The Immuno*Signature*™ as a Tool for Autoimmune Disease Diagnosis, Therapeutic Response Prediction, Monoclonal Antibody Characterization and Biomarker Discovery

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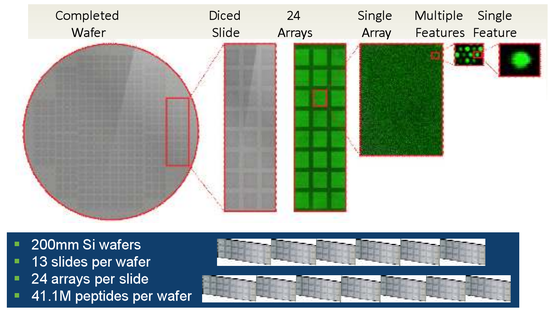
Introduction

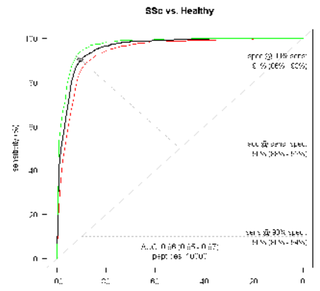
An individual's Immuno*Signature*—the quantitative profile of antibody binding specificity—varies over time due to environmental and genetic influence, acute and latent infection, disease and cancer status, as well as treatment with immunomodulatory therapeutics. This multifactorial yet fine-tuned B cell response allows for the diagnosis of complex biological states not amenable to traditional diagnostic approaches, such as the identification of autoimmune disease, prediction of response to therapeutic agents or their associated adverse events, and cancer progression. Here, we demonstrate that HealthTell's unique approach to peptide microarray fabrication, combining silicon wafer-based photolithographic methods with quality controlled peptide synthesis, permits high-throughput ImmunoSignaturing with exceptional scalability, reproducibility and accuracy, making it broadly-applicable to diagnostic, clinical and discovery applications. These peptide sequences broadly cover amino acid chemical space, representing linear epitopes and conformational mimotopes without bias towards a single organism's proteome, allowing the Immuno*Signature* to recapitulate in vivo binding and identify specific protein targets. Additional examples presented here include this application of the Immuno*Signature* to biomarker identification, as well as to the unsupervised prediction of the protein targets and binding epitopes of monoclonal antibodies.

Photolithography-based peptide array synthesis



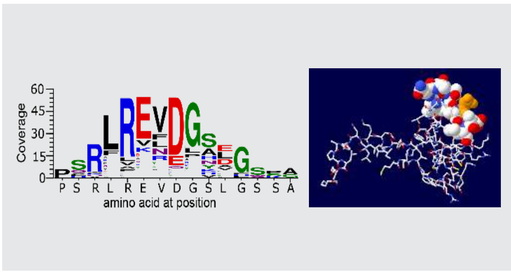
Peptide arrays are synthesized on silicon wafers using BOC chemistry, with photolithography-directed deprotection, to generate spatially-indexed micron-scale features with defined amino acids sequences. Wafers are diced into slides that can be reassembled into an SBS 96-well footprint, allowing for assay automation with standard liquid handling tools and robotics. Excess regions of the wafer are used for quality control, with features suitable for routine MALDI-based peptide analysis, as well as ellipsometry-based surface thickness change determination.

Immuno*Signature*-based discrimination between biological states:



The unique combination of increases and decreases in peptide binding allows a subset of features to discriminate between any biological contrast that yields a consistent antibody response, including:

- *Disease vs healthy*
- *Disease severity*
- *Adverse event*
- *Disease subtypes*
- *Drug response*
- *Progression/relapse*



Biomarker discovery, target discovery, epitope mapping and ant body specificity:

Alignment of peptides bound by a monoclonal can, for linear epitopes, predict the protein target de novo. Likewise, key contact regions of a conformational epitope can be predicted based on pairwise-alignment of bound peptides.

For serum samples, peptides that contrast between two different biological states can be aligned to the proteome to discover biomarkers for that contrast, which have the potential to be therapeutically-relevant.

Monitoring Immune Response

In a longitudinal study of influenza vaccine response, following IRB approval, serum was collected from 17 volunteers from Arizona and California at various timepoints from two weeks before vaccination through two months post-vaccination. 134 array peptides increased in intensity following vaccination in most volunteers, as shown in the Figure to the right.

These results are in concordance with studies[1,2] that rely on the much more laborious and inconsistent Hemagglutination Inhibition (HI) assay. In these studies, a typical vaccine response is a ~4-fold increase in HI activity in ~two-thirds of patients.

