Anti-BCMA CAR-T cells therapy for a patient with extremely high membrane BCMA expression: a case report

Dan Li,[1,2] Yimei Que,[1] Shengnan Ding,[1,2] Guang Hu,[3] Wen Wang,[3] Xia Mao,[1,2] Ying Wang,[1,2] Chunrui Li ![ORCID],[1,2] Liang Huang ![ORCID],[1,2] Jianfeng Zhou,[1,2] Wei Zhang,[1,2] Min Xiao ![ORCID] [1,2]

Correspondence to
Dr Min Xiao;
xiaomin@tjh.tjmu.edu.cn

Dr Wei Zhang;
260606299@qq.com

ABSTRACT

B cell maturation antigen (BCMA)-directed CAR-T cell therapy is a disruptive approach for treating relapsed/refractory multiple myeloma (R/R MM); however, optimization is necessary to maximize patient benefit. We report the case of a 61-year-old woman with primary refractory MM who presented with high expression of membrane BCMA and low expression of soluble BCMA (sBCMA), experienced grade 4 cytokine release syndrome, and died from severe pneumonia after receiving anti-BCMA CAR-T (CT103A) therapy. This case highlights the importance of assessing the expression range of BCMA for its efficacy and safety in patients receiving BCMA CAR-T therapy. For patients who present with extremely high membrane BCMA expression and extremely low sBCMA expression, the presence of γ-secretase-related gene mutations should be considered. Special attention should also be paid to the prevention and treatment of cytokine release syndrome in such patients.

INTRODUCTION

B cell maturation antigen (BCMA, also referred to as TNFRSF17 or CD269) is expressed almost exclusively in plasma and B cells, and its overexpression and activation are associated with multiple myeloma (MM) progression. BCMA-directed immunotherapies, especially BCMA CAR-T cell therapy, have shown remarkable activity in relapsed/refractory MM (R/R MM).[1 2] Despite significant efficacy, the use of CAR-T cell therapy is limited by potentially severe toxicities. The most frequent life-threatening adverse events following CAR-T cell infusion are cytokine release syndrome (CRS) and CAR-related encephalopathy syndrome. The γ-secretase complex cleaves membrane-bound BCMA from the surface of plasma cells to a soluble form (sBCMA).[3] Recent studies have shown that γ-secretase inhibitors improve the recognition of anti-BCMA CAR-T cells, increase surface BCMA levels, and enhance antitumor activity.[4] Nevertheless, there have been no reports on the effect of BCMA expression

on anti-BCMA CAR-T cell therapy. In this report, we present an in-depth analysis of the high surface expression of BCMA in an R/R MM patient who had severe adverse events after receiving anti-BCMA CAR-T (CT103A) therapy.

