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Ibrutinib improves the efficacy of anti-CD19-CAR T-cell therapy in patients with refractory non-Hodgkin lymphoma

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Abstract

The efficacy and side effects of the second-time humanized CD19 chimeric antigen receptor (CD19-CAR) T-cell therapy after unsuccessful first-time anti-CD19-CAR T-cell therapy and subsequent ibrutinib salvage treatment were observed in patients with refractory B-cell lymphoma. In our study, 3 patients with refractory mantle cell lymphoma (MCL) and 4 patients with refractory follicular lymphoma (FL) reached stable disease (SD), partial remission (PR), or progression of disease (PD) after first-time humanized anti-CD19-CAR T-cell therapy. They received ibrutinib as a salvage treatment and kept an SD in the following 7-16 mo, but their disease progressed again during ibrutinib salvage treatment. All 7 patients received a second-time humanized anti-CD19-CAR T-cell therapy, which was the same as their first-time anti-CD19-CAR T-cell therapy. In total, 3 MCL patients and 3 FL patients reached complete response (CR) with the second-time anti-CD19-CAR T-cell therapy combined with ibrutinib, whereas 1 FL patient reached PR. There were no differences in the transduction efficiency and proliferation between the 2 instances of anti-CD19-CAR T-cell therapy. However, the second-time anti-CD19-CAR T-cell therapy led to higher peaks of anti-CD19-CAR T cells and anti-CD19-CAR gene copies, but also to higher grades of cytokine release syndrome (CRS) and more serious hematological toxicity. The successful outcome of the second-time anti-CD19-CAR T-cell therapy might suggest that the previous ibrutinib treatment improved the activities of anti-CD19-CAR T cells.

KEYWORDS

chimeric antigen receptor, cytokine release syndrome, ibrutinib, non-Hodgkin lymphoma, programmed death-1

1 | INTRODUCTION

B-cell non-Hodgkin lymphoma (B-NHL) is a lymphoproliferative disorder. Although R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) regimens achieve high overall response rates (ORRs), most patients still face inevitable relapse and poor prognosis.[1-3] As a Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib has been approved in chronic lymphocytic leukemia

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Trial registration: *ChiCTR-ONN-16009862*.

(CLL) and mantle cell lymphoma (MCL).[4-6] As a single agent, ibrutinib has achieved 30%-55% ORRs in early-phase clinical trials in patients with relapsed/refractory follicular lymphoma (FL).[7,8] BTK inhibitors have greatly improved the outcomes in patients with MCL, but patients who experience a disease progression during BTK inhibitor therapy face a poor prognosis.[9,10] Moreover, despite marked curative effect of BTK inhibition as monotherapy, the resistance might develop, which should not be ignored.[11]

Recently, CD19-CAR T-cell therapy has shown significant curative effect in R/R B-NHL patients.[12-14] However, complete response (CR) rates of R/R B-NHL patients treated with anti-CD19-CAR T-cell therapy were relatively low.[14,15] Patients with R/R large B-cell lymphoma who received anti-CD19-CAR T-cell therapy showed an ORR rate and CR rate of 82% and 54%, respectively.[13] R/R MCL and FL also have a good response to anti-CD19-CAR T-cell therapy.[16,17] The tumor microenvironment (TME), which is characterized by dysfunctional blood vessels, could hinder the delivery of immunotherapeutic agents and cause immunosuppression.[18] The presence of bulky masses might hinder the infiltration of T lymphocytes, thereby reducing the effectiveness of anti-CD19-CAR T-cell therapy.[19] Our previous study provided evidence that ibrutinib could improve the curative effect of anti-CD19-CAR T cells in Raji cell subcutaneous tumorigenic mice other than in the tail vein tumorigenic model. It suggested that ibrutinib might have a synergistic effect with anti-CD19-CAR T cells by improving the TME.[20]

In this clinical study, 7 R/R B-NHL patients who had achieved stable disease (SD), partial remission (PR), or progression of disease (PD) with their first-time anti-CD19-CAR T-cell therapy received salvage ibrutinib treatment for 7-16 mo. CR was achieved in 6 patients and PR in 1 patient in their second-time anti-CD19-CAR T-cell therapy after 7-16 mo of ibrutinib salvage therapy. We compared the efficacy and adverse events (AEs) between the first-time and second-time anti-CD19-CAR T-cell therapies. The second-time anti-CD19-CAR T-cell therapy showed better efficiency and anti-CD19-CAR T-cell amplification than the first-time therapy. Nevertheless, the AEs were more severe in the second-time anti-CD19-CAR T-cell therapy than in the first-time therapy. Ibrutinib might play an important role in the success of the second-time anti-CD19-CAR T-cell therapy.

2 | PATIENTS AND METHODS

2.1 | Medical history of patients before anti-CD19-CAR T-cell therapy

The 7 refractory B-NHL patients of the observational case series were admitted to the Department of Hematology in Tianjin First Central Hospital between January 2017 and January 2019. Three of them were refractory MCL patients (ibrutinib was not yet available in China at the time they were diagnosed with refractory B-NHL). Four patients were refractory FL patients. Before they were diagnosed as refractory B-NHL, they all received previously at least third-line treatment, including rituximab combination regimens. The cutoff date was September 30, 2020.

