Generation and Characterization of Human Placental-derived CD19 CAR-T Cells Using Viral Vectors

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Introduction

Celularity Inc. is developing a CD19 CAR-T cell therapy using an allogeneic platform derived from postpartum human placental cells. To augment the CD19 CAR expression and activity of Placental-derived T (P-T) cells, we have evaluated the use of lentiviral (LV) vectors, as well as a retroviral (RV) CD19 CAR construct (provided by Sorrento Therapeutics, Inc) and assessed the effects of each virus on the phenotypic characteristics and activity of the P-T cells. In addition to mouse (Ms) scFv CD19 CAR constructs, three human (Hu) scFv CD19 CAR constructs were also tested, as fully humanized scFv CD19 constructs have been shown to be less immunogenic with improved persistence and efficacy in patients (Sommermeyer, et. al. Leukemia, 2017).

Herein we demonstrate the ability to generate P-T CD19 CAR cells through the transduction of P-T cells with a variety of viral vectors containing the 4-1BB costimulatory domain. We report on the similarities in CAR expression and *in vitro* activity observed between CAR constructs/ viral vectors, while highlighting differences detected in phenotype and *in vivo* anti-tumor activity of these cells.

Methods

Gene Modification: P-T CD19 CAR cells were generated through transduction of human placental T cells using lentiviral and retroviral vectors carrying Mouse (Ms) and Human (Hu) scFv anti-CD19 CAR sequences according to table below:

Construct	Viral Vector	scFv Sequence	Costimulatory Domain	# of Donors (N)	Additional Details
JL4.19	Lentivirus	Mouse	4-1BB	4	Based on mouse Ab FMC63
JL	Lentivirus	Human	4-1BB	2	Identical to JL4.19, except Hu scFv
JK1	Lentivirus	Human	CD28	2	Modified in hinge & transmembrane domains from JL with CD28 costimulatory domain
JK2	Lentivirus	Human	4-1BB	2	Same as JK1, but with 41BB costimulatory domain
Sorrento	Retrovirus	Mouse	4-1BB	7	Proprietary

Phenotypic Characterization: The phenotype and T cell differentiation status of P-T cells were determined using flow cytometry. The cells were stained for CD3, CD56, CD4, CD8, CD25, CD127, CD45RA, CCR7, CD27, PD-1, TIM-3, and CD57 expression. The viability was assessed using 7AAD or FVS staining. CD19 CAR Expression was quantified using a recombinant CD19 Fc-Fitc labeled protein.

Cytotoxicity Assay: The *in vitro* anti-tumor functional activity of P-T CD19 CAR cells against CD19+ Burkitt's Lymphoma (Daudi) and Acute lymphoblastic Leukemia (NALM6) cell lines was assessed using the kinetic ACEA –based cytotoxicity assay at an E:T ratio of 2.5:1. K562 cells were included as a CD19- negative control.

Cytokine Release Assay: The *in vitro* functional activity of P-T CD19 CAR cells against CD19+ Burkitt's Lymphoma (Daudi) and Acute lymphoblastic Leukemia (NALM6) cell lines cell was assessed by co-culturing P-T cells at an E:T ratio of 1:1 for 24-hours and quantifying the levels of proinflammatory cytokines and effector proteins in the supernatant using the Meso Scale Discovery (MSD) platform.

In vivo Anti-Tumor Model: The Disseminated Daudi (lymphoma) xenograft model was established in NSG mice. NSG mice were preconditioned with busulfan (30 mg/kg, intraperitoneal injection) on Day -7 and inoculated with 3 x 10^6 Daudi-luc cells intravenously (IV) on Day 0. Vehicle, P-T Ms CD19-CAR LV and RV T cells, and PBMC Ms CD19-CAR RV T cells were IV administered on Day 7 according to their CD8+ CD19 CAR+ frequencies (low dose:0.6MM cells and high dose:2MM cells). Bioluminescence imaging was measured once per week. The surviving P-T CD19 CAR-treated mice were then re-challenged on Day 122 with an additional inoculation of 3 x 10^6 Daudi-luc cells. Age-matched (6-month-old) naïve NSG mice were included as new vehicle controls.

