374 DISCOVERY OF A PUBLIC HPV16-E6 DIRECTED T CELL RESPONSE THAT IS ASSOCIATED WITH OVERALL AND PROGRESSION FREE SURVIVAL

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Background Human papillomavirus (HPV) infection causes at least 650,000 anogenital and oropharyngeal cancers (OPC) worldwide annually. Despite the viral immunological target, immune checkpoint inhibitors produce responses only in a minority of patients, and cancer therapies that directly target HPV-antigens represent an attractive alternative therapeutic approach. With this work, we introduce a high-throughput and epitope-agnostic pipeline for HPV16-reactive T cell discovery and validation.

Methods Using the FEST assay [1], T cell responses against HPV16 were assessed in 3 patients with HPV16-positive OPC that participated in an open-label phase I clinical trial studying the safety and immunogenicity of vaccination with a DNA-based HPV16-E7 vaccine. T cell receptor (TCR) motifs were constructed using GLIPH2 [2]. Enrichment of motifs in tumors and HLA alleles among patients was determined using Wilcoxon signed-rank test and Fisher Exact test, respectively. Association between TCR motifs and survival was assessed using a log-rank test. Single cell sequencing (sc-seq) was performed using the 10x platform. Expression of TCRs in effector cells was achieved using electroporation. All studies were approved by MD Anderson IRB (PA17-0149, PA19-0470, and 2019-1059).

Results Sixteen HPV16-reactive TCRs were identified in 3 patients with HPV16-positive OPC, and responses were found against most HPV16 proteins. Five clones were associated with commonly shared TCR motifs identified in our combined patient cohort. A TCR motif against HPV16-E6 (E6-TCR-motif) was found enriched in tumors ($p=0.007$; figure 1) of patients who expressed a common HLA allele ($p=0.0019$; figure 2). Strikingly, no patients that naturally harbor the E6-TCR-motif died ($p=0.04$) nor progressed ($p=0.023$; figure 3). Recognition of a region within E6 by a T cell belonging to the E6-TCR-motif was validated via transgenic TCR expression in Jurkat cells co-cultured with HLA-matched peptide-pulsed target cells, and eventually the minimal epitope was determined. Impressively, an interaction between TCR and epitope was still detectable at 10^{-15} M peptide concentration, a factor 1000 lower compared to our positive control TCR [3] against HPV16-E6 (figure 4).

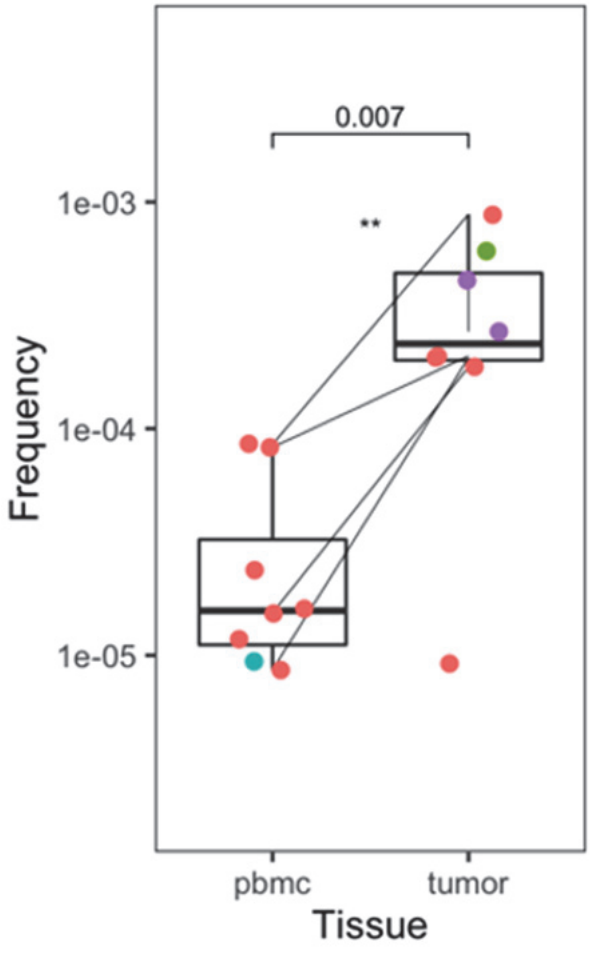
Conclusions We have identified a common HPV16-E6 specific TCR motif shared among patients with HPV16-positive OPC cancer that is associated with survival. We are currently working to translate this finding to the clinic as an adoptive cellular therapy. Given the success of this approach, we are working to map the antigenic/TCR landscape in patients with HPV16-positive tumors, with the goal of developing a suite of TCR-based therapies restricted to common HLA alleles and to construct a therapeutic vaccine against persistent HPV16 infection.

