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SHORT REPORT

Stable engraftment, as well as graft versus host disease-free and relapse-free survival brought by the combination of CD7 targeted universal chimeric antigen receptor-T, and donor hemopoietic stem cells: Indication of a case report

Shiqi Li[1] | Zhongtao Yuan[1] | Lin Liu[1] | Yu Li[1] | Le Luo[1] | Lihui Peng[1] | Mengli Xu[1] | Ping Yin[1] | Yingnian Chen[1] | Xiaoping Li[1] | Qingying Zang[1] | Xi Zhang[2] | Sanbin Wang[1]

[1]Department of Hematology, 920th Hospital of Joint Logistics Support Force of People's Liberation Army of China, Kunming, China

[2]Department of Hematology, Xinqiao Hospital, Army Medical University, Chongqing, China

Correspondence
Xi Zhang, Medical Center of Hematology, Xinqiao Hospital Postal address: No.83 Xinqiao Street, Shapingba District, 400038, Chongqing, China.
Email: zhangxxi@sina.com

Sanbin Wang, Department of Hematology, 920th Hospital of Joint Logistics Support Force of People's Liberation Army of China, Postal address: No.212 Daguan Road, Xishan District, Kunming, Yunnan Province, 650100, China.
Email: Sanbin1011@163.com

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Abstract

CD7 targeted CAR-T has demonstrated potential in the treatment of T cell malignancies but no study has been reported about its potential in the prophylaxis of GVHD in allo-HSCT. Here we reported a special case that a boy diagnosed with refractory acute T lymphoblastic leukemia (T-ALL) was treated with universal CD7 targeted CAR-T (CD7 UCAR-T) and parent-derived peripheral blood stem cells (PBSCs). Complete remission and full engraftment of donor was observed. In the later four months of follow-up, in the absence of any immunodepression treatment, no signs of GVHD were observed. This case initially demonstrates the potential of CD7 UCAR-T in the prophylaxis of GVHD.

KEYWORDS
allogeneic hemopoietic stem cell transplantation, graft versus host disease, refractory, T cell acute lymphoblastic leukemia, universal CD7 targeted CAR-T

1 | BACKGROUND

Graft versus host disease (GVHD) greatly affects the clinical outcomes of allogeneic hemopoietic stem cell transplantation (allo-HSCT). To prevent GVHD a huge amount of immunodepression interventions are used, leading to increased infection and transplant failure [1]. Severe GVHD occurs in some patients even in the presence of immunodepression interventions.

Recently CD7 targeted chimeric antigen receptor-modified T (CAR-T) has drawn the attention of researchers around the world. We previously reported the first-in-human clinical study about the universal CD7 targeted CAR-T (CD7 UCAR-T), which showed high efficacy and led to long-term survival in some patients with acute T lymphoblastic leukemia (T-ALL) [2, 3]. After that time many other types of CAR-T

Shiqi Li, Zhongtao Yuan, and Lin Liu contribute equally to this work.

directed to CD7 had been reported [4–7] and had demonstrated good clinical potential in the treatment of T cell malignancies. However, the potential of CD7 UCAR-T to avoid GVHD in allo-HSCT has never been reported.

Here, we first report a case indicating the potential role of CD7 UCAR-T in allo-HSCT as a preconditioning intervention to eradicate the risk of GVHD.

2 | CASE PRESENTATION

2.1 | Before CAR-T

A 13-year-old boy was diagnosed with T-ALL alone with the MLL-AF10 fusion gene in Kunming. Bone marrow smear showed 82.5% blasts with normal karyotype. After diagnosing he was treated with vincristine, daunorubicin, cyclophosphamide, L-asparaginase, and prednisone (VDCLP regimen) as induction. Bone marrow smear showed 33.5% blasts after the completion of VDCLP. Another VDCLP was given but the bone marrow smear still showed 26% blasts. Then the patient moved to Shanghai and was enrolled in a clinical trial about parent-derived CD7-targeted CAR-T. He first received an infusion of his father-derived CAR-T but showed no response. Again his mother-derived CAR-T was infused but his bone marrow smear showed 75% blasts 16 days after infusion. Homoharringtonine and Vinaikra were given as salvage therapy but no response was observed still. For further treatment, the patient came to our department, the 920th Hospital.

