555 CHALLENGES IN PREDICTING ICI RESPONSE IN HPV+ HNSCC: INSIGHTS INTO THE TUMOR MICROENVIRONMENT

[1]Anastasiia Nikitina*, [1]Anastasia Sobol, [1]Evgeniia Alekseeva, [1]Andrey Tyshevich, [1]Anna Love, [2]Adam J Luginbuhl, [3]Alban J Linnenbach, [2]Joseph M Curry, [1]Vladimir Kushnarev, [1]Christopher JH Davitt, [1]Nikita Kotlov, [1]Alexander Bagaev, [1]Nathan Fowler. *[1]BostonGene Corp., Waltham, MA, USA; [2]Thomas Jefferson University Hospitals, Philadelphia, PA, USA; [3]Sidney Kimmel Medical College, Philadelphia, PA, USA*

Background The relationship between HPV status and immune checkpoint inhibitor (ICI) response in head and neck squamous cell carcinoma (HNSCC) is not fully understood. Previously, we showed that immune-inflamed TMEs are associated with ICI response in HPV- HNSCC.[1] However, HPV+ tumors have distinct biological characteristics that influence the immune landscape. Therefore, our objective was to characterize the tumor microenvironment (TME) in HPV+ HNSCC relative to ICI response.

Methods Comparative analysis of RNA-seq data from 74 HNSCC patients (40 HPV+ and 34 HPV-) receiving anti-PD-1 therapy and two public cohorts, TCGA-HNSC (n = 504)[2] and GSE127165 (n = 112)[3] was performed. Functional gene expression signature (FGES) analysis[4] and Kassandra cellular deconvolution[5] were performed. T and B cell receptor (TCR, BCR) repertoires were reconstructed using MiXCR.6 GATK Pathseq[7] and Vi-Fi[8] were used to identify viral reads and viral-host chimeric reads, respectively.

Results Cellular deconvolution of 594 HPV- and 164 HPV+ samples indicated HPV+ samples had higher immune (NK, CD8+ T, CD4+ T, and B) cell content, while HPV- samples were enriched with stromal components (fibroblasts and endothelial cells, $p < 0.001$). Congruent with these findings, HPV + samples had stronger checkpoint inhibition, antitumor cytokine, and MHCII FGES and weaker CAF, extracellular matrix, and angiogenesis FGES ($p < 0.001$) compared to HPV- samples. Moreover, HPV+ tumors contained larger and more diverse TCR and BCR repertoires ($p < 0.001$).

Pre-treatment HPV+ samples from two nivolumab-treated cohorts with 25 responders and 14 nonresponders were analyzed, but no associations were observed between ICI response and immune and stromal cell content in the TME, analyzed FGES, TCR and BCR diversity or clonality, viral transcript expression, or host genome integration. Interestingly, longitudinal analysis of 40 pre- and 37 post-treatment samples showed higher levels of EMT, CAF, macrophage, and checkpoint inhibition FGES in ICI responder and nonresponder post-treatment samples ($p < 0.05$), suggesting these TME dynamics were unrelated to outcomes.

Conclusions Our study highlighted the disparity in underlying mechanisms of ICI response between HPV- and HPV+ HNSCC patients. While factors like an immune-enriched TME and robust expression of checkpoint inhibition FGES are reportedly influential in HPV- cases, these and other factors were not associated with therapy response in HPV+ HNSCC patients. These results are likely due to the predominantly immune-enriched TME characteristic of HPV+ HNSCC. Our findings underscored the importance of considering HPV status when investigating the molecular determinants of ICI response in HNSCC.

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