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Single VHH-directed BCMA CAR-NK cells for multiple myeloma

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Abstract

Natural killer (NK) cells are promising alternatives for the production of "off-the-shelf" CAR products, posing a lower risk of cytokine release syndrome (CRS) than CAR-T cells. We synthesized four single VHH-directed anti-BCMA CARs, incorporating various intracellular regions (2B4 *versus* CD28) and hinge domains (CD28 *versus* IgG1) and ectopically producing IL-15. NK cells derived from peripheral blood (PB) were expanded ex vivo by K562-mbIL21 feeder cells. Stable CAR transduction was obtained through lentiviral transduction with the BaEV-Rless pseudotyped lentiviral vector. BCMA-CD28-IL15 CAR-NK cells with ectopic expression of IL-15 exhibited superior cytotoxicity were compared to BCMA-CD28 CAR-NK cells lacking IL-15 and BCMA-hIgG1-IL15 CAR-NK cells with an IgG1 hinge domain. We further assessed the cytotoxic capabilities of BCMA-2B4-IL15 CAR-NK cells with 2B4 intracellular domain. The BCMA-CD28-IL15 CAR-NK cells revealed stronger cytotoxicity and higher cytokine secretion against $BCMA^+$ tumor cells than BCMA-2B4-IL15 CAR-NK cells in vitro. In the MM.1S-Luc mouse model, BCMA-CD28-IL15 CAR-NK inhibited the growth of tumor cells and prolonged mouse survival. These results show that the single VHH-directed BCMA CAR-NK cells exhibited remarkable specific killing ability, making them a potential candidate for immunotherapy in multiple myeloma treatment.

Keywords BCMA, CAR-NK, Multiple Myeloma, VHH

To the Editor:

Natural killer (NK) cells are promising alternatives for the production of "off-the-shelf" CAR products, posing a lower risk of cytokine release syndrome (CRS) than CAR-T cells [1, 2]. Nanobodies (Nbs), or "single domain antibodies" (sdAbs) or "variable domains of heavy chain of heavy-chain antibodies" (VHHs), are naturally occurring antibodies that lack light chains in Camelidae and shark species peripheral blood [3]. Our previous studies have confirmed that PRG1801 CAR-T cells using a single VHH targeting one BCMA epitope are effective in the clinical treatment of multiple myeloma (MM) [4]. In this study, we prepared single VHH-directed anti-BCMA CAR-NK cells and evaluated their cytotoxic properties.

First, we confirmed our optimized protocol for ex vivo expansion of NK cells derived from peripheral blood (PB). Using K562-mIL-21 as feeder cells can make NK cells rapidly expand and obtain high-purity NK cells [5, 6] (Supplemental methods and data). The NK cells achieved approximate 4,000-fold expansion with a final purity of more than 90%. We used BaEV-Rless envelope pseudotyped lentiviral vector, which can bind to the

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amino-acid-transporter receptors ASCT1 and ASCT2 that are highly expressed on activated NK cells [5, 7], in the lentiviral package system. Stable CAR transduction efficiency was obtained.

We then selected MM.1S, Daudi, NCI-H929, and RS4;11 cell lines with different BCMA expression levels as target cells for in vitro cytotoxicity validation (Supplementary Fig. S2D). The CAR ectopically produced IL-15 allowing NK cells to prolong in vivo proliferation [8, 9]. We encoded the IL-15 gene in the CAR construct and verified the functional impact of IL-15 expression. A BCMA-CD28-IL15 CAR with ectopic IL-15 expression and a BCMA-CD28 CAR without IL-15 were synthesized (Fig. 1A). There was a statistically significant difference in the cytotoxic activity of BCMA-CD28-IL15 CAR-NK cells toward MM.1S and Daudi cells, compared to BCMA-CD28 CAR-NK and NK cells ($P<0.0001$, n=3) (Fig. 1D). Furthermore, the secretion levels of IFN-γ, granzyme B, and IL-15 were increased in