2.2 | Transduction and function detection of anti-CD19-CAR T cells

Peripheral blood mononuclear cells (PBMCs) were collected from all the refractory B-NHL patients and isolated by Ficoll density gradient centrifugation. The procedures for CAR T-cell production and quality-control assays were conducted in accordance with procedures described in the literature.[21] The anti-CD19-CAR transduction efficiency was analyzed by flow cytometry (FCM) on the 12th day of cultivation. 2-5 $\times$ 10^6 total anti-CD19-CAR T cells were isolated and stained with antibodies (1:200) for 15 min at room temperature. The ratio of anti-CD19-CAR T cells in $CD3^+$ T cells was determined using anti-CD19-CAR-phycoerythrin (PE; 1:200; Shanghai Genbase Biotechnology Co., Ltd) and anti-CD3-allophycocyanin (APC; 1:200; Catalog number GMP-10977-H001, Miltenyi Biotec, Inc). The release of interferon-gamma (IFN-γ) was detected by enzyme-linked immunosorbent assay (ELISA) following co-culture of the 2 times anti-CD19-CAR T cells and JEKO-1 cells for 48 h. Cytotoxicity assay was conducted at a 1:1 effector target ratio of the 2 times anti-CD19-CAR T cells and JEKO-1 cells, and a 10:1 effector target ratio of T cells and JEKO-1 cells for 24 h in the absence of supplemented cytokines. Cytotoxicity was detected using a lactate dehydrogenase (LDH) cytotoxicity test kit (Dojindo Molecular Technologies, Inc) at 490 nm. Then, release of IFN-γ and cytotoxicity of the second-time anti-CD19-CAR T cells in the presence of ibrutinib in vitro were detected under the same conditions. The dose of ibrutinib was set at 5 μmol/L based on our previous research.[20] The expression of PD-1 in $CD3^+$ T cells in peripheral blood was observed by FCM before 2 times anti-CD19-CAR T-cell production and on days 0, 7, 14, 21, and 28 after CAR T-cell infusion. Then, PD-1 expression was observed every 2 mo until the day of cutoff date or death.

2.3 | Clinical response criteria

At 1 mo and 2 mo after the first-time and second-time anti-CD19-CAR T-cell therapy, we evaluated the efficacy in all patients. CR, PR, SD, and PD were defined in accordance with the Lugano Revised Criteria for Response assessment.[22]

2.4 | The first-time anti-CD19-CAR T-cell therapy and ibrutinib therapy

In total, 7 patients were diagnosed with refractory B-NHL and received their first-time anti-CD19-CAR T-cell therapy. They were enrolled in a clinical trial of humanized anti-CD19-CAR T-cell therapy (*ChiCTR-ONN-16009862*) in our hospital. They signed informed

consent before the clinical trial. PBMCs for this anti-CD19-CAR T-cell therapy were collected using leukapheresis and isolated by Ficoll density gradient centrifugation. Fludarabine (30 mg/m^2) and cyclophosphamide (400 mg/m^2) from day 4 to day 2 as a lymphodepleting chemotherapy were administered to all patients. On the 12th day of cultivation, transduction efficiencies of anti-CD19-CAR were analyzed by flow cytometry (FCM). Autologous anti-CD19-CAR T cells were infused on day 0 (2×10^6 cells/kg).

Patients reached PR, SD, or PD after the first-time humanized anti-CD19-CAR T-cell therapy. Next, all patients received ibrutinib treatment 1 mo later as a salvage therapy after all AEs of CAR T-cell therapy. Patients Nos. 2-7 received 420 mg ibrutinib per day, considering their body weight of 60-80 kg, whereas the patient No. 1 received 560 mg ibrutinib per day given that their weight was 115 kg. During 7-16 mo of salvage ibrutinib treatment, they had a SD. The duration of ibrutinib in patients Nos. 1-7 was 14, 8, 16, 7, 9, 15, and 13 mo, respectively.

2.5 | The second-time anti-CD19-CAR T-cell therapy and ibrutinib therapy

Patients received ibrutinib as a salvage treatment after the first-time anti-CD19 CAR T-cell therapy until their disease progressed again. However, the disease progressed again without stopping their ibrutinib therapy. Therefore, they were administered the same anti-CD19-CAR T-cell therapy (*ChiCTR-ONN-16009862*) again combined with ibrutinib therapy in our hospital because they had no other treatment options. PBMCs for the second-time anti-CD19-CAR T-cell therapy were collected by the second leukapheresis from all 7 patients.

After the second-time anti-CD19 CAR T-cell therapy, all 7 patients received ibrutinib as a maintenance treatment until death or occurrence of intolerable side effects.

2.6 | Anti-CD19-CAR T-cell proportion and CAR 19 gene level in peripheral blood

Proportion of anti-CD19-CAR T cells and level of anti-CD19-CAR gene was examined using quantitative polymerase chain reaction (qPCR) on days 0, 7, 14, 21, and 28 after CAR T-cell infusion.

2.7 | Adverse events observation in the anti-CD19-CAR T-cell therapy and ibrutinib therapy

The levels of interleukin-6 (IL-6), IL-2R, IL-8, and tumor necrosis factor-α (TNF-α) were measured by ELISA on days 0, 7, 14, 21, and 28 after anti-CD19-CAR T-cell infusion. The AEs were observed throughout the anti-CD19-CAR T-cell therapy. Cytokine release syndrome (CRS) grades were evaluated in accordance with the National Cancer Institute Common Terminology Criteria for AE v.4.03 in CAR T-cell therapy.[23] Immune effector cell-associated neurotoxic syndrome (ICANS) grades were evaluated in accordance with the American Society for Blood and Marrow Transplantation (ASBM) ICANS Consensus Grading for Adults.[24] In two stages of ibrutinib therapy, the associated responses to ibrutinib were slight and controllable.

2.8 | Statistical analysis

All statistical analyses were performed using GraphPad Prism software. Differences between the first-time and second-time anti-CD19-CAR T-cell therapy were analyzed by paired nonparametric test (Wilcoxon matched-pairs test) or unpaired nonparametric test (Mann-Whitney *U*-test). A *P*-value of less than .05 was considered significant.

3 | RESULTS

3.1 | Characteristics of all patients

The baseline patient characteristics, disease status, and disease burden in 7 patients with refractory B-NHL before the 2 times of anti-CD19-CAR T-cell therapy are listed in Table 1. Median age of the patients was 55 y (range 37-67 y). No patients received hematopoietic stem-cell transplantation before the study.

