

Update on Biomarkers to Predict Responders to Allergen Immunotherapy

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Abbreviations AIT Allergen immunotherapy · BMK Biomarker · DC Dendritic cell · EPIT Epicutaneous immunotherapy · IL Interleukin · ILC Innate lymphoid cell · OIT Oral immunotherapy · SCIT Subcutaneous immunotherapy · SLIT Sublingual immunotherapy · VIT Venom immunotherapy

Opinion statement

Most investigations aiming to identify biomarkers (BMKs) have focused on immunological changes reflecting our understanding of allergen-specific immunotherapy (AIT) mechanisms of action. The upregulation of allergen-specific regulatory CD4⁺ T cells, the downregulation or anergy of Th2 cells, the induction of specific “blocking” IgG4s, and the decrease of basophil activation are all thought to contribute to AIT efficacy. To this date, however, none of these immunological alterations can be used to predict AIT efficacy. Changes in the blood at the level of dendritic cells (DCs) reflecting the induction of regulatory DCs concomitantly with a downregulation of pro-allergic DC2s are the only parameters which have been correlated at an individual patient level with AIT efficacy. In this context, the search for BMKs should be extended beyond currently existing boundaries.

To this aim, the combined use of “Omics” technologies, encompassing comprehensive genome, transcriptome and proteome-wide analyses, is being implemented to compare biological samples obtained from clinical responders and non-responders. Such a non-hypothesis driven approach, referred to as the “panorOmic” view, will lead to the discovery of completely novel molecules. In order to predict the patient’s specific response to AIT, future BMKs will likely emerge as algorithms combining information on the patient’s disease, as well as his/her immune status and exposome.

Introduction

AIT addresses the cause of the disease by reorienting inappropriate Th2 immune responses to allergens toward a Th1/regulatory T cell profile [1–3]. As a consequence, AIT is the only approach altering the natural evolution of type I allergic diseases (involving food, venom, or aero-allergens), in contrast to pharmacological treatment [4–6]. Immunotherapy is being commonly performed via either the subcutaneous or sublingual route, with other exploratory routes of immunization (e.g., epicutaneous, intradermal, intralymphatic, oral...) being currently investigated not only to treat respiratory allergies, but also in new clinical indications such as allergic asthma, food allergies, and atopic dermatitis [7, 8].

The search for BMKs reflects a trend toward stratified or precision medicine, in which patients are classified based upon their distinctive biological characteristics in

order to apply treatments tailored to their disease or physiology. This new patient-centric approach departing from the previously prevailing “one drug fits all” credo relies upon an integrated use of diagnostic and therapies, which may even imply a co-development of a companion diagnostic with the targeted therapy [9, 10••, 11].

Biomarkers are defined by The National Institutes of Health Biomarkers Definitions Working Group as “characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [12•]. Allergen immunotherapy could benefit from various types of BMKs in relationship with efficacy or safety (Table 1). When available, such BMKs should both improve the patient’s compliance to

Table 1. BMKs useful for allergen immunotherapy

Types of marker	Applications	Examples
Diagnostic/pronostic	To stratify patients based on disease severity and/or risk of progression.	- Patterns of IgE sensitization (allergic rhinoconjunctivitis or asthma). - Markers in breath condensate to predict risk of asthma exacerbation.
Predictive of AIT safety	- To select before treatment patients more likely to exhibit serious adverse events during AIT. - To reduce risk and/or severity of adverse events.	- Patterns of IgE sensitization (LTPs, Ara h 2). - Tryptase (follow-up of anaphylaxis) or PDG2 (risk of systemic reaction) levels in serum.
Predictive of AIT efficacy	- To select before treatment patients more likely to benefit for AIT. - To reduce the size of the cohort needed for clinical development. - To increase the cost-effectiveness of AIT.	- High levels of specific IgEs relative to total IgEs. - High blood basophil responsiveness to the allergen(s). - High level of sialyl fetuin A in the blood (grass pollen-allergic patients receiving SLIT).
Follow-up of AIT efficacy	- To confirm or not the early onset of efficacy after few weeks of AIT by documenting whether immune protection has been reached. - To adjust AIT modalities (e.g., dosing, immunization scheme). - To support decision to pursue or stop AIT. - To confirm lasting protection after stopping AIT (supporting decision to resume or not AIT). - To improve patient compliance.	↗ Tregs, ↗ Th1, ↘ Th2 (correlation with clinical benefit at a cohort level). ↗ DCregs, ↘ DC2s (correlation with clinical benefit at an individual patient level). ↘ IgE, ↗ IgG4, ↗ IgA, ↗ blocking antibodies (correlation with clinical benefit at a cohort level).

treatment, support decision-making by the physician before, during and after AIT and guide manufacturers during development [13•]. The performance of a BMK should thus be assessed in part in terms of health economics that is looking for evidence that it carries a positive cost-benefit value.

Herein, I review the status of BMKs associated with AIT efficacy, with a focus on candidate markers allowing to (i) predict before initiating AIT which patients are more likely to benefit from it (predictive BMKs) and (ii) identify responder patients after AIT has being initiated (follow-up BMKs).

Therapy

Which biomarkers of efficacy do we want?

BMKs could represent a molecular signature reflecting either the allergic condition or a protective biological mechanism elicited by AIT [13•, 14, 15, 16]. Those can be derived from either cellular parameters, DNA variants or mutants, RNAs, proteins, metabolites.... Irrespective of the source, the ideal BMK should persist in biological fluids to be used as a smoking gun of residual allergic inflammation or treatment efficacy [13•]. It should also be objectively measured using simple and well-standardized analytical methods (such as ELISA or PCR assays) in easily accessible body fluids (e.g., in the blood), while reflecting physiological or pathological processes occurring in target organs [13•, 14, 15, 16]. Alternatively, bronchoalveolar lavages, nasal secretions, saliva, exhaled air condensates, sputum, or tissue biopsies can also be used to document inflammatory processes locally in the airways. In all circumstances, relevant threshold values for positivity should be established in order to support decision-making.

