HLA-matched allogeneic anti-CD19 CAR-T therapy in treating a relapsed/refractory acute lymphoblastic leukemia patient with high tumor burden

Yue Huang MD, Qin Yu PhD, Guoqing Wei PhD, He Huang MD, Yue Huang, Qin Yu, and Elaine Tan Su Yin contributed equally to this work.

Trial registration: ChiCTR, ChiCTR1800017669. Registered 8 April 2018 - Retrospectively registered, http://www.chictr.org.cn/showproj.aspx?proj = 29243

Funding information
the 973 Program, Grant/Award Number: 2015-CB964900; the Natural Science Foundation of China, Grant/Award Numbers: 81770201, 81730008

Abstract
The genetically engineered chimeric antigen receptor T cell (CAR-T) therapy has shown remarkable clinical efficacy in the treatment of hematological malignancies. Nonetheless, it is difficult to harvest adequate autologous T cells to manufacture potent CAR-T cell products in patients with high tumor burden and prior tumor-reductive treatment. Here we reported a relapsed/refractory acute lymphoblastic leukemia patient with high leukemia burden and central nervous system (CNS) involvement. The patient responded to donor-derived HLA-matched allogeneic CAR-T treatment, with the achievement of quick complete remission. And for the first time, we revealed the development of a cerebral CRS in situ after allogeneic CAR-T therapy.

KEYWORDS
acute lymphoblastic leukemia, allogeneic chimeric antigen receptor T cells, central nervous system leukemia, cytokine release syndrome, HLA-matched

1 | INTRODUCTION

Chimeric antigen receptor T cell (CAR-T) therapy, a newly established adoptive T cell therapy, specifically redirects genetically modified immune cells to fight against hematologic malignancies. According to recent clinical trials, CAR-T therapy has a complete remission (CR) rate of 80%–90% for patients with relapsed/refractory acute B-cell lymphoblastic leukemia (r/r B-ALL) and a 50% of CR rate in relapsed/refractory B-cell lymphoma patients. Though autologous CAR-T therapy has gained outstanding success in treating hematological malignant patients, there are several clinical limitations worth noting.
Generally, the manufacturing process of CAR-T cells is rather tedious and challenging as it is much dependent on the quantity and quality of one’s autologous T cells. However, in some patients with high tumor burden and prior tumor-reductive treatment, they might experience prolonged and severe lymphopenia. This condition would complicate the CAR-T cell production as it is more difficult to harvest and obtain enough T cells to manufacture potent CAR-T cell products in these patients. Furthermore, autologous CAR-T cell production is costly and time-consuming. Lastly, CAR-T therapy is an individualized therapy for patients, and thus, it might not be a feasible treatment plan for some patients with advanced diseases.

‘Off-the-shelf’ allogeneic CAR-T therapy has the potential to overcome these limitations. Moreover, CAR expression of allogeneic cell types like NK cells is also currently under exploration. Nonetheless, this plan may take several years to be clinically and technologically mature. Concerning convenience and clinical practicability, HLA-matched allogeneic CAR-T cells might be the most appropriate strategy during this transition period.

To date, a small number of patients with B-cell malignancies, especially those who relapsed post-transplantation, were treated by donor-derived CAR-T cells. As a result, most of them have achieved CR or partial remission (PR) with a relatively low incidence of graft-versus-host disease (GVHD) and toxicity. With this, we report a case of an R/R ALL patient with high disease burden and central nervous system (CNS) infiltration, which had been successfully treated by donor-derived HLA-matched allogeneic CAR-T treatment.

## CASE PRESENTATION

A 53-year-old female patient was admitted to our hospital due to R/R B-ALL. The initial bone marrow (BM) smear revealed the diagnosis of the L2 subtype of acute lymphoblastic leukemia (ALL-L2). The flow cytometry analysis showed 91.6% of the lymphoblasts expressed CD123+, CD33+, CD34+, CD10+, CD19+, and lacked CD20. Besides, the karyotyping analysis displayed 45,−6, XX, Ph(cp9)/46, while 7.4x10E9/L copies of infusion gene BCR-ABL(p190) was detected, along with NPM1-gene mutation. The presence of the unfavorable prognostic factors, such as IK6 isoform of the IKZF1 gene mutation and immunoglobulin heavy chain (IgH) rearrangement, indicated a dismal prognosis in this patient.

