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CD19/CD22 bispecific CAR-T cells for MRD-positive adult B cell acute lymphoblastic leukemia: a phase I clinical study

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Dear Editor,

Measurable residual disease (MRD) is now considered as one of the most important prognostic factors for B cell acute lymphoblastic leukemia (B-ALL), even for patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1, 2]. Early achievement of MRD negative is imperative, as many studies consistently demonstrated that the patients with a negative MRD during the early time of treatment, especially after induction, had a superior survival [3]. Chimeric antigen receptor T (CAR-T) cells targeting CD19 or CD22 has been demonstrated as the most effective salvage therapy for refractory/relapsed B-ALL [4, 5]. Considering the facts that a lower disease burden was associated with a higher safety profile and dual targeting of CD19/CD22 might lower relapse risk, we designed a phase I study to evaluate the safety and efficacy of CD19/CD22 bispecific targeted CAR-T cells for MRD-positive adult B-ALL patients, especially for primary patients with MRD persistence after early consolidation therapy.

Adult MRD positive B-ALL patients were enrolled into the clinical trial (NCT: 03919526). Primary patients with MRD persistence after induction and at least 2 courses of consolidation therapy were defined as first-line consolidation group; while recurrent patients with MRD-positive complete remission (CR) or CR with incomplete hematological recovery (CRi) after salvage therapy or patients with MRD relapse were defined as relapsed group. The details of inclusion/exclusion criteria were shown in Supplementary Table 1.

The study was an open, phase I clinical trial with a sample size of 16–18. The primary objective was to assess the safety of CD19/CD22 bispecific CAR-T cells for MRD positive B-ALL, and the secondary was to evaluate its efficacy. We performed traditional $1+1+3+3$ dose escalation to determine the optimal single dose of CAR-T cells, which corresponded to four dose levels of 1×10^6 cells/kg, 2×10^6 cells/kg, 3×10^6 cells/kg, and 5×10^6 cells/kg, respectively. The study design was summarized in supplementary materials,methods, and Supplementary Fig. 1.

Fifteen out of 19 patients completed CAR-T cell infusion from March 2019 to May 2022. Four did not due to disease progression (2/4) and complete MRD response to graft versus host disease (2/4). The baseline characteristics of the 15 patients were summarized in Table 1 and Supplementary Table 2. Transduction efficiency and infused doses of CAR-T cells were shown in the Supplementary table 3. The adverse events (AEs) within 28 days after infusion were shown in Supplementary table 4. All the 15 patients experienced grade 3 or higher AEs and the most common were cytopenia. A total of 4 patients (26.7%, 4/15) developed grade 1 or 2 cytokine-release syndrome (CRS) and all were in 5×10^6/kg CAR-T cell dose group. Two patients (NO. 007 and NO. 015) with grade 2 CRS were treated with tocilizumab and recovered quickly. The median time of CRS onset was 2 (range, 1–8) days after infusion, and the median duration was 1 (range, 1–3) day. One patient (No.009) developed delayed- neurotoxicity on day 230 after CAR-T infusion and recovered quickly with prednisone (15 mg/d) (Details were described in supplementary materials).

As shown in Fig. 1, the median follow-up time was 15.5 months (range, 2.5–33). Five patients achieved MRD-negative CR before infusion (after lymphodepletion). The overall MRD response rate was 100% at day 28 with 93.9% (14/15) MRD-negative CR and 6.7% (1/15) MRD-negative CRi. Eleven remained MRD-negative until the end of the follow-up. Five patients experienced morphological or MRD relapse (3 in the first-line consolidation group and 2 in the relapsed group) at follow-up (Details in supplementary materials).

For total patients, the median relapse free survival (RFS) and overall survival (OS) were not reached (Supplemental Fig. 2A, B). The 12-month RFS and OS were 77% (95% CI, 55–99) and 86% (95% CI, 68–104), respectively, while the 24-month estimated RFS and OS were the same as those of the 12-month. Median RFS and OS were similar between patients with/without subsequent transplantation after CAR-T therapy ($P=0.735$ for RFS, $P=0.671$ for OS) (Supplementary Fig. 2A–B) and patients in the first-line consolidation /relapsed groups ($P=0.803$ for RFS, $P=0.369$ for OS) (Supplementary Fig. 3). Neither MRD status before CAR-T cells nor Ph-positive/negative affected the survival (Supplementary Fig. 4A–D).

