Background: B7-Homolog 6 (B7-H6) is a B7 family member and the natural ligand for NK cell-activation receptor, NKp30. B7-H6 is expressed on multiple tumor types but has limited expression in normal tissues. Given this tumor specificity, B7-H6 represents an attractive target for CAR T therapy. CAR T cell therapy is associated with high clinical response rates in hematologic malignancies, but opportunities for improved efficacy in solid tumors remain. γδ T cells, whose solid tumor infiltration has demonstrated a significant correlation with survival, combine innate and adaptive mechanisms to recognize and kill tumors. In addition, γδ T cells engineered with CARs have shown enhanced tumoricidal activity and compelling clinical efficacy. Here, we evaluated the antitumor activity of γδ T cells modified with a set of novel scFv-based CARs targeting B7-H6, potentially applicable against multiple cancer indications for which natural tissue tropism of γδ T cells may offer advantages.

Methods: Phage-display libraries were used to identify scFvs against B7-H6 epitopes. To confirm activation upon target engagement, scFvs formatted into CARs were evaluated in a Jurkat-Lucia™ NFAT reporter cell line. PBMCs from healthy donors were used to activate, expand, and engineer Vδ1 T cells to express libraries of CAR constructs representing permutated scFv arrangements. Vδ1 CAR T cells were assessed for phenotype and in vitro activity using flow cytometry and cell-based assays. Tumor xenograft models were further used to evaluate and assess CAR candidates for in vivo efficacy.

Results: Phage-display derived scFvs showed a diverse range of affinities against multiple B7-H6 epitopes. Increased NFAT activity post-activation and minimal tonic signaling was observed in the majority of CAR-modified Jurkat-Lucia™ cells. Vδ1 CAR T cells manufactured from healthy PBMC donors demonstrated a predominant naïve-like phenotype with low levels of exhaustion-associated markers. Upon in vitro stimulation with B7-H6+ cell lines, leading Vδ1 CAR T cells inhibited tumor cell growth, demonstrated antigen associated proliferation, and released proinflammatory cytokines. Robust tumor growth inhibition was also observed for a subset of active constructs against tumor xenografts in immunodeficient mice (figure 1).

Conclusions: In summary, we present the preclinical discovery, optimization, and generation of allogeneic γδ CAR T cells targeting B7-H6 with potential applications across a broad set of cancer indications. Based on these data, continued preclinical development of a clinical lead candidate is ongoing for future evaluation in the clinic.

Ethics Approval: All mouse experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and followed all institutional and national guidelines with appropriate protocol review and approval.