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C3aR costimulation enhances antitumor efficacy of CAR-T through promoting Th17 expansion and memory T phenotype

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Short Report

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Abstract

Chimeric antigen receptor (CAR)-modified adoptive T-cell therapy is a promising immunotherapy for hematologic malignancies. However, the efficacy in extramedullary leukemia is still limited and new strategies with improved anti-tumor activity are highly needed. Since C3aR activation builds a bridge between innate immune and adaptive immune and it could trigger Th17 response, we hypothesized that C3aR incorporation as costimulatory domain would augment anti-tumor activity of CAR-T. To address this possibility, we introduced a genetically engineered construct, comprising the domain of C3aR into CAR-T cells. The generated BB-ζ-C3aR CAR-T exhibited potent cytolytic ability to eradicate various tumor cells expressing CD19 or BCMA *in vitro*. When administrated intravenously into the xenografts leukemia NCG mice who received CD19 + or BCMA + expressing tumor cells, BB-ζ-C3aR CAR-T cells reduced the tumor burden and improved the survival of mice. Of note, these BB-ζ-C3aR CAR-T cells could effectively eradicate subcutaneous CD19 + tumor cells, highlighting the potential therapy for extramedullary leukemia. Mechanistically, the BB-ζ-C3aR CAR-T cells preferred to exhibit tumor-killing Th17 phenotype and suppressed the tumor-tolerated Treg function. In addition, the induction of memory T cells phenotype in BB-ζ-C3aR CAR-T group indicated their long duration effects. Taken together, our findings suggest that the application of C3aR costimulation to boost CAR-T-cell activity is efficacious against aggressive tumor cells via Th17 expansion and memory T cell induction.

To The Editor

Genetic engineering of T cells to express chimeric antigen receptors (CARs) is recognized as a promising new approach for relapsed/refractory acute lymphoblastic leukemia[1]. However, its activity in extramedullary leukemia has not been well-characterized[2]. The efforts are underway to find strategies that can increase its anti-tumor efficacy, which is highly dependent on the optimal molecular design of the CAR. The CARs are fusion proteins with well-defined functional domains, among which the costimulatory molecule domains are required for activation, expansion and survival of CAR-T cells. Several costimulatory molecules such as CD28, 4-1BB, ICOS, OX-40, CD27, TLR2 and so on have been tried to incorporated into CAR and can influence the function of CAR-T cells[2,3]. C3aR, the G protein-coupled receptor for the complement fragment C3a, plays a role in the induction and regulation of effector CD4 and CD8 T cells responses[4]. Previous studies found C3aR signaling direct CD4 T cells differentiation towards tumor-killing Th17 cells and against the regulatory effect on Treg commitment, highlighting the potential role of C3aR[5,6]. Here we introduced the C3aR domain to the 3’ end of CD3ζ, which was followed 4-1BB domain, to generate a new BB-ζ-C3aR CAR-T cell.

Then we would like to evaluate whether C3aR costimulation incorporation play a role in the efficacy of CAR-T cell therapy regardless of targeted antigen. Here the BB-ζ-C3aR CAR-T cell were established with targeting CD19-specific or BCMA-specific tumor antigen (Additional file 1: Fig. S1). The CAR constructs contain the CD19-scFv or BCMA-scFv and CD3ζ signal transduction domain with the 4-1BB and C3aR

signaling domains in tandem (Additional file 1: Fig. S1a, c). All CAR vectors were efficiently transduced and the CAR-GFP were effectively expressed in transduced T cells (Additional file 1: Fig. S1b, d).

Initially, we detected the activity of the novel 19-BB-ζ-C3aR CAR-T cells *in vitro*. NALM6, Raji, and K562.CD19 cell lines were applied as tumor model in vitro. Incubation of CAR-T cells with these tumor cells resulted in significant potent cytotoxicity at indicated ratios of effector:target. Both 19-BB-ζ-C3aR and 19-BB-ζ CAR-T cells lysed CD19-expressing NALM6, Raji, and CD19-K562 compared with mocked T cells, with the cytotoxicity was significantly higher in 19-BB-ζ-C3aR CAR-T cells (Fig. 1a). Of note, we further found that C3aR incorporation improved the generation of IL-17-expressing Th17 cells while suppressed the differentiation of Foxp3 + CD25 + CD4 + Treg cells compared to the mock transduced T or 19-BB-ζ CAR-T cells (Fig. 1b, Additional file 2: Fig. S2a, b).