3.2 | Transduction and function, infusion of the anti-CD19-CAR T cells

In the first-time anti-CD19-CAR T-cell therapy, mean efficiency of anti-CD19-CAR transduction in all patients was 42.27% ± 15.63%. We present the 2 times anti-CD19-CAR transduction FCM data of 1 MCL patient and 1 FL patient (Nos. 2 and 4) in Figure S1. The mean quantity of anti-CD19-CAR T cells in all patients on the harvest date was $8.57 \pm 3.43 \times 10^6$ cells/kg. They received a dose of $2.12 \pm 0.23 \times 10^6$ cells/kg humanized anti-CD19-CAR T-cell infusion on day 0. In the second-time anti-CD19-CAR T-cell therapy, the mean efficiency of anti-CD19-CAR transduction in all patients was 63.41% ± 14.85%. The mean quantity of anti-CD19-CAR T cells in the patients on the harvest date was $11.03 \pm 4.73 \times 10^6$ cells/kg. Next, the patients received a dose of $2.08 \pm 0.31 \times 10^6$ cells/kg anti-CD19-CAR T-cell infusion on day 0. There were no differences in the CD19-CAR transduction efficiency, proliferation of anti-CD19-CAR T cells on the harvest date, and anti-CD19-CAR T-cell infusion dose between the 2 times of anti-CD19-CAR T-cell therapy (Figure 1A). The IFN-γ release following co-culture with JEKO-1 cells at 48 h was higher with the second-time anti-CD19-CAR T-cell group than the first-time anti-CD19-CAR T-cell group ($P = .0156$, Figure 1B). The first-time and second-time anti-CD19-CAR T cells showed significant cytotoxicity for JEKO-1 cells at 24 h, and T cells also showed cytotoxicity at high effective target ratio. There was no difference in cytotoxicity between the 2 times of T cells or anti-CD19-CAR T cells (Figure 1C,D). In addition, ibrutinib could not improve the release

TABLE 1 Baseline characteristics, disease status, and disease burden of the refractory patients before the 2 times of CAR T-cell therapy

Patient	Age	Sex	Molecular subtype		Stage before CAR T		Previous response before CAR T		ECOG status before CAR T		Primary lines before CAR T		Maximum tumor diameter (cm) before CAR T		LDH (IU/L)	
			I	II	I	II	I	II	I	II	I	II	I	II	I	II
Pt 1	55	Male	MCL	MCL	II	III	Refractory	Refractory	3	3	2	4	8.5	9.2	113.3	124.8
Pt 2	56	Male	MCL	MCL	III	III	Refractory	Refractory	2	3	3	5	12.3	14.6	138.4	317.4
Pt 3	63	Female	MCL	MCL	IV	IV	Refractory	Refractory	3	3	2	4	4.3	3.9	180.5	217.2
Pt 4	67	Male	FL	FL	III	IV	Refractory	Refractory	2	2	3	5	4.1	5.6	211	430.7
Pt 5	50	Male	FL	tFL	IV	IV	Refractory	Refractory	4	4	3	5	3.4	12.6	257.3	306.4
Pt 6	37	Female	tFL	tFL	III	IV	Refractory	Refractory	3	4	2	4	8.3	15.6	190.5	185.9
Pt 7	42	Male	FL	FL	III	IV	Refractory	Refractory	2	3	2	4	3.6	6.4	188	385.9

Abbreviations: FL, follicular lymphoma; MCL, mantle cell lymphoma; tFL, diffuse large B-cell histologic transformation FL.

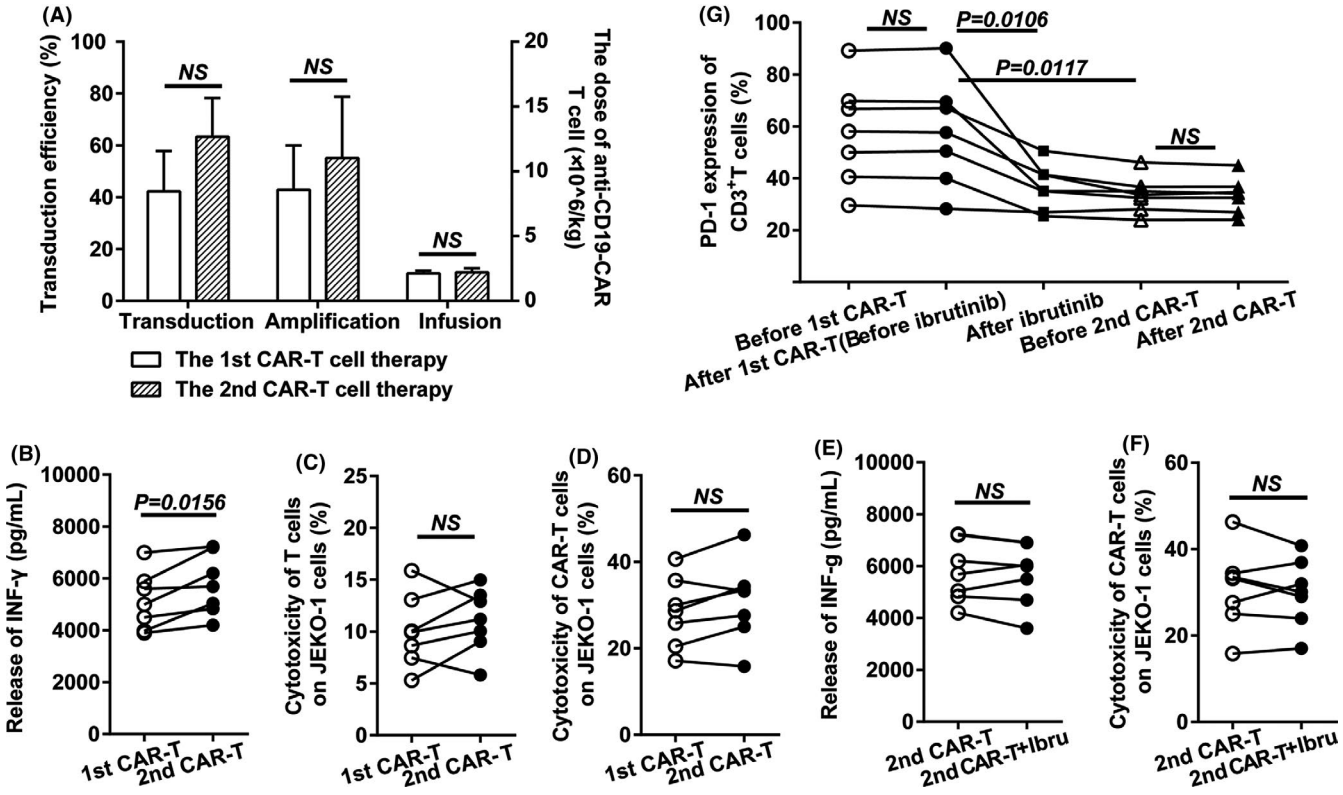

FIGURE 1 Transduction and function, infusion of the anti-CD19-CAR T cells. A, There were no differences in the anti-CD19-CAR transduction efficiency, proliferation of anti-CD19-CAR T cells, and CAR T-cell infusion doses between the 2 times of anti-CD19-CAR T-cell therapy. B-D, The second-time anti-CD19-CAR T cells released more IFN-γ. However, there was no difference in cytotoxicity between the 2 times of T cells or anti-CD19-CAR T cells. E, F, Ibrutinib cannot improve IFN-γ release and cytotoxicity of the second-time anti-CD19-CAR T cells for JEKO-1 cells in vitro. G, PD-1 expression declined in all 7 patients after the maintenance or salvage therapy with ibrutinib. PD-1 expression was lower in the second-time than in the first-time therapy in these patients

