

# A phase I study of CAR-T bridging HSCT in patients with acute CD19<sup>+</sup> relapse/refractory B-cell leukemia

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**Abstract.** Chimeric antigen receptor (CAR)-T cell therapy is a novel cellular immunotherapy for relapsed/refractory (R/R) B acute lymphoblastic leukemia (B-ALL). However, the survival duration of CAR-T cells *in vivo* is noteworthy, and in some cases recurrence occurs following CAR-T cell therapy. There is controversy over the benefits of bridging to allo-HSCT after CAR-T cell therapy. The present study explored the efficacy and safety of CD19 chimeric antigen receptor (CAR) T-bridged allogeneic hematopoietic stem cell transplantation (allo-HSCT) treatment in relapsed/refractory B-cell acute lymphocytic leukemia (R/R B-ALL). A total of 9 patients with B-ALL treated at The First Affiliated Hospital of Wenzhou Medical University between December 2016 and November 2017 were included. The results demonstrated that the total response rate on day 28 after receiving CD19-CAR T-cell therapy was 100% (9/9) and all patients exhibited complete remission. The 1-year overall survival (OS) rate for 5 patients who received CAR-T bridged HSCT was 100%, the 1-year DFS rate was 100%; the 1-year OS rate for the 4 patients who received

CAR-T therapy was 75%, and the 1-year DFS rate was 75%. Patients who received CAR-T bridged to HSCT had no significant prolongation of myeloid and platelet engraftment median time compared with patients who received CAR-T alone, and the incidence of acute graft-versus-host disease or extensive chronic graft-versus-host disease did not increase. Overall, the present clinical trial demonstrated that CAR-T therapy bridging to HSCT is a feasible, safe and effective method to treat adult patients with R/R B-ALL.

## Introduction

Adult patients with acute B cell lymphoblastic leukemia (B-ALL) often relapse following chemotherapy alone, and the long-term survival rate is ~30% (1,2). Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a radical treatment, and 50% of adult patients exhibit long-term disease-free survival (DFS) after transplantation (3,4). However, it is difficult to achieve remission via traditional treatment methods in patients with relapsed/refractory (R/R) B-ALL due to a high tumor burden and poor patient physical condition, and these patients lose the best chance of receiving transplantation. Previously, only 5% of patients with R/R B-ALL who underwent conventional chemotherapy were able to undergo allo-HSCT (5,6). Minimal residual disease (MRD) status has been demonstrated to be a strong prognostic factor for adult patients with ALL (7). Logan *et al* (7) reported that MRD monitoring is useful for determining the clinical indications for allogeneic HSCT in the treatment of ALL in CR1. Patients with (MRD<sup>+</sup>) possess a number of risks for transplantation, and the outcome for patients with MRD<sup>+</sup> who undergo allo-HSCT is worse compared with that of patients without MRD (MRD<sup>-</sup>) (8-10).

Chimeric antigen receptor (CAR)-T cell therapy is a novel cellular immunotherapy that uses genetically engineered T cells that express tumor-specific CARs and combines the antigen-antibody binding mechanism with the killing effect of T cells (11,12), thus specifically killing tumors. Anti-CD19 CAR-T (CART19) cell therapy can significantly improve the remission and survival rate of patients with B-ALL (13,14). The remission rate of CART19 cell therapy for patients with B-ALL can be as high as 70-90% (15-17), but the duration period of CAR-T cells *in vivo* is noteworthy, and in some cases recurrence occurs soon after CAR-T cell therapy (18). There is controversy over the benefits of bridging to allo-HSCT after CAR-T cell therapy. For example,

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**Abbreviations:** CAR-T bridged, chimeric antigen receptor T-bridged; HSCT, hematopoietic stem cell transplantation; allo-HSCT, allogeneic hematopoietic stem cell transplantation; OS, overall survival; DFS, disease free survival; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; R/R B-ALL, relapse/refractory B lymphoblastic leukemia; PBMC, peripheral blood mononuclear cells; B-ALL, acute B lymphoblastic leukemia; CR, complete remission; MRD, minimal residual disease; CNSL, central nervous system leukemia; ATG, thymoglobulin; FISH, fluorescence *in situ* hybridization; FCM, flow cytometry; CRS, cytokine release syndrome

**Key words:** CAR-T bridging HSCT, CD19<sup>+</sup>, relapse, refractory, acute B-cell leukemia

Park *et al* (19) evaluated the 19-28z CAR-T cell infusion in adult patients with R/R B-ALL; 12/44 patients underwent allo-HSCT, and the results demonstrated that allo-HSCT had no significant effect on survival after infusion of CAR-T cells. In addition, Summers *et al* (20) evaluated the efficacy of SCRI-CAR19v1 for treating pediatric and young adult patients with B-ALL; 17/50 patients underwent allo-HSCT, and the results suggested that these patients had improved leukemia-free survival.

The aim of the present study was to investigate the feasibility, safety and efficacy of CART19 cell therapy bridging to HSCT in adult patients with R/R B-ALL.

## Patients and methods

**Study design and patients.** The present study was a phase I open-label clinical trial aiming to evaluate the feasibility, safety and efficacy of the CART19 bridging regimen followed by allo-HSCT for the treatment of patients with R/R B cell leukemia (clinical trial no. NCT03110640). Patients were screened and treated at The First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) between December 2016 and November 2017. A total of 4 patients were not eligible for the CART19 cell clinical trial due to liver dysfunction (n=2), abnormal renal function (n=1) and pneumonia (n=1). The median age of the patients was 34.1 years (range, 16-57 years), and 4 patients were male and five were female. The inclusion criteria were as follows: i) 5-70 years old and ii) histologically confirmed CD19<sup>+</sup> leukemia. Patients also had to meet one of the following conditions: i) received at least two prior combination chemotherapy regimens (not including single agent monoclonal antibody therapy) and failed to achieve complete remission (CR) or have disease recurrence; ii) creatinine expression levels <2.5 mg/dl; iii) Alanine aminotransferase/Aspartate aminotransferase <3x normal levels; iv) bilirubin levels <2.0 mg/dl. In addition, female patients of a reproductive age had to have a negative serum or urine pregnancy test within 48 h before treatment infusion. Patients who met any of the following criteria were excluded: i) Symptoms of central nervous system, including central nervous system-leukemia; ii) another malignant tumor; iii) active hepatitis B or C, or HIV; iv) other diseases that may affect the outcome of the trial, such as acute myocardial infarction; v) severe cardiovascular or respiratory disease; vi) poorly controlled hypertension; vii) a history of poorly controlled mental illness; viii) patients who had taken immunosuppressive agents within one week of the start date due to organ transplantation or another disease requiring long-lasting treatment; ix) unstable pulmonary embolism, deep vein thrombosis or other major arterial/venous thromboembolic events 30 days prior to the study; x) patients reaching a steady dose of anticoagulant therapy 3 months ago before the study start date; xi) pregnant or lactating women; and xii) patients with a disease affecting their ability to provide informed consent. The protocol was approved by The First Affiliated Hospital of Wenzhou Medical University Institutional Review Board (approval no. 2016-220). All patients provided written informed consent prior to enrollment. Consent was obtained from the patients or their parents/legal guardians if the patients were <18 years. All patients underwent CART19 cell infusion after the standard lymphodepletion regimen with fludarabine (25 mg/m<sup>2</sup>/day on days -4, -3 and -2) and cyclophosphamide

(1,000 mg/m<sup>2</sup> on day -4). The post-infusion responses and toxicities were closely monitored daily. The expansion and persistence of CD19 CAR-T cells (the copies of CD19 CAR DNA and the percentage of CD19 CAR T cells), as well as MRD, were measured using flow cytometry (FCM) and quantitative (q)PCR assays on days +1, +3, +9, +14, +21 and +28 and every 30 days after CD19 CAR-T cell infusion. When no CART19 cells could be detected in the blood and/or MRD recurrence was observed, patients began HSCT therapy. Salvage therapy for no. 4 patient was FLAG-IDA regimen which included fludarabine, cytarabine, idarubicin, and G-CSF.