To test the *in vivo* activity of 19-BB-ζ-C3aR CAR-T cells, NCG (NOD-SCID-IL2rg$^{-/-}$) mice were injected intravenously with NAML6-ffLuc and treated with twice i.v. injections of indicated CAR-T cells (1×10^5 via tail vein injection on Day 2 and Day 8). These mice were then followed with serial bioluminescence imaging (Fig. 1c). As expected, mice treated with mock transduced T cells succumbed quickly to disease, whereas mice treated with CAR-T cells showed significant reduction of leukemia. Of note, the 19-BB-ζ-C3aR CAR-T cells infusion got a more pronounced effect of tumor eradication than that of 19-BB-ζ control (Fig. 1e). Consistently, the mice received 19-BB-ζ-C3aR CAR-T cells exhibited a better survival advantage (Fig. 1d). Furthermore, 19-BB-ζ-C3aR CAR-T cells showed the most potent ability to eradicate the CD19 blasts in blood, whereas no difference on the accounts of GFP + CAR-T cells, CD4 + T, and CD8 + T cells in circulation were observed among groups (Fig. 1f-i).

As we known, extramedullary relapse is an aggressive type of leukemia and the conventional CAR-T cells had shown little evidence of antitumor activity against it[7]. It is important to validate the therapeutic potential of the novel CAR-T cells to eradicate extramedullary leukemia cells. We therefore established a subcutaneous leukemia model and examined whether 19-BB-ζ-C3aR CAR-T cells harbored cytolytic activity against extramedullary leukemia. The immunodeficient NCG mice received a subcutaneous injection of 2×10^5 NALM-6 cells that were tagged with GFP and luciferase (Fig. 1j). The Bioluminescence imaging (BLI) results revealed that tumors were significantly suppressed in mice treated with 19-BB-ζ-C3aR in comparison to 19-BB-ζ CAR-T cells (Fig. 1l). The tumor volume was examined at indicated time points and the extracted tumors were measured. Although there was no significant statistic difference of tumor volume and weight between 19-BB-ζ CAR-T and 19-BB-ζ-C3aR CAR-T cell group (Fig. 1k,m), more tumor-bearing mice after 19-BB-ζ-C3aR CAR-T cell infusion attained thorough removal (Fig. 1l) and less CD19-expressing tumor cells were detected (Fig. 1n), highlighting the potent efficacy of 19-BB-ζ-C3aR CAR-T cells against extramedullary leukemia. Taken together, this novel CAR with C3aR incorporation displayed enhanced anti-leukemia efficacy particularly in the subcutaneous leukemia-bearing xenografts.

Similarly, the activity and efficacy of the BB-ζ-C3aR CAR-T cells targeting BCMA antigen were evaluated *in vitro* and *in vivo*. The IM9, MM1S, K562, and K562.BCMA cell lines were utilized as BCMA-expressing tumor model *in vitro*. Strikingly, the BCMA-BB-ζ-C3aR CAR-T cells exhibited the most potent cytotoxicity,

particularly in the MM1S culture system (Additional file 3: Fig. S3a). These BCMA-BB-ζ-C3aR CAR-T cells preferred to present with Th17 phenotype and away from tumor-tolerated Treg phenotype (Additional file 3: Fig. S3b). *In vivo*, the NCG mice was utilized to establish the multiple myeloma model by intravenous injection of IM9-ffLuc. At the day of 8 and 12, these mice received twice corresponding T cell therapies and monitored by serial BLI (Fig. 2a). As expected, the mice receiving BCMA-BB-ζ-C3aR CAR-T cells showed a lowest tumor burden (Fig. 2b) and a longest survival time (Fig. 2c) without any BCMA-expressing tumor cells (Fig. 2d). Thus, these data suggested that BB-ζ-C3aR CAR-T cells targeting BCMA possessed potent anti-tumor activity.

Finally, to evaluate the underlying mechanism of C3aR incorporation-mediated anti-tumor activity, the Th17 and Treg phenotypes were examined in the peripheral blood from NCG tumor mice after individual CAR-T cell therapy. A significant elevation of Th17 cells was observed in the 19-BB-ζ-C3aR CAR-T cell-treated mice, whereas the 19-BB-ζ signaling did not favor the expansion of Th17 cells compared to the mock control (Additional file 4: Fig. S4a). In contrast, the 19-BB-ζ-C3aR CAR-T cells infusion resulted in lowest percentage of Treg cells (Additional file 4: Fig. S4a), which facilitated tumor progression. Similarly, the BCMA-BB-ζ-C3aR CAR-T cell also promoted Th17 cell percentage and suppressed Treg percentage in tumor-bearing mice (Fig. 2e).