Noteworthy, the predictive value of a BMK does not necessarily require that a causal association exists with disease severity or treatment efficacy, but having some information on the function of the BMK is useful. Two additional critical attributes of candidate BMKs relate to the specificity and sensitivity with which they help distinguishing treatment responders from non-responders [13•, 15]. Receiver operating curve (ROC) representations of those two parameters are commonly used, which further allow to define combinatorial algorithms to increase the overall predictive value by using several BMKs. The use of multiple data sources and the management of big data derived from those sources represent significant challenges to be faced in the identification of BMKs informing the physician's decisions [9, 10••, 13•].

Challenges associated with the identification of candidate BMKs of AIT efficacy

Several specific hurdles make it difficult to identify BMKs of AIT efficacy. First of all, AIT is known to be associated with a significant placebo effect [17]. Obviously, BMKs should reflect those protective biological responses related to clinical benefit beyond such a placebo effect. However, in the literature, biological changes induced by AIT are often documented in studies lacking a placebo group, making it impossible to correlate such changes with treatment-driven clinical improvement. In addition, objective quantification of allergy symptoms, for example, in association with respiratory allergies, is difficult, as it commonly relies upon a subjective auto-evaluation by the patient of his/her nasal and ocular symptoms using a grading system [18, 19]. Nasal, conjunctival, bronchial provocation tests, as well as oral food challenges or skin prick tests can be performed to obtain quantitative clinical read-outs, with the

limitation that the allergens and the procedures should be well standardized [20]. A medication score is also often used to reflect the potential reduction in the need for symptomatic treatments following AIT. Health authorities recommend a combined symptom and medication score to measure AIT efficacy, but as of today, no reference algorithm for such a combination has been broadly established [18, 19].

As discussed below, most candidate BMKs of efficacy are being investigated at a cohort level. However, to be useful to the physician, a BMK should ideally reflect a correlation between a biological change and a clinical benefit at an individual patient level. To this aim, clinical studies can be performed in allergen challenge chambers under well-standardized conditions, with respect to both allergen exposure conditions and measurement of clinical symptoms [21]. Specifically, this setting allows a baseline evaluation of allergic symptoms during a pre-treatment challenge, making it possible to document clinical improvement following AIT for each patient. Candidate BMKs identified in such challenge chambers should obviously be validated in the context of larger efficacy studies conducted under natural exposure conditions.

BMK predictive of AIT efficacy

As of today, no BMK is available to assess before treatment the likelihood of AIT efficacy in patients with respiratory or food allergies [13•]. Nonetheless, BMKs predictive of disease severity and/or progression could be considered to select patients for AIT, and even pave the ground for primary or secondary prophylaxis (Table 1). For example, studies conducted by the MeDALL (Mechanisms of the Development of ALLergy) consortium have linked co-morbidities of rhinitis, asthma, and atopic dermatitis with patterns of IgE sensitization [22]. Interestingly, young children with high titres of IgE to pathogenesis-related class 10 allergens appear more likely to develop allergic rhinoconjunctivitis and/or asthma in their adolescence or young adulthood [23]. In vitro allergen-specific IgE reactivity testing is currently being explored to establish patterns of IgE sensitization in relationship with subsequent AIT outcomes. In line with the observation that patients with severe rhinoconjunctivitis are usually good responders to AIT, both high titers of specific IgEs (relative to total IgEs) and high basophil responsiveness to the allergen before treatment have both been proposed to identify patients likely to benefit from AIT [24–27]. Component-resolved diagnostic approaches have suggested that patients with broad patterns of IgE reactivity to multiple minor allergens, or to cross-reactive panallergens such as profilins or polcalcins, respond poorly to AIT [13•, 28]. In our hands, however, grass pollen immunotherapy was shown to be equally efficacious in all patients irrespective of their IgE sensitization profiles [29].

BMKs are also actively investigated to predict responsiveness to biological or pharmacological treatments of allergic asthma, given the known heterogeneity of this disease with multiple existing endotypes. Genome-wide gene-environment interaction studies coupled to mass-spectrometry-based metabolomic analyses of exhaled breath condensates are being implemented to identify candidate markers predicting the risk for severe asthma exacerbations in asthmatic children [30••, 31, 32, 33]. With evidence that Th2-mediated asthmas

exhibit therapeutic responses to anti-IL13 antibodies or corticosteroids, prediction of clinical responders can be made on the basis of high serum levels of either periostin, elevated eosinophil counts in sputum, or high values of FeNO (fraction of exhaled nitric oxide) [34–36]. Transcriptomic and epigenetic signatures obtained from peripheral blood leukocytes or nasal epithelial cells are also being actively explored to identify therapy-resistant asthmatic children [37, 38].

Recently, we identified a sialylated form of the fetuin A (FetA) molecule which appears to be the first candidate BMK predictive of AIT efficacy in patients with allergic rhinoconjunctivitis. Specifically, sialyl FetA is expressed at high levels in pre-treatment sera from grass pollen-allergic patients who subsequently benefit from SLIT, whereas non-responders rather have unsialylated forms of this molecule in their blood. FetA has been previously reported as a molecule involved in various inflammatory conditions associated with either metabolic, infectious, as well as cardiovascular diseases and cancers [39]. Although no link between FetA and allergy had been established before, we documented that this molecule can either amplify or downregulate allergic inflammation depending upon its post-translational modifications (our unpublished results). Importantly, the identification of sialyl FetA as a potential candidate BMK predictive of AIT efficacy confirms the power of “Omics” technologies in identifying novel molecules.

