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### REVIEW

## Predictive biomarkers in allergen specific immunotherapy



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According to the definition made by Dr. Shashi Amur, Scientific coordinator for CDER's Biomarker Qualification Program, "A biomarker is a characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or biological responses to a therapeutic intervention".

Since the simultaneous discovery of sIgE in 1967 by Ishizaka and Johansson, the quantification of specific antibodies of IgE isotype has been the pivotal biomarker to define allergic diseases. Now we have a broader view of abnormal Th2 responses to innocuous antigens and although some relatively new pathologies, such as eosinophilic esophagitis or local rhinitis, cannot be diagnosed by serological sIgE measurement, its determination continues to be pivotal in clinical allergy.

sIgE diagnosis has been based on extract-based preparations used for both "in vivo" and "in vitro" methods for decades. The progressive characterization of the relevant allergenic molecules eased the development of a new diagnostic methodology called component-resolved diagnosis (CRD)<sup>1</sup> and a new body of knowledge, molecular allergology.<sup>2</sup> The introduction of new diagnostic algorithms based on CRD

has significantly increased the precision when identifying relevant sensitizers.<sup>3–6</sup> About 30% of patients were wrongly diagnosed by extract-based diagnosis.<sup>7</sup>

Component-resolved diagnosis enabled epidemiological studies to be conducted that were essential to understand allergen/patient interaction and identify clinical phenotypes associated with variable allergen exposure.<sup>3</sup> In spite of a clear association between particular profiles and responses to IT,<sup>8,9</sup> sIgE titers have a limited individual predictive value. This can be easily explained by the fact that the effect or potential of the sIgE depends on many factors, including T cell status, level of tolerance and interference of other antibody subclasses or the coexistence of other sensitizations.<sup>10</sup>

Part of the positive effect of specific immunotherapy (SIT) is to induce the development of an sIgG4 response. IgG4 will interfere with sIgE for example by inhibiting binding to the low affinity receptor (CD23), down regulating sIgE synthesis. Facilitated antigen binding inhibition assay, which is a functional way of evaluating the effect of sIgG4, has proved to be a valuable tool in the assessment of SIT effect.<sup>11</sup> However, the complexity of the experimental set-up limits its potential use as a widespread biomarker.

Different systemic biomarkers have been developed for evaluating systemic inflammation, such as measurement of exhaled nitric oxide (FeNO)<sup>12</sup> or periostin<sup>13,14</sup> or, more recently, the so-called electronic nose<sup>15</sup> that could be

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of potential in SIT. Nonetheless, although they could be good monitoring tools they have a low individual predictive capacity.

## Specific immunotherapy

Although used for more than 100 years, it is only recently that the mechanisms underlying its effects are coming to light.<sup>16</sup> Until the beginning of the present century, most of the evidence for SIT effect was based on clinical trials underpowered in both size and duration, which were jointly reviewed in a meta-analysis and presented a high level of variability.<sup>17</sup> Only a few exploratory trials investigated sustained effect or disease prevention, clinical characteristics that should be considered as pivotal for SIT intervention.<sup>18</sup>

With the advent of a new generation of fully documented allergen-based vaccines, this situation started to change and, more importantly, offered new tools to analyze and thus understand the underlying mechanisms and potential associated biomarkers.

GRAZAX is the best documented allergen vaccine. It was formulated as a fast-dissolving formulation of a grass (*Phleum pratense*) extract for oral administration. The product must be administered over three consecutive years, maintaining its clinical benefit for at least two years after discontinuation.<sup>19</sup>

With about 25 documented clinical trials, it is known that two months of therapy are required to detect a significant clinical benefit and to reduce symptom scores during unrelated pollen seasons.<sup>20,21</sup>

## Immunological mechanisms

With the known product clinical profile, a clinical trial was designed to identify potential immunological mechanisms associated with the different clinical effects. The study, that lasted for 5 years was initially covered by a program called MEICA funded by the "Fundación Genoma", and later by ALK-Abello.