Considering the persistence of CAR-T cells is dependent on the establishment of CAR + memory T subsets[8,9]. We therefore assessed the expressions of CD45RO and CCR7 in the CD4 and CD8 T subsets to detect the CD45RO + CCR7 + T central memory cells (Tcm) and CD45RO + CCR7- T effector memory cells (Tem) in the blood from CAR-T cells-infused mice. In the CD4 + compartment, the enrichment of Tcm cells was higher in the 19-BB-ζ-C3aR CAR group in comparison to the 19-BB-ζ group (Additional file 4: Fig. S4b). Consistently, 19-BB-ζ-C3aR signaling improved the establishment of the Tcm population in the CD8 + compartment (Additional file 4: Fig. S4b). In contrast, both groups yielded a similar proportion of Tem phenotype, indicating that Tcm was predominantly required in the anti-tumor effect of 19-BB-ζ-C3aR CAR-T cells. Interestingly, the BCMA-BB-ζ-C3aR CAR-T cells induced a Tem phenotype in both CD4 and CD8 compartments, and a significant elevation of Tcm phenotype was only observed in CD8 cells (Fig. 2f). Together, these data suggested that C3aR incorporation promoted the memory feature of CAR-T cells.

In summary, we provide the unexpected observation that C3aR incorporation, a novel costimulatory domain for T cells function, had significantly enhanced the potency of CAR-T anti-tumor abilities in vitro and in vivo, particularly for treating extramedullary leukemia. The BB-ζ-C3aR CAR-T cells enhanced the tumor eradication activity through Th17 expansion and induced memory T phenotype for the long-term effect. The results not only highlight the importance of optimizing CAR engineering but also provide evidences that BB-ζ-C3aR CAR-T cells may be efficient for refractory tumors such as extramedullary leukemia and solid tumor.

Abbreviations

CAR

Chimeric antigen receptor
CAR-T
Chimeric antigen receptor T cell
C3aR
Complement C3aR
NCG
NOD-SCID-IL2rg$^{-/-}$
i.v.
Intravenous
BLI
Bioluminescence imaging
Tem
T effector memory cells
Tcm
T central memory cells
Treg
Regulatory T cells

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article or its supplementary information files. The raw datasets are available from the corresponding authors on reasonable request.

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Contributions

PLL, XD, JYW and DQP conceived the study and designed the experiments. PLL, XMC, YLW, JHW and SXG performed the experiments. PLL, XMC and YLW analyzed the data. PLL, JYW, YCZ, PL and DQP prepared the paper. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

All the animal procedures were performed in accordance with the rules of the IACUC in the Guangdong Provincial People's Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, Gao L, Wen Q, Zhong JF, Zhang C, Zhang X. Recent advances in CAR-T cell engineering. J Hematol Oncol. 2020;13(1):86.
2. Weng J, Lai P, Qin L, Lai Y, Jiang Z, Luo C, Huang X, Wu S, Shao D, Deng C, Huang L, Lu Z, Zhou M, Zeng L, Chen D, Wang Y, Chen X, Geng S, Robert W, Tang Z, He C, Li P, Du X. A novel generation 1928zT2 CAR T cells induce remission in extramedullary relapse of acute lymphoblastic leukemia. J Hematol Oncol. 2018;11(1):25
3. Nishino M, Ramaiya NH, Hatabu H, Hodi FS. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nat Rev Clin Oncol. 2017;14(11):655-668.
4. Sacks SH. Complement fragments C3a and C5a: the salt and pepper of the immune response. Eur J Immunol. 2010;40(3):668-670.
5. Jugel W, Aigner A, Michen S, Hagstotz A, Ewe A, Appelhans D, Schackert G, Temme A, Tietze S. Targeted RNAi of BIRC5/Survivin Using Antibody-Conjugated Poly(Propylene Imine)-Based Polyplexes Inhibits Growth of PSCA-Positive Tumors. Pharmaceutics. 2021 8;13(5):676.
6. Bowers JS, Nelson MH, Majchrzak K, Bailey SR, Rohrer B, Kaiser AD, Atkinson C, Gattinoni L, Paulos CM. Th17 cells are refractory to senescence and retain robust antitumor activity after long-term ex vivo expansion. JCI Insight. 2017 Mar 9;2(5):e90772.
7. Wan X, Yang X, Yang F, Wang T, Ding L, Song L, Miao Y, Wang X, Ma Y, Luo C, Tang J, Gu L, Chen J, Tang Y, Lu J, Li B. Outcomes of Anti-CD19 CAR-T Treatment of Pediatric B-ALL with Bone Marrow and Extramedullary Relapse. Cancer Res Treat. 2021. doi: 10.4143/crt.2021.399.
8. Tian Y, Li Y, Shao Y, Zhang Y. Gene modification strategies for next-generation CAR T cells against solid cancers. J Hematol Oncol. 2020;13(1):54.
9. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. Nat Biotechnol. 2018;36(4):346-351.