Summary

- Isolated P-T cells consisted mostly of naïve T cells, with a small proportion of central memory T cells (Tcm)
- P-T cells could be readily expanded following 15 days in culture (research-scale); highest fold expansion of 483-fold was achieved transducing P-T cells with Ms CD19 CAR LV and lowest fold expansion of 132-fold was obtained with Hu JK1 CD19 CAR LV
- CD19 CAR transduction efficiency was high in P-T cells transduced with all CAR constructs containing the 4-1BB costimulatory domain (Avg: 42% CD19 CAR+; $p \leq 0.0085$ vs. NT), but not with the CD28 costimulatory domain (Hu JK1 LV)
- Observed distinct phenotypic differences between P-T's transduced with RV vs. LV:
  - **RV**: Greater frequency of CD8+ T cells, equal expression of CAR between CD4 and CD8 T cells, greatest frequency of CD8+ CD19 CAR+ (of CD3+) T cells, and less differentiated phenotype with highest frequency of CAR+ T scm/ naïve cells
  - **LV**: Greater frequency of CD4+ T cells, greater frequency of CD4+ CD19 CAR+ (of CD3+) T cells (esp. Ms LV), lowest frequency of CD8+ CD19 CAR+ (of CD3+) T cells, and more differentiated phenotype with lower frequency of CAR+ T scm/ naïve cells (esp. Hu JK2 LV)
- All CD19 CAR+ P-T cells expressed higher frequency of CD45RA, CCR7, CD27, and lower frequency of PD-1, TIM-3, and the exhaustion marker CD57, as compared to PBMC-derived CD19 CAR+ T cells
- With the exception of Hu JK1 CD19 CAR LV, all P-T CD19 CAR cells specifically lysed CD19+ Daudi ($p \leq 0.0091$ vs. NT) and Nalm6 ($p \leq 0.0029$ vs. NT) targets, but not CD19- K562 cells in the ACEA kinetic *in vitro* cytotoxicity assay; *in vitro* cytotoxic activity was comparable across all CD19 CAR constructs
- When P-T CD19 CAR cells were co-cultured with CD19+ Daudi/ Nalm6 target cells for 24-hours, all CD19 CAR constructs secreted pro-inflammatory cytokines and effector proteins in an antigen-specific manner, with greatest overall secretion observed with Ms CD19 CAR RV
- *In vivo*, all P-T CD19 CAR cells were well tolerated, significantly reduced tumor burden, and improved survival compared to vehicle control
  - Low/ single dose of P-T CD19 CAR LV cells performed as well as PBMC CD19 CAR RV (high dose); high/ multi-dose enhanced tumor burden reduction and survival
  - P-T CD19 CAR RV cells: Only treatment to eliminate tumor and result in 100% survival out to120 days, in addition to managing tumor following Daudi re-challenge (on Day 122), and extending survival out to 215 days
- Current, on-going *in vivo* efficacy study is evaluating P-T cells transduced with Ms LV vs. Ms RV, as well as with Hu JL LV, and Hu JK2 LV in the Disseminated Daudi Mouse Model

RESULTS

Figure 1. Isolated P-T Phenotype

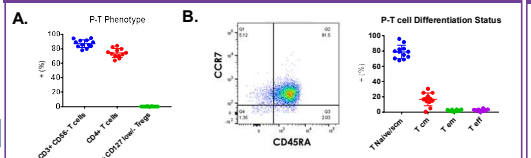

Figure 1. (A) Phenotype and (B) T cell differentiation status of isolated, starting material P-T cells (Mean with SD, n=12).

Figure 2. Fold Expansion and Phenotype of Day 15 P-T CD19 CAR Cells

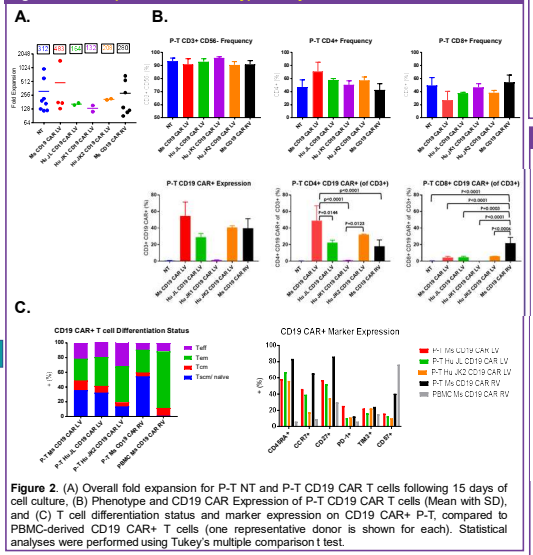

Figure 2. (A) Overall fold expansion for P-T NT and P-T CD19 CAR T cells following 15 days of cell culture, (B) Phenotype and CD19 CAR Expression of P-T CD19 CAR T cells (Mean with SD), and (C) T cell differentiation status and marker expression on CD19 CAR+ P-T, compared to PBMC-derived CD19 CAR+ T cells (one representative donor is shown for each). Statistical analyses were performed using Tukey's multiple comparison t test.

