Background While immunotherapy is profoundly efficacious in certain cancers, its success is limited in cancers with lower mutational burdens, such as gliomas. Therefore, investigating neoantigens beyond those from somatic mutations can expand the repertoire of immunotherapy targets. Recent studies identified alternative-splicing (AS) events in various cancer types that could potentially translate into tumor-specific proteins.

Our study investigates AS within glioma to identify novel MHC-I-presented neoantigens through an integrative transcriptomic and proteomic computational pipeline, complemented by an extensive spatiotemporal analysis of the AS candidates.

Methods Bulk RNA-seq of high tumor purity TCGA-GBM/LGG (n=429) were analyzed through a novel systematic pipeline, and tumor-specific splicing junctions (neojunctions) were identified in silico by cross-referencing with bulk RNA-seq of GTEx normal tissue (n=9,166). Two HLA-binding prediction algorithms were subsequently incorporated to predict peptide sequences with a high likelihood for HLA presentation. Investigation of the tumor-wide clonality and temporal stability of the candidates was performed on extensive RNA-seq data from our spatially mapped intratumoral samples and longitudinally collected tumor tissue RNA-seq. Proteomic validation was conducted through mass-spectrometric analysis of the Clinical Proteomic Tumor Analysis Consortium (CPTAC)-GBM repository (n=99).

Results Our analysis of TCGA-GBM/LGG bulk RNA-seq identified 249 putative neoantigens that translate into 222 cancer-specific peptide sequences encoding 21,489 tumor-specific n-mers (8–11 amino acids in length, figure 1). Both prediction algorithms concurrently identified 271 n-mers likely to bind and be presented by HLA-A*0101, HLA-A*0201, HLA-A*0301, HLA-A*1101, or HLA-A*2402. We confirmed the expression of 17 out of 74 HLA-A*0201-binding candidates in RNA-seq of two HLA-A*0201+ patient-derived glioma cell lines with a subset of candidates found tumor-wide (figure 2). Analysis of CPTAC-GBM mass-spec data detected 42 tumor-specific peptides generated specifically from 23 GBM-specific splicing events (figure 3). 4 candidates were selected for downstream immunogenicity analysis and were selected based on high in silico HLA-presentation scores and detection in RNA sequencing data from our spatially mapped intratumoral samples and longitudinally collected tumor tissue RNA-seq. Proteomic validation was conducted through mass-spectrometric analysis of the Clinical Proteomic Tumor Analysis Consortium (CPTAC)-GBM repository (n=99).

Conclusions Tumor-specific neoantigens identified in our unique integrative pipeline present novel candidate immunotherapy targets for gliomas and offer a new avenue in neoantigen discovery across cancer types.

REFERENCES
Peptide-level validation of NJ-derived neoantigens

Protein-level expression of the neojunctions was detected by analyzing mass-spectrometry data from CPTAC. Mass spectrometry data of GBM patients (n=99) detected 42 peptides that are uniquely derived from 23 unique neojunctions.

NJ-derived neoantigens elicit CD8+ T-cell response

Four neojunction-derived neoantigen candidates demonstrated high HLA-presentation scores and were detected on both an RNA- and peptide-level. Neoantigen sensitization was performed on donor-derived PBMCs, and neojunction-derived mutant-RPL22 elicited a CD8+ T-cell-specific immune response.