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Title: Basophil Activation Test : A diagnostic, predictive and monitoring assay for Allergen Immunotherapy

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For decades, the approach to clinical care has involved the assessment of a fairly small set of patients' signs and symptoms, sampled over a short period of time with limited attention given to individual variations in aetiology and pathophysiology. Subsequently, a therapy is assigned predominantly with a "one shoe fits all" approach. Precision medicine approaches are now starting to change ways to test, treat and develop new therapies for allergic and immunological disorders. (Figure 1).

One of the first examples for such a personalized approach of predictive enrichment is with Herceptin, approved by the FDA in 1998 for HER2-positive metastatic breast cancer (1). The *HER2* gene is amplified in 20–30% of early-stage breast cancers, and by measuring *HER2* expression in the tumour, proper patient selection can be made for the use of Herceptin. From 1998 to 2000, the percentage of patients who underwent HER2 evaluation increased from 12 to 94% along with a 5-fold increase in this targeted therapy after 2004. Despite the success story in biomarker-driven treatment selection with Herceptin, there has been a significant gap in the development of such companion diagnostics in other indications, partly due to the difficulties in either identifying or not knowing the signals that induce biomarkers relevant for predicting treatment outcomes. To tackle this gap for allergic patients, Hoffmann and colleagues (2) now introduce “Time” as a 3rd dimension, to static and induced biomarkers. They elegantly show, using a functional basophil assay, how changes over a short period of 3 weeks after initiation of therapy, can predict long-term clinical outcomes following SCIT.

The authors monitored the basophil sensitivity response every three months during treatment, where they are able to monitor the changes in the allergic response over time. Basophil surface expression of CD63 showed changes shortly after the start of the allergen immunotherapy (AIT) and correlated with

long term clinical outcomes based on *in vivo* allergen challenge. Further experimentation into mechanisms using blocking factor and antigen presentation assays showed that humoral factors contributed to the reduction in the basophil responses. Collection of their data in a clinical care setting with an open control arm further strengthens this study as real-world-data, and real-world-evidence is playing more of a role in clinical decision making as well as by regulatory agencies for device, test and drug approvals. Having such objective measures of response to therapy is critical in a disease where clinical decisions are generally made by subjective reports of patient measured outcomes. Early identification of proper patients that will benefit from the continuation of the allergen immunotherapy is an unmet need for the treatment of allergic disorders (Figure 2).

Basophil Activation Test (BAT) can be a very effective predictive and a monitoring tool. As a predictive assay, the biggest utility of BAT is before oral food challenges. Along these lines, BAT has been referred to as an oral food challenge in a test tube. The value to BAT in predicting outcomes of food challenges is well established for a wide variety of allergens. In some cases, BAT has proven useful as a diagnostic test for venom allergies, even more sensitive than intradermal skin testing (3). Moreover, increase basophil responsiveness has been associated with adverse effects arising from venom immunotherapy. In a more recent study, the magnitude of the CD63 response to peanut allergen before start of peanut food immunotherapy showed correlation with outcomes, where those with a lower CD63 upregulation were more likely to achieve sustained unresponsiveness after peanut oral immunotherapy (4).

As a monitoring assay, determining basophil response to allergens is a powerful tool when monitoring the effects of AIT and other immunomodulatory treatments, such as anti-IgE (5). Basophil sensitivity (as measured by CD-sens) has shown to be reduced following AIT to aeroallergens such as birch pollen, timothy grass, and following AIT to insect venom (6). A drop-in basophil sensitivity has also been reported following treatment with omalizumab across a range of allergies including allergy to peanut, cat and *Aspergillus*. This finding is accompanied by increased in the expression of basophil spleen tyrosine kinase (Syk), an enzyme essential for IgE-mediated histamine release and can be used

as a potential biomarker for monitoring the clinical efficacy of omalizumab (7). The utility of basophil activation test as a biomarker of efficacy immunotherapy not only includes the use of surface markers such as CD63 and CD203c, but also more novel markers such as diamine oxidase (DAO), which can bind to intracellular histamine in basophils and mast cells. One of the earliest studies reporting the use of basophil histamine release as a novel biomarker of grass pollen allergen immunotherapy was described in 2015 (8). The study showed a superior clinical correlation of measuring histamine release by basophils using fluorochrome-conjugated DAO when used in combination with basophil surface markers. Additional studies have shown the use of this approach as a measure of the efficacy of grass pollen subcutaneous and sublingual immunotherapy treatments. Following two years of subcutaneous or sublingual allergen immunotherapy, both treatments suppressed grass pollen-induced basophil responsiveness as indicated by surface CD63 expression (9). Measurement of CD63 expression on basophils as an indication of allergen threshold sensitivity has also been reported in other immunotherapy models. In a study involving birch pollen immunotherapy, basophil sensitivity response was found to be reduced in a time-dependent manner (10). The study also reported that this decrease correlated with serum IgG antibodies level, as well as an improvement in clinical symptoms.

