allergens.

The survey concluded that most physicians believed that the majority of their polysensitised patients' needs could be met without allergen mixtures, either by using sequential single allergen immunotherapies or parallel single immunotherapies, but that, on average, 14% of all patients might still require tailored allergen mixtures (63,64).

The British Society for Allergy and Clinical Immunology guidelines note that 'single allergen immunotherapy may be effective against the relevant allergen (source) in polysensitised patients and that a single allergen used for immunotherapy as part of a multi-allergen mixture may retain efficacy against the relevant allergen' (65). Despite this recommendation and the fact that many of the physicians questioned in the survey (63,64) take this approach, there are some questions that should be addressed concerning the use of multiple sequential or parallel injections/ingestions of single allergens. In particular, are there any safety issues to be considered? A particular concern might be the overall dose of aluminium that patients were receiving in multiple injections of allergens. Should there be a limit to the number of single agents a patient should receive in this way?

The British guidelines do not recommend the use of mixtures of allergens to treat multiple allergies due to lack of evidence (65). The EU's Committee for Medicinal Products for Human Use (CHMP) provides little guidance on conducting clinical studies with mixtures of allergens, recommending that manufacturers request scientific advice if mixtures of allergen extracts or allergens are used (66). Currently, it is hard to see how a trial with allergen mixtures could be designed to pass the scrutiny of the EMA; however, it is time for this challenge to be addressed, to ensure that polysensitised patients have the same access to documented allergens that patients with single allergies enjoy. To prevaricate any longer on this issue, to go on using mixtures that have not been demonstrated to be safe and effective, is to call the integrity of allergology into question.

Use of allergen exposure chambers in Phase 3 studies

Although allergen exposure chambers (AECs) may be used to generate data in Phase 2 studies, and have delivered reliable dose-finding and onset of action studies, (53,60,67–70) the 'Guideline on the Clinical Development of Products for Specific Immunotherapy for The Treatment of Allergic Diseases (CHMP/EWP/18504/2006)' of the European Medicines Agency (EMA) requires Phase 3 studies to be conducted in real-life situations (field trials) under natural allergen exposure, rather than in an AEC facilities (66). However, several disadvantages are associated with this methodological approach.

First, there remains a lack of internationally recognised and standardised harmonisation on the definition of pollen seasons based on pollen count data. Therefore, an EAACI task force initiative has been initiated and has published an EAACI position paper with consensus based definitions on 'pollen season', 'high pollen season' (or 'peak pollen period') and 'high pollen days' for different pollen species (71), (Table 4).

Table 4. Overview on proposed definitions of periods of pollen exposure times for analysis of outcomes in allergen immunotherapy in allergic rhinoconjunctivitis due to pollen. Daily mean pollen concentration (pollen/m^3) is used for these definitions. Reproduced with permission from John Wiley & Sons Ltd from Pfaar et al, 2017 (71)

	Pollen season	**High pollen season (or 'Peak pollen period')†**	**High pollen days**
Birch (*Betula* sp.)	Start of season: 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥10 pollen/m^3 and with a sum of these 5 days of ≥100 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥10 pollen/m^3 and with a sum of these 5 days of ≥100 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥100 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥100 pollen/m^3	The day(s) with at least 100 pollen/m^3
Grasses (Poaceae)	Start of season: 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥50 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥50 pollen/m^3	The day(s) with at least 50 pollen/m^3
Cypress (*Cupressus* sp.‡)	Start of season is defined as 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥20 pollen/m^3 and with a sum of these 5 days of ≥200 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥20 pollen/m3 and with a sum of these 5 days of ≥200 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥100 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥100 pollen/m^3	The day(s) with at least 100 pollen/m^3
Olive (*Olea* sp.)	Start of season is defined as 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥20 pollen/m^3 and with a sum of these 5	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥100	The day(s) with at least 100 pollen/m^3

	days of ≥200 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥20 pollen/m^3 and with a sum of these 5 days of ≥200 pollen/m^3	pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥100 pollen/m^3	
Ragweed (*Ambrosia* sp.)	Start of season is defined as 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥50 pollen pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥50 pollen pollen/m^3	The day(s) with at least 50 pollen/m^3

†Multiple peak pollen periods may occur during one pollen season. ‡The definition for this pollen is only valid for Mediterranean areas where *Cupressus* species dominate the Cupressaceae concentrations.

