Preclinical Evaluation of Human Placental-derived Allogeneic CD19 CAR-T Cells Against B Cell Malignancies

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Abstract #3222

Introduction

Celularity, Inc. is developing a CD19 CAR-T Cell therapy using an allogeneic platform derived from postpartum human placental cells. T cells isolated from placenta/ umbilical cord blood and genetically modified to express CD19 chimeric antigen receptor (CAR), termed Placental-derived (P-) CD19 CAR T cells, are in development for the treatment of CD19+ B cell malignancies. Unlike adult peripheral blood mononuclear cell (PBMC)-derived T cells, P-T cells are mostly naïve (CD45RA+) and can be readily expanded while maintaining an earlier differentiation phenotype, such as greater expression of naïve/ memory markers, lower expression of effector/ exhaustion markers, allowing for greater proliferative potential of these cells *in vivo* [1-2]. These cells are also known to have greater immune tolerance to HLA mismatch and display impaired allogeneic activation, contributing to lower incidences of severe graft-verse-host disease (GvHD) [3-4], making them an attractive cell population for use as an allogeneic, adoptive cell therapy.

A process for the isolation, transduction, and expansion of placental-derived T cells to generate off-the-shelf allogeneic P-CD19 CAR T cells has been developed. These cells exhibit potent anti-tumor activity both *in vitro* and *in vivo* with little evidence of acute GvHD induction, highlighting their potential as an allogeneic, adoptive cell therapeutic agent.

Methods

Gene Modification: P-T CD19 CAR cells were generated through transduction of human placental T cells using retroviral vector carrying anti-CD19 CAR (provided by Sorrento Therapeutics, Inc.). Additional gene modification included a CRISPR-mediated T-cell receptor α constant (TRAC) knockout (KO) step as a supplementary risk-mitigation strategy to circumvent any potential GvHD stemming from expression of endogenous T cell receptor.

Phenotypic Characterization: The phenotype and T cell differentiation status of P-T cells was determined using flow cytometry. The cells were stained for CD3, CD56, CD4, CD8, CD25, CD127, CD45RA, CCR7, CD27, PD-1, TIM-3, CD57, and TCR α/β 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 at various effector to target (E:T) ratios using a 4-hour PKH26/ TO-PRO-3 FACS-based method and a kinetic ACEA –based cytotoxicity assay.

Cytokine Release Assay: The *in vitro* functional activity of P-T CD19 CAR cells against CD19+ Burkitt's Lymphoma (Daudi) cell line 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 protein in the supernatant using MSD.

In vivo Anti-Tumor Model: 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, PBMC CD19-CAR (7x10^6) or P-T CD19-CAR cells (14x10^6) were IV administered on Day 7 according to their CD8+ CD19 CAR+ frequencies. 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.

In vivo Xenogeneic GvHD Model: NCG mice were IV administered with 30 x 10^6 PBMC or Day 21 expanded, non-modified P-T cells from three donors on Day 0. Body weight was measured, and blood was collected to evaluate CD3+ T cells by FACS

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 to 283-370-Fold following 15 days in culture (research-scale)
- CD19 CAR transduction efficiency was high in P-T cells (40% CD19 CAR+), with even distribution of CD19 CAR on both CD4+ and CD8+ T cells
- Following expansion, P-T CD19 CAR cells expressed high levels of naïve / memory markers and low levels of inhibitory molecules/ exhaustion markers
- P-T CD19 CAR cells specifically lysed CD19+ Daudi/ Nalm6 targets in both 4-hour endpoint FACS and ACEA kinetic *in vitro* cytotoxicity assays
- When P-T CD19 CAR cells were co-cultured with CD19+ Daudi target cells for 24-hours, they secreted pro-inflammatory cytokines and effector proteins in an antigen-specific manner
- *In vivo*, P-T CD19-CAR significantly reduced tumor burden and improved survival compared to the vehicle control and PBMC CD19-CAR cells (out to Day 120)
- Upon *in vivo* tumor re-challenge on Day 122, P-T CD19 CAR continued to reduce tumor burden (lower BLI) and improve survival out to Day 151, as compared to the vehicle control; Study is still ongoing
- Expanded, non-modified P-T cells did not induce Xenogeneic GvHD *in vivo*, whereas PBMC did, as evidenced by significant weight loss, death of all mice, and increase in detection of human CD3+ T cells in PBMC treated mice by Day 28 post infusion
- CRISPR-mediated TRAC KO efficiency was high in P-T CD19 CAR cells (>97% TCR α/β-) and did not affect CD19 CAR expression or *in vitro* cytotoxic activity
- Future *in vivo* GvHD studies will include assessment of both CD19 CAR and TRAC KO genetically modified P-T cells

