Background Pre-operative immunotherapy results in pathologic tumor responses (pTR) for some patients with head and neck squamous cell carcinoma (HNSCC), but response mechanisms remain poorly defined.1,2 We evaluated T cell profiles and clonal dynamics associated with pTR in a phase II trial of two doses of neoadjuvant pembrolizumab.

Methods 29 patients with stage III/IV HPV-unrelated HNSCC were enrolled in a multicenter phase 2 clinical trial of anti-PD-1 antibody pembrolizumab (2 doses, Q3 weeks) as neoadjuvant immunotherapy over 5 weeks prior to surgery. pTR to PD-1 blockade was assessed based on histologic reduction of tumor cell-fraction, as previously published, with responders defined as pTR of >10%.1 We profiled tumor-infiltrating lymphocytes (TILs) from 14 tumor biopsies from 4 Responders (Rs) and 4 Non-Responders (NRs), collected either before or after PD-1 blockade through single-cell RNA (scRNA-seq) and T-cell receptor sequencing (scTCR-seq). Quality and quantity of TILs were assessed with multiplex immunofluorescence. Data were validated via comparison with a similar cohort of 36 HNSCC patients receiving single-dose neoadjuvant pembrolizumab.

Results pTR was detected in surgical specimens from 15 patients (53%), with two-year overall survival (OS) and progression-free survival (PFS) rates of 92.2% (95% CI: 72.1-97.9) and 92.3% (95% CI: 72.6-98.0%), respectively. Single-cell analysis of CD8+ TILs identified 12 transcriptionally-defined clusters. The microenvironment of Rs compared to NRs dominated by T TE-CTX, a subpopulation with characteristics of cytotoxicity and high expression of ZNF683, suggesting a tissue-resident memory (TRM) program.4,5 Multiplex immunofluorescence of pre-treatment biopsies confirmed that Rs were more highly infiltrated with CD3+ TILs with a TRM phenotype, identified through CD103 co-expression (figure 1b).4 Multiplex immunofluorescence of pre-treatment biopsies confirmed that Rs were more highly infiltrated with CD3+ TILs with a TRM phenotype, identified through CD103 co-expression (figure 1b).

Conclusions A larger pre-treatment proportion of T Ex-TILs retaining cytotoxic potential and a TRM signature are associated with pTR in HNSCC. Expanded T Ex-CTX clones were diminished in number after immunotherapy treatment, consistent with release of their anti-tumor activity and subsequent contraction due to antigen clearance.

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Ethics Approval This study was approved by the Institutional Review Boards of Dana-Farber/ Harvard Cancer Center (DFCI# 16-385), Washington University (#201412118) and Memorial Sloan-Kettering Cancer Center (MSKCC) (#18-379).

Abstract 503 Figure 1 Dynamics of exhausted CD8 T cells expressing markers of cytotoxicity and a tissue-resident memory program in HNSCC tumors. a. Frequencies of principal phenotypes among CD8+ TILs collected from Responders (R, circles, n=3) or Non-Responders (NR, diamonds, n=4) at pre-treatment timepoints (Pre). Box plots – median percentage of TILs with phenotypes corresponding to CD8+ non-exhausted memory cell states (TExM, blue), exhausted states (Tex, red), or unclassified clusters (Other, grey). Whiskers: min-max values;
b, Multiplexed Immunofluorescence of tumor biopsies collected prior to treatment from 3 Rs (left) and 3NRs (right) patients. The representative images demonstrate the pre-existing high levels of tissue resident memory-like (CD103+) and exhausted (PD1) TILs (CD3+) in Rs within the tumor bed, marked by expression of cytokeratin (Cytok). c, Bidimensional plot quantifying the expression ZNF683 expression (x axis) and cytotoxicity genes (summarized in a score [3], y axis) in CD8+ TILs with TEx-TCR clonotypes. Cells are colored according to the size of the TCR clonotype family they belong to. The percentage of cells in each quadrant is calculated based on thresholds representing the average values of variables (vertical and horizontal lines), as measured in the entire dataset of CD8+ TILs.