Durable remission achieved in pediatric patients with *TCF3-HLF*-positive relapsed/refractory B-cell acute lymphoblastic leukemia by dual CD19- and CD22-targeted chimeric antigen receptor T-cell therapy

The chromosomal translocation t(17;19), resulting in the oncogenic fusion transcription factor *TCF3-HLF*,[1] defines a rare cytogenetic subtype of B-cell precursor (BCP) ALL (<1% of pediatric ALL). It is reported to be associated with relapse and death within 2 years from diagnosis, even with the most intensive conventional chemotherapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT).[2,3] Given the strong homogeneous expression of CD19 in *TCF3-HLF*-positive ALL, CD19-directed immunotherapeutic approaches will be under consideration.[4] With the rapid development of chimeric antigen receptor T-cell (CAR T) therapy in recent years, unprecedented success has been achieved in early trials of anti-CD19 CAR T cells for relapsed and/or refractory B-cell malignancies, with many patients obtaining long-term remission and possibly even a cure.[5-7] Of note, CD19-negative relapse is the predominant cause of treatment failure in patients (Pt.) treated with anti-CD19 CAR T cells as a stand-alone therapy, occurring in 25-42% of responding patients.[5,8] Similarly, reduced CD22 antigen density at relapse was observed in a phase I study of anti-CD22 CAR T-cell therapy, suggesting that escape by CD22 downregulation is also possible.[9,10] Therefore, co-administration of CD19 and CD22 CAR T cells was decided in our study. This report precisely focused on long-term analysis of safety and efficacy outcomes in 16 patients with *TCF3-HLF*-positive B-ALL. Clinical characteristics, treatment responses, toxicity and outcomes were analyzed retrospectively. Consequently, 13 of 16 patients (81.25%) are alive. Surprisingly, only one Pt. was observed with CD19-negative relapse after CAR T infusion and durable remissions lasted for more than 24 months in four treated Pt. The 12-month overall survival (OS) and 12-month relapse-free survival (RFS) were 93.33% and 76.58%, respectively. It suggests that CAR T might provide an attractive stand-alone treatment without HSCT for *TCF3-HLF*-positive childhood B-ALL Pt. This ongoing phase II trial (*Chinese clinical trial registry: ChiCTR2000032211*) evaluates bispecific CD19/CD22 CAR T-cell therapy in children with relapsed/refractory B-cell malignancies and was approved by the ethics committee of Shanghai Children's Medical Center (Institutional Review Board number: SCMCIRB-K2016067-1), with updated outcomes recently reported.[11] Written informed consent was obtained from Pt. or their guardians. T cells were transduced with CD19-specific or CD22-specific CAR lentiviral vectors with 4-1BB co-stimulatory and CD3-ζ signaling domains. We combined anti-CD19 CAR T cells with anti-CD22 CAR T cells whose scFv was derived from the FMC63 and the m5/44 clone, respectively. Three Pt. in this cohort were previously reported by our center for early responses;[12] the current sub-study extends follow-up (December 2019 to July 2024) to assess long-term efficacy in *TCF3-HLF*-positive Pt. treated with autologous CAR T cells. Pt. previously treated with anti-CD19 CAR T therapy were excluded from this study (*Online Supplementary Figure S1A*). The median follow-up was 15.45 months (range, 2.1-56.3 months) to a data cutoff of September 1, 2024. The cohort (N=16) had a median age of 10.7 years (range, 3.2-14.9) and received a median of one prior line of therapy (range, 1-3). Three Pt. had prior blinatumomab exposure, two were post-allo-HSCT relapses, and 16 had high-risk disease (6 first relapse, 10 refractory). Pre-lymphodepletion (day -7), five Pt. exhibited measurable residual disease (MRD) >15% and five had extramedullary disease (EMD). All patients received protocol-defined fludarabine/cyclophosphamide conditioning. Autologous CD19/CD22 CAR T cells were infused at a median dose of 8.3×10^6/kg (range, $3.5\text{-}11.4\times10^6$/kg), with seven receiving cryopreserved products. Median CAR transduction efficiencies were 44.2% (CD19; range, 26-67.1%) and 54.7% (CD22; range, 24.3-73%) (Table 1).