Follow-up BMKs of AIT efficacy

Markers associated with the polarization of allergen-specific CD4⁺ T cells

Changes in the polarization of allergen-specific CD4⁺ T cells are thought to represent the central mechanism supporting AIT efficacy. Specifically, successful AIT leads to the downregulation of allergen-specific CD4⁺ Th2 cells together with the induction of IFN- γ -secreting Th1 and IL-10-producing regulatory CD4⁺ T cells [1–3]. Given the dramatic differences in patterns of cytokines produced by these distinct subsets of CD4⁺ T cells, such changes explain as well the impact of AIT on both allergen-specific antibody responses and on the recruitment and activation of proinflammatory innate cells in target organs [1–3].

SCIT, SLIT, OIT, and EPIT performed with aero or food allergens have all been reported to elicit various types of regulatory CD4⁺ T cells, including Foxp3⁺ regulatory T cells, IL-10-producing Tr1 cells, as well as TGF- β -secreting Th3 cells [27, 40–45]. These regulatory T cells have been mostly detected in the patients’ blood, but few studies have also documented their presence within the oral and nasal mucosae following SCIT or SLIT [1–3]. Mechanistically, such Tregs differentiate from naive T cells in lymphoid organs draining the administration site, likely following epigenetic modifications (such as hypomethylation of Foxp3 CpG sites) induced during AIT [45, 46]. AIT is known as well to elicit allergen-specific IFN- γ -producing Th1 cells, in parallel with a decline in Th2 CD4⁺ T lymphocytes mediated at least in part by apoptosis [47–49]. Recently, OIT in peanut allergic patients was shown to induce as well the anergy of such Th2 cells [50••].

Although alterations of allergen-specific CD4⁺ T cells are broadly considered as being critical to AIT success, their use as a BMK for efficacy remains difficult. Correlations between changes in patterns of allergen-specific CD4⁺ T cells and clinical improvement have been reported in the blood at a cohort, but not an individual patient level [21, 51]. In addition, whereas modifications in the balance between Th2 and regulatory T lymphocyte cells are often observed after long (>6 months) AIT regimens, evidence obtained in the first few months of AIT is less convincing [2, 51]. Noteworthy, AIT for respiratory allergies has been shown to decrease symptoms within 1 week and venom immunotherapy within hours, whereas no major modulation of T cell responses is observed in such a timeframe [2, 21]. Furthermore, several clinical studies have failed to document any induction of regulatory T lymphocytes, or even alterations in peripheral CD4⁺ T cell responses during AIT despite evidence for clinical improvement in some patients [51–53]. Such discrepancies with the aforementioned studies may be explained by the inherent capacity of CD4⁺ T cells to circulate throughout the body, making the quantitative analysis of “in transit” T cells in the blood inappropriate to document pathophysiological mechanisms occurring in target organs [13•]. Difficulties in the standardization of quantitative T cell assays may also explain those results [13•]. For those reasons combined, quantification of allergen-specific Th2 or Treg cells with the methods currently available cannot be used as a BMK of AIT efficacy.

Changes in allergen-specific antibody responses

During AIT, allergen-specific IgE serum levels are usually boosted in the first few months in most patients prior to a progressive decline observed after 6 months to 1 year of AIT [1–3]. The latter likely reflects the time needed to downregulate long-lived bone marrow-resident IgE-secreting plasma cells. In contrast, AIT induces allergen-specific IgG4 secretion by IL-10-producing regulatory B cells as early as after 2 weeks, and such IgGs are still detected in serum for more than a year after stopping treatment [54–57]. Among those, IgG4s with a high affinity for the allergen appear to be the most relevant for AIT efficacy, since they mediate a blocking activity by competing with IgEs to bind the allergen [57, 58]. Such blocking antibodies can inhibit histamine release by basophils when exposed to the allergen, as well as CD23-dependent allergen presentation to T cells. IgGs can also engage FcγRIIB (CD32) inhibitory receptors, thereby downregulating B cell, mast cell, and basophil activation [58]. In murine asthma models, the efficacy of SLIT conducted with allergens formulated in vector systems is associated with a strong induction of IgAs in respiratory mucosae [59], and such antibodies likely acting as blocking antibodies at mucosal surfaces have also been detected in patients undergoing AIT [54, 60].

The induction of specific IgG4s and further, of blocking antibodies following AIT have been correlated with clinical improvement at a cohort level. Follow-up studies in cohorts of patients having received 2 years of SCIT in mite or grass pollen-allergic patients show that within a year,

following treatment cessation allergen-specific IgG4s reverts back to pre-treatment levels. Interestingly, an IgE-blocking activity persists in post-treatment sera, which better correlates with sustained clinical responses than IgG4 levels [57, 61]. In a 4-month SLIT study in grass pollen-allergic patients, we observed that clinical responders encompass both “immunoreactive” patients with strong allergen-specific IgE, IgG, and IgG4 responses and blocking antibodies, but also “non immunoreactive” patients without any such detectable antibody responses [29]. This result was confirmed in a large cohort of mite-allergic patients receiving a 1-year-SLIT course, with documented induction of specific IgG4 antibodies only in a subset (i.e., 15–20%) of patients, irrespective of clinical benefit [62]. Collectively, it can be concluded that whereas allergen-specific IgG4 (and IgA) responses likely contribute to tolerance induction in some, but not all patients through their IgE-blocking activity, such antibody responses cannot be used as a marker of efficacy for AIT at an individual patient level.

