CD8 T CELL REPERTOIRE ANALYSIS OF PATIENTS WITH RESECTABLE HEAD AND NECK CANCER ENROLLED IN A PHASE II NEOADJUVANT STUDY OF α-PD1 ADMINISTERED ALONE OR IN COMBINATION WITH α-CTLA4 OR α-LAG3

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Background Head and neck squamous cell carcinomas (HNSCC) are caused by alcohol and/or tobacco-derived exposure to carcinogens or by malignant transformation following oncogenic HPV infection. Standard of care regimen for HNSCC involve surgical resection followed by radiation or chemoradiation. Recently, monoclonal antibodies against immune checkpoint inhibitors (ICI) have been approved for patients with unresectable or metastatic disease. However, response rate for ICI remains low in HNSCC and other solid tumors (20-40%). Thus, there is a need to better understand the mechanisms involved in heterogeneous responses to ICI and to identify new targets that may sensitize HNSCC to combinatorial treatments. As part of a Phase II study examining the safety and tolerability of α-PD1 administered alone or in combination with α-CTLA4 or α-LAG3, the alterations in T cell receptor (TCR) clonotypes and changes in transcription profiles in CD8 T cells from blood and tumor following mono- and combinatorial immune checkpoint blockade (ICB) in treatment naïve HNSCC patients were analyzed.

Methods Subjects with resectable, stage III/IVa HNSCC were stratified based on HPV, PD-L1, and LAG3 status and randomized into 3 treatment arms: α-PD1 alone, α-PD1+α-CTLA4, or α-PD1+α-LAG3 prior to surgical resection. Blood and tumor samples were collected at baseline and on the day of surgery (21-35 days after ICB) and processed to generate single cell suspensions as previously described. FACS-sorted CD45+CD3+ cells were used to generate single cell RNA (scRNA) 5' gene expression libraries and TCR libraries using 10x Genomics workflow. Libraries were sequenced and data was processed using Cellranger 5.0.0 (10x Genomics). Data analysis was performed using R (v4.2.0) packages Seurat (v4.1.1), scRepertoire (v1.6.0) and Immunarch (v0.6.9).

Results To date, 34/60 patients have been enrolled in the trial. Based on scRNA data available from accrued patients (n=25), the proportion of CD8 T cells in the tumor increased after treatment in patients randomized to α-PD1+α-CTLA4 arm. Patients treated with α-PD1+α-LAG3 show an increase in CD8 TCR repertoire diversity as measured by Gini-Simpson index and had more TCR clones occupying 50% of the repertoire in post-treatment tumors compared to baseline. Overlap analysis on the number of TCR clones shared between post-treatment versus baseline did not show any significant differences across treatment arms. Trial accrual is still in progress and further analysis is currently ongoing to 1) correlate these data with clinical outcomes and 2) further investigate changes in transcriptional profiles of expanded clones in tumors across different treatment arms and by response.

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Trial Registration NCT04080804

REFERENCES

Ethics Approval This study was approved by University of Pittsburgh’s Institutional Review Board; approval number HCC 18-139/CA224-056.