144 NOVEL PUBLIC AND TUMOR-WIDE NEOANTIGENS ARISING FROM CLONAL ABERRANT SPLICING EVENTS DRIVE TUMOR-SPECIFIC T-CELL RESPONSES ACROSS DIVERSE CANCER TYPES

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Background Immunotherapy in gliomas is limited by tumor heterogeneity and low mutational burden. We developed a novel comprehensive *in silico* pipeline for detecting tumor-specific splicing events (neojunctions) across multiple cancer types, and in gliomas, we successfully identified tumor-wide, public, alternatively spliced neoantigens (ASNs) that elicit CD8+ T-cell-mediated immune responses.

Methods Our pipeline identified recurring public neojunctions expressed in TCGA RNA-seq (positive sample rate (PSR) > 10%) and not in GTEx normal tissue RNA-seq data (PSR < 1%) across six cancer types. To characterize intratumorally-conserved neojunctions, we utilized available multi-site RNA sequencing across diverse cancer types. With gliomas, our in-house dataset comprised of 56 patients with approximately 10 maximally-distanced intratumoral biopsy sites per patient (n=535). Tumor-wide public neojunction expression was subsequently validated in RNA-sequencing and mass spectrometry (MS) data from patient-derived cell lines (n=68) and samples (n=99). Two independent algorithms then predicted peptide processing likelihood and HLA-binding affinity of ASN candidates. *In vitro* sensitization (IVS) of healthy donor-derived CD8+ T-cells against high-confidence ASN candidates, followed by 10x VDJ scRNA-seq, was performed to identify ASN-specific TCR sequences. TCRs were transduced into triple-reporter Jurkat76s and co-cultured with ASN and HLA-expressing COS7 cells and glioma cell lines to evaluate TCR functionality.

Results Pan-cancer analysis identified large subsets of neojunctions that were interpatiently and intratumorally conserved. In particular, our glioma-specific analysis identified 249 public neojunctions with varying intratumoral heterogeneity. 4 ASNs were concurrently identified in transcriptomic and proteomic glioma data and predicted to be presented by HLA-A*02:01 with high confidence. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) performed on COS7 cells transfected with HLA-A*02:01 and full-length mRNA containing the neojunction validated proper translation and processing of the full-length mutant peptide into its HLA-bound n-mer. IVS and subsequent 10x VDJ scRNA-seq on expanded PBMC-derived CD8+ populations cultured against ASN-pulsed dendritic cells identified TCR clonotypes reactive against neojunctions in *RPL22* (n=7) and *GNAS* (n=1), the latter being highly intratumorally-conserved (detected in > 90% of spatially-mapped biopsies across 17/56 patients (26.78%)). TCR-transduced T-cells demonstrated recognition and immunogenic activation against endogenously processed and presented neoantigens in both glioma and transfected COS7 cells. Pan-cancer analysis revealed the detection of both *RPL22* and *GNAS* neojunctions in various tumor types beyond gliomas.

Conclusions Our unique integrative pipeline identified novel public tumor-wide splice-derived neoantigen candidates and ASN-specific TCRs, offering a promising off-the-shelf immunotherapy approach for diverse cancer types. Furthermore, characterization of novel intratumorally-conserved neoantigens addresses the critical challenge of intratumoral heterogeneity in immunotherapy resistance.

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