Changes at the level of the innate immune system

Following changes in the cytokine milieu elicited during AIT, the recruitment and activation of proinflammatory basophils, eosinophils, neutrophils, and mast cells are significantly reduced in target mucosae [1–3]. In the context of venom immunotherapy (VIT) performed via the subcutaneous route, several alterations of innate immune parameters are seen within hours, including a decrease in basophil activation (with a downregulation of FcεRI and c-Syk and the upregulation of the H2R histamine receptor), the induction of immunoglobulin-like transcript ILT3 and ILT4 molecules, as well as IL-10 and intracellular cAMP production by monocytes [63, 64].

The impact of AIT on blood basophil responsiveness to the allergen(s) has been investigated following SCIT or SLIT for respiratory allergies or OIT for peanut allergy. In summary, a decrease in basophil activation has been fairly consistently observed after long-term (>6 months) AIT, together with a decrease in specific IgE levels [65–68], even if a 1-year OIT study in peanut allergic patients failed to correlate tolerance induction with changes in basophil responsiveness [45]. In our hands, we also failed to relate clinical improvement with alterations of basophil activation during a 4-month SLIT course in grass pollen patients [26].

We documented as well an impact of AIT on blood dendritic cells, with evidence that SLIT in grass pollen-allergic patients elicits within 2-month regulatory dendritic cells (DCregs) expressing high levels of the C1Q molecule [69••]. The C1Q molecule itself was subsequently shown to exhibit a powerful anti-inflammatory activity against Th2 responses in a murine asthma model, suggesting that DCregs not only support the differentiation of regulatory T cells, but also mediate a direct anti-inflammatory activity by themselves [70]. Furthermore, this induction of blood DCregs was paralleled by a decrease in the blood of markers such as CD141 and OX40L associated with pro-allergic DC2s (i.e., DCs supporting the differentiation of Th2 cells) [71]. These changes in the DCreg/DC2 balance were noticeable by quantitative PCR only in the peripheral blood of those

patients exhibiting clinical responses, further corroborating at the level of the innate immune system, the paradigm that a reorientation of immune responses from a Th2 to a regulatory profile is critical to the success of AIT [69••, 71]. Together with other studies reporting an impact of AIT on the phenotype and function of blood DCs, these results suggest that the induction of DCregs is an interesting candidate marker of AIT efficacy [13•, 72].

Recently, the impact of AIT on a new family of lymphocyte-like cells termed innate lymphoid cells (ILCs) has been investigated. These cells encompass three distinct subsets, ILC1s, ILC2, and ILC3 based upon their patterns of cytokine production, which match those of polarized CD4⁺ T lymphocytes [73]. ILCs play a role in the initiation and persistence of allergic inflammation, with evidence that numbers of circulating ILC2 cells are increased in the blood of asthmatic patients, and that ILC3s can contribute to asthma associated with obesity [74–76]. Recently, a study conducted in a small cohort of grass pollen-allergic patients suggested that SCIT can blunt the upregulation of ILC2 cells elicited in the blood during the pollen season [77]. In our hands, a short course SLIT regimen had no detectable effect on circulating ILC2s [76]. The potential heterogeneity of ILCs, their role in allergic diseases as well as the impact of long-term AIT on ILC2 frequencies and activation need to be further investigated.

Perspectives

The interest of BMKs to adjust treatment modalities stems from the inherent heterogeneity of allergic patients. These patients differ quite significantly in their patterns of IgE sensitization (i.e., to seasonal versus perennial allergens, to a single or multiple allergen sources), their genetic backgrounds, their exposomes, and their microbiota. The patients' immune status also varies dramatically beyond the fact that they share the atopic condition. For example, most patients with pollinosis or food allergy exhibit bona fide Th2 responses to the allergen(s), whereas patients chronically exposed and allergic to house dust mites develop mixed allergen-specific Th2/Th1, Th2/Th17, or even Th1/Th17 immune responses [78]. Future investigations should clarify as to whether AIT is equally efficacious in those various subsets of patients, and further, whether BMKs of efficacy differ between AIT approaches, when conducted using distinct routes of administration in grass pollen, food, or house dust mite-allergic patients. Also, as we better understand the role of mucosal microbiota in shaping local immune responses, the analysis of differences between individuals in terms of flora composition and the study of profiles associated with positive or negative AIT outcomes should be pursued [79].

This review was focused on BMKs allowing to predict or monitor AIT efficacy, but there is a significant need as well to identify BMKs allowing to predict the occurrence of severe or systemic reactions to AIT. In this regard, serum mast cell tryptase is being used to help to the diagnosis of anaphylaxis, and an elevation of a PGD2 metabolite in serum has been proposed as a read-out predictive of systemic reactions to AIT [80, 81]. Baseline allergic rhinitis asthma and skin reaction to peanut allergens have been proposed to predict gastrointestinal adverse events and systemic reactions occurring during OIT [82]. Also, allergic patients with high serum IgE levels to either plant food

non-specific lipid transfer proteins or to the peanut Ara h 2 allergen appear to exhibit a higher risk of systemic reactions when exposed to those allergens [83, 84]. Additional studies are necessary to define molecular signatures allowing to assess in pre-treatment biological samples the risk to develop unwanted adverse events during AIT.

Compliance with Ethical Standards

Conflict of Interest

Dr. Philippe Moingeon is an employee at Stallergenes SAS, outside of the submitted work.

