Interrogating real world tumor-infiltrating T-cell repertoires to identify antigen enriched TCRs in a large pan-cancer clinical cohort

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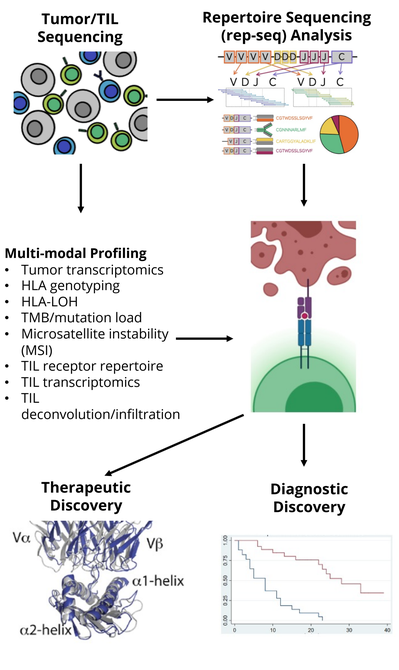
INTRODUCTION

- TCR repertoire profiling can provide a useful window into the complex interactions between tumor cells and infiltrating lymphocytes.
- Despite recent advances in repertoire sequencing methods, the characterization of tumor-infiltrating T-cell repertoires has been limited by access to large, real-world patient cohorts with genomic, TCR, and clinical profiling.
- In this study, we analyzed a large, real-world, clinico-genomic database of over 130k patients - with T-cell repertoire data covering a diverse landscape of HLA genotypes and tumor neoantigens - to identify public TCRs associated with HLA-specific neoantigens and viral epitopes.

METHODS

- TCR sequences were detected using the Tempus xR assay, a hybrid-capture whole exome RNA assay optimized with probes for capture of TCR genes.
- Repertoire profiling reads were aligned, assembled, and annotated against IMGT reference sequences. Analyses focus on TCRβ due to the increased diversity of the VDJ region.
- Neoantigens were identified from paired RNA and DNA samples using the Tempus xT targeted DNA panel covering 648 cancer-associated genes.
- HPV status was determined using a panel of probes to E6 and E7 genes in HPV16, 18 and 33 and captured by both the RNA and DNA assays.

Workflow:

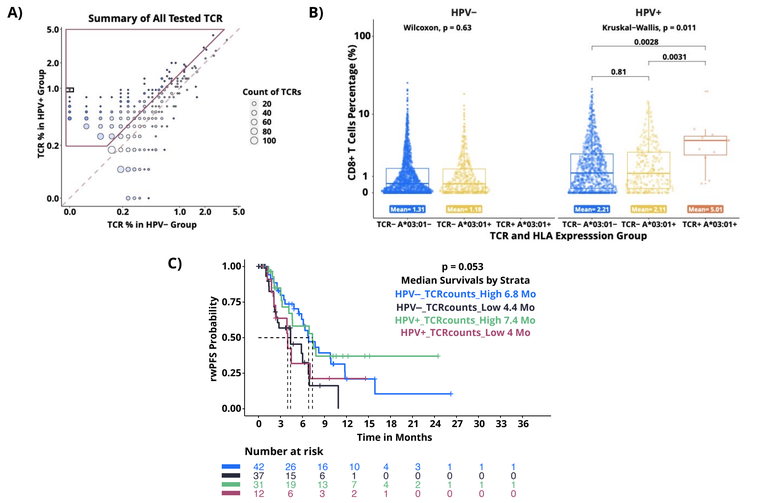


SIGNIFICANCE

- By incorporating routine TCR repertoire profiling into a high-volume clinical genomic sequencing program, we have developed a **rich, multi-modal resource** for studying the complex tumor-immune interaction.
- Analysis of this resource revealed a subset of **TCRs enriched within specific viral and neo-antigen contexts**.
- This dataset is a **valuable resource for TCR therapeutic discovery and** can help identify naturally occuring TCRs that may minimize on-target, off-tumor toxicity.

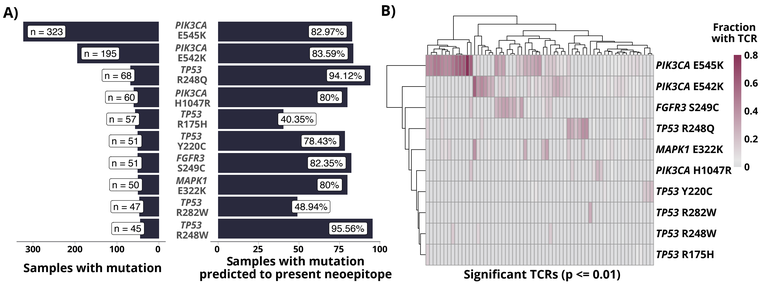
RESULTS

Figure 1. Characterization of HPV Associated TCRs and the Associated Tumor Microenvironment



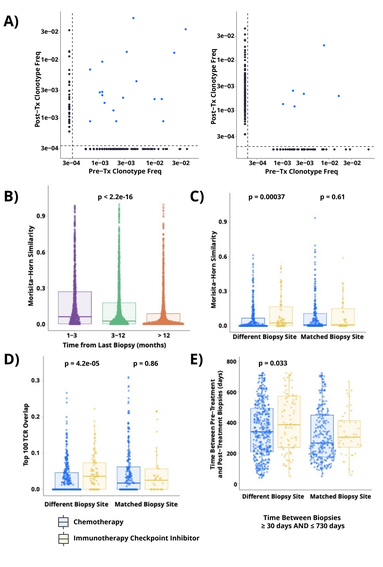
HPV positive tumors have a high prevalence in cervical and head and neck cancer. Here we characterized 1151 HPV+ and 2118 HPV- tumors across the two cancer types. **(A)** TCRβs were tested for association with HPV. Enriched TCRβs were specifically seen in HPV+ tumors (Fisher p<0.05 and odds ratio > 1). **(B)** Interrogation of one TCRβ [black box in Fig 1A], specific to A*03:01 demonstrates that presence of this TCRβ is seen in more CD8 infiltrated tumors. **(C)** Within HNSCC patients receiving immunotherapy, pre-treatment high total TCRβ counts shows a longer median rwPFS compared to low TCRβ abundance, independent of HPV status.

Figure 2. Identification of Neoantigen Specific TCRs



(A) Within the cohort of cervical and head and neck cancer patients we identified the most prevalent pathogenic neoantigens, along with what fraction present those neoantigens, integrating HLA typing, demonstrating variable prevalence of predicted immunogenicity. **(B)** Fisher exact tests were performed on all TCRβs present in at least 5 individuals to identify TCRβs enriched within specific neoantigen contexts. *PIK3CA* E542K and E545K have among the highest number of associated TCRβs.

Figure 3. Tumor Immune Repertoire Changes Over Time Can Capture Treatment Associated Responses



Comparisons of samples over time can lend insights into repertoire dynamics based on regimen and biopsy site. **(A)** Illustrative example of two patients' repertoires over time. **(B)** Repertoire similarity decreases over time due to both temporal and treatment associated changes. **(C)** Comparison of chemotherapy and immunotherapy associated repertoire changes, demonstrates that treatment with immunotherapy results in a more similar repertoire when sampled from distinct biopsy sites. **(D)** This result was consistent when quantifying just the top 100 most frequent clones in each sample. **(E)** Sample timing did not differ within these groups in the direction that would bias this result.

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