Engineering High Affinity and Cleavage Resistant CD16 to Augment ADCC of Placental Hematopoietic Stem Cells-Derived Natural Killer Cells

Abstract #1576

Xuan Guo[1], Srinivas Somanchi[1], Shuyang He[1], Qian Ye[1], Andrea DiFiglia[1], Salvatore Rotondo[1], Hemlata Rana[1], Weifang Ling[1], Robert Hariri[1] and Xiaokui Zhang[1]

[1]Celularity, Inc, Warren, NJ

INTRODUCTION

Celularity, Inc. is developing human placental hematopoietic stem cells-derived, cryopreserved, off-the-shelf, ex-vivo expanded and allogenic natural killer (NK) cells for treatment of various hematological malignancies and solid tumors.[1]

NK cells play a central role in antibody dependent cell mediated cytotoxicity (ADCC) through Fc receptor CD16 in monoclonal antibody mediated anti-tumor therapies.

Two allelic forms of CD16 have been identified with the 158Val/Val form shown to have higher IgG binding affinity comparing with the 158Phe/Phe form.[2] The high IgG binding allele are found in about 10-20% of the normal population.[3, 4] In addition, activation of NK cells induces CD16 shedding by matrix metalloprotease ADAM17 at 197Ser, thus limiting ADCC responses. A single mutation (Ser197Pro) prevents CD16 shedding and increases ADCC activity in NK cells.[5]

To augment the effector functions of the placental hematopoietic stem cell derived NK cells and to sustain their tumor-killing potential, we expressed a high affinity (158Val) and proteinase cleavage resistant (197Pro) CD16 variant (CD16VP). Here we report the *in vitro* and *in vivo* phenotypic and functional evaluation of CD16VP cells.

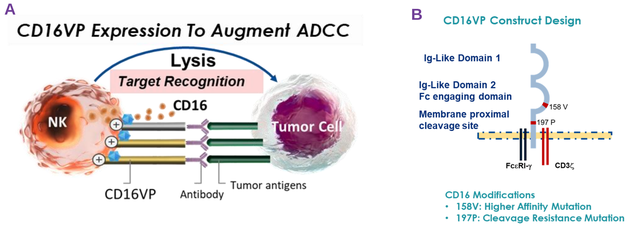

Figure 1. (A) Schematic representation of CD16VP-mediated ADCC. (B) Engineered construct of CD16VP with high affinity and proteinase cleavage resistant mutations to be expressed on placental hematopoietic stem cell derived NK cells.

MATERIALS AND METHODS

- Cell culture: Human placental CD34+ cells were isolated and cultured in the presence of cytokines including thrombopoietin, SCF, Flt3 ligand, IL-7, IL-15 and IL-2, for 35 days to generate NK cells.
- Cell Expansion and Characterization: Cell expansion was recorded during the culture process. On day 35, CD16VP cells were evaluated for NK surface markers CD56+/CD3-, and CD16, using flow cytometry.
- CD16VP Shedding Assay: Expression of CD16VP was evaluated by activating cells with PMA/ionomycin to induce CD16 cleavage followed by immunostaining with CD16 antibody and analyzed using flow cytometry.
- *In vitro* ADCC Assay: ADCC activity of CD16VP cells was assessed against Daratumumab (anti-CD38) or Rituximab (anti-CD20) opsonized lymphoma cell lines at various effector to target (E/T) ratios. IgG was used as ADCC control. In sustained ADCC assay, CD16VP cells were treated with PMA/ionomycin and then evaluated for ADCC activity as described above.
- Animal Study: *In vivo* anti-tumor activity was assessed in a Daudi disseminated Xenograft model in NSG mice. Luciferase-expressing Daudi cells ($3x10^6$) were intravenously (IV) administered at day 0, followed by CD16VP cells ($10x10^6$) IV at day 1 and day 3, and Daratumumab at day 3. Tumor burden in mice was monitored by Bioluminescence Imaging (BLI).
- Statistical Analysis: Statistical analysis was performed using Prism/Excel program. Data are presented as mean ± standard deviation. Paired or unpaired two-tailed Student's test were used for comparing two groups.