For the 11 patients in the first-line consolidation group, the median RFS and OS were not reached (Supplementary Fig. 2C, D), the 12-month RFS and OS were 77.8% (95% CI, 51–105) and 80.8% (95% CI, 57–105). Except for one patient with subsequent transplantation after CAR-T cells, the 10 patients had a 12-month RFS of 77.8% (95% CI, 51–105) and OS of 88.9% (95% CI, 68–109). The 24-month estimated RFS and OS for all patients and patients except one with subsequent transplantation were the same as those of 12 months. Five out of the 11 patients had a RFS of more than 18 months (Fig. 1). Stratified survival analysis showed no significant differences in RFS and OS between patients with Ph-positive/negative B-ALL ($P=0.432$ for RFS, $P=0.417$ for OS), and different MRD status before infusion ($P=0.379$ for RFS, $P=0.593$ for OS) (Supplementary Fig. 5A–D).

Peripheral blood CAR T-cell expansion was observed in all 15 patients, with a median time to reach the peak CAR-T cell concentration (Cmax) of 10 days (range, 7–14). Cmax and AUC_{0-28} of CAR T-cell expansion were higher in patients with sustained remission than that with relapse ($P=0.048$ for Cmax and $P=0.018$ for AUC_{0-28d}, respectively). CAR-T cell persistence in peripheral blood with >100 copies/μg DNA lasted for more than 60 and 90 days in 7 and 3 patients, respectively and decreased significantly in the rest patients within 28 days after infusion. The details of pharmacokinetics and pharmacodynamics were shown in supplementary materials and Supplementary Fig. 6A, B.

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Table 1. Patient characteristics.

Baseline characteristics	All patients evaluable ($N = 15$)	First-line consolidation ($N = 11$)	Relapsed group ($N = 4$)	*P*
Median age, years (range)	51 (23–70)	45 (23–70)	54.5 (31–61)	0.447
Age ≥ 35 years, *n* (%)	10(66.7%)	7(63.6%)	3(75%)	0.68
Male, *n* (%)	6 (40%)	5 (45.5%)	1 (25%)	0.348
ECOG performance status score of 0–1, *n* (%)	15 (100%)	11 (100%)	4 (100%)	>0.99
Median time since diagnosis, months (range)	6.5 (1–41.5)	6.5 (3–18)	7 (1–41.5)	0.851
Median Cycle Number of chemotherapy, *n* (range)	4(3–8)	4(3–5)	3.5 (3–8)	0.949
Ph-positive	7(46.7%)	3(27.3%)	4(100%)	0.026
Disease burden				
Before lymphodepletion				
MRD ≥ 10^{-2}	2(13.3%)	1(9.1%)	1(25%)	0.4
MRD ≥ 10^{-3}–<10^{-2}	5(33.3%)	3(27.3%)	2(50%)	
MRD ≥ 10^{-4}–<10^{-3}	8(53.3%)	7(63.6%)	1(25%)	
Before infusion				
MRD ≥ 10^{-2}	3(21.4%)	1(9.1%)	2(50%)	0.183
MRD ≥ 10^{-3}–<10^{-2}	5(35.7%)	5(45.5%)	0	
MRD ≥ 10^{-4}–<10^{-3}	1(7.1%)	1(9.1%)	0	
MRD Negative	5(35.7%)	3(27.3%)	2(50%)	
Follow-up time	15.5(2.5–33)	15.5(2.5–33)	15.25(10.5–20.5)	0.949