**Generation of retroviral vectors, CAR-T cell production and quality control of CART19 cells.** The present study constructed  $\gamma$ -retroviral vectors encoding second-generation BB $\zeta$ CD19-targeting CAR molecules (4-1BB-engineered CAR-T cells) (Fig. 1C). To evaluate the functionality of these CAR constructs, human primary T cells from 9 patients were transduced with CAR vectors. All CAR PBMC populations were expanded under conditions to generate CAR-T cells. Activated PBMCs were resuspended in 50 IU/ml recombinant human IL-2 and incubated at 37°C for 7-14 days. During *ex vivo* expansion, culture medium was replenished, and the density of T cells was maintained at 0.5-1x10<sup>6</sup> cells/ml. Routine screening of CART19 cell cultures was negative before infusion. The methods for the generation of retroviral vectors, CAR-T cell production and quality control of CART19 cells before infusion are presented in our previous study (21). In this study, the PBMCs were from the patient but not from the healthy donors, which was different from our previous study (21). The methods for the generation of retroviral vectors, CAR-T cell production and quality control of CART19 cells before infusion are accordant with our previous study (21).

**CART19 cell treatment.** The CART19 cell infusion day was set as study day 0. Prior to CAR-T cell infusion, patients were administered 1,000 mg/m<sup>2</sup> cyclophosphamide on day -4 and 25 mg/m<sup>2</sup>/day fludarabine on days -4, -3 and -2. On day 0, CART19 cells at a target dose of 1x10<sup>6</sup> T cells/kg were intravenously injected directly into the enrolled patients.

**Management of cytokine release syndrome (CRS).** Hemodynamic parameters, vital signs and peripheral blood cytokine levels, including interferon  $\gamma$  (IFN- $\gamma$ ), interleukin 2 (IL-2), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), IL-6, IL-10 and C-reactive protein (CRP), were closely monitored on days +1, +3, +9, +14, +21 and +28 and every 30 days after CD19 CAR-T cell infusion. Supportive care for patients receiving CAR T cells was also provided. If a patient met CRS criteria for tocilizumab treatment (90 mmHg systolic blood pressure could not be maintained with norepinephrine, oxygen requirement of  $\geq 50\%$  FiO<sub>2</sub> for  $\geq 2$  h continuously, severe dyspnea potentially requiring mechanical ventilation), 4-8 mg/kg tocilizumab was administered over 1 h with the overall dose  $\leq 800$  mg. If a patient met CRS criteria for corticosteroids (grade 3 neurological toxicity, with the exception of headaches, lasting continuously for  $\geq 24$  h or grade 4 neurologic toxicity of any duration), 10 mg dexamethasone was administered for 6 h via intravenous injection until toxicities improved to grade 1 or lower. All toxicities occurring within 30 days of CAR-T cell therapy were graded for all 9 patients.

**Assessment of CART19 cell expansion and persistence.** Genomic (g)DNA was isolated from peripheral blood mononuclear cell (PBMC) samples using a Mini BEST Universal Genomic DNA Extraction kit (Takara Biotechnology Co., Ltd.), quantified using a spectrophotometer and stored at -80°C. The presence, expansion and persistence of CAR-T cells in the blood and bone marrow were assessed using quantitative (q)PCR (21). The analysis of CAR-T cell percentages in PBMCs and bone marrow samples was performed using multiparametric FCM (21). The CAR-T cells were stained for CD45RA, CD45RO, CD3, CD4, CD8 and CD62L with fluorescence-labelled antibodies (BioLegend). CAR detection was monitored with polyclonal goat anti-mouse F(ab)2 antibodies labelled with biotin (Jackson Immunoresearch Laboratories, Inc.) and streptavidin labelled with BV421 (BioLegend) (21).

**Assessment of serum cytokines, immune effector molecules and MRD.** Measurements of IL-6, granzyme B, TNF- $\alpha$  and IFN- $\gamma$  were performed by FCM using a BD Cytometric Bead Array (BD Biosciences) according to the manufacturer's instructions. IL-15 and CRP expression levels were analyzed using the cytokines ELISA kit (cat. no. ABIN4883305) from R&D Systems. MRD assessment was measured at regular intervals using FCM and qPCR assays. MRD in the bone marrow of all patients was assessed at the following time points. The parameters varied with patients, owing to the difference of their immunophenotype at initial diagnosis: On enrolment, on day 1 and every 30 days after CD19 CAR-T cell infusion. In addition, MRD in the peripheral blood of all patients at days +1, +3, +9, +14, +21 and +28 and every 30 days after CD19 CAR-T cell infusion was analyzed. RT-PCR was performed to assess BCR-ABL1 (p190) for Ph<sup>+</sup> B-ALL (22).

**Donor selection.** A matched sibling donor was chosen as the first treatment option. Patients were eligible for haploidentical SCT if a matched sibling donor was unavailable. Donors and patients were assessed for the degree of HLA matching. HLA-A and HLA-B typing were performed by intermediate resolution DNA typing, whereas HLA-DRB1 typing was performed using high-resolution DNA techniques. The donor inclusion criteria were as follows: The donor was HLA-matched with the patient, and the donor had no serious heart, liver and kidney disease. Among the five donors, two were sibling donors, and the other three were haploidentical HSCT donors. The median age of the donors was 38 years (age range, 24-49 years), four men and one woman. Leukapheresis was performed for patients 4-5 days after starting G-CSF based on the peripheral blood CD34<sup>+</sup> cell count (PB CD34). All donors tolerated the stem cell (from bone marrow or/and peripheral blood) harvest and leukapheresis procedures and had no severe side effects.

**Conditioning regimen and graft-versus-host disease (GVHD) prophylaxis.** When no CART19 cells could be detected in the blood and/or MRD recurrence was observed, patients began to receive HSCT. The conditioning therapy for patients undergoing haploidentical HSCT was a modified busulfan/cyclophosphamide (BUCY) regimen plus thymoglobulin (ATG) consisting of cytarabine (4 g/m<sup>2</sup>/day intravenously on days -10 and -9), BU (3.2 mg/kg/day on days -7 to -4), CY (1.8 g/m<sup>2</sup>/day intravenously on days -5 and -4), Methyl-CCNU semustine (250 mg/m<sup>2</sup> orally

once on day -3) and ATG (5 mg/kg/day intravenously on days -5 to -2). The conditioning therapy for patients undergoing sibling HSCT was BUCY, consisting of BU (3.2 mg/kg/day on days -7 to -4), CY (1.8 g/m<sup>2</sup>/day intravenously on days -5 and -4) and Me-CCNU (250 mg/m<sup>2</sup> orally once on day -3). All patients received cyclosporine A (5 mg/kg.d) and short-term methotrexate 15 mg/m<sup>2</sup> +1, 10 mg/m<sup>2</sup> +3,6,11 for GVHD prophylaxis.

