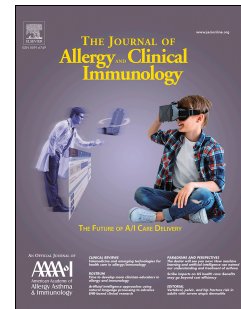


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# Transcriptomic drug-response gene signatures are informative for the stratification of patients for clinical trials

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Asthma is a chronic heterogenous inflammatory disorder of the airways with mixed lymphocytic and myelogenous infiltrates. Cellular characterisation of this disorder has identified 4 phenotypes; eosinophilic (T2), neutrophilic (T1), mixed eosinophilic/neutrophilic and paucigranulocytic (1). Supporting this classification patients can also be stratified, at a molecular and transcriptional level, into T2-high, T2-low and/or T1 based on their gene signatures (2)(3). Collectively, CD4<sup>+</sup> T-helper-2 lymphocytes (Th2 cells) and group-2 innate lymphoid cells (ILC2), regulate the T2 allergic inflammatory response through the secretion of cytokines that modulate the interleukin-(IL)-5/eosinophil and the IL-4/IL-13/IL-4 receptor axes. T1 asthma is more complex with both Th1- and Th17-like (T17) pathways linked to the regulation of innate immune responses, which orchestrate neutrophilia and macrophage activation in severe disease (4).

The classification of Th responses into subtypes has significantly aided in our understanding of the host response to infection, in the pathogenesis of chronic inflammatory conditions and for the development of approaches to new treatments. Indeed, biologics targeting the IL-5/eosinophil, IL-4R-alpha and IgE pathways have significantly improved the management of T2 asthma. However, patients treated with these biologics still exacerbate indicating that alternative pathways may operate, which may be linked to the type of exacerbation trigger (e.g., allergen, viral, bacterial or fungal infection). By contrast there are currently no specific treatments targeting T1 asthma, which is often resistant to corticosteroid therapy. Notably using bronchial biopsies from mild to severe asthmatics Bullone et al., identified a neutrophil high subtype in 30% of patients (4). These patients had a significant T17 signature with increased levels of IL-17A<sup>+</sup> and IL-22<sup>+</sup> cells and IL-17F protein (also known as IL-25). The increased levels of these factors correlated with elevated levels of airway neutrophils. It is worth noting

that Th17 cells do not always express IL-22 and there is also a distinct lineage of IL-22 producing T-helper cells (Th22 cells) (that do not express IL-17). This further adds to the complexity of the regulation of non-T2 asthma. Early attempts at therapeutic intervention showed a benefit from azithromycin, a macrolide antibiotic, in non-eosinophilic asthma (5). By contrast blocking the actions of IL-17 did not improve asthma (6), however these patients were not stratified into the non-eosinophilic subtypes of asthma.

So how do we advance our understanding of the pathogenesis of asthma and identify treatable traits at a molecular level, i.e. asthma endotypes? Presentation of atopic dermatitis (AD) (initially T2/IgE) can often be a predictor for the development of asthma (the atopic march (AM)). The concept of the AM suggests that the pathognomonic process in the skin may also reflect the origins of T2 inflammation at other tissue/mucosal sites. The IL-22/Th22 pathway is prominent in AD and is also found in asthma. Further, the emergence of IL-22-producing T-cells that promote airway inflammation and airway hyperresponsiveness following antigen challenge in epicutaneously sensitized mice, suggests a direct role for T-cell derived IL-22 in driving the AM (7).

