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

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Running head CD19 and CD22 CAR-T in relapsed B-ALL post-HCT

This study was registered on Chinese Clinical Trial Registry/WHO International Clinical Trial Registry (ClinicalTrials#: ChiCTR-ONC-17013648).

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Author 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 CARs; CT and SL designed and guided the study.

Competing Interests

Alex H. Chang is a founding member of Shanghai YaKe Biotechnology Ltd., a biotechnology company focusing on research and development of tumor cellular immunotherapy. The remaining authors declare no conflict of interest.

Data Availability Statement

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

To the editor,

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 (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 out of 27 patients (85%) obtained complete remission (CR) after first CD19 CAR-T treatment; subsequently, 21 cases undertook a second CD22 CAR-T therapy, for whom the event-free survival (EFS) and OS rates at 18-month 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 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 second CD22 CAR-T therapy, with intervals of 1.5–6.5 months between two cell infusions. Patient-derived donor lymphocytes and lentiviral vectors encoding second generation CARs composed of CD3ζ and 4-1BB were used for manufacturing of CAR-T cells. The details of the CAR-T 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 overall survival and event-free survival 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 three cases who achieved complete remission after second CD22 CAR-T was calculated from the 2nd 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 (Trial registration: ChiCTR-ONC-17013648), written informed consents were obtained from all patients including three cases with MRD relapse.

The baseline patient characteristics are summarized in Supplemental Table S1−S2. The study cohort included 20 adults (67%) and 10 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 twenty-four patients administered with both CD19 and CD22 CAR-T therapies, over a median follow-up period of 64.4 (95% CI, 50.6 to 68.6) months, twelve patients stayed in continuous complete remission after first CD19 CAR-T (n=11) or second CD22 CAR-T (n=1, patient 27); the other twelve 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 (one 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 in 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 event-free survival and overall survival estimated by Kaplan–Meier methods were 54% (95% CI, 33 to 71) and 79% (95% CI, 57 to 91), respectively, while the 5-year EFS and OS were 50% (95% CI, 29 to 68) and 75% (95% CI, 53 to 88), respectively (Figure 2A−B).

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 had a significantly worse prognosis (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 extramedullary diseases (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 did not influence treatment outcomes. Nevertheless, patients who achieved PR or relapsed shortly after first CD19 CAR-T therapy had a significantly worse EFS probability, therefore, other treatments following CD22 CAR-T (such as second allo-HCT) should be considered to prolong remission.

Among the six patients without receiving CD22 CAR-T cells, four died and two remained alive (Supplemental 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 to 66) and 70% (95% CI, 50 to 83) at 3 years, and 46% (95% CI, 28 to 63) and 67% (95% CI, 47 to 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 one 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 eleven pneumonia (ten grade 3 and one 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 (Supplemental Table S3). In the context of intermittent intravenous infusion of immunoglobulin for patients with IgG <5.0g/L (they usually could not strictly follow our advice to receive regular immunoglobulin infusion once a month), eight of 13 severe infections occurred in patients with low immunoglobulin levels.

In this cohort of patients with more than 70% of HLA-haploidentical transplantation from family members, six cases experienced CAR-T