of IFN-γ or cytotoxicity of the second-time anti-CD19-CAR T cells in vitro (Figure 1E,F). There was no difference in the PD-1 expression in $CD3^+$ T cells in peripheral blood before or after their 2 times of anti-CD19-CAR T-cell therapies in any of the patients. However, after salvage treatment with ibrutinib, the PD-1 expression in all these 7 patients declined to varying degrees ($P = .0106$; Figure 1G). The PD-1 expression levels in $CD3^+$ cells in peripheral blood before 2 times of anti-CD19-CAR T-cell therapy were compared. We found that expression was lower before the second-time anti-CD19-CAR T-cell therapy than that after the first-time anti-CD19-CAR T-cell therapy ($P = .0117$; Figure 1G). To date, the expression of PD-1 has maintained at a low level in the 6 patients who continued the CR

status of their disease. The exact values of each parameter of all patients associated with Figure 1 are summarized in Table S1.

3.3 | Clinical responses to the first-time anti-CD19-CAR T-cell therapy

At 1 mo and 2 mo after the first-time anti-CD19-CAR T-cell infusion, we evaluated the efficacy in all patients with refractory B-NHL. Two FL patients (Nos. 4 and 7) obtained PR, 2 MCL patients (Nos. 2 and 3) achieved SD, while 1 MCL patient and 2 FL patients (Nos. 1, 5, and 6) experienced PD (Figure 2). The tumor size in the neck of patient No. 2 enlarged, and the tumor size in the abdominal cavity of patient No. 4 shrank slightly after the first-time anti-CD19-CAR T-cell treatment (Figure 3).

3.4 | Ibrutinib salvage therapy

All 7 refractory B-NHL patients who achieved PR, SD, or PD from the anti-CD19-CAR T-cell therapy were given ibrutinib as a salvage therapy after the first-time CAR T-cell therapy during the following

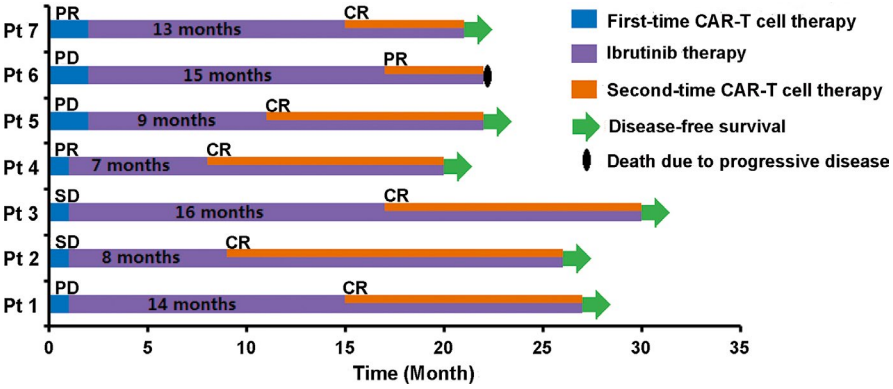

FIGURE 2 Seven patients achieved PR, SD, or PD from the first-time anti-CD19-CAR T-cell therapy. After maintenance or salvage therapy with ibrutinib, all patients received the second-time anti-CD19-CAR T-cell therapy when the ibrutinib treatment was ineffective or the disease progressed again. Six patients reached CR and 1 FL patient obtained PR with the second-time anti-CD19-CAR T-cell therapy combined with ibrutinib

