**TUMOR-INTRINSIC P38 SIGNALING AS A THERAPEUTIC TARGET TO OVERCOME NON-T CELL-INFLAMED TUMORS AND IMMUNOTHERAPY RESISTANCE**


**Background**

We previously identified WNT/β-catenin (CTNNB1) and an atlas of molecular alterations that drive the non-T cell-inflamed phenotype and immune-checkpoint inhibitor (ICI) resistance across cancers. To refine our analysis, we have subsequently separated tumor types by clinically relevant stratifications, such as human papilloma virus (HPV) in head and neck squamous cell carcinoma (HNSCC), to identify immune-exclusion mechanisms associated with specific patient populations. P38 MAPK is a known regulator of dendritic cells (DCs) and myeloid cells however a tumor-intrinsic immunomodulatory role has not been previously described.

**Methods**

Using the T cell-inflamed gene expression signature we previously defined, we integrated tissue RNAseq from 395 HPV- HNSCCs in The Cancer Genome Atlas (TCGA) with single-cell RNAseq from two independent HNSCC studies. We detected differentially expressed genes in non-T cell-inflamed *versus* inflamed tumors by limma voom (v3.36) in concert with causal network prediction, followed by validation in two independent HNSCC cohorts. scRNAseq data was processed by Cellranger (v6) and Seurat (v3.99). We quantified pathway expression levels in tumor cells, DCs, macrophages, and other main cell types in the tumor microenvironment. Single-cell pathway comparisons were further conducted using generalized linear mixed-effects models (GLMM) and protein-protein interaction networks constructed by STRING. Pharmacologic inhibition of p38 MAPK was explored in combination with ICI in two syngeneic murine models. Patients with ICI refractory tumors were treated with p38 inhibitor plus ICI.

**Results**

We identified 67 pathways as activated in non-T cell-inflamed tumors from the HPV- cohort of HNSCC, 59 of which were independently validated. This included CTNNB1 from our prior work and p38 MAPK, the therapeutic target in our ongoing clinical trial (NCT04074967). CTNNB1 and p38 pathway molecules both showed inverse correlation with CD8A protein abundance from the Clinical Proteomic Tumor Analysis Consortium. We observed a significant enrichment of pathway expression only in tumor cells (p<0.05) from both HNSCC scRNAseq studies and dominantly in non-T cell-inflamed tumors. Using an accumulative scoring system integrating bulk tissue and single cell sequencing data, we prioritized seven pathways as strongly connected in non-inflamed tumors. EMT6 and CT26 syngeneic murine models demonstrated improved survival with the addition of p38 inhibitor to ICI relative to ICI monotherapy. Major and durable clinical responses have been observed in patients with anti-PD1 refractory tumors.

Conclusions p38 is a novel tumor-intrinsic mechanism that drives immune exclusion. P38 inhibition enhances ICI and can overcome anti-PD1 resistance in patients.

**Ethics Approval**

Our study gained approval from IRB, HCC#19-097. Any/all participants in human studies gave informed consent before participating.