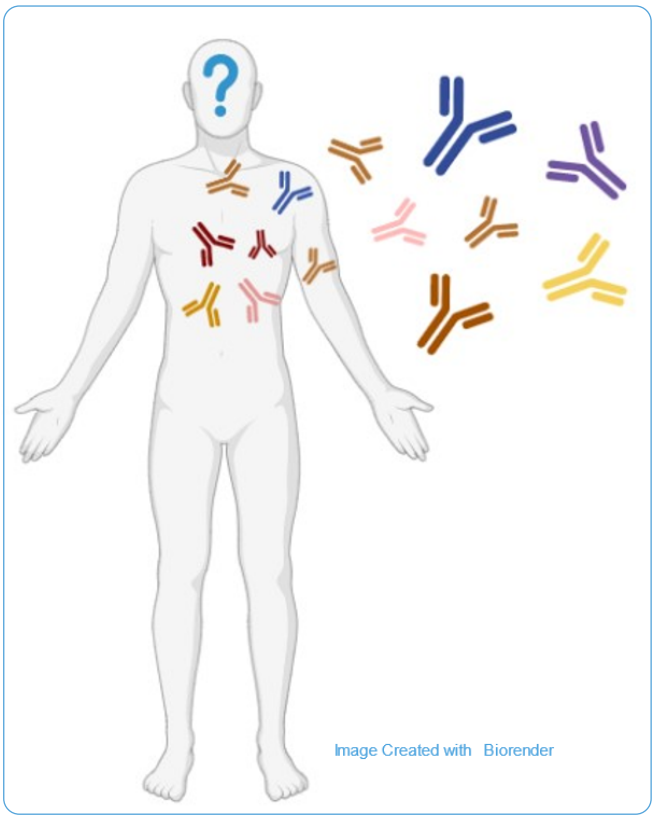
Antibodies: Immunoprofiling to decode the information content in the humoral immune system

Chronic diseases such as autoimmune, cancer and neurological diseases are complex, difficult to diagnose, and problematic to treat due to their inherent heterogeneity. As a result, patient responses to current therapeutics are highly variable, with many not responding or exhibiting severe adverse reactions.

Biomarkers can be valuable tools to aid in early detection, disease endotyping, stratifying responders and non-responders, predicting adverse outcomes, and enriching clinical trials. While any objectively measured biological molecule could be a biomarker, antibodies are a direct measure of disease as compared to frequently employed measures such as DNA, RNA and proteins which could be the result of any process occurring in the body. Antibodies are an early and direct consequence of illness. Decoding the information content in the humoral immune system with antibody immunoprofiling offers direct insight into the disease state.

Antibodies are ideal biomarkers because they are direct manifestations of disease, occurring early, often before symptoms, and persisting through the duration of the disease. Self-proteins can become autoantigenic when disease-associated changes occur, such as genetic mutation or ectopic expression. Unexpected post-translational modifications, splice variants and neoantigens, among other variations, can also be autoantigenic. For example, diseases such as cancer and ALS activate aberrant host protein expression, initiating the production of antibodies. TDP-43 tangles result in antibody production in ALS (Conti et al., 2021), and tumor associated antigens in cancer can become autoantigenic. (Aziz & Blackburn, 2018; Sexauer et al., 2022). Antibodies appear early, are target specific, abundant, and easy to obtain from sera. In fact, antibodies could flag illness much earlier than many current diagnostic tools. In 2003, Arbuckle et. al. examined blood samples from U.S. Military personnel diagnosed with lupus and discovered antibodies present in the sera up to 9 years before the lupus diagnosis (Arbuckle et al., 2003). Antibodies also demonstrate great prognostic potential (Bizzaro, 2007; Kathrikolly et al., 2022; Zaenker & Ziman, 2013). Patel et. al. (Patel et al., 2022) conducted an antibody screen in a cohort of non-small cell lung cancer patients, identifying a panel of 13 antibodies highly predictive of poor 5-year survival rates.

Chronic diseases effect multiple organs. The humoral immune system is always surveilling the entire body and produces antibodies associated with disease expressed



in different tissues. Because the antibody repertoire varies across individuals, antibody panels – combinations of predictive antibodies - yield higher diagnostic and prognostic value compared with single antibodies, DNA or RNA (Damoiseaux et al., 2015; Kathrikolly et al., 2022). Further, antibodies may also uncover potential disease related protein pathways useful for target deconvolution and drug discovery.

Antibodies are highly specific, recognizing small discontinuous antigen epitopes (Barlow et al., 1986; Muro et al., 1994). Disease and antigen specificity makes them excellent biomarkers. Therefore, in order to best capture antibodies, protein arrays require full length, properly folded proteins that display specific, biologically relevant epitopes. The Sengenics i-Ome protein microarray utilizes the patented KREX protein folding technology to print more than 1600 correctly folded proteins on an array. This technology not only accurately and reliably identified a novel panel of 13

antibodies correlating with poor survival among non-small cell lung cancer patients, but also demonstrated high reproducibility. In a second cohort, the same panel exhibited no significant difference from the original cohort while retaining high specificity and sensitivity.(Patel et al., 2022).

Because the humoral immune system is constantly surveilling the body and marking out changes with antibodies, immunoprofiling can provide valuable information about the disease state and serve as an important source of biomarkers.

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