HPV16 E1 AND E2 ELICIT A ROBUST CYTOTOXIC IMMUNE RESPONSE IN VIRALLY DRIVEN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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Background Human papillomavirus (HPV) infection is a risk factor for oropharyngeal squamous cell carcinoma (OPSCC). HPV+ OPSCC is characterized by distinct biology, a heterogeneous immune landscape, and accounts for approximately 14,400 new diagnoses per year in the U.S. For patients with locoregional recurrence or distant metastasis, systemic treatment options beyond immune checkpoint inhibitors are limited. Though a decline in HPV+ HNSCC is expected due to HPV vaccination campaigns, incidence is currently increasing, prompting a wave of therapeutic development. While most therapeutic efforts have been focused on eliciting a cellular response to HPV early antigens E6 and E7, we sought to identify additional highly prevalent, immunogenic targets in both early stage as well as in relapsed/refractory HPV+ HNSCC.

Methods Treatment-naïve HPV+ OPSCC patients (n=19) were prospectively enrolled under an institutional review board (IRB)-approved tissue collection protocol (DF/HCC#09–472) for collection of surgical specimens. Tumor tissue from a biopsy or definitive oncologic transoral robotic-assisted resection specimen was collected prospectively for analysis. Multiplexed, barcoded peptide-MHC-I tetramer libraries containing epitopes derived from the HPV16 genome were used to probe dissociated tumors followed by single cell RNA sequencing using the 10x Genomics platform. SingleR and published gene marker sets were used to phenotype and perform broad lineage assignment.

Results We found broad T-cell reactivity to several HPV proteins across multiple HLA alleles and epitopes. E1 and E2-reactive T-cells were cytotoxic and highly expanded, whereas E5, E6 and E7-reactive T-cells were rare and displayed exhausted or naïve phenotypes. Cytotoxic E2 T-cell responses were associated with loss of E2 expression in at least one tumor suggesting functional capacity of these T-cells. We also identified evidence of pseudo-public TCR sequences, as TCR alpha, beta or paired CDR3 sequences associated with E2 reactivity were shared across multiple patients with common HLAs. TCR specificity was confirmed for several TCR-epitope interactions using recombinant TCRs and cognate peptide.

Conclusions We employed high-throughput, single-cell immune synapse profiling to characterize the cellular response to HPV + HNSCC across common HLA haplotypes and identified new candidates for immunotherapeutic intervention. We found that HPV16 proteins E1 and E2 induce robust, effective cytotoxic responses in HPV16-driven OPSCC. These findings indicate HPV16 E1 and E2-directed immunotherapy may be effective among patients with OPSCC expressing these antigens.

Ethics Approval Patients were consented and enrolled under an institutional review board (IRB)-approved tissue collection protocol (DF/HCC#09–472).