Background: CD4 T helper (Th) cells play a key role in orchestrating immune responses, but the identity of the CD4 Th cells involved in the immune response against cancer remains to be defined.

Methods: To better define the composition of the CD4 Th cells infiltrating human solid tumors, we analyzed the phenotype of CD4 T cells in 22 patients with head and neck squamous cell carcinoma and 16 patients with microsatellite stable colorectal cancer by high dimensional flow cytometry. In addition, we determined their spatial location and cellular interactions in the tumor microenvironment by multiplex IHC to understand their role in the anti-tumor immune response. We also assessed the capacity of the CD4 Th cell populations sorted and expanded based on the expression of PD-1 and ICOS to recognize tumor-associated antigens and tumor-specific neoantigens. Finally, we investigated whether the presence of PD-1+ICOS+ CD4 Th cells in the tumor was associated with disease-free survival in HNSCC patients.

Results: Following t-SNE analysis, we identified a subset of CD4 Th cells distinct from FOXP3+ regulatory T cells that co-expressed PD-1 and ICOS. This cell population, which was present in the tumor but absent in the periphery, exhibited features of chronic stimulation and displayed characteristics of tissue resident memory T cells. PD-1+ICOS+ tumor-infiltrating (TIL) CD4 Th cells were located primarily in the tumor stroma in proximity to MHC class II+ cells and were proliferating, suggesting local antigen recognition. Interestingly, both PD-1+ICOS+ CD4 Th cells and CD39+CD103+ tumor-reactive CD8 T cells were enriched for cells secreting CXCL13, a chemokine involved in the recruitment of B cells. PD-1+ICOS+ CD4 Th TIL were shown to recognize both tumor-associated antigens and tumor-specific neoantigens, which were distinct from the epitopes recognized by the CD8 T cells from the same patients. Finally, higher frequencies of PD-1+ICOS+ CD4 Th TIL in patients with HNSCC was associated with better disease-free survival.

Conclusions: Our findings provide an approach for isolating tumor-reactive CD4 Th TIL directly ex vivo that will help define their role in the anti-tumor immune response and potentially improve future adoptive T-cell therapy approaches.

Ethics Approval: All surgical tumor samples and blood samples used in this study were taken from individuals treated at the Providence Cancer Center. All patients signed written informed consent approved by the Providence Portland Medical Center Institutional Review Board (IRB protocol no. 06-108A) and the study was conducted in accordance with the ethical standards established by the Declaration of Helsinki.