Development of CD38 CAR Engineered Human Placental Hematopoietic Stem Cell Derived Natural Killer Cells (PNK-CAR38) As Allogeneic Cancer Immunotherapy

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Abstract #2070

INTRODUCTION

Natural killer (NK) cells exhibit innate anti-tumor activity owing to the expression of a multitude of activating and inhibitory receptors that orchestrate NK cell responses. It is thus possible to use NK cells from allogeneic sources without the risk of graft-vs-host disease[1], making them very attractive for developing "off-the-shelf" cellular therapies. The anti-tumor responses of NK cells can be further enhanced by expressing Chimeric Antigen Receptors (CARs)[2,3].

Celularity has developed a GMP process for generating off-the-shelf, allogeneic human Placental Hematopoietic Stem Cell (HSC) derived Natural Killer cells (PNK)[4]. The placental HSC source is vast, and Celularity process is streamlined to yield large quantities of differentiated and activated NK cells that have been well characterized. Here, we report the development of a tumor targeted approach through engineering PNK cells to express CAR against tumor antigen CD38.

CD38 is a glycoprotein and ectoenzyme highly expressed in hematological malignancies notably in lymphoma[5,6] and multiple myeloma[7], making it an attractive target for antibody and CAR based therapies.

We have demonstrated here that the PNK cells expressing CD38 CAR (PNK-CAR38) have enhanced anti-tumor function against CD38+ lymphoma and multiple myeloma (MM) cell lines in pre-clinical studies. Despite expression of CD38 on healthy lymphocytes and hematopoietic progenitor cells, PNK-CAR38 cells do not exhibit on-target off-tumor cytotoxicity as assessed against healthy CD38+ T cells and CD34$^+$CD38$^+$ progenitor cells.

Methods

Gene Modification and PNK Culture: PNK-CAR38 cells were generated through transduction of human placental CD34+ cells using retroviral vector carrying anti-CD38 CAR (CAR2-anti-CD38 A2; CD38scFv-CD28CD3ζ) followed by expansion and differentiation to NK cells in presence of cytokines including thrombopoietin, SCF, Flt3 ligand, IL-7, IL-15 and IL-2.

Phenotypic Characterization: The purity of PNK non-transduced cells (PNK-NT) and PNK-CAR38 was determined using flow cytometry. The cells were stained for CD56, CD3, CD19, CD16 and CD38 CAR expression. The viability was assessed using 7AAD staining.

Cytotoxicity Assay: The anti-tumor activity of PNK-NT and PNK-CAR38 cells was assessed against Lymphoma lines - Daudi, Raji, HS Sultan and SUDHL6 and Multiple Myeloma cell lines Molp8, LP1 and OPM2, at various effector to target (E:T) ratios using a 4-hour PKH26 flow cytometry-based method.

In Vivo Anti-Tumor Model: Disseminated Daudi (lymphoma) xenograft model was established in NSG mice. By i.v. inoculation of 3×10^6 luciferase-expressing Daudi cells on Day 0, followed by IV injection of PBS, PNK-NT or PNK-CAR38 cells (10×10^6) on Days 1 and 3. Recombinant IL-15 was given to mice every other day for 15 days. Tumor burden was assessed weekly by Bioluminescence Imaging (BLI).

Summary

- Retroviral transduction of placental CD34+ cells was efficient and generated PNK-CAR38 cells with an average 64% CD38 CAR expression at end of expansion
- A robust expansion was achieved at median of 28,800-fold for PNK-CAR38 cells
- PNK-NT and PNK-CAR38 cells showed comparable differentiation and NK cell phenotype
- PNK-CAR38 cells showed significantly higher anti-lymphoma and anti-MM cytolytic function in vitro compared to PNK-NT
- PNK-CAR38 cells with 35% CD38 CAR expression lysed >50% of Daudi cells
- PNK-CAR38 cells did not display on-target off-tumor cytotoxicity against healthy activated T cells and hematopoietic progenitor cells from unrelated donors
- PNK-CAR38 cells showed a 49% reduction in BLI 10 days after PNK-CAR38 cell administration in vivo indicating anti-lymphoma function, but did not demonstrate improvement in survival in the current model
- Further in vivo evaluation of PNK-CAR38 is ongoing
- PNK-CAR38 developed by Celularity is a promising allogeneic approach with low potential for on-target off-tumor toxicity and presents an opportunity for developing CD38 targeted therapy for lymphoma in addition to MM