**Engraftment, chimerism, MRD and GVHD evaluation.** For VHD evaluation, skin rash <25% within +100 days was defined as grade 1 skin aGVHD. The day of engraftment was defined as maintenance of an absolute neutrophil count >500/UI for 3 consecutive days after HSCT. Chimerism was evaluated by DNA-based HLA typing (for mismatched loci), PCR DNA fingerprinting of short tandem repeats and chromosomal fluorescence *in situ* hybridization (FISH) for the Y chromosome. MRD was assessed by FISH and FCM. The MRD parameters by FCM varied with patients, owing to the difference of their immunophenotype at initial diagnosis, and MRD parameters by FISH for Y chromosome when the donor and patient sex are different. MRD and chimerism were evaluated on days +30, +60, +90 and +180 and years +1, +3 and +5. The methodology for DNA-based HLA typing (for mismatched loci) was as follows: Genomic DNA was extracted from human whole blood. PCR products were gel purified and sequenced. Sequences were imported into the Sequencing Based Typing software, GenDx SBTengine<sup>®</sup> to analyze heterozygous nucleotide positions throughout the amplified region. The methodology for PCR DNA fingerprinting of short tandem repeats was as follows: DNA was isolated from the skin biopsies as well as from a donor blood. PCR amplification of nine highly polymorphic short tandem repeats was performed for each specimen and a unique DNA 'fingerprint' was obtained from each. Thorough analysis confirmed GVHD. The methodology for chromosomal fluorescence *in situ* hybridization (FISH) for the Y chromosome was as follows: Before hybridization, the DNA probe was labeled by various means. The labeled probe and the target DNA were denatured. Combining the denatured probe and target allows the annealing of complementary DNA sequences.

**Statistical analysis.** All statistical analyses were performed using SPSS version 19 (SPSS, Inc.) and data are presented as the mean  $\pm$  standard deviation. Differences between two groups were analyzed using unpaired two-tailed Student's t-tests. Differences among multiple groups were analyzed using one-way ANOVA and Bonferroni's correction. OS and progression-free survival rate were analyzed using Kaplan-Meier curves and log-rank tests. P<0.05 was considered to indicate a statistically significant difference. All experiments were performed in triplicate.

## Results

**Characterization of patients.** Between November 2016 and November 2017, a total of 13 patients with CD19<sup>+</sup> R/R B-ALL were recruited. A total of 4 patients were excluded from receiving lymphodepletion chemotherapy and CART19 cell infusion (Fig. 1). The other 9 patients received CAR-T cell infusion. As presented in Table I, all patients had R/R B-ALL, and 6 patients had detectable leukemic cells in the

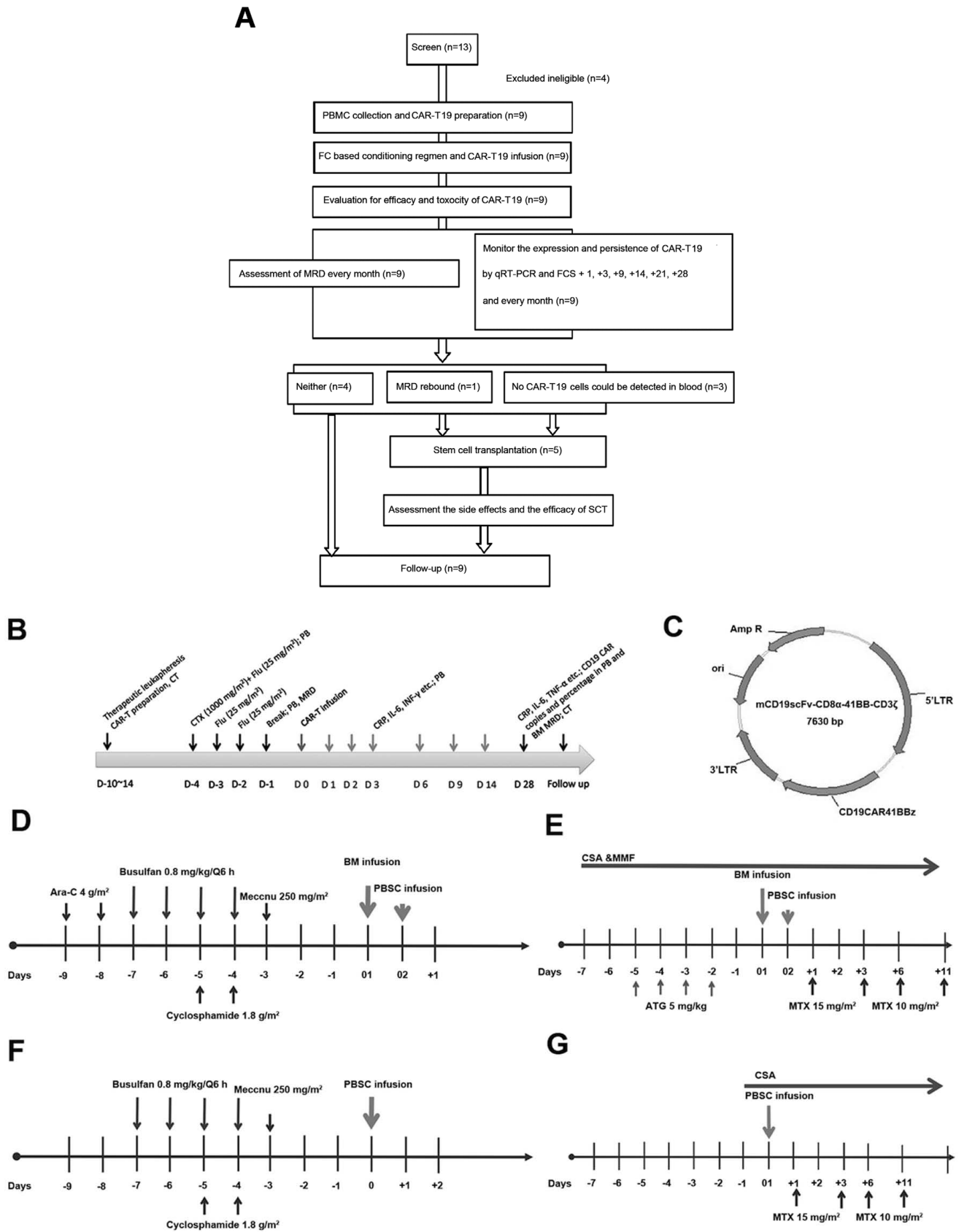


Figure 1. Study schema for the clinical trial of CAR-T bridging HSCT in the treatment of CD19<sup>+</sup> relapse/refractory B-cell lymphoblastic leukemia. (A) Patient enrollment flow chart; a total of 13 patients were screened and four ineligible patients were excluded. (B) Clinical treatment protocol for CAR-T therapy. (C) Clinical treatment protocol for haplo-HSCT therapy (BUCY base conditioning regimen and stem cell infusion). (D) Clinical treatment protocol for haplo-HSCT therapy (administration of immunosuppressive agents). (E) Clinical treatment protocol for HLA-matched sibling-HSCT (BUCY base conditioning regimen and stem cell infusion). (F) Clinical treatment protocol for HLA-matched sibling-HSCT (administration of immunosuppressive agents). (G)  $\gamma$ -retroviral vectors encoding the second-generation CD19-targeting CAR molecules was constructed. CART, chimeric antigen receptor T; HSCT, hematopoietic stem cell transplantation; CAR, chimeric antigen receptor; PBMCs, peripheral blood mononuclear cells; PBSC, peripheral blood stem cells; FC, fludarabine and cyclophosphamide; MRD, minimal residual disease; RT-qPCR, reverse transcription quantitative PCR; BUCY, busulfan/cyclophosphamide.

Table I. Response, toxicities and prognosis after CART19 therapy and SCT of patients with relapsed/refractory B-cell acute lymphocytic leukemia.

