Background Nasopharyngeal carcinoma (NPC) is an EBV-driven cancer endemic to East and Southeast Asia with poor survival rates and limited treatment options. While EBV infection plays a key role in NPC pathogenesis, little is known about EBV-specific T cells in the context of immune response to NPC. We, therefore, interrogated the role of EBV-specific T cell responses in NPC immunopathology with the goal of better understanding how the tumor evades immune targeting and identifying new targets for immunotherapy.

Methods We profiled the phenotypes and antigenicity of EBV-specific CD8 T-cells in NPC patient peripheral blood mononuclear cells (PBMCs) from a 51-patient new diagnosis Singaporean NPC cohort using a combined mass cytometry (CyTOF) and multiplexed peptide-MHC tetramer panel. UMAP and clustering analyses were performed to analyze these highly dimensional data and then phenotypic clusters were correlated to patient clinical parameters to identify clinically relevant, NPC-associated EBV-specific phenotypes. Additionally, we performed single-cell multi-omics sequencing (10X Genomics/CITE-seq) on NPC patient PBMCs to dissect the role of peripheral T cells with NPC-associated phenotypes on tumor pathogenesis.

Results We identified that peripheral EBV-specific CD8 T cells in NPC patients are phenotypically distinct from other antigen-specific T cells and differentially express activation, exhaustion, and homing markers, such as CD38, HLA-DR, CD39, and CD103. We found that the frequencies of EBV-specific T cells with these phenotypes are correlated with clinical parameters (i.e., EBV-DNA titer, tumor volume, stage etc.), and observed similar correlations with bulk CD8 T cells with these phenotypes. Finally, we assessed differential gene expression profiles of CD8 T cells with surface expression of NPC-associated phenotypic markers.

Conclusions Overall, we show that the phenotypic profiles of peripheral EBV-specific T cells may be reflective of NPC anti-tumor immune responses, and these phenotypic profiles could potentially be used as biomarkers for immunotherapeutic response.

Ethics Approval All patient samples were obtained from patients who provided informed consent at the National Cancer Centre Singapore, and de-identified patient information from this cohort was obtained through approval by the institutional review board at the Fred Hutchinson Cancer Research Center (IR File#: 6007-1053).