2749. Preclinical assessment of chimeric antigen receptor (CAR) T persistence and functionality in the disseminated NALM6-Luc human B cell acute lymphoblastic leukemia (ALL) model

David Draper, Derrik Germain, Stacey Roys, Olivia Nelson and Scott Wise; Labcorp Early Development Laboratories Inc., Ann Arbor, MI

Introduction

- Establishing long-term CAR T persistence and efficacy remains a barrier to broader application of CAR T therapies in the clinic, thus development of robust platforms that can provide longitudinal assessments of CAR T persistence and functionality is paramount.
- Using the NALM6-Luc ALL model, Labcorp Drug Development has developed a flow cytometry platform that provides quantitative analysis of CAR T cells over time as well as surface markers that are documented to correlate with sustained T cell persistence, activation and exhaustion *in vivo*.

Methods

- Human peripheral blood mononuclear cells (hPBMCs) were transduced with lentivirus, expanded in culture and cryopreserved for future use.
- Flow cytometry featuring a CAR-specific monoclonal antibody (mAb) or anti-CD3 mAb was used to determine transduction efficiency and persistence, respectively.
- To assess *in vitro* activity, co-cultures of T cells and CD19-expressing NALM6-Luc-mCh-Puro cells were used to measure cytotoxicity. Human MV-4-11-Luc-mCh-Puro AML cells were used as a negative control.
- For evaluation of *in vivo* efficacy of anti-CD19 CAR T cells, activity against the disseminated NALM6-Luc-mCh-Puro ALL model in female *NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}*/SzJ (NSG) mice was determined.
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.
- For flow cytometric analysis, blood was collected 24h after CAR T cell infusion and weekly thereafter. After RBC lysis, single cell suspensions were labeled with fluorescent antibodies. Cells/µL of blood were quantified using Precision Count Beads™ (BioLegend). Data was acquired on an Attune NxT flow cytometer (Thermo Fisher Scientific) and analyzed using FlowJo® software (BD). Where indicated, statistical analysis was performed using a Student's T-test (* p<0.05).

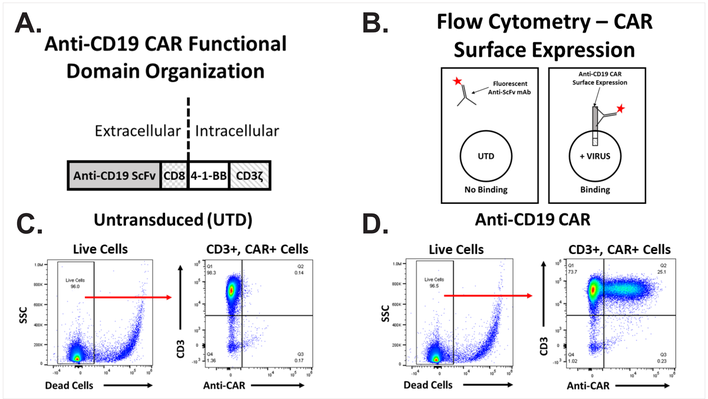

Figure 1. Anti-CD19 CAR T Cell Generation and Assessment of CAR Surface Expression by Flow Cytometry. **A.** Anti-CD19 CAR functional domain organization. **B.** Diagram depicting flow cytometry staining to identify positive transformants. UTD = untransduced **C.** UTD T cells are 96% viable and single positive for human CD3 (hCD3+CAR-, Q1, 98.3%). UTD T cells do not express anti-CD19 CAR (hCD3+CAR+, Q2, 0.14%). **D.** Anti-CD19 CAR T cells are 96.5% viable and express anti-CD19 CAR (hCD3+CAR+, Q2, 25.1%).