In our department, we gave the patient vinaikra, azacitidine, cytosine arabinoside, aclarubicin, recombinant human granulocyte-stimulating factor, prednisone regimen (VACAG-P), and daletumab as salvage therapy. However, as in the previous treatment, no response was observed. Flow cytometric (FCM) analysis of his bone marrow showed 51.36% blast with high expression of CD7 (99.3%), dim expression of CD3 and CD5, and no expression of CD2, CD34+1a, cTdT, CD4, CD8, CD38, CD19, and CD56. Then the patient was enrolled in our CD7 UCAR-T clinical trial (Figure 1A).

2.2 | CAR-T therapy

As is in most CAR-T therapies, a three-day FC preconditioning chemotherapy was given before CAR-T infusion: fludarabine 30 mg/M^2 × 3 days, cyclophosphamide 500 mg/M^2 × 3 days. Then a total dose of 4 × 10^8 (4.81 × 10^6/kg) CD7 UCAR-T cells was infused by vein at a time. Response evaluation was set at day 14 and day 28 ± 2 days (Figure 1B). At day 0 before infusion, FCM analysis of bone marrow showed 29.69% leukemic cells and the expression of CD7 was 100%.

2.3 | Response to treatment

At day 14 after CAR-T infusion, blasts in the bone marrow were undetectable by bone marrow smear but the flow cytometry analysis showed 0.43% minimal residue disease (MRD). At day 27 the MRD was undetectable by FCM (Figure 1B). CR was verified 4 months after the CAR-T infusion. Long-term follow-up is ongoing.

Robust expansion of CD7 UCAR-T was detected by FCM and quantitative polymerase chain reaction (qPCR) along with quick elimination of CD3+CD7+ lymphocytes and natural killer (NK) cells. The peak of CAR-T expansion was around days 7–14, of which the proportion was over 95% lymphocytes. The persistence of CAR-T was verified over 4 months by CAR transgene. Recovery of B cells was accompanied by ongoing undetectable CD3+CD7+ lymphocytes and NK cells in the 4th month after CAR-T infusion. The portion of CD3+CD7- lymphocytes gradually increased and accounted for 42.9% of lymphocytes (Figure 2A).

2.4 | Allo-HSCT

Due to frequent and heavy previous treatment and uncontrolled disease, myelosuppression was severe and persistent for the patient. There were no signs of hematopoietic recovery even one week after when bone marrow remission was verified. To support the hematopoietic recovery of the patient we gave him an infusion of his father's peripheral blood stem cells (PBSCs) from day 21 to day 23. The human leukocyte antigen matching was 7/12 and the total infused dose in the three days was 16.6 × 10^8/kg mononuclear cells (7 × 10^6/kg CD34+ cells).

On day 32 after CAR-T infusion engraftment of neutrophile granulocyte and platelet was observed (Figure 2C). At day 36 the chimerism of the donor was 98.45% detected by STR—PCR [8]. The full chimerism of the donor was kept stable for up to 120 days after CAR-T infusion, which is the latest follow-up time (Figure 2A).

