Five-year outcome of CD19 followed by CD22 chimeric antigen receptor T-cell therapy in B-cell acute lymphoblastic leukemia patients who relapsed after allo-transplantation

Before the era of chimeric antigen receptor (CAR) T-cell therapy, patients with acute lymphoblastic leukemia (ALL) who relapsed after allogeneic hematopoietic cell transplantation (allo-HCT) had a very poor prognosis. The overall survival (OS) rate was shown to be 15% at 3 years in children,[1] and 8±1% at 5 years in adults.[2]

Previously, we reported a phase I clinical trial (*Chinese Clinical Trial Registry/WHO International Clinical Trial Registry: ChiCTR-ONC-17013648*) concerning the sequential treatment of CD19 followed by CD22 CAR T cells for post-HCT relapsed B-cell lymphoblastic leukemia.[3] In the trial with both adults and children, 23 of 27 patients (85%) obtained complete remission (CR) after first CD19 CAR T-cell treatment; subsequently, 21 cases undertook a second CD22 CAR T-cell therapy, for whom the event-free survival (EFS) and OS rates at 18 months were 67.5% and 88.5%, respectively. Here, the 5-year long-term outcomes were followed. Apart from the 27 reported patients with hematologic relapse and/or extramedullary diseases (EMD), additional three cases, who were not eligible for the trial (only with minimal residual disease [MRD]) but who also received CD19/CD22 CAR T cells during the same time period and following the same protocol, were included in this long-term observation.

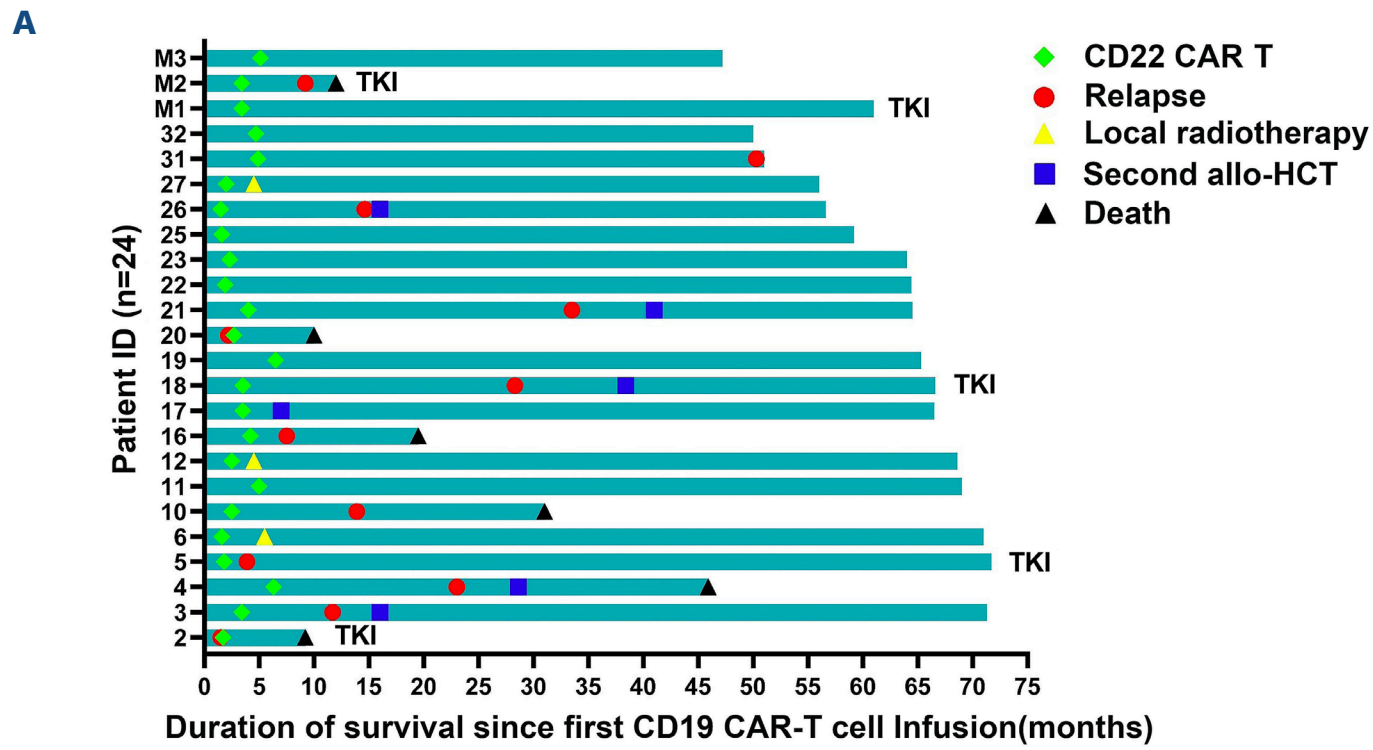
The expression of CD19/CD22 antigen on lymphoblasts was detected using multiparameter flow cytometry (FCM), all involved patients were confirmed to have both CD19 and CD22 expression before cell treatment. A total of 30 adult and pediatric B-ALL patients who relapsed after allo-HCT received a first CD19 CAR T-cell therapy, and 24 of them received a second CD22 CAR T-cell therapy, with intervals of 1.5–6.5 months between the two cell infusions. Patient-derived donor lymphocytes and lentiviral vectors encoding second generation CAR composed of CD3ζ and 4-1BB were used for manufacturing of CAR T cells. The details of the CAR T-cell treatment protocol and disease evaluation are described in our previous work.[3] The first CD19 CAR T-cell infusions were performed during the period from December 2017 to October 2019, the last follow-up visit was December 31, 2023.