References

1. Okas, et. al. Journal of Immunotherapy, 2010
2. Frumento, et. al. Journal of Transplantation, 2013
3. Barker, et. al. Blood, 2001
4. Chen, et al. Biology of Blood and Marrow Transplantation, 2006

Disclosure

KKM: SH: KT: WL: Celularity Inc: Employment; GK: Sorrento Therapeutics, Inc.: Employment, Equity Ownership, Patents & Royalties. JZ: Sorrento Therapeutics Inc: Employment, Equity Ownership. HJ: Sorrento Therapeutics Inc: Employment, Equity Ownership, Patents & Royalties; Celularity, Inc.: Equity Ownership, Membership on an entity's Board of Directors or advisory committees. RH: Celularity Inc: Employment. XZ: Celularity Inc: Employment.

RESULTS

Figure 1. Isolated P-T Phenotype

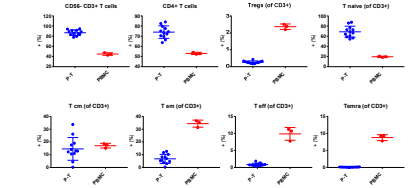

Figure 1. Phenotype and T cell differentiation status of isolated, starting material P-T cells (Mean with SD, n=12), compared to PBMC.

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

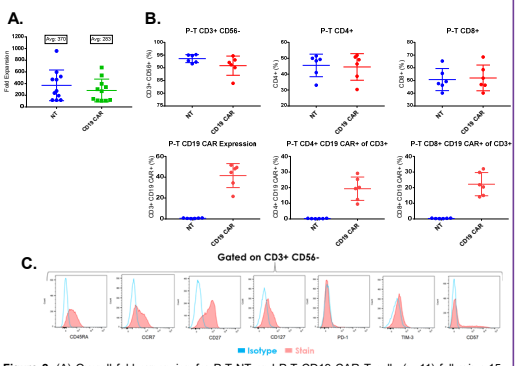

Figure 2. (A) Overall fold expansion for P-T NT and P-T CD19 CAR T cells (n=11) following 15 days of cell culture (B) Phenotype and CD19 CAR Expression of P-T CD19 CAR T cells (n=6) (C) Representative flow histograms for expression of various markers on P-T CD19 CAR cells.

Figure 3. P-T CD19 CAR Cells Specifically Lyse CD19+ Targets *in vitro*

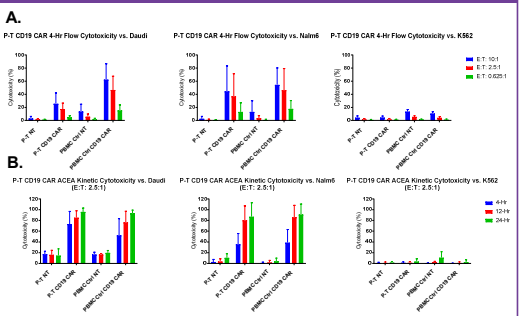

Figure 3. (A) 4-Hour endpoint Flow-based cytotoxicity assay vs. CD19+ Daudi and Nalm6 targets and CD19- K562 cells (Mean with SD, n=6) (B) ACEA Kinetic cytotoxicity assay vs. CD19+ Daudi and Nalm6 targets and CD19- K562 cells (Mean with SD, n=6).