CASE PRESENTATION

A 61-year-old Chinese woman was diagnosed with IgG lambda MM (DS IIIA, ISS II, and R-ISS II) in April 2018. RB-1 (93%+) was detected by fluorescence in situ hybridisation (FISH), but no other myeloma-associated chromosomal abnormalities were detected. Over the following 8 months, the patient received three chemotherapy regimens: VRD (bortezomib, lenalidomide, and dexamethasone), VCD (bortezomib, cyclophosphamide, and dexamethasone), and VDCEP (bortezomib, dexamethasone, etoposide, cisplatin, and cytarabine), but never achieved partial remission (figure 1A). The best response was the minimal response. In January 2019, after the failure of three prior lines of treatment, the patient was enrolled in the CT103A clinical trial (phase I). Before CAR-T cell infusion, serum protein electrophoresis revealed an immunoglobulin level of 46.34 g/dL. Bone marrow cytology showed 26% plasma cells, and 2.46% of cells were considered monoclonal plasma cells in flow cytometry. FISH analysis revealed 1q21 amplification, Rb1 deletion, MAF deletion, and a few tetraploid karyotype complex changes. Notably, high surface expression of BCMA (mean fluorescence intensity=51 025) was observed by flow cytometry, whereas significantly decreased serum sBCMA (sBCMA=7.12 ng/mL) was detected by ELISA (figure 1B). At enrolment, the patient was assigned to the high-dose group. After lymphodepletion with fludarabine and cyclophosphamide, a target dose
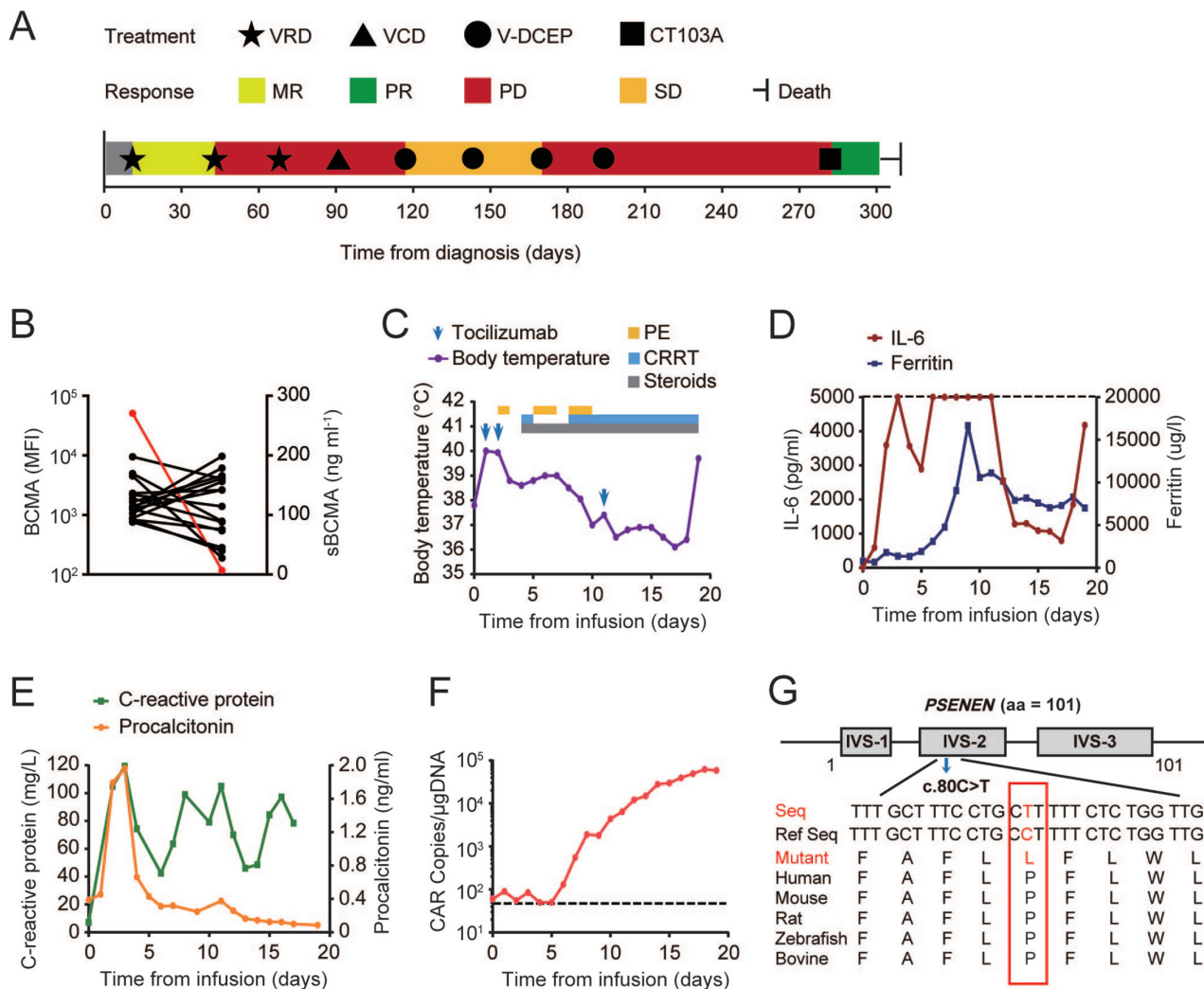
Figure 1 Clinical course of the patient. (A) Timeline of treatment and response. StAR, triangle, circle, and square markers indicate the start time of VRD, VCD, V-DCEP, and CT103A regimen chemotherapy, respectively. (B) Paired plots diagrams of MFI of BCMA and sBCMA concentrations from an MM patient cohort. The red plot indicates the patient with extremely high BCMA expression and with low sBCMA levels. (C) Body temperature and treatment for CRS of the patient after CAR-T cell infusion. (D) Levels of IL-6 and ferritin after CAR-T cell infusion. The horizontal line denotes the higher limit of quantitation (5000 pg/mL). (E) Levels of procalcitonin and C reactive protein after CAR-T cell infusion. (F) Human CAR BCMA transgene copy numbers detected by ddPCR. The horizontal line denotes the lower limit of quantitation (50 copies/μg). (G) Location of the monoallelic *PSENEN* missense mutation c.80G>T, p.Pro27Leu. Each gray rectangle indicates an exon of the *PSENEN* gene. The red font illustrates the altered codon and protein. CAR, chimeric antigen receptor; CRRT, continuous renal replacement therapy; CRS, cytokine release syndrome; ddPCR, droplet digital PCR; GC, glucocorticoid; MFI, mean fluorescence intensity; MR, minimal response; PD, progression disease; PE, plasma exchange; PR, partial response; sBCMA, soluble B cell maturation antigen; SD, stable disease.