Patient no.	Patient			Infused CART19 cells			CRS			BM leukemia cell clinical response		Stem cell transplantation			1-year outcome
										Before CART19, %	After 1 month, %	SCT	Type	Side effects	
				Sex	Type	Age, years	CART/CD3 <sup>+</sup> T cells, %	Dose, x10 <sup>6</sup> /kg	Grade						
1	M	Ph <sup>-</sup>	24	59.3	1	1	N	N	63.90	<0.01	Y	haplo-HSCT	cGVHD	CR	
2	M	Ph <sup>-</sup>	38	46.5	1	1	N	N	10.60	<0.01	Y	haplo-HSCT	cGVHD	CR	
3	F	Ph <sup>-</sup>	43	61.6	1	0	N	N	5.20	<0.01	Y	Sibling SCT	N	CR	
4	F	Ph <sup>-</sup>	18	26	1	1	N	N	3.00	<0.01	Y	Sibling SCT	N	CR	
5	M	Ph <sup>-</sup>	16	60	1	2	N	N	1.09	<0.01	Y	haplo-HSCT	aGVHD grade I skin	CR	
6	F	Ph <sup>+</sup>	49	62	1	4	Y	Y	5.00	<0.01	N	N	NA	CR	
7	F	Ph <sup>-</sup>	40	50	1	3	Y	N	6.80	<0.01	N	N	NA	CR	
8	F	Ph <sup>+</sup>	57	62	1	2	N	N	4.60	<0.01	N	N	NA	CR, Imatinib maintenance	
9	M	Ph <sup>-</sup>	22	65.6	1	2	N	N	11	<0.01	N	N	Relapse	Relapsed and died on day 123	

CART, chimeric antigen receptor T; M, male; F, female; ALL, acute lymphoblastic leukemia; BM, bone marrow; CRS, cytokine release syndrome; NA, not available; Tocili, tocilizumab; Y, yes; N, no; cGVHD, chronic graft-versus-host disease; MRD, minimal residual disease; CR, complete remission; PR, partial remission; HSCT, hematopoietic stem cell transplantation; SCT, stem cell transplantation.

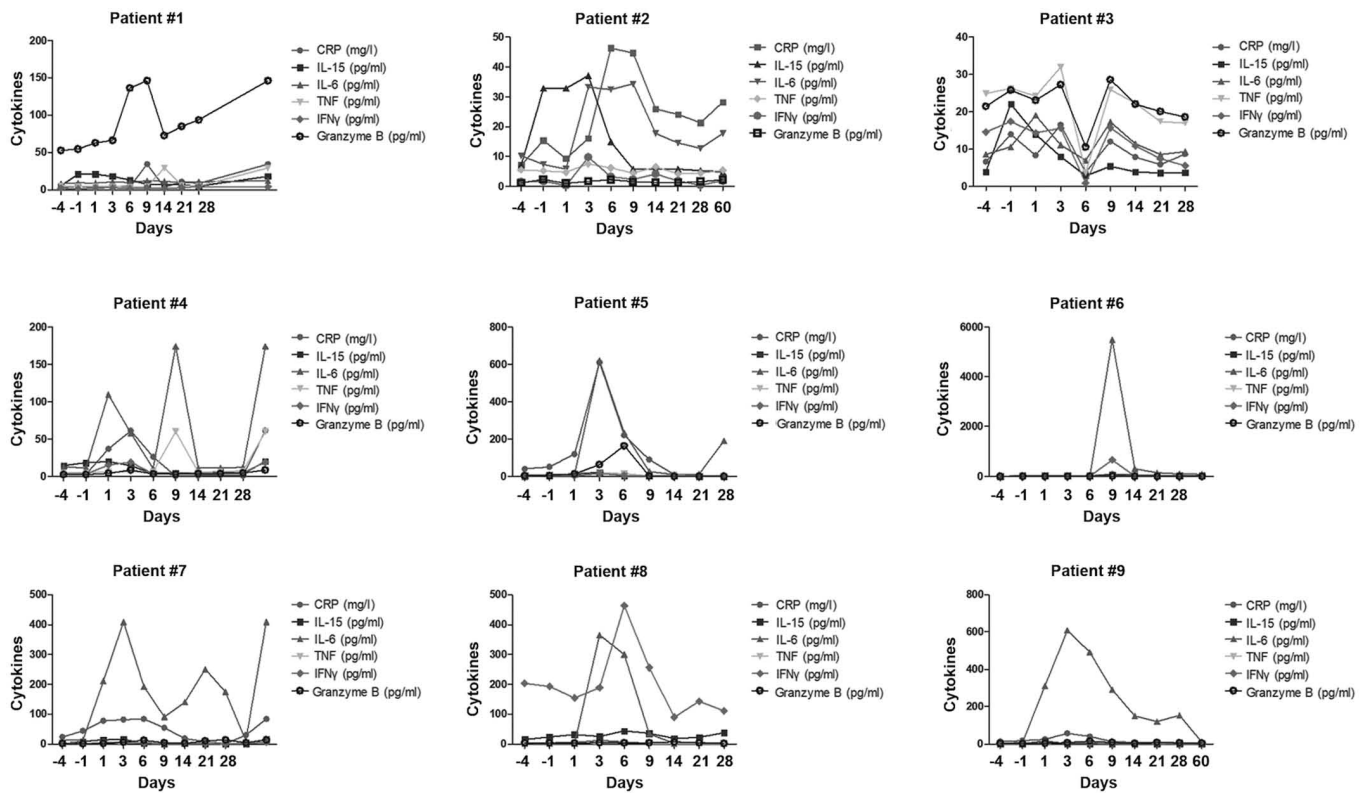


Figure 2. Cytokine levels in each patient. Intermittent monitoring of serum levels of cytokines IL15, IL6, granzyme B, TNF, IFN- $\gamma$  and CRP were assessed during CRS on day -4, -1, +1, +3, +6, +9, +14, +21 and +28 following chimeric antigen receptor T therapy with or without hematopoietic stem cell transplantation for relapse/refractory B-cell lymphoblastic leukemia. CRS, cytokine release syndrome; IL, interleukin; TNF, tumor necrosis factor; IFN- $\gamma$ , interferon  $\gamma$ ; CRP, C-reactive protein.

bone marrow prior to CART19 cell infusion. The median percentage of leukemic blasts in the bone marrow was 12.35% (range, 1.09-63.9%) of marrow blasts (Table I). One patient (patient 6) had Ph<sup>+</sup> ALL [relapsed with G250E and V299L mutations and an additional abnormal karyotype: del(16),t(1,16)(q21,q12)]. Patient 6 did not achieve remission despite steroid-based chemotherapy and TKI-targeted therapy, including dasatinib and nilotinib. Two patients had asymptomatic central nervous system leukemia (CNSL) (patients 6 and 7) before CART19 cell infusion.

**Preclinical evaluations of CAR-T cells.** As presented in Table I, a median of 54.78% (range, 26-65.6%) of CD3<sup>+</sup> T cells expressed CAR constructs equipped with signaling domains consisting of 4-1BB. All 9 patients received the CART19 cell infusion at a dose of  $1 \times 10^6$  cells/kg.

**Efficacy assessment of CAR-T cell infusion.** All patients achieved CR within 28 days of infusion of CAR-T cells. One patient with Ph<sup>+</sup> ALL who was resistant to both dasatinib and nilotinib also achieved MRD<sup>-</sup> remission. The 2 patients who had asymptomatic CNSL maintained a blast-negative status in the cerebrospinal fluid and MRD<sup>-</sup> remission in the bone marrow (BM).

