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Potent anti-leukemia activities of humanized CD19-targeted Chimeric antigen receptor T (CAR-T) cells in patients with relapsed/refractory acute lymphoblastic leukemia

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Abstract

Chimeric antigen receptor T (CAR-T) cell therapy has shown promising results for relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL). The immune response induced by murine single-chain variable fragment (scFv) of the CAR may limit CAR-T cell persistence and thus increases the risk of leukemia relapse. In this study, we developed a novel humanized scFv from the murine FMC63 antibody. A total of 18 R/R ALL patients with or without prior murine CD19 CAR-T therapy were treated with humanized CD19-targeted CAR-T cells (hCART19s). After lymphodepletion chemotherapy with cyclophosphamide and fludarabine, the patients received a single dose (1×10^6/kg) of autologous hCART19s infusion. Among the 14 patients without previous CAR-T therapy, 13 (92.9%) achieved complete remission (CR) or CR with incomplete count recovery (CRi) on day 30, whereas 1 of the 3 patients who failed a second murine CAR-T infusion achieved CR after hCART19s infusion. At day 180, the overall and leukemia-free survival rates were 65.8% and 71.4%, respectively. The cumulative incidence of relapse was 22.6%, and the nonrelapse mortality rate was 7.1%. During treatment, 13 patients developed grade 1-2 cytokine release syndrome (CRS), 4 patients developed grade 3-5 CRS, and 1 patient experienced reversible neurotoxicity. These results indicated that hCART19s could induce remission in patients with R/R B-ALL, especially in patients who received a reinfusion of murine CAR-T.

1 | BACKGROUND

The adoptive transfer of chimeric antigen receptor T (CAR-T) cell represents a highly promising strategy to fight against multiple cancers.[1–5] Although CARs are generated for a variety of cell surface molecules, such as HER2, mesothelin and carbonic anhydrase IX (CAIX),[6–8] the most impressive therapeutic effect is observed in the use of CD19-targeted CAR-T cells in treating B cell malignancies.[9–14] The available results show that 70%-90% of relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL) patients who receive CD19 CAR-T therapy achieve complete remission (CR)[15] and that the regression of leukemia cells is closely associated with the proliferation and persistence of CD19 CAR-T cells. However, the disappearance or loss of CAR-T cells is often accompanied by the recovery of normal B cells and an increased risk of recurrent CD19^{+} leukemia. Several factors might be involved in the inactivation of CD19 CAR-T cells in vivo. One of these factors is the HLA-restricted T cell-mediated immune response, resulting from targeting specific epitopes that specifically bind to antigens

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derived from a murine single-chain variable fragment (scFv) in the CAR structure.[8,16] Humanized scFv is expected to reduce the immunogenicity of CAR, thereby promoting the survival time and improving the therapeutic efficacy of CAR-T cells.[17]

The scFvs of CD19 CAR in published clinical trials are primarily derived from murine FMC63 or SJ25C1 antibodies. Clinical studies on the humanized CD19 CAR-T treatment of R/R B-ALL are currently limited. In the present study, 18 patients with R/R B-ALL were treated with humanized CD19 CAR-T cells (hCART19s) derived from the murine FMC63 antibody to evaluate the efficacy and safety profiles and pursue an optimal CAR-T-based therapeutic strategy in R/R B-ALL patients.

2 | METHODS

2.1 | Study design and patient selection

We performed a pilot phase I study to assess the efficacy and safety of the infusion of autologous T cells expressing humanized CD19 CAR/4-1BB/CD3-ζ/T2A-EGFRt for the treatment of R/R B-ALL (ClinicalTrials. gov # NCT02782351). The following inclusion criteria were used: (1) Age less than 70 years; (2) R/R CD19$^+$ B-ALL patients who had not been treated with CAR-T cells infusion or had no response or relapsed after prior murine CD19 CAR-T therapy; and (3) measurable disease and adequate performance status and organ function. The protocols were approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University. All patients enrolled and treated on this trial signed written informed consents before participation. All clinical investigations were conducted according to the Declaration of Helsinki principles.

2.2 | hCART19s generation

The humanized scFv sequence specific for CD19 was derived from Clone FMC63 as previously described.[18] The humanized scFv sequence was inserted in tandem with the human CD8 transmembrane, CD8 hinge, 4-1BB costimulatory domain, CD3ζ intracellular regions and T2A-EGFRt sequence.[16,19] The hCART19s were acquired through the transfection of lentivirus carrying a CAR sequence of humanized anti-CD19 scFv into peripheral CD3$^+$ T cells. Detailed information is provided in Supporting Information methods.