While these previous studies have confirmed basophil responsiveness as a useful biomarker of AIT efficacy, findings from the study by Hoffman et al. published in this issue highlight the importance of using basophil sensitivity in the context of “time” as a potential candidate of predicting biomarker of AIT outcome. The current study by Hoffman et al., not only re-validates the aforementioned studies but expands the current understanding of basophil responsiveness as a tool to predict responses to AIT.

The burden of AIT on the healthcare costs can become very high in the absence of proper patient selection as well as monitoring tools, especially with approximately \$11 billion annual medical costs of allergic rhinitis that affects up to 40% of the population in the United States, with inconsistencies in patient compliance observed in up to 15% of patients (11). One can hypothesize that this non-compliance can be the result of reduced efficacy of therapy. As functional basophil assays become more widely available to better select and monitor patients starting allergen immunotherapy,

reduction in the cost of care is expected. Having said this, however, further studies are still warranted to fully implement the use of BAT in routine clinical practice. Disease monitoring is an essential part of management in many areas of medicine; glucose monitoring in diabetes, Holter monitoring in arrhythmias, and thyroid hormone levels in hypothyroidism, to name a few. Such monitoring, however, has not lived up to what it should be in allergic and immunological disorders. An example is an overall lack of monitoring antibody deficiency patients in the clinic, using flow cytometry, for progression into combined immune deficiency. As new forms of allergen-specific therapies are entering the market, physicians need better guidance on patient selection. This, in turn, can not only tie into better clinical outcomes but also impact on the cost of healthcare that can extend to reduced drug pricing.

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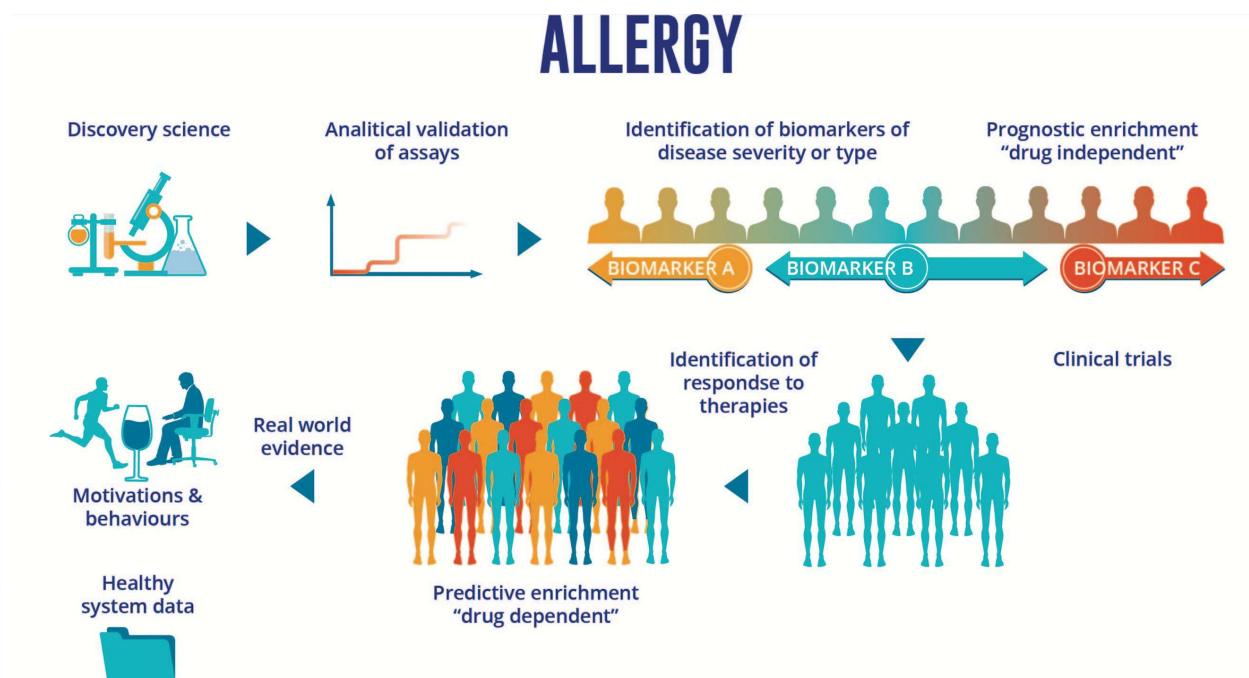
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Figure 1