**Safety evaluation of CAR-T cell infusion.** A total of 8 patients experienced CAR-T cell-related adverse effects of any grade, with grade 3 and grade 4 events reported in 1 (11.1%) patient each. Three patients experienced grade 2 CRS. CRS was

fully reversible in all 5 patients and was well managed with supporting therapy alone (n=3), supporting therapy plus the anti-IL6 receptor antibody tocilizumab (n=1) and supporting therapy plus tocilizumab and corticosteroids (n=1) (Table I). The patient who experienced grade 4 CRS had severe diffuse alveolar hemorrhage after CAR-T cell infusion and recovered one week after high-dose corticosteroid therapy, mechanical ventilation, tocilizumab and supportive care (blood transfusion and oxygen inhalation.). The patient achieved CR with MRD<sup>-</sup> on day 21 and had maintained CR for 203 days at the end of the follow-up. Serum levels of the cytokines IL15, IL6, granzyme B, TNF, IFN- $\gamma$  and CRP were assessed during CRS (Fig. 2). The results showed that serum levels of cytokine IL-15 ranged from 3-43 pg/ml, while those of cytokine IL-6 ranged from 4-609 pg/ml. Serum levels of cytokine granzyme B ranged from 1-156 pg/ml, while those of TNF ranged from 3.2-59.1 pg/ml. Serum levels of cytokine IFN- $\gamma$  ranged from 1-482 pg/ml, while those of CRP ranged from 0-601 mg/l, and there was no significant difference in the peak levels between the bridging HSCT group and the non-HSCT group (Fig. 3).

There was no significant difference in the peak levels in the BM/PB between the bridging HSCT group and the non-HSCT group (Fig. 4A-D). After treatment with CART19 cell infusion, all patients achieved CR and 2 out of 9 (22.2%) patients had severe complications (grade 4 CRS), but CRS in these patients was reversed after administration of IL-6 receptor antagonists and glucocorticoid [1 of the 2 patients received allo-HSCT and exhibited long-term survival; the other patient with Ph<sup>+</sup> ALL also had BCR-ABL1 (p190)<sup>+</sup> B-ALL

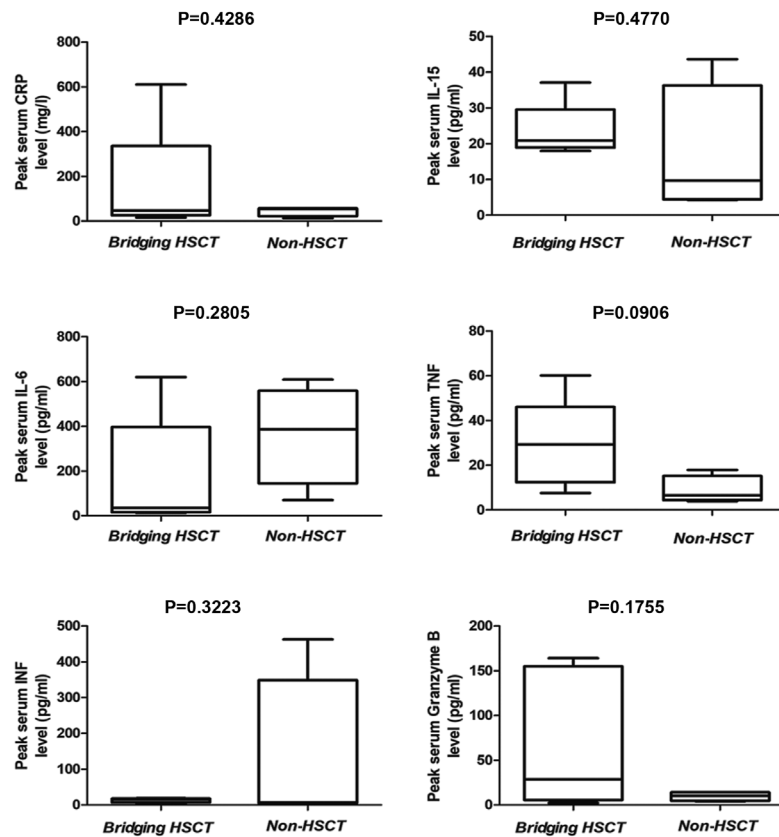


Figure 3. Box plot of the association between peak serum expression levels of cytokines IL15, IL6, granzyme B, TNF, IFN- $\gamma$  and CRP between the bridging HSCT group and non-HSCT group. HSCT, hematopoietic stem cell transplantation; IL, interleukin; TNF, tumor necrosis factor; IFN- $\gamma$ , interferon  $\gamma$ ; CRP, C-reactive protein.

and secondary resistance to imatinib]. Steroid-based mutation detection of the ABL gene revealed the presence of G250E and V299L mutations of one patient. Chromosomal banding analysis of the bone marrow revealed additional abnormal karyotypes, including deletion (16),t(1,16)(q21,q12). Although the treatment regimen was changed to dasatinib (800 mg/day) and a steroid-based regimen (idamycin 10 mg/m<sup>2</sup>, d1-3, vincristine 4 mg d1,8,15,22 and prednisone 60 mg d1-28) for 3 months, patients were unable to achieve remission. Grade 4 CRS and diffuse alveolar hemorrhage occurred after CD19 CAR-T cell infusion, and the patient recovered within 1 week after high-dose corticosteroid therapy, mechanical ventilation, tocilizumab and supportive care. Imatinib was orally administered in the absence of CAR-T cells after 3 months of CD19 CAR-T cell infusion, and this patient also survived at the end of follow-up. The remaining 7 (77.7%) patients had complications of CRS of grades 0-2, and 2 patients with severe complications had higher MRD before the infusion compared with the other patients and multiple relapses in the past.

**Expansion and persistence of CART19 cells in the peripheral blood and bone marrow.** In all 9 patients, the *in vivo* expansion of CAR-T cells was monitored by qPCR and FCM. As presented in Fig. 4, all patients had detectable circulating CAR-T cells. The peak in CAR-T cell expansion ranged between days 6 and 9 (Fig. 4). The median peak of CAR DNA copies was  $2.61 \times 10^5$  (range,  $3.16 \times 10^4$ - $6.34 \times 10^5$ ) copies/ $\mu$ g gDNA in the blood and  $6.48 \times 10^3$  (range,  $302$ - $2.13 \times 10^4$ ) copies/ $\mu$ g gDNA

in the bone marrow. The median peak of CAR DNA copies was  $3.92 \times 10^5$  (range,  $1.78 \times 10^4$ - $6.34 \times 10^5$ ) copies/ $\mu$ g gDNA in the blood and  $10.9 \times 10^3$  (range,  $2.61 \times 10^3$ - $21.3 \times 10^3$ ) copies/ $\mu$ g gDNA in the bone marrow in the non-HSCT group, whereas the median peak of CART DNA copies was  $1.57 \times 10^5$  (range,  $3.16 \times 10^5$ - $4.87 \times 10^5$ ) copies/ $\mu$ g gDNA in the blood and  $2.88 \times 10^3$  (range,  $302$ - $5.48 \times 10^3$ ) copies/ $\mu$ g gDNA in the bone marrow in the bridging HSCT group. In addition, CART19 cells were detectable in the blood and marrow between 3 and 6 months. When no CART19 cells could be detected in the blood and/or MRD recurrence was observed, patients began to receive HSCT. Patient 4, who still had detectable levels of CAR-T cells in bone marrow analyzed using qPCR (117 copies/ $\mu$ g DNA) at day +90 following CAR-T cell infusion, relapsed with CD19<sup>+</sup> leukemia (>20% blasts in the bone marrow) at the same time. This patient then received salvage therapy (FLAG-IDA regimen which included fludarabine, cytarabine, idarubicin and G-CSF) and matched sibling donor SCT and maintained MRD<sup>-</sup> CR post-HSCT until the end of the follow-up period.

**Patient receiving SCT and donor characteristics.** Among the 5 patients who received allo-HSCT, there were 4 patients with no detectable CART19 cells in the blood/bone marrow on day +90 after CAR-T cell therapy and 1 patient who relapsed with CD19<sup>+</sup> leukemia even with detectable levels of CAR-T cells in the bone marrow analyzed using qPCR on day +90 following CAR-T cell infusion (Table I).