2.3 | Quality control of hCART19s prior to infusion

To evaluate the therapeutic potential of hCART19s, we conducted preclinical assessments of the effect of hCART19s in vitro and on xenograft animal models (see Supporting Information methods). All mouse studies were performed in accordance with the guidelines for the Welfare Of Animals In Experimental Neoplasia by the United Kingdom Coordinating Committee on Cancer Research (UKCCCR). In addition, bacteria, fungi, chlamydia, mycoplasma, and endotoxin were detected twice to prevent potential contamination before hCART19s infusion.

2.4 | hCART19s infusion

Peripheral blood mononuclear cells (PBMCs) were obtained from patients by leukapheresis for hCART19s preparation on day −11, and the first day of hCART19sinfusion was set on day 0. To remove endogenous lymphocytes before hCART19s infusion, patients received FC-based lymphodepletion chemotherapy consisting of fludarabine (30 mg/m^2/day, days −5 to −2) and cyclophosphamide (750 mg/m^2, day −5). On day 0, the patients received a single dose of autologous hCART19s infusion at 1×10^6 EGFRt$^+$ cells/kg.

2.5 | Clinical response assessment

To assess the efficacy of hCART19s, peripheral blood (PB) and bone marrow samples from each patient were obtained every 1–2 months before and after infusion. Cytokine release syndrome (CRS) grading and other toxicities were assessed as previously described.[20] CR, no remission (NR) and CR with incomplete count recovery (CRi) were defined according to the National Comprehensive Cancer Network (NCCN) guideline, version 1.2016. Minimal residual disease (MRD) negative was defined as the absence of immunophenotypically abnormal blasts in BM, which was determined by flow cytometry (FACS) (limit of detection 1:10 000).

2.6 | Assessment of hCART19s expansion and persistence

Blood samples were obtained from patients before and at intervals after hCART19s infusion, and FACS was used to identify the persistence of hCART19s. Circulating hCART19 numbers per microliter were calculated on the basis of absolute CD3$^+$ T lymphocyte counts. In addition, CAR DNA copies were assessed by quantitative real-time PCR (QPCR), as described in Supporting Information methods.

2.7 | Measurement of serum and cerebrospinal fluid (CSF) cytokine concentrations

The concentrations of IL-2, IL-6, IL-10, IFN-γ and TNF-α in serum and CSF were measured by ELISA according to the manufacturer's instructions (R&D systems, Minneapolis).

2.8 | Statistics

Comparisons of continuous variables and risk factors that may influence variations in severe CRS development were performed using the Mann-Whitney U test for two groups. The Kaplan–Meier approach was performed to estimate time-to-event analyzes. All statistical analyzes were performed using SPSS, and P values less than .05 were considered significant.

3 | RESULTS

3.1 | Preclinical evaluation of hCART19s

Based on mouse anti-human CD19 antibody (FMC63), we obtained the humanized anti-human CD19 antibody (Clone H3L2) with high affinity (Supporting Information Figure S1A). In vitro experiments demonstrated that hCART19s carrying the CAR sequence of humanized

anti-CD19 scFv could efficiently eliminate CD19-expressing cells (Supporting Information Figure S1B,C). The secretion of cytokines by hCART19s, IL-2 and IFN-γ, was dramatically increased following engagement with the target cells (Supporting Information Figure S1D, E). Moreover, in a human leukemia xenograft animal model, we found that hCART19s were capable of eradicating Nalm6 cells in NOD/SCID mice (Supporting Information Figure S1F). These data showed that hCART19s targeting human CD19 antigen were functional both in vitro and in vivo.

3.2 | Patient characteristics

From May 2016 to August 2017, 18 R/R CD19$^+$ B-ALL patients, aged from 3 to 57 years old, with a median age of 14 years old, were enrolled into the present trial (Table 1). All patients received 2 to 17 intensive therapies, with a median number of 9. Patients No.1–15 with R/R B-ALL had not previously received CAR-T therapy, whereas patients No.16–18 did not respond to a secondary infusion of murine CD19 CAR-T therapy after relapse from the first infusion. Additionally, 17/18 patients presented leukemia cells in the bone marrow before hCART19s infusion, and 1 patient showed no leukemia cells in bone marrow but was MRD positive and experienced extramedullary relapse. A total of 4 patients had central nervous system leukemia (CNSL), and 1 had testicular leukemia (TL).

