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RESEARCH ARTICLE

Phase I study of CBM.CD19 chimeric antigen receptor T cell in the treatment of refractory diffuse large B-cell lymphoma in Chinese patients

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Abstract **Anti-CD19 chimeric antigen receptor (CAR) T cell therapy has shown impressive efficacy in treating B-cell malignancies. A single-center phase I dose-escalation study was conducted to evaluate the safety and efficacy of T cells transduced with CBM.CD19 CAR, a second-generation anti-CD19 CAR bearing 4-1BB costimulatory molecule, for the treatment of patients with refractory diffuse large B-cell lymphoma (DLBCL). Ten heavily treated patients with refractory DLBCL were given CBM.CD19 CAR-T cell (C-CAR011) treatment. The overall response rate was 20% and 50% at 4 and 12 weeks after the infusion of C-CAR011, respectively, and the disease control rate was 60% at 12 weeks after infusion. Treatment-emergent adverse events occurred in all patients. The incidence of cytokine release syndrome in all grades and grade ⩾ 3 was 90% and 0, respectively, which is consistent with the safety profile of axicabtagene ciloleucel and tisagenlecleucel. Neurotoxicity or other dose-limiting toxicities was not observed in any dose cohort of C-CAR011 therapy. Antitumor efficacy was apparent across dose cohorts. Therefore, C-CAR011 is a safe and effective therapeutic option for Chinese patients with refractory DLBCL, and further large-scale clinical trials are warranted.**

Keywords CAR-T cell therapy; refractory diffuse large B-cell lymphoma; cytokine release syndrome; dose-limiting toxicity

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma (NHL) and accounts for approximately 30%–40% of NHL cases [1]. "One shot disease" is usually a typical presentation of DLBCL; although this illness is sensitive to first-line treatment, the therapeutic effect is not satisfactory once the patient is exposed to second-line or salvage chemotherapy [2,3]. Patients with refractory DLBCL who are failed by primary or salvage immunochemotherapy or those who relapsed after autologous stem cell transplantation (ASCT) usually have a dismal prognosis with a median overall survival (OS) of approximately 6 months [4]. According to the SCHOLAR-1 study, patients in the primary refractory DLBCL and high-risk international prognostic index (IPI) subgroups had the lowest response rates of 20% and 18%, respectively after salvage therapy, and those who relapsed within 12 months post-ASCT (34%) and were refractory to second-line or later-line therapy (26%) responded slightly better; this finding indicated that the majority of patients with refractory DLBCL lack curative treatment options [5]. Therefore, novel therapeutic approaches with different mechanisms for these patients are urgently needed.

The adoptively transferred chimeric antigen receptor (CAR) T cell therapy is a personalized immunotherapy for cancers using genetically modified T cells that can recognize and target specific tumor cell surface antigens in a major histocompatibility complex (MHC)-independent manner. The introduction and application of CAR-T cell therapy for the treatment of B cell neoplasms including chronic lymphocytic leukemia (CLL) [6,7], B cell precursor acute lymphoblastic leukemia (B-ALL) [8,9], and B cell NHL [10–12] has yielded encouraging antitumor effectiveness. These advances led to the approval of two autologous CD19 CAR-T products for the treatment of relapsed or refractory B-ALL

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(tisagenlecleucel) and large B cell lymphoma (tisagenlecleucel and axicabtagene ciloleucel) by the US Food and Drug Administration (FDA). Another anti-CD19 CAR-T cell product, lisocabtagene ciloleucel, has recently showed therapeutic potential for relapsed or refractory aggressive NHL cases in a large clinical trial and thus may soon be approved by the FDA [13]. Owing to the therapeutic effect of this promising immunotherapy, many clinical trials are under way to use CAR-T cells in the treatment of hematological malignancies and promote CAR-T cell therapy as part of the mainstream cancer therapy to provide a potential cure for patients [14,15].

In B cell malignancies, CD19 is an attractive therapeutic target because of its restricted expression on the surface of normal and most malignant, transformed B cells. Autologous T cells genetically engineered to express CD19-targeted CARs on the cell membrane can eliminate tumor cells expressing CD19. Compared with the first-generation CARs harboring only a CD3ζ intracellular signaling domain, the second-generation CARs incorporate a costimulatory signal domain that is derived from CD28 or 4-1BB, can continuously stimulate T cells, and greatly enhance the antitumor activity [14,16].

The National Cancer Institute (NCI) and other institutions reported that second-generation anti-CD19 CAR-T cells can induce responses with a manageable toxicity profile in patients with refractory DLBCL [10,17,18]. This work presents the preliminary results of a phase I dose-escalation study conducted in Chinese patients with refractory DLBCL who were treated with CBM.CD19 CAR-T cells (C-CAR011) bearing 4-1BB costimulatory domains.

Patients and methods

Study design

The clinical trial was an open-label single-center phase I study that aimed to evaluate the safety and feasibility and explore the maximum tolerable dose of C-CAR011 in the treatment of refractory DLBCL and was registered with ClinicalTrials.gov (NCT02976857). All patients provided written informed consent at the time of enrollment in the trial in accordance with the *Declaration of Helsinki*, and the study protocol was approved by the ethics committee of Jiangsu Province Hospital.