The detailed information for each patient is shown in Figure 1. On day 30 after dual-targeted CAR T infusion, all Pt. achieved complete response or complete response with incomplete bone marrow recovery. The rate of complete molecular remission (MRD <0.01% as assessed with flow cytometry) was 100% at 2 months. One Pt. underwent consolidative HSCT while in complete remission, and four Pt. maintained complete remission without any additional treatment at 12 months after infusion and three of them still persisted at 24 months. Four Pt. have relapses with rising MRD (N=3, $CD19^+CD22^+$ relapse; N=1, $CD19^-CD22^+$ relapse). Three Pt. received a second CAR T infusion. Pt.05 received a combined humanized CD19 and CD22 CAR T cells and achieved MRD-negative CR at day 12 after infusion. Pt.04 and Pt.06 received the donor-derived dual CD19/CD22 CAR T product. Both of them achieved MRD-negative complete remission (CR) by day 30 post-re-infusion. Then allogeneic HSCT had been performed in Pt.04 and Pt.06, who maintain CR until now. However, patient Pt.05 died of uncontrollable infections before bridging to HSCT. As of the cut-off date, 14 of 16 Pt. are alive. The RFS rate as the time from CAR T

Table 1. Patient and CAR T products' characteristics.

Patient ID	Sex	Age, years	Genetic variations	Indication	Blinatumomab prior to CAR T	Allogenic HSCT prior to CAR T	Relapse sites	MRD before CAR T infusion, %	Total CAR-T cell dose, E6/kg	Single or split dose	19 CAR$^+$, %	22 CAR$^+$, %	Disease Status on day 30
01	M	9.78	*TCF3-HLF*	R1	N	N	BM, Testis	1	3.48	Single	49.00	67.00	MRD neg
02	M	8.0	*TCF3-HLF*	Refractory	Y	Y	BM	Neg	10.92 #	split dose: day0 and day 16	38.30	35.40	MRD neg
03	M	11.83	*TCF3-HLF, PAX5* exon5 del	Refractory	N	N	BM	28.83	3.62	Single	40.00	41.70	MRD neg
04	M	11.73	*TCF3-HLF, ZEB2* Q1072R	Refractory	N	N	BM, Bone	0.40	3.7	Single	40.00	46.00	MRD neg
05	M	10.35	*TCF3-HLF, NR3C1* G172fs	Refractory	N	N	BM	40.2	3.78	Single	26.00	26.00	MRD neg
06	M	3.18	TCF3-HLF	Refractory	N	N	BM	48.90	7	Single	31.00	26.70	MRD neg
07	M	14.90	*TCF3-HLF, NRAS* G12D	Refractory	N	N	BM	0.38	7	Single	33.60	24.30	MRD neg
08	F	5.59	*TCF3-HLF*	R1	N	N	BM	5.45	8	Single	57.00	73.00	MRD neg
09	F	4.79	*TCF3-HLF, TCF3* L472fs; *NRAS* G13D	R2	N	N	BM, bone, kidney	0.33	8	Single	67.10	71.50	MRD neg
10	M	11.70	*TCF3-HLF, RB1-LINC00462, BTG1*-chr12	R1	N	N	BM, bone	8.81	8.6	Single	51.70	56.70	MRD neg
11	F	11.04	*TCF3-HLF, CDKN2A-chr12, MTAP*-chr9, *NRAS* G12D	Refractory	Y	Y	BM	Blast 78	8.69#	Single	59.00	67.50	MRD neg
12	M	14.12	*TCF3-HLF, PAX5-ZCCHC7, ZCCHC7-PAX5, NR3C1*-chr5, *NRAS* G13V	Refractory	N	N	BM	2.00	9#	Single	42.70	64.40	MRD neg
13	F	5.54	*TCF3-HLF, BCOR* S1223fs	Refractory	N	N	BM	75	9.7#	Single	45.70	52.60	MRD neg
14	F	14.88	*TCF3-HLF,* FLT3-PAN3, *PAX5-IGK*	Refractory	N	N	BM	1.58	10.5	split dose: day 0 and day 4	45.80	60.40	MRD neg
15	M	12.90	*TCF3-HLF*	R1	N	N	BM, bone	6.30	10.93 #	split dose: day 0 and day 43	55.10	61.40	MRD neg
16	F	6.00	*TCF3-HLF*	R1	Y	N	BM	0.82	11.4#	Single	33.70	43.00	MRD neg

