Preclinical Discovery and Evaluation of Allogeneic “off-the-shelf” γδ CAR T Cells Targeting B7-H6+ Tumors

Spike-in soluble B7-H6 (20 ng/mL) expression were cytotoxic in 24 and 48% while killing 72% of the tumor cells.

In contrast to the robust killing observed in Jurkat cells, recombinant CAR1, 2 and 3 were evaluated against B7-H6 expressing HeLa and HeLa KO cell lines. CARs demonstrated high activity against the B7-H6 expressing HeLa cell line (21 ± 7, 12 ± 3, and 44 ± 2 MFI, respectively) with no significant difference in activity observed between CAR1 and 2 or CAR3 and 4 (p > 0.05). The CAR3, demonstrated the most robust activity against HeLa B7-H6 KO cell line (25 ± 2 MFI) while CAR1 and 2 were only marginally less cytotoxic (10 ± 2 and 11 ± 2 MFI, respectively).

CONCLUSIONS
- Polyfunctional killing capacity of CAR T cells is critical to cell activity.
- CARs targeting B7-H6 can serve as attractive targets for adoptive cell therapy.
- Determination of the optimal CAR construct for B7-H6 targeting is required.

In summary, we present the initial preclinical discovery and generation of allogeneic γδ CAR T cells targeting B7-H6 with potential applications across numerous indications.

Figures:
- Figure 1: Schematic representation of expression of γδ T cells using recombinant γδ ScFvs derived from tumor mass.
- Figure 2: Generation of B7-H6 CAR V5.1 T cells shows robust expansion from donor PBMCs.
- Figure 3: B7-H6 CAR V5.1 T cells exhibit a naive-like T cell memory phenotype, robust proliferation, and polyfunctional cytokine production.
- Figure 4: 
- Figure 5: B7-H6 CAR V5.1 T cells maintain cytotoxicity against HCT-15 cells in the presence of soluble B7-H6.
- Figure 6: Heatmap showing survival, phenotype, and polyfunctionality of γδ CAR T cells. The heatmaps demonstrate differentiation of γδ CAR T cell populations into different functional subsets.
- Figure 7: Heatmap showing survival, phenotype, and polyfunctionality of γδ CAR T cells. The heatmaps demonstrate differentiation of γδ CAR T cell populations into different functional subsets.

Methods:
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