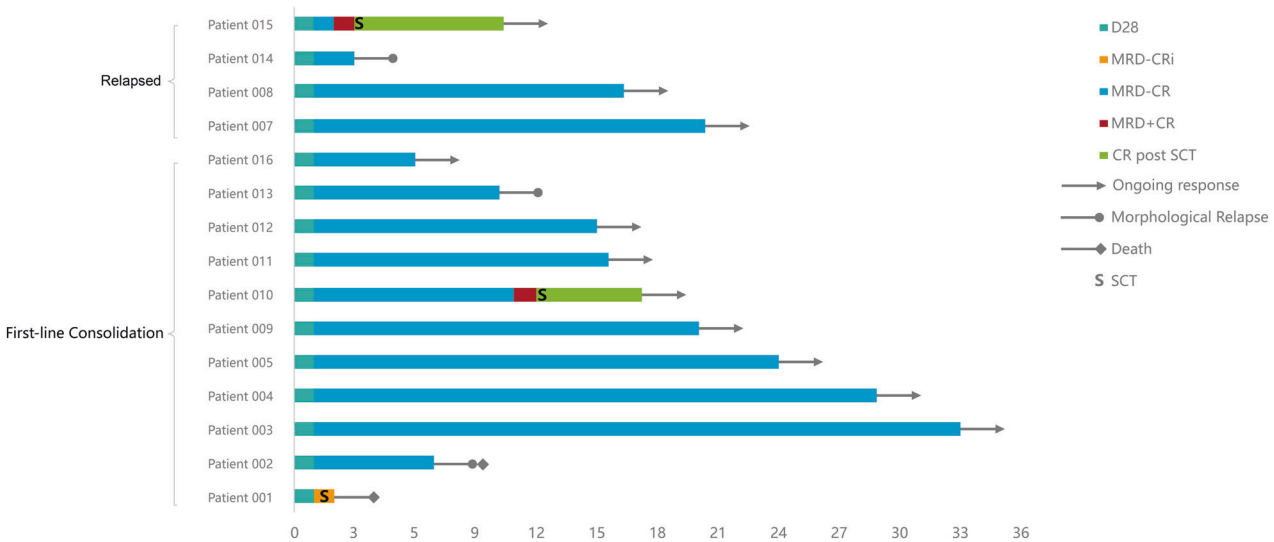

Fig. 1 Clinical outcomes of patients with MRD-positive B-ALL after CD19/CD22 bispecific CAR-T cells. The bar chart shows the clinical response and follow-up of patients during CAR-T therapy. Each bar represents an individual patient and the study number. The different colors represent different disease status.

Cytokines were routinely detected after CAR-T cell infusion (Supplementary Table 5). The elevated levels of cytokines (CRP, IL-6, etc.) had no significant differences between patients with and without CRS, as well as between patients with relapse and sustained remission ($P > 0.05$) (Supplementary Fig. 7A–F). The lymphocyte subtype numbers of all patients at different times pre- and post-infusion were shown in Supplementary Fig. 8 and Supplementary Table 6.

In the present study, only 26.7% of patients developed CRS without severe CRS and early neurotoxic effects, although a high dose of CD19/22 CAR-T cells of 5×10^6/kg was given to most patients, which might be due to the relatively lower disease burden and peak values of CAR-T cell expansion [6]. In other reports, CD19/22 dual targeting CAR-T cells [7, 8] and CD19/22 CAR-T cell cocktail therapy [9] did not increase the risk of severe CRS and neurotoxic effects. It was also reported that bispecific anti-CD20/CD19 CAR-T cells for relapsed B cell malignancies developed lower incidences of grade 3–4 CRS with 5%(1/22) and grade 3–4 neurotoxicity with 14% (3/22) [10]. Although the hematologic toxicity was the most common AE, it recovered quickly in the present study. These results suggested that the CD19/22 bispecific CAR-T cells for MRD-positive B-ALL patients had a higher safety.

Although the Cmax of CD19/CD22 CAR-T cell expansion was relatively lower, but it still had a good efficacy, which was consistent

with the report by Park et al [11]. In their study, they found that a higher ratio of peak values of CAR-T cells expansion to tumor burden significantly correlated with EFS and OS. A higher dose of CAR-T cells for the majority of patients in our study might result in a higher ration of peak values of CAR-T cells expansion to tumor burden. In our study, CD19/CD22 bispecific CAR-T cell therapy resulted in an overall MRD response rate of 100% at day 28 assessment, indicating that the bispecific CAR-T cells had a rapid and efficient response rate for MRD-positive patients. The relapse incidence in our study was lower than that in other reports for relapsed/refractory B-ALL patients [7, 8], likely due to the enrolled patients with a lower disease burden, although three patients eventually experienced morphological relapse and 2 MRD relapse. For the 10 patients without subsequent transplantation after CAR-T in the first-line consolidation group, the 24-month RFS and OS were 77.8% and 88.9%; while for patients ≥45 years old, the 24-month RFS and OS were 77% and 88%. The result was similar to the report by Schultz LM et al. using Tisagenlecleucel for the patients with low disease burden of 1-year OS of 85% and EFS of 72%, respectively [12]. Allo-HSCT could eradicate MRD persistence in B-ALL patients, but 5-year OS was only 33% due to non-relapse mortality and relapse [13]. The immune reconstitution kinetics for $CD3^+$, $CD4^+$, and $CD8^+$ were similar to other studies [14]. Till the end of the follow-up, 9 patients (4 Ph-negative and 5 Ph-positive) were with ongoing MRD negative remission and no history of allo-HSCT post CAR-T cell infusion. During to the relatively-shorter follow-up, we only propose that CD19/CD22 bispecific CAR-T therapy might be an optimal consolidation treatment modality for adult patients with MRD persistence after early consolidation chemotherapy, but it remains to be verified in further phase 2 clinical trials with larger sample size and a longer follow-up.

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DATA AVAILABILITY

The data that support the findings of the study are available on request from the corresponding author.

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AUTHOR CONTRIBUTIONS

XS designed and supervised the clinical study; HW and XD supervised the CAR T-cell production; XS conducted preclinical validation and quality control; JN, HQ, and FX collected clinical data; JN, FX, LZ, HQ, JY, CH, KZ, YT, YC, BD, YL, XS, LW, XD, HW, XS enrolled patients and took care of the patients; JN contributed to response monitoring of the patients.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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