The treatment protocol of the clinical trial consisted of three stages, including a 3-week screening period, a 3-day treatment period, and a 12-week follow-up period for safety and effectiveness as depicted in Fig. 1. After signing the informed consent, the patients were screened and underwent an apheresis to obtain peripheral blood mononuclear cells (PBMCs) for the preparation of CAR-T cells, which required a period of 10–14 days. The median time from apheresis to final product was 9 days. The baseline disease condition of patients was assessed a week prior to C-CAR011 infusion. Conditioning chemotherapy with fludarabine (at a dose of 30 mg/m^2 per day) and cyclophosphamide (at a dose of 30 mg/m^2 per day) was administered on days −5 through −3. On days 0 to 2, the

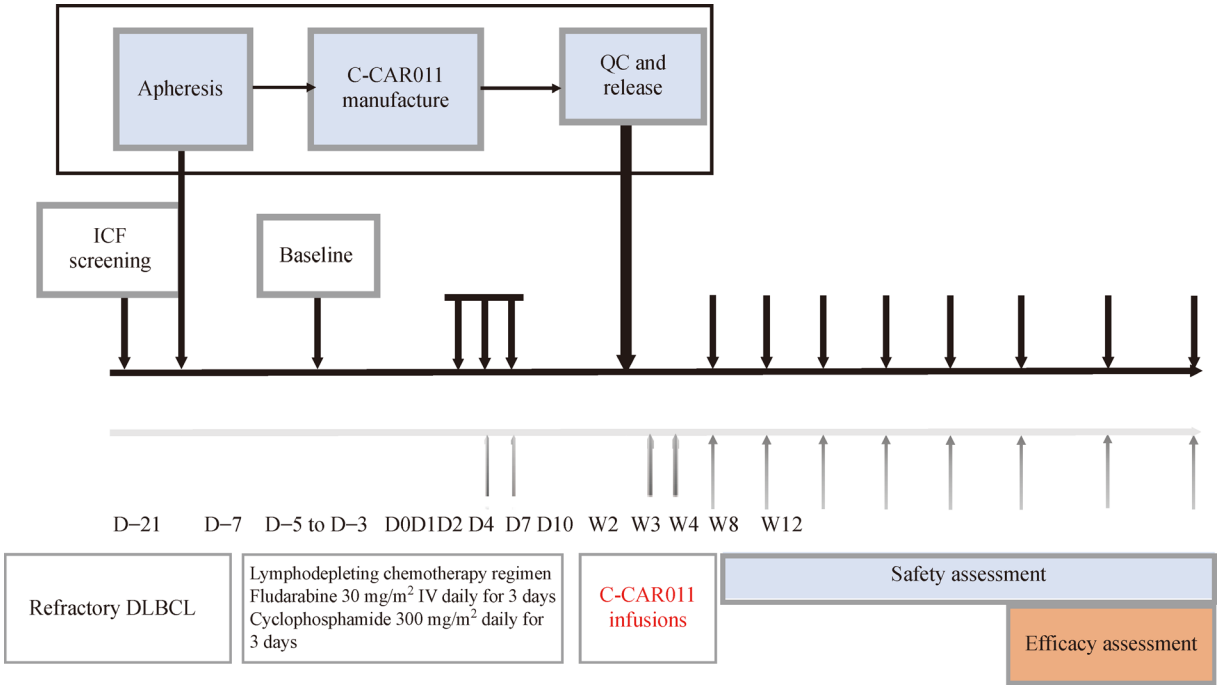

Fig. 1 Schematic of study design. D, day; W, week.

patients were grouped into low-dose, middle-dose, and high-dose cohorts and received 0.8×10^6, 2.5×10^6, and 5×10^6 C-CAR011 cells/kg, respectively, according to the dose-escalation schedule following a standard $3 + 3$ design. The total CAR-T cells were split into 3 consecutive days of intravenous infusions with 10% of cells infused on day 1, 30% on day 2, and 60% on day 3.

The patients were required to be hospitalized for approximately 1 month after infusion and were followed up through systematic assessment including physical examination, vital signs, laboratory and imaging examinations, and therapeutic efficacy evaluation. The safety of CAR-T cells was assessed at days 4, 7, and 10 and weeks 2, 3, 4, 8, and 12, and their effectiveness was evaluated at weeks 4 and 12 after the first infusion.

Generation and transduction of C-CAR011

The second-generation CBM.CD19 CAR used in this study contained an extracellular segment of anti-CD19 single-chain variable fragment that was derived from FMC63 monoclonal antibody, a hinge and trans-membrane regions that were derived from CD8α, and an intracellular segment that consists of the 4-1BB costimulatory signaling domain and CD3ζ T cell activation molecule and can recognize and eliminate lymphoma cells in a CD19-specific manner.

Approximately 1×10^9 to 1×10^{10} PBMCs were obtained from the patients through leukapheresis and then delivered to the manufacture center through a qualified biological cold chain. T lymphocytes with high purity were obtained by magnetic bead sorting. After simulation, the T lymphocytes were transduced with lentivirus carrying CBM.CD19 CAR. The cells expressing CBM.CD19 CAR were amplified, cultured, and cryopreserved at a temperature below −135 °C. After the standard quality requirements were satisfied, the CAR-T cells were transported via the cold chain to our clinical center, where they were resuscitated and prepared for infusion.