In addition a high variation in pollen count data has been found between different regions and across the different years analysed, therefore a large degree of patient's individual pollen exposure during the pollen season can be assumed (67,72). In addition a high (yearly) variation of allergenicity of pollen grains has been demonstrated in preliminary analyses (73). These factors may confound the proof of efficacy of AIT intervention in phase III trials (67,74–76). The problem is not restricted to pollen, regional differences in the prevalence of (and hence exposure to) other aero allergens such as mite species (77) also impacts the outcomes of trials.

AECs may bypass some of these methodological restrictions (Box 2) (67). An increasing number of facilities (including mobile chambers) of various sizes exist across the world, and have been used in multiple Phase 2 trials (Table 5).

Box 2. Advantages of AECs (adapted from Devillier et al., 2011 (67)**)**

Advantages of allergen exposure chambers

- Exposure to a defined, controlled allergenic environment (particles/m3, temperature, air-pressure and humidity)
- Absence of confounding allergens (especially in polysensitised patients)
- No bias of rescue-medication intake
- No impact of patient's individual activities (outdoor vs indoor activities) or poor compliance
- Good supervision of patients
- More efficient collection of biological samples from participants
- Dose ranging and onset of action can be analysed
- Requires fewer subjects (positive impact on costs, ethical benefit)

Table 5. Examples of AEC-based Phase 2 studies in AIT currently listed on clinicaltrials.gov and clinicaltrialsregister.eu (as per 25th February 2017*)

Allergen	Route	Sponsor	AEC	Registry Number
Betula verrucosa	SLIT (tablet)	ALK-Abelló A/S	Inflamax Research Inc.	NCT02481856
Birch	SCIT	Anergis	Inflamax Research Inc.	NCT02271009
Cat	SLIT (liquids)	ALK-Abelló A/S	Cetero Research	NCT00987909
Grasses	SCIT	Allergy Therapeutics	Inflamax Research Inc.	NCT02582073
Grasses	SCIT	Biomay AG	Vienna Challenge Chamber	NCT02643641
Grasses	Intradermal	Circassia Limited	Kingston General Hospital	NCT01385800
Grasses	Intradermal	Circassia Limited	Kingston General Hospital	NCT01923779
Grasses	SCIT	LETI Pharma GmbH	Fraunhofer, ITEM	EudraCT 2007-004255-10
Grasses	SCIT	LETI Pharma GmbH	Fraunhofer, ITEM	EudraCT 2006-005269-20
Grasses	SCIT	Allergopharma GmbH & Co. KG	Inflamax Research Inc.	NCT02297490
Grasses	Intradermal	Circassia Limited	Kingston General Hospital	NCT02292875

Mite	Intradermal	Circassia Limited	Cetero Research	NCT01447784
Mites	Intradermal	Circassia Limited	Inflamax Research Inc.	NCT01923792
Phleum Pratense	SCIT	Allergopharma GmbH & Co. KG	Fraunhofer, ITEM	EudraCT 2011-000674-58
Phleum Pratense	SCIT	LETI Pharma GmbH	Fraunhofer, ITEM	EudraCT 2014-004732-19
Phleum Pratense	SLIT (liquids)	HAL Allergy	Inflamax Research Inc.	NCT02556801
Phleum Pratense	SCIT	ALK-Abelló A/S	Fraunhofer, ITEM	Eudra CT 2013-005130-38
Ragweed	SCIT	Allergy Therapeutics	Allied Research International Inc.	NCT00110786
Ragweed	SCIT	Allergy Therapeutics	Allied Research International Inc.	NCT00325338
Ragweed	Intradermal	Circassia Limited	Cetero Research	NCT01198613
Ragweed	Intradermal	Circassia Limited	Cetero Research	NCT01448603
Ragweed	Intradermal	Circassia Limited	Inflamax Research Inc.	NCT02061709

AEC, allergen exposure chamber; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.
*Without claiming completeness.

