#615 Spatially resolved expression of T cell receptors elucidates spatial relationships between T cells, immune infiltration, and cancer-associated pathways

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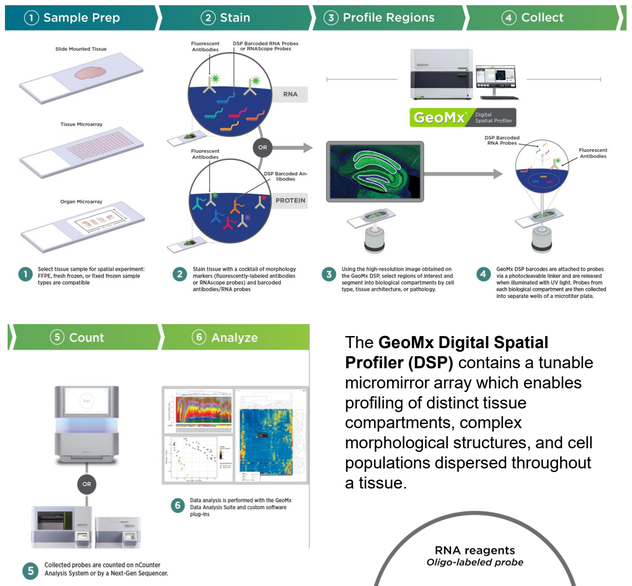
Abstract

Spatial distribution of T cells is key in understanding the escape of tumors from immune surveillance via the adaptive immune response, including interactions between immune cells and the surrounding tumor microenvironment. T cells are critical to the adaptive immune response to pathogens and cancers, mediating an antigen-specific response through both specificity and diversity of T cell receptor (TCR) clonotypes. Many methods exist to determine specific clonotypes and overall TCR diversity present from bulk tissues or sorted cell populations; however, nearly all fail to capture spatial orientation and arrangement of T cells engaging with their microenvironment, and most require large amounts of starting material from precious samples. Here, we present a TCR expression profiling panel for the GeoMx® Digital Spatial Profiler that can be combined with the GeoMx Cancer Transcriptome Atlas (CTA) or Human Whole Transcriptome Atlas (WTA) on archival formalin-fixed paraffin embedded (FFPE) tissue specimens. This represents the first commercial spatial expression profiling assay for the simultaneous quantification of TCR constant, variable, and joining segments *in situ*.

We show reliable sensitivity and specificity (>90%) with respect to orthogonal sequencing and robust detection of TCR chains with evidence of clonal expansion and CD8 infiltration across tumor regions in colorectal cancer tissue. These events also corresponded to increased signatures of exhaustion from the T cells and suggest that the T cells resident in or near the tumor are tumor-specific and poised for activation via checkpoint blockade. Signaling pathways and tumor-specific signatures were also evaluated to look for mechanisms through which tumor cells respond to T cell infiltration. We further validated the performance of the TCR probe pool in cell pellet arrays with orthogonal TCR sequencing, tonsil and colorectal cancer tissues.

Together, the combination of our TCR add-on panel with the CTA or WTA illuminates T cell phenotypes, signaling pathways, population dynamics, and transcriptomic changes, yielding an unparalleled view of the T cell response in any context.

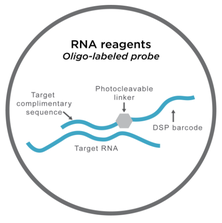
GeoMx® DSP enables direct i*n situ* expression profiling



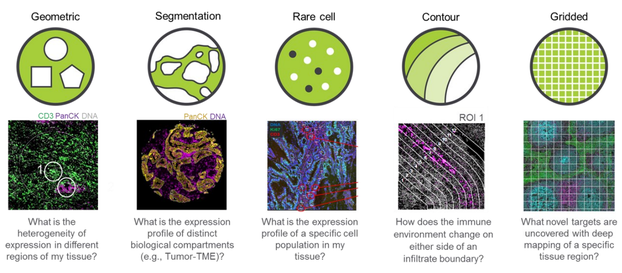
The **GeoMx Digital Spatial Profiler (DSP)** contains a tunable micromirror array which enables profiling of distinct tissue compartments, complex morphological structures, and cell populations dispersed throughout a tissue.

Tagged Oligonucleotide Chemistry

GeoMx Digital Spatial Profiler (DSP) uses oligonucleotides which hybridize to target mRNAs to quantitatively read out DNA tags which are selectively released *in situ* by specifically shining UV light into certain regions of the tissue.



GeoMx DSP enables a *tailored* profiling approach to tackle specific questions



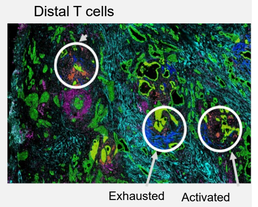
A variety of pre-set tools already exist to create Regions of Interest (ROIs) to your specifications. ROIs may also be hand-drawn, creating limitless possibilities for sampling designs.