Figures

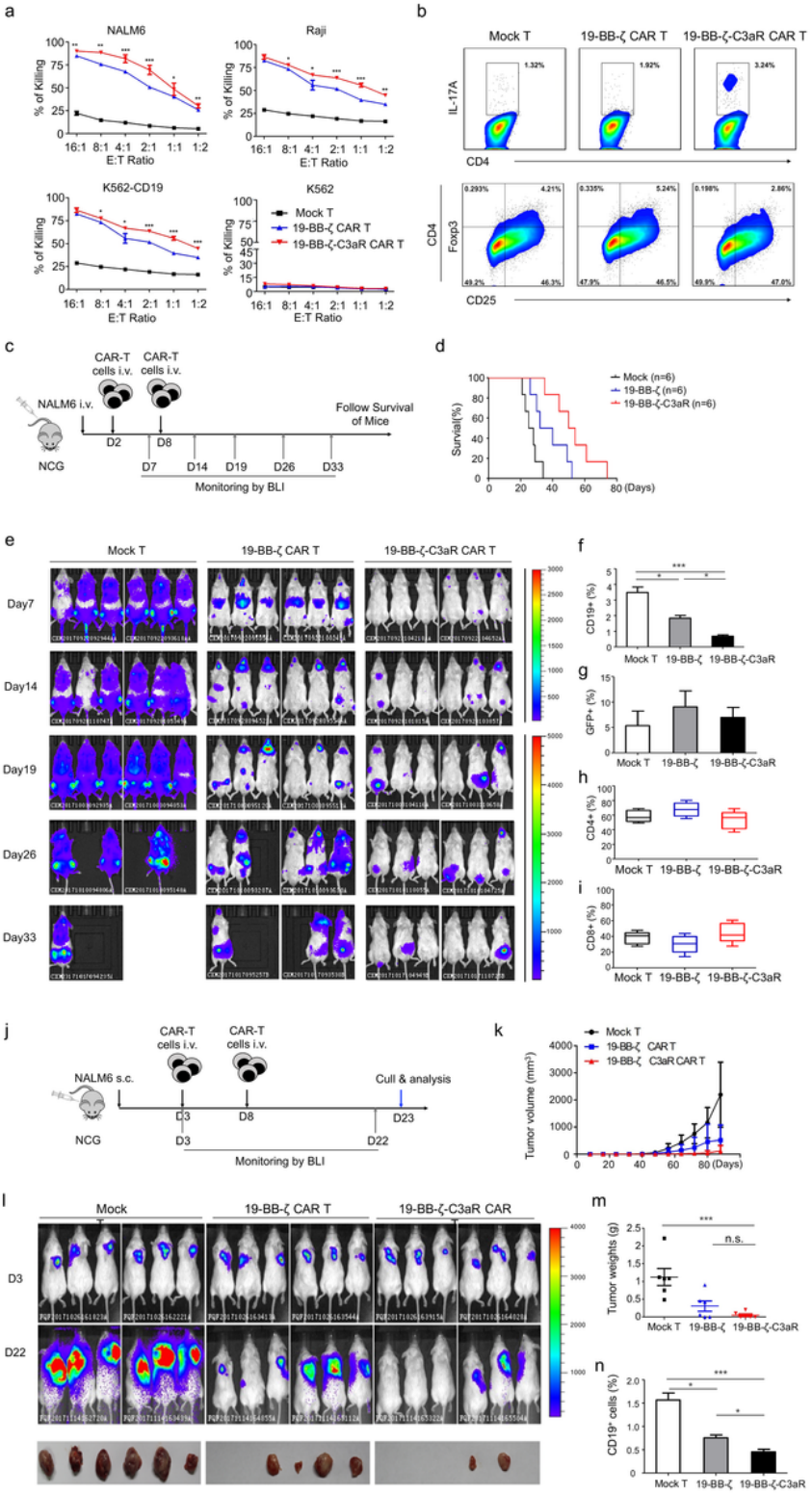

Figure 1

19-BB-ζ-C3aR CAR-T cells displayed potent anti-leukemic activity in vitro and in vivo, particularly in the xenografts extramedullary leukemia model.