RESULTS

Figure 2. Placental CD34+ Cells Expanded and Differentiated to NK Cells

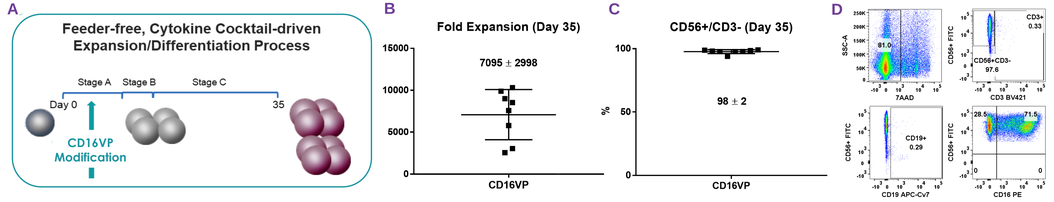

Figure 2. (A) Process schema (B,C) Total fold expansion of 35-day cultures and confirmation of NK phenotype of CD16VP cells (n=8 donors). (D) Examples of phenotypic characterization of CD16VP cells.

Figure 3. High Expression of CD16VP Sustained During Culture and Was Resistant to Shedding

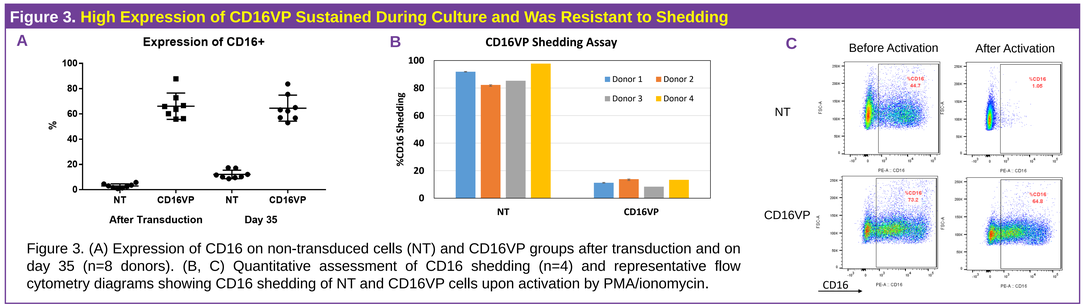

Figure 3. (A) Expression of CD16 on non-transduced cells (NT) and CD16VP groups after transduction and on day 35 (n=8 donors). (B, C) Quantitative assessment of CD16 shedding (n=4) and representative flow cytometry diagrams showing CD16 shedding of NT and CD16VP cells upon activation by PMA/ionomycin.

Figure 4. CD16VP Cells Demonstrated Augmented ADCC against Tumor Cell Lines *In Vitro*