In its guideline, the EMA proposes specific requirements for pivotal Phase 3 studies in AIT in which the primary objective includes measurement of combined symptom and medication scores as e.g., standardized by the EAACI (78), under natural allergen exposure (66). Moreover, it is clearly stated that Phase 3 trials in AIT challenge tests (such as allergen challenges in AEC facilities) or paraclinical outcomes 'can give additional information but are no surrogate markers and cannot replace the measurement of clinical symptoms' (66). However, it is acknowledged that an AEC can be seen as a 'promising tool for the evaluation of efficacy', although the guideline also emphasises the clinical unmet need of further validation of these facilities in natural conditions during e.g., pollen season. This has been mirrored in a position paper of the EAACI on outcome measures in AIT trials (78).

In their subsequent review on the technical and clinical validation of international AEC facilities, Rösner-Friese et al. summarised the current status of technical validation data and found a high body of evidence regarding the different chamber models (72). However, the authors also emphasised the need for further clinical validation of AECs and grouped these clinical questions into the four domains 'sensitivity/specificity of Allergic Rhinitis (AR) symptoms', 'reproducibility of AR symptoms and AIT treatment outcomes', 'correlation of AECs versus natural exposure regarding AR symptoms and AIT treatment outcomes' and 'seasonal priming effects on AR symptoms and AIT treatment outcomes'. On the basis of this work, an interdisciplinary EAACI task force initiative comprising clinicians, regulators and AEC vendors has elaborated an EAACI Position Paper outlining i)

minimal standards regarding technical requisites (including standard operating procedures for operating and cleaning the chamber, use of GMP-grade materials and known quantities of allergens); ii) recommendations for model validation for conducting validation trials (including the use of standardised and clinically-relevant outcome measures and defined safety measures); and iii) prerequisites for clinical validation of AIT studies (primarily reproducibility) (76). The task force acknowledged that several of these minimal requirements have already been met (76,79–83) but still important unmet needs for the future remain.

One important task will be the proof of inter-chamber comparability regarding results on AIT efficacy. Another important task is to investigate the correlation of AIT treatment effect sizes as demonstrated in AEC with those as found in a field trial under natural exposure in a 'hybrid' study design (76).

Requirements for studies in children

The right for children to receive evidence-based medicine was enshrined in EU Directive 1901/2006 EC (84), which mandated that any MA application for a new medicinal product should include either the results of studies conducted in accordance with an agreed paediatric investigation plan (PIP) or an EMA decision on a waiver or deferral. Directive 2001/83 EC (62) also contains this provision.

The EMA has stated that it expects AIT to act in the same way in both adults and children, but it is possible that the magnitude of the effect and the safety profile could differ (66,85). Thus, the EMA emphasised that dose finding efforts for safety and efficacy performed in adults, do not have to be duplicated with children or adolescents. It also believes that children with allergic rhinoconjunctivitis may ultimately derive greater benefit from AIT than adults because it can prevent progression to asthma (7,86,87). For these reasons and due to specific demands of the EMA Pediatric Committee (PDCO), the agency needs to see evidence from at least one long-term (3 years double blind, placebo controlled and 2 years blinded follow-up) study to demonstrate long-term effects (i.e. in allergic rhinoconjunctivitis) or prevention of allergy (and development of asthma) (66,85). The types of studies which are recommended are randomised, placebo-controlled double-blind parallel group trials to evaluate efficacy, safety and tolerability. Children aged from 5 to <12 years should be assessed separately from those aged 12 to <18 years.