3.3 | Clinical response after hCART19s infusion

On day 30 after infusion, the treatment response was evaluated in 17/18 patients (Table 1), as 1 patient (No. 5) died in the early phase of treatment due to cerebral hemorrhage (grade 5). Among the 14 patients (No. 1–4 and 6–15) without CAR-T therapy previously, 13/14 (92.9%) patients achieved CR or CRi on day 30, in which 11 patients were MRD negative and 2 patients were MRD positive. Among the 13 patients who achieved remission (CR or CRi), 3 patients (No. 6, 11, and 12) with extramedullary relapse also achieved CR, as confirmed by CSF and ultrasound examinations. Patient No. 14 had no response to hCART19 therapy due to the lack of activation and expansion of CAR-T cells. Three patients (No. 16–18) received murine CD19 CAR-T therapy twice, and only patient No. 16 achieved CR with MRD negative after hCART19s infusion.

3.4 | In vivo expansion and persistence of hCART19s

The amplification and survival of peripheral hCART19s were monitored by FACS and QPCR analyzes (Figure 1). In 18 patients, the amplification of hCART19s peaked on days 7–14 after infusion, followed by a decrease in the cell number and copies of hCART19s with a prolonged follow-up period. FACS analysis showed that hCART19s was not detectable on day 30 in 15 patients, with only 2 patients presenting few hCART19s on day 60 (Figure 1A,B). In the PB of patients with grade 3–5 CRS, the median count of hCART19s was higher than that in patients with grade 0–2 CRS, 406 per μl (95% CI 183–596) VS 109 per μl (95% CI 76–142) ($P = .0049$) respectively (Figure 1C). Due to the limited sensitivity of FACS analysis, we also used QPCR to detect the presence of hCART19s. QPCR analysis demonstrated the existence of hCART19s in most patients on day 60 with the longest survival time being 1 year after infusion (Figure 1D,E). The median CAR DNA copy number in patients with grade 3–5 CRS was higher than that in patients with grade 0–2 CRS, 118 100 per μg (95% CI 60 700-201 900) VS 64,430 per μg (95% CI 43 760-76 220) respectively ($P = .0035$) (Figure 1F). Notably, 3 patients (No. 16, 17, and 18) achieved CR after the first infusion of murine CD19 CAR-T cells and QPCR assay showed that the CAR DNA copies were amplified. However, the secondary murine CAR-T therapy was ineffective when these patients relapsed again, and no amplification of the CAR DNA copies was detected by QPCR. Next, we tried to eliminate CD19$^+$ leukemia cells through hCART19s infusion. After infusion of hCART19s, patient No. 16 displayed an increase in CAR DNA copies and achieved CR on day 30 (Figure 1G). However, patient No.17 did not show any increase in the copy number of hCART19s after infusion or in anti-leukemia activity (Figure 1H). Interestingly, patient No.18 showed an increase in the copy number of hCART19s but without anti-leukemia activity (Figure 1I).

3.5 | Prognosis

The median follow-up time was 244 days (ranging from 105 to 624 days) in these 17 patients, except patient No. 5. In the 14 patients receiving hCART19s infusion who achieved CR or CRi, there were 7 patients maintaining CR with MRD negative (Table 1). Patient No. 6 received HLA matching unrelated donor allogeneic hematopoietic stem cell transplantation (allo-HSCT) after achieving CR. However, this patient died from severe graft-versus-host disease (GVHD) and infections on day 59 after transplantation. Patient No. 7 received HLA matching sibling allo-HSCT after CR and is currently still in CR with MRD negative. Patients No. 10 and 12 refused to accept allo-HSCT after CR, considering the transplantation-associated risks, but received autologous HSCT (auto-HSCT) and are currently in CR with MRD negative. A total of 4/14 patients with CR at 1 month subsequently relapsed, among whom 3 showed CD19^{high} blasts (Patients No. 1, 4 and 9, Supporting Information Figure S2A) and 1 showed both CD19^{high} and CD19^{dim} blasts (Patient No. 3, Supporting Information Figure S2B). In the enrolled patients, at 180 days, the overall survival (OS) rate was 65.8% (95% CI 39.1%-83.0%) (Figure 2A), and the leukemia-free survival (LFS) rate was 71.4% (95% CI 40.6%-88.2%) (Figure 2B). The cumulative incidence of relapse (CIR) was 22.6% (Figure 2C), and the nonrelapse mortality (NRM) rate was 7.1% (Figure 2D).