SAS version 9.4 and GraphPad Prism 7 were used for statistical analyses. The probabilities of OS and EFS were estimated using the Kaplan-Meier method. The time-to-event analysis for each patient was calculated from the first CD19 CAR T-cell infusion (the EFS of 3 cases who achieved complete remission after second CD22 CAR T-cell therapy was calculated from the second cell infusion) to the date of last follow-up, relapse or death. The risk factors associated with EFS were evaluated using univariate Cox regression analysis.

This study was approved by the institutional review board of Beijing Boren Hospital (*Chinese Clinical Trial Registry/WHO International Clinical Trial Registry: ChiCTR-ONC-17013648*), written informed consents were obtained from all patients including three cases with MRD relapse.

The baseline patient characteristics are summarized in *Online Supplementary Tables S1, S2*. The study cohort included 20 adults (67%) and ten children (33%) aged <18 years, with a median age of 20.5 (range, 1.6-55) years. Most patients (70%, 21/30) relapsed with higher disease burden (blasts in bone marrow/blood ≥20% or with EMD), 73% (22/30) of the patients underwent HLA-haploidentical transplantation, and four cases presented with mild chronic graft-*versus*-host disease (GvHD).

Three patients with MRD relapse became MRD-negative after the first CD19 CAR T-cell treatment, and received the following CD22 CAR T cells. In 24 patients administered with both CD19 and CD22 CAR T-cell therapies, over a median follow-up period of 64.4 (95% confidence interval [CI]: 50.6-68.6) months, 12 patients stayed in continuous complete remission after the first CD19 CAR T-cell infusion (N=11) or second CD22 CAR T-cell therapy (N=1, patient 27); the other 12 patients relapsed, half of the relapses appeared within 12 months of first T-cell infusion, and the late relapses (>2 years) occurred in three cases (25%, 3/12) at 28.3, 33.5 and 50.3 months, respectively (Figure 1A). In 11 patients with FCM data (1 relapsed only with *BCR/ABL*$^{+}$), all showed CD19/CD22 expression except for two with CD19 negativity (18.2%, 2/11) and one with CD22 partial expression. Of 12 relapsed patients, six remained alive after receiving other treatments, and six died from disease progression. Among seven patients who did not undergo second allo-HCT, five died within 2.7-19.6 months after relapse, whereas five patients who proceeded to second allo-HCT survived for additional 22.9-59.6 months after relapse. Before the second allo-HCT, four of five patients were in CR and one was with EMD (who relapsed again and died) after treatment with salvage therapies including the reinfusion of CAR T cells, blinatumomab or inotuzumab ozogamicin and chemotherapy. The 3-year EFS and OS estimated by Kaplan–Meier methods were 54% (95% CI: 33-71) and 79% (95% CI: 57-91), respectively, while the 5-year EFS and OS were 50% (95% CI. 29-68) and 75% (95% CI: 53-88), respectively (Figure 2A, B).