Two interconnected problems arise from these requirements. Five years is a long time in the life of a child, and half of the children participating in a study are randomised to the placebo arm. Physicians who have been involved in paediatric studies have found it difficult to recruit and retain patients once this is explained to parents. There are also ethical issues associated with continuing to give children placebo (particularly with the possibility that they could develop asthma in the long term) when it is clear that treatment is effective, as was the case in a recent study (7,86,87).

The difficulties in including patients will be possibly compounded by the numbers of children required to be recruited into trials – although the EMA has not provided guidance on this. To meet the requirements of recent and current MA applications, more than 1000 children will need to be screened for each individual study (7).

The feasibility of randomised controlled trials in the context of the Paediatric Investigator Plan (85) to provide evidence on which to base treatment decisions has been questioned (88,89). It has been proposed that observational studies and clinical registers could provide additional evidence. This is

highly pertinent to AIT in children and should prompt further discussions between physicians, manufacturers and regulators about how data requirements can be met. The EMA allows the extrapolation of dose finding data from adult trials to children (85). For an indication in children the Paul-Ehrlich-Institut (PEI) (Langen, Germany) accepts short term studies in children. These studies may start as soon as efficacy and safety in a short term Phase III study in adults has been shown. These studies can be performed in parallel with studies or deferrals according to an approved PIP. If efficacy and safety is shown in children in the first year, the PEI can grant the indication "treatment of allergic symptoms" also for children, provided there is still compliance with the PIP (personal communication S.Vieths).

The current environment for PIPs in AIT is very unsatisfactory, with a number of important questions – not least concerning the ethics of current requirements – necessary to be resolved. It must be in the interests of all stakeholders – especially children and their parents – for ongoing discussions to arrive at concrete solutions.

Summary

In this era of evidence-based medicine, a clear demand of both regulatory bodies and physicians requires that products for AIT have to thoroughly meet modern methodological standards for proving clinical efficacy and safety. However, AIT differs from pharmacotherapy. The introduction of Directives designed to bring AIT into line with the requirements for small molecule drugs has been met with a number of difficulties. Ongoing discussions between all stakeholders about the minimal requested level of evidence needed that is necessary to ensure that patients – especially children – continue to have access to AIT are essential.

The future of diagnostic allergens for *in vivo* testing

The enactment of EU Directive 2001/83 EC (62) classified allergens as medicines, differentiating between their use as test allergens and therapeutic allergens (90,91). It has also created a dichotomy where foodstuffs, for example, are considered safe under the relevant laws for consumption, but not for use in diagnostic tests. In fact, even a licensed drug would require extended registration for use as a diagnostic.

In spite of the EU directive, the current regulatory situation for in vivo diagnostics for allergy testing is still highly heterogenous. In many EU countries, diagnostic allergens are not licensed, but available on the national markets, and in some countries certain tests (e.g. nasal provocations tests [NPTs]) are regulated as devices (92). Without the provision of exemptions when the Directive is transposed into national law (as in Germany, for example), manufacturers need to undergo the process of obtaining marketing authorisations for their products in countries where they don't have authorisations. This requires clinical trials demonstrating the specificity and sensitivity of the test substances and individual marketing applications in each country where the product is to be marketed, as there is currently no centralised process for licensing allergy diagnostics (93). The first step for all new drugs is to licence it either centrally through the EMA or by mutual recognition inside the EU. This is possible for diagnostic allergens as well. The second step, as is the case for all drugs, would be the local market access in the individual countries and registration. International procedures such as the Mutual Recognition Procedure (MRP) of the Decentralised Procedure (DCP) could be applied to test allergens in the same way as for therapy allergens. Two test allergens have

in fact been authorised via an MRP. Test allergens containing biotechnological products such as recombinant allergens would be authorised via the centralised procedure. In addition, manufacturers will also need to provide reports of process variations, results of stability testing, periodic safety update reports (PSURs) and data on safety and efficacy during routine use (94,95). As diagnostic allergens are the cornerstone of allergology, this is a matter of great concern to manufacturers, clinicians and patients.