Once the diagnosis was confirmed, she received a series of chemotherapies but all therapies failed to achieve persistent CR as shown in Figure 1. Since October 2018, she received a cycle of induction chemotherapy with VICEP (vindesine, idarubicin, cyclophosphamide, and methylprednisolone), combined with dasatinib for 1 month. After induction therapy, the BM examination revealed this patient had achieved CR with no IKZF1-gene point mutation (IK6 isoform) was detected. Subsequently, from November 2018 to February 2019, three cycles of VP (vindesine and prednisone) combined with dasatinib were administered uneventfully. In the routine follow-up after the chemotherapy, she relapsed with a minimal residual disease (MRD) of 84%, positive BCL-ABL fusion, IK6 isoform of IKZF1 mutation, and T315I mutation. As a salvage therapy, the patient received another cycle of HVP (homoharringtonine, vindesine, and methyl-prednisolone) with dasatinib, but she did not respond to the given chemotherapy. Worse still, the leukemia cells in BM rose to 85%, and the serum lactate dehydrogenase (LDH) level was 2341 U/L.

Soon, one additional cycle of VP combined with ponatinib were administered. The subsequent BM examination strongly suggested a PR as it showed 6% of lymphoblast and 0.022% of BCR-ABL gene. Unfortunately, shortly after obtaining PR, the patient developed hyperleukocytosis with a WBC level of 201x10E9/L and hypothyroidemia with 86% leukemia cells in the BM in August 2019. The patient immediately received leukoreduction treatment for the prevention of leukostasis.

Given that the tumor burden at the relapse time was as high as 85%, we expected high failure rate of manufacturing autologous CAR-T cells of the patient due to the inadequate quantity. To avoid treatment delay, the HLA-matched allogeneic CAR-T strategy was considered instead. The patient was then recruited in our clinical trial (ChiCTR1800017669). Shortly after a cycle of Hyper-CVAD Part A chemotherapy (cyclophosphamide, epirubicin, vinorelbine, and dexamethasone), the patient accepted HLA-matched sister’s allogeneic anti-CD19 CAR-T cells infusion at the dose of 3.3 × 10^6/kg with preceding lymphodepletion using fludarabine and cyclophosphamide. Soon after the CAR-T cell infusion, the patient developed recurrent fevers (shown in Figure 2A) and severe headache. To add on, Figure 2C showed markedly elevated cytokine levels in patient’s serum, including interleukin (IL)−2, IL-6, IL-10, interferon-γ (IFN-γ), C-reactive protein, D-dimer, and ferritin. Both CAR-T cells and non-CAR-T cells of this patient expanded exponentially (shown in Figure 2B).

The patient was diagnosed with Grade 2 cytokine release syndrome (CRS) according to CARTOX criteria, with no signs and symptoms of GVHD such as hepatocellular injury, diarrhea, or rash. Following 10 days of supportive care and antibiotics and antifungal therapy, her serum cytokines have restored to the normal range. Nevertheless, the patient still complained of a headache and nausea though no symptoms of Immune effector cell-associated neurotoxicity syndrome (ICANS) was detected.

The patient received lumbar puncture on day 10. The cerebrospinal fluid (CSF) analysis revealed that the level of leukemia cells was at 1/ul as shown in Figure 2F, confirming the diagnosis of CNSL. Further CSF analysis showed 200 WBCs/µl with 75% karyocytes, 2.040 g/µl of protein. The CSF cytokine level was much higher than that in the serum, as indicated by increased levels of IL-6 (9451.02 vs. 7.36 pg/ml), IL-10 (597.30 vs. 2.64 pg/ml), and IFN-γ (904.01 vs. 4.66 pg/ml). Moreover, the flow cytometry analysis also indicated effective CAR-T cell expansion in CSF on 10th day (shown in Figure 2D and E). Then antiemetic drugs and mannitol were included in the treatment regimen. On the 14th day, no leukemia cells was found in CSF (Figure 2G). The patient was declared to be in CR with minimal residual disease (MRD)-negative, BCR-ABL negative, and T315I mutation-negative. She was discharged from the hospital 15 days after CAR-T cell infusion, and prepared for the upcoming treatment of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Nonetheless, after 35 days of receiv-
revealed that allogenic CAR-T cells.

The inability of allogeneic T cells to HLA-matched allogeneic CAR-T treatment satisfied the disease as the MRD level had increased to 53.703% through flow cytometry analysis see supporting information.

3 | DISCUSSION

In this study, we reported an R/R ALL (L2 subtype) patient with CNSL. The healthy sibling-derived CAR-T treatment had displayed an extraordinary efficacy in treating hematological malignancy as our patient achieved BM remission within 14 days after infusion.