References

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Disclosure

SS: XG: SH: AD: HR: 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. Retroviral (RV) CD38 CAR Construct, PNK Transduction and Expansion Process

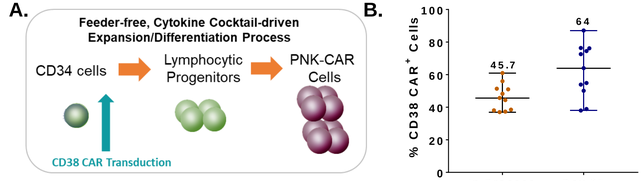

Figure 1. (A) Schema of Placental CD34+ cells were expanded and differentiated after RV transduction early in the process (B) Expression of CD38 CAR in process and at end of process (Median and Range, n=11)

Figure 2. Fold Expansion and Phenotype of PNK-CAR38 Cells

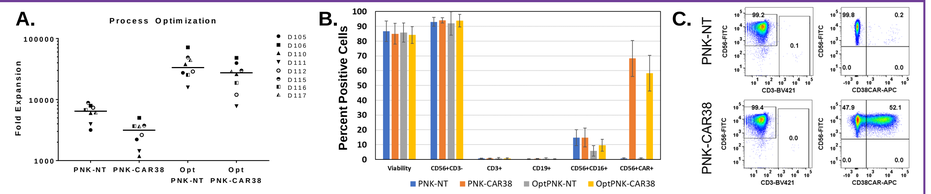

Figure 2. (A) Overall fold expansion: Optimized process yielding higher and comparable median fold expansion for PNK-NT and PNK-CAR38 cells (n=8) (B) The differentiation efficiency of PNK-CAR38 was comparable to PNK-NT (C) Representative flow plots demonstrating differentiation and percent CD38 CAR expression on PNK-CAR38 cells compared to PNK-NT. Staining for CAR expression was performed using recombinant CD38-his protein followed by anti-His PE antibody.

Figure 3. PNK-CAR38 Cells Enhance Cytotoxicity Against MM Lines In Vitro

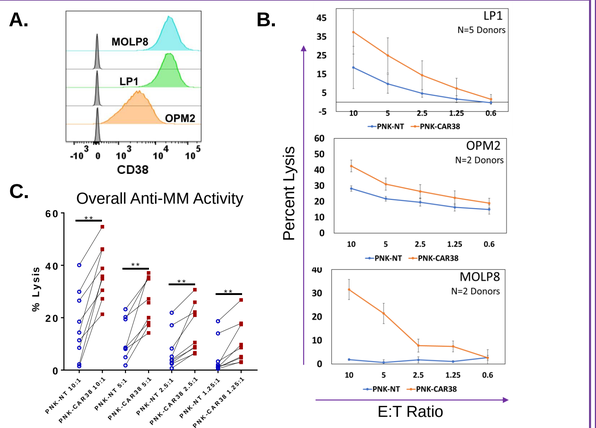

Figure 3. (A) CD38 expression on MM cell lines (B) Lysis of MM cell lines by PNK-CAR38 compared to PNK-NT (Mean ± Std Dev) (C) Combined anti-Multiple Myeloma cytotoxicity of PNK-CAR38 cells compared to PNK-NT (**p<0.004). Statistical analysis is performed by Wilcoxon matched-pairs two tailed t test.