The situation presents a deep dilemma for allergists. Of course, they want the reassurance that the extracts they are using for testing are of good quality, safe and effective: however, simple economics dictates that rare allergens and forms of test other than the widely-used skin prick tests are less likely to be supported by manufacturers because the cost of registration (see Table 6) will exceed the value of sales (or, alternatively, the price increase needed to cover the costs will make the test non-viable for payers) (94,96). It is expected that many currently used tests will not be available in the future. In France, Germany and the Netherlands, already there have been sharp reductions in the numbers of available diagnostic tests (Figure 4). In the Netherlands, clinicians now only have six authorised prick test diagnostic allergens (grasses, grass mixture, tree mixture, cat, *Alternaria*, mites and histamine). They cannot officially test in vivo for reactions to any other allergens.

Table 6. Estimated costs* for registering a skin prick test in the EU**

Item	**Cost (€)**
Phase 1/2 clinical trials (20 patients)	20,000
Phase 3 clinical trials (100 patients)	150,000
Paediatric investigation plan (PIP; 50 patients)	100,000
Quality development (to demonstrate compliance with GMP)	80,000
Total cost of submission package	**300,000**
Additional costs	
Mean cost of registration per EU country	35,000

* Estimates come from internal discussions. **In addition, manufacturers will need to bear the costs of pharmacovigilance (periodic safety update reports) every 6 months during the first 2 years after marketing authorisations; every 12 months in years 3 and 4; every 3 years thereafter) –preparation and fees for submission. EU, European Union.

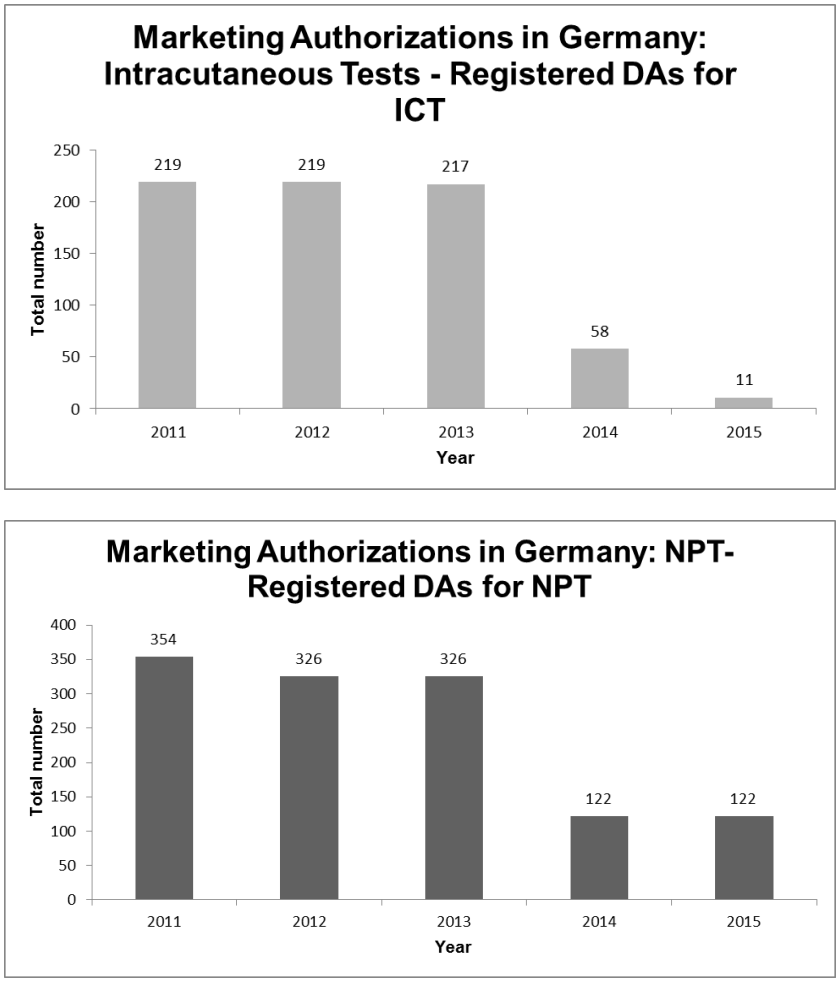

Figure 4. Fall in marketing authorisations of in vivo diagnostic tests for allergy in Germany since transposition of Directive 2001/83 EC (62) **into national law** (90,97)**. DAs, diagnostic allergens; ICT, intracutaneous tests; NPT, nasal provocation test.**

