222 DEVELOPING AN ADOPTIVE CELL TRANSFER IMMUNOTHERAPY FOR PEDIATRIC HIGH-GRADE GLIOMAS

Stephen Frederico*, Chaim Sneiderman, Ian Pollack, Gary Kohanbash. *University of Pittsburgh, Pittsburgh, PA, USA*

Background Pediatric high-grade gliomas (HGGs) are one of the deadliest brain tumors that arise in children, with an average five-year survival for this disease being less than 20%.[1] HGGs expresses glioma-associated antigens (GAAs) which can be targeted by the immune system. These include IL-13Ra2, Survivin, and EphA2 [2]. Previously, our research group conducted a clinical trial where HLA-A2 positive pediatric patients were vaccinated with these GAA epitopes when newly diagnosed with HGG [2]. Our group observed many patients enrolled in the study showed positive anti-GAA immune responses to IL-13Ra2, EphA2, and Survivin. The findings from this trial highlighted that the sparsity of T-cells within the tumor microenvironment may pose a major challenge to improving immuno-therapeutic outcomes.

Methods In this study we engaged in identifying TCR sequences targeted to IL-13Ra2, Survivin, or EphA2. To accomplish this, a single cell-suspension of CD8 T-cells tetramer stained for Survivin, IL13Ra2, or EphA2 were obtained using FACS from PBMCS taken from individual patients who underwent vaccination. Single-cell RNA-sequencing (ScRNA-Seq) was performed on these samples to determine the phenotype of the T-cells. We also assessed T-Cell expansion and obtained the nucleotide sequence for the CDR3 region. Following acquisition of the nucleotide sequence, we developed retroviral TCR-vectors and viral particles to enable transduction of T cells. We confirmed the presence of TCRs on the T-cell surface via tetramer staining and flow cytometric analysis. We then performed in vitro killing assays by co-culturing transduced T-cells with U87 cells and assessed for LDH within our samples.

Results ScRNA-seq allowed us to identify a cluster of T-cells consisting of PRF1+ (Perforin) & GZMB+ (Granzyme b), as well as a cluster of PDCD1+ (PD-1) & TIGIT+ cells. Our tetramer staining confirmed the presence of our TCRs on the T-cell surface. In vitro killing assays demonstrated T-cell cytotoxicity as T-cells with U87 cells in 10:1, 4:1 and 1:1 ratios had a percentage cytotoxicity of 30.95%, 22.59% and 9.37% respectively. When pairing T-cells with U87 cells while blocking HLA-A2, there was a marginal decrease in cytotoxicity.

Conclusions TCRs targeted to IL-13Ra2, Survivin, or EphA2 were positively identified on the surface of transduced T-cells and demonstrated a cytotoxic response during *in vitro* killing assays. We now intend to grow these cells into large numbers and adoptively transfer them into tumor-bearing mice in hopes this will provide a survival benefit.

Acknowledgements This research was supported by the American Brain Tumor Association Jack & Fay Netchin Medical Student Summer Fellowship in memory of Jeffrey Ragan Frost.

REFERENCES

1. Huang TY, Piunti A, Qi J, Morgan M, Bartom E, Shilatifard A, Saratsis AM. Effects of H3.3G34V mutation on genomic H3K36 and H3K27 methylation patterns in isogenic pediatric glioma cells. *Acta Neuropathol Commun*. 2020 Dec 7;**8**(1):219. doi: 10.1186/s40478-020-01092-4. PMID: 33287886; PMCID: PMC7722426.
2. Pollack IF, Jakacki RI, Butterfield LH, Hamilton RL, Panigrahy A, Potter DM, Connelly AK, Dibridge SA, Whiteside TL, Okada H. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and nonbrainstem gliomas. *J Clin Oncol*. 2014 Jul 1;**32**(19):2050-8. doi: 10.1200/JCO.2013.54.0526. Epub 2014 Jun 2. PMID: 24888813; PMCID: PMC4067943.

Ethics Approval All experiments were carried out in conformity with the principles set out in the World Medical Association's Declaration of Helsinki as well as the Department of Health and Human Services Belmont Report. The University of Pittsburgh Institutional Review Board approved sample use (PRO08030085). Informed written consent was provided by all patients prior to inclusion in the study.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0222