2.5 | Toxicity and GVHD monitor

Grade 2 cytokine release syndrome (CRS) was observed. On day 0, the patient developed a fever (39.9) with chill after CAR-T infusion, which was quickly resolved by Ruxolitinib and a low dose of dexamethasone, as is what we had previously reported [3]. The oxygen saturation dropped below 90% but a low-flow nasal cannula (3 L/min) could maintain the oxygen saturation above 95%. Intermittent fever occurred during the following days after day 0 and was well controlled. A significant change in blood pressure was not observed. Elevated liver enzymes were observed on day 7 and were controlled after anti-CRS treatment. Hemorrhagic cystitis accompanied by high copies of urine BKV DNA (1.16 × 10^9) occurred on day 10. After anti-BKV treatment and adequate hydration and alkalization of urine hemorrhagic cystitis was resolved on day 14. On day 32 the quantification of Epstein-Barr virus was 4.23 × 10^3 copies/mL and gradually increased to 4.83 × 10^5 copies/mL on day 35. Cytomegalovirus copies increased from 1.32 × 10^3 copies/mL to 2.45×10^4 copies/mL between day 46 to day 69. Anti-virus therapies were given and both viruses were controlled well. Immune effector cell-associated

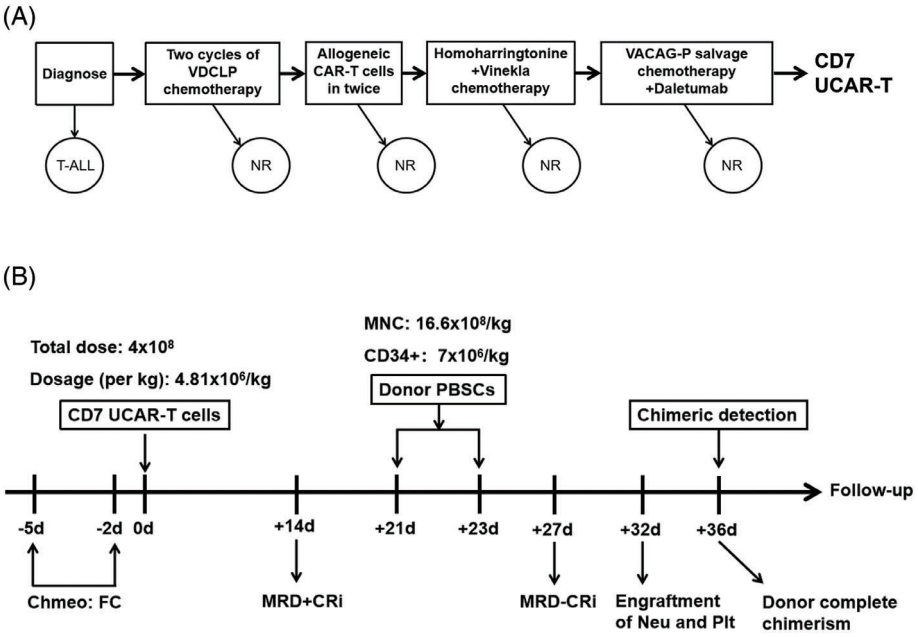

FIGURE 1 (A) Treatments before CD7 targeted chimeric antigen receptor-T (CD7 UCAR-T). (B) Protocol of CD7 UCAR-T treatment and peripheral blood stem cells (PBSCs) infusions.