The limitations of deploying patient derived, autologus T cells include harvest and manufacturing failures, disease progression during manufacture, tumor cell contamination, and, especially, T cell dysfunction. In our case, the patient had received eight lines of intense chemotherapies, which suggested inadequate T cell count and high proportion of exhausted T cells. Actually, T cell dysfunction can occur in leukemia patients even in the absence of therapy as exemplified in recent study. HLA-matched allogeneic CAR-T treatment satisfactorily solved these problematic issues of autogenous CAR-T therapy.

Throughout this treatment process, the adverse effect was manageable. We posted a concern that the donor-derived non-CART cell expansion was induced by the cytokines released during the CRS process and might act against host tissue to cause GVHD. However, no GVHD symptoms were observed in this patient after the infusion of allogeneic CAR-T cells, which was similar to that reported in the previous studies. Generally, the occurrence of GVHD was associated with concurrent stimulation of alloreactive T cells via TCR in response to allogeneic B cells. Ghosh et al. revealed that allogenic CAR-T cells with certain co-stimulatory domain had decreased potential to cause GVHD, which resulted from cumulative CAR and alloreactive TCR signaling, leading to exhaustion and eventual deletion of the alloreactive CAR T cells. As shown in our case, the short maintenance of CAR-T and non-CART cells in the host’s body may explain the low incidence of GVHD.

Both CNSL and CAR-T expansion were confirmed through CSF flow cytometry analysis. The level of the CAR-T cell was at approximately 8/ul, and leukemia cells were about 1/ul in the CSF at day 10. Though neurotoxicity occurred in three of eight patients after HLA-matched allogeneic CAR-T cell infusion in a recent case series study, there is a paucity of data regarding the CNS penetration of allogeneic CAR-T cells. It is noteworthy that there was a considerable discrepancy in cytokine distributions between CSF and serum, which is consistent with our previous study. Since CNS cells showed absence of CD19 expression, the reason for CNS symptoms is less likely attributable to direct interactions between CD19+ normal cells and anti-CD19 CART cells. A similar moderate cerebral CRS with inflammatory cytokines was presumably generated by blood-brain-barrier (BBB) penetrating CAR-T cells. One of the possible mechanisms is that BBB penetrating CAR-T cells may be activated by CNS-entering potent minimal residual CD19+ leukemia cells which are less than detection limits.

4 | OUR DATA FIRST REVEALED THE DEVELOPMENT OF A CEREBRAL CRS IN SITU BY ALLOGENIC CAR-T THERAPY

However, this patient experienced relapsed disease only 35 days after receiving allogeneic CAR-T cell therapy, albeit the fact that this patient did achieve CR for 21 days. The donor CAR-T cells maintained for a shorter period in the patient’s body than the autologous CAR-T cells. Several cases, including haploidentical or matched CAR-T, have reported similar quick relapse. The inability of allogeneic T cells to maintain long-term immune surveillance might be attributed to the
FIGURE 2  Biochemical and immunological kinetics after allogeneic CAR-T cell infusions. (A) Wave changes in body temperature after CAR-T infusion, with a maximum temperature per 24-h period indicated by the squares. (B) The flow cytometry results showed that the count of CAR-T cells increased significantly in the peripheral blood of the patient, arriving the peak at day 9 and diminishing quickly. (C) The kinetics of inflammatory cytokines and ferritin revealed a mild CRS after the CAR-T infusion, the adverse effect was manageable. The serum cytokines fell into normal range within 10 days of supportive care. (D and E) The flow cytometry results demonstrated the portion of CAR-T cells in CSF lymphocytes at 8.09% on day 10 and at 18.7% on day 14, indicating effective expansion of CART cells in CSF. (F and G) The flow cytometry results revealed the portion of CD19+ CD22+ leukemia cells in CSF lymphocytes at on day 10 and day 14. (H) CSF and serum cytokine levels (IL-6, IL-10, IFN-γ) at the Days 10 and 14 after CART cell infusion.
recognition and elimination by the host immune cells. It might also associate with severe lymphopenia, the number of allo-CART cells, and the degree of HLA-matching. Some research studies suggested that donor-derived CAR-T cells be part of the conditioning regimen prior to HSCT.\textsuperscript{25,26} Besides, some cases reported of successful sequential or combined allo- and auto-CART therapy in chemo-refractory patients with high tumor burden.\textsuperscript{27} In short, the short-term persistence of donor-derived CAR-T cells is a significant hurdle to the broader application of allogeneic CAR-T therapy in hematological malignancies. Therefore, timing of subsequent treatment options like HSCT should be considered as soon as possible worth further exploration.