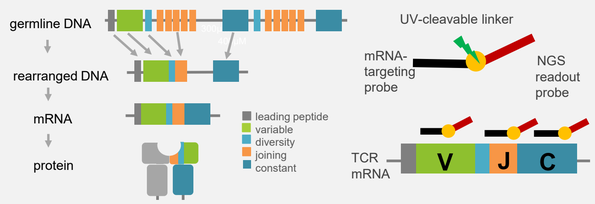
Add-on Panel enables *in situ* expression profiling of TCRs

The human TCR Add-on panel covers **variable** and **joining** gene segments for alpha, beta, gamma, delta chains, and:

- Addresses spatial heterogeneity of TCR segments & tissue response in FFPE
- Is designed to be added to other NGS readout panels (CTA or WTA)
- Has minimal target site cross-reactivity

	TCR profiling panel (Alpha program)
TCR chains	α, β, γ, δ
TCR segments	V & J
Total TCR targets	146
Positive controls	6 T cell markers
Negative controls	50 probes

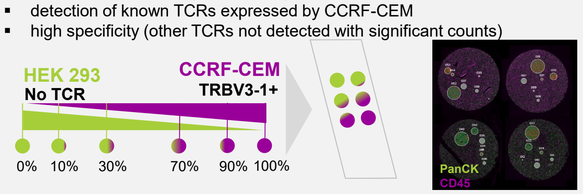




TCR Add-on panel is *specific* and *spatial*

Profiling a cell pellet array containing a dilution of the T cell lymphoma cell line (CCRF-CEM) into an epithelial cell line demonstrated

- detection of known TCRs expressed by CCRF-CEM
- high specificity (other TCRs not detected with significant counts)



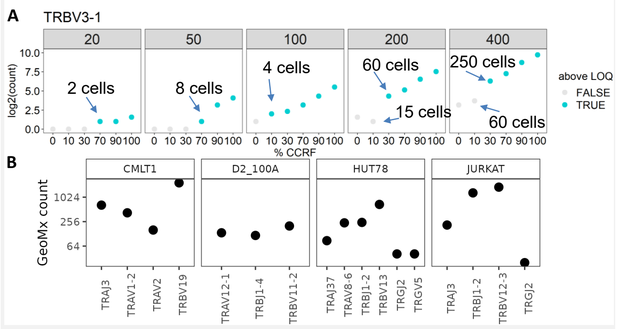


Figure 1: **A) Detection of TRBV3-1 in a titrated T cell lymphoma line shows counts are proportional to the number of T cells present.** Counts of TRBV3-1 in a titrated cell pellet array as percent of CCRF-CEM cells increases across ROIs 20, 50, 100, 200, and 400uM in diameter. Cell numbers shown are approximate. **B) TCR Add-On Panel is sensitive and specific.** T cell segments are detected with > 90% sensitivity and >95% specificity when benchmarked with orthogonal sequencing on commercial and experimentally produced T cell lines.

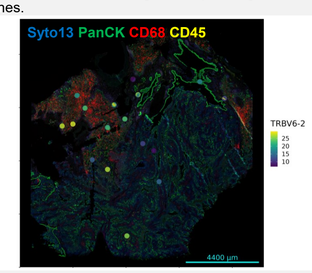
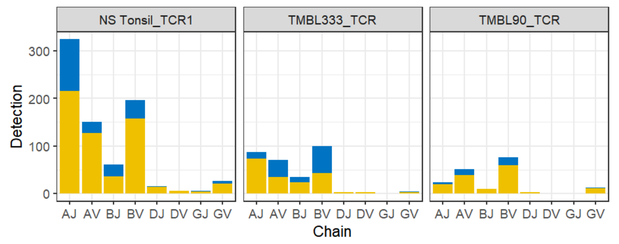


Figure 2: **Detection of TRBV6-2 in lung cancer tissue shows expression is not uniform over space.** Normalized counts of TRBV6-2 in a lung cancer tissue show expression is higher in CD68 rich regions of the tissue.

Robust detection of TCR chain utilization across normal and malignant tissue



- Highest expression observed in tonsil samples
- Alpha/Beta TCR chain utilization detected in tumor samples identified within malignant tissue
- Several logs of dynamic range observed for TCRs across samples

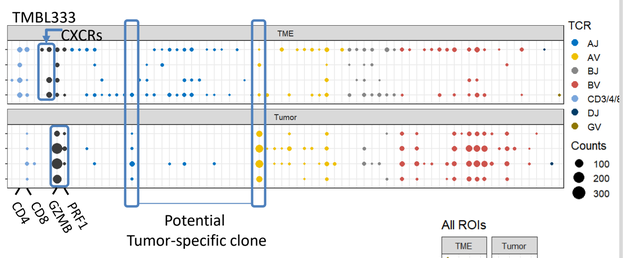
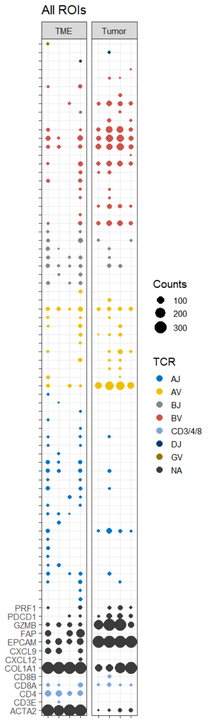


Figure 3: **Evidence of clonal expansion and CD8 infiltration across tumor regions.** Potential tumor-specific TCR clonotype detected preferentially within the tumor bed associated with local expression of perforin and granzyme B.