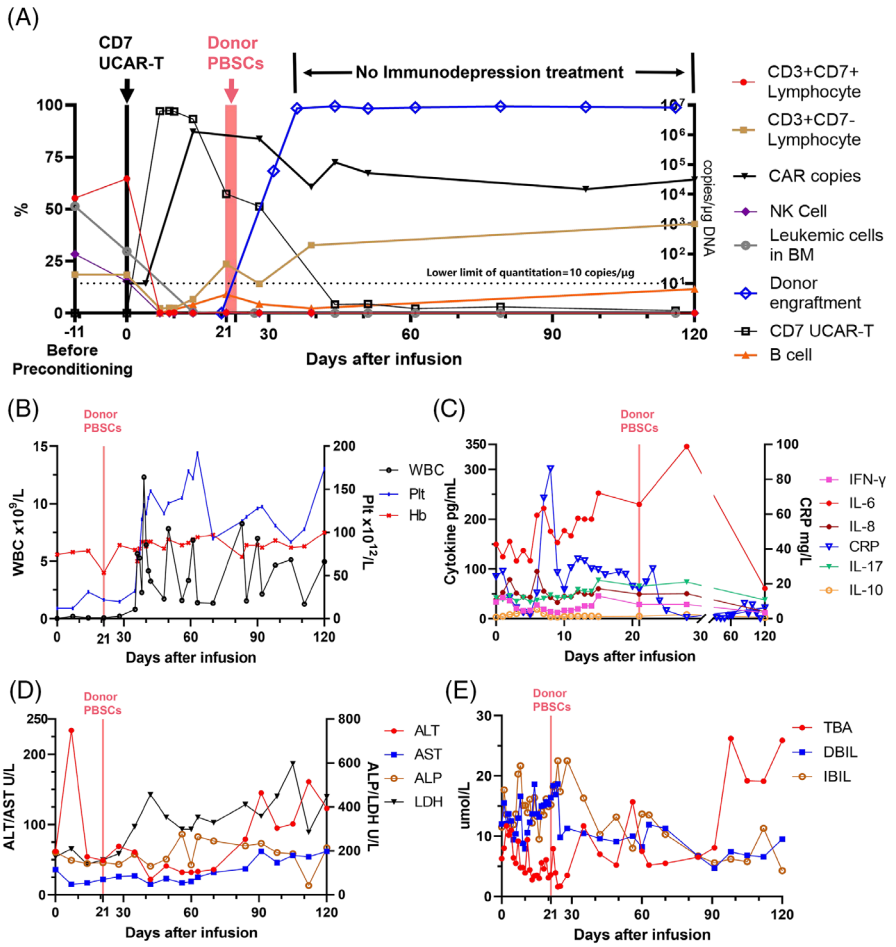

FIGURE 2 Clinical responses monitored. (A) Changes of each cell type and donor chimerism. (B) Changes in white blood cells, hemoglobin, and platelet. (C) Serum levels of cytokines. (D and E) Levels of biochemical indices of the liver. ALP, alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate aminotransferase; DBIL, direct bilirubin; IBIL, indirect bilirubin; LDH, Lactate dehydrogenase; TBA, total bile acid.

neurotoxicity syndrome or any signs of bacteria or fungal infection were not observed.

C-reactive protein (CRP) and 12 cytokines (interleukin [IL]-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, interferon [IFN]-α, IFN-γ, and tumor necrosis factor [TNF]-α) of serum were detected before and after CAR-T infusion. Significant elevation was only observed in IL-6 and CRP. However, the level of IL-6 and CRP was higher than normal before CAR-T infusion. The peak level of IL-6 was below 350 pg/mL, much lower than what was reported in severe CRS. IFN-γ, IL-8, IL-10, and IL-17 were elevated in a small range (Figure 2C). Other cytokines detected had very slight changes after infusion and were not shown.

Due to the worry that immunodepression intervention might have an influence on UCAR-T, we didn't give the patient any immunodepression intervention after engraftment of his father's PBSCs. We closely monitored the occurrence of GVHD and planned to interfere at any time. Beyond our expectation, no signs of GVHD were observed up to 3 months after engraftment (Figure 2D,E). We used a six-factor GVHD predicting system by detecting serum levels of IL-6, IL-8, sTNFR1, sST2, Reg3α, and Elafin to evaluate the GVHD risk at day 102 [9, 10]. The result was low GVHD risk. No signs of other complications of transplantation were observed. Longer time follow-up is ongoing.

3 | DISCUSSION AND CONCLUSIONS

CD7 is widely expressed in T cells and NK cells and their precursors [11], making it an ideal target for T/NK cell-derived malignancies. Elimination of CD7+ T cells is natural and common in CD7-targeted therapies. The lack of CD7+ T cells may be persistent accompanied by long-term persistence of CAR-T. The compensatory proliferation of CD7- T cells after CD7 targeted CAR-T had been observed by our and other's previous reports [6, 12].

