

## DEFINING T CELL EXHAUSTION AND MEMORY CORRELATES OF GD2 CAR T CELL EXPANSION IN PEDIATRIC PATIENTS WITH SOLID TUMOR MALIGNANCIES

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**Background** Chimeric antigen receptor T cells (CAR-Ts) hold promising therapeutic potential for solid tumors but have yet to produce consistent durable responses in patients. A major limitation to response in solid tumors remains the lack of CAR-T expansion, persistence, and anti-tumor cytotoxicity. Identifying molecular markers that correlate with CAR-T activity could elucidate key biological pathways and T cell populations central to the success of CAR-Ts in patients.

**Methods** A phase I trial (NCT02107963) was conducted to determine the feasibility of producing and safety of administering escalating doses of a third generation GD2 CAR-T (GD2-CAR.OX40.28.z.ICD9) in children and young adults with GD2+ solid tumors, including neuroblastoma and osteosarcoma. To understand biological differences correlating with CAR-T cell activity in patients, patient apheresis, CAR-T product, and post-treatment peripheral blood samples were analyzed for immune phenotype by mass cytometry (CyTOF), transcriptomic profile by RNA-sequencing (RNA-seq), and epigenetic landscape with Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq).

**Results** Across 4 dose levels, 15 patients (8-28 years old) were enrolled, of which 13 patients were infused. At Day 28 following GD2 CAR-T infusion, 23.1% (3/13) of evaluable patients had progressive disease and 76.9% (10/13) had stable disease (SD), but all patients eventually progressed. Despite limited GD2-CAR-T persistence, half of the patients had expansion similar to that seen in clinically active CD19 and CD22 CAR-Ts. Since a major barrier to CAR-T efficacy is inadequate CAR-T expansion, we comprehensively evaluated patient immune profiles to identify determinants of CAR-T expansion. Good CAR-T expansion was found to be associated with increased abundance of naïve CD8+ T cells in apheresis by CyTOF. Similarly, RNAseq demonstrated enrichment of naïve memory T cell pathways in the apheresis samples of good expanders. ATACseq identified epigenetic differences in apheresis that may predict good CAR-T expansion in patients. CAR-T products across all patients, regardless of CAR-T expansion, expressed activation/exhaustion markers by CyTOF. RNAseq of CAR-T products revealed an enhanced exhaustion signature in poor compared to good expanders. At post-treatment timepoints, poor expanders demonstrated increased expression of T cell exhaustion markers.

**Conclusions** Comprehensive analyses of patients' apheresis, product, and post-treatment timepoints enable characterization of the T cell immune compartment before CAR-T treatment, after CAR-T manufacturing, and after CAR-T infusion. We identified phenotypic, transcriptomic, and epigenetic T cell signatures correlating with CAR-T expansion. These data suggest key mechanisms of underlying T cell biology that may contribute to CAR-T activity in pediatric solid tumors.

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**Trial Registration** NCT02107963

**Ethics Approval** The phase I study protocol conformed to the Declaration of Helsinki, Good Clinical Practice guidelines, and was approved by the NCI Institutional Review Board (14-C-0059) and the FDA. All patients or their legal guardians signed a document of informed consent indicating their understanding of the investigational nature and risks of this study.

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