The results of the first two years indicate that an exacerbation of allergy (increase in both sIgE and IL4 secreting cells) is detected after one month of therapy that is progressively reduced thereafter. A progressive induction of sIgG4 precedes the onset of activated memory T cell regulatory response that is consolidated after two years of therapy. The onset of this response correlates with an early decline in IL4 production.<sup>22</sup> Interestingly, specific immunotherapy has an effect on gamma interferon production by innate immune cells, suggesting a pivotal mechanism to understand preventive effect. It is increasingly evident that there is a complex relationship between innate and adaptive immunity to allergens. Understanding this interaction network will be crucial to help define intervention and predictive strategies.<sup>23</sup>

The results of the five year study are currently in the publication process and support a key role for this regulatory response in sustained clinical benefit.

Interestingly, no peripheral mechanism could explain the early immunotherapy effect.<sup>20,24</sup>

## Oral mucosa and early effect

In project PI13/00477 funded by ISCIII we aimed to understand why grass pollen overexposed allergic patients developed severe profilin food-related reactions with a relatively high frequency compared to patients residing in other areas.

We formulated the hypothesis that these extreme phenotypes could suffer an oral mucosa impairment and that profilin, an allergen that is quickly inactivated, would exert its effect by penetrating the oral mucosa and gaining access to the effector cells.

The results of this study are under publication, but initial data presented in the EAACI annual meeting celebrated in Vienna<sup>25,26</sup> fully support this hypothesis. There is an entry gate to the immune system through the oral mucosa. This access might be critical to understand sublingual IT mechanisms and, in particular, to formulate a new hypothesis about the short-term effect of sublingual therapy.

Early dendritic cell interactions that will govern immunological shift to tolerance are pivotal and the subject of active research,<sup>27</sup> constituting a very promising strategy to identify early response biomarkers.

It is documented that for a short-term effect, the doses for sublingual therapy must be relatively high. In fact, early sublingual trials that were performed at lower doses usually only showed an effect after several years of therapy. The accumulation of side effects during the first days of therapy is consistent with this hypothesis and suggests that effector cell desensitization is key to understanding early effect, being a separate mechanism to T cell regulation and governing clinical benefit during the first phase of Immunotherapy. After this period, regulatory pathways will lead the effect, and maintenance of the regulatory response will be crucial for sustained clinical benefit.

## Individual patient variability

Knowing individual patient variability is essential in the design of biomarker strategies. Assuming sIgE increase in the short-term to be an indicator of IT detection, a small fraction of patients (10–15%) do not alter sIgE levels, suggesting a lack of response to the therapy. Around 85% of patients develop a regulatory response, but the individual kinetics are quite different. Using sIgG4 as a marker, some patients boost sIgG4 levels in the first four months, while in other cases this might take more than two years. In any case, the boost in both sIgE and sIgG4 is followed by a decline that is quite sharp in some patients, making it difficult to define standard screening times.

## New biomarker approaches

We are working on strategies to identify biomarkers associated with inflammation.

Using clinical models of severe allergic-related pathologies and applying metabolomics and transcriptomic unbiased approaches, we aim to identify new biomarkers associated with allergy and use them to predict or monitor intervention effect.

Metabolomic analysis is revealing to be a very promising approach and initial data suggest that it could represent a breakthrough for the identification of new diagnostic strategies.

The validation of these new biomarkers should be performed in collaborative frameworks. In relation to this, a newly created network of cooperative research funded by the Instituto de Salud Carlos III in Spain, RETIC ARAYDAL, will be pivotal. With three research programs covering basic aspects, biomarkers and interventions, and the cooperation of more than 20 basic and clinical groups, this has the potential of becoming a reference framework for the future of allergy management.

## Conflict of interest

The Dr. Escribese declare no conflict of interest.

The Dr. Barber declare having acted as Scientific Advisor in the last 12 months for the following Companies: ALK-Abello and AIMMUNE.

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