related graft-versus-host disease (four with and two without pre-existing 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. Another patient without pre-existing cGVHD presented with skin GVHD from 2-month 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 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 have achieved higher CR rates (Blinatumomab, 44%-63%;[4-5] CD19 CAR-T, 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. 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 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, 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 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 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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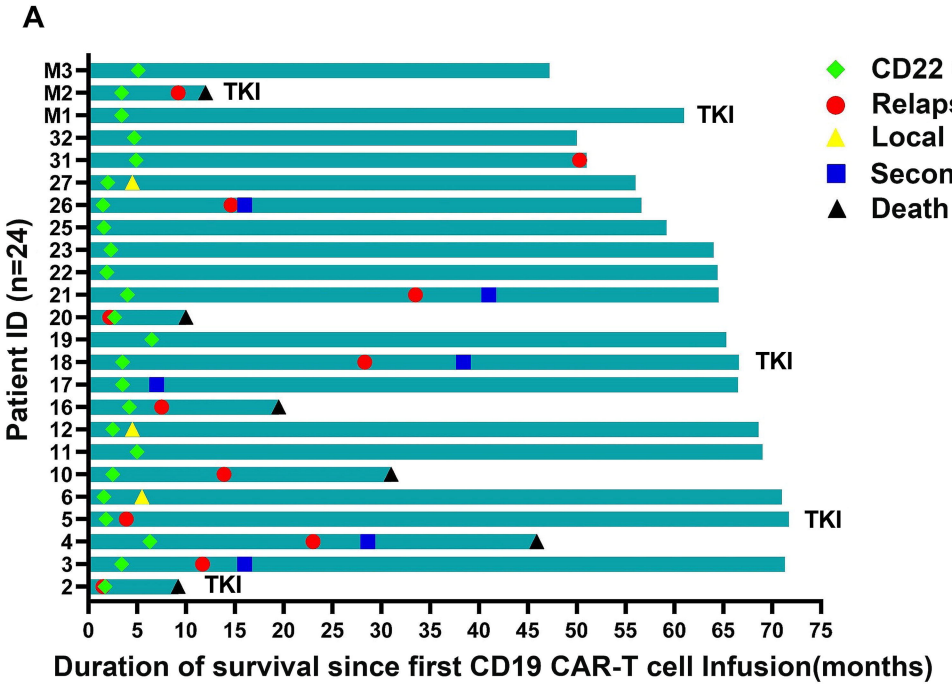
Figure Legends

Figure 1. The follow-up information in 24 patients treated with CD19/ CD22 CAR-T. (A) Survival duration of each patient (Pt.), calculated from the first CD19 CAR-T cell infusion. M1–M3 were three patients only with minimal residual disease. After first CD19 CAR-T, three 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 completer remission after second CD22 CAR-T. Among 11 relapsed cases with flow cytometry data (Pt.5 relapsed only with BCR/ABL+), two (Pt.3 and 20) showed CD19 negativity and others were CD19-positive; all patients showed CD22 normal expression (>80% of positive blasts) except for one (Pt.20) with partial expression (20%-80% of positive blasts). After CD22 CAR-T treatment, five cases with BCR-ABL transcript had taken tyrosine kinase inhibitors (TKIs); three ladies with extramedullary disease in the breast received local irradiation; and one 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 five cases (no data of B-cell recovery <6 months here because there were only three cases in this sub-group). Abbreviations: EMD, extramedullary diseases; 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 CAR-T cells (n=24). (C) OS and (D) EFS among the intention-to-treat population (n=30) that includes six patients who did not receive the second CD22 CAR-T therapy.[3]