of $6*10^6$/kg of CAR+T cells was infused. The patient was febrile for 10 days, with a maximum temperature of 40.0°C on the first day postinfusion (figure 1C). Serum IL-6 (>5000 pg/mL) and ferritin (16 680 μg/L) levels peaked on the 3rd and 10th day postinfusion, respectively (figure 1D). C reactive protein and procalcitonin (PCT) levels continued to increase at an early stage, while PCT levels returned to normal on the fifth day (figure 1E). Cellular kinetic analysis showed substantial expansion and persistence of CAR-T cells (figure 1F). The patient experienced grade 4 CRS, requiring steroids, tocilizumab, plasma exchange, and continuous renal replacement therapy during the entire treatment period. Eventually,

the patient achieved a partial response according to the current International Myeloma Working Group guidelines, but died 20 days after infusion caused by severe pneumonia.

To elucidate the molecular mechanism underlying severe CRS, we performed whole-genome sequencing on the MM cells of the patient, which revealed a single-base missense mutation and deletion of *Psenen* alleles (figure 1G). To simulate the state of the patient based on the deletion of *Psenen* alleles, we constructed a Psenen knockout (KO) model in MM.1S and RPMI 8226 cell lines using CRISPR/Cas9 technology (online supplemental figure S1). Gene expression results were confirmed at
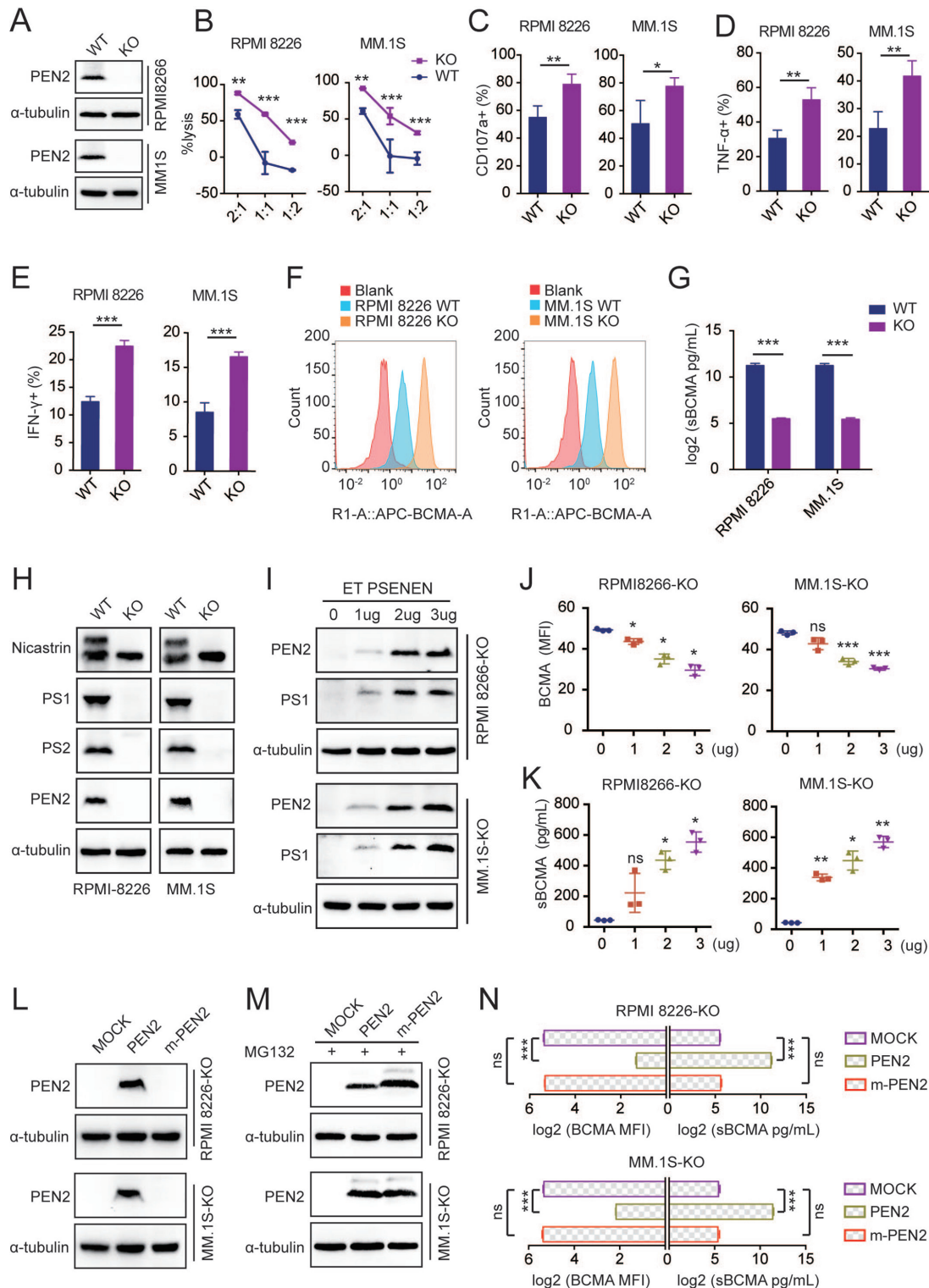
Figure 2 Phenotypic alterations associated with PSENEN deletion and missense mutation. (A) Pen2 protein levels detected by Western blotting. KO cell lines had no Pen2 protein expression. (B) Cytotoxicity of CAR-T cells at different effector-to-target ratios. (C–E) degranulation markers CD107a, TNF-A, and IFN-γ at the indicated effector-to-target ratio. (F) FACS analyses for membrane BCMA expression. (G) sBCMA concentrations on wild-type (WT) cells and Psenen KO cells. (H) Effects of PEN2 KO on the protein levels of different γ-secretase subunits. (I) Protein levels of PEN2 and PS1 in KO cell lines after plasmid electrotransfection. (J, K) MFI of BCMA and sBCMA concentrations in KO cell lines after plasmid electrotransfection. (L, M) expression of Pen2 protein in PSENEN and mPSENEN overexpressing cell lines. Proteasome inhibitor MG132 was added before protein extraction. Pen2 protein in mPSENEN-overexpressing cells could be detected only with MG132. (N) Pairing diagrams of MFI of BCMA and sBCMA concentrations in PSENEN and mPSENEN overexpression cell lines. BCMA, B cell maturation antigen; MFI, mean fluorescence intensity; ns, not significant; KO, sBCMA, soluble BCMA; WT, soluble BCMA. *p < 0.05, **p < 0.01, and ***p < 0.001.