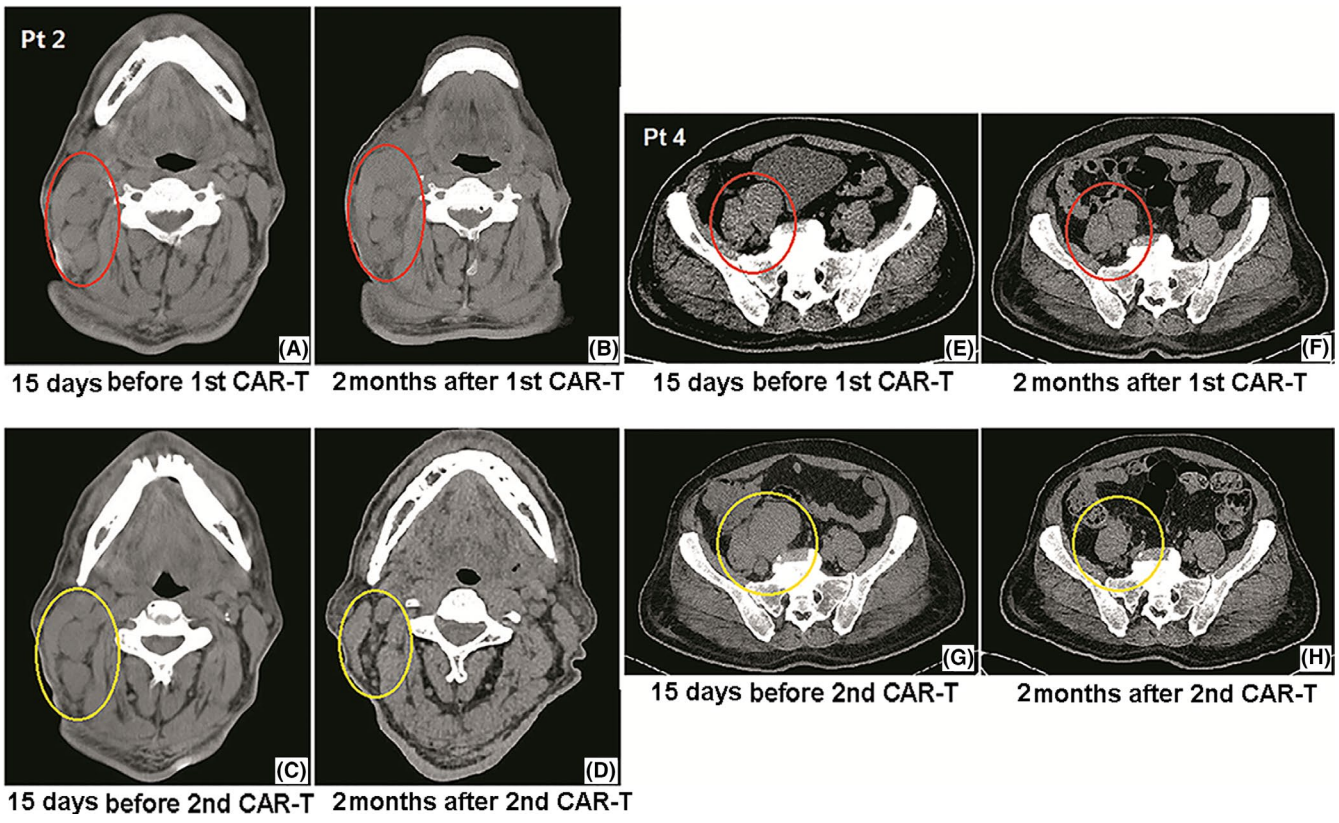

FIGURE 3 Evaluation of anti-CD19-CAR T-cell therapy effects by computed tomography in patients Nos. 2 and 4. A, E, Tumor sizes before the first-time anti-CD19-CAR T-cell therapy. B, F, Tumor size enlarged in patient No. 2 and shrank slightly in patient No. 4 after the first-time anti-CD19-CAR T-cell therapy. C, G, Tumor size increased again during therapy with ibrutinib. D, H, Tumors of patients Nos. 2 and 4 disappeared after second-time anti-CD19-CAR T-cell therapy

7-16 mo. During ibrutinib therapy, no-one discontinued the therapy or withdrew from treatment because of ibrutinib toxicity. However, the disease progressed again during ibrutinib therapy in all 7 patients.

3.5 | Clinical responses to the second-time anti-CD19-CAR T-cell therapy

The patients were enrolled in the same clinical trial again in our hospital as their disease progressed again during ibrutinib salvage treatment. Ibrutinib treatment was continued during the second-time anti-CD19-CAR T-cell therapy. Three refractory MCL patients (Nos. 1, 2, and 3) and 3 FL patients (Nos. 4, 5, and 7) achieved CR from the second-time anti-CD19-CAR T-cell therapy combined with ibrutinib (Figure 2). The tumors in patients Nos. 2 and 4 disappeared after second-time anti-CD19-CAR T-cell therapy (Figure 3). Patient No. 6 with FL reached PR with this combination therapy only (Figure 2). Maintenance therapy of ibrutinib was continued after the second-time anti-CD19-CAR T-cell therapy. The CR status was maintained for 12, 17, 13, 12, 11, and 6 mo in patients Nos. 1-5 and patient No. 7 until September 30, 2020. The disease in patient No. 6, who had obtained PR from the second-time anti-CD19-CAR T-cell therapy, progressed again later, and she died 6 mo after the second-time anti-CD19-CAR T-cell therapy.

3.6 | Proportion of anti-CD19-CAR T cell, and expression levels of anti-CD19-CAR DNA in peripheral blood

The proportions of anti-CD19-CAR T cells and the expression levels of anti-CD19-CAR DNA were detected in the 2 times of