Human and Animal Rights and Informed Consent

This review article does not contain any studies performed with human or animal subjects performed by the author.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Shamji MH, Durham SR. Mechanisms of immunotherapy to aeroallergens. *Clin Exp Allergy*. 2011;41:1235–46. doi:[10.1111/j.1365-2222.2011.03804.x](#).
2. Moingeon P. Update on immune mechanisms associated with sublingual immunotherapy: practical implications for the clinician. *J Allergy Clin Immunol Pract*. 2013;1:228–41. doi:[10.1016/j.jaip.2013.03.013](#).
3. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*. 2014;133:621–31. doi:[10.1016/j.jaci.2013.12.1088](#).
4. Canonica GW, Bousquet J, Casale T, Lockey RF, Baena-Cagnani CE, Pawankar R, et al. Sub-lingual immunotherapy: world allergy organization position paper 2009. *Allergy*. 2009;64(Suppl 91):1–59. doi:[10.1111/j.1398-9995.2009.02309.x](#).
5. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol*. 2011;127:S1–55. doi:[10.1016/j.jaci.2010.09.034](#).
6. Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M, et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. *J Allergy Clin Immunol*. 2013;131:1288–96. doi:[10.1016/j.jaci.2013.01.049](#).
7. Moingeon P, Mascarell L. Novel routes for allergen immunotherapy: safety, efficacy and mode of action. *Immunotherapy*. 2012;4:201–12. doi:[10.2217/imt.11.171](#).
8. Passalacqua G, Compalati E, Canonica GW. Sublingual immunotherapy: other indications. *Immunol Allergy Clin N Am*. 2011;31:279–87. doi:[10.1016/j.iac.2011.02.011](#).
9. Trusheim MR, Burgess B, Hu SX, Long T, Averbuch SD, Flynn AA, et al. Quantifying factors for the success of stratified medicine. *Nat Rev Drug Discov*. 2011;10:817–33. doi:[10.1038/nrd3557](#).
10. • Willis JC, Lord GM. Immune biomarkers: the promises and pitfalls of personalized medicine. *Nat Rev Immunol*. 2015;15:323–9. doi:[10.1038/nri3820](#).
This is a review introducing the concept of personalized medicine and the use of BMKs to stratify patients
11. Topol EJ. Individualized medicine from prewomb to tomb. *Cell*. 2014;157:241–53. doi:[10.1016/j.cell.2014.02.012](#).
12. • Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69:89–95. doi:[10.1067/mcp.2001.113989](#).
This article presents a reference definition of a BMK
13. • Moingeon P. Biomarkers for allergen immunotherapy: a “Panoromic” view. *Immunol Allergy Clin N Am*. 2016;36:161–79. doi:[10.1016/j.iac.2015.08.004](#).
This review describes the challenges and opportunities to identify BMKs of AIT efficacy