3.6 | Toxicities after hCART19s infusion

In our study, 17 of the 18 patients (94.4%) experienced CRS. Among these patients, 13 had grade 1–2 CRS, and 4 had grade 3–5 CRS (Figure 3A). CRS primarily occurred within a median of 6 days after infusion (range 1 to 9 days) and lasted for a median of 7 days (range 2 to 14 days). Fever with higher temperature was observed in all 17 patients and lasted longer period for patients with grade 3–5 CRS

TABLE 1 The characteristics of patients and clinical responses after hCART19s infusion

| Patient No. | Age (yr) | Prior intensive therapies | Before hCART19 cell therapy | | | Infused hCART19 cells | | CRS | | | Neurotoxicity | Disease response at 1 month | Outcomes (follow-up time, days) |
			Extramedullary disease	BM blasts (%)	MRD (%)	Percent transduced (%)	Dosage (10^6/kg)	Grade	Tocili	Steroid			
1	19	3	N	3	8.01	31.7	1	1	N	N	Y	CR, MRD-	Relapsed on day 119 and died (381)
2	13	15	N	2	5.32	22.9	1	1	N	N	N	CR, MRD-	MRD-negative (624)
3	3	7	N	82	72.21	43.6	1	2	Y	N	N	CR, MRD+	Relapsed on day 131 and died (244)
4	50	4	N	70	75.65	36.4	1	3	Y	N	N	CRi, MRD+	Relapsed on day 100 and died (176)
5	34	12	N	75	70.43	25.7	1	5	Y	N	N	NA	Died of intracranial hemorrhage on day 14 (14)
6	20	3	CNSL, TL	80	71.85	38.2	1	3	Y	N	N	CR, MRD-	Underwent allo-HSCT on day 46 and complicated with GVHD and died of infection (105)
7	52	16	N	61	68.45	32.1	1	1	N	N	N	CR, MRD-	MRD-negative (429)
8	21	14	N	25	31.5	23.7	1	1	N	N	N	CR, MRD-	MRD-negative (414)
9	6	13	N	60	40.0	38.5	1	2	N	N	N	CR, MRD-	Relapsed on day 205 (414)
10	5	2	N	18	13.3	41.8	1	2	Y	N	N	CR, MRD-	Underwent auto-HSCT on day 138 (350)
11	27	7	CNSL	0	0.5	35.4	1	1	N	N	N	CR, MRD-	MRD-negative (350)
12	13	16	CNSL	12	12.2	25.2	1	1	N	N	N	CR, MRD-	Underwent auto-HSCT on day 84 (289)
13	15	2	N	16	18.1	36.3	1	1	N	N	N	CR, MRD-	Underwent allo-HSCT on day 30 (207)
14	7	5	N	80	79.5	55.4	1	1	N	N	N	NR	Underwent salvage chemotherapy and died (125)
15	57	4	N	65	60.2	38.3	1	1	N	N	N	CR, MRD-	MRD-negative (179)
16	9	17	N	86	83.4	37.1	1	1	N	N	N	CR, MRD-	MRD-negative (168)
17	7	11	CNSL	85	85.4	50.2	1	0	Y	N	N	NR	Underwent salvage chemotherapy and died (169)
18	7	15	N	22	24.16	41.6	1	3	Y	Y	N	NR	Underwent salvage chemotherapy and died (145)