CAR: chimeric antigen receptor; M: male; F: female; N: no; Y: yes; #: frozen products; BM: bone marrow; R1: first remission; R2: second remission; MRD neg: measurable residual disease negative.

infusion to morphological relapse or death was 93.33% at 6 months and 76.58% at 12 months for all treated Pt. (*Online Supplementary Figure S1B*). The OS rate was 93.33% at both 6 months and 12 months (*Online Supplementary Figure S1C*). Table 2 summarized the toxicity caused by CAR T cells. Thirteen of 16 Pt. developed CRS at a median of 5 days (range, 2-17 days) after infusions. CRS was relatively mild in most cases, and grade >3 cytokine release syndrome (CRS) was only observed in two Pt. Of 14 Pt. treated with tocilizumab, nine Pt. were combined with corticosteroid. The peak of interleukin (IL)-6 and interferon(IFN)-γ was usually detected around day 7 (*Online Supplementary Figure S2A*). Four Pt. had CAR-related neurotoxicity (Table 2). The copy of CAR T cells in blood always reached peak levels before day 14 after treatment. Within 28 days after infusion, the highest peak copies of CD22 CAR T and CD19 CAR T were observed in Pt.10 on day 6 and Pt.06 on day 3, respectively (*Online Supplementary Figure S2B*). For long-term follow-up, CAR T cells were still detectable by quantitative polymerase chain reaction in Pt.06, Pt.10, Pt.11, Pt.13, and Pt.16's peripheral blood at 6 months post-CAR T-cell infusion. However, CAR T cells in Pt.08 steeply dropped between 1 and 3 months after infusion while patient Pt.4 maintained a low level of CAR T cells both within 14 days and 3 months post-CAR T-cell infusion (*Online Supplementary Figure S2C*). B-cell recovery was detected in three of 12 Pt. at a median time of 89 (range, 44-89) days. B cells remained undetectable in other nine Pt. until the last visit. Pt.14 had persistent B-cell aplasia over 26 months while Pt.06 relapsed after 24 months with CD19-negative disease with detectable CAR T copies and ongoing B-cell aplasia (Table 2). However, this study is limited by incomplete CAR T persistence and cytokines data, primarily due to sample availability and technical constraints. While sensitivity analyses support the validity of our findings, future studies should prioritize standardized longitudinal sampling to minimize such gaps. This might be the first study providing a median follow-up of 15.45 months in 16 pediatric participants with *TCF3-HLF*-positive ALL treated by dual CD19- and CD22-directed immunotherapy, four of whom were followed up more than 30 months. We showed durable remissions lasted for more than 24 months in four of 16 treated Pt. The 6-month RFS and 12-month RFS were 93.33% and 76.58%, respectively. CD19 antigen loss would be regarded as one of the main causes of CD19-directed immunotherapy failure. A European cohort reported that four of nine *TCF3-HLF*-positive ALL Pt. remained in molecular remission treated by blinatumomab as a bridge to HSCT with a median follow-up of 324 days and in one Pt. a CD19-negative relapse was observed.[13] Dual-targeted CAR T was regarded as a promising strategy to reduce this kind of relapse, thus co-administration was chosen by our group. In our

