INTEGRATED IMMUNOPROFILING AND RNA SEQUENCING (RNA-SEQ) FOR ANTI-PD-1 RESPONSE PREDICTION IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS (HNSCC)

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Abstract 222-K Figure 1 Classification of immune cells of the blood into five immunotypes

Background While PD-1 inhibitors are promising therapies for HNSCC, better methods are needed to predict response. We used integrated immunoprofiling of peripheral blood mononuclear cells (PBMCs) and RNA-seq of tumor tissue to identify novel predictors of anti-PD-1 response in HNSCC.

Methods Immunoprofiling using multiparameter flow cytometry was applied to PBMCs collected from a large internal cohort of cancer patients and healthy donors (n=850). Unsupervised clustering of normalized cell population frequencies from batched flow cytometry data utilizing a common backbone and variable functional staining panels was used to classify patients into different immunotypes. We then analytically validated populations by cellular deconvolution with Kassandra™ from the same specimens. PBMCs from previously untreated stage II-IV HNSCC patients (n=36) were analyzed at baseline and on-treatment with the anti-PD-1 inhibitor nivolumab ± an IDO inhibitor as a validation cohort. RNA-seq was retrospectively performed on tumors at baseline and on-treatment, along with transcriptome-based tumor microenvironment (TME) subtyping1 and cellular deconvolution with Kassandra™.2 All disease sites were assigned a pathologic Treatment Response (pTR)3 and analysis was completed based on primary site response alone and overall response (OR) based on all disease sites.

Results Blood immunoprofiling of the internal cohort revealed five conserved immunotypes enriched in certain cell types (G1-naive T and B cells; G2-central memory CD4+ T cells; G3-transitional memory CD8+ T cells; G4-effector memory CD8+ T cells; G5-monocytes/granulocytes; figure 1), with immunotypes clustering to different disease states in these patients. We then stratified the 36 HNSCC patients treated with nivolumab into the same G1-G5 immunotypes as a validation cohort. At baseline, the G2 group had higher OR rates than other groups (p=0.02; figure 2). Baseline primary tumors showed OR correlated with PD-L1 and PD-L2 expression, interferon responsive genes, T-cell trafficking, and MHC class I pathway (higher values in Responders versus Non-responders, p<0.05; figure 3). Cell deconvolution showed greater CD8+ T cells in the TME correlated with primary site response (p<0.01). All 12 patients with immune-desert TMEs showed no primary site response (p=0.003); 4/5 patients with an immune-enriched TME showed a primary site response (p=0.002; figure 4). Primary tumors with fibrotic TMEs showed no response. However, in patients with a fibrotic TME and a positive OR, indicated by a significant pTR, the G2 immunotype was identified (figure 5).

Conclusions Our results suggest that this integrated approach shows potential for the development of more accurate prediction of response to ICI therapies for HNSCC.

REFERENCES

Ethics Approval The cohort from Thomas Jefferson University was collected under ClinicalTrials.gov identifier NCT03854032.
Abstract 222-K Figure 2  
Immunotype analysis of the HNSCC cohort (n = 36) and treatment response (R) and non-response (NR) to nivolumab; Fisher’s exact test was used for statistical comparison.

Abstract 222-K Figure 3  
Tumor expression-based biomarkers of response (R) and non-response (NR) to nivolumab. *p < 0.05; **p < 0.01; ***p < 0.001.
Abstract 222-K Figure 4  Transcriptome-based classification of primary tumor samples of responders (R) and non-responders (NR) from the HNSCC cohort (n = 36) into four TME subtypes (Fibrotic; Immune Desert; Immune-Enriched, Fibrotic; Immune-Enriched, Non-fibrotic).

Abstract 222-K Figure 5  Association of TME subtypes (Fibrotic; Immune Desert; Immune-Enriched, Fibrotic; Immune-Enriched, Non-fibrotic) at baseline with primary and overall response to nivolumab. R - Response; NR - Non-response

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