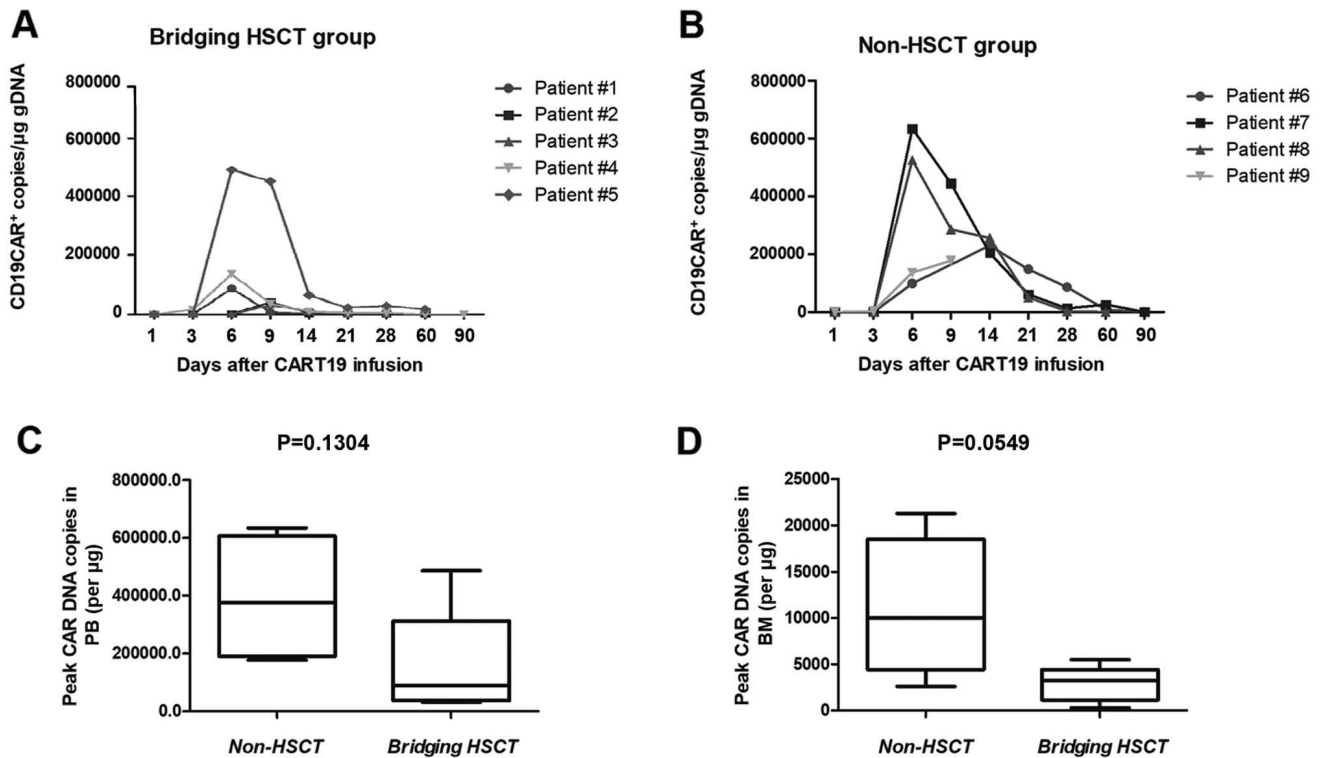


Figure 4. CART19 engraftment, expansion and persistence *in vivo*. (A) Expression levels of CART19s in the PB assessed using quantitative PCR on days 1, 3, 6, 9, 14, 21, 28, 60 and 90 after infusion of CART19s in bridging HSCT group (patients 1-5). (B) Expression levels of CART19s in PB assessed by RT-qPCR on days 1, 3, 6, 9, 14, 21, 28, 60 and 90 after infusion of CART19s in non-HSCT group (patients 6-9). (C) Box plot of the association between peak expression levels of CART19s and CAR DNA copies in the PB between the bridging HSCT and non-HSCT groups. (D) Box plot of the association between peak expression levels of CART19s and CAR DNA copies in the bone marrow between the bridging HSCT and non-HSCT groups. CART, chimeric antigen receptor T; PB, peripheral blood; CAR, chimeric antigen receptor; HSCT, hematopoietic stem cell transplantation.

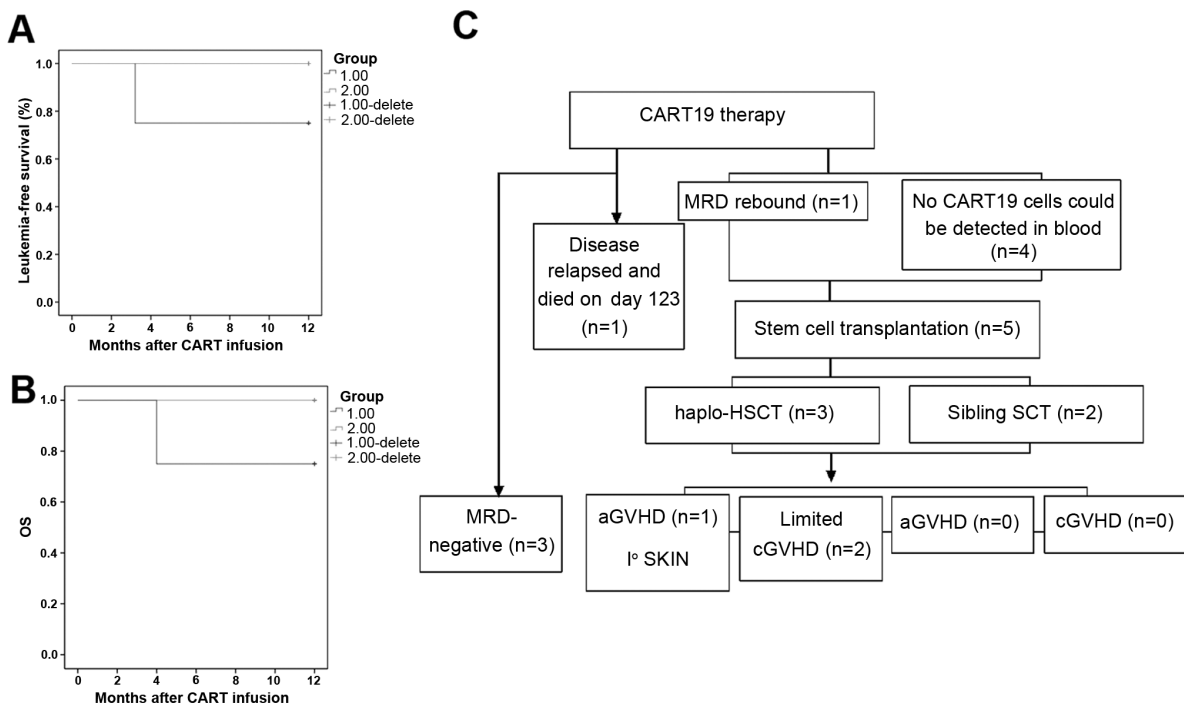


Figure 5. Outcome after CAR-T19 bridging with HSCT. (A) Leukemia-free survival of all 9 patients. Survival fractions were calculated using the Kaplan-Meier method, and lines indicate censored patients. Group 1, Bridging HSCT. Group 2, non-HSCT. (B) OS of all patients. Survival fractions were calculated using the Kaplan-Meier method, and lines indicate censored patients. Group 1, bridging HSCT. Group 2, non-HSCT. (C) Flow chart of the outcome of all 9 patients, including stem cell transplantation, donor and transplantation complications. The patient who suffered relapse 3 months after CAR-T therapy died 1 month later, thus the leukemia-free survival changed at 3 months while the OS curve changed at 4 months. CART, chimeric antigen receptor T; MRD, minimal residual disease; HSCT, hematopoietic stem cell transplantation; SCT, stem cell transplantation; a, acute; c, chronic; GVHD, graft-versus-host disease; OS, overall survival.