Results

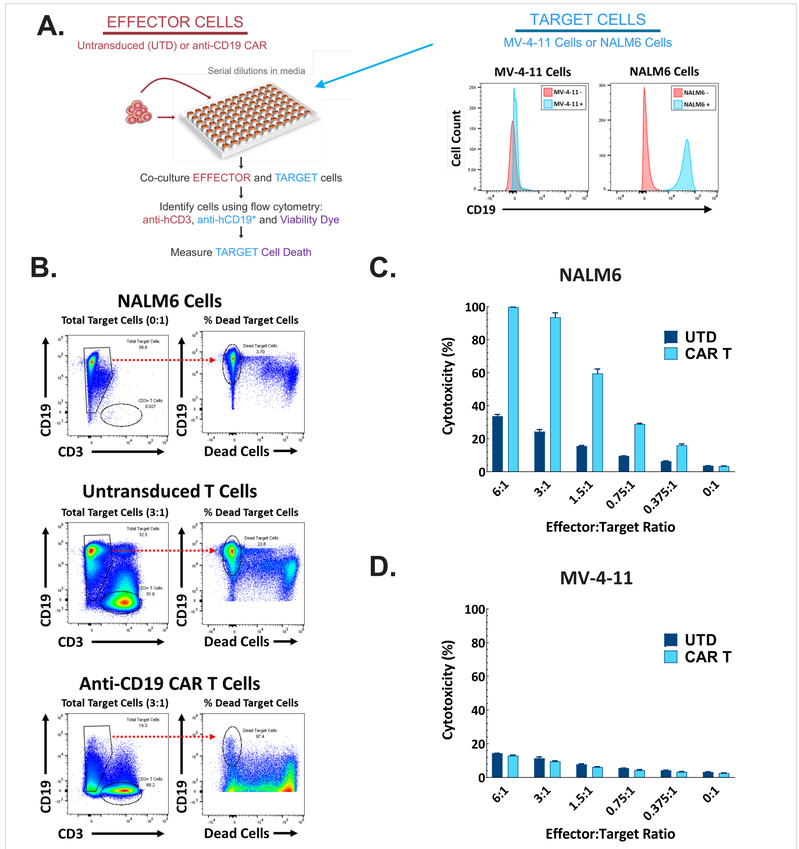

Figure 2. CAR T Cell Cytotoxicity Assay. A. Cytotoxicity assay set-up and histograms show MV-4-11 and NALM6 CD19 expression levels in the absence (-) and the presence (+) of an anti-CD19 mAb. **B.** Total target cells are analyzed for viability dye uptake. Dead Target Cells gate numbers represent the % of cells outside the live cell gate. 0:1 = 0 T cells for 1 target cell, 3:1 = 3 T cells for 1 target cell. **C. and D.** Quantification of cell killing assay. Data is representative of triplicate wells for each T cell:Target cell ratio.

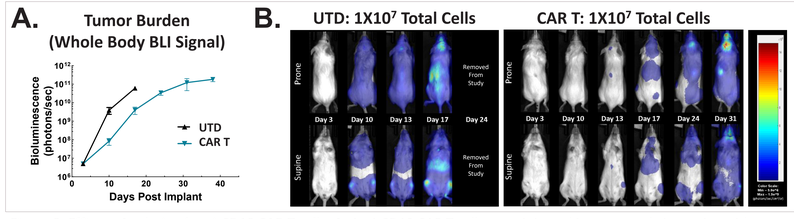

Figure 3. Efficacy Analysis of anti-CD19 CAR T cells. A. Anti-CD19 CAR T cells were injected intravenously into mice with established NALM6 disease. Disease progression and *in vivo* efficacy were monitored by bioluminescence (BLI) imaging on a weekly schedule. **B.** Representative BLI images.

Results (continued)

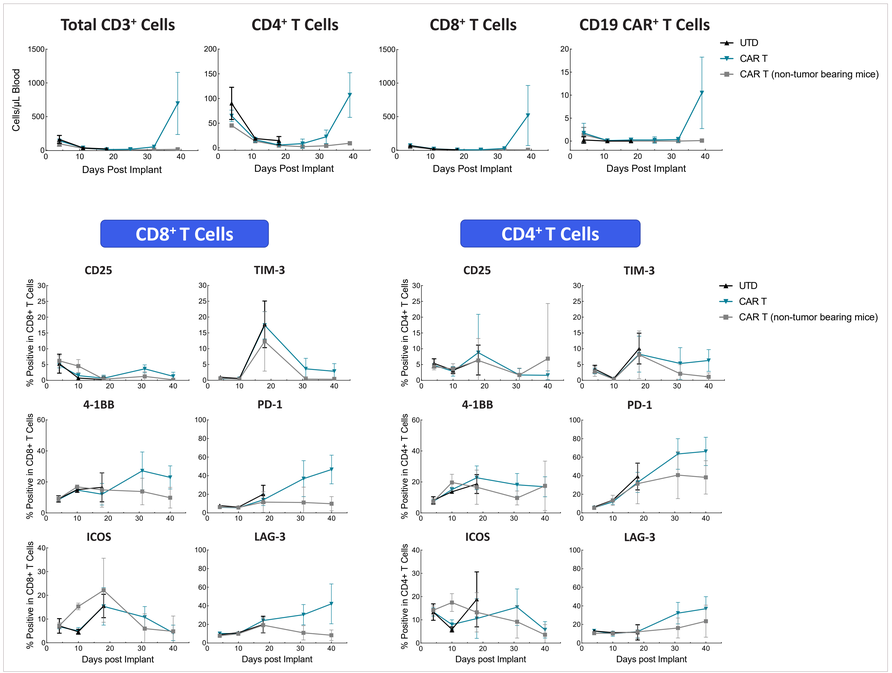

Figure 4. Assessment of Anti-CD19 CAR T Cell *In Vivo* Persistence and Phenotype. Whole blood samples were drawn weekly from NALM6 tumor-bearing and non-tumor bearing mice following adoptive cell therapy treatment. Flow cytometry was performed to measure: **A.** Human T cell persistence. **B**. Expression levels for selected T cell activation and exhaustion markers.

Conclusions

- After transduction of hPBMCs with lentivirus, approximately 25% of T cells were CD3$^+$/CAR$^+$.
- Anti-CD19 CAR T cells show specificity in a cell killing assay towards CD19 expressing NALM6 cells compared to CD19-null MV-4-11 cells.
- Anti-CD19 CAR T cells delayed tumor growth in the systemic NALM6-Luc ALL model but mice eventually succumbed to disease.
- Uncontrolled disease progression coincided with increased exhaustion marker expression (PD-1 and LAG-3) in CD8$^+$ T cells.
- Treatment-induced effects observed are likely independent of graft versus host disease because the CAR T cell phenotype was not recapitulated in non-tumor bearing mice.

DOWNLOAD THIS POSTER

AACR Annual Meeting, April 14-19, 2023, Orlando, FL

labcorp Drug Development