Research objectives and assessments

The primary objective was to evaluate the safety and feasibility of C-CAR011, especially the incidence of grade $\geqslant 3$ cytokine release syndrome (CRS) and neurotoxicity. The secondary objectives were to assess the efficacy of C-CAR011 for the treatment of refractory DLBCL and to explore the recommended dose for phase II clinical trials. The exploratory objectives were to determine the *in vivo* expansion of C-CAR011 and the persistence and ability of CAR-T cells to eliminate peripheral B cells.

Safety evaluations include dose-limiting toxicities (DLTs) and the incidence of treatment-emergent adverse events (TEAEs). Efficacy was evaluated by the overall response rate (ORR) at 4 and 12 weeks after infusion and the disease control rate (DCR) at 12 weeks after infusion. DLTs were defined in accordance with the criteria used by Lee *et al*. [19] and Locke *et al*. [20]. CRS was evaluated mainly according to the CRS revised grading system proposed by Lee *et al*. [21,22] and in reference to the criteria raised by Neelapu *et al*. [23]. Other TEAEs, including neurotoxicity, were graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.03). Details regarding the toxicity and response criteria are provided in the supplementary material.

Patient eligibility

Inclusion criteria were as follows: patients aged 18–70 years with histologically confirmed measurable refractory DLBCL, which was defined as (1) progressive disease (PD) as the best response to the latest standard chemotherapy, (2) stable disease (SD) after receiving at least four cycles of first-line treatment or two cycles of second-line or later treatment, or (3) recurrence or progression within 12 months after ASCT. All patients must have received standard treatment containing anti-CD20 monoclonal antibody and anthracycline-contained chemotherapy according to the National Comprehensive Cancer Network (NCCN) guidelines. Eligible patients must meet the requirements of the Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, left ventricular ejection fraction (LVEF) $\geqslant 50\%$, and adequate organ and bone marrow functions, including the serum level of albumin $\geqslant 30$ g/L, total bilirubin $\leqslant 25.7$ μmol/L, creatinine $\leqslant 132.6$ μmol/L, alanine aminotransferase and aspartate aminotransferase less than three times of the normal upper limit, and the absolute neutrophil count $\geqslant 1.0 \times 10^9$/L and platelet $\geqslant 50 \times 10^9$/L in the peripheral blood.

Exclusion criteria were as follows: patients who previously received CAR-T treatment or other genetically modified T cell therapies; those who relapsed after allogeneic stem cell transplantation; those with extranodal lesions involving the central nervous system, skeleton, lung or gastrointestinal tract; and those with a history of active systemic autoimmune/immunodeficiency disease requiring immunosuppressive therapy, cardiac insufficiency of grade III or IV per the New York Heart Association (NYHA) classification, or seropositive hepatitis B or C virus.

Results

Patient characteristics

Among the 18 patients screened for eligibility, 12 were enrolled in this study. Two patients withdrew from the

clinical trial prior to C-CAR011 administration. Finally, the 10 patients classified in three dose-escalation groups received conditioning chemotherapy and completed CAR-T cell infusion as specified in the protocol. Their demographic and baseline characteristics are provided in Table 1. The patients consisted of 7 males and 3 females with a mean age of 44.5 ± 13.2 years (range, 25.4–69.2 years). The mean age of patients at the time of diagnosis of DLBCL was 44.7 ± 14.9 years (range, 24.8–68.5 years), and the mean time to pathological diagnosis was −386.4 ± 124.6 days (range, −600.0 days to −230.0 days). According to the modified Ann Arbor staging system, eight patients were classified as stage III (n = 3) or IV (n = 5) prior to enrollment. Eight patients (80%) previously received more than three lines of treatment, and the best response of seven patients (70%) to prior treatment was SD (30%) or PD (40%). After CAR-T cell infusion was completed, eight of the patients were followed up for 12 weeks, and the remaining two voluntarily withdrew from the study after 4 weeks.

Safety

Among the 10 patients who received CAR-T cell infusion, no DLTs were observed at any dose level. The incidence of TEAEs and adverse reactions (ARs) after treatment was 100% as listed in Table 2. All 10 patients had at least one TEAE that met the criteria of NCI CTCAE ⩾ 3, and these TEAEs were reversible. Only one serious TEAE occurred in a patient from the low-dose (0.8×10^6 CAR^+ T cells/kg)

Table 1 Baseline demographic and disease characteristics of patients who received C-CAR011 treatment