As all diagnostic tests are manufactured involving an industrial process, there is no option for them to be the subject of NPPs. In some cases, it will be possible to replace these in vivo diagnostics with laboratory tests (e.g. enzyme-allergosorbent tests (EASTs), serum specific IgE (ImmunoCAP or other) component-resolved diagnostics, basophil activation tests); these have proved very useful for food allergies (98). They are, however, expensive to conduct and are not appropriate for all situations.

EAACI has established a task force to coordinate its response to the introduction of the Directive (62). The first action of the task force was to evaluate current practice in allergy diagnosis in Europe and how this might be affected by transposition of the Directive in countries where this has not already occurred. This was made possible by surveying all affiliated national societies inside and outside the EU. In total, 31 countries responded (99). The national societies were questioned about the types of tests used by their members and their awareness of national legislation pertaining to in

vivo diagnostic tests and changes that might occur as a result of the transposition of Directive 2001/83 EC (62). Worryingly, the survey revealed that some national associations knew little or nothing about these changes.

The EAACI survey concentrated on IgE-dependent allergies and so did not include patch testing for contact allergies. As patch testing is the only option for contact allergy, this is a particular concern in the light of Directive 2001/83 EC (62). It has been suggested that these tests could be covered by the EU's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) programme, under which each new chemical introduced into the EU must be assessed for safety (which includes patch testing [http://ec.europa.eu/growth/sectors/chemicals/reach]).

EAACI has drafted a position statement that, in addition to items proposed for discussion by the EMA, will include proposals to reduce (or even waive) registration and testing fees for allergen-based in vivo tests; acceptance of data from small study populations for applications for rare allergens; consideration of the principle of granting registration for homologous groups of tests (a principle applied to therapeutic allergens), and of the use of therapeutic allergens as companion diagnostics (94,100).

This advocacy has found open ears at the EMA, which has recognised that there is a problem for manufacturers, clinicians and patients who need access to the >250 drug substances and >3000 allergen products that are affected by the Directive. It has established a working group within the CHMP to identify the issues facing interested parties with a view to revising the current guidelines, and to propose legislative actions, if needed. Issues under discussion include: the introduction of specific facilitated pathways for obtaining MAs for allergens, and distinguishing between their use in AIT and as diagnostics; defining rare allergens, reviewing pharmacovigilance requirements; and harmonizing registration in all Member States.

Further definition is required on the nature of the data presented to regulatory authorities. There is no absolute definition of sensitivity and specificity for these tests, and this is made more complicated by the fact that there is no guidance on whether the test should diagnose sensitisation to the allergen or clinically-relevant sensitisation (i.e. allergic disease). Usually, diagnostic tests only reveal sensitisation to the allergen, doctors must use their skill and experience to discriminate between sensitisation and allergic disease.

EAACI also has successfully drawn allergen manufacturers into the conversation. It has proposed that manufacturers consider 'exchanging' some allergens – particularly rare allergens – in some markets, so that all manufacturers are not faced with supporting all allergens in all markets. Another conversation that needs to take place concerns acceptance by health authorities, insurers and patients that costs for these tests will rise.

Summary

There is general agreement that skin tests and provocation tests are the cornerstones of the diagnosis of allergic diseases, and some would go as far as to say that without them the allergy community risks losing its credibility – even its very existence. The issue is to overcome the challenges posed by Directive 2001/83 EC, either by amending current legislation or, if necessary, introducing new legislation – and this is in hand. It is essential that EAACI, manufacturers, the EMA

and national regulators continue to work together to resolve the issues raised by Directive 2001/83 EC in order to ensure the continued availability of in vivo allergen diagnostic tests in the EU.

