Spatially-resolved measurement of intratumoral T-cell Receptor diversity in Melanoma with the

GeoMx Digital Spatial Profiler



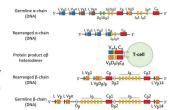
GeoMx enables spatially resolved determination of TCR chain utilization and estimated clonality



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Rationale for developing TCR specific readout for GeoMx DSP

- Diversity & clonality of the T-cell Receptor (TCR) repertoire is an important biomarker of active immune response in cancer
- The physical distribution of T-cells within the microenvironment reflects the status of the ongoing immune response and potential to respond to immunotherapy
- GeoMx digital spatial profiling can potentially be used to characterize TCR utilization by profiling constant and variable regions of α, β, v, δ TCRs in addition to gene expression
- We show proof-of-principle data on the sensitivity and specificity of our approach using a series of cell pellet arrays
- Further, we demonstrate detection of multiple TCR variable regions in a series of melanoma tumors, sampling from multiple regions within the tissues yielding insights about the TCR repertoires prevalence and distribution within tumors



TRBV3-1 in top expressors of all

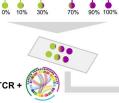
TCR targets

TCR Targets

200 um ROIs

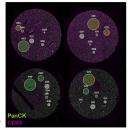


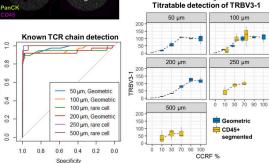
Sensitive and specific detection of TCR variable chains from cell pellet arrays CCRF-CEM TRBV3-1+ No TCR 70% 90% 100% 10% 30%





Testing dilution of CCRF-CEM T-ALL cells into HEK-293 cells. Different sizes were collected so that sensitivity of detection of TCR clones could be assessed for cell lines with defined clonotypes (above). We observe specific detection of TRBV3-1, the known TCRβ variable chain expressed by CCRF-CEM cells, lack of expression of non-specific TCR variable chains. and titration with size profiled. HEK lines should be negative for all TCR variable chain expression, CD45+ ROIs had higher sensitivity for detection than geometric ROIs of similar

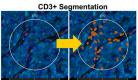


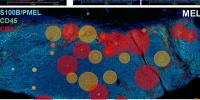


Clonality and Diversity Assessment by GeoMx TCR chain utilization was characterized for 3 T-cell infiltrated primary melanoma

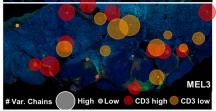
samples using circular ROIs with or without segmentation based on CD3+ staining. ROIs were placed in regions with either high CD3+ staining, low CD3+ staining or tumor regions with no CD3+ staining to assess TCR chain expression. Bubbles show low (0) to high (13) unique TCR variable chains detected within the ROI.









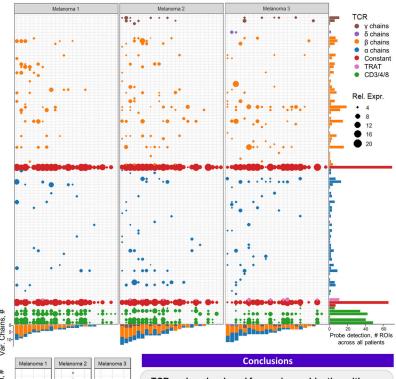


Defining Patient-specific TCR clonotypes

TCR probes against all variable and constant segments, as well as targets for CD3, CD4, CD8, and TRAT1 were assessed relative to background (top right). For each ROI (x-axis) we observe consistent detection of constant regions & CD3/4/8 targets, but unique patterns of TCR variable regions across patients, with common variable chains restricted to each patient.

We detect specific expression of TCR chains in CD3 high or low ROIs relative to tumor ROIs (P = 0.004, right), supporting the specificity of the assay in these samples

Unique TCR fingerprint detected across ROIs within each Patient



TCR probes developed for use in combination with GeoMx NGS assays:

- Show strong concordance with known cell line expression
- · Have low background signal across cell lines and in tumor only ROIs
- · Identify diverse variable chain distributions across distinct regions in melanoma samples
- · Enable characterization of TCR chain utilization in a spatially resolved manner