**Engraftment.** All 5 patients achieved sustained myeloid engraftment. Full donor chimerism was achieved in all 5 patients after transplantation. The median time for myeloid recovery was 14 days (range, 12-16 days), and the median time for platelet recovery was 15 days (range, 13-17 days).

**GVHD.** All patients with engraftment were eligible for the assessment of acute (a)GVHD. One patient had grade 1 skin aGVHD (Skin rash <25% within +100 days). A total of 5 patients survived >100 days after transplantation and were evaluated for cGVHD. Two of these patients developed limited cGVHD, whereas no patients developed extensive cGVHD.

**Outcome.** As presented in Fig. 5, eight of the 9 patients with a median follow-up time of 338 days (range, 123-365 days) maintained CR. The 1-year allo-HSCT group had an OS rate of 100% and a DFS rate of 100%. Two of the 4 patients in the untransplanted group had Ph<sup>+</sup> B cell acute lymphoblastic leukemia. Imatinib was administered orally in the absence of CAR-T cells 3 months after CD19 CAR-T cell infusion, and the patients had sustained remission. One patient refused transplantation, and the bone marrow continued to recover. One patient refused to undergo allo-HSCT, relapsed 6 months after CD19 CAR-T cell infusion, refused further chemotherapy and other treatment, and died after 9 months. The 1-year OS rate of the untransplanted group was 75%, and the DFS rate was 75%.

## Discussion

The present study aimed to investigate the feasibility, safety and efficacy of CD19 CAR-T cell therapy as a bridge to allo-HSCT in the treatment of adult patients with R/R B-ALL. The clinical outcomes of the enrolled patients were positive.

The present study demonstrated that it is feasible to determine the timing of transplantation by simultaneously monitoring MRD and the CD19 CAR-T cell ratio or qPCR values. Consistent with the results of previous studies (15,23-25), strong expansion and long-term persistence of CD19 CAR-T cells were observed in the present experiments. The duration was 3-6 months, and CD19 CAR-T cell amplification peaked between days 6 and 9. Patients with MRD<sup>-</sup> CR received allo-HSCT at a median time of 104 days after infusion. Of note, CD19 CAR-T cells in the PB of patients cannot be detected using FCM, but the CD19 CAR DNA copy number can be detected using qPCR. A possible explanation for this is that CARs antigen on the surface shed from the cell surface which can't be detected by FCM, whereas CAR DNA of transduced T was existent which can be detected via qPCR analysis (16). These findings are consistent with those of Maude *et al* (16), who reported that 3 patients did not respond to CTL019 after CAR-T cell infusion, but CTL019 expression levels peaked at 6-14 days and were detected using qPCR, whereas circulating CTL019 was not detected by FCM. In the present study, at day +90 after CAR-T cell infusion, there levels of CAR-T cells in the bone marrow were still detectable using qPCR.

MRD<sup>-</sup> was a feasible predictor of survival in the present study. This was consistent with the findings of Park *et al* (17), who used multiparameter FCM to evaluate MRD in

BM samples from days 14-28 after CAR-T cell infusion after 19-41BB treatment and demonstrated that post-treatment MRD status was a strong predictor of OS; the 6-month OS rate was 76% in the MRD<sup>-</sup> CR group and 14% in the MRD<sup>+</sup> CR group. The use of MRD monitoring to determine the timing of transplantation requires analysis of CD19 antigen expression levels and must also take into account the fact that B-ALL leukemia-initiating cells with stem cell-like characteristics that do not express CD19 also affect the therapeutic effect (26). In the present study, the leukemia cells of the patient who had response after CART19 cell treatment but relapsed after 3 months did not express CD19, and the leukemia cells had transformed into acute myeloid leukemia cells. The reason for recurrence may be that the leukemia stem cell differentiation patterns in ALL are mostly branched, rather than a single linear pattern (27,28), and there may have been at least two CD18<sup>+</sup> and CD19<sup>-</sup> leukemia cell clones in the patient. After CAR-T cell therapy killed CD19<sup>+</sup> dominant clones in large numbers, the original unaffected non-predominant myeloid leukemia cell clones became the dominant clones, and the proliferation of these cells led to leukemia recurrence. Another possibility is that antigenic shift or antigenic drift occurred in the CD19<sup>+</sup> leukemia cells. The appearance of stem cell markers (for example CD34<sup>+</sup>) and myeloid cell markers would suggest that the leukemia cells had escaped immune attack and surveillance, leading to leukemia recurrence. Therefore, after CAR-T cell treatment, MRD monitoring should not be limited to CD19<sup>+</sup> cell populations. It is particularly important to focus on the immunophenotypic changes of CD19<sup>-</sup> cell populations and other leukemia cell populations.

The results of the present study were consistent with the findings of Hu *et al* (18), who confirmed that in CART19 cell treatments for patients with R/R ALL in China, MRD and the number of previous relapses after the pre-treatment regimen were associated with the high risk of grade 3 CRS, while other risk factors included age, sex and therapies prior to the FC pre-treatment regimen (chemotherapy or allo-HSCT). CART19 cell dosage and MRD were not associated with the risk of grade 3 CRS in the present study. It was also observed that serum levels of CRP were associated with the severity of CRS. Patients with grade 3 or 4 CRS had higher peak CRP expression levels after infusion of CART19 cells compared with patients without CRS or with grade 1 or 2 CRS. In the case of patient 6, CRS was effectively improved without a significant effect on CART19 cell expansion and persistence using tocilizumab. These results are consistent with recent published findings (18).

Luznik *et al* (6) reported that following the treatment of hematologic malignancies with HLA-haploidentical HST, platelet and median granulocyte engraftment times were 15 and 24 days, respectively, and that 13% of patients experienced surgical failure. The cumulative incidence of non-relapse mortality for aGVHD was 15% at 1 year, and the recurrence rate was 51%. The OS rate at 2 years after transplantation was 36%. In the present study, patients who received allo-HSCT had a shorter platelet recovery period and no graft failure, no aGVHD or extensive cGVHD. Compared with previous stem cell transplantation studies (29-31), the patients who underwent bridging transplantation after CART19 cell

infusion had a similar engraftment time and a similar incidence of aGVHD or extensive cGVHD. The cumulative incidence of non-relapse mortality for aGVHD was lower (0%) at 1 year, and the recurrence rate was lower (0%). The OS at 1 year after transplantation was higher (100%).

The results of the present study were consistent with those of Summers *et al* (20), who evaluated the efficacy of SCRI-CAR19v1 treatment in pediatric and young adult patients with B-ALL, and 17/50 patients underwent allo-HSCT, suggesting an improvement in leukemia-free survival rate in the patients who underwent allo-HSCT compared with those who did not.

There were limitations to the present study. Due to the short duration, the number of cases was small, and the follow-up time was short. Despite the results already achieved, the long-term effects of CAR-T bridged allo-HSCT treatment remain to be determined. Determining the overall efficacy of this therapy in R/R B-ALL requires a large number of patients and comparison with the CD19 CAR-T cell treatment alone group by statistical analysis. In addition, all patients received the same chemotherapy regimen combination, dosage power and intensity (FC regimen, 1,000 mg/m<sup>2</sup> cyclophosphamide on day -4 and 25 mg/m<sup>2</sup>/day fludarabine on days -4, -3 and -2). In the future, it would be important to investigate the mortality and morbidity rates and adverse side effects of other variations of CAR-T therapy conditions, such as chemotherapy regimen combinations, dosage power and intensity.