allo-HSCT, allogeneic hematopoietic stem cells transplantation; auto-HSCT, autologous hematopoietic stem cells transplantation; BM, bone marrow; CR, complete remission; CRi, CR with incomplete count recovery; CRS, cytokine release syndrome; CNSL, central nervous system leukemia; GVHD = graft-versus-host disease; MRD, minimal residual disease; N, no; NA, not available; NR, no remission; TL, testicular leukemia; Tocili, tocilizumab; Y, yes.
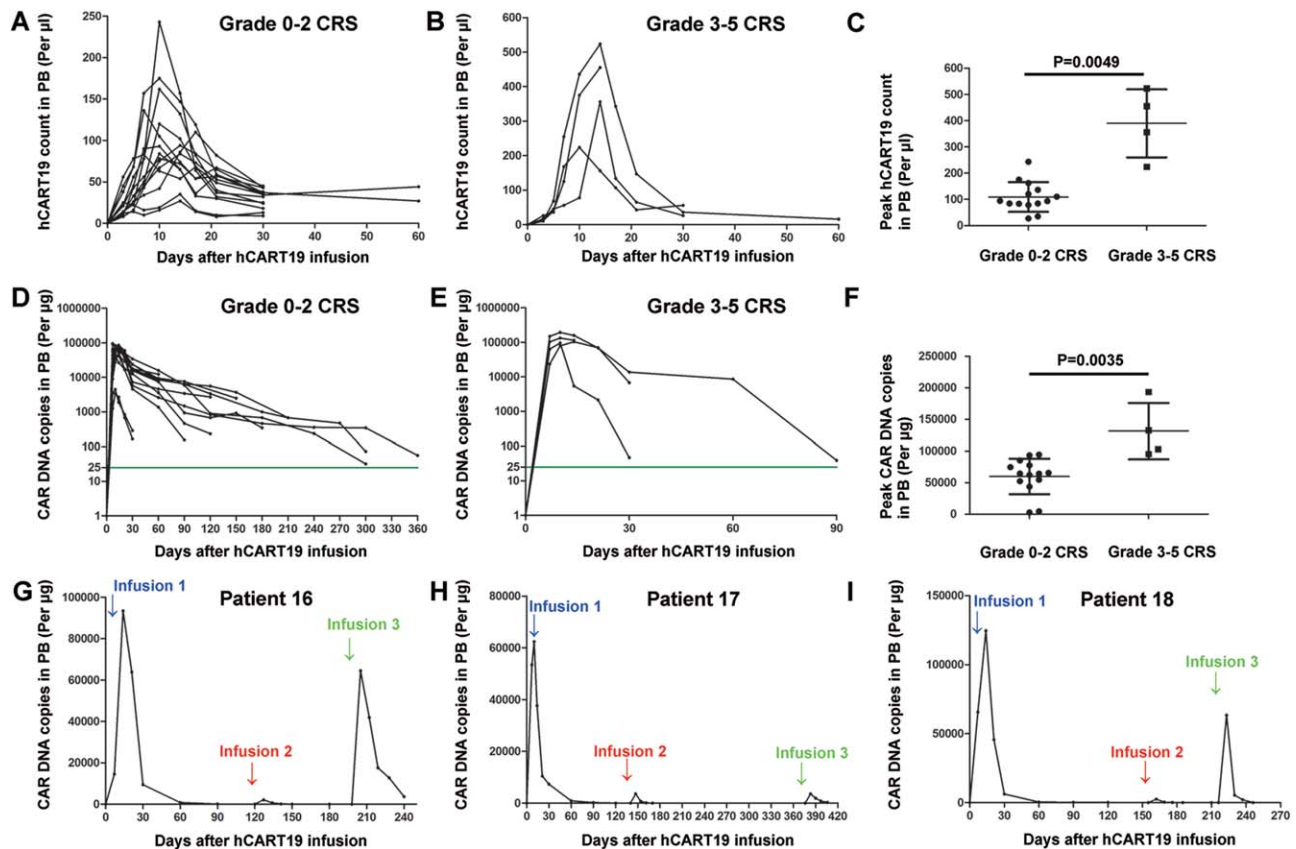
FIGURE 1 hCART19s engraftment, expansion and persistence in vivo. (A-B) Counts of hCART19s in peripheral blood (PB) were, respectively, assessed by FACS at serial time points before and after infusion of hCART19s in patients who developed grade 0–2 CRS and grade 3–5 CRS. (C) Peak counts of hCART19s in the grade 0–2 CRS group and the grade 3–5 CRS group. (D-E) CAR DNA copies of hCART19s in PB were, respectively, assessed by QPCR at serial time points before and after infusion of hCART19s in patients who developed grade 0–2 CRS and those who developed grade 3–5 CRS. The horizontal line at 25 copies per microgram of DNA represents the lower limit of quantification of this assay. (F) Peak CAR DNA copies of hCART19s in the grade 0–2 CRS group and the grade 3–5 CRS group. The data represent the means ± SD. The Mann-Whitney U test was used for statistical analysis. (G-I) Engraftment after each infusion was analyzed by QPCR. Each graph shows the number of CAR DNA copies detected in PBMCs collected at the indicated times after the first, second, and third CAR-T cell infusions. The times of the first (blue), second (red) and third (green) CAR-T cell infusions are noted on the graphs [Color figure can be viewed at wileyonlinelibrary.com]

compared to patients with grade 1–2 CRS (Figure 3B,C). Coagulopathy, elevated prothrombin and partial-thromboplastin periods as well as severe hypofibrinogenemia were observed in 3 patients with severe CRS and 1 patient with grade 2 CRS. In addition, we measured the plasma levels of IL-2, IL-6, IL-10, IFN-γ, TNF-α, and ferritin as well as CRP in patients with CRS. We found that the levels of IL-6, IFN-γ, ferritin and CRP were increased during CRS and that the peak value in patients with grade 3–5 CRS was significantly higher than that in patients with grade 1–2 CRS (Figure 3D-G). However, CRS was reversible in most patients, except for patient No. 5. The CRS-associated symptoms were relieved in 12 patients after supportive treatment, 3 patients after supportive treatment and IL-6 receptor monoclonal antibody, and 1 patient after supportive treatment, tocilizumab and corticosteroids. In 17 patients with CRS, 14 patients achieved CR or CRi. Patients No. 14 and 18 developed grade 1 and 3 CRS, respectively, but they did not achieve CR. A previous study demonstrated the association of higher tumor burden with the severity of CRS. However, our study showed no correlation of the risk of severe CRS with age, gender, previous treatment times or tumor burden (data not shown).

