Clinical Case Report

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Preventive infusion of donor-derived CAR-T cells after haploidentical transplantation

Two cases report

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Abstract

Rationale: Relapse is the main cause of death after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Unfortunately, there are no efficient methods to prevent relapse after allo-HSCT. Chimeric antigen receptor T (CAR-T) cells have achieved favorable outcomes in the treatment of refractory/relapsed acute lymphoblastic leukemia (ALL) because of their strong anti-leukemia activity. However, it is unclear whether the CAR-T cells constructed using viral systems can be used as preventive infusions to prevent relapse after haploidentical HSCT.

Patient concerns: Two patients with ALL with high risk received haploidentical HSCT.

Diagnoses: Two patients were diagnosed with ALL with high risk.

Interventions: Patients received preventive infusion of donor-derived CAR-T cells constructed using viral systems on day 60 after haploidentical HSCT.

Outcomes: The CAR-T cells were continually detected, and no graft versus host disease developed. The two patients survived with disease-free for 1 year and 6 months, respectively.

Lessons: Preventive infusion of donor-derived CAR-T cells after haploidentical HSCT may be safe and that immunosuppressors may not affect the proliferation of CAR-T cells.

Abbreviations: ALL = acute lymphoblastic leukaemia, allo-HSCT = allogeneic hematopoietic stem cell transplantation, CAR-T cells = chimeric antigen receptor T cells, CRS = cytokine release syndrome, DLI = donor lymphocyte transfusion, GVHD = graft-versus-host disease, GVL = graft-versus-leukemia, Hyper-CVAD = cyclophosphamide, vincristine, doxorubicin, and dexamethasone.

Keywords: acute lymphoblastic leukemia, CAR-T cells, donor-derived CAR-T cells, haploidentical hematopoietic stem cell transplantation, preventive infusion

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1. Introduction

Approximately 50% patients with acute lymphoblastic leukemia (ALL) who undergo allogeneic hematopoietic stem cell transplantation (allo-HSCT) will relapse, which is the main cause of death for these patients.[1] Donor lymphocyte transfusion (DLI) is the main method used to prevent relapse in patients with ALL after allo-HSCT through the graft-versus-leukemia (GVL) effect, but it has limited efficacy.[2] Moreover, approximately one-third of patients who receive DLI develop severe acute graft-versus-host disease (GVHD), resulting in 6–11% treatment-related mortality. Therefore, it is vital to explore new, safer treatment strategies that prevent disease relapse post-transplantation.

Chimeric antigen receptor T (CAR-T) cell therapy is an emerging method of immunotherapy that has produced favorable outcomes in the treatment of refractory/relapsed B-ALL due to strong anti-leukemia activity.[3,4] At present, there are two ways to accomplish gene incorporation in the construction of CAR-T cells: viral vector systems and non-viral systems.[3] Viruses are the major vectors used for gene therapy in basic research and clinical study because of their high transfer efficiency, the availability of different viruses with different expression characteristics and the relatively short time needed to reach the clinically necessary

numbers of cultured T cells.[5] A recent study used CAR-T cells made with the non-viral Sleeping Beauty system for infusion in patients after transplantation.[6] However, it is still unclear whether donor-derived CAR-T cells constructed with viral systems can be used as preventive infusions after haploidentical HSCT with the long-term use of immunosuppressors in order to prevent relapse and improve long-term survival. Here we report on patients with refractory ALL who received CAR-T cells constructed with a viral system as part of a conditioning regimen for haploidentical transplantation.[7] The CAR-T cells were able to proliferate despite the continual use of immunosuppressors, which may inhibit the proliferation of CAR-T cells or kill them. In addition, patients with a low disease burden had markedly enhanced remission duration and survival after CAR-T cell treatment.[8] Therefore, we suggest that donor-derived CAR-T cells constructed with a viral system can be used as a preventive infusion after haploidentical transplantation. In this study, we report two patients with high-risk ALL who received preventive infusion of donor-derived CD19-CAR-T cells constructed with a viral system on day 60 after haploidentical HSCT. The production, detection and quantification of the CD19-CAR-T cells were performed according to our previous report.[7] This study was approved by the Institutional Review Board of Xinqiao Hospital (Ethical approval number: CHiECRCT-20160022 and 2018-063-01) and was conducted in accordance with the Declaration of Helsinki. Patients have provided written informed consent for publication of the cases.