In summary, apart from the autologous CAR-T therapy, allogeneic CAR-T treatment is one of the best treatment options for patients with hematological malignancies. This approach circumvents the difficulty of harvesting adequate and optimal T cells from patients with severe lymphopenia with a relatively low incidence rate of GVHD and mild CRS toxicity. Despite its advantages, the short maintenance period and the high relapse rate of allo-CART therapy should be improved in future studies. All in all, allogeneic CAR-T cell therapy is definitely worth further investigation and exploration.

ACKNOWLEDGEMENTS

The study design, materials, and data analyses used in this study were supported by the 973 Program (2015-CB964900), the Natural Science Foundation of China (81770201, 81730008).

CONFLICT OF INTEREST

The authors have no relevant conflicts of interest to report.

ORCID

Yue Huang MD https://orcid.org/0000-0001-8014-313X
Elaine Tan Su Yin MD https://orcid.org/0000-0001-7268-8455
He Huang PhD https://orcid.org/0000-0002-2723-1621

REFERENCES

1. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. New Eng J Med. 2018;378(5):439–448. https://doi.org/10.1056/nejmoa1709866
2. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, et al. Long-term follow-up of CD19 CAR T therapy in acute lymphoblastic leukemia. New Eng J Med. 2018;378(5):449–459. https://doi.org/10.1056/nejmoa1709919
3. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. New Eng J Med. 2019;380(1):45–56. https://doi.org/10.1056/nejmao1804980
4. Depil S, Duchateau P, Grupp SA, Multi G, Poirot L. ‘Off-the-shelf’ allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov. 2020;19(3):185–199. https://doi.org/10.1038/s41573-019-0051-2
5. Köhl U, Arsenieva S, Holzinger A, Abken H. CAR T cells in trials: Recent achievements and challenges that remain in the production of modified T cells for clinical applications. Hum Gene Ther. 2018;29(5):559–568. https://doi.org/10.1089/hum.2017.254
6. Jensen MC, Riddell SR. Design and implementation of adoptive therapy with chimeric antigen receptor-modified T cells. Immunol Rev. 2014;257(1):127–144. https://doi.org/10.1111/imr.12139
7. Salmikangas P, Kinsella N, Chamberlain P. Chimeric antigen receptor T-cells (CAR T-cells) for cancer immunotherapy – Moving target for industry? Pharm Res. 2018;35(8):152. https://doi.org/10.1007/s11095-018-2436-z
8. Lin JK, Muffy LS, Spinner MA, Barnes JI, Owens DK, Goldhaber-Fiebert JD. Cost effectiveness of chimeric antigen receptor T-cell therapy in multiply relapsed or refractory adult large B-cell lymphoma. J Clin Oncol. 2019;37(24):2105–2119. https://doi.org/10.1200/jco.18.02079
9. Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med. 2017;9(374): https://doi.org/10.1126/scitranslmed.aaj2013
10. Osborn MJ, Webber BR, Knipping F, Lonetree C, Tnis N, DeFeo AP, et al. Evaluation of TCR Gene Editing Achieved by TALENs, CRISPR/Cas9, and megTAL Nucleases. Mol Ther. 2016;24(3):570–581. https://doi.org/10.1038/mt.2015.197
11. Poirot L, Philipp B, Schiffer-Mannioui C, Le Clère D, Chion-Sotinel I, Derianne S, et al. Multiplex Genome-Edited T-cell Manufacturing Platform for “Off-the-Shelf” Adoptive T-cell Immunotherapies. Cancer Res. 2015;75(18):3853–3864. https://doi.org/10.1158/0008-5472.can-14-3321
12. Liu EL, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. New Eng J Med. 2020;382(6):545–553. https://doi.org/10.1056/nejmoa1910607
13. Zhang C, Wang XQ, Zhang RL, Liu F, Wang Y, Yan ZL, et al. Donor-derived CD19 CAR-T cell therapy of relapse of CD19-positive B-ALL post allotransplant. Leukemia. 2021;35(6):1563–1570. https://doi.org/10.1158/0005-6456.2021-01056-6
14. Smith M, Zakrzewski J, James S, Sadelain M. Posttransplant chimeric antigen receptor therapy. Blood. 2018;131(10):1045-1052. https://doi.org/10.1182/blood-2017-08-752121
15. Jin X, Cao YQ, Wang LQ, Sun R, Cheng L, He XY, et al. HLA-matched and HLA-haploidentical allogeneic CD19-directed chimeric antigen receptor T-cell infusions are feasible in relapsed or refractory B-cell acute lymphoblastic leukemia before hematopoietic stem cell transplantation. Leukemia. 2020;34(3):909. –913. https://doi.org/10.1136/leukemia-2019-006020
16. Mullighan CG, Su XP, Zhang JH, Radtke I, Phillips L.A.A, Miller CB, et al. Deletion of KIF14 and Prognosis in Acute Lymphoblastic Leukemia. N Engl J Med. 2009;360(5):470. –480. https://doi.org/10.1056/nejmoa0808253
17. Moreira LB, P, Queiróz RP, Suazo VK, Perna E, Brandalise SR, Yunes JA, et al. Detection by a simple and cheaper methodology of Ik6 and Ik10 isoforms of the IKZF1 gene is highly associated with a poor prognosis in B-lineage paediatric acute lymphoblastic leukaemia. Br J Haematol. 2019;187(3): https://doi.org/10.1111/bjh.16172
18. Kyoda K, Nakamura S, Matano S, Ohtake S, Matsuda T. Prognostic significance of immunoglobulin heavy chain gene rearrangement in patients with acute myelogenous leukaemia. Leukemia. 1997;11(6):803–806. https://doi.org/10.1038/sj.leu.2400662
19. Neelapu SS, Tummala S, Kebrabai E, Wierda W, Gutierrez C, Locke F, et al. Chimeric antigen receptor T-cell therapy – assessment and management of toxicities. Nat Rev Clin Oncol. 2018;15(1):47–62. https://doi.org/10.1038/nrclinonc.2017.148
20. Lacey SF, Xu J, Ruella M, Barrett DM, Kastdir S, Ambrose DE, et al. Cars in Leukemia: Relapse with Antigen-Negative Leukemia Originating from a Single B Cell Expressing the Leukemia-Targeting CAR. Blood. 2016;128(22):281–281. https://doi.org/10.1182/blood.2018.128.22.281.281
21. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med. 2018;24(5):563–571. https://doi.org/10.1038/s41573-019-0051-2
22. Brudno JN, Somerville RPT, Shi V, Rose JJ, Halverson DC, Fowler DH, et al. Allogeneic T Cells That Express an Anti-CD19 Chimeric Antigen Receptor Induce Remissions of B-Cell Malignancies That Progress After Allogeneic Hematopoietic Stem-Cell Transplantation Without Causing Graft-Versus-Host Disease. J Clin Oncol. 2016;34(10):1112–1121. https://doi.org/10.1200/jco.2015.64.5929