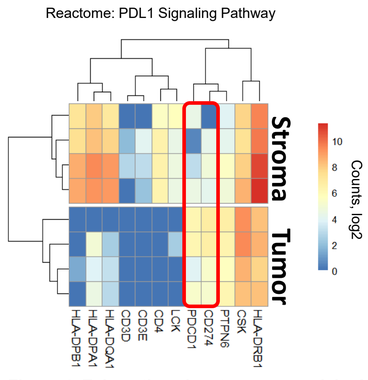


Figure 4: **Exhaustion signatures are enriched within the tumor microenvironment of this sample.** *PDCD1* (encodes programmed cell death protein 1) is expressed in activated T cells. *CD274* (PDL1) binds with PD1, commonly found on T-cells.

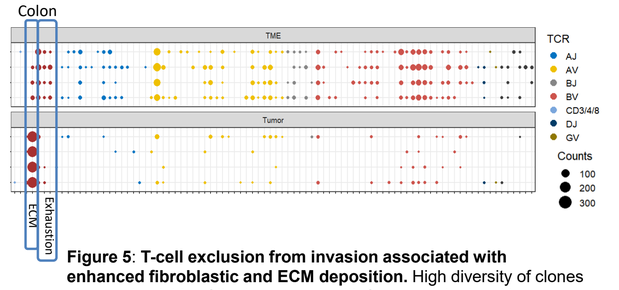
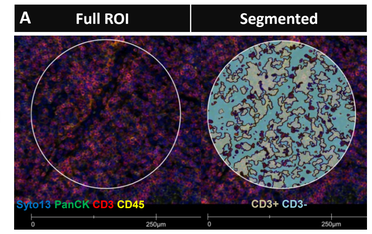
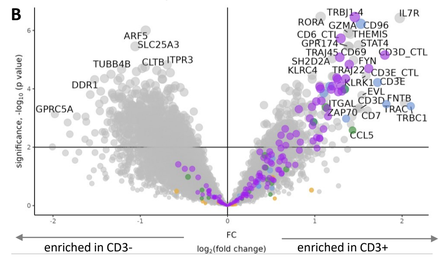


Figure 5: **T-cell exclusion from invasion associated with enhanced fibroblastic and ECM deposition.** High diversity of clones observed outside of the tumor nest, with few consistent and low detected clones observed within the tumor bed.

Segmentation on CD3 selects for T cells

Figure 6: A) Segmentation on an ROI from a colorectal tissue enables discrete transcriptomic profiles for CD3+ cells and CD3- cells **B) CD3+ segments in colorectal cancer tissue selects for T cells and T cell markers.** Genes on the right of x=0 are enriched in CD3+ samples;. Genes to the left of x=0 are enriched in CD3- samples.





TCR Add-on panel
T cell marker
B cell marker
Tumor inflammation signature (TIS)

Transcriptomic characterization of γ/δ-derived subset within T cell lymphoma cohort

A cohort of T cell lymphomas (n=68) were profiled with the TCR Add-on panel

- 184 ROIs selected across all cores
- Enrichment of γ/δ TCR chains and depletion of α/β chains observed in a subset of samples

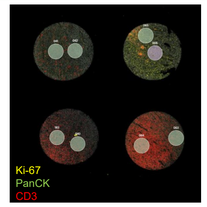


Figure 7: Correlation of TCR constant chains with TCR Add-on control genes. Circled samples are enriched for gamma/delta constant chains and depleted for alpha/beta constant chains.

Figure 8: Cores enriched for gamma/delta show enrichment for related genes. Genes on the right of x=0 are enriched in gamma/delta-rich samples; for example, *DNTT* is involved in T-cell maturation and V(D)J recombination. Genes to the left of x=0 are enriched in alpha/beta-rich samples.

Conclusions

- Together, the TCR Add-On panel and GeoMx DSP RNA atlas panels enable *in situ* profiling of specific cell types and their neighboring cells
- T-cell receptor gene segments were confirmed to be sensitively and specifically captured by GeoMx DSP
- Diverse T-cell populations detected from melanoma, along with sample-restricted detection of γ/δ T-cells
- GeoMx simultaneously differentiated α/β- from γ/δ-derived T cell lymphomas on a tissue microarray and identified genes enriched in one subtype vs. the other