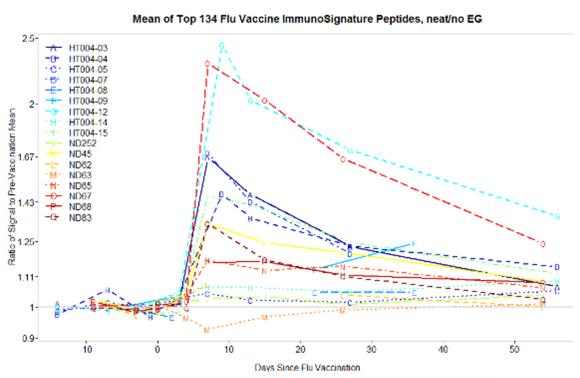
However, unlike the HI functional assay which requires serial dilutions and overexpressed HA, the Immuno*Signature* simultaneously measures antibodies against all flu proteins, while providing sequence info and requiring only patient serum in a single well.

Thus, the Immuno*Signature* assay could be used for novel vaccine development for diseases in which a functional assay has not been developed, or in which likely immunogens are unknown. No a priori knowledge is required to determine if a vaccine prep elicits an immune response.

[1] Sacadura-Leite E1, Sousa-Uva A, Rebelo-de-Andrade H. Vaccine. 2012 Jan 5;30(2):436-41 Epub 2011 Nov 4.

[2] Wright PF1, Sannella E, Shi JR, Zhu Y, Ikizler MR, Edwards KM. Pediatr Infect Dis J. 2008 Nov;27(11):1004-8

Response to flu vaccine

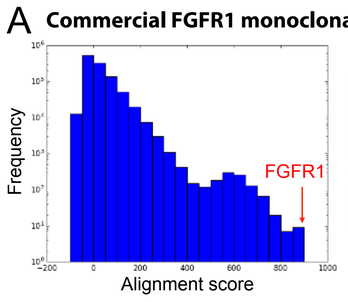


Pre- vs post-vaccination discrimination

AUC = 0.90 (0.86-0.94)

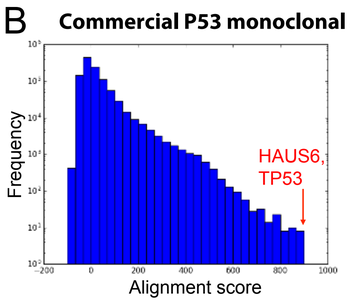
De Novo Antibody Target Identification, Specificity and Epitope Mapping

A Commercial FGFR1 monoclonal antibody



Protein	Epitope start	Score
FGFR1	820	900.4757
FREM1	610	881.7643
ZC3H4	550	867.873
NOX4	240	867.5873
PRDM4	220	865.7568
ARFGEF1	230	859.5755
ABCB11	700	858.2205
HN1L	180	854.8035
DNAH1	710	850.7618
TBC1D2	230	850.7296

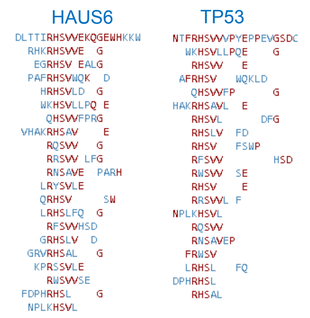
B Commercial P53 monoclonal antibody



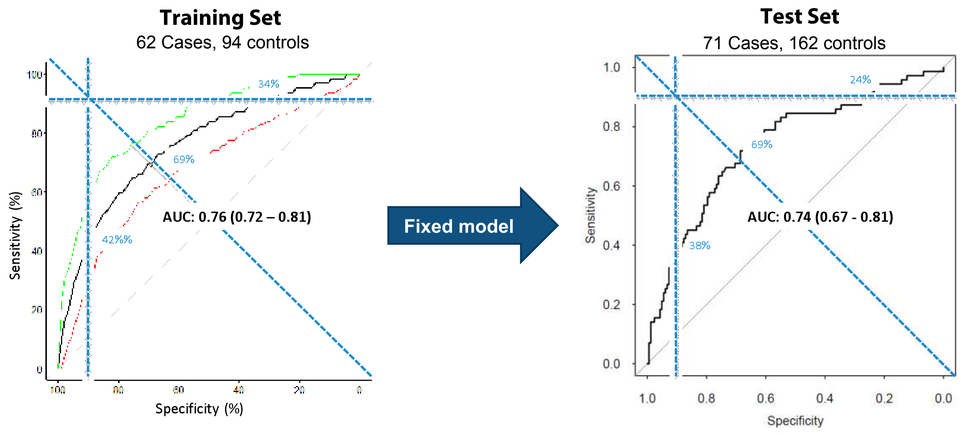
Protein	Epitope start	Score
HAUS6	360	1078.809
TP53	170	973.4539
TRMT61A	40	940.2552
NMRK1	80	935.122
NMRK1	70	931.6747
ZNF695	80	929.9102
LARP1	950	910.9402
TP53	160	897.1248
LARP1	960	883.9961
OBSCN	7820	879.0043

The target of a monoclonal antibody can be identified with no a priori knowledge by aligning bound array peptides to the proteome, with corrections for array and proteome composition as well as repeated motifs. In (A), the array peptides bound by a commercial FGFR1 antibody are aligned to the human proteome, yielding the histogram of alignment scores show, with detailed results for the top 10. An epitope near the C-terminus of FGFR1 shows the highest alignment score, matching the antibody's immunogen.