14. Alam R. Biomarkers in asthma and allergy. *Immunol Allergy Clin N Am*. 2012;32:xi–xii. doi:[10.1016/j.iac.2012.06.013](https://doi.org/10.1016/j.iac.2012.06.013).
 15. Shamji MH, Ljorring C, Wurtzen PA. Predictive biomarkers of clinical efficacy of allergen-specific immunotherapy: how to proceed. *Immunotherapy*. 2013;5:203–6. doi:[10.2217/imt.13.6](https://doi.org/10.2217/imt.13.6).
 16. Popescu FD. Molecular biomarkers for grass pollen immunotherapy. *World J Methodol*. 2014;4:26–45. doi:[10.5662/wjm.v4.i1.26](https://doi.org/10.5662/wjm.v4.i1.26).
 17. Narkus A, Lehnigk U, Haefner D, Klinger R, Pfaar O, Worm M. The placebo effect in allergen-specific immunotherapy trials. *Clin Transl Allergy*. 2013;3:42. doi:[10.1186/2045-7022-3-42](https://doi.org/10.1186/2045-7022-3-42).
 18. Agency EM. Guideline on the clinical development of products for specific immunotherapy for the treatment of allergic diseases. CHMP/EWP/18504/2006. 2008.
 19. Devillier P, Chassany O, Vicaud E, de Beaumont O, Robin B, Dreyfus JF, et al. The minimally important difference in the rhinoconjunctivitis total symptom score in grass-pollen-induced allergic rhinoconjunctivitis. *Allergy*. 2014;69:1689–95. doi:[10.1111/all.12518](https://doi.org/10.1111/all.12518).
 20. Scadding G, Hellings P, Alobid I, Bachert C, Fokkens W, van Wijk RG, et al. Diagnostic tools in rhinology EAACI position paper. *Clin Transl Allergy*. 2011;1:2. doi:[10.1186/2045-7022-1-2](https://doi.org/10.1186/2045-7022-1-2).
 21. 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. doi:[10.1016/j.jaci.2009.06.006](https://doi.org/10.1016/j.jaci.2009.06.006).
 22. Anto JM, Pinart M, Akdis M, Auffray C, Bachert C, Basagana X, et al. Understanding the complexity of IgE-related phenotypes from childhood to young adulthood: a Mechanisms of the Development of Allergy (MeDALL) seminar. *J Allergy Clin Immunol*. 2012;129:943–54 e4. doi:[10.1016/j.jaci.2012.01.047](https://doi.org/10.1016/j.jaci.2012.01.047).
 23. Westman M, Lupinek C, Bousquet J, Andersson N, Pahr S, Baar A, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J Allergy Clin Immunol*. 2015;135:1199–206. doi:[10.1016/j.jaci.2014.10.042](https://doi.org/10.1016/j.jaci.2014.10.042).
 24. Howarth P, Malling HJ, Molimard M, Devillier P. Analysis of allergen immunotherapy studies shows increased clinical efficacy in highly symptomatic patients. *Allergy*. 2012;67:321–7. doi:[10.1111/j.1398-9995.2011.02759.x](https://doi.org/10.1111/j.1398-9995.2011.02759.x).
 25. Di Lorenzo G, Mansueto P, Pacor ML, Rizzo M, Castello F, Martinelli N, et al. Evaluation of serum-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2009;123:1103–10. doi:[10.1016/j.jaci.2009.02.012](https://doi.org/10.1016/j.jaci.2009.02.012).
 26. 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. doi:[10.1111/j.1398-9995.2011.02696.x](https://doi.org/10.1111/j.1398-9995.2011.02696.x).
 27. Fujimura T, Yonekura S, Horiguchi S, Taniguchi Y, Saito A, Yasueda H, et al. Increase of regulatory T cells and the ratio of specific IgE to total IgE are candidates for response monitoring or prognostic biomarkers in 2-year sublingual immunotherapy (SLIT) for Japanese cedar pollinosis. *Clin Immunol*. 2011;139:65–74. doi:[10.1016/j.dim.2010.12.022](https://doi.org/10.1016/j.dim.2010.12.022).
 28. Harwanegg C, Laffer S, Hiller R, Mueller MW, Kraft D, Spitzauer S, et al. Microarrayed recombinant allergens for diagnosis of allergy. *Clin Exp Allergy*. 2003;33:7–13.
 29. Baron-Bodo V, Horiot S, Lautrette A, Chabre H, Drubert AS, Danze PM, et al. Heterogeneity of antibody responses among clinical responders during grass pollen sublingual immunotherapy. *Clin Exp Allergy*. 2013;43:1362–73. doi:[10.1111/cea.12187](https://doi.org/10.1111/cea.12187).
 - 30.●● Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. *J Allergy Clin Immunol*. 2015;135:299–310. doi:[10.1016/j.jaci.2014.12.1871](https://doi.org/10.1016/j.jaci.2014.12.1871).
- This is a comprehensive review on the heterogeneity of asthma and ongoing efforts to relate endotypes with response rates to specific treatments
31. Carraro S, Rezzi S, Reniero F, Heberger K, Giordano G, Zanconato S, et al. Metabolomics applied to exhaled breath condensate in childhood asthma. *Am J Respir Crit Care Med*. 2007;175:986–90. doi:[10.1164/rccm.200606-769OC](https://doi.org/10.1164/rccm.200606-769OC).
 32. Martinez-Lozano Sinues P, Kohler M, Zenobi R. Human breath analysis may support the existence of individual metabolic phenotypes. *PLoS One*. 2013;8:e59909. doi:[10.1371/journal.pone.0059909](https://doi.org/10.1371/journal.pone.0059909).
 33. Bos LD, Sterk PJ, Fowler SJ. Breathomics in asthma and COPD. *J Allergy Clin Immunol*. 2016. doi:[10.1016/j.jaci.2016.08.004](https://doi.org/10.1016/j.jaci.2016.08.004).
 34. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol*. 2014;133:388–94. doi:[10.1016/j.jaci.2013.07.036](https://doi.org/10.1016/j.jaci.2013.07.036).
 35. Cowan DC, Taylor DR, Peterson LE, Cowan JO, Palmay R, Williamson A, et al. Biomarker-based asthma phenotypes of corticosteroid response. *J Allergy Clin Immunol*. 2015;135:877–83. doi:[10.1016/j.jaci.2014.10.026](https://doi.org/10.1016/j.jaci.2014.10.026).
 36. Sordillo JE, Kelly R, Bunyavanich S, McGeachie M, Qiu W, Croteau-Chonka DC, et al. Genome-wide expression profiles identify potential targets for gene-environment interactions in asthma severity. *J Allergy Clin Immunol*. 2015;136:885–92. doi:[10.1016/j.jaci.2015.02.035](https://doi.org/10.1016/j.jaci.2015.02.035).
 37. Persson H, Kwon AT, Ramilowski JA, Silberberg G, Soderhall C, Orsmark-Pietras C, et al. Transcriptome analysis of controlled and therapy-resistant childhood asthma reveals distinct gene expression profiles. *J Allergy Clin Immunol*. 2015;136:638–48. doi:[10.1016/j.jaci.2015.02.026](https://doi.org/10.1016/j.jaci.2015.02.026).