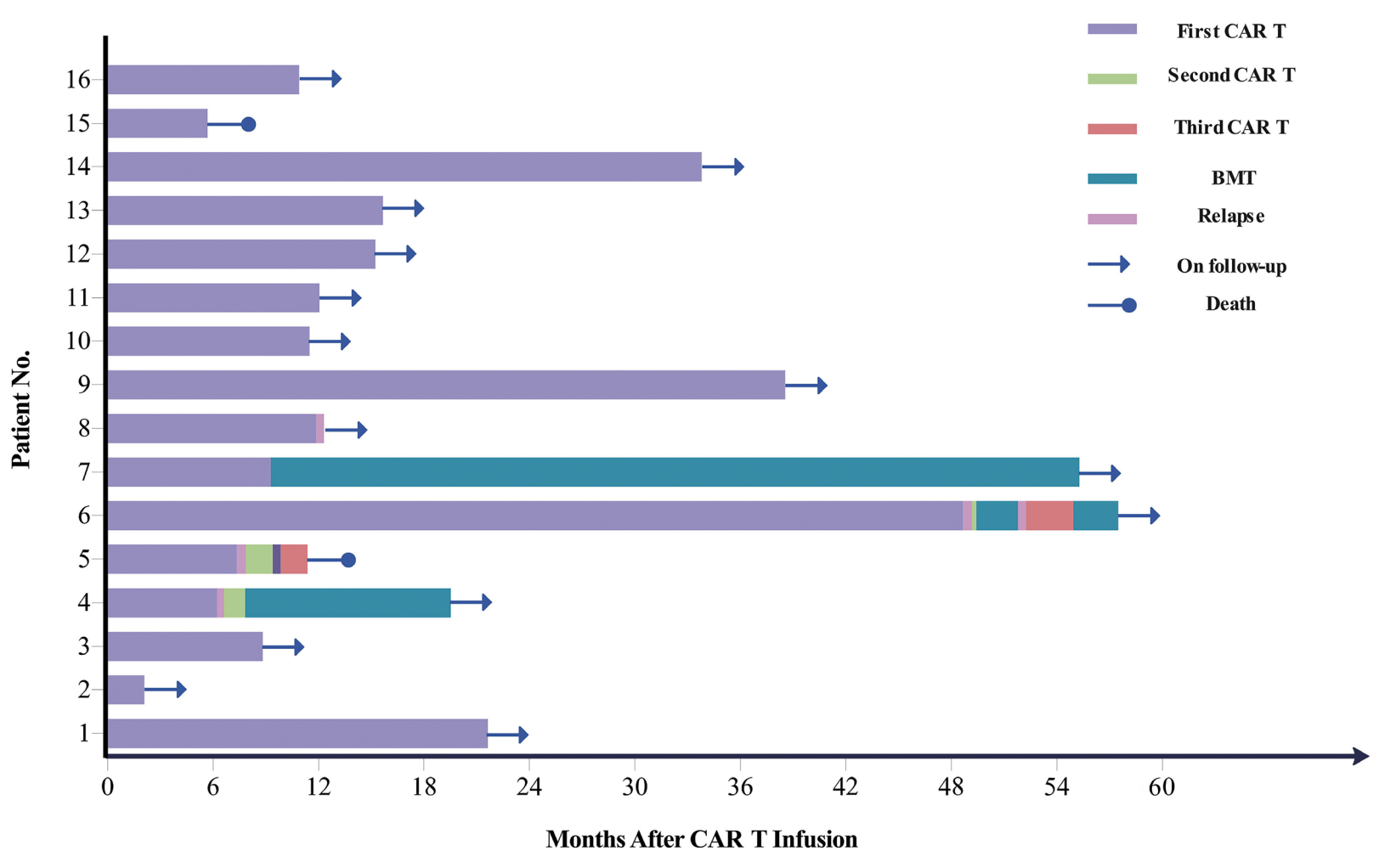

Figure 1. Clinical response and outcomes. Swimmer plot shows the clinical response and follow-up of individual *TCF3-HLF*-positive patients, as indicated with different colors in the swimmer lanes. The length of each bar represents a patient's survival time (months) from the date of chimeric antigen receptor (CAR) T-cell infusion to the date of death or last follow-up. Patient (Pt.) 15 died of epileptic seizure, which was unrelated with CAR T-cell therapy. BMT: bone marrow transplant.

Table 2. CAR T toxicity and clinical outcomes.

Patient ID	CRS grade	Duration of CRS, days	ICANS grade	Duration of ICANS, days	Received toxilizumab	Received corticosteroid	B-cell aplasia until last FU, days	Final status
01	3	5	2	1	Y	Y	189	Alive with CCR
02	3	10	0	0	N	N	88	Alive with CCR
03	3	6	0	/	Y	Y	59	Alive with CCR
04	0	/	0	/	N	N	44	Alive after 2nd CAR T
05	3	N/A	0	/	Y	Y	NA	Death
06	3	4	2	/	Y	Y	587	Alive after 3rd CAR T
07	2	3	0	/	Y	N	N/A	Alive with CCR
08	3	2	0	/	Y	N	89	Alive with Relapse after CAR T
09	3	7	0	/	Y	Y	N/A	Alive with CCR
10	4	4	0	/	Y	Y	187	Alive with CCR
11	3	7	0	/	Y	N	181	Alive with CCR
12	1	/	0	/	Y	N	N/A	Alive with CCR
13	2	17	0	/	Y	Y	326	Alive with CCR
14	0	/	0	/	N	N	793	Alive with CCR
15	4	4	3	N/A	Y	Y	N/A	Death
16	1	10	0	/	Y	N	188	Alive with CCR