A
CD22 CAR-T
Relapse
Local radiotherapy
Second allo-HCT
Death
Patient ID (n=24)
M3
M2
M1
32
31
27
26
25
23
22
21
20
19
18
17
16
12
11
10
6
5
4
3
2
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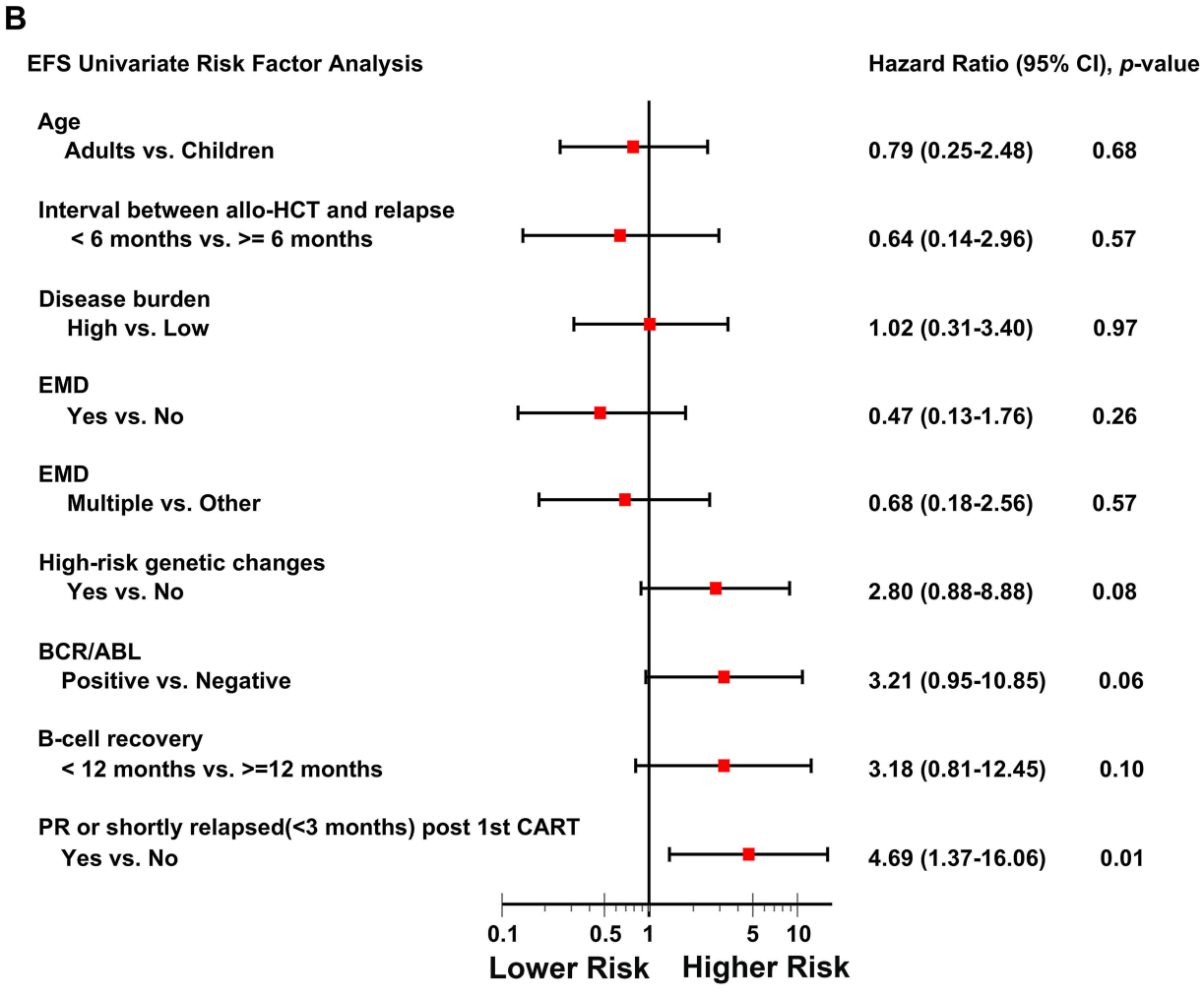
TKI
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Duration of survival since first CD19 CAR-T cell Infusion(months)


B
EFS Univariate Risk Factor Analysis
Hazard Ratio (95% CI), p-value
Age
Adults vs. Children
0.79 (0.25-2.48)
0.68
Interval between allo-HCT and relapse
< 6 months vs. >= 6 months
0.64 (0.14-2.96)
0.57
Disease burden
High vs. Low
1.02 (0.31-3.40)
0.97
EMD
Yes vs. No
0.47 (0.13-1.76)
0.26
EMD
Multiple vs. Other
0.68 (0.18-2.56)
0.57
High-risk genetic changes
Yes vs. No
2.80 (0.88-8.88)
0.08
BCR/ABL
Positive vs. Negative
3.21 (0.95-10.85)
0.06
B-cell recovery
< 12 months vs. >=12 months
3.18 (0.81-12.45)
0.10
PR or shortly relapsed(<3 months) post 1st CART
Yes vs. No
4.69 (1.37-16.06)
0.01
0.1 0.5 1 5 10
Lower Risk Higher Risk