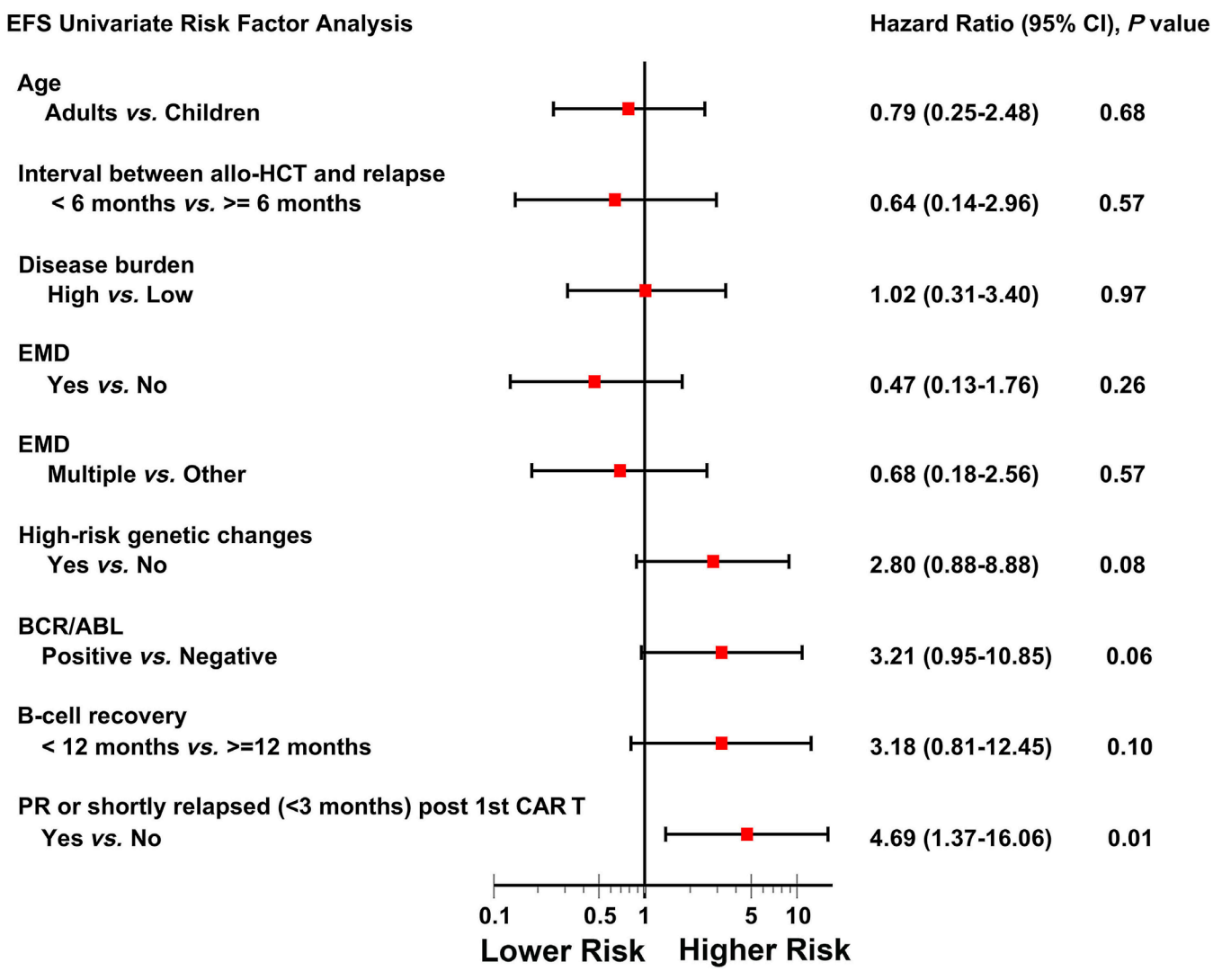


Figure 1. The follow-up information in 24 patients treated with CD19/ CD22 CAR T-cell therapy. (A) Survival duration of each patient (Pt.), calculated from the first CD19 chimeric antigen receptor (CAR) T-cell infusion. M1-M3 were 3 patients only with minimal residual disease. After first CD19 CAR T-cell therapy, 3 cases (Pt. 10, 16 and 27) with multifocal extramedullary disease (EMD) obtained partial remission (their EMD had not been completely eliminated) and all of them achieved complete remission after second CD22 CAR T-cell therapy. Among 11 relapsed cases with flow cytometry data (Pt. 5 relapsed only with *BCR/ABL*$^+$), 2 (Pt. 3 and 20) showed CD19 negativity and others were CD19-positive; all patients showed normal CD22 expression (>80% of positive blasts) except for 1 (Pt. 20) with partial expression (20-80% of positive blasts). After CD22 CAR T-cell treatment, 5 cases with *BCR-ABL* transcript had taken tyrosine kinase inhibitors (TKI); 3 female patients with EMD in the breast received local irradiation; and 1 case (Pt. 17) underwent a second allogeneic hematopoietic cell transplantation (allo-HCT) for further consolidation in remission status. (B) The univariate Cox regression analysis of risk factors for event-free survival (EFS). To make analysis more credible, each group included at least 5 cases (no data of B-cell recovery <6 months here because there were only 3 cases in this sub-group). PR: partial remission.