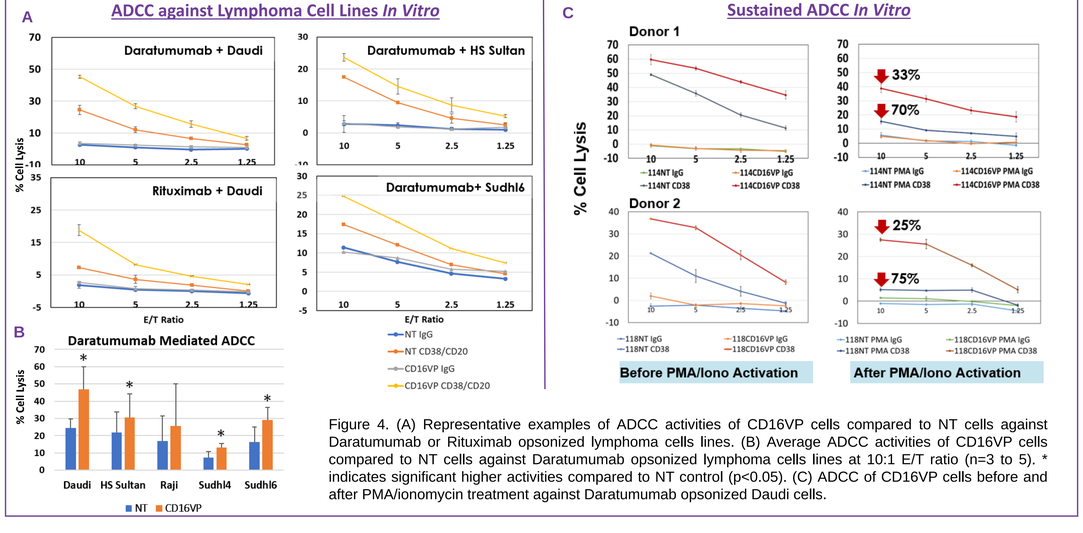

Figure 4. (A) Representative examples of ADCC activities of CD16VP cells compared to NT cells against Daratumumab or Rituximab opsonized lymphoma cells lines. (B) Average ADCC activities of CD16VP cells compared to NT cells against Daratumumab opsonized lymphoma cells lines at 10:1 E/T ratio (n=3 to 5). * indicates significant higher activities compared to NT control ($p<0.05$). (C) ADCC of CD16VP cells before and after PMA/ionomycin treatment against Daratumumab opsonized Daudi cells.

Figure 5. CD16VP Cells Demonstrated *In Vivo* Anti-Tumor Activities

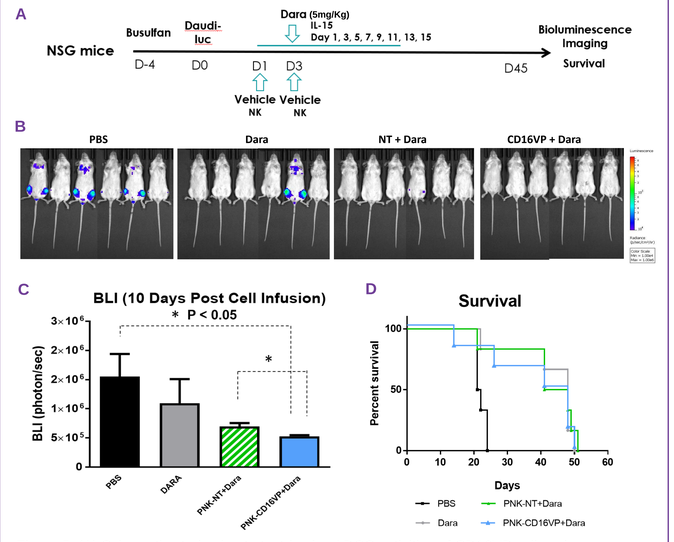

Figure 5. (A) Schematic study plan to test *in vivo* ADCC activities of CD16VP cells using a disseminated Daudi Xenograft model. (B) Bioluminescence images of mice from each group after 10 days of cell infusion. (C) Analysis of BLI after 10 days of cell infusion. * indicates a significant difference in between groups (n=6, $p<0.05$). (D) Survival of mice through the course of the study.

SUMMARY

- In this study, we genetically modified placental CD34+ cells to over-express CD16VP and evaluated the phenotype and functions of CD16VP cells regarding enhancement of ADCC and cleavage resistance.
  - ~7000-fold expansion and >90% NK purity were achieved by the process.
  - ~65% of CD16VP expression was maintained during the culture.
  - CD16VP expression was shown to be resistant to shedding after activation.
- CD16VP cells demonstrated enhanced ADCC *in vitro* against lymphoma cell lines in combination with Daratumumab or Rituximab.
- CD16VP resistance to activation induced shedding supported sustained killing *in vitro*.
- CD16VP cells showed *in vivo* anti-tumor activities at early time points in an ADCC lymphoma model.
- CD16VP provides a promising approach to augment the anti-tumor activities in combination with monoclonal antibodies. Further investigation is perused to support development of CD16VP in combination with therapeutic antibodies for various hematological malignancies and solid tumors.

References

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Disclosure

XG: SS: SH: QY: AD: SR: HR: WL: RH: XZ: Celularity Inc: Employment