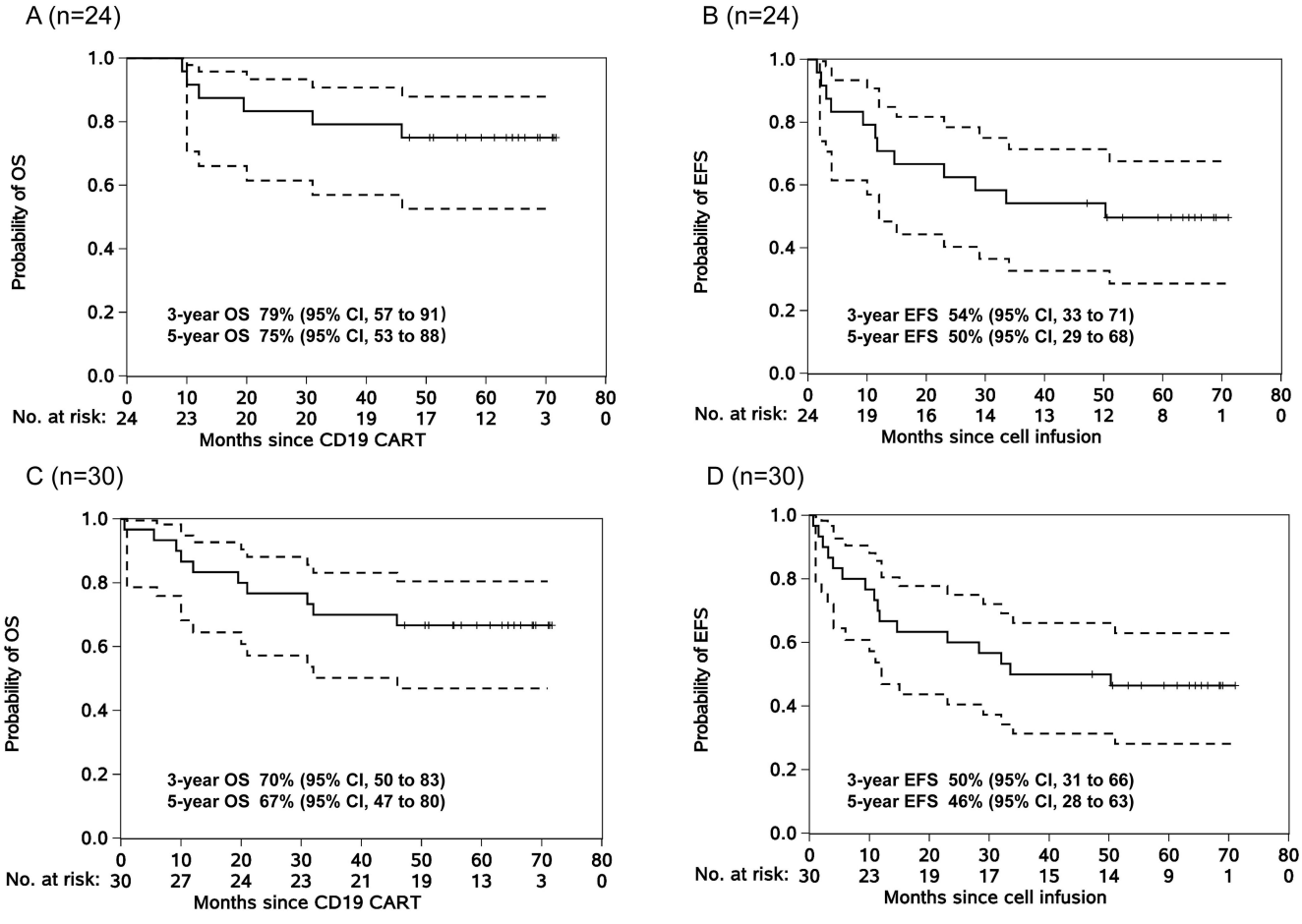

A (n=24)
Probability of OS
1.0
0.8
0.6
0.4
0.2
0.0
3-year OS 79% (95% CI, 57 to 91)
5-year OS 75% (95% CI, 53 to 88)
0 10 20 30 40 50 60 70 80
No. at risk: 24 23 20 20 19 17 12 3 0
Months since CD19 CART
B (n=24)
Probability of EFS
3-year EFS 54% (95% CI, 33 to 71)
5-year EFS 50% (95% CI, 29 to 68)
No. at risk: 24 19 16 14 13 12 8 1 0
Months since cell infusion
C (n=30)
Probability of OS
3-year OS 70% (95% CI, 50 to 83)
5-year OS 67% (95% CI, 47 to 80)
No. at risk: 30 27 24 23 21 19 13 3 0
Months since CD19 CART
D (n=30)
Probability of EFS
3-year EFS 50% (95% CI, 31 to 66)
5-year EFS 46% (95% CI, 28 to 63)
No. at risk: 30 23 19 17 15 14 9 1 0
Months since cell infusion