the protein level using western blotting (figure 2A). Psenen KO induced high proliferation and a reduction in bortezomib-triggered apoptosis when compared with wild-type (WT) cells (online supplemental figures S1B and S1C). Rescue experiments were performed to re-express Psenen in the KO cells. Restoring Psenen expression reinhibited the proliferation and rescued the apoptosis induced by bortezomib owing to Psenen knockdown (online supplemental figures S1D and S1E). This suggests that the deletion of Psenen may reflect a higher malignant potential of MM cells, which concurs with the clinical characteristics of primary refractory disease. Notably, Psenen KO cells were more sensitive to CAR-T cell-mediated killing at different effector-to-target ratios (figure 2B). Higher lysis was positively correlated with the release of CD107a, TNF-α, and IFN-γ in BCMA CAR-T cell cocultures (figure 2C–E). γ-secretase acts as a multisubunit protease that directly cleaves BCMA from plasma cells, consisting of nicastrin, presenilin 1 or 2 (PS1 or PS2), Aph-1, and presenilin enhancer protein (PEN2, Psenen-encoded protein).[5] Some studies have shown that PEN2 is required to stabilize the γ-secretase complex.[6] Considering the essential role of PEN2, we assessed BCMA expression in the KO and WT cells. We found that KO cells expressed significantly higher levels of membrane BCMA and lower levels of sBCMA than WT cells (figure 2F,G). Moreover, knockdown of Psenen resulted in the downregulation of other γ-secretase subunits (figure 2H). To further verify the association between Psenen and BCMA, we conducted rescue experiments using plasmid electrotransfer in Psenen KO cells. Interestingly, we found that this markedly increased the expression of PEN2 and PS1 in a dose-dependent manner, concurrently and significantly decreased surface BCMA levels, and increased sBCMA concentrations with dose escalation (figure 2I–K). Given that surface BCMA is a determinant of tumor cell recognition by CAR-T cells, we speculate that the deletion of Psenen might affect the killing efficiency of CAR-T cells by regulating BCMA expression through γ-secretase.