38. Xiao C, Biagini Myers JM, Ji H, Metz K, Martin LJ, Lindsey M, et al. Vanin-1 expression and methylation discriminate pediatric asthma corticosteroid treatment response. *J Allergy Clin Immunol*. 2015;136:923–31. doi:[10.1016/j.jaci.2015.01.045](https://doi.org/10.1016/j.jaci.2015.01.045).
 39. Mori K, Emoto M, Inaba M. Fetuin-A: a multifunctional protein. *Recent Pat Endocr Metab Immune Drug Discov*. 2011;5:124–46.
 40. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol*. 2003;111:1255–61.
 41. Bohle B, Kinaciyan T, Gerstmayr M, Radakovic A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol*. 2007;120:707–13. doi:[10.1016/j.jaci.2007.06.013](https://doi.org/10.1016/j.jaci.2007.06.013).
 42. Eifan AO, Akkoc T, Yildiz A, Keles S, Ozdemir C, Bahceciler NN, et al. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy*. 2010;40:922–32. doi:[10.1111/j.1365-2222.2009.03448.x](https://doi.org/10.1111/j.1365-2222.2009.03448.x).
 43. Mondoulet L, Dioszeghy V, Puteaux E, Ligouis M, Dhelft V, Plaquet C, et al. Specific epicutaneous immunotherapy prevents sensitization to new allergens in a murine model. *J Allergy Clin Immunol*. 2015;135:1546–57. doi:[10.1016/j.jaci.2014.11.028](https://doi.org/10.1016/j.jaci.2014.11.028).
 44. O'Hehir RE, Gardner LM, de Leon MP, Hales BJ, Biondo M, Douglass JA, et al. House dust mite sublingual immunotherapy: the role for transforming growth factor-beta and functional regulatory T cells. *Am J Respir Crit Care Med*. 2009;180:936–47. doi:[10.1164/rccm.200905-0686OC](https://doi.org/10.1164/rccm.200905-0686OC).
 45. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol*. 2014;133:500–10. doi:[10.1016/j.jaci.2013.12.1037](https://doi.org/10.1016/j.jaci.2013.12.1037).
 46. Swamy RS, Reshamwala N, Hunter T, Vissamsetti S, Santos CB, Baroody FM, et al. Epigenetic modifications and improved regulatory T-cell function in subjects undergoing dual sublingual immunotherapy. *J Allergy Clin Immunol*. 2012;130:215–24. doi:[10.1016/j.jaci.2012.04.021](https://doi.org/10.1016/j.jaci.2012.04.021).
 47. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol*. 1995;154:4187–94.
 48. Wambre E, DeLong JH, James EA, LaFond RE, Robinson D, Kwok WW. Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during specific immunotherapy. *J Allergy Clin Immunol*. 2012;129:544–51. doi:[10.1016/j.jaci.2011.08.034](https://doi.org/10.1016/j.jaci.2011.08.034).
 49. Suarez-Fueyo A, Ramos T, Galan A, Jimeno L, Wurtzen PA, Marin A, et al. Grass tablet sublingual immunotherapy downregulates the TH2 cytokine response followed by regulatory T-cell generation. *J Allergy Clin Immunol*. 2014;133:130–8. doi:[10.1016/j.jaci.2013.09.043](https://doi.org/10.1016/j.jaci.2013.09.043).
 50. Ryan JF, Hovde R, Glanville J, Lyu SC, Ji X, Gupta S, et al. Successful immunotherapy induces previously unidentified allergen-specific CD4+ T-cell subsets. *Proc Natl Acad Sci U S A*. 2016;113:1286–95. doi:[10.1073/pnas.1520180113](https://doi.org/10.1073/pnas.1520180113).
- This is a comprehensive study of the impact of OIT for peanut allergy on CD4⁺ T cell subsets
51. 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. doi:[10.1111/cea.12015](https://doi.org/10.1111/cea.12015).
 52. Rolinck-Werninghaus C, Kopp M, Liebke C, Lange J, Wahn U, Niggemann B. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol*. 2005;136:134–41. doi:[10.1159/000083320](https://doi.org/10.1159/000083320).
 53. Dehlink E, Eiwegger T, Gerstmayr M, Kampl E, Bohle B, Chen KW, et al. Absence of systemic immunologic changes during dose build-up phase and early maintenance period in effective specific sublingual immunotherapy in children. *Clin Exp Allergy*. 2006;36:32–9. doi:[10.1111/j.1365-2222.2006.02400.x](https://doi.org/10.1111/j.1365-2222.2006.02400.x).
 54. Scadding GW, Shamji MH, Jacobson MR, Lee DI, Wilson D, Lima MT, et al. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy*. 2010;40:598–606. doi:[10.1111/j.1365-2222.2010.03462.x](https://doi.org/10.1111/j.1365-2222.2010.03462.x).
 55. Bahceciler NN, Arikan C, Taylor A, Akdis M, Blaser K, Barlan IB, et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol*. 2005;136:287–94. doi:[10.1159/000083956](https://doi.org/10.1159/000083956).
 56. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol*. 2013;131:1204–12. doi:[10.1016/j.jaci.2013.01.014](https://doi.org/10.1016/j.jaci.2013.01.014).
 57. James LK, Shamji MH, Walker SM, Wilson DR, Wachholz PA, Francis JN, et al. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol*. 2011;127:509–16. doi:[10.1016/j.jaci.2010.12.1080](https://doi.org/10.1016/j.jaci.2010.12.1080).
 58. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol*. 2004;4:313–8.

