UNCOVERING THE DARK IMMUNOPEPTIDOME OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC): RELEVANCE FOR UNIVERSAL CANCER VACCINES, IMMUNOLOGICAL MONITORING AND TIL THERAPY

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Background
Discoveries of the last two years have initiated a renaissance in our understanding of the targets T cells recognize on cancer cells. Identification of HLA-presented non-canonical or cryptic peptides that are non-mutated and have high interpatient sharing in AML [PMID: 33740418], coupled with their absence from the thymus, led them to be designated as alternative neoantigens with potential for being universal cancer vaccines [PMID: 33852826]. These cryptic genes, in some cases, appear to play a role in promoting malignancy, further strengthening the rationale for their identification and use as targets for immunotherapy. Our group seeks to identify the Dark Immunopeptidome of HNSCC. Over the last several months we have switched from exome-capture RNA-Seq, which fails to detect non-canonical gene sequences, to ribo-Seq, which can capture these cryptic transcripts.

Methods
HNSCC cells were lysed and recovered lysates were treated with detergent in the presence of protease inhibitor. HLA peptides were collected from HLA complexes purified by anti-HLA-I antibody (w6/32). Recovered HLA peptides were analyzed by the microLC-QTOF MS (LCMS-9030, Shimadzu Corporation). Ribo-Seq was performed on HNSCC and full cryptic protein databases were generated using a modified version of the pipeline described in Scull et al. [PMID: 34509645]. Libraries were aligned to the GRCh38 reference genome using STAR [PMID: 23104886] in two-pass mode and both a reference guided assembly and a de-novo assembly were generated using Cufflinks [PMID:22383036]. Variant calling was performed using GATK Best Practices Workflow ‘RNASeq short variant discovery (SNPs + Indels)’ utilizing MuTect2 [PMID:23396013] for variant calling and subsequent filtering. The reference guided assembly, de-novo assembly, and the variant calling output were turned into transcriptome assemblies utilizing gffread [PMID: 32489650], and underwent three frame translation using triple_translate (Scull et al.). The resulting three-frame translated fasta files were merged and had any duplicates or redundancies removed using squish (Scull et al.) for database generation to be used in PEAKS [PMID: 14558135].

Results
We have completed ribo-Seq on 2 HNSCC cell lines and are preparing to interrogate the microLC-QTOF MS-generated spectra for HLA-presented peptides of the HNSCC cell lines.

Conclusions
While data are still being generated and evaluated using different assessment tools, our group considers this proteogenomic approach to assess the Dark Immunopeptidome for HNSCC holds substantial promise for uncovering targets of anti-cancer immunity. In addition to applications for immunological monitoring, this has potential therapeutic application for cancer vaccines or as vaccine boosters for patients receiving TIL.

Acknowledgements

Ethics Approval
The study was approved by the institutional review board of the Providence Portland Medical Center (06-108).