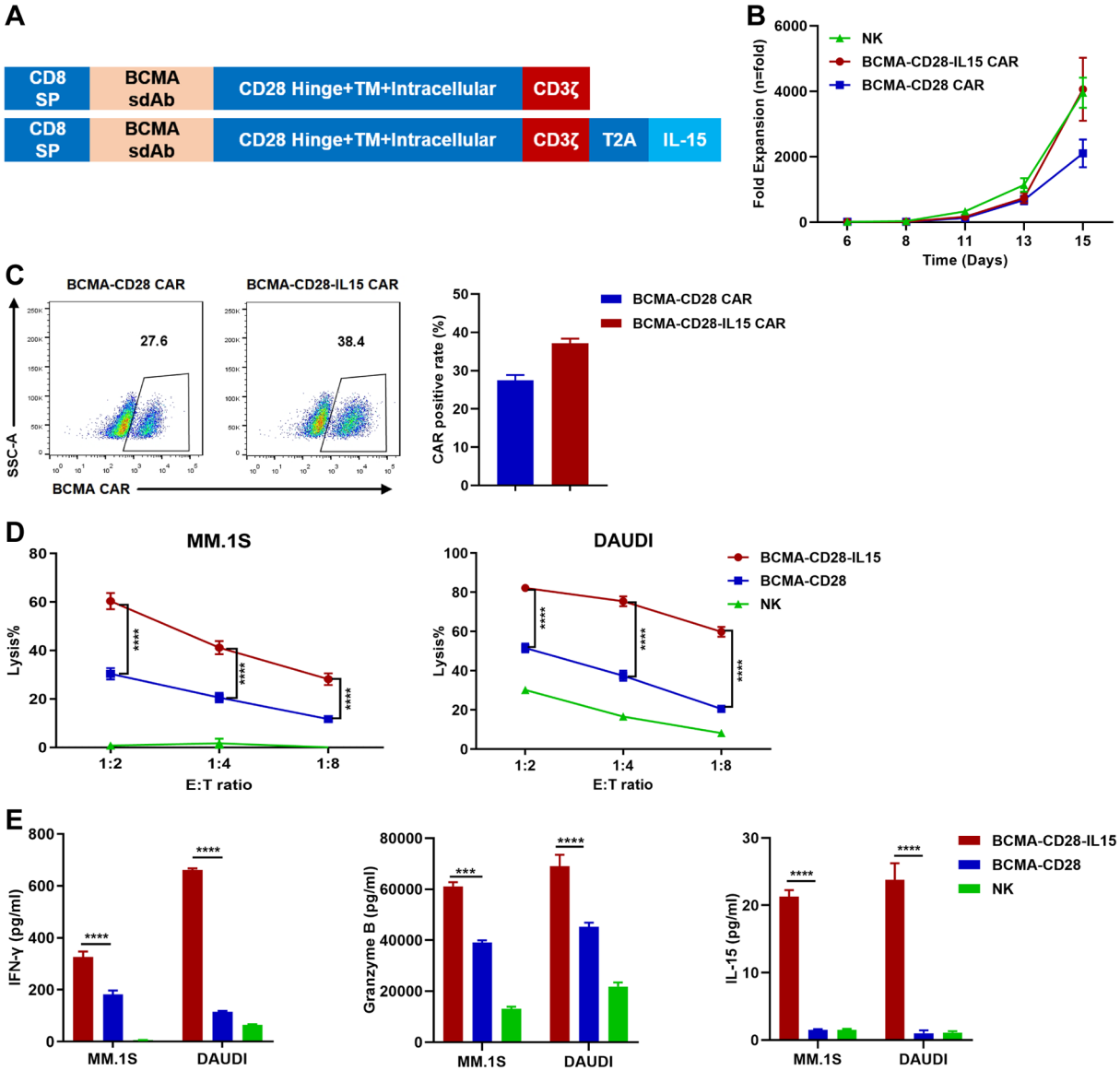

Fig. 1 BCMA-CD28-IL15-engineered NK cells showed enhanced antitumor activity compared to BCMA-CD28 CAR-NK cells. **(A)** Schematic diagrams of BCMA CAR-NK constructs. **(B)** The fold expansion curve of NK cells, BCMA-CD28-IL15 CAR-NK cells, and BCMA-CD28 CAR-NK cells. **(C)** BCMA CAR expression of NK cells on day 14. **(D)** The cytotoxic activity of BCMA-CD28-IL15 CAR-NK cells vs. BCMA-CD28 CAR-NK cells and ex vivo-expanded NK cells against MM.1S and Daudi cells using a lactate dehydrogenase release assay (n=3; ****, $P<0.0001$). The numbers of effector cells were calculated as CAR-positive cells. NK cells were used to adjust the different CAR-positive cells. **(E)** MM.1S and Daudi cells were cocultured with BCMA-CD28-IL15 CAR-NK cells, BCMA-CD28 CAR-NK cells, or NK cells at an E:T ratio of 1:2 for 24 h. IFN-γ, granzyme B, and IL-15 secretion in supernatants was measured by ELISA (n=3; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$)