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

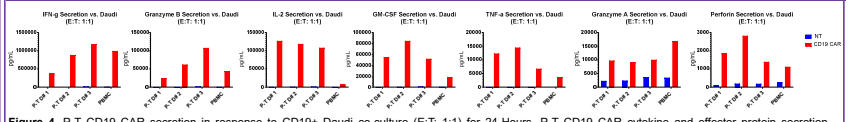

Figure 4. P-T CD19 CAR secretion in response to CD19+ Daudi co-culture (E:T: 1:1) for 24-Hours. P-T CD19 CAR cytokine and effector protein secretion quantified using MSD and compared to PBMC-derived CD19 CAR T cells.

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

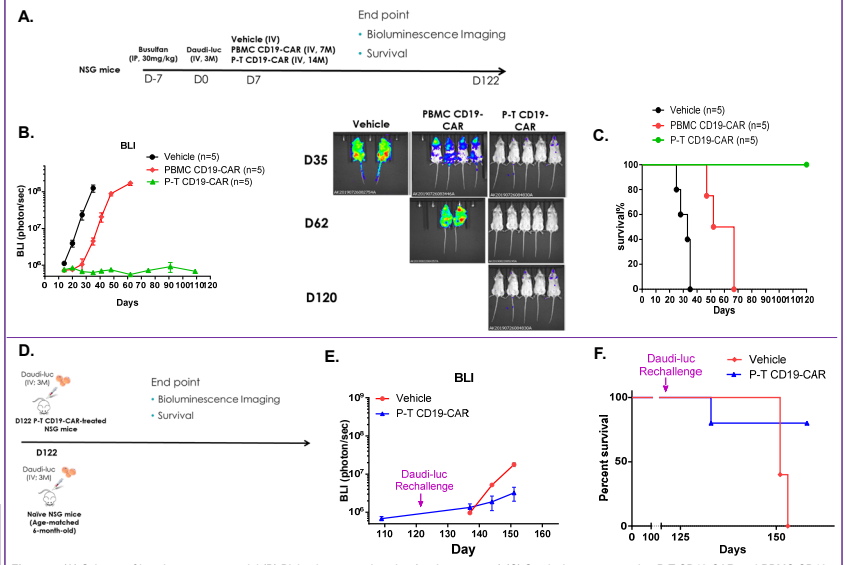

Figure 5. (A) Schema of lymphoma tumor model (B) Bioluminescence imaging (each group n=5) (C) Survival curve comparing P-T CD19 CAR and PBMC CD19 CAR with PBS control (D) Schema of Day 122 lymphoma tumor re-challenge (E) Tumor re-challenge Bioluminescence imaging (each group n=5) (F) Tumor re-challenge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

Figure 6. P-T Cells Do Not Induce Xenogeneic GvHD *in vivo*

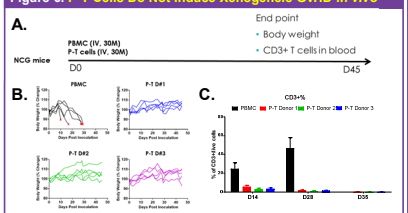

Figure 6. (A) Schema of Xeno-GvHD model (B) Body weight change and survival (x=death) (each group n=5) (C) Flow-based *ex vivo* human CD3+ T cell detection/ expansion in mice.

Figure 7. TRAC KO Efficiency is High in P-T CD19 CAR Cells and Does Not Affect CD19 CAR Expression or *in vitro* Cytotoxic Activity

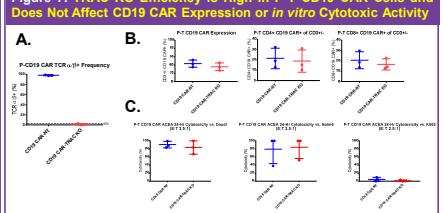

Figure 7. (A) TRAC KO efficiency (TCR α/β expression) in P-T CD19 CAR cells (Mean with SD, n=3) (B) Effects of TRAC KO on CD19 CAR expression (C) Effects of TRAC KO on ACEA 24-Hr cytotoxic activity.