Harnessing the Signalosome to Enhance Ex-Vivo Generation of Tumor Antigen-Specific T Cells

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The adoptive transfer of tumor antigen-specific T cells (ASTs) can serve as an effective immunotherapy against pediatric solid tumors of the central nervous system (CNS). However, current methods of T cell engineering are time-inefficient. With prolonged inactivity, tumoral diversification may occur, resulting in worsened medical outcomes. Understanding this, attempts were undergone at hastening cell therapy manufacturing techniques through signalosome ligands (SLs). It was anticipated that by providing correct stimuli to T cells, they may be developed as memory-trained, producing an immune response. CMV-pp65 was of focus. Donor-derived PBMCs of varying CMV serostatus were expanded with CMV-pp65 and SLs. Cells were stimulated in-vitro with subsequent analysis of IFN-y secretion. Short-term expansion of CMV seropositive cells with CCL21 resulted in CMV-ASTs. Short-term expansion with pp65/ICAM-1 and isolated pp65 additionally produced ASTs. Thus, time-based immunotherapeutic manufacturing efficiency increased significantly with SLs. Phenotypic characterization via flow cytometry revealed a relatively higher proportion of CD8 T cells in expanded CMV seropositive cells. Demonstrating the ability to effectively stimulate ASTs in CMV, RNA-seq differential expression analysis was performed to determine antigens of interest, targeting CNS tumors. Data was stratified by tumor reference specificity to examine differential expression between tumor site-specific and generalized healthy tissue of the CNS. Predictive MHC-peptide bond strength analysis was subsequently undergone to determine respective magnitudes of immunogenicity in upregulated genes. TOP2A and CCNB2 were identified as promising antigens of interest in AST development for pediatric tumors of the CNS.