the BCMA-CD28-IL15 CAR-NK cells ($P<0.0001$, n=3) (Fig. 1E).

Subsequently, a BCMA-hIgG1-IL15 CAR with an immunoglobulin G-based (IgG1) hinge was synthesized to verify the functional impact of different hinge regions. After coculture with MM.1S cells for 16 h, the BCMA-CD28-IL15 CAR-NK cells exhibited superior cytotoxic lysis over BCMA-hIgG1-IL15 CAR-NK cells at all tested E:T ratios ($P<0.0001$, n=3). The BCMA-CD28-IL15 CAR-NK cells secreted significantly more IFN-γ against MM.1S cells than BCMA-hIgG1-IL15 CAR-NK cells ($P<0.0001$, n=3) (Supplementary Fig. S1).

The optimal intracellular domain in CAR-NK is not fully understood [10]. A BCMA-2B4-IL15 CAR with costimulator 2B4 was also constructed for in vitro functional comparison. 2B4 is an NK cell-specific receptor. CAR constructs with 2B4 costimulatory domains have shown superior antitumor efficacy [11, 12]. The fold expansion and CAR-positive rate of the BCMA-2B4-IL15 CAR-NK cells and BCMA-CD28-IL15 CAR-NK cells are shown in Supplementary Figure S2B, C. The BCMA-CD28-IL15 CAR-NK cells demonstrated higher levels of cell lysis than the BCMA-2B4-IL15 CAR-NK cells against MM.1S and NCI-H929 cells ($P<0.01$, n=3). However, in terms of antitumor activity in the Daudi and RS4;11 cells, no significant differences were observed. Both CAR structures showed minor cytotoxic effects against BCMA-negative RS4;11 cells (Supplementary Fig. S2E). The secretion levels of IFN-γ, granzyme B, and IL-15 by BCMA-CD28-IL15 CAR-NK cells, with the exception of the RS4;11 cells, were higher