The present study demonstrated that in patients with R/R B-ALL, the 1-year OS and DFS rates of the CD19 CAR T+HCT group were improved compared with those of the CD19 CAR T therapy alone group. For the patients with R/R B-ALL, whose CAR-T cells *in vivo* do not survive for a long period, their recurrence commonly occurred soon after CAR-T cell therapy. The bridging to allo-HSCT after CAR-T cell therapy is feasible, safe and efficient for these patients.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Authors' contributions

SJ and KY designed the study. SJ, KY, SZ, HF and YM performed the research. SJ, KY and YM wrote the manuscript. SJ, KY and YM performed the statistical analysis and interpreted the data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The protocol was approved by The First Affiliated Hospital of Wenzhou Medical University Institutional Review Board (Wenzhou, China; approval no. 2016-220). All patients provided written voluntary informed consent.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Iacobucci I and Mullighan CG: Genetic basis of acute lymphoblastic leukemia. *J Clin Oncol* 35: 975-983, 2017.
2. Terwilliger T and Abdul-Hay M: Acute lymphoblastic leukemia: A comprehensive review and 2017 update. *Blood Cancer J* 7: e577, 2017.
3. Styczynski J, Tallamy B, Waxman I, van de Ven C, Milone MC, Shaw LM, Harrison L, Morris E, Satwani P, Bhatia M, *et al*: A pilot study of reduced toxicity conditioning with BU, fludarabine and alemtuzumab before the allogeneic hematopoietic SCT in children and adolescents. *Bone Marrow Transplant* 46: 790-799, 2011.
4. Xu LP, Zhang XH, Wang FR, Mo XD, Han TT, Han W, Chen YH, Zhang YY, Wang JZ, Yan CH, *et al*: Haploidentical transplantation for pediatric patients with acquired severe aplastic anemia. *Bone Marrow Transplant* 52: 381-387, 2017.
5. Chang J, Graves SS, Butts-Miwongtum T, Sale GE, Storb R and Mathes DW: Long-term tolerance toward Haploidentical Vascularized Composite Allograft Transplantation in a Canine Model Using Bone Marrow or Mobilized Stem Cells. *Transplantation* 100: e120-e127, 2016.
6. Luznik L, O'Donnell PV and Fuchs EJ: Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. *Semin Oncol* 39: 683-693, 2012.
7. Logan AC, Zhang B, Narasimhan B, Carlton V, Zheng J, Moorhead M, Krampf MR, Jones CD, Waqar AN, Faham M, *et al*: Minimal residual disease quantification using consensus primers and high-throughput IGH sequencing predicts post-transplant relapse in chronic lymphocytic leukemia. *Leukemia* 27: 1659-1665, 2013.
8. Bassan R, Spinelli O, Oldani E, Intermeoli T, Tosi M, Peruta B, Rossi G, Borlenghi E, Pogliani EM, Terruzzi E, *et al*: Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood* 113: 4153-4162, 2009.
9. Maude S and Barrett DM: Current status of chimeric antigen receptor therapy for haematological malignancies. *Br J Haematol* 172: 11-22, 2016.

10. Pan J, Yang JF, Deng BP, Zhao XJ, Zhang X, Lin YH, Wu YN, Deng ZL, Zhang YL, Liu SH, *et al*: High efficacy and safety of low-dose CD19-directed CAR-T cell therapy in 51 refractory or relapsed B acute lymphoblastic leukemia patients. *Leukemia* 31: 2587-2593, 2017.
11. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, *et al*: Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med* 377: 2531-2544, 2017.
12. Tiberghien P, Deconinck E and Adotevi O: More on Anti-CD19 CAR T Cells in CNS Diffuse Large-B-Cell Lymphoma. *N Engl J Med* 377: 2101-2102, 2017.
13. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, Bartido S, Stefanski J, Taylor C, Olszewska M, *et al*: CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med* 5: 177ra38, 2013.
14. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, Chung SS, Stefanski J, Borquez-Ojeda O, Olszewska M, *et al*: Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 6: 224ra25, 2014.
15. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, *et al*: T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A phase 1 dose-escalation trial. *Lancet* 385: 517-528, 2015.
16. Maude SL, Teachey DT, Porter DL and Grupp SA: CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood* 125: 4017-4023, 2015.
17. Park JH, Riviere I, Gonen M, Wang X, Sénéchal B, Curran KJ, Sauter C, Wang Y, Santomasso B, Mead E, *et al*: Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. *N Engl J Med* 378: 449-459, 2018.
18. Hu Y, Wu Z, Luo Y, Shi J, Yu J, Pu C, Liang Z, Wei G, Cui Q, Sun J, *et al*: Potent Anti-leukemia Activities of Chimeric Antigen Receptor-Modified T Cells against CD19 in Chinese Patients with Relapsed/Refractory Acute Lymphocytic Leukemia. *Clin Cancer Res* 23: 3297-3306, 2017.
19. Park J, Riviere I, Wang XY, Bernal Y, Purdon T, Halton E, Wang Y, Curran KJ, Sauter CS, Sadelain M, *et al*: Implications of Minimal Residual Disease Negative Complete Remission (MRD-CR) and Allogeneic Stem Cell Transplant on Safety and Clinical Outcome of CD19-Targeted 19-28z CAR Modified T Cells in Adult Patients with relapsed, Refractory B-Cell ALL. *Blood* 126: 682, 2015.
20. Summers C, Annesley C, Bleakley M, Dahlberg A, Jensen MC and Gardner R: Long Term Follow-up after SCRI-CAR19v1 Reveals Late Recurrences As Well As a Survival Advantage to Consolidation with HCT after CAR T Cell Induced Remission. *Blood* 132 (Supp. 1): 967, 2018.
21. Cheng Z, Wei R, Ma Q, Shi L, He F, Shi Z, Jin T, Xie R, Wei B, Chen J, *et al*: In vivo expansion and antitumor activity of coinfused CD28- and 4-1BB-engineered CAR-T cells in patients with B cell leukemia. *Mol Ther* 26: 976-985, 2018.
22. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, Stetler-Stevenson M, Phan GQ, Hughes MS, Sherry RM, *et al*: B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 119: 2709-2720, 2012.
23. Harb JG, Chyla BI and Huettner CS: Loss of Bcl-x in Ph<sup>+</sup> B-ALL increases cellular proliferation and does not inhibit leukemogenesis. *Blood* 111: 3760-3769, 2008.
24. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, *et al*: Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 371: 1507-1517, 2014.
25. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, Bagg A, Marcucci KT, Shen A, Gonzalez V, *et al*: Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med* 7: 303ra139, 2015.
26. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, *et al*: Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 368: 1509-1518, 2013.
27. Bozic I, Antal T, Ohtsuki H, Carter H, Kim D, Chen S, Karchin R, Kinzler KW, Vogelstein B and Nowak MA: Accumulation of driver and passenger mutations during tumor progression. *Proc Natl Acad Sci USA* 107: 18545-18550, 2010.
28. Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, Taylor C, Yeh R, Bartido S, Borquez-Ojeda O, *et al*: Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* 118: 4817-4828, 2011.
29. Bachanova V, Sandhu K, Yohe S, Cao Q, Burke MJ, Verneris MR and Weisdorf D: Allogeneic hematopoietic stem cell transplantation overcomes the adverse prognostic impact of CD20 expression in acute lymphoblastic leukemia. *Blood* 117: 5261-5263, 2011.
30. Huang XJ, Zhu HH, Chang YJ, Xu LP, Liu DH, Zhang XH, Jiang B, Jiang Q, Jiang H, Chen YH, *et al*: The superiority of haploidentical related stem cell transplantation over chemotherapy alone as postremission treatment for patients with intermediate- or high-risk acute myeloid leukemia in first complete remission. *Blood* 119: 5584-5590, 2012.
31. Villalobos IB, Takahashi Y, Akatsuka Y, Muramatsu H, Nishio N, Hama A, Yagasaki H, Saji H, Kato M, Ogawa S, *et al*: Relapse of leukemia with loss of mismatched HLA resulting from uniparental disomy after haploidentical hematopoietic stem cell transplantation. *Blood* 115: 3158-3161, 2010.