Considering that the patient had a monoallelic Psenen deletion and a missense mutation (c.80C>T, p.Pro27Leu) on the remaining allele, we constructed an m-PEN2 cell line with single-base mutations using lentiviral overexpression technology in KO cells. However, while both DNA electrophoresis and real-time qPCR showed that the mutant Psenen (m-Psenen) was successfully transferred (online supplemental figures S2A and S2B), western blotting indicated that mutated PEN2 protein could not be expressed normally (figure 2L). Indeed, recent studies indicate that PEN2 is degraded via the proteasome pathway.[7] We observed that mutated PEN2 was normally detected by western blotting only in the presence of the proteasome inhibitor MG132 (figure 2M). Furthermore, m-PEN2 cells expressed significantly higher levels of membrane BCMA and lower levels of sBCMA than the PEN2 cells (figure 2N). These results suggest that both the deletion and missense mutation of Psenen are inactivating mutations, which increase BCMA expression in tumor cells and decrease sBCMA in peripheral blood.

DISCUSSION AND CONCLUSIONS

CAR-T immunotherapies are a disruptive approach for treating hematological malignancies, but optimization is necessary to maximize patient benefit. Although the causes and consequences of BCMA decrease have been described in CAR-T cell therapy studies,[8 9] the mechanisms and clinical outcomes of high BCMA expression have rarely been reported. Here, we report a case of a patient with very high membrane BCMA expression and very low sBCMA expression who experienced grade 4 CRS after high-dose BCMA CAR-T cell infusion. We conclude that BCMA expression should be concerned not only with the lower boundary, but also the upper line, to ensure the efficacy and safety of patients receiving BCMA CAR-T therapy. For patients with γ-secretase-related gene mutations who may present with extremely high expression of membrane BCMA and extremely low expression of sBCMA, special attention should be paid to the prevention and treatment of CRS.

Clinical manifestations of CRS after CAR-T-cell therapy include fever, hypotension, and coagulopathy, which range from non-specific to severe life-threatening symptoms.[10] Currently, tocilizumab, steroids, and plasma exchange have been used to treat CRS once the symptoms become severe.[10] However, despite the use of these regimens, the levels of cytokines, such as IL-6 and ferritin, increased after infusion and remained elevated in the patient experiencing CRS, while inflammatory markers, such as PCT, returned to normal on the fifth day. This indicates that the patient developed uncontrolled CRS rather than an infection.

BCMA is actively cleaved from the tumor cell surface by the multisubunit γ-secretase complex, which reduces the ligand density on tumor cells for CAR-T cell recognition and releases an sBCMA fragment capable of inhibiting CAR-T cell function.[4] We further verified that both the deletion and the single-base missense mutation of Psenen resulted in γ-secretase inactivation, which in turn increased membrane BCMA expression and decreased sBCMA. Previous studies have shown that increasing BCMA surface expression on myeloma cells with small-molecule γ-secretase inhibitors correlates with increased CAR-T cell effector function in vitro, including cytokine release and proliferation, and improved in vivo antitumor activity in preclinical models.[4] This is consistent with the CRS severity described above. For patients with γ-secretase-related gene mutations such as deletions or missense mutations in Psenen, special attention should be paid to the prevention and treatment of CRS when receiving anti-BCMA CAR-T therapy.

Author affiliations
[1]Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

²Immunotherapy Research Center for Hematologic Diseases of Hubei Province, Immunotherapy Research, Wuhan, Hubei, China
³Nanjing IASO Biotherapeutics Ltd, Nanjing, Jiangsu, China

ORCID iDs
Chunrui Li http://orcid.org/0000-0001-5134-7133
Liang Huang http://orcid.org/0000-0002-8370-3232
Min Xiao http://orcid.org/0000-0001-9421-6344

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