Cytokine release syndrome (CRS) grade 1, N=1 patient; CRS grade 2-3, N=10; immune effector cell-associated neurotoxicity syndrome (ICANS) grade 2, N=2 patients; ICANS grade 3-4, N=2. ICANS: CAR: chimeric antigen receptor; CR: complete remission; CCR: continuous CR; FU: follow-up; NA: not available; Y: yes; N: no; /: none.

cohort of Pt. enrolled with *TCF3-HLF*-positive r/r B-ALL, only Pt.06 was observed with CD19-negative relapse but CD22 positivity was conserved over 43 months after CAR T-cell infusion. Moreover, Pt.04, Pt.05, and Pt.06 received a second CAR T therapy and achieved CR again, as well as Pt.04 and Pt.06 who underwent HSCT and kept the status of CR. No cases of dual CD19/CD22-negative relapse were observed, and we found the peak expansion of CD22-CAR T cells was higher than that of CD19-CAR T cells (peaked at median: 825.74 *vs.* 495.78 CAR T copies/ng of genome DNA) in eight Pt. tested. This may support the hypothesis that dual targeting mitigates antigen loss.

The better efficacy in our cohort may refer to the high transduction efficiency and high dose of CAR T cells infused, which can result in longer CAR T-cell persistence *in vivo*. The median transduction efficiency of $CD19^-$ and $CD22^-$ CAR T was 44.2 % and 54.65%. In five Pt. with detectable CAR T copies at 6 months, all received over $7x10^6$/kg CAR T cells, and Pt.16 was infused with a single dose of $11.4x10^6$/kg CAR T cells without dose-limiting toxicity. B-cell aplasia was still not recovered after 26 months in Pt.14, who received $10.5x10^6$/kg CAR-T cells. In a phase I trial, 15 Pt. accepted AUTO3 CAR T cells transduced by a bicistronic retroviral vector encoding humanized anti-CD19 and CD22 CAR. The 1-year event-free survival was reported to be 32% and the median transduction efficiency of this product was reduced by 18%, which correlated with its shorter persistence.[14] Anti-CD19 CAR T therapy bridging to allo-HSCT has been announced to prolong the event-free survival and OS of r/r B-ALL in several clinical trials,[15,16] but the risk of graft-*versus*-host disease (GVHD) remains underappreciated. The occurrence of CAR T-related GVHD was found in 23% of B-ALL Pt. who experienced relapse after allo-HSCT and were subsequently treated with sequential CD19 and CD22 CAR T cells.[17] In our cohort, CAR T-cell therapy seems to be a stand-alone treatment without SCT for childhood B-ALL patients with *TCF3-HLF*-positive gene. Ten of 16 Pt. maintain ongoing complete remission without HSCT or additional treatments. Notably, three of these Pt. have sustained this remission status for over 2 years. Thus, for Pt. with high-risk genetic features (e.g., *TCF3-HLF*), first-line CAR T or bispecific antibodies could be studied in parallel with reduced-intensity chemotherapy to minimize cumulative toxicity while preserving efficacy.

In conclusion, our study presented durable efficacy of dual CD19 and CD22-targeted CAR T cells in a subset of 16 Pt. with *TCF3-HLF*. An impressive survival rate and CAR T-cell persistence were achieved in our study with a long-term follow-up. In order to decide whether this strategy can be stand-alone for *TCF3-HLF*-positive Pt., our phase II trial of dual targeting CD19 and CD22 CAR T therapy will be ongoing, which may provide additional supportive clues.

Authors

Xinyu Wan,[*] Tianyi Wang,[*] Jing Yang,[*] Xiaomin Yang, Wenjie Li, Lixia Ding, Liu Yang, Jing Zhang, Meng Su, Kang An, Zichao Zhou, Yue Li, Xiang Wang, Hua Zhu, Chengjuan Luo, Longjun Gu, Jing Chen, Lili Song,[#] Yanjing Tang[#] and Benshang Li[#]

Department of Hematology and Oncology, Key laboratory of Pediatric Hematology and Oncology Ministry of Health, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[*]XWan, TW and JY contributed equally as first authors.
[#]LS, YT and BL contributed equally as senior authors.