Characteristic	Low-dose cohort (N = 3)	Middle-dose cohort (N = 3)	High-dose cohort (N = 4)	Total (N = 12[a])
Age (year)				
Mean±SD	50.3±5.8	28.8±3.2	52.9±11.0	44.5±13.2
Range	43.7–53.0	25.4–31.6	45.4–69.2	25.4–69.2
Gender, n (%)				
Male	2 (66.7)	3 (100.0)	2 (50.0)	9 (75.0)
Female	1 (33.3)	0	2 (50.0)	3 (25.0)
Height (cm)				
Mean±SD	169.0±4.4	174.3±4.9	168.5±6.1	170.5±4.93
Range	164–172	171–180	163–176	163–180
Weight (kg)				
Mean±SD	69.7±9	73±7	67.9±12.2	69.6±8.3
Range	64–80	68–81	60–86	60–86
Days to pathological diagnosis[b]				
Mean±SD	−440.5±21.9	−415.0±261.6	−331.3±61.8	−386.4±124.6
Range	−456 to −425	−600 to −230	−399 to −278	−600 to −230
Modified Ann Arbor stage before enrollment, n (%)				
I or II	0	1 (33.3)	1 (25.0)	4 (33.35)
III or IV	3 (100.0)	2 (66.7)	3 (75.0)	8 (66.7)
Symptom A or B, n (%)				
A	3 (100.0)	3 (100.0)	1 (25.0)	9 (75.0)
B	0	0	3 (75.0)	3 (25.0)
Number of prior lines of treatment, n (%)				
1–2	0	0	0	1 (8.3)[c]
3	1 (33.3)	1 (33.3)	1 (25.0)	3 (25.0)
>3	2 (66.7)	2 (66.7)	3 (75.0)	8 (66.7)[d]
Best response to previous regimens, n (%)				
CR	0	0	1 (25.0)	1 (8.3)
PR	0	2 (66.7)	0	2 (16.7)
SD	1 (33.3)	1 (33.3)	1 (25.0)	4 (33.3)
PD	2 (66.7)	0	2 (50.0)	5 (41.7)

[a] The total population including two patients who withdrew from the clinical trial prior to C-CAR011 infusions (Patient 001 and Patient 009).
[b] The time span from the enrollment in this clinical trial to the initial diagnosis.
[c] Patient 001.
[d] Including Patient 009.
CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

Table 2 TEAE after treatment (Safety Analysis Set, SAS)

Factor, *n* (%)	Low-dose cohort (*N* = 3)	Middle-dose cohort (*N* = 3)	High-dose cohort (*N* = 4)	Total (*N* = 10)
Patients with ⩾ 1 TEAE	3 (100%)	3 (100%)	4 (100%)	10 (100%)
Patients with ⩾ 1 TEAE (NCI CTCAE ⩾ 3)	3 (100%)	3 (100%)	4 (100%)	10 (100%)
Patients with ⩾ 1 Serious TEAE	1 (33.3%)	0	0	1 (10%)

TEAEs, treatment-emergent adverse events; *n*/*N*, number of patients.
No patient had ⩾ 1 TEAE leading to withdrawal, dose adjustment/discontinuation, re-use after suspension, dose decrease or study discontinuation. No deaths or other ARs occurred apart from TEAEs.

cohort and who developed grade 3 erysipelas 77 days after CAR-T cell infusion. After evaluation, this serious TEAE was confirmed to be unrelated to the conditioning chemotherapy or C-CAR011 infusion. In summary, the incidence of serious TEAEs was 10% with no TEAEs or adverse reactions leading to the withdrawal of patients or dose adjustment/discontinuation. No TEAE-related deaths were noted.

According to the summary of system organ class (SOC) and preferred term (PT) in Table S1, the TEAEs were widely distributed in various organs systems. Metabolic and nutritional disorders, respiratory, thoracic and mediastinal diseases, general disorders, and administration site reactions were the major sources of toxic effects. TEAEs with the highest incidence (10/10, 100%) were multiple hemocytopenia including B lymphocytes, white blood cells and neutrophils, decrease in immunoglobulin A, and increase in C-reactive protein.

CRS and neurotoxicity are two common toxicities for patients who received CAR-T cell therapies. Description of cytokine release syndrome was shown in Table 3. During the study period, nine of the 10 patients (90%) experienced CRS, including three in the low-dose cohort, two in the middle-dose cohort, and four in the high-dose cohort. Only one patient in the high-dose cohort developed grade 2 CRS that had manifested as fever, anorexia, vomiting, hypoxemia, and elevated transaminase. No episodes of severe CRS defined as grade 3 or higher were noted. Except for one case with self-limited CRS in the low-dose cohort, the other eight patients with CRS received only supportive treatment, and their symptoms were relieved within five days. No patients received tocilizumab or corticosteroids, and no deaths were attributed to CRS. Neurotoxicity was not observed.

Efficacy

Efficacy was evaluated in the full analysis set (FAS) as

Table 3 Description of cytokine release syndrome

Cohort	Patient	Symptom description	Level	Use of tocilizumab Yes/no	Use of corticosteroids Yes/no	Other medication Yes/no	Action to study product	Withdrawal Yes/no
Low-dose cohort	Patient 001	Fever/chills/ elevated transaminase	1	No	No	Yes	Not applicable	No
	Patient 002	Elevated transaminase	1	No	No	No	Not applicable	No
	Patient 003	Fever/diarrhea	1	No	No	Yes	Continue	No
Middle-dose cohort	Patient 005	Elevated transaminase	1	No	No	Yes	Not applicable	No
	Patient 006	Diarrhea/ constipation	1	No	No	Yes	Continue	No
High-dose cohort	Patient 007	Fever	1	No	No	Yes	Not applicable	No
	Patient 008	Fever/anorexia/ vomiting/ hypoxemia /elevated transaminase	2	No	No	Yes	Continue	No
	Patient 009	Fever/elevated transaminase	1	No	No	Yes	Not applicable	No
	Patient 010	Fever/fatigue	1	No	No	Yes	Continue	No

