TUMOR-INFILTRATING CXCR6-EXPRESSING TISSUE-RESIDENT MEMORY HPV-SPECIFIC CD8+ T CELLS ARE TERMINALLY EXHAUSTED IN HPV-ASSOCIATED HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background Tissue-resident memory T cells (TRM) are important for controlling both infection and tumor growth. We performed comprehensive immunophenotyping of HPV-specific and non-HPV-specific viral bystander (EBV, CMV, IAV, and SARS2-CoV2) CD8+ TRM cells in HPV-associated HNSCCs.

Methods We assessed HPV-specific and non-HPV-specific viral bystander CD8+ T cell frequency and functional states in the primary tumor, tumor-involved lymph node (LN), and peripheral blood mononuclear cells (PBMC) from 34 HPV-associated HNSCC patients. CD8+ T cells were isolated and probed by 89 different pMHC tetramers across three HLA alleles (A*02:01, A*01:01 and A*24:02) using multiplexed combinatorial peptide-MHC (pMHC) tetramer staining and spectral flow cytometry. To interrogate HPV-specific responses, we analyzed 61 HPV16 E6 and E7 epitopes. To compare non-HPV bystander virus-specific T cells, we investigated 11 bystander virus (EBV, CMV and IAV) and 17 SARS2-CoV-2 (spike protein) epitopes. Tetramer+ CD8+ T cells were immunophenotyped by co-staining 20 cellular markers to probe T cell exhaustion and tissue resident phenotypes.

Results 76.4% (26 of 34) of HPV-associated HNSCCs had detectable HPV-specific T cells within their tissue and 82% (28 of 34) had bystander virus-specific T cells. Overall, we identified 26 HPV- (10 HPV16-E6 and 16 HPV16-E7), 11 bystander (non-HPV) virus-, and 8 SARS-CoV-2 spike-specific CD8+ T cell populations. We observed a broader HPV epitope repertoire within LN and PBMC as compared to tumors. However, the frequency of HPV-specific T cells infiltrating the tumor was significantly higher compared to the LN and PBMC. Within the tumor, 13 HPV- (6 HPV16-E6 and 7 HPV16-E7), 6 bystander (non-HPV) virus-, and 3 SARS-CoV-2 spike-specific CD8+ T cell populations were identified. The frequency of tumor-infiltrating HPV-specific T cells ranged from 0.02-3.01% for E6, 0.013-10.9% for E7, 0.013-1.93% for bystander (non-HPV) virus-specific T cells, and 0.014-11.5% for SARS-CoV-2 spike-specific T cells. High-dimensional analysis identified four distinct TRM subsets. Tumor-infiltrating HPV-specific T cells were enriched for two CXCR6+ TRM subsets that were also PD-1hiTIM-3+, suggesting they were terminal exhausted. In contrast, tumor- and LN-infiltrating bystander non-HPV-specific T cells were CXCR5+PD-1int TRM and not exhausted.

Conclusions We report a high frequency of HPV and bystander viral epitopes within HPV-associated HNSCCs, and identified two distinct CXCR6+ terminal exhausted HPV-specific TRM subsets within the tumor. Spatial localization of these two distinct CXCR6+ terminally exhausted TRM to myeloid populations in the primary tumor and tumor draining lymph nodes are ongoing to better understand tumor-specific T cell priming within these two sites.