In this issue Badi et al use an established cohort of AD patients to interrogate the transcriptome (Fig 1) of lesional and non-lesional skin biopsies (8). Gene signatures were obtained before and after 12 weeks of treatment with the anti-IL-22 inhibitor Fezakinumab. In those patients clinically responding to therapy a differentially expressed set of genes were identified. The authors then employed Gene Set Variation Analysis (GSVA) to generate enrichment scores (ES) of the AD and AD-Fezakinumab response signatures in their U-BIOPRED asthma cohort. This cohort contains patients stratified by transcriptome associated cluster (TAC) into 3 groups: TAC1, a T2 high gene expression signature with high levels of sputum eosinophils; TAC2, with increased expression of inflammasome associated pathways and high sputum neutrophilia; and TAC3, characterised by high levels of macrophages and predominantly paucigranulocytic. Notably, the AD proinflammatory gene signature (112 up-regulated genes) contained genes associated with Th2 and Th17/Th22 pathways, and these factors were enriched in the sputum and blood of asthmatics with increasing disease severity. Further the AD gene signature was most prominent in those patients characterised as neutrophilic or mixed granulocytic subtypes. Similar results were found after analysis of a second asthma cohort, known as the Airway Disease Endotyping for Personalized Therapeutics.

Treatment with Fezakinumab resulted in 296 down regulated genes. This gene signature was enriched in asthmatic blood, and again was most prominent in the sputum of neutrophilic and mixed granulocytic subtypes. Gene pathway analysis demonstrated a correlation between the AD-Fezakinumab gene signature and the IL-22/Th22 gene signature. The ES of the IL-22/Th22 signature significantly correlated with the AD-Fezakinumab down ES in asthmatic sputum, bronchial and nasal brushings, and blood cells. Importantly protein analysis demonstrated that sputum IL-22 levels were increased in TAC2 asthma compared to levels in TAC1. Increased levels of IL-22 have been previously reported in the bronchoalveolar lavage fluid and serum in severe asthma, and enhanced staining for IL-22 positive cells was found in the airways of neutrophilic asthmatics. However, while the IL-22/Th22 gene signature contains 16 genes that are regulated by IL-22 and is indicative of a contribution from Th22 cells (e.g. expression of CCR10) it cannot be discounted that other IL-22 producing cells (Th17 cells, gamma/delta T cells, ILC3 and neutrophils) do not contribute; as specific markers for Th22 cells have yet to be firmly delineated. The identification of Th22-specific markers and unique druggable targets to modulate Th22 function in non-T2 asthma patients will likely require single-cell multi-omics approach including ScRNA-seq and CyTOF. Furthermore, assigning T-cell phenotypes to

asthma subtypes is further complicated by the plasticity of Th cells in inflamed tissues; particularly following repeated antigen exposure and during exacerbations. Indeed the Th17 and Th22 lineages exhibit bi-directional plasticity in models of infection. Badi et al data also demonstrates that other factors associated with the IL-1 family and IL-17 pathways are enriched and are possibly other targetable pathogenic pathways for TAC2 asthma.

In this paper Badi et al provide excellent proof-of-principle data on how a transcriptomic drug-response gene signature at the site of lesions in one inflammatory condition can be employed to stratify patients with another inflammatory condition. Thus, identifying potential responder groups for subsequent clinical trials. Failure to stratify patients can result in false-negative clinical trials and early discontinuation of drug development. Examples of this include early trials of mepolizumab (9) and a negative trial with brodalumab (anti-IL-17 receptor mab) in asthma (6). Was the latter study a false negative? Badi et al approach may be particularly relevant to diseases that have similar aetiological origins and pathological evolution. For example, both AD and asthma are identified as predominantly T2 diseases early in life, however due to infection they may become more T1/T17 and neutrophil dominant. As disease progresses the T2 response may wane due to negative regulation by T1/T17 inflammatory programs. It also raises the possibility of the need to target multiple pathways to provide optimal disease control.

It is important to note that IL-22 is a bi-functional cytokine that can have either pro-inflammatory or tissue protective roles depending on cellular source, inflammatory context and cytokine milieu. In models of T2 asthma IL-22 has been shown to be pathogenic and protective (10). The factors which determine the functional role of IL-22 and Th22 cells are yet to be fully delineated. It will be very interesting to determine the therapeutic efficacy of Fezakinumab for the treatment of severe neutrophilic asthmatics and potentially for exacerbations that are not attenuated by current type-2 biologics.

