Background The low mutational burden and immunologically ‘cold’ microenvironment of mutant IDH1 low-grade gliomas (LGG) are considerable challenges in immunotherapy for these tumor types. However, we hypothesize that LGG-targeting T-cells may exist at low frequency and with limited regional infiltration within the tumor. Through multi-region tumor sampling coupled with high-throughput T-cell receptor (TCR) profiling, we identified tumor-wide neoantigens and corresponding neoantigen-specific T-cells regionally infiltrating the tumor and persisting in peripheral blood.

Methods Maximally-distanced anatomical sampling of at least 10 distinct tumor regions was performed at the initial resection for three WHO Grade II diffuse astrocytoma patients for exome-based prediction of clonally and subclonally expressed neoantigens, RNAseq analysis of regional immune cell composition, and TCR beta deep sequencing. We used these predictions to generate a barcoded library of patient-specific peptide-HLA multimers loaded with predicted neoepitopes. With this library, neoantigen-specific CD8+ T-cells were captured and isolated from patients' peripheral blood. Single-cell TCR sequencing allowed us to identify the neoantigen-reactive TCR clonotypes which were transduced subsequently into Jurkat76 cell lines for functional validation.

Results We screened patient-derived peripheral blood drawn two years after initial resection in 3 mutant IDH1 LGG patients and detected a total of 20 TCR clonotypes recognizing neoepitopes derived from truncal, tumor-wide mutations in CNTNAP1 (n=8), TP53 (n=3), and MRPL46 (n=2) as well as subclonal mutations in PRMT5 (n=1) and ZDHHC5 (n=6). Jurkat76 cells transduced with the mutant-PRMT5-specific TCR demonstrated dose-dependent neoantigen-specific immune responses when co-cultured with mutant-PRMT5 pulsed-antigen presenting cells expressing HLA-A*0201.

Conclusions Our study demonstrates the existence and persistence of neoantigen-targeting T-cells within the blood and tumor of mutant IDH1 LGG patients. We identified a TCR clonotype that successfully recognizes and induces an immune response against mutant-PRMT5. These findings suggest a feasible methodology to develop personalized T-cell-based immunotherapies for patients with mutant IDH1 LGGs.