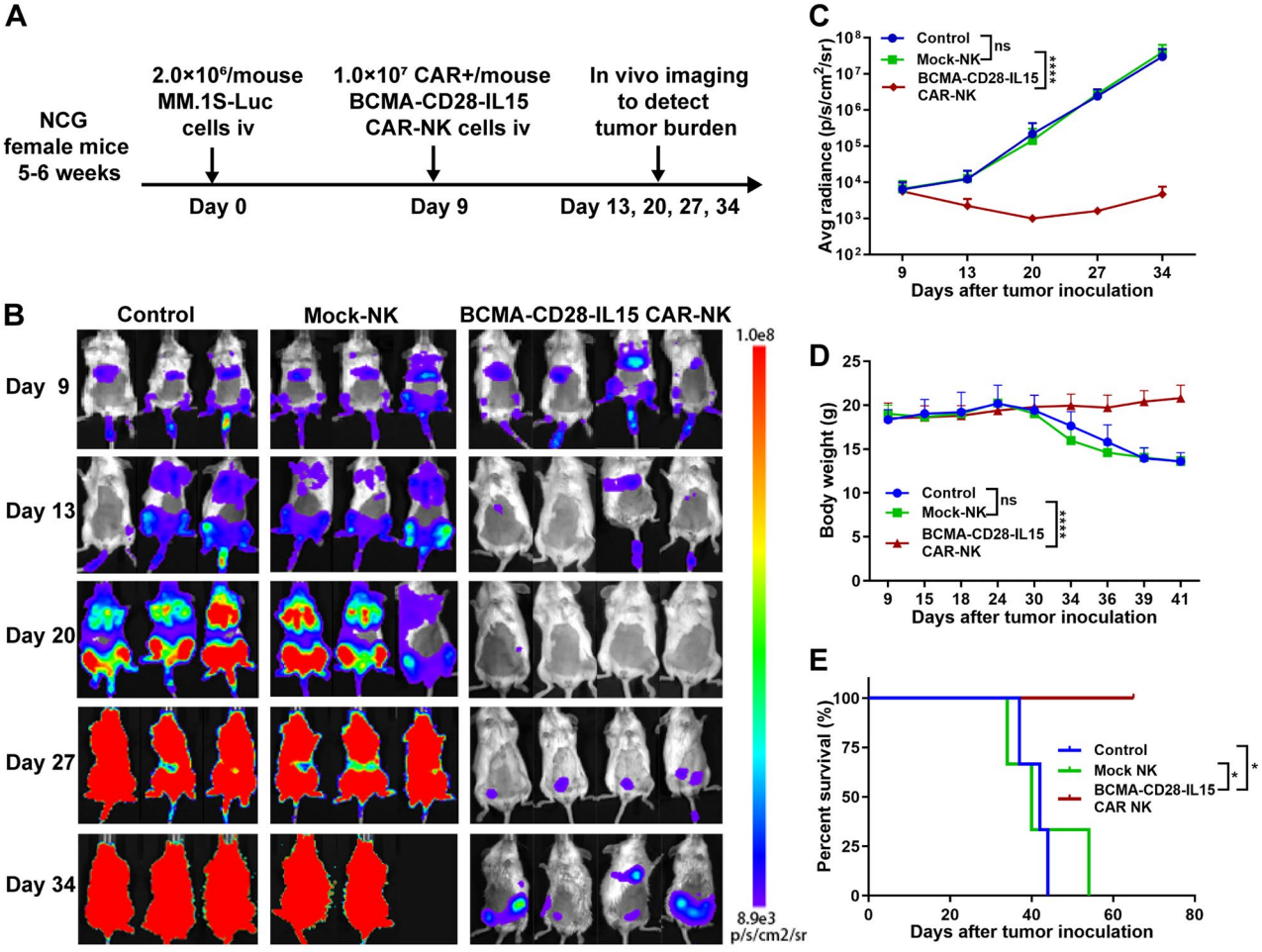

Fig. 2 In vivo antitumor activity of BCMA-CD28-IL15 CAR-NK cells in the MM.1S-Luc transplanted NCG mouse model. **(A)** Schematic diagram of the mouse in vivo study. The NCG mice received an *IV* injection of 2.0×10^6 MM.1S-Luc cells on day 0. Nine days after tumor inoculation, the mice were randomly divided into three groups (n=3 for the control and mock-NK groups; n=4 for the BCMA-CD28-IL15 CAR-NK group) according to the average radiance of the bioluminescence imaging. The mice were intravenously administered cryoprotectant (a solvent control), mock-NK cells, and BCMA-CD28-IL15 CAR-NK cells on day 9. **(B)** Bioluminescence images on days 9, 13, 20, 27, and 34. **(C)** Statistical analysis of the bioluminescence intensity on different days (****, $P<0.001$; ns, no significance). **(D)** Body weight of each group measured on different days (****, $P<0.0001$; ns, no significance). **(E)** Kaplan–Meier survival curves of mice in vivo. A statistical analysis of survival between groups was performed using the log-rank test. Statistical significance in survival rates was obtained after Bonferroni correction for multiple comparisons (*, $P<0.05$)

than those in the BCMA-2B4-IL15 CAR-NK cells with the indicated cell lines ($P<0.05$, n=3) (Supplementary Fig. 2F).