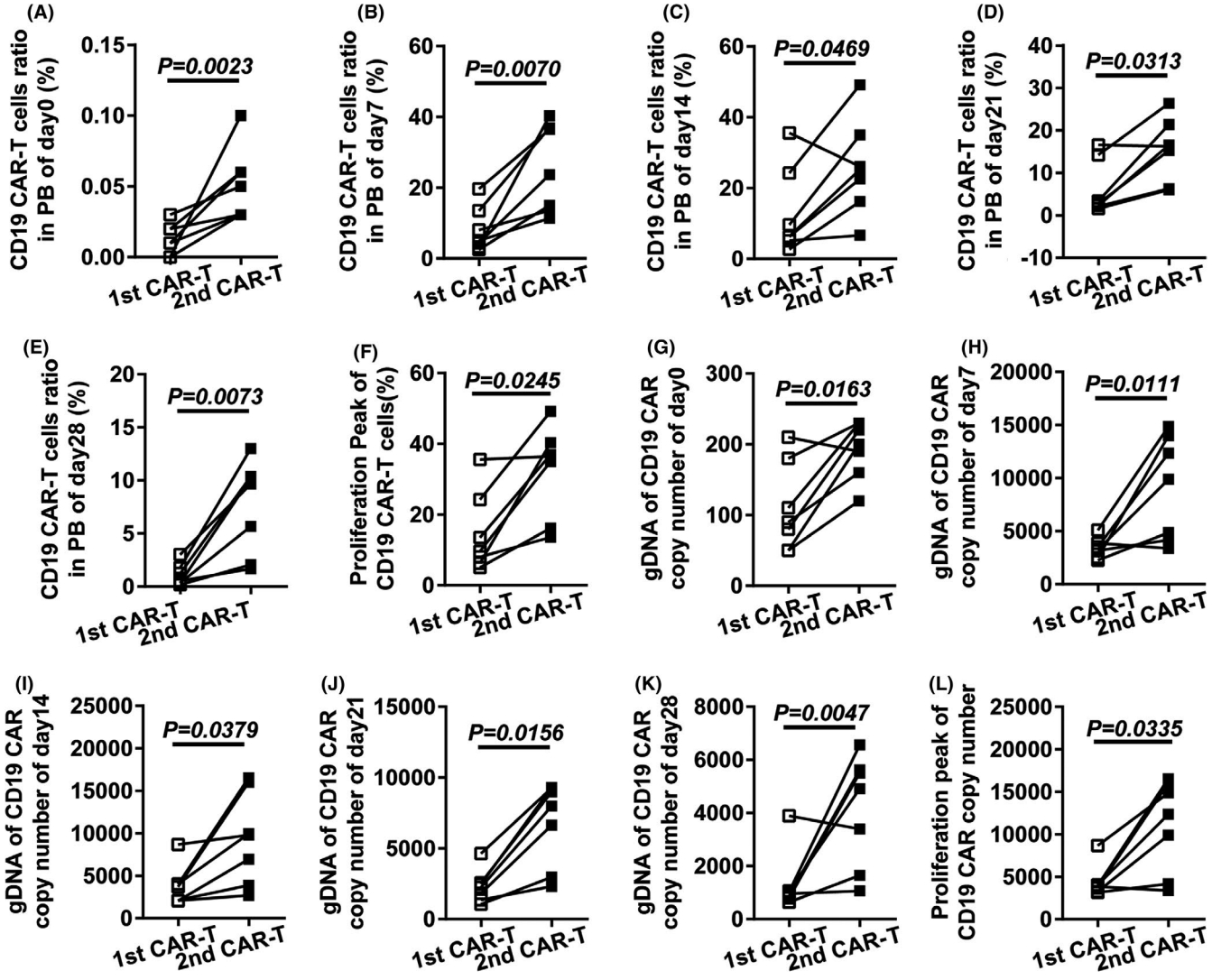

FIGURE 4 CD19-CAR T-cell and the CD19-CAR DNA level in peripheral blood in the 2 times of anti-CD19-CAR T-cell therapy. A-E, Proportions of anti-CD19-CAR T cells in 2 times of anti-CD19-CAR T-cell therapy. G-K, DNA levels of anti-CD19-CAR gene in 2 times of anti-CD19-CAR T-cell therapy. F, L, Median peak of the anti-CD19-CAR T cells and copy of anti-CD19-CAR gene were higher in the second-time therapy than in the first-time therapy

anti-CD19-CAR T-cell therapy on days 0, 7, 14, 21, and 28 post infusion. The median peak of anti-CD19-CAR T cells in the $CD3^+$ T cells was 20.34% ± 10.70% (range 5.04%-35.59%) on day 10.5 ± 3.56 after infusion in the first-time anti-CD19-CAR T-cell therapy, whereas the median peak was 32.11% ± 13.28% (range 13.56%-49.18%) on days 9.33 ± 3.40 after infusion in the second-time anti-CD19-CAR T-cell therapy (Figure 4A-E). The expression levels of anti-CD19-CAR DNA showed the same trends. The median peak of anti-CD19-CAR DNA was 6698.7 ± 3251.62 (range 3160-8680) copies/μg in the first-time anti-CD19-CAR T-cell therapy, and 10 200.0 ± 5142.28 (range 3380-16 550) copies/μg in the second-time therapy (Figure 4G-K). The median peaks of the anti-CD19-CAR T cells and anti-CD19-CAR gene copy of the 7 patients were higher in the second-time therapy than in the first-time therapy ($P = .0245$ and $P = .0335$; Figure 4F,L). Table S2 shows the exact values of each parameter of all patients associated with Figure 4.

3.7 | Adverse effects

Patients showed pyrexia with or without chills, fatigue, nausea, decreased appetite, edema, and hypoalbuminemia after the anti-CD19-CAR T-cell infusion (Table 2). Notable CRS were grades 0-2 in the first-time, and grades 2-4 in the second-time anti-CD19-CAR T-cell therapy (Figure 5A,C). No patients were diagnosed with ICANS in the first-time anti-CD19-CAR T-cell therapy, but 1 patient with MCL was diagnosed with grade 2 ICANS (Table S3). Ibrutinib treatment was continued for a median duration of 13 mo (range 7-16 mo) at a daily dose of 420 mg or 560 mg. Adverse events related to ibrutinib are summarized in Table S4. Hematological toxicity was graded as 1-3 in the first-time and graded 3 and 4 in the second-time anti-CD19-CAR T-cell therapy (Figure 5B,D). AEs occurred 5-7 d after anti-CD19-CAR T-cell infusion and recovered 14-16 d after the anti-CD19-CAR T-cell infusion. Two patients were diagnosed with Gram-negative bacterial infections in the second-time anti-CD19-CAR T-cell therapy. These patients were cured by antibiotics with supportive treatment. No patients were diagnosed with invasive fungal disease.

Levels of IL-6, IL-2R, TNF-α, and IL-8 were observed in the 2 times of anti-CD19-CAR T-cell therapy. The TNF-α and IL-8 peaks were higher in the second-time anti-CD19-CAR T-cell therapy than in the first-time therapy. Peaks of another 2 cytokines were not significantly different between the 2 times of anti-CD19-CAR T-cell therapy, although they tended to be higher in the second-time anti-CD19-CAR T-cell therapy than in the first-time therapy (Figure 5E-H). The patients received antipyretic drugs and methylprednisolone to overcome the AEs. No patients received tocilizumab. The AEs were relieved 14-16 d after the anti-CD19-CAR T-cell infusion. No patients died of CRS or ICANS in our study. The absolute lymphocyte count (ALC) was reduced significantly after pretreatment with fludarabine and cyclophosphamide lymphodepletion regimen, and the ALC levels could not be changed by the ibrutinib therapy (Figure 5I).

TABLE 2 CRS and hematological toxicity after the 2 times of anti-CD19-CAR T-cell therapy

