1037 SINGLE CELL EVALUATION OF ANTIGEN SPECIFICITY, CLONAL DYNAMICS AND TRANSCRIPTIONAL SIGNATURES OF CD8+ T CELLS IN HEAD AND NECK SQUAMOUS CARCINOMA

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Background Head and neck squamous cell carcinoma (HNSCC) is caused by high tobacco and alcohol consumption and/or human papillomavirus (HPV) associated.[1-3] Anti-PD-1 immunotherapy only benefits 15-20% of HNSCC patients[4, 5] Thus, there is a need to identify mechanisms involved in resistance to immune checkpoint blockade. Several studies have described the transcriptional phenotypes of tumor infiltrating lymphocytes (TIL) and correlated it with clinical response to ICB.[6-8] It has also been shown that majority of neoantigen-specific CD8+ T cells from the tumor have an exhausted-like phenotype [9-11], but specific targetable epitopes and whether anti-viral T cells dominate in HPV-associated HNSCC immunotherapy have not been determined. Collectively, these studies do not address neoantigen and HPV-antigen specificities of evaluated T cells and their transcriptional and functional status in the HNSCC TME, nor whether these cells re-circulate and demonstrate restoration of function once in the peripheral circulation.

Methods Single cell suspensions were prepared from tumor and blood of 21 treatment-naïve HNSCC patients. T cells were FACS-sorted using barcoded mAbs (CITE-seq) and used to generate single cell RNA sequencing (scRNA), T cell receptor (TCR) and ADT libraries using 10x Genomics workflow. A cell-based method for epitope discovery (SABRs) was used to characterize antigen specificity of selected CD8+ TILs.[12]

Results Clonal expanded CD8 T cells in the tumor express high levels of T cell exhaustion markers (PDCD1, HAVCR2, ENTPD1, LAG3). GSEA analysis show that highly expanded CD8+ TILs share gene signatures with neoantigen-specific CD8+ TILs identified from other cancers. There was no significant overlap observed between TCRs of CD8 T cells with high expression of immune checkpoint receptors in the tumor with peripheral blood CD8+ T cells. TCR analysis show that T cell clones shared between tumor and peripheral blood are found in a cluster of CD8 T cells with an effector memory phenotype expressing distinct granzyme expression in activated versus exhausted tumor reactive cells.

Conclusions In this study, we'd like to evaluate 1). Antigen specificities and transcriptional phenotypes of CD8+ T cells. We found that potential tumor reactive cells express high checkpoints, implying most of tumor reactive cells are dysfunctional within tumor. 2). Clonal dynamics of tumor reactive cells. We observed low clonal overlap between peripheral and tumor resident TCRs, suggesting tumor resident population expanded locally. Shared T cell clones between peripheral CD8+ T cells in effector memory population may reflect an active recruitment and/or exhange of T cells between periphery and tumor.

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Ethics Approval Peripheral blood and tumor tissues from treatment-naïve HNSCC patients were collected with their written consent in accordance with the Declaration of Helsinki, under the University of Pittsburgh Cancer Institute Review Board–approved protocol (99-069).

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