Figure 4. PNK-CAR38 Cells Enhance Cytotoxicity Against Lymphoma Lines In Vitro

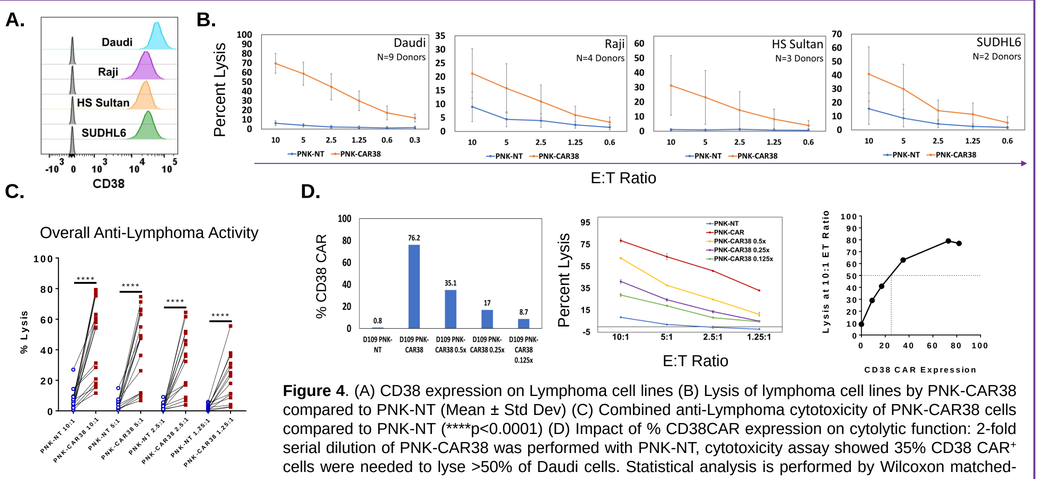

Figure 4. (A) CD38 expression on Lymphoma cell lines (B) Lysis of lymphoma cell lines by PNK-CAR38 compared to PNK-NT (Mean ± Std Dev) (C) Combined anti-Lymphoma cytotoxicity of PNK-CAR38 cells compared to PNK-NT (****p<0.0001) (D) Impact of % CD38CAR expression on cytolytic function: 2-fold serial dilution of PNK-CAR38 was performed with PNK-NT, cytotoxicity assay showed 35% CD38 CAR$^+$ cells were needed to lyse >50% of Daudi cells. Statistical analysis is performed by Wilcoxon matched-pairs two tailed t test.

Figure 5. PNK-CAR38 Cells Do Not Exhibit On-Target Off-Tumor Cytotoxicity

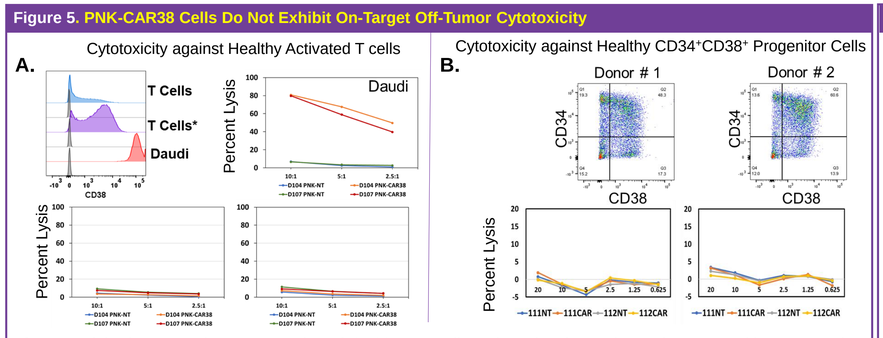

Figure 5. (A) Histogram shows expression of CD38 on T cells activated 5 days prior to the assay with TransAct (T cell*). Cytotoxicity was assessed against activated T cells and Daudi cells as control (B) CD34$^+$CD38$^+$ hematopoietic progenitors (from 2 unrelated donors) were tested for sensitivity to lysis by PNK-CAR38.

Figure 6. PNK-CD38CAR Reduce Lymphoma Tumor Burden In Vivo

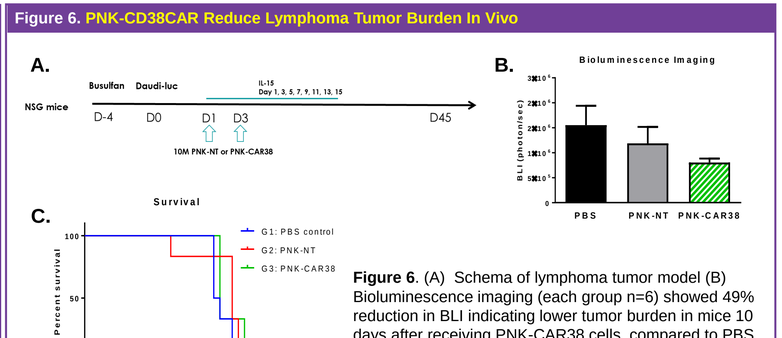

Figure 6. (A) Schema of lymphoma tumor model (B) Bioluminescence imaging (each group n=6) showed 49% reduction in BLI indicating lower tumor burden in mice 10 days after receiving PNK-CAR38 cells compared to PBS or PNK-NT controls (C) Survival curve comparing PNK-NT and PNK-CAR38 with PBS control