2. Case description

2.1. Case 1

A 27-year-old male was admitted to our hospital due to fever and abnormal hemogram (white blood cell count 40.05×10^9/L and platelet count 81×10^9/L). The patient was diagnosed Ph-negative ALL with 83% blast cells. Flow cytometry analysis revealed 81.7% of cells were positive for CD19, 72.5% for CD22, 72.5% for CD79a, 8.1% for CD10(+), 82.1% for HLA-DR, and 90.5% for CD34. Karyotyping was normal. No other high-risk genes or mutations were detected. The patient received chemotherapy consisting of cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD). After chemotherapy, the patient achieved complete remission with negative minimal residual disease (MRD). A medium-high dose of methotrexate was used as consolidation chemotherapy for two cycles. The patient received haploidentical HSCT from his father as the donor with the conditioning regimen and GVHD prophylaxis described in our previous reports.[1,9] The patient engrafted on day 15 with full donor chimerism. The patient then received preventive infusion of donor-derived CD19-CAR-T cells at a dosage of 0.5×10^6/kg divided between 2 days on days 60 and 61 after allo-HSCT. No cytokine release syndrome (CRS) or GVHD developed (Fig. 1a). The CAR-T cells could continually be detected (Fig. 1b). The patient survived for 1-year disease-free.

2.2. Case 2

A 5-year-old male was admitted to our hospital due to refractory/relapsed B-cell ALL. He had a history of thirteen cycles of chemotherapy that were conducted in accordance with the guidelines for treating childhood ALL in other hospitals. A bone marrow smear revealed 71.5% blasts. Flow cytometry analysis revealed 84.9% were positive for CD19, 53.5% for CD22, 51.9% for CD79a, 73.9% for CD10(+), and 92.4% for CD38. Karyotyping revealed 57 XY, add (1) (q44), add (2) (q37), +2, +4, +6, add (7) (q36), +8, +10, +11, add (13) (q34), +15, +18, +18, +21, +22.[5] The patient was treated with fludarabine, etoposide, pegylated liposomal doxorubicin, vindesine, and dexamethasone again for induction. However, 66.5% blast cells were observed by flow cytometry after chemotherapy. The patient then received haploidentical HSCT with his father as the donor with the conditioning regimen and GVHD prophylaxis described in our previous reports.[1,9] The patient engrafted on day 13 with full donor chimerism. The bone marrow tested negative for MRD. The patient received preventive infusion of donor-derived CD19-CAR-T cells at a dosage of 0.5×10^6/kg divided between 2 days on days 60 and 61 after allo-HSCT. No CRS or GVHD developed (Fig. 1c). The CAR-T cells could continually be detected (Fig. 1d). The patient survived for 6-month disease-free.

3. Discussion

In this study, we report that two patients successfully received preventive infusions of CD19-CAR-T cells post-HSCT without serious complications, and the CAR-T cells survived despite immunosuppression. This is first report on the preventive infusion of CD19-AR-T cells constructed with a viral system after haploidentical HSCT with the long-term use of immunosuppressors.

Relapse is the main cause of death for patients with ALL, even if they undergo allo-HSCT. Although DLI are currently the most commonly used treatments in clinics, the outcomes have proven unsatisfactory for patients with ALL and the related complication and death rates are high.[2] CAR-T cells have shown powerful anti-leukemia effects and may be ideal for preventing relapse post-transplantation. However, GVHD is one of the main risks associated with the infusion of donor-derived CAR-T cells. In addition, the question of when to infuse CAR-T cells is of vital importance. The time at which acute GVHD is most likely to develop is generally 6 weeks after transplantation, and some reports showed that the optimal time for administering immunotherapy is from days 55 to 200 post-transplantation.[4,6] Accordingly, in this study we infused the donor-derived CAR-T cells on day 60 post-transplantation and the cells were administered in two doses over the course of 2 days. No GVHD was observed.