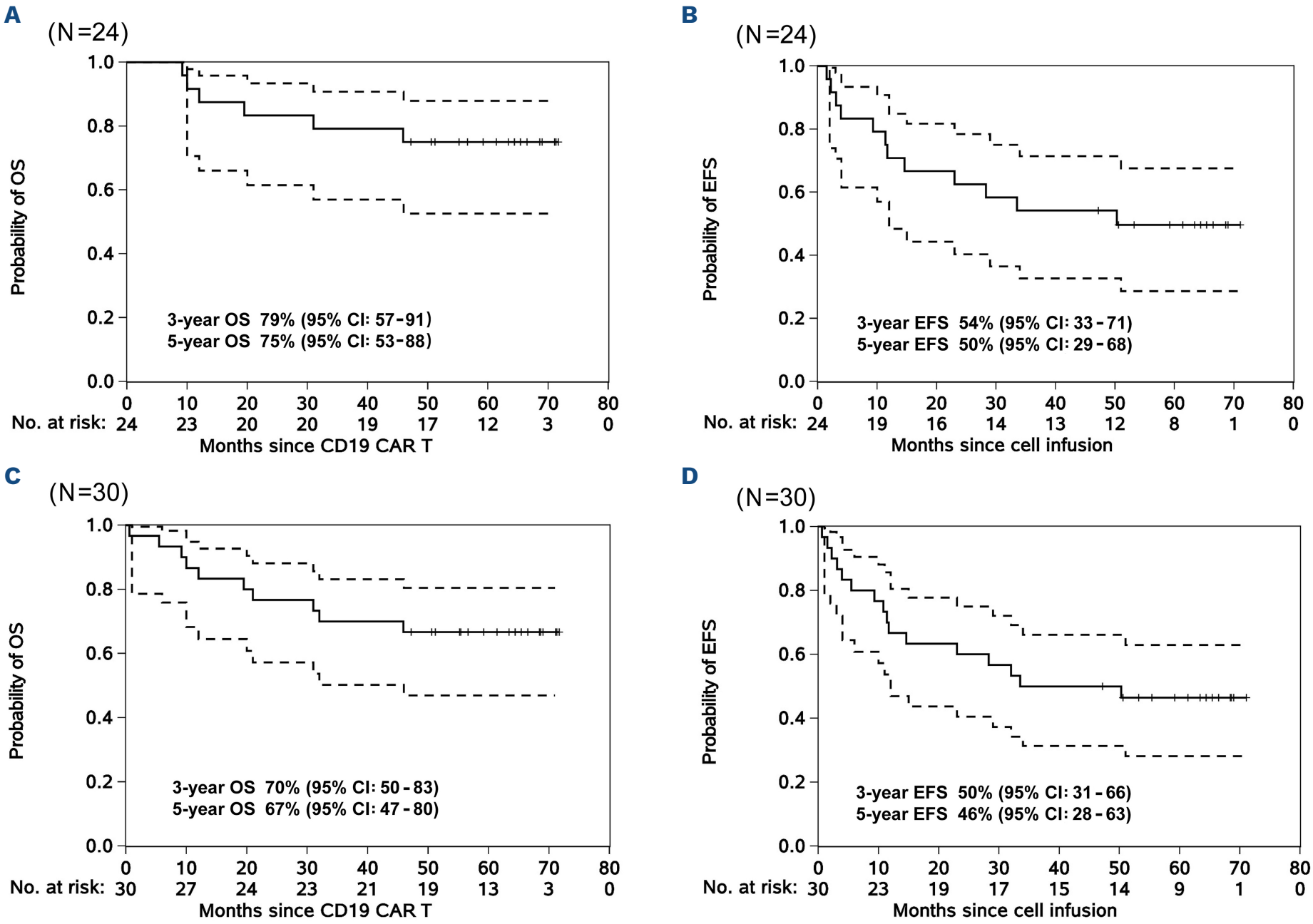


Figure 2. The long-term survival estimated by Kaplan-Meier curve. (A) Overall survival (OS) and (B) event-free survival (EFS) among the patients treated with CD19/CD22 chimeric antigen receptor (CAR) T cells (N=24). (C) OS and (D) EFS among the intention-to-treat population (N=30) that includes 6 patients who did not receive the second CD22 CAR T-cell therapy.[3] CI: confidence interval.

The risk factor analysis for EFS based on univariate Cox regression revealed that patients who obtained partial remission (PR, whose EMD had not been completely eliminated) or shortly relapsed (<3 months) after their first CD19 CAR T-cell therapy had a significantly worse prognosis (hazard ratio [HR] =4.69, 95% CI: 1.37-16.06; *P*=0.01). Patients with high-risk cytogenetic changes (*BCR-ABL* or *MLL-AF4* fusion gene/complex karyotype) and those with B-cell recovery of less than 1 year displayed a trend towards worse EFS (HR=2.80, 95% CI: 0.88-8.88; *P*=0.08; and HR=3.18, 95% CI: 0.81-12.45; *P*=0.10). Age (children or adults), time interval between the first allo-HCT and relapse (<6 months or ≥6 months), disease burden (higher or low), and EMD (with or without EMD) were not associated with EFS. In our previous follow-up at 18 months, the multifocal EMD was related to poor prognosis; however, the later relapses of four patients without EMD alleviated this impact, and the multifocal EMD was no longer associated with long-term EFS (HR=0.68, 95% CI: 0.18-2.56; *P*=0.57) (Figure 1B).

These data showed that being an adult, early relapse after first allo-HCT, and a higher disease burden before CAR T-cell therapy did not influence treatment outcomes. Nevertheless, patients who achieved PR or relapsed shortly after first CD19 CAR T-cell therapy had a significantly worse EFS probability, therefore, other treatments following CD22 CAR T-cell therapy (such as second allo-HCT) should be considered to prolong remission.

Among the six patients who did not receive CD22 CAR T cells, four died and two remained alive (*Online Supplementary Table S3*). In the intention-to-treat population with 30 patients including these six, the EFS and OS probabilities were shown to be 50% (95% CI: 31-66) and 70% (95% CI 50-83) at 3 years, and 46% (95% CI: 28-63) and 67% (95% CI: 47-80) at 5 years (Figure 2C, D), respectively.