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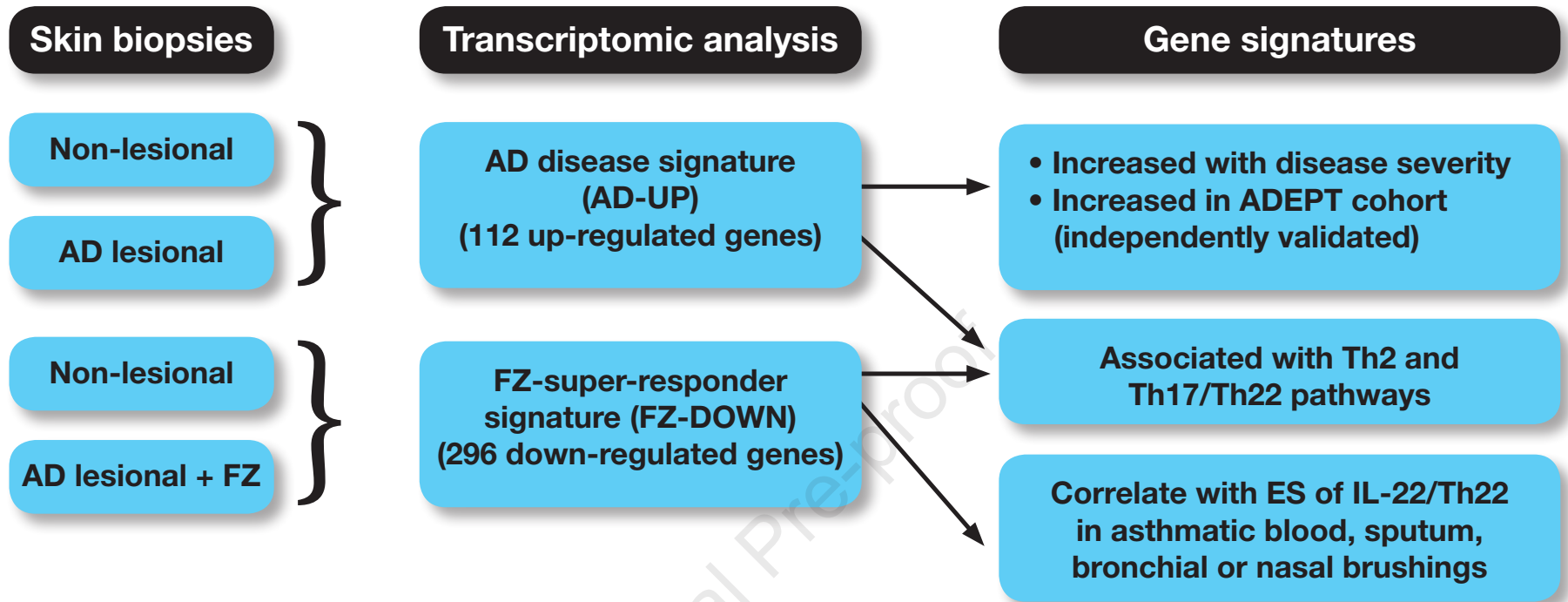
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**FIG 1.** Transcriptomic drug-response gene signatures in atopic dermatitis (AD) identify specific asthma cohorts for clinical trials. A. AD Gene signatures were obtained before and after 12 weeks of treatment with the anti-IL-22 inhibitor Fezakinumab (FZ) and differentially expressed sets of genes were identified in clinically responding patients. The AD proinflammatory gene signature (112 up-regulated genes) contained genes associated with Th2 and Th17/Th22 pathways, and these factors were enriched in the sputum and blood of asthmatics with increasing disease severity. B. Gene Set Variation Analysis (GSVA) was employed to generate enrichment scores (ES) of the AD and AD-FZ response signatures in the U-BIOPRED asthma cohort and confirmed in the Airway Disease Endotyping for Personalized Therapeutics (ADEPT) cohort. The analysis identified transcriptome associated cluster (TAC)-2 and neutrophilic asthma as a potential stratified asthma cohort for examining the therapeutic benefit of FZ treatment. HC; healthy control, P; pauci-granulocytic, E; eosinophilic, N; neutrophilic and M; mixed.

A



B