Events	First-time CAR T-cell therapy	Second-time CAR T-cell therapy
General condition		
Temperature ≥38°C (fever)	2/7 (28.57%)	6/7 (85.71%)
Chills	1/7 (14.29%)	5/7 (71.43%)
Muscular weakness	2/7 (28.57%)	5/7 (71.43%)
Rash	0/7 (0.00%)	1/7 (14.29%)
Systolic blood pressure <90 mm Hg (hypotension)	0/7 (0.00%)	3/7 (42.86%)
Needing oxygen for SaO_2 >90% (hypoxia)	0/7 (0.00%)	2/7 (28.57%)
Fatigue	3/7 (42.86%)	5/7 (71.43%)
Organ toxicities		
Cardiac		
Tachycardia	1/7 (14.29%)	4/7 (57.14%)
Arrhythmias	0/7 (0.00%)	1/7 (14.29%)
Heart block	0/7 (0.00%)	0/7 (0.00%)
Respiratory		
Hypoxia	0/7 (0.00%)	2/7 (20.00%)
Dyspnea	0/7 (0.00%)	3/7 (42.86%)
Cough	1/7 (14.29%)	2/7 (28.57%)
Pleural effusion	1/7 (14.29%)	4/7 (57.14%)
Gastrointestinal		
Nausea	1/7 (14.29%)	3/7 (42.86%)
Vomiting	0/7 (0.00%)	2/7 (28.57%)
Decreased appetite	2/7 (28.57%)	4/7 (57.14%)
Hepatic		
Increased serum ALT, AST	0/7 (0.00%)	3/7 (42.86%)
Increased serum bilirubin levels	0/7 (0.00%)	2/7 (28.57%)
Renal		
Acute kidney injury (increased serum creatinine levels)	0/7 (0.00%)	2/7 (28.57%)
Oliguria	0/7 (0.00%)	4/7 (57.14%)
The electrolyte		
Hypokalemia	0/7 (0.00%)	2/7 (28.57%)
Hypocalcemia	0/7 (0.00%)	0/7 (0.00%)
Hyperglycemia	0/7 (0.00%)	1/7 (14.29%)
Coagulopathy		
Disseminated intravascular coagulation	0/7 (0.00%)	1/7 (14.29%)
Hematological		
Neutropenia (grade 3/4) ($<1 \times 10^9$/L)	1/7 (14.29%)	4/7 (57.14%)
Anemia (grade 3/4) (<80 g/L)	0/7 (0.00%)	3/7 (42.86%)
Thrombocytopenia (grade 3/4) ($<50 \times 10^9$/L)	0/7 (0.00%)	3/7 (42.86%)

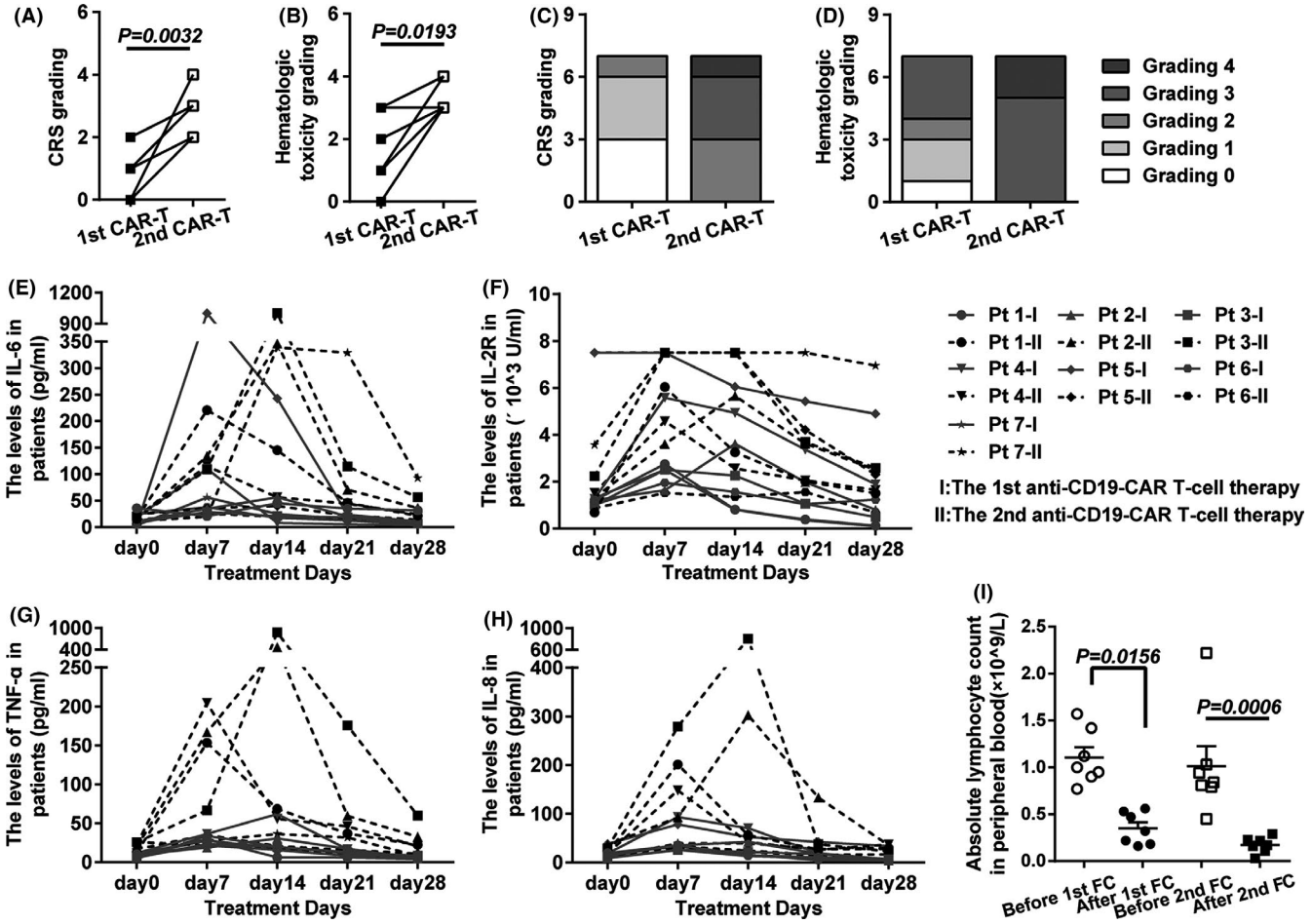

FIGURE 5 A, C, Notable AEs were graded 0-2 CRS in first-time anti-CD19-CAR T-cell therapy, and 2-4 CRS in the second-time anti-CD19-CAR T-cell therapy. B, D, Hematological toxicity was grades 1-3 in first-time anti-CD19-CAR T-cell therapy and grades 3 and 4 in the second-time anti-CD19-CAR T-cell therapy. E-H, Serum levels of IL-6, IL-2R, TNF-α, and IL-8 were observed in the 2 times of anti-CD19-CAR T-cell therapy. I, ALC was reduced significantly after pretreatment of fludarabine combined with cyclophosphamide lymphodepletion regimen, and the ALC levels could not be changed by the ibrutinib therapy