Figure 3. P-T CD19 CAR Cells Specifically Lyse CD19+ Targets *In Vitro*

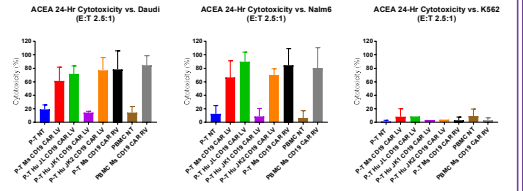

Figure 3. ACEA Kinetic cytotoxicity assay vs. CD19+ Daudi and Nalm6 targets and CD19- K562 cells (Mean with SD), compared to PBMC Ms CD19 CAR RV (n=6).

Figure 4. P-T CD19 CAR Cells Secrete Pro-inflammatory Cytokines and Effector Proteins in Response to CD19+ Target *In Vitro*

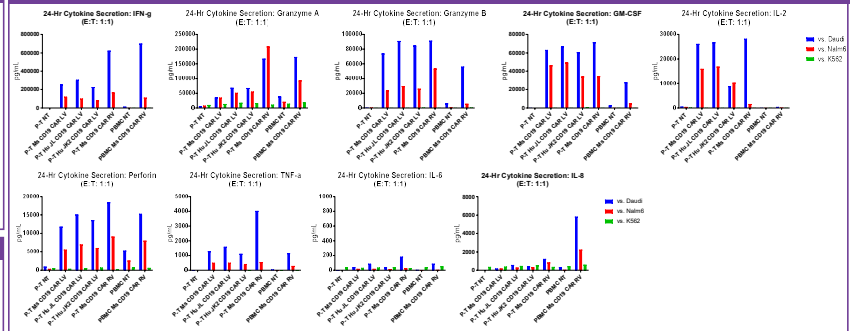

Figure 4. P-T CD19 CAR secretion in response to co-culture (E:T: 1:1) with CD19+ Daudi and Nalm6 target cells, and CD19- K562 cells for 24-Hours. P-T CD19 CAR cytokine and effector protein secretion quantified using MSD and compared to PBMC-derived CD19 CAR T cells (one representative donor is shown for each).

Figure 5. P-T CD19 CAR Cells Significantly Reduce Lymphoma Tumor Burden and Improve Survival *In Vivo*

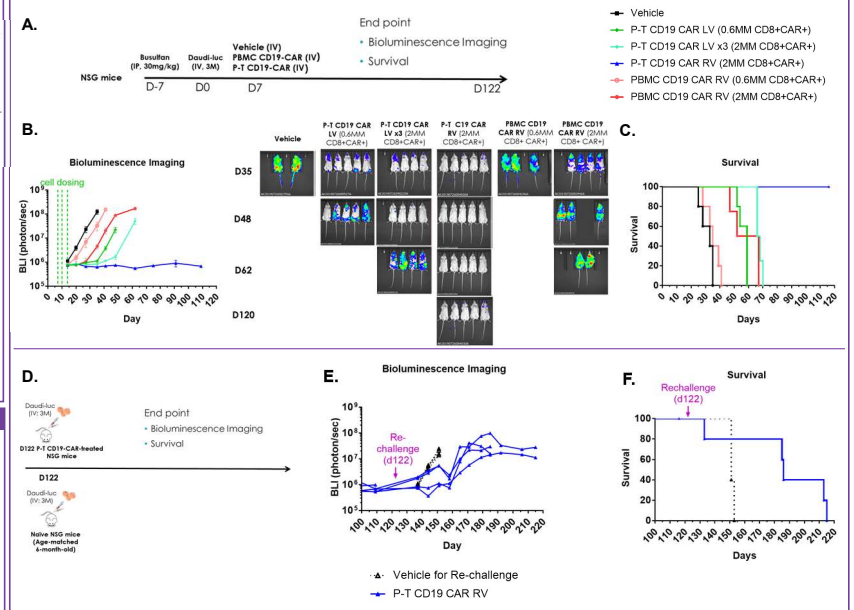

Figure 5. (A) Schema of lymphoma tumor model, (B) Bioluminescence imaging (Mean with SEM, each group n=5), (C) Survival curve comparing P-T Ms CD19 CAR LV, P-T Ms CD19 CAR RV, and PBMC Ms CD19 CAR RV with PBS control. (D) Schema of Day 122 lymphoma tumor re-challenge, (E) Tumor re-challenge Bioluminescence imaging (each group n=5), and (F) Tumor re-challenge survival curve.