a The cytotoxic abilities of CAR-T cells were detected by flow-cytometry of CFSE and PI staining. Both CAR-T cells specifically targeted CD19+ tumors. Of note, the 19-BB-ζ-C3aR CAR-T cells have significant

increased ability to lyse CD19+ leukemia cells compared to 19-BB-ζ CAR-T cells. Result of cytotoxicity assay were repeated at least three independent experiments. **b** Flow-cytometry result revealed that enhanced expansion of Th17 cells and reduced Tregs phenotype of 19-BB-ζ-C3aR CAR-T cells compared to 19-BB-ζ and mock-transduced T cells. **c** Xenograft leukemic model using CD19-specific CAR-T cells to eradicate NAML6-ffLuc in NCG mice. 0.5×10^6 $ffLuc^+$ NAML6 cells were administered i.v. into NCG mice to establish the ALL model. Then the mice were randomized to treatment with 2×10^6 indicated T cell on Day 2 and Day 8. NAML6 tumor growth was then monitored by Xenogen imaging. **d** Kaplan-Meier analysis of survival for each group of xenograft leukemia model. Log-rank (Mantel-COX) tests were used to perform statistical analyses of survival between groups. N =6. **e** Representative bioluminescence images of NCG mice at Days 7, 14, 19, 26, and 33 are depicted for each group. **f** Peripheral blood CD19+ ALL blast cell counts were measured in some of the xenograft leukemic mice on Day 22. The 19-BB-ζ-C3aR CAR-T group have significantly lower blast counts than Mock and 19-BB-ζ CAR-T groups. **g** On Day 22, the detectable GFP positive T cells were similar in three groups. **h,i** There were no differences in CD4+ and CD8+ T cells between the indicated T cell populations. **j** Xenograft extramedullary leukemic model was established by subcutaneous injection of 2×10^5 NALM-6 cells. The indicated CAR-modified T cells with 2×10^6 dose were i.v. injected twice on Day 3 and Day 8, respectively. **l** NAML6 subcutaneous tumor growth was monitored by Xenogen imaging. Representative bioluminescence images of NCG mice at Days 3 and 22 are depicted for each group. The tumor was extracted on Day 23 and the specimen were showed. **k,m** Although there were no significant statistic differences, the tumor mass in the mice treated with 19-BB-ζ-C3aR CAR-T cells seem to be smallest and lightest. n = 6. **n** Peripheral blood CD19+ ALL blast cell counts were measured in some of the xenograft leukemic mice on Day 22. The 19-BB-ζ-C3aR CAR-T group have the lowest blast counts. ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, N.S. no significant.