Similarly, in (B) array peptides that bind a commercial P53 antibody were aligned to the human proteome. However, the known target is actually the second hit—the top hit is a nearly identical epitope within the protein HAUS6. This suggests that non-specific binding of this antibody to HAUS6 could be a concern.



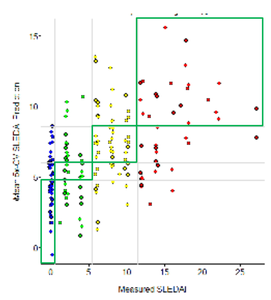
Ovarian Cancer Versus Benign Mass in a Blinded Study



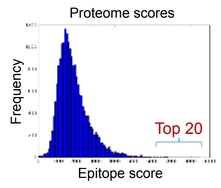
Two different sets of ovarian cancer and control (benign, surgical, healthy) serum samples were obtained: the first set is non-blinded and was used to train a Support Vector Machine classifier to create an Immuno*Signature* that differentiates cancer from benign masses. The second set is blinded—HealthTell reported predictions to a third party and received an AUC score. Performance on the blinded set matched performance on the training set, with this performance rivaling many new biomarkers under evaluation in the literature.

Lupus (SLE) Disease Activity and Biomarker Identification

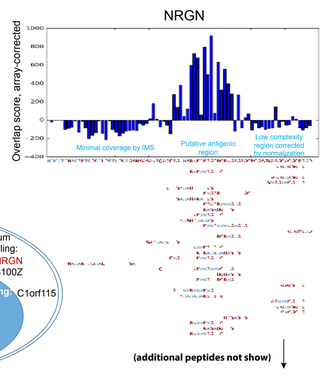
A Cross-validated Predictions of Elastic Net Model Using 76 Peptides



B Distribution of Mappings to the Proteome



Peptides Mapped to NRGN



A non-blinded serum sample set was used to develop an Immuno*Signature* that discriminates Lupus patients from healthy volunteers[3]. As shown in (A), this model has equivalent performance in a blinded testing set. Within the Lupus patient population, a number of disease subtypes and severities exist, as commonly measured by a SLEDAI score. Immuno*Signature* peptides recapitulate this score (B), while providing orthogonal information. Additionally, as summarized in (C), the Lupus Immuno*Signature* peptides can be mapped back to the human proteome, yielding known and potentially novel disease biomarkers and entry points for therapeutic intervention

[3] Chaim Putterman, Michael Rowe, Joseph Barten Legutki, Theodore M. Tarasow, Kathryn Sykes. 2016 ACR/ARHP Annual Meeting, September 28, 2016

Autoimmune Disease Identification

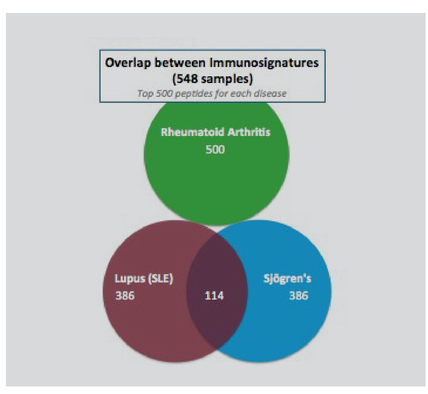
Classification Performance

Autoimmune Disease	Contrast	AUC (95% CI)
Lupus (SLE)	Healthy	0.95 (0.93-0.97)
Sjogren's Sbdroome	Healthy	0.92 (0.9-0.94)
Rheumatoid Arthritis	Healthy	0.97 (0.96-0.98)
Ulcerative Colitis	Healthy	0.84 (0.8-0.88)
Scleroderma	Healthy	0.96 (0.95-0.97)
GAVE 2° to Scleroderma	Scleroderma without gastric involvement	0.77 (0.64-0.84)
Scleroderma	Dermatomyositis	0.77 (0.74-0.8)
Sjogren's Syndrome	Lupus (SLE)	0.98

Sera samples from patients with a variety of autoimmune conditions, as well as healthy volunteers, were used to develop diagnostic Immuno*Signatures*.

100 iterations of 5-fold cross-validation were used to evaluate Support Vector Machine models of disease versus healthy. The AUCs for this discrimination, with associated confidence intervals, are shown in the table to the left.

Thus, the Immuno*Signature* is a powerful and broadly applicable method for the diagnosis of complex disease states.



Conclusions

HealthTell has developed a novel Immuno*Signature* technology that has the ability to quantitatively profile the immune system's response to various diseases from a very small volume of blood, plasma, serum, or many other biological fluids. Drug development and diagnostic applications include:

Diagnostic

- Disease Diagnosis
- Disease Severity
- Disease Progression and Relapse
- Drug Response
- Patient Stratification
- Adverse Event Prediction

Therapeutic Discovery

- Monoclonal Characterization
- Target Identification
- Epitope Mapping
- Antibody Specificity