Due to limited knowledge of CD7- T cells, it is not clear what the compensatory proliferation of CD7- T cells and the lack of CD7+ T cells will bring. In our recent report, the expansion of CD3- T cells was accompanied by the loss of UCAR-T [12], indicating the CD7- T cells may play a role in eliminating UCAR-T cells.

As we know, there is no study about the role of CD7- T cells in allo-HSCT. For allo-HSCT in the treatment of hematological malignancies, an ideal scenario would be to achieve rapid and stable implantation in the absence of either GVHD or relapse. We speculate that CD7 UCAR-T as preconditioning may theoretically achieve that ideal scenario. Due to the broad expression of CD7 in T cells and NK cells, CD7 UCAR-T can eradicate most T cells and NK cells from both the donor and the receptor, creating a good environment for implantation and reducing the risk of GVHD. CD7 UCAR-T can kill the CD7+ malignant cells while the graft versus leukemia (GVL) effect can kill the CD7- malignant cells, decreasing the relapse rate.

The severe pancytopenia was heavy for the patient after CD7 UCAR-T. We think there are two major potential causes of severe pancytopenia: one is the heavy treatments the patient received before, another is the uncontrolled disease. The two factors may make the patient's hematopoietic function so poor and need donor's PBSCs to support hematopoietic recovery.

A recent study reported that patients underwent allogeneic stem-cell with GVHD prophylaxis at which point any persisting CD7 UCAR-T cells were depleted by the conditioning regimen used before transplant. GVHD was observed in that study [13]. In this report, three goals were achieved in at least 4 months: no GVHD, no relapse, and smooth implantation. The patient experienced no GVHD in the absence of immunodepression interventions after engraftment.

We think the possible mechanism of this treatment may be from two aspects: First, CD7-targeting CAR-T removes all CD7 + cells, which means that most of the T cells and NK cells from donor and recipient are removed, which reduces the possibility of bidirectional rejection between donor and recipient. Secondly, we speculate the CD7- T cells may not drive the bidirectional rejection between donor and recipient, which needs to be proved by future exploration. Interestingly, in a recent study, CD7-deleted hematopoietic stem cells were proven to restore the immunity of mice after UCART7 treatment [14], indicating the possibility of CD7-deleted hematopoietic stem cell transplantation.

It is not yet clear whether the persistent absence of CD7+ T cells may lead to increased post-transplant relapse and/or infections. And we still don't know if the CD7- T cells reserve the ability of GVL and anti-infection. We are doing further studies to answer these questions.

AUTHOR CONTRIBUTIONS

Sanbin Wang conceived and designed the study; Xi Zhang provided research instructions and checked the manuscript; Shiqi Li and Xi Zhang analyzed and interpreted the data; Shiqi Li wrote the manuscript; Zhongtao Yuan and Lin Liu provided the study materials or patients; Yu Li, Le Luo, Lihui Peng, Xiaoping Li, and Mengli Xu collected and checked the original material and data; Yingnian Chen and Qingying Zang provided nursing support; Ping Yin performed the experiments and detection. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

This clinical trial was approved by the Ethics Committee of the 920th Hospital and was registered at the International Standard

Randomized Controlled Trial Number system(https://www.isrctn.com/) as ISRCTN19144142. The trial was designed and conducted in accordance with the Declaration of Helsinki. The participant's guardians were well-informed and provided written informed consent. The clinical data reported in the manuscript was from the 920th Hospital.

PATIENT CONSENT STATEMENT

The authors have confirmed patient consent statement is not needed for this submission.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

ORCID

Xiaoping Li https://orcid.org/0000-0002-9215-9611
Xi Zhang https://orcid.org/0000-0002-8548-2832
Sanbin Wang https://orcid.org/0000-0002-4802-0292

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