23. Ghosh A, Smith M, James SE, Davila ML, Velardi E, Argyropoulos KV, et al. Donor CD19 CAR T cells exert potent graft-versus-lymphoma activity with diminished graft-versus-host activity. Nat Med. 2017;23(2):242–249. https://doi.org/10.1038/nm.4258

24. Hu YX, Sun J, Wu Z, Yu J, Cui Q, Pu CF, et al. Predominant cerebral cytokine release syndrome in CD19-directed chimeric antigen receptor-modified T cell therapy. J Hematol Oncol. 2016;9(1):https://doi.org/10.1186/s13045-016-0299-5

25. Cai B, Guo M, Wang Y, Zhang YJ, Yang J, Guo YL, et al. Co-infusion of haplo-identical CD19-chimeric antigen receptor T cells and stem cells achieved full donor engraftment in refractory acute lymphoblastic leukemia. J Hematol Oncol. 2016;9(1):https://doi.org/10.1186/s13045-016-0357-z

26. Zhang C, Kong PY, Li SQ, Chen T, Ni X, Li YY, 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(6):306–311. https://doi.org/10.1097/cji.0000000000000233

27. Zhang JP, Zhang R, Tsao ST, Liu YC, Chen XC, Lu DP, et al. Sequential allogeneic and autologous CAR-T-cell therapy to treat an immune-compromised leukemic patient. Blood Adv. 2018;2(14):1691–1695. https://doi.org/10.1182/bloodadvances.2018017004

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Huang Y, Yu Q, Yin ETS, Wei G, Wu W, Chang AH, et al. HLA-matched allogeneic anti-CD19 CAR-T therapy in treating a relapsed/refractory acute lymphoblastic leukemia patient with high tumor burden. ImmunoMedicine. 2022;2:e1032. https://doi.org/10.1002/imed.1032