59. Razafindratsita A, Saint-Lu N, Mascarell L, Berjont N, Bardon T, Betbeder D, et al. Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. *J Allergy Clin Immunol*. 2007;120:278–85. doi:[10.1016/j.jaci.2007.04.009](https://doi.org/10.1016/j.jaci.2007.04.009).
60. Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol*. 2007;178:4658–66.
61. Shamji MH, Ljorring C, Francis JN, Calderon MA, Larche M, Kimber I, et al. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy*. 2012;67:217–26. doi:[10.1111/j.1398-9995.2011.02745.x](https://doi.org/10.1111/j.1398-9995.2011.02745.x).
62. Baron-Bodo V, Batard T, Nguyen H, Frereux M, Horiot S, Harwanegg C, et al. Absence of IgE neosensitization in house dust mite allergic patients following sublingual immunotherapy. *Clin Exp Allergy*. 2012;42:1510–8. doi:[10.1111/j.1365-2222.2012.04044.x](https://doi.org/10.1111/j.1365-2222.2012.04044.x).
63. Bussmann C, Xia J, Allam JP, Maintz L, Bieber T, Novak N. Early markers for protective mechanisms during rush venom immunotherapy. *Allergy*. 2010;65:1558–65. doi:[10.1111/j.1398-9995.2010.02430.x](https://doi.org/10.1111/j.1398-9995.2010.02430.x).
64. Cellesnik N, Vesel T, Rijavec M, Silar M, Erzen R, Kosnik M, et al. Short-term venom immunotherapy induces desensitization of FcepsilonRI-mediated basophil response. *Allergy*. 2012;67:1594–600. doi:[10.1111/all.12044](https://doi.org/10.1111/all.12044).
65. Ceuppens JL, Bullens D, Kleinjans H, van der Werf J, Group PBES. Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects. *Clin Exp Allergy*. 2009;39:1903–9. doi:[10.1111/j.1365-2222.2009.03379.x](https://doi.org/10.1111/j.1365-2222.2009.03379.x).
66. Shamji MH, Layhadi JA, Scadding GW, Cheung DK, Calderon MA, Turka LA, et al. Basophil expression of diamine oxidase: a novel biomarker of allergen immunotherapy response. *J Allergy Clin Immunol*. 2015;135:913–21. e9 doi:[10.1016/j.jaci.2014.09.049](https://doi.org/10.1016/j.jaci.2014.09.049).
67. Zidarn M, Kosnik M, Silar M, Bajrovic N, Korosec P. Sustained effect of grass pollen subcutaneous immunotherapy on suppression of allergen-specific basophil response; a real-life, nonrandomized controlled study. *Allergy*. 2015;70:547–55. doi:[10.1111/all.12581](https://doi.org/10.1111/all.12581).
68. Aasbjerg K, Backer V, Lund G, Holm J, Nielsen NC, Holse M, et al. Immunological comparison of allergen immunotherapy tablet treatment and subcutaneous immunotherapy against grass allergy. *Clin Exp Allergy*. 2014;44:417–28.
- 69.●● 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. doi:[10.1016/j.jaci.2012.02.014](https://doi.org/10.1016/j.jaci.2012.02.014).
This is the first study reporting the induction of regulatory DCs in patients responding to AIT, with correlates of efficacy established at an individual patient level
70. Mascarell L, Airouche S, Berjont N, Gary C, Gueguen C, Fourcade G et al. The regulatory dendritic cell marker C1q is a potent inhibitor of allergic inflammation. *Mucosal Immunol*. 2016; In press. doi:[10.1038/mi.2016.87](https://doi.org/10.1038/mi.2016.87).
71. Gueguen C, Bouley J, Moussu H, Luce S, Duchateau M, Chamot-Rooke J, et al. Changes in markers associated with dendritic cells driving the differentiation of either TH2 cells or regulatory T cells correlate with clinical benefit during allergen immunotherapy. *J Allergy Clin Immunol*. 2016;137:545–58. doi:[10.1016/j.jaci.2015.09.015](https://doi.org/10.1016/j.jaci.2015.09.015).
72. Angelini F, Pacciani V, Corrente S, Silenzi R, Di Pede A, Polito A, et al. Dendritic cells modification during sublingual immunotherapy in children with allergic symptoms to house dust mites. *World J Pediatr*. 2011;7:24–30. doi:[10.1007/s12519-011-0242-3](https://doi.org/10.1007/s12519-011-0242-3).
73. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol*. 2011;12:21–7. doi:[10.1038/ni.1962](https://doi.org/10.1038/ni.1962).
74. Barlow JL, McKenzie AN. Type-2 innate lymphoid cells in human allergic disease. *Curr Opin Allergy Clin Immunol*. 2014;14:397–403. doi:[10.1097/ACI.0000000000000090](https://doi.org/10.1097/ACI.0000000000000090).
75. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J Allergy Clin Immunol*. 2014;134:671–8. doi:[10.1016/j.jaci.2014.06.024](https://doi.org/10.1016/j.jaci.2014.06.024).
76. Lombardi V, Beuraud C, Neukirch C, Moussu H, Morizur L, Horiot S, et al. Circulating innate lymphoid cells are differentially regulated in allergic and nonallergic subjects. *J Allergy Clin Immunol*. 2016;138:305–8. doi:[10.1016/j.jaci.2015.12.1325](https://doi.org/10.1016/j.jaci.2015.12.1325).
77. Lao-Araya M, Steveling E, Scadding GW, Durham SR, Shamji MH. Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol*. 2014;134:1193–5. doi:[10.1016/j.jaci.2014.07.029](https://doi.org/10.1016/j.jaci.2014.07.029).
78. Wambre E, Bonvalet M, Bodo VB, Maillere B, Leclert G, Moussu H, et al. Distinct characteristics of seasonal (Bet v 1) vs. perennial (Der p 1/Der p 2) allergen-specific CD4(+) T cell responses. *Clin Exp Allergy*. 2011;41:192–203. doi:[10.1111/j.1365-2222.2010.03641.x](https://doi.org/10.1111/j.1365-2222.2010.03641.x).
79. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, et al. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORγ(+) T cells. *Science*. 2015;349:989–93. doi:[10.1126/science.aac4263](https://doi.org/10.1126/science.aac4263).
80. Brown SG, Blackman KE, Heddle RJ. Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas*. 2004;16:120–4. doi:[10.1111/j.1742-6723.2004.00562.x](https://doi.org/10.1111/j.1742-6723.2004.00562.x).
81. Rank MA, Kita H, Li JT, Butterfield JH. Systemic reactions to allergen immunotherapy: a role for measuring

- a PGD2 metabolite? *Ann Allergy Asthma Immunol*. 2013;110:57–8. doi:[10.1016/j.anai.2012.10.009](https://doi.org/10.1016/j.anai.2012.10.009).
82. Virkud YV, Burks AW, Steele PH, Edwards LJ, Berglund JP, Jones SM, et al. Novel baseline predictors of allergic side effects during peanut oral immunotherapy. *J Allergy Clin Immunol*. 2016; doi:[10.1016/j.jaci.2016.07.030](https://doi.org/10.1016/j.jaci.2016.07.030).
83. Ballmer-Weber BK, Lidholm J, Fernandez-Rivas M, Seneviratne S, Hanschmann KM, Vogel L, et al. IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall study. *Allergy*. 2015;70:391–407. doi:[10.1111/all.12574](https://doi.org/10.1111/all.12574).
84. Romano A, Scala E, Rumi G, Gaeta F, Caruso C, Alonzi C, et al. Lipid transfer proteins: the most frequent sensitizer in Italian subjects with food-dependent exercise-induced anaphylaxis. *Clin Exp Allergy*. 2012;42:1643–53. doi:[10.1111/cea.12011](https://doi.org/10.1111/cea.12011).