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RAPID COMMUNICATION

Could a patient's prior immune history impact your future clinical trial?

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What if it were possible to survey a patient's own unique antigen-specific immune history versus the entire human proteome or the universe of all known viruses? What could you discover?

Today's most heralded advances in immuno-oncology work by manipulating systemic immunologic processes with little or no knowledge of the specific underlying molecular features visible to a patient's own unique and evolving adaptive immune system. However, evidence is growing that baseline or post-treatment antigen-specific immunity may predict the success of immunotherapies or even predict the likelihood of autoimmune adverse events. What if it were possible to survey a patient's own unique antigen-specific immune history versus the entire human proteome or the universe of all known viruses? What could you discover?

The antibody fingerprint: impacting cancer immunotherapy
On average, >20% of the entire human proteome is targeted by a very unique fingerprint of baseline autoantibodies in healthy individuals[1]. This landscape of autoantibodies is very specific to the individual person, can remain stable for many years, and contains unique features reported in association with cancer, autoimmunity, infection, and neurologic conditions[1-8]. Women harbor more autoantibodies than men, and cancer patients more than healthy people[1]. Such pre-existing or "natural" baseline antibody landscapes are thought to be the result of prior adaptive immunity to similar peptide mimics found in commensal microbes, foods, environmental exposures, infections, and autologous proteins. Alterations in the microbiome – thought to be a major source of this baseline antibody fingerprint – are well known to have a dramatic impact on the outcomes of cancer immunotherapy[9-10]. Could important impacts of the microbiome on antigen-specific immunity be visible via an imprint on serum immunoglobulins? It has already been hypothesized that similar cross-reactive microbial T cell immunity creates baseline recognition for some viruses[11]. Mark Davis and colleagues have reported that adults often harbor HLA-restricted memory T cells recognizing viral peptides from diseases they have never been exposed to[12].

Baseline autoantibodies can directly manipulate immune signaling to have dramatic associations with clinical outcomes. A survey of AIRE-deficient patients reported in Cell demonstrated that some patients harbored unique high-affinity neutralizing autoantibodies to type 1 interferons - especially IFNα[3]. Those patients with the naturally occurring IFNα neutralizing autoantibodies were protected from type-1 diabetes – a major complication of AIRE-deficiency[3]. This study demonstrates that major changes in global immunity can be created by natural antibodies that inadvertently target and manipulate immunologic pathways. Could similar mechanisms explain why some patients fail checkpoint-blockade immunotherapy? A recent report using CDI HuProt™ arrays has already shown that preexisting

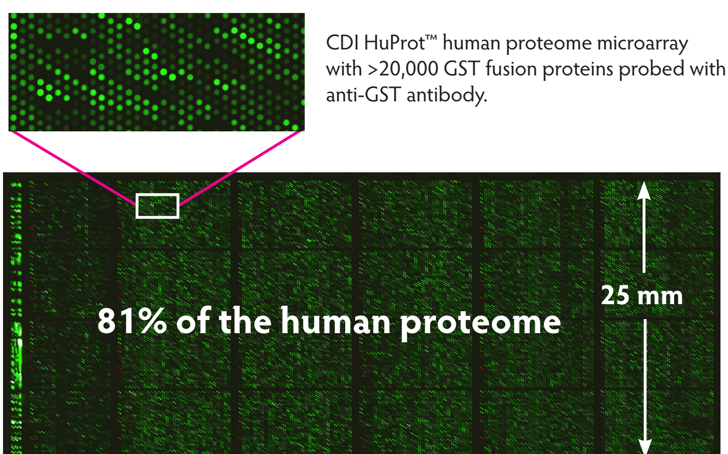

Figure 1: You won't get antigen-specific data from TCR sequencing. It isn't practical to run thousands of parallel T cell assays. But what about antibodies? The technology is already available to perform seromic profiling with technologies like HuProt™.