Table 4 Evaluation of efficacy (Full Analysis Set, FAS)

Response	Low-dose cohort (N = 3)	Middle-dose cohort (N = 3)	High-dose cohort (N = 4)	Total (N = 10)
Week 4, *n* (%)				
CR	0	0	0	0
PR	1 (33.3%)	0	1 (25.0%)	2 (20.0%)
SD	1 (33.3%)	3 (100.0%)	2 (50.0%)	6 (60.0%)
PD	1 (33.3%)	0	1 (25.0%)	2 (20.0%)
NA	0	0	0	0
ORR[a]	1 (33.3%)	0	1 (25.0%)	2 (20.0%)
95% CI	0.8%–90.6%	0.0%–70.8%	0.6%–80.6%	2.5%–55.6%
Week 12, *n* (%)				
CR	2 (66.7%)	0	1 (25.0%)	3 (30.0%)
PR	0	1 (33.3%)	1 (25.0%)	2 (20.0%)
SD	0	1 (33.3%)	0	1 (10.0%)
PD	0	1 (33.3%)	1 (25.0%)	2 (20.0%)
NA	0	0	0	0
ORR[a]	2 (66.7%)	1 (33.3%)	2 (50.0%)	5 (50.0%)
95% CI	9.4%–99.2%	0.8%–90.6%	6.8%–93.2%	18.7%–81.3%
DCR[b]	2 (66.7%)	2 (66.7%)	2 (50.0%)	6 (60.0%)
95% CI	9.4%–99.2%	9.4%–99.2%	6.8%–93.2%	26.2%–87.8%

N, number of patients; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; DCR, disease control rate; CI, confidence interval.
[a]ORR = (CR + PR)/Total *N*.
[b]DCR = (CR + PR + SD)/Total *N*.

shown in Table 4. At 4 weeks post-infusion of C-CAR011, the overall response rate was 20%, with two patients achieving partial remission (PR). The CAR-T therapy yielded an ORR of 50% at 12 weeks after infusion. Complete remission (CR) was obtained by three patients, and PR was achieved by two patients. For the different dose groups, the ORR was 66.7% in the 0.8×10^6 CAR-T cells/kg cohort, 33.3% in the 2.5×10^6 CAR-T cells/kg cohort, and 50% in the 5×10^6 CAR-T cells/kg cohort. Disease control rate at week 12 was 60% and was 66.7%, 66.7%, and 50% in the low-, middle-, and high-dose cohorts, respectively.

Among the 10 patients receiving treatment, two had withdrawn from the study after 4 weeks, for whom the efficacy at week 12 was not available. However, the therapeutic efficacy of C-CAR011 therapy for DLBCL based on the per-protocol set (PPS) was consistent with that based on the FAS.

Exploratory results

After the infusion of C-CAR011 cells, the DNA copy number of CAR-T cells targeting CD19 in peripheral blood increased rapidly. The number of CAR copies reached a peak level in the blood around 10 days after infusion and remained at a high level in most patients until 12 weeks (Fig. 2A). In four patients, the number of CD19-positive B cells in peripheral blood decreased significantly after the infusion of C-CAR011 (Fig. 2B). This finding indicated that the CAR-T cells can effectively proliferate and expand in patients and have the ability to remove CD19-positive B cells.

The serum levels of cytokines were detected one week prior to C-CAR011 infusion and at multiple time points after infusion (Fig. 3). The level of serum interleukin (IL)-2, IL-4, and tumor necrosis factor (TNF)-α in peripheral blood were lower than the detection limit (IL-2, 17.55 pg/mL; IL-4, pg/mL; TNF-α, 16.00 pg/mL) at all time points. In particular, the serum IL-6 in nine patients and the interferon (IFN)-γ in two patients increased briefly after treatment and then returned to normal levels. Four patients had elevated serum IL-10 level after CAR-T cell infusion, and this trend was detected prior to infusion in three of these patients. Further analysis was conducted on the correlation between serum cytokine levels and CRS, and a significant correlation was found between temperature (T) and IL-6 (P = 0.0073) on day 4, between C-reaction protein and IL-6 (P = 0.0341) on day 4, and between temperature and IFN-γ (P = 0.0245) on day 10.

Discussion

In this study, the safety and efficacy of C-CAR011 therapy in the treatment of refractory DLBCL in Chinese patients was reported. Ten patients with refractory DLBCL in three dose cohorts received conditioning chemotherapy and

three consecutive days of CAR-T cell infusion. Except for age, no substantial differences in demographic and baseline characteristics were noted among the different dose cohorts.

As a novel cellular immunotherapy, the safety and toxicity profile of CD19 CAR-T cell therapy have been widely studied, especially the most common CRS and neurotoxicity [24,25]. The costimulatory domains of second-generation CARs enhance the cell proliferation ability and simultaneously produce high cytotoxicity [26]. The results reveal that the use of CBM.CD19 CAR harboring 4-1BB costimulatory domain is generally safe and feasible. Among the 10 patients who received treatment, the infusions of C-CAR011 were well tolerated without DLTs observed at any dose level. Only one patient in the low-dose cohort developed serious TEAE, which was evaluated and confirmed to be unrelated to the CAR-T therapy. The TEAEs in other patients were mild and clinically manageable, and no TEAE-related deaths were observed.