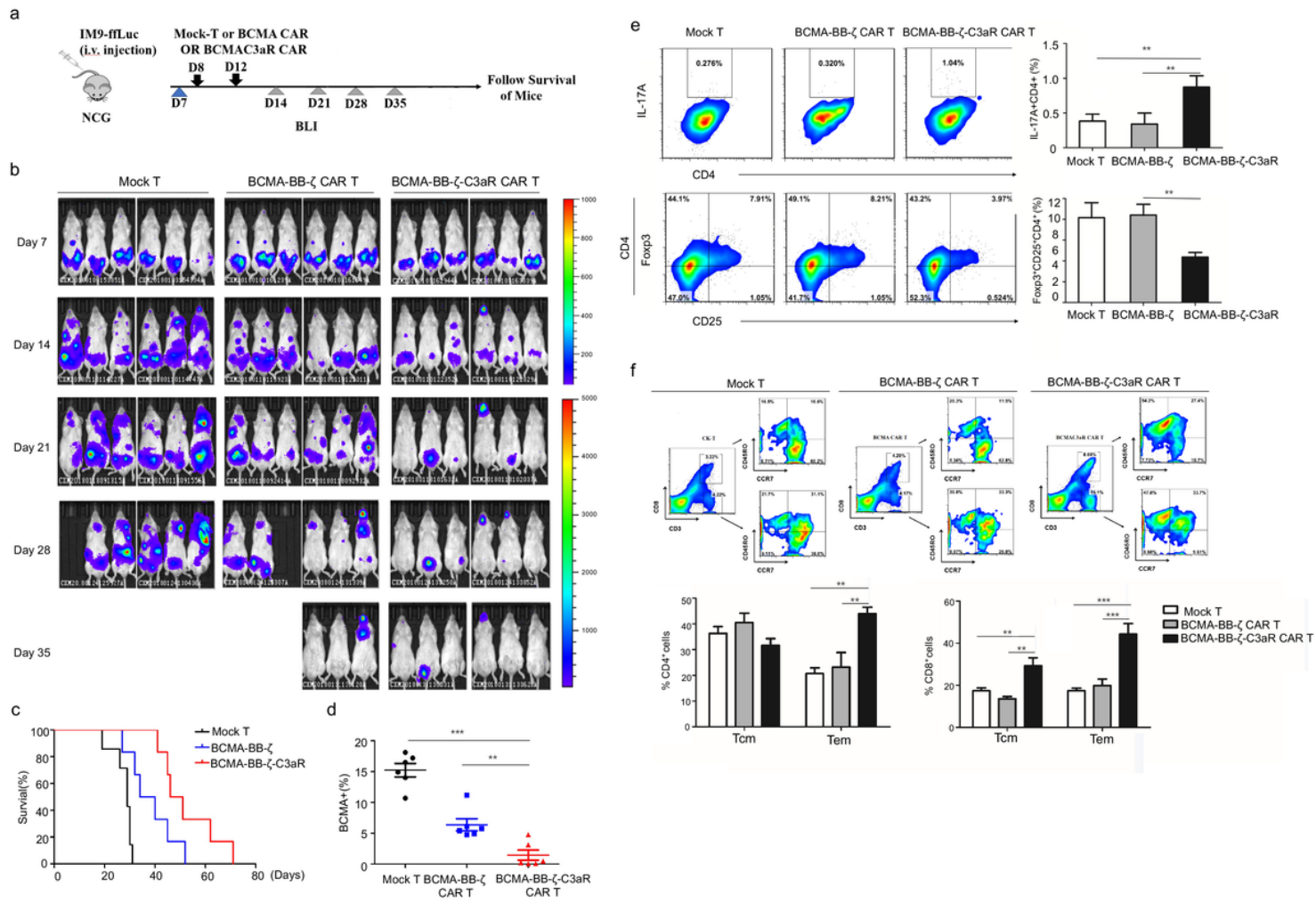

Figure 2

The BB-ζ-C3aR CAR-T cells could also significantly eradicate BCMA-expressing tumor with favoring Th17 cells expansion and memory phenotypes.

a 0.5×10^6 ffLuc$^+$ IM9 cells were administered i.v. into NCG mice to establish the MM model. Then the mice were randomized to treatment with 2×10^6 indicated T cell populations on Day 8 and Day 12. IM9 tumor growth was then monitored by Xenogen imaging. **b** Representative bioluminescence images of NCG mice at Days 7, 14, 21, 28, and 35 are depicted for each group. **c** Kaplan-Meier analysis of survival showed longest survival time in BCMA-BB-ζ-C3aR CAR-T cells group. **d** Peripheral blood BCMA+ tumor cell counts were measured and little was detected in BCMA-BB-ζ-C3aR CAR-T cells group. **e** In the xenograft MM mice, the BCMA-BB-ζ-C3aR CAR-T cells promoted the generation of IL-17-expressing Th17 cells and reduced the Tregs compared to the BCMA-BB-ζ CAR-T cells group. **f** Representative T cell immunophenotyping of CD4+ and CD8+ T cell subsets. A fraction of the CD4+ cells exhibited features of central memory cells (Tcm), notably high expression of CCR7 and CD45RO. These Tcm cells were increased in the BCMA-BB-ζ-C3aR CAR-T-treated mice compared to BCMA-BB-ζ controls. In addition, the percentage of CD45RO+CCR7- effector memory cells (Tem) in both CD4+ and CD8+ compartment was

significantly increased in the BCMA-BB-ζ-C3aR CAR-T cells. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, N.S. no significant.

Supplementary Files

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