Reversible neurotoxicity was only observed in 1 patient (5.6%). Headache, vomiting and transient muscle clonus in limbs were observed in patient No. 1 on day 14 after infusion, without abnormality after head screening by computed tomography and magnetic resonance imaging. CSF examination showed an increase in IFN-γ (1598 pg/mL) and IL-6 (3455 pg/mL) compared with PB (126 pg/mL for IFN-γ and 29 pg/mL for IL-6). The number of EGFRt$^+$ CAR-T cells was 9.6% relative to CD3$^+$ T cells in CSF, as demonstrated by FACS (Supporting Information Figure S3A), and QPCR analysis showed 98754 CAR copies/μg DNA in CSF and 54678 CAR copies/μg DNA in PB. After mannitol treatment, neurotoxicity was ameliorated on day 19.

CD19$^+$ cells were measured by FACS to reflect the development of B-cell aplasia (Supporting Information Figure S3B). A total of 14 patients with CR or CRi after hCART19s infusion all developed B-cell aplasia, with a median duration of 111 days. At day 180, the probability
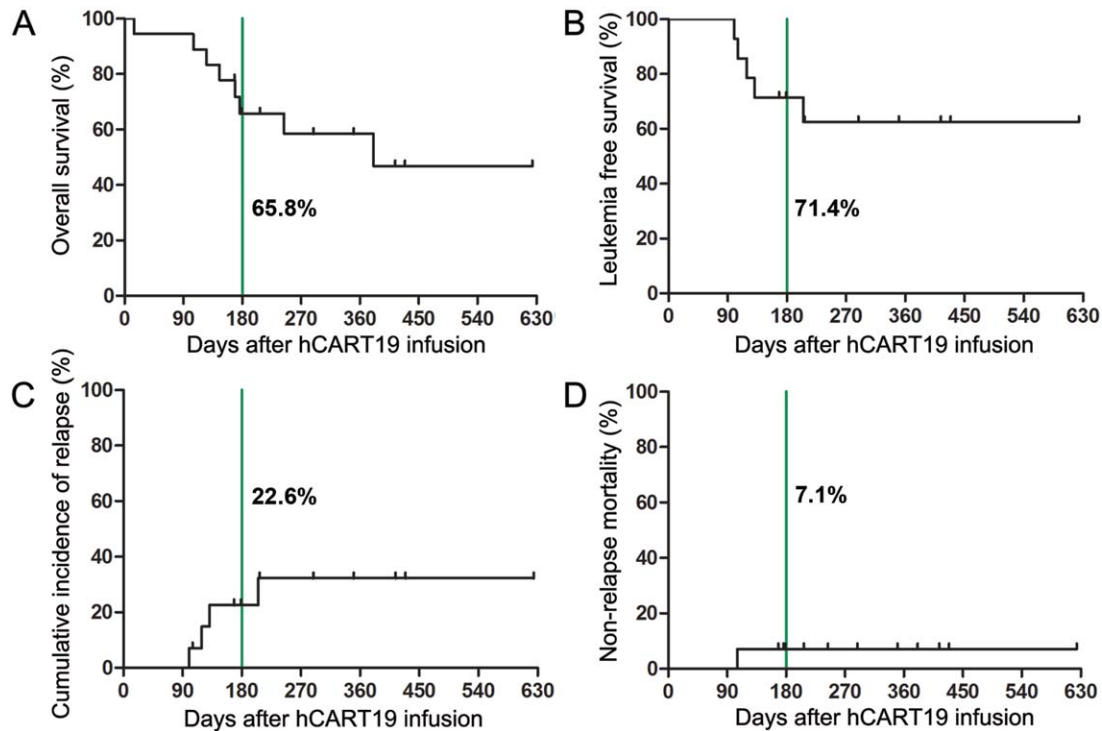
FIGURE 2 Prognosis of patients after hCART19s therapy. (A) The overall survival (OS) for all 18 patients treated in this study is shown. (B) The leukemia-free survival (LFS) of 14 patients is shown. (C) The cumulative incidence of relapse (CIR) of 14 patients is shown. (D) The non-relapse mortality (NRM) of 14 patients is shown. The survival fractions were calculated by the Kaplan–Meier method, and the lines indicate censored patients [Color figure can be viewed at wileyonlinelibrary.com]

of B-cell aplasia was 55% (Supporting Information Figure S3C). A total of 4 patients (No. 1, 3, 4, and 9) relapsed after the recovery of $CD19^+$ B cells, and 4 patients (No. 2, 7, 8 and 11) were still in CR with MRD negative, even with the recovery of $CD19^+$ B cells which were later demonstrated by FACS to have a normal phenotype. Through an infusion of immunoglobulin to maintain the level of immunoglobulin G (IgG), these patients did not develop any other adverse events, such as infections.