Acknowledgements This research was supported by The University MD Anderson HPV-Related Cancers Moon Shot, the MD Anderson Oropharynx Cancer Program, and Intramural Startup funds. FEST assays were performed by the FEST and TCR Immunogenomics core at Johns Hopkins University. We would also like to thank Drs. Alexandre Reuben and Minying Zhang for sharing reagents.

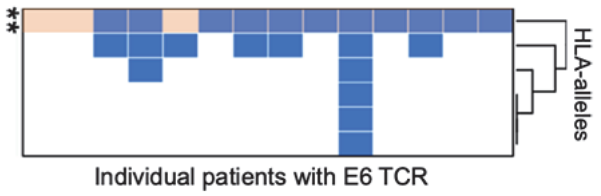
REFERENCES

1. Danilova L, Anagnostou V, Caushi JX, Sidhom JW, Guo H, Chan HY, Suri P, Tam A, Zhang J, Asmar ME, Marrone KA, Naidoo J, Brahmer JR, Forde PM, Baras AS, Cope L, Velculescu VE, Pardoll DM, Housseau F, Smith KN. The mutation-associated neoantigen functional expansion of specific T cells (MANAFEST) assay: A sensitive platform for monitoring antitumor immunity. *Cancer Immunol Res*. 2018;**6**:888-899.
2. Huang H, Wang C, Rubelt F, Scriba TJ, Davis MM. Analyzing the Mycobacterium tuberculosis immune response by T-cell receptor clustering with GLIPH2 and genome-wide antigen screening. *Nat Biotechnol*. 2020; **38**:1194-1202.
3. Draper LM, Kwong ML, Gros A, Stevanović S, Tran E, Kerkar S, Raffeld M, Rosenberg SA, Hinrichs CS. Targeting of HPV-16+ epithelial cancer cells by TCR gene engineered T cells directed against E6. *Clin Cancer Res*. 2015; **21**:4431-4439.

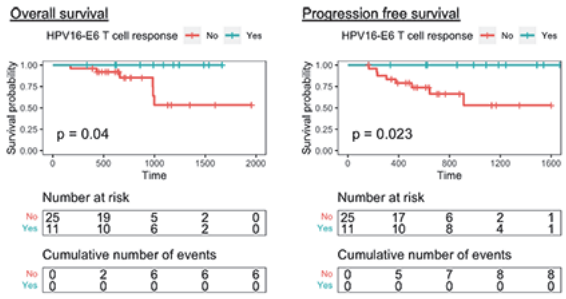
Ethics Approval All studies were approved by MD Anderson IRB (PA17-0149, PA19-0470, and 2019-1059).



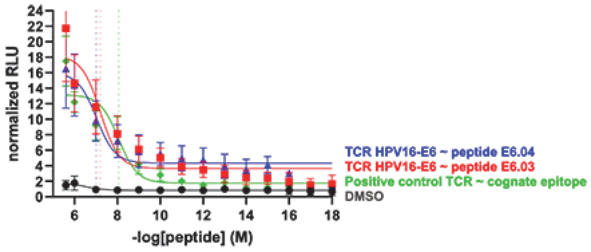
Abstract 374 Figure 1 HPV16-E6 TCR motif is enriched in tumors of patients with HPV16-positive oropharyngeal cancer. Frequency of T cell clones that belong to the HPV16-E6 TCR motif constructed using the GLIPH2 algorithm is shown. Statistical significance was determined using a Wilcoxon signed-rank test. Color represents data source (not shown). Line indicates paired tumor and blood data.



Abstract 374 Figure 2 Specific HLA-allele is enriched among patients that harbor HPV16-E6 TCR motif. Rows represent a specific HLA allele, columns represent individual patients. Enriched HLA allele is highlighted in orange (top row). Statistical significance was calculated using Fisher Exact test; ** indicate p-value < 0.01.



Abstract 374 Figure 3 Harboring the HPV16-E6 TCR motif is associated with survival. Natural presence of T cells from the HPV16-E6 TCR motif in tumors and/or blood was associated with overall (left) and progression free (right) survival in HPV16-positive oropharyngeal cancer patients that express the shared HLA allele.



Abstract 374 Figure 4 HPV16-E6 TCR motif T cells recognize their cognate epitope with high sensitivity. A full-length paired TCR belonging to the E6-TCR motif was expressed in Jurkat T cells without endogenous TCR expression and that has been genetically modified to produce luciferase upon TCR engagement. Peptide-pulsed and HLA-matched CD3 PBMC was used as target cells. DMSO (negative control) is shown in black and a positive control TCR that recognize the HPV16-E629-38 epitope [3] is shown in green. Blue and red represent the HPV16-E6 TCR we have discovered when pulsed with two different 15 amino acid long peptides that share a region of 11 amino acids; the minimal epitope reside in the overlapping region.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0374