Table S1. Patient characteristics

Characteristics	No. (n=30)	% of patients
Age (years)		
Median	20.5 (range, 1.6-55)	
Children (<18)	10	33
Adults	20	67
Sex		
Male	17	57
Female	13	43
Type of transplant		
HID	22	73
MSD	4	13
MUD	3	10
UCB	1	3
Interval between HCT and relapse (months)		
Median	9 (range, 3-32)	
< 6	8	27
6-12	12	40
>12	10	33
Disease status at enrollment[a]		
High disease burden (blasts in BM/blood≥20% or with EMD[b])	21	70
Low disease burden (isolated BM blasts < 20%)	9	30
EMD distribution		
Overall	11	37
Multiple sites[c]	5 (1 with CNSL)	17
Single site	6	20
CNS	4	
Others (breast, mediastinum)	2	
High-risk genetic changes		
Complex karyotype	7 (1 with BCR/ABL)	23
Fusion gene	8	27
BCR/ABL	7 (3 with T315I mutation)	
MLL/AF4	1	
Pre-existing mild cGVHD		
Yes	4	13
No	26	87

[a]Disease status was no longer evaluated in most patients before CD19 CAR-T cell infusion because all infusions were performed within a month (80% ≤15 days) since enrollment (it only took 5-8 days to produce CAR-T cells); and debulking chemotherapy was short-term and non-intensive. [b]Involved sites of EMD included central nervous system (CNS), lymph nodes, bones, soft masses, breast, mediastinum, pharynx and kidney. [c]Multiple sites were defined as ≥2 sites of EMD.

Abbreviations: HID, haploidentical donor; MSD, matched sibling donor; MUD, matched unrelated donor; UCB, unrelated cord blood; HCT, hematopoietic cell transplantation; BM, bone marrow; EMD, extramedullary disease; CNSL, central nervous system leukemia; cGVHD, chronic graft versus host disease.

Table S2. Basic information of individual patients at enrollment (*n* =30)

Pt.No.	Age(years)	Sex	Type of transplant	Duration from HCT to relapse(months)	Blasts in BM(%)[a]	EMD	Complex karyotype	Fusion gene	pre-existing cGVHD
2	9	M	HID	4	66	-	-	BCR/ABL(with T315I mutation)	
3	10	M	MUD	6	14.5	-	+	-	
4	18	M	MSD	12	-	Multiple[b]	-	-	mild(mouth)
5	21	F	HID	12	11	-	-	BCR/ABL(with T315I mutation)	
6	27	F	HID	12	75.5	Breast	-	-	
7	15	M	HID	6	36.5	-	-	-	mild(skin and liver)
9	29	M	HID	12	28	-	-	-	mild(skin and liver)
10	29	F	HID	15	0.38	Multiple	-	-	
11	18	M	HID	6	19	-	+	E2A/PBX1	mild(skin)
12	28	F	MUD	7	2.66	Multiple	-	-	
13	29	M	HID	3	86.5	-	+	-	
15	43	M	HID	9	-	CNS	-	BCR/ABL	
16	20	M	HID	31	-	Multiple(with CNS)	-	-	
17	16	F	HID	9	0.85	Mediastinum	-	TEL/AML1	
18	55	F	HID	30	32	-	-	BCR/ABL	
19	31	F	HID	9	15.5	-	-	-	
20	18	F	HID	4	91	-	+	-	
21	5	F	MUD	11	55(PB)	-	+	-	
22	1.6	M	HID	3	13	CNS	N/A	MLL/AF4	
23	6	M	HID	18	54	-	-	-	
25	12	F	HID	5	0.41	CNS	-	-	
26	9	F	HID	11	85.5	-	N/A	-	
27	31	F	MSD	4	-	Multiple	-	-	
28	38	M	HID	4	16	-	+	-	
29	41	F	MSD	8	81	CNS	+	BCR/ABL(with T315I mutation)	
31	31	F	UCB(5/6)	9	17	-	-		
32	22	M	HID	3	21.5	-	-	-	
M1	20	M	MSD	13	0.04	-	-	BCR/ABL(with T315L mutation)	
M2	9	M	HID	9	0.98	-	-	BCR-ABL(with F317L mutation)	
M3	22	M	HID	32	0.8	-	-		

[a] Blasts were determined by flow cytometry when morphologic count was<5%. [b]Multiple was defined as ⩾2 sites of EMD, involved sites included central nervous system (CNS), lymph nodes, bones, soft masses, breast, mediastinum, pharynx and kidney.

Abbreviations: Pt.No., patient number, M1-M3 were three cases only with minimal residual disease; HCT, hematopoietic cell transplantation; BM, bone marrow; EMD, extramedullary disease; cGVHD, chronic graft-versus-host disease; HID, haploidentical donor; MUD, matched unrelated donor; MSD, matched sibling donor; UCB, unrelated cord blood; CNS, central nervous system.