Correspondence:
B. LI - libenshang@scmc.com.cn

https://doi.org/10.3324/haematol.2024.287023

Received: December 2, 2024.
Accepted: May 9, 2025.
Early view: May 22, 2025.

Disclosures

No conflicts of interest to disclose.

Contributions

LS, YT and BL conceived and designed the analysis. XWan, TW, JY, XY and WL collected the data. LD, LY, JZ, MS, KA, ZZ, YL, XWang, HZ, CL, LG and JC provided administrative, technical or material support. XWan and TW performed the analysis. XWan, TW and JY wrote the paper. All authors reviewed the manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (81670174 to BL) and the National Outstanding Youth Science Fund Project of National Natural Science Foundation of China (81900158 to LS and 82402624 to TW).

Data-sharing statement

The datasets of this article are available from the corresponding author on reasonable request.

References

1. Inaba T, Roberts WM, Shapiro LH, et al. Fusion of the leucine zipper gene HLF to the E2A gene in human acute B-lineage leukemia. Science. 1992;257(5069):531-534.
2. Fischer U, Forster M, Rinaldi A, et al. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options. Nat Genet. 2015;47(9):1020-1029.
3. Inukai T, Hirose K, Inaba T, et al. Hypercalcemia in childhood acute lymphoblastic leukemia: frequent implication of parathyroid hormone-related peptide and E2A-HLF from translocation 17;19. Leukemia. 2007;21(2):288-296.
4. Velasquez MP, Bonifant CL, Gottschalk S. Redirecting T cells to hematological malignancies with bispecific antibodies. Blood. 2018;131(1):30-38.
5. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439-448.
6. Park JH, Riviere I, Gonen M, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med. 2018;378(5):449-459.
7. Gardner RA, Finney O, Annesley C, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood. 2017;129(25):3322-3331.
8. Ghorashian S, Kramer AM, Onuoha S, et al. Enhanced CAR T cell expansion and prolonged persistence in pediatric patients with ALL treated with a low-affinity CD19 CAR. Nat Med. 2019;25(9):1408-1414.
9. Shah NN, Highfill SL, Shalabi H, et al. CD4/CD8 T-cell selection affects chimeric antigen receptor (CAR) T-cell potency and toxicity: updated results from a phase I anti-CD22 CAR T-cell trial. J Clin Oncol. 2020;38(17):1938-1950.
10. Fry TJ, Shah NN, Orentas RJ, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat Med. 2018;24(1):20-28.
11. Wang T, Tang Y, Cai J, et al. Coadministration of CD19- and CD22-directed chimeric antigen receptor T-cell therapy in childhood B-cell acute lymphoblastic leukemia: a single-arm, multicenter, phase II trial. J Clin Oncol. 2023;41(9):1670-1683.
12. Wang T, Wan X, Yang F, et al. Successful treatment of TCF3-HLF-positive childhood B-ALL with chimeric antigen receptor T-cell therapy. Clin Lymphoma Myeloma Leuk. 2021;21(6):386-392.
13. Mouttet B, Vinti L, Ancliff P, et al. Durable remissions in TCF3-HLF positive acute lymphoblastic leukemia with blinatumomab and stem cell transplantation. Haematologica. 2019;104(6):e244-e247.
14. Cordoba S, Onuoha S, Thomas S, et al. CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial. Nat Med. 2021;27(10):1797-1805.
15. Jiang H, Li C, Yin P, et al. Anti-CD19 chimeric antigen receptor-modified T-cell therapy bridging to allogeneic hematopoietic stem cell transplantation for relapsed/refractory B-cell acute lymphoblastic leukemia: an open-label pragmatic clinical trial. Am J Hematol. 2019;94(10):1113-1122.
16. Chen W, Ma Y, Shen Z, et al. Humanized anti-CD19 CAR-T cell therapy and sequential allogeneic hematopoietic stem cell transplantation achieved long-term survival in refractory and relapsed B lymphocytic leukemia: a retrospective study of CAR-T cell therapy. Front Immunol. 2021;12:755549.
17. Liu S, Deng B, Yin Z, et al. Combination of CD19 and CD22 CAR-T cell therapy in relapsed B-cell acute lymphoblastic leukemia after allogeneic transplantation. Am J Hematol. 2021;96(6):671-679.