4 | DISCUSSION

The adoptive infusion of CD19-targeted CAR-T cells showed promising treatment effects for R/RB-ALL. Despite in vivo CAR-T cell expansion and the achievement of CR, relapses still occur after treatment in some patients with $CD19^+$ leukemia cells as a result of CAR-T loss or inhibitory tumor microenvironment.[21] Transgene-specific immune responses
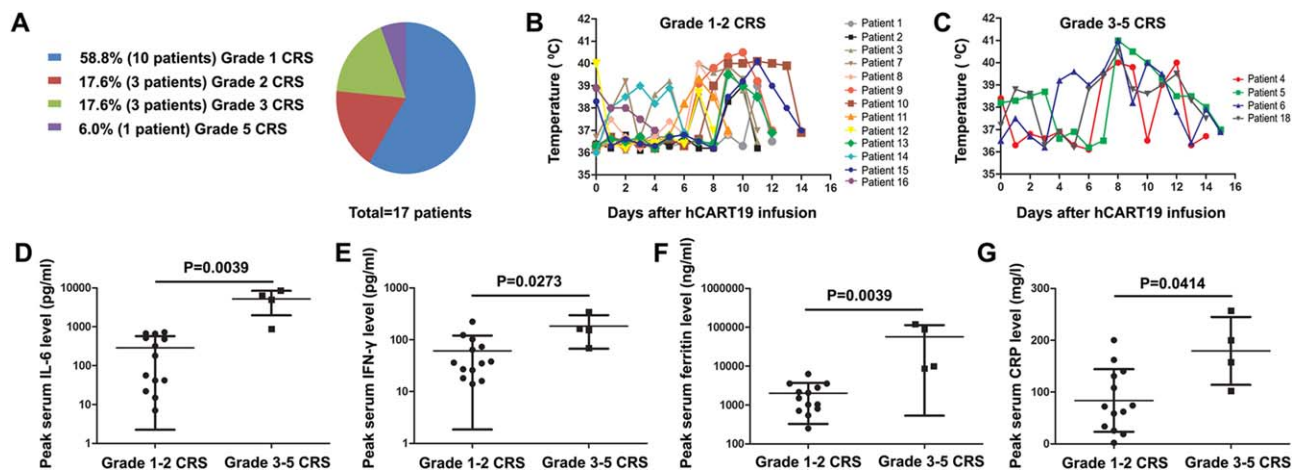

FIGURE 3 CRS complication after hCART19s therapy. (A) CRS grade distribution in 17 patients. (B-C) Fever developed in all CRS patients after infusion of hCART19s. The maximum daily temperature of each CRS patient is shown. Patients with grade 3–5 CRS had a higher temperature that lasted a longer period than those with grade 1–2 CRS. (D-G) Peak serum levels of IL-6, IFN-γ, ferritin and CRP in patients who developed grade 3–5 CRS ($n = 4$) compared with those with grade 1 or 2 CRS ($n = 13$). The data represent the means ± SD. The Mann-Whitney U test was used for statistical analysis [Color figure can be viewed at wileyonlinelibrary.com]

were demonstrated to limit the in vivo persistence of adoptively trans-ferred herpes-simplex-virus thymidine-kinase modified donor T cells, and a recent study showed that some patients treated with anti-CD19 or anti-CAIX CAR-T cells developed an immune response specific for epitopes in murine scFv and rendered subsequent T-cell infusions inef-fective.[8,16,22] Reducing the immunogenicity of CARs by using human-ized scFv may improve the longevity of CAR-T cell persistence and enhance their therapeutic efficacy in patients. In this study, we obtained humanized anti-CD19 antibodies with a similar affinity to murine antibodies. The hCART19s targeting human CD19 antigen were demonstrated to effectively eliminate leukemia cells both *in vitro* and *in vivo*. To evaluate the safety and efficacy of hCART19s, 18 patients with R/R B-ALL were recruited into this clinical trial.

