Utilizing the Autoantibody Immune Response to Tumor Antigens for Kidney Cancer Early Detection

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Background: Kidney cancer (renal cell carcinoma, RCC), the 8th most common U.S. cancer, is in need for better cure rates through early detection (5-year survival for stage I RCC: ~95%; for stage IV RCC ~19%). Autoantibodies are common in cancer and result from the altered expression, localization, or post-translational modification of endogenous proteins in tumor cells (autoantigens) and from the expression of mutated genes that give rise to new proteins (neoantigens). In contrast to cellular immune responses in cancer, autoantibodies are less well characterized, yet hold promise to enable cancer early detection by immune amplification of the 'cancer signal' while retaining specificity to cancer types including RCC. Autoantibodies may therefore be useful for kidney cancer early detection and diagnosis.

Objective: Our goal was to profile the autoantibody repertoire in blood from patients with clear cell RCC (ccRCC), the most common form of RCC, in order to: 1) determine if autoantibodies can be detected in patients with early stage and late stage ccRCC; 2) identify common epitopes amongst ccRCC patients that could suggest common RCC antigens; and 3) determine specificity and sensitivity of potential autoantibody biomarkers for ccRCC vs. other non-cancer conditions.

Methods: We use the SERA platform (https://serimmune.com/publications/) to compare putative autoantibody signal in blood from 154 treatment naïve patients with ccRCC of four stages, 23 with benign kidney lesions, and 1,519 healthy controls who are 41 years old or older. SERA utilizes a random bacterial display 12mer peptide library of 10^{10} diversity in conjunction with next generation sequencing to ascertain epitope enrichment across the entire human proteome.

Results: We find significant differences in epitope repertoires in ccRCC compared to the healthy human cohort. Patients with ccRCC exhibit a rich repertoire of rare, enriched epitopes which may comprise putative autoantibody signal. This epitope signal is present with high abundance in all ccRCC stages, including stage I ccRCC. In contrast, healthy controls or patients with benign kidney lesions demonstrate more restricted repertoires. We are exploring evidence of common ccRCC antigens although our initial results suggest that each patient may develop an individualized tumor-associated antibody response. Whether measuring epitope diversity in a patient's blood could be useful, without needing to identify specific epitopes, warrants further study.

BACKGROUND

Renal cell carcinoma (RCC):

- 8th most commonly diagnosed cancer in the U.S.
- early stage disease curable through surgery
- 40% of patients diagnosed with advanced disease

Ideal biomarker for RCC early detection:

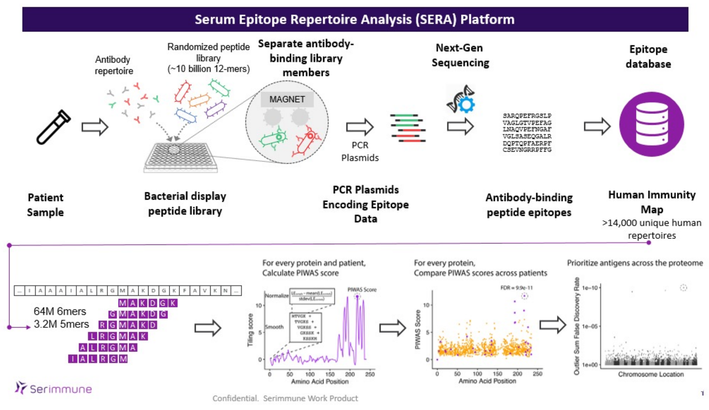
- highly <u>sensitive</u>: detects RCC when tumors are small
- highly <u>specific</u>: detects 'bad-acting' RCC, but not 'good-acting' RCC or benign tumors
- <u>cost-effective</u>: cheap test for screening, ideally population-wide

GOAL

To profile the autoantibody repertoire in blood from patients with clear cell RCC (ccRCC)

- Detect autoantibodies in patients with early and late stage ccRCC
- Identify common epitopes suggestive of common RCC antigens
- Determine sensitivity and specificity of biomarkers for ccRCC

AIMS & STUDY DESIGN



Renal Lesion Patient Characteristics (N = 200)	
Characteristic	**Number**
Age range (years)	41-84
Sex	
Male	132
Female	68
Clear cell RCC (total)	177
Stage I	51
Stage II	8
Stage III	32
Stage IV	63
Benign Renal Mass (total)	23
Oncocytoma	13
Angiomyelolipoma	6
Other	4

Figure 1: **SERA assay and PIWAS method.** The serum epitope repertoire analysis (SERA) assay has been previously published (Haynes et al. 2021). Serum samples were incubated with a bacterial display peptide library, antibody-bound bacteria were selected, and the peptides were amplified by PCR and sequenced. A PIWAS value was calculated per sample per antigen, by 5 and 6-mers enrichment values compared with controls. A sum-of-PIWAS value for all antigens per sample was calculated to estimate the overall autoantigen immune response per sample (Dorff et al. 2021).