Finally, we assessed the in vivo antitumor activity of the BCMA-CD28-IL15 CAR-NK cells in the immunocompromised NCG mouse model (Fig. 2A). The MM.1S-luc cell injection was defined as day 0, and BCMA-CD28-IL15 CAR-NK cells were injected nine days after tumor inoculation. After BCMA-CD28-IL15 CAR-NK cells administration, the BCMA-CD28-IL15 CAR-NK group had a lower bioluminescence intensity, compared with the control or mock-NK groups ($P<0.0001$, n=4) (Fig. 2B, C), which demonstrated the ability to inhibit tumor cells in vivo. We further found that the mice in the BCMA-CD28-IL15 CAR-NK group maintained their weight throughout the observation period, while the mice in both the control and mock-NK groups, losing large amounts of weight from day 27 until death, reflecting the degree of disease progression (Fig. 2D). The BCMA-CD28-IL15 CAR-NK group had significantly prolonged survival compared with the control and mock-NK groups ($P<0.05$, n=4) (Fig. 2E). The median survival time of the BCMA-CD28-IL15 CAR-NK group was undefined until being euthanized on day 65. The median survival time was 42 days for the control group and 40 days for the mock-NK group. There was no significant difference in survival between the control and mock-NK groups ($P=0.8355$, n=3). However, a limitation of the mouse model is that we did not include the BCMA-2B4-IL15 CAR-NK group.

In conclusion, the single VHH-directed BCMA CAR-NK cells exhibited remarkable specific killing ability, making them a potential candidate for immunotherapy for MM.

Abbreviations

CAR	Chimeric antigen receptor
BCMA	B-cell maturation antigen
CRS	Cytokine release syndrome
VHH	Variable domain of heavy chain of heavy-chain antibody
PB	Peripheral blood
BaEV	Baboon retrovirus envelope pseudotyped lentivectors

Supplementary Information

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Supplementary Material 1

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Authors' contributions

YPS and JSZ designed the study. QR and YLZ drafted the manuscript and prepared the figures. QR performed the experiments. HCS, QML, BX and YPL provided the resources. All authors participated in the process of drafting and revising the manuscript. All the authors have read and approved the final manuscript.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All mouse experiments were performed and approved by the Institutional Animal Care and Use Committee (IACUC) of ANLING Laboratories (Shenzhen, China). Blood samples were collected from healthy donors with written consent.

Consent for publication

All authors critically reviewed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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References

1. Zhang Y, Zhou W, Yang J, Yang J, Wang W. Chimeric antigen receptor engineered natural killer cells for cancer therapy. Exp Hematol Oncol. 2023;12:70.
2. Mazinani M, Rahbarizadeh F. New cell sources for CAR-based immunotherapy. Biomarker Res. 2023;11:49.
3. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature. 1993;363(6428):446–8.
4. Han L, Zhang J-S, Zhou J, Zhou K-S, Xu B-L, Li L-L, et al. Single VHH-directed BCMA CAR-T cells cause remission of relapsed/refractory Multiple Myeloma. Leukemia. 2021;35(10):3002–6.
5. Colamartino ABL, Lemieux W, Bifsha P, Nicoletti S, Chakravarti N, Sanz J, et al. Efficient and robust NK-Cell Transduction with Baboon Envelope Pseudotyped Lentivector. Front Immunol. 2019;10:2873.
6. Liu S, Galat V, Galat Y, Lee YKA, Wainwright D, Wu J. NK cell-based cancer immunotherapy: from basic biology to clinical development. J Hematol Oncol. 2021;14:7.
7. Wang X, Yang X, Yuan X, Wang W, Wang Y. Chimeric antigen receptor-engineered NK cells: new weapons of cancer immunotherapy with great potential. Exp Hematol Oncol. 2022;11:85.
8. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-Transduced Natural Killer cells in CD19-Positive lymphoid tumors. N Engl J Med. 2020;382(6):545–53.
9. Liu E, Tong Y, Dotti G, Shaim H, Savoldo B, Mukherjee M, et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia. 2018;32(2):520–31.
10. Gong Y, Klein Wolterink RGJ, Wang J, Bos GMJ, Germeraad WTV. Chimeric antigen receptor natural killer (CAR-NK) cell design and engineering for cancer therapy. J Hematol Oncol. 2021;14(1):73.
11. Xu Y, Liu Q, Zhong M, Wang Z, Chen Z, Zhang Y, et al. 2B4 costimulatory domain enhancing cytotoxic ability of anti-CD5 chimeric antigen receptor engineered natural killer cells against T cell malignancies. J Hematol Oncol. 2019;12(1):49.
12. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-Derived natural killer cells Engineered with chimeric Antigen receptors enhance anti-tumor activity. Cell Stem Cell. 2018;23(2):181–92. e5.

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