On day 30 after hCART19 infusion, a high CR rate was observed in enrolled patients. In patients who had not previously received CD19 CAR-T therapy, 13 patients (92.9%) obtained CR or CRi, among whom 11 patients achieved CR with MRD negative, and the CR rate was com-parable or even superior to those in previous studies using murine scFv-derived CAR-T cells.[9–11,23] A total of 3 patients with CNSL and/or TL also obtained CR, suggesting that hCART19s, similar to murine-derived CD19 CAR-T cells, can migrate into the tumor site and exert an anti-tumor effect in bone marrow, central nervous system and the testis.[20] Additionally, the increased amplification and persistent existence of hCART19s were observed in B-ALL patients with CR or Cri, as demon-strated by FACS and QPCR. More importantly, we showed that a CD19^{+} ALL patient, who did not respond to a secondary infusion of murine CD19 CAR-T cells after relapse from the first infusion, was ben-efited from hCART19s infusion and achieved CR. This result brings hope to relapsed patients with CD19^{+} leukemia cells who did not respond to multiple infusions of murine CAR-T cells. For patients receiv-ing multiple infusions of murine CAR-T cells, we believed that the T cell immune response to the antigen encoded by murine scFv occurred after the first infusion and caused the rapid clearance of CAR-T cells after the secondary infusion, which has been demonstrated by previous studies. These data showed that humanized CD19 CAR-T cells could be used to effectively treat pediatric or adult R/R B-ALL and would benefit patients who did not respond to multiple infusions of murine CAR-T cells.

CRS is the most common acute toxicity of CAR-T cells, which is con-sidered as a systemic inflammatory response resulting from CAR-T cell acti-vation and proliferation.[24–27] In our study, 94.4% patients experienced the signs and symptoms of CRS, with 22.2% of the patients showing severe CRS, which is consistent with previous studies. Neurotoxicity is another side effect associated with CAR-T cell therapy and is often accompanied by the occurrence of CRS. Once CRS was relieved, neurotoxicity was also ameliorated.[9–11] However, neurotoxicity does not always occur with the presence of systemic CRS, and the mechanism remains unclear. In our study, reversible neurotoxicity was only observed in 1 patient (5.6%) after hCART19s infusion, and the incidence rate of neurotoxicity was lower as compared to reported data by other studies.[16,23,28–30] In addition, elevated amplification of CAR-T cells and increased cytokines were detected in CSF, which is in accordance with other reports.[9,10,23,31] However, whether increased cytokines in CSF are associated with neurotoxicity need to be illustrated in prospective studies and with more samples.

Relapse remains a major problem for CD19 CAR-T cell therapy, despite a promising high CR rate. In line with other studies, loss of CAR-T cells is one of the causes for relapse. We found 4 patients relapse between 100–250 days after hCART19s infusion because of limited persistence and loss of hCART19s in vivo. Although humanized modification may decrease the immunogenicity, prolong CAR-T cell persistence and improve therapeu-tic efficiency, the limitation is still occurred in the present study. Neverthe-less, allo-HSCT is a potential approach to reduce leukemia relapse in patients who achieved CR after CD19 CAR-T therapy. Some studies showed that CAR-T cells might effectively serve as a "bridge to transplant" that potentially could improve the outcome of allo-HSCT for these patients.[10,30,32] However, a previous study in MSKCC showed that the 6-month OS did not significantly differ between patients who underwent allo-HSCT after achieving CR with CD19 CAR-T cells and those who did not.[33] In the present study, 2 patients received allo-HSCT after achieving CR by hCART19s therapy, among whom 1 patient is currently in CR with MRD-negative and the other patient died from GVHD and severe infec-tion. With a limited sample size and follow-up period, these results showed that allo-HSCT can reduce the leukemia relapse for patients who achieved CR after hCART19s therapy; however, transplantation-associated compli-cations might affect the disease-free survival. More importantly, for the first time, we reported 2 patients receiving auto-HSCT maintained CR with MRD negative after achieving CR through hCART19s therapy. These results showed that auto-HSCT may be another strategy to reduce leuke-mia relapse for patients with R/R B-ALL who cannot receive allo-HSCT after achieving CR with CAR-T therapy.

5 | CONCLUSIONS

Taking the results together, this study demonstrated that hCART19s have high therapeutic efficacy in children and adults with R/R B-ALL. More importantly, hCART19s was confirmed to exert anti-leukemic activities in patients who did not respond to multiple murine CAR-T infusions. In patients with R/R B-ALL, an in-depth study of the persist-ence of hCART19s and the anti-CAR immune response will be critical to improve the sustained remission rate.

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