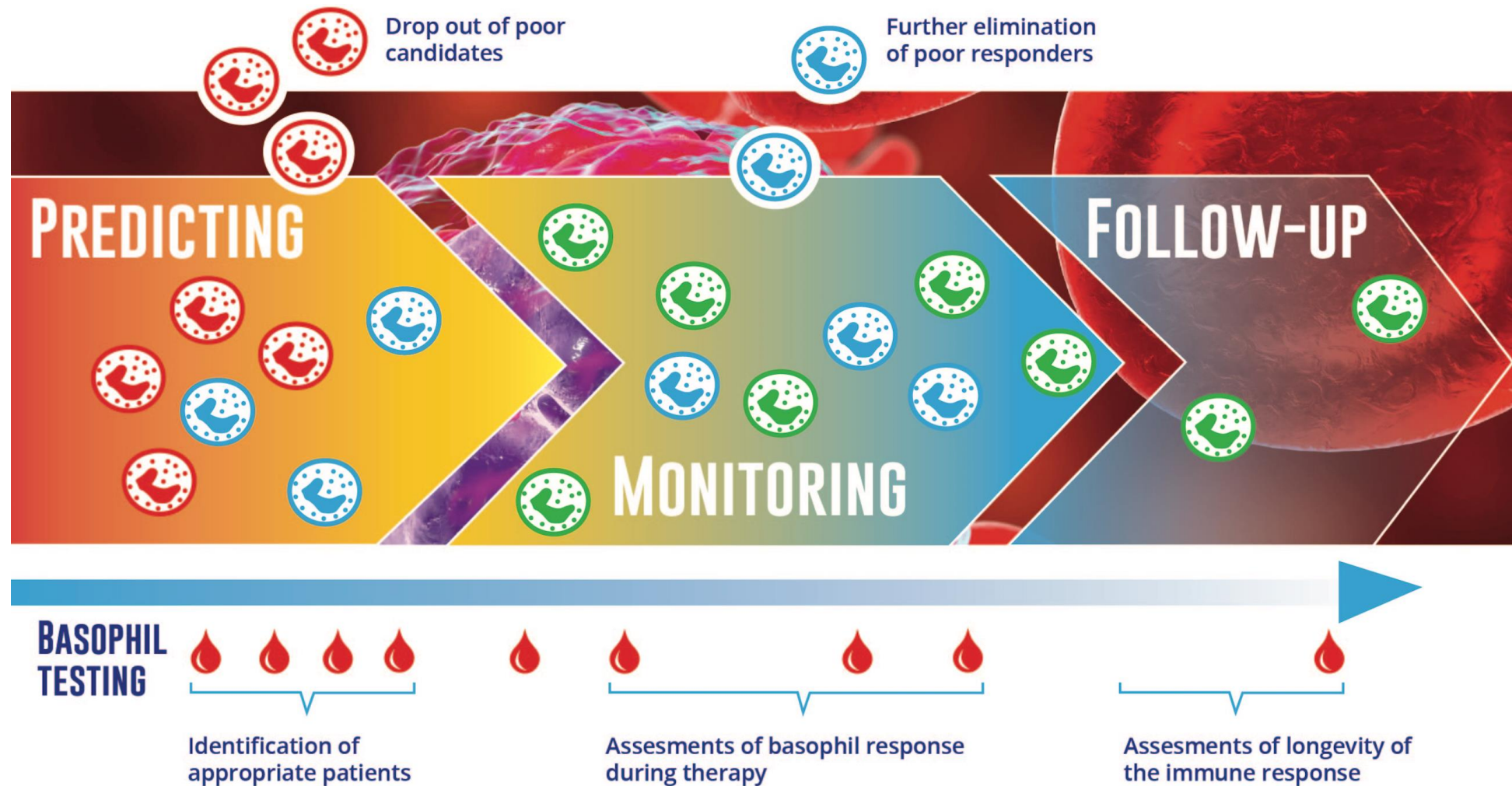


Figure 2: Keeping track of patients on Allergen Immunotherapy. The utility of BAT in patient selection, immune monitoring, predicting response and immune tolerance induced by allergen immunotherapy remain to be further validated.