4 | DISCUSSION

Although anti-CD19-CAR T-cell therapy could be a salvage therapy for R/R B-NHL patients, some patients could not benefit from this therapy.[25-27] Some reports have demonstrated that patients with MCL and CLL who progressed during BTK inhibitor treatment had a poor survival, and B-NHL patients who were resistant to BTK inhibitor faced a therapeutic challenge.[9,10,28,29] Multiple preclinical and clinical studies have proven that ibrutinib could improve anti-CD19-CAR T-cell therapy in CLL and MCL,[30-33] but clinical studies on the effect of ibrutinib treatment on the efficacy of 2 anti-CD19-CAR T-cell treatments in the same patients are limited. In our study, 7 patients with refractory B-NHL (MCL and FL) received ibrutinib as a salvage treatment and kept a stable state of their disease during the following 7-16 mo after the failed anti-CD19-CAR T-cell therapy. When their disease progressed again despite the continued ibrutinib treatment, they received the second-time anti-CD19-CAR T-cell therapy. We evaluated and compared the efficacy and safety of the 2 times anti-CD19-CAR T-cell therapy in 7 patients.

It has been shown previously that 60 R/R MCL patients with a prior BTK inhibitor treatment achieved a 93% ORR and a 67% CR rate following anti-CD19-CAR T-cell therapy.[30] At a median follow-up of 12.3 mo, 57% patients in the primary efficacy analysis were in remission, while the grade 3 or higher CRS and neurologic events occurred in 15% and 31% of patients, respectively.[30] This efficacy was superior to that previously reported for anti-CD19-CAR T-cell therapy in patients with R/R B-NHL.[13,14] In previous studies, more than 3-5 cycles of ibrutinib treatment improved the expansion of anti-CD19-CAR T cells and decreased the expression of the PD-1 in T cells in CLL patients.[32,34] Furthermore, it was confirmed that the expansion of anti-CD19-CAR T cells from 3 CLL patients who had been treated with ibrutinib more than 12 mo improved significantly compared with the anti-CD19-CAR T cells before ibrutinib treatment.[32] Our previous study observed that PD-1 expression of anti-CD19-CAR T cells increased when co-cultured with lymphoma cells in vitro, and ibrutinib could weaken this effect.[20] In this study, the mean expression of PD-1 on $CD3^+$ T cells in peripheral blood declined after 7-16 mo of ibrutinib salvage treatment, which is consistent with the

previous studies that showed that function of T cells and defective microenvironment in CLL patients were improved through several cycles of ibrutinib therapy.[32,35-37] Therefore, this might be one of the mechanisms for an improved efficacy of the second-time anti-CD19-CAR T-cell therapy in our study. The successful outcome of the second-time anti-CD19-CAR T-cell therapy compared with the first-time anti-CD19-CAR T-cell therapy before ibrutinib salvage therapy might confirm the role of ibrutinib in improving the activity of anti-CD19-CAR T cells in our study.

Ibrutinib could improve T-cell function by increasing the persistence of activated T cells, decreasing the Treg/$CD4^+$ T-cell ratio, and diminishing the immune-suppressive properties through BTK-dependent and BTK-independent mechanisms in CLL patients.[35] In addition, inhibition of IL-2-inducible T-cell kinase (ITK) activity would lead to the inhibition of Th2 cell differentiation and promotion of a Th1 cell immune response.[38] Our previous study suggested that ibrutinib might have a synergistic effect with anti-CD19-CAR T cells by improving the TME.[20] In our study, these 7 patients were all R/R B-NHL patients with unsatisfactory efficacy of the first-time anti-CD19-CAR T-cell therapy. CR was achieved in 6 patients and PR occurred in 1 patient after ibrutinib salvage therapy, which improved their T-cell function in the second-time anti-CD19-CAR T-cell therapy. It is worth mentioning that the median peak of the anti-CD19-CAR T cells and copy of the anti-CD19-CAR gene were higher in the second-time therapy compared with the first-time therapy. However, the CRS grades and hematological toxicity grades were also higher in the second-time therapy. Higher tumor burden and higher amplification of anti-CD19-CAR T cells may bring about more intense cytotoxicity and bone marrow suppression, which may lead to higher grades in CRS and hematological toxicities. Moreover, the increased anti-CD19-CAR T-cell function might lead to higher CRS levels during anti-CD19-CAR T-cell therapy, although some studies reported that anti-CD19-CAR T-cell therapy in R/R CLL was very well tolerated and with low levels of CRS.[39,40] Other studies have demonstrated that ibrutinib could inhibit the occurrence of CRS in an MCL mouse model with anti-CD19-CAR T-cell therapy.[41] Although the CRS grades and hematological toxicity grades were greater than in the first-time anti-CD19-CAR T-cell therapy, the second-time anti-CD19-CAR T-cell therapy was relatively safe and practicable.

The number of cases in our study was relatively small, so more cases are needed to confirm this conclusion. The period of ibrutinib therapy might play an important role in the success of the second-time anti-CD19-CAR T-cell therapy. Moreover, the mechanisms of the combination of anti-CD19-CAR T cells with ibrutinib and how to manage the more serious AEs need further research.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

DQ: conception and design. DQ and ZZQ: study supervision. LMJ and DHB: drafting or review of the manuscript. LMJ, DHB, MJ, LQ, JYY, and PYD: acquisition of data. JYL: analysis and interpretation of data. All authors: writing, review, and/or revision of manuscript.

ETHICAL APPROVAL

This study was approved by the Medical Ethics Committee of the Department of Hematology, Tianjin First Central Hospital (Tianjin, China) (Approved No. of Ethics Committee: 2015002X). The clinical trial in our study was registered at http://www.chictr.org.cn/index.aspx as ChiCTR-ONN-16009862. The patients gave their written informed consent in accordance with the Declaration of Helsinki. All the 7 patients provided informed consent for receiving anti-CD19-CAR T-cell therapy. In addition, the patients agreed to the use of their specimens and data for our study. The studies involving human participants were reviewed and approved by Tianjin First Central Hospital (Tianjin, China). The patients/participants provided their written informed consent to participate in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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