547 REPROGRAMMING OF TYPE I INTERFERON-RESPONDING TUMOR-REACTIVE T LYMPHOCYTES ASSOCIATES WITH IMPROVED OUTCOMES TO NEOADJUVANT DOUBLET IMMUNOTHERAPY IN HNSCC

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Background While immune checkpoint inhibitors (ICI) have shifted the paradigm of cancer treatment, the mechanisms that drive tumor rejection remain elusive. Neoadjuvant ICI therapies provide a unique window of opportunity to study temporal changes of immune responses to ICI at the tumor site.[1] We interrogated transcriptional and TCR clonal dynamics of head and neck squamous cell carcinoma (HNSCC) patients who received neoadjuvant nivolumab (anti-PD1) ± ipilimumab (anti-CTLA4) or relatlimab (anti-LAG3). The aim was to elucidate temporal changes of T cells associated with favorable clinical response.

Methods Fresh pre- and post-treatment tumor and peripheral blood specimens from HNSCC patients (n=41) were processed by 10x Genomics 5' single-cell RNA-seq, TCR-seq, and cellular indexing of transcriptomes and epitopes (CITE)-seq. Of 41 patients, 35 have so far completed sequencing and were analyzed using Seurat (v4.0.0). Canonical T cell marker genes, GSEA and RNA velocity were used to define cellular states, enriched signaling pathways, and estimate transcriptional dynamics. FFPE tissue sections were used to perform multispectral imaging to evaluate T cell composition changes in tumor. Pathologic response was scored using standard criteria.[2]

Results A type I interferon (IFN-I) response gene signature was strongly enriched in CD8+ TIL from responders at baseline. Interestingly, this IFN-I response signature was lost solely in the CD8+ TIL from patients that responded to nivolumab +relatlimab therapy. Responder CD8+ TIL co-expressed tumor-reactive markers CD39/*ENTPD1* and CD103/*ITGAE* at baseline and underwent clonal expansion post therapy. Clonal TCR tracing of expanded CD8+CD39^{high} TIL from patients treated with nivolumab+relatlimab therapy displayed decreased gene sets associated with T cell exhaustion, including decreased LAG3 and CD39 expression, and increased effector gene programs post therapy. Novel TCR clonotypes identified from post-treatment, responder tumors had smaller clone and population size. *LAG3* mRNA expression by CD8+ TIL at baseline correlated with pathological response only in patients treated with nivolumab+relatlimab (n= 11, R=0.59, p=0.058), similar to LAG3 immunohistochemistry. Multispectral imaging showed that intratumoral CD8+ T cell density was significantly increased after treatment in major responders (n=7, p=0.019).

Conclusions Transcriptional reprogramming of tumor-reactive CD8+ TIL from a hyper IFN-I responsive cell state to effector cell phenotype, potentially driven by LAG3 signaling inhibition, is a novel mechanism that may be required for tumor rejection in the context of neoadjuvant nivolumab+relatlimab therapy. Novel TCR clonotypes from post-treatment, responder tumors only occupied minor fraction of CD8 TIL population, which suggests that reprogramming and maintenance of resident tumor-reactive cells present at baseline is a major driver of anti-tumor immunity.

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