Cytokine release syndrome and neurotoxicity have been described in the previous report of ours.[3] Late adverse effects 1 month post-T-cell infusion were observed as well. After each cell infusion, the CR/PR patients who presented with incomplete blood cell count at the 1-month evaluation achieved a recovery of cell count at a median

of 2 (range, 1.3-6.7) months. Thirteen severe infections (greater than or equal to grade 3, graded according to CTCAE version 5.0) within 2 years were found in 11 patients, including 11 pneumonia (10 grade 3 and 1 grade 4) and two skin infections (grade 3) caused by varicella-zoster virus; most of these infections (84.6%, 11/13) occurred within a year of T-cell infusion (*Online Supplementary Table S3*). In the context of intermittent intravenous infusion of immunoglobulin for patients with immunoglobulin (Ig) G <5.0 g/L (they usually could not strictly follow our advice to receive regular Ig infusion once a month), eight of 13 severe infections occurred in patients with low Ig levels.

In this cohort of patients with more than 70% of HLA-haploidentical transplantation from family members, six cases experienced CAR T-cell-related GvHD (4 with and 2 without pre-existing chronic GvHD [cGvHD] prior to T-cell infusion[3]). Three patients recovered, and three persisted until disease recurrence (N=1) or death (N=2). One patient with pre-existing cGvHD died from extensive GvHD at 5.5 months post CD19 CAR T-cell therapy. Another patient without pre-existing cGvHD presented with skin GvHD from 2 months onward, and developed lung GvHD at 9 months. He had recurrent pulmonary infections and finally died of acute respiratory failure associated with lung GvHD and infection at 32 months (both had not received the second CD22 CAR T-cell therapy owing to unsolved GvHD).

No secondary T-cell leukemia or malignancy was found in these patients.

Although CD19-directed immunotherapies including blinatumomab and CAR T-cell therpay have achieved higher CR rates (blinatumomab, 44-63%;[4-5] CD19 CAR T cells, 81-90%[6-8]) in relapsed/refractory B-ALL, many patients relapse again and cannot maintain a long-lasting remission. The reported EFS rates at 6 months were 31%[4] in patients treated with blinatumomab and 50-73%[6-8] in those treated with CD19 CAR T cells. The 1-year OS probability among patients without following allo-HCT was 29% after blinatumomab.[5] Regarding the long-term survival after CD19 CAR T-cell therapy, recent investigations showed an EFS of 44% and OS of 63% at 3 years[9] in young adult and pediatric patients, and an EFS of <30% and OS of <60% at 2 years in adults.[10]

Here, our study revealed that, in post-HCT relapsed B-ALL patients, the combination of CD19 and CD22 CAR T-cell therapy significantly improved long-term survival, and half of the patients could be cured. More importantly, among these patients, most were adults (63%, 15/24), and only one received a second allo-HCT for further consolidation during remission.

Of the 12 patients who relapsed post CAR T cells, five underwent a second allo-HCT and survived for additional 22.9-59.6 months after relapse, whereas five of the seven patients who did not undergo a second transplantation died 2.7-19.6 months after relapse. This finding indicates that patients who relapse post-CD19/CD22 CAR T-cell therapy can still benefit from a second allo-HCT, which is therefore recommended as a priority treatment for these patients.

GvHD has emerged as an adverse effect in post-HCT patients after treatment with CAR T cells.[11,12] Our data provided evidence that more and severe CAR T-cell-associated GvHD could occur in the patients with prior haploidentical transplantation and in those with pre-existing cGvHD, which requires more attention from clinicians.

In conclusion, among B-ALL patients relapsed after allo-HCT, CD19 followed by CD22 CAR T-cell therapy resulted in an OS of 75% and an EFS of 50% at 5 years, significantly improved long-term survival and could be a curative approach for these patients with extremely poor prognosis.

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Disclosures

AHC is a founding member of Shanghai YaKe Biotechnology Ltd., a biotechnology company focusing on research and development of tumor cellular immunotherapy. The remaining authors have no conflicts of interest to disclose.

Contributions

SL analyzed data and wrote the manuscript. LA and ZY collected data and made the tables. SL, LA, ZY, YL, ZL, DZ and TW treated patients. BD conducted CAR T-cell manufacturing. XY and QZ performed flow cytometry and molecular analysis. AHC provided CD-19 and CD-22 CAR. CT and SL designed and guided the study.

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Data-sharing statement

For detailed original data, please contact Dr. Lihong An at anlh@gobroadhealthcare.com or Dr. Shuangyou Liu at liusy@gobroadhealthcare.com.

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