CRS is the most prominent and severe toxic effect of anti-CD19 CAR-T cell therapy and is accompanied by dramatic elevations of multiple serum cytokines including IL-2, IL-6, IL-8, IL-10, and IFN-γ [27]. In this study, the incidence of CRS of all grades was 90%, which was similar to those of tisagenlecleucel and axicabtagene

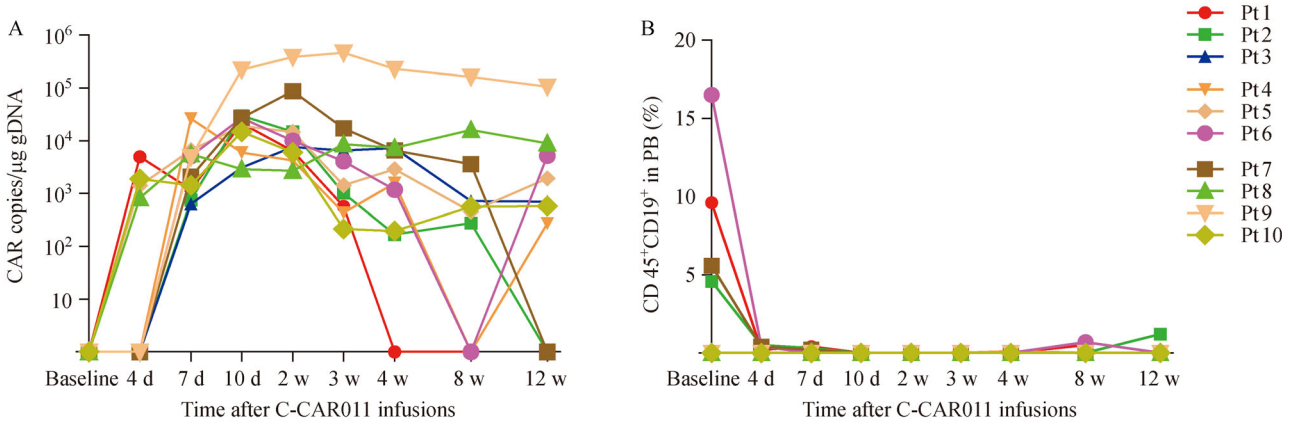

Fig. 2 (A) Persistence of C-CAR011 in peripheral blood (PB); (B) Percentage of $CD45^{+}CD19^{+}$ cells in PB. d, day; w, week; Pt, patient.

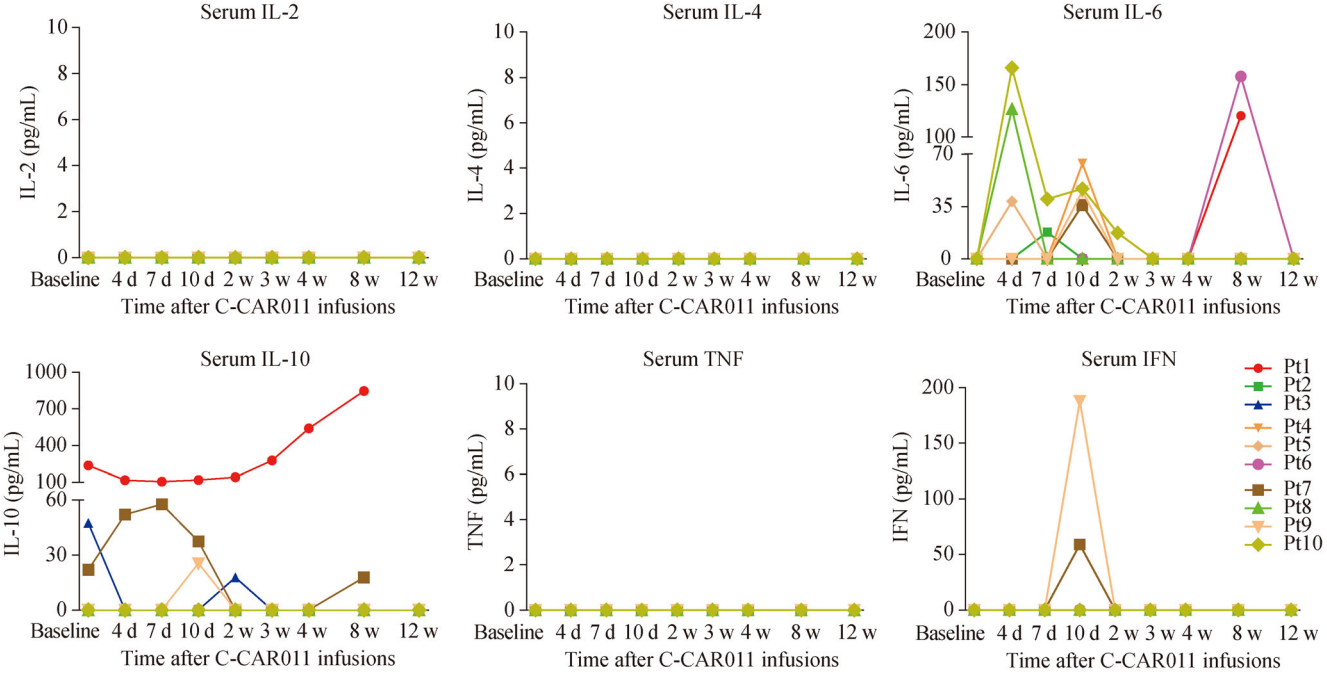

Fig. 3 Levels of serum cytokines in peripheral blood at baseline and after C-CAR011 infusions. d, day; w, week; Pt, patient.