The other main question concerning CAR-T cells is whether immunosuppressors may inhibit their proliferation or kill them. A recent study showed that low-dose dexamethasone ($\leq$6 mg/day) did not diminish CAR-T cell anti-tumor activity in-vivo.[10] It is unclear whether the other immunosuppressors in common long-term use post-transplantation, such as tacrolimus and cyclosporin A, may affect the proliferation of CAR-T cells. Our previous study showed that the immunosuppressors used during transplantation did not affect the proliferation of CAR-T cells used as part of conditioning regimens.[7] In this study, the donor-derived CD19-CAR-T cells continually proliferated despite the use of immunosuppressors post-transplantation. Although a report showed that the CAR-T cells were infused post-transplantation, the immunosuppressors were tapered after 3 months and discontinued by 6 months post-transplantation.[6] On the other hand, this system uses a synthetic DNA transposon for nonviral somatic gene transfer, which may affect the survival of CAR-T cells in the body and the survival of patients because of the lack of consistency and preciseness for electroporation.[11] So

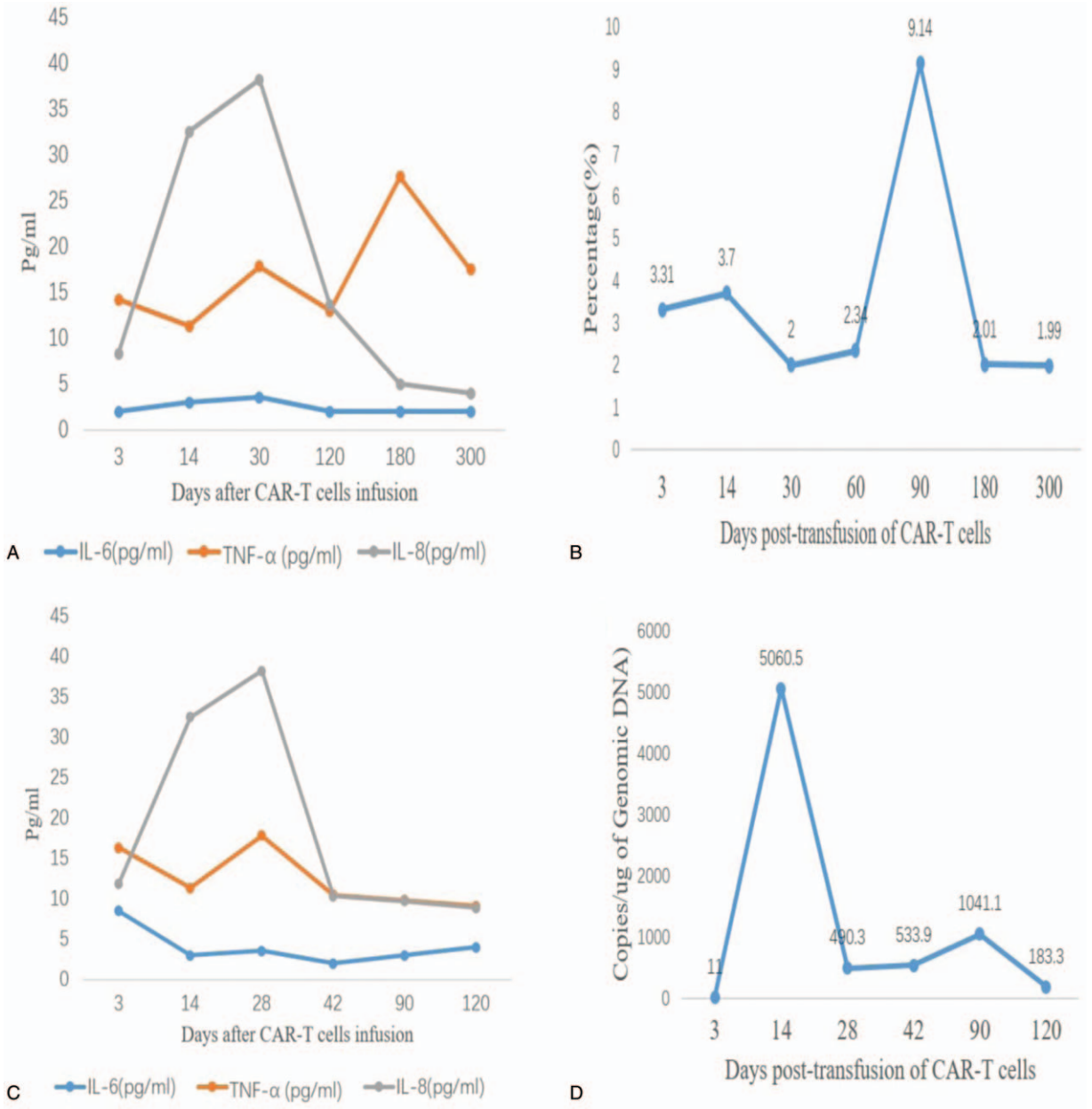

Figure 1. The proliferation of CAR-T cells after allogeneic hematopoietic stem cell transplantation under immunosuppression. CAR-T cells continually proliferated post-transplantation although immunosuppressors were used. No serious cytokine release syndrome was observed. (A) Cytokine release level for Case 1. (B) The proliferation of CAR-T cells observed in Case 1. (C) Cytokine release level for Case 2. (D). The proliferation of CAR-T cells for Case 2.

the averagely survival time of CAR-T cells was 51 days in the reported study. In our study, the immunosuppressors were tapered after 6 months and discontinued by 1 year post-transplantation, the CAR-T cells constructed using viral systems can be continually detected.

CRS is the most common and potentially severe toxicity associated with CAR-T cell treatment, and can cause patient death. In this study, the patients did not experience CRS. The use of immunosuppressors may have inhibited the release of cytokines after the infusion of CAR-T cells.

4. Conclusion

