Background The composition of T-cell subsets within the tumor microenvironment (TME) can impact TCR diversity and immune therapy response. Studies have shown that the presence of tumor-infiltrating lymphocytes (TILs), particularly CD8+ cytotoxic T cells, is associated with improved outcomes in HNSCC patients. TCR diversity in tumor is difficult to obtain by T-cell receptor (TCR) sequencing with formalin-fixed paraffin-embedded (FFPE) tissue due to RNA degradation. Spatial biology platforms, such as the NanoString GeoMx Digital Spatial Profiler (DSP), have enabled high resolution transcriptomic studies of TME. We used a new 139 gene TCR spike-in panel combined the current whole transcriptome DSP panel to assess T-cell activity and microenvironment changes associated with checkpoint inhibition.

Methods We selected a cohort of 22 HNSCC patients whose tumors were resected then treated with chemotherapy. Upon progression, patients were treated with checkpoint inhibitor therapy (ICI). Matched biopsies collected before and after ICI treatment were profiled with GeoMx DSP whole transcriptome atlas (WTA) panel spiked with the TCR add-on panel. We stained tissues with fluorophore-labeled antibodies to CD3, CD20 and cytokeratin for region of interest selection. We then used the exquisite targeting features of GeoMx to profile CD3+ T cells and compared them to adjacent tumor and CD3- stroma. Tissues were also stained by immunohistochemistry (IHC) for CD3, CD8 and the positive cells were quantified; whole-genome RNA transcriptomics was also performed on the bulk tissue using HTG EdgeSeq.

Results Spatially resolved transcriptomics allows probing of TME by cell type. We applied the new capability to assess T-cell diversity in situ to calculate the Simpson Diversity Metric using α/β V/J gene frequencies. Higher diversity in the TCRα-V genes in T-cells adjacent to tumor was positively associated with partial response to ICI. In contrast, IHC counts of CD3 and CD8 cells alone in the biopsy were not predictive of response. We compared TCR V/J gene frequency within patient samples (pre/post ICI) and between patients; each patient had a distinct pattern of V/J subunit usage, consistent with literature. Tertiary lymphoid structures have been positively associated with response to ICI. We profiled T-cells in regions enriched for B-cells and found increased expression of pro-inflammatory markers across the cohort. In non-T-cell stroma, immune cell signature analysis suggested dendritic cells were elevated in partial responders; TGFβ was associated with non-responders.

Conclusions High resolution profiling of T-cell diversity in the TME can uncover local mechanisms important to checkpoint inhibitor therapy response.

REFERENCES

4. Raay K, Reeves J, Piazza E, Kaplan H, Vivian J, Fernandez F, Hoang M, Beechem J Spatially resolved expression of T cell receptors elucidates spatial relationships between T cells, immune infiltration, and cancer-associated pathways. AACR Annual Meeting 2023; Poster # 615

Ethics Approval Subjects provided informed consent to Capital Biosciences, Inc. (Gaithersburg, MD) for genetic and protein analysis

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