Table S3. CAR-T treatments, late adverse effects and long-term outcomes

Pt.No.	First CD19 CAR-T(n=30)					Second CD22 CAR-T(n=24)				B-cell aplasia(months after CD19 CAR-T)	Survival/relapse/death
	Disease burden at enrollment*		Treatment response on day 30	Months after cell infusion		Disease status before CAR-T	Teatment response on day 30	Months after cell infusion			
	Blasts in BM(%)	EMD		Blood cell count recovery	Severe infections (site, grade)			Blood cell count recovery	Severe infections (site, grade)		
2	66	-	CR			MRD+	PD				died of disease progression
3	14.5	-	CRi	2.0		CR	CR			6.2	relapsed and alive
4	-	Multiple	CR		3.5(lung, III)	CR	CR		7.0(lung, III)	7.6	relapsed and died
5	11	-	CR		3(skin VZV, III)	CR	CRi	3.0		3.9	relapsed and alive
6	75.5	Breast	CRi	1.4		CR	CRi	3.0		4.8	CCR
7	36.5	-	CRi	5.5(not yet before death)	4.9(lung, III)		N/A			5.5	died of extensive GVHD
9	28	-	CRi	1.8	4.1(lung, III)		N/A			10.8	relapsed and died
10	0.38	Multiple	PR	1.0	3.9(lung, III)	PR	CR			6	relapsed and died
11	19	-	CRi	5(not yet before 2nd CAR-T)		CRi	CRi	5	6.5(skin VZV, III); 21.5(lung, III)	60(no further data)	CCR
12	2.66	Multiple	CRi	2.5(not yet before 2nd CAR-T)		CRi	CRi	6.7	16.2(lung, III)	30.8	CCR
13	86.5	-	CRi	2.3	6.5(lung, III)#		N/A			20.1(no further data)	died of acute respiratory failure associated with lung GVHD and infection
15	-	CNS	CRi	3.4			N/A			27.5	CCR
16	-	Multiple(with CNS)	PR	1.0		PR	CRi	1.6		2	relapsed and died
17	0.85	Mediastimum	CR			CR	CR			6.3	CCR
18	32	-	CRi	2.0		CR	CR			28.3	relapsed and alive
19	15.5	-	CRi	1.5		CR	CR			16	CCR
20	91	-	CRi	2.0		BM blasts 11.5%	CRi	2.5(not yet before 2nd relapse)			relapsed and died
21	55(PB)	-	CRi	2.0		CR	CR			15.3	relapsed and alive
22	13	CNS	CR			CR	CR		5.3(lung, III)	12	CCR
23	54	-	CRi	1.3		CR	CRi	2.0		51	CCR
25	0.41	CNS	CRi	1.6(not yet before 2nd CAR-T)		CRi	CRi	4.0	6.1(lung, III)	34.6(no further data)	CCR
26	85.5	-	CRi	1.5(not yet before 2nd CAR-T)		CRi	CR			9.6	relapsed and alive
27	-	Multiple	PR	1.6		PR	CR			6.4	CCR
28	16	-	CR		2.2(lung, IV)		N/A			12.9(no further data)	CCR
29	81	CNS	Died				N/A				died of severe CRS(on day 17)
31	17	-	CR			CR	CR			12.6	relapsed and alive

32	21.5	-	CRi	4.7(not yet before 2nd CAR-T)		CRi	CRi	4.0		47	CCR
M1	0.04	-	CR			CR	CR			9	CCR
M2	0.98	-	CR			CR	CR			6.8	relapsed and died
M3	0.8	-	CR			CR	CRi	2.0		11.3	CCR

*Disease status was no longer evaluated before CD19 CAR-T cell infusion since all infusions were finished in a month (80% ≤15 days) since enrollment.

#Patient 13 had anothr twice severe infections related to lung GVHD (he developed lung GVHD from 9-month on), we only count this isolated infection here.

Abbreviations: Pt.No., patient number, M1-M3 were three cases only with minimal residual disease; BM, bone marrow; EMD, extramedullary disease; CR, complete remission; MRD, minimal residual disease; PD, progressive disease; CRi, CR with incomplete blood count recovery; VZV, varicella-zoster virus; CCR, continuous complete remission; N/A, not applicable; GVHD, graft-versus-host disease; PR, partial response; CNS, central nervous system; CRS, cytokine release syndrome.