This study showed that it is safe to infuse donor-derived CD19-CAR-T cells after haploidentical transplantation, and the immunosuppressors used post-transplantation did not affect the proliferation of the CAR-T cells. Clinical trials should be performed to further investigate this protocol.

Author contributions

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References

[1] Chen XH, Zhang C, Zhang X, et al. Role of antithymocyte globulin and granulocyte-colony stimulating factor-mobilized bone marrow in allogeneic transplantation for patients with hematologic malignancies. Biol Blood Marrow Transplant 2009;15:266–73.
[2] Wang YX, Li YH. Efficacy of donor lymphocyte infusion for treating relapsed high-risk leukemia patients after allogeneic hematopoietic stem cell transplantation. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2015;23:982–8.
[3] Zhang C, Liu J, Zhong JF, et al. Engineering CAR-T cells. Biomark Res 2017;5:22.
[4] Liu J, Zhong JF, Zhang X, et al. Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies. J Hematol Oncol 2017;10:35.
[5] Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 2013;5:177ra38.
[6] Kebriaei P, Singh H, Huls MH, et al. Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells. J Clin Invest 2016;126:3363–76.
[7] Zhang C, Kong PY, Li S, et al. Donor-derived CAR-T cells serve as a reduced-intensity conditioning regimen for haploidentical stem cell transplantation in treatment of relapsed/refractory acute lymphoblastic leukemia: case report and review of the literature. J Immunother 2018;41:306–11.
[8] Park JH, Rivière I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med 2018;378:449–59.
[9] Gao L, Zhang Y, Hu B, et al. Phase II multicenter, randomized, double-blind controlled study of efficacy and safety of umbilical cord-derived mesenchymal stromal cells in the prophylaxis of chronic graft-versus-host disease after HLA-haploidentical stem-cell transplantation. J Clin Oncol 2016;34:2843–50.
[10] Brown CE, Aguilar B, Starr R, et al. Optimization of IL13Rα2-targeted chimeric antigen receptor T cells for improved anti-tumor efficacy against Glioblastoma. Mol Ther 2018;26:31–44.
[11] Anita G, Julie G. What you always needed to know about electroporation based DNA vaccines. Hum Vaccin Immunother 2012;8:1694–702.