ciloleucel (58% and 93%, respectively), two CD19 CAR-T regimens approved by the FDA [18,28]. The CRS in our patients was less severe than that in previous reports. Only one case of grade 2 CRS was noted, and no grade $\geqslant 3$ CRS occurred. By contrast, the incidence of grade $\geqslant 3$ CRS in patients receiving tisagenlecleucel and axicabtagene ciloleucel was 22% and 13%, respectively [18,28]. In all patients with CRS in this study, the symptoms were either self-limiting or clinically manageable with only supportive treatment. No patients received tocilizumab or corticosteroids, and no CRS led to the withdrawal of patients or dose adjustment/discontinuation. The levels of serum cytokines, including IL-6, IL-10, and IFN-γ, were temporally increased in some patients after treatment and returned to normal a few days later. The high level of IL-6 on day 4 and IFN-γ on day 10 after initial CAR-T cell infusion was substantially associated with CRS. No neurotoxicity occurred in any dose cohort after C-CAR011 infusion, indicating that safety of this treatment is remarkably superior to other CAR-T therapies with high incidence rates of neurotoxicity [29].

The results meet the primary objective of this clinical trial regarding safety and feasibility. Each dose of C-CAR011 exhibited a favorable safety profile for the treatment of patients with refractory DLBCL. The data implied that the safety profile of C-CAR011 is similar to or slightly better than that of FDA-approved CD19 CAR-T drugs. However, the sample size of this study is relatively small, and the safety profile related to CRS and cytokines in CAR-T therapy requires further investigation.

According to the international SCHOLAR-1 study, patients with DLBCL that was aggressive or resistant to primary or salvage chemotherapy and those who relapsed within 12 months after ASCT had ORR and CR rate of 26% and 7%, respectively, and the median OS was 6.3 months with currently available therapies [5]. In the present work, high-risk patients without curative therapeutic options were enrolled. At baseline, 80% of the patients presented with an advanced stage DLBCL (modified Ann Arbor stage III or IV), 80% had previously experienced three or more lines of treatment, and 70% had achieved only SD or PD as the best response to prior treatment. Although the primary endpoint of this clinical trial was not the efficacy parameter, the preliminary efficacy results were encouraging. In summary, the administration of C-CAR011 treatment could yield an ORR of 50%, including 30% CR and 20% PR rate. Approximately 60% of patients achieved disease control at 12 weeks after CAR-T cell infusion, suggesting its promising anti-tumor effect in patients with refractory DLBCL. These results were roughly consistent with the two FDA-approved CD19 CAR-T products, that is, 50% ORR, 43% CR, and 7% PR for tisagenlecleucel [17] and 82% ORR, 54% CR, and 28% PR for axicabtagene ciloleucel [18].

In accordance with the observed clinical responses, the number of T cells expressing the CAR gene rapidly increased to a peak period of approximately 10 days after infusion and remained at a high level until 12 weeks in most patients. Meanwhile, the number of CD19-positive B cells in peripheral blood decreased substantially after C-CAR011 infusion. These results indicate that C-CAR011 cells can effectively proliferate in patients and eliminate CD19-positive B cells.

The results from this phase I clinical trial confirm the safety and feasibility of C-CAR011 therapy in the treatment of Chinese patients with refractory DLBCL. The three dose cohorts of C-CAR011, 0.8×10^6, 2.5×10^6, and 5.0×10^6 CAR-T cells/kg were well tolerated by patients with no observed DLTs or unexpected side effects, and the episodes of CRS were generally manageable and reversible. The response rates were comparable with that reported in other studies of CD19 CAR-T therapy in predominant Caucasian patients. All dose cohorts showed antitumor effect, thus supporting the need for further clinical studies in a large phase II clinical trial with a recommended dose of 5.0×10^6 CAR-T cells/kg. Continuous follow-up will be conducted for these patients to assess long-term efficacy and patient survival.

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Compliance with ethics guidelines

Lei Fan, Li Wang, Lei Cao, Huayuan Zhu, Wei Xu, and Jianyong Li declare no conflict of interests. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the *Helsinki Declaration* of 1975 as revised in 2000 (5). Informed consent was obtained from all patients.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-021-0843-8 and is accessible for authorized users.