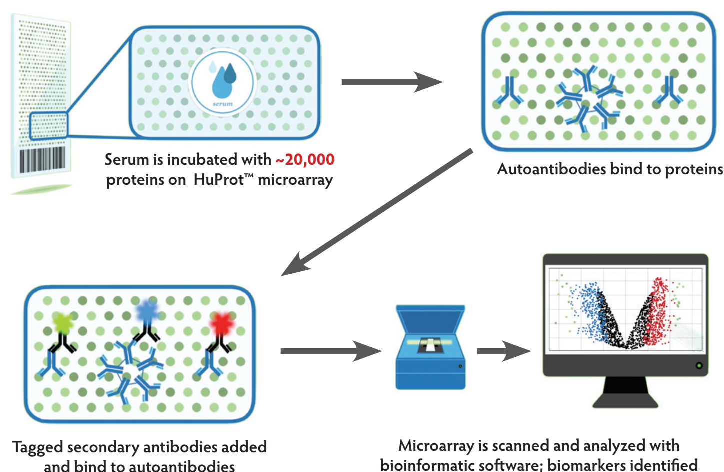

Figure 2: High-throuput serum profiling workflow using the HuProt™ human proteome microarray.

autoantibodies to specific proteins – some of which are cytokines – can identify patients more likely to have severe autoimmune side-effects from checkpoint blockade immunotherapies[13].

IgG autoantibody may aid in priming antigen-specific anti-tumor CD8+ T cells, sculpting the T cell repertoire to approximately mirror background antibody. This was clearly demonstrated in a clinical paper

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where patients receiving anti-EGFR (Cetuximab®) antibody developed new CD8+ T cell responses to EGFR peptides through increased antigen-uptake and cross-presentation by antigen-presenting cells via Fc receptors[14] In a more natural setting, another group demonstrated that in archived blood samples from patients eventually diagnosed with lung cancer – antibodies could be seen to lung cancer proteins years before diagnosis. After diagnosis – those patients harbored antigen-specific T cells that recognized HLA-restricted peptides from those proteins[15]. Finally in a mouse model, animals had stronger baseline IgG antibody signals to neoantigen peptides than their wild-type counterpart peptides – and were more likely to develop MHCI-restricted CD8+ T cell responses to peptide and live tumor if those antigens had stronger baseline IgG antibody signals[16]. Together, these data suggest that some features of T cell immunity – including CD8+ immunity – may be indirectly observable via antibodies as a surrogate.

Antibodies can directly help kill tumor cells via antibody-dependent cell-mediated cytotoxicity (ADCC) – directly impacting therapeutic outcomes. Most clearly visible via therapeutic antibody therapies such as anti-HER-2 (Trastuzumab®), ADCC is antibody-bound Fc-receptor directed killing of tumor cells – often aided by macrophages, monocytes, eosinophils, or natural killer cells directed via Fc receptors. This process is well established, varies in strength across antibody isotypes, and can be strongly directed against cancer by isotypes such as IgE that are less commonly screened for in cancer studies[17].

Insights into antibody analytics

HuProt™ Arrays are made of folded 3-dimensional proteins – but your epitopes of interest may be linear or internal to the protein. It is possible to denature the arrays with urea and DTT without damaging the amino acid sequences. This allows for screening for additional linear epitopes on a denatured protein array[18].

HuProt™ Arrays are made of folded 3-dimensional proteins – some of which are members of protein complexes – and may have protein-protein interactions with other proteins in your serum. This means you may have false hits from antibodies bound to a complex or other interacting protein binding to printed target – and not antibodies directly binding the target itself. Comparing folded to denatured array assays can help control for this, and eventually bioinformatics tools may be developed to tease out these interactions. Profiling of interacting partners and membership in such complexes is rapidly improving by mass spectrometry[19].

Your target antibody of interest may be in a different antibody subtype – or present as a target visible via a ratio of those subtypes. IgG1 is the most common of the circulating antibodies and perhaps the isotype most likely to aid T cell priming, but IgG2 secretion is thought to be triggered by cytokines more relevant to T cells and immuno-oncology such as IFNγ and IL-2[20] – suggesting a ratio between the two may lead to deeper antigen-specific insights into the type of immunity these antibodies target. Perhaps your result on interest is only visible in IgM, IgE, or IgA. HuProt™ arrays can scan for all of these isotypes simultaneously in a single assay – all it takes is additional secondary antibodies and channels on your chip reader.

Forward strategies using existing data

Is there public data you could integrate with your antibody profiling? The Cancer Genome Atlas and MSK-IMPACT have made broad mutation and mRNA expression profiling databases available to researchers on http://www.cbioportal.com[21]. Have you tried aligning what is known about your tumor of interest broadly – or even data from your specific patients – alongside seromic profiling?

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