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


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