RESULTS

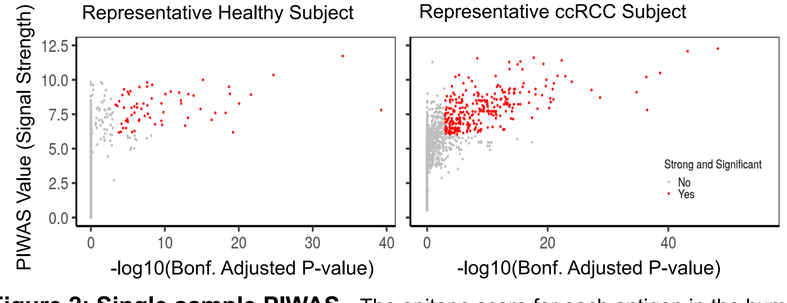


Figure 2: Single sample PIWAS. The epitope score for each antigen in the human proteome for an individual is compared to the scores in a control population (n=1,519). Antigens with significant outlier scores are seen in the upper right quadrant of the graph in red. Comparison between a representative healthy individual (left) and a representative patient with RCC (right) is shown.

RESULTS

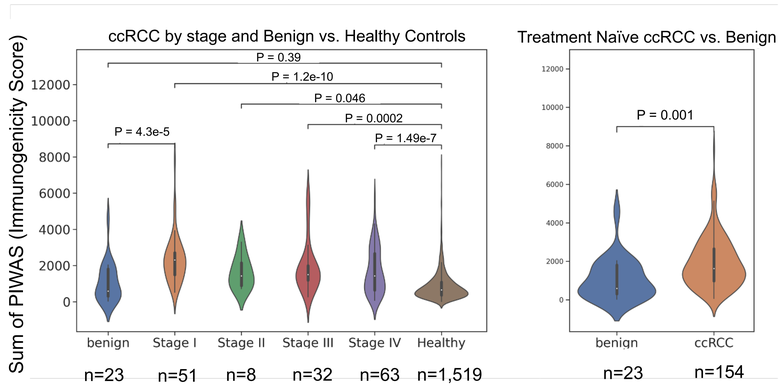


Figure 3: Cohort comparison of Sum of PIWAS scores. For each individual, the sum of PIWAS scores for all antigens that are elevated (PIWAS > 6, which represents a Z-score using a healthy population control) and statistically significant (p-value < .05 with Bonferroni correction) is computed (Dorff et al. 2021). P-values shown in the figure are calculated using two-sided Welch's t-tests. A comparison of scores for cohorts with RCC vs. healthy vs. benign shows a statistically significant elevation of Sum of PIWAS scores for subjects with RCC. This indicates more putative outlier antigen signals for subjects in the RCC cohorts relative to the comparator groups.

CONCLUSIONS

- Our results demonstrate an increased overall epitope signal in putative antigens in subjects with RCC using a Sum of PIWAS metric.
  - This is consistent with findings from previous studies that demonstrate increased immune activity in RCC tumors.
  - The signal for benign tumors appears also to be slightly elevated, but not statistically different than controls.
- To explore immune signal further, we are performing analyses to characterize signal against frameshifted antigens as RCC has been shown to have a high frequency of indels. We are also exploring signal against potential microbiome proteomes.

REFERENCES

- Haynes, W., et al. "Protein-based Immunome Wide Association Studies (PIWAS) for the discovery of significant disease-associated antigens." *Frontiers in Immunology* 12 (2021): 1404.
- Dorff, T, et al. "Phase Ib study of patients with metastatic castrate-resistant prostate cancer treated with different sequencing regimens of atezolizumab and sipuleucel-T." *Journal for immunotherapy of cancer* 9.8 (2021).

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