EAACI guidelines

In many countries, awareness among health professionals – and the general population – of the potential of AIT to affect a cure for allergies is very poor. This can result in inequalities in access to treatment and/or reimbursement. To address this issue, and to foster awareness and better national policies, EAACI started to draft its 'Guidelines for Clinical Practice for AIT' in 2015 (101). The guidelines should assist clinical decision-making, educate individuals or groups in how to assess and ensure the quality of care, and, ultimately, to influence the allocation of healthcare resources for allergy patients.

The involvement of all stakeholders, as well as independence, transparency and disclosure of methods, disclosure of financial and non-financial conflicts of interest, and an external peer-review process are essential to producing high quality guidelines. With this in mind, EAACI included clinicians, immunologists, GPs, patient organizations, allied health representatives, and members of regulatory bodies in the seven multidisciplinary working teams established to draft the guidelines (101). These groups worked within a well-established pre-defined process to identify, critique and synthesize the evidence from a series of systematic literature reviews on the use of allergen immunotherapy for the prevention and clinical management of allergic rhinoconjunctivitis, asthma, allergies to insect venom and foods, as well as primary and secondary prevention of allergic diseases. Key initial steps in the process were the identification of a core group responsible for the creation, update, implementation and dissemination of the guidelines, and the appointment of a team responsible for methodology.

The project group has published systematic reviews of the current available evidence, and two papers highlighting the role of regulatory authorities and of primary care clinicians caring for allergy patients, respectively (102–110). The guidelines will highlight the benefits of AIT at the public and policy makers level, too, illustrating potential savings in direct and indirect costs of treating allergic disease. Dissemination and implementation of the guidelines is essential to achieving EAACI's ultimate ambition of implementing excellence in the care of allergic diseases (101). These guidelines have the potential to reduce variations in clinical practice and, most importantly, form a basis for continuous collaborations between centres of excellence and primary and secondary care. Such vertical and horizontal networks will be instrumental not only in identifying gaps and barriers that can be overcome by more education, networking and research, but also in driving changes in policies and legislation affecting patients and allergy specialists at the EU and national level. Allergies are a public health issue, thus there is a need for national programs in addition to AIT guidelines, such as the Finnish Allergy Programme 2008-2018 (111). Involving all the above mentioned stakeholders in the implementation of a national program, we can replace the avoidance strategy with a tolerance induction strategy where AIT is the key tool.

Conclusions

The 2017 FASIT Workshop provided a platform for fruitful and interesting discussion and debate between all groups of global stakeholders in the field of AIT. It covered a broad range of topics covering basic research, field testing and regulatory issues and addressed some specific, current challenges that will dictate how AIT continues to respond to patients' needs in the future. This workshop also highlighted the forthcoming EAACI guidelines for best practice in AIT. These guidelines will set new standards for awareness of the principles of the only disease-modifying treatment option for patients with allergic diseases as well as clinical practice. This supplement presents an insight into those discussions and highlights unmet needs and possible solutions to them for the future.

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Author contribution

All authors took part at the Future of the Allergists and Specific Immunotherapy (FASIT) meeting, held February 2017 in Hamburg, critically reviewed the manuscript, approved the final version for submission and accept overall accountability for accuracy and integrity of the manuscript.

MJ, TJ, JKT, OP and UW developed the topics to be discussed during the FASIT meeting and proposed the experts to participate in the meeting.

MJ, TJ, OP and UW chaired the two half-day sessions of the FASIT meeting.

JKT and PD chaired the two panel discussions.

MS, SK, EV, MJ, CSW, ML, UW, OP in cooperation with PZ, MK, AM, TZ, LK in cooperation with VC gave introductory lectures to the topics to be discussed during the meeting.

MJ, PK, ML, KS, SB, VC, LK, CS, TZ took part in the panel discussions.

Conflicts of Interest

S Bonini, A Muraro, K Simonsen, C Somoza, E Valovirta and U Wahn have nothing to disclose.

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