References

1. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. Blood 2015; 125(1): 22–32
2. Van Den Neste E, Schmitz N, Mounier N, Gill D, Linch D, Trneny M, Milpied N, Radford J, Ketterer N, Shpilberg O, Dührsen U, Ma D, Brière J, Thieblemont C, Salles G, Moskowitz CH, Glass B, Gisselbrecht C. Outcome of patients with relapsed diffuse large B-

cell lymphoma who fail second-line salvage regimens in the International CORAL study. Bone Marrow Transplant 2016; 51(1): 51–57

3. Gisselbrecht C, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, Bosly A, Ketterer N, Shpilberg O, Hagberg H, Ma D, Brière J, Moskowitz CH, Schmitz N. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol 2010; 28(27): 4184–4190
4. Nagle SJ, Woo K, Schuster SJ, Nasta SD, Stadtmauer E, Mick R, Svoboda J. Outcomes of patients with relapsed/refractory diffuse large B-cell lymphoma with progression of lymphoma after autologous stem cell transplantation in the rituximab era. Am J Hematol 2013; 88(10): 890–894
5. Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, Link BK, Hay A, Cerhan JR, Zhu L, Boussetta S, Feng L, Maurer MJ, Navale L, Wiezorek J, Go WY, Gisselbrecht C. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood 2017; 130(16): 1800–1808
6. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 2011; 365(8): 725–733
7. Turtle CJ, Hay KA, Hanafi LA, Li D, Cherian S, Chen X, Wood B, Lozanski A, Byrd JC, Heimfeld S, Riddell SR, Maloney DG. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. J Clin Oncol 2017; 35(26): 3010–3020
8. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, Mahnke YD, Melenhorst JJ, Rheingold SR, Shen A, Teachey DT, Levine BL, June CH, Porter DL, Grupp SA. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371(16): 1507–1517
9. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Bader P, Verneris MR, Stefanski HE, Myers GD, Qayed M, De Moerloose B, Hiramatsu H, Schlis K, Davis KL, Martin PL, Nemecek ER, Yanik GA, Peters C, Baruchel A, Boissel N, Mechinaud F, Balduzzi A, Krueger J, June CH, Levine BL, Wood P, Taran T, Leung M, Mueller KT, Zhang Y, Sen K, Lebwohl D, Pulsipher MA, Grupp SA. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018; 378 (5): 439–448
10. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, Yang JC, Phan GQ, Hughes MS, Sherry RM, Raffeld M, Feldman S, Lu L, Li YF, Ngo LT, Goy A, Feldman T, Spaner DE, Wang ML, Chen CC, Kranick SM, Nath A, Nathan DA, Morton KE, Toomey MA, Rosenberg SA. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 2015; 33(6): 540–549
11. Kochenderfer JN, Somerville RPT, Lu T, Shi V, Bot A, Rossi J, Xue A, Goff SL, Yang JC, Sherry RM, Klebanoff CA, Kammula US, Sherman M, Perez A, Yuan CM, Feldman T, Friedberg JW, Roschewski MJ, Feldman SA, McIntyre L, Toomey MA, Rosenberg SA. Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum interleukin-15 levels. J Clin Oncol 2017; 35(16): 1803–1813
12. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, Lin Y, Braunschweig I, Hill BT, Timmerman JM, Deol A, Reagan PM, Stiff P, Flinn IW, Farooq U, Goy A, McSweeney PA, Munoz J, Siddiqi T, Chavez JC, Herrera AF, Bartlett NL, Wiezorek JS, Navale L, Xue A, Jiang Y, Bot A, Rossi JM, Kim JJ, Go WY, Neelapu SS. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. Lancet Oncol 2019; 20(1): 31–42
13. Abramson J, Gordon L, Palomba M, Lunning M, Arnason J, Forero-Torres A, Wang M, Maloney D, Sehgal A, Andreadis C, Purev E, Solomon SR, Ghosh N, Albertson TM, Xie B, Garcia J, Siddiqi T. Updated safety and long term clinical outcomes in TRANSCEND NHL 001, pivotal trial of lisocabtagene maraleucel (JCAR017) in R/R aggressive NHL. J Clin Oncol 2018; 36(15_suppl): 7505
14. Salter AI, Pont MJ, Riddell SR. Chimeric antigen receptor-modified T cells: CD19 and the road beyond. Blood 2018; 131(24): 2621–2629
15. Kallam A, Vose JM. Recent advances in CAR-T cell therapy for non-Hodgkin lymphoma. Clin Lymphoma Myeloma Leuk 2019; 19 (12): 751–757
16. Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, Kamble RT, Bollard CM, Gee AP, Mei Z, Liu H, Grilley B, Rooney CM, Heslop HE, Brenner MK, Dotti G. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest 2011; 121(5): 1822–1826
17. Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak Ö, Brogdon JL, Pruteanu-Malinici I, Bhoj V, Landsburg D, Wasik M, Levine BL, Lacey SF, Melenhorst JJ, Porter DL, June CH. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med 2017; 377(26): 2545–2554
18. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM, Stiff PJ, Friedberg JW, Flinn IW, Goy A, Hill BT, Smith MR, Deol A, Farooq U, McSweeney P, Munoz J, Avivi I, Castro JE, Westin JR, Chavez JC, Ghobadi A, Komanduri KV, Levy R, Jacobsen ED, Witzig TE, Reagan P, Bot A, Rossi J, Navale L, Jiang Y, Aycock J, Elias M, Chang D, Wiezorek J, Go WY. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017; 377(26): 2531–2544
19. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroncek D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS, Mackall CL. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015; 385(9967): 517–528
20. Locke FL, Neelapu SS, Bartlett NL, Siddiqi T, Chavez JC, Hosing CM, Ghobadi A, Budde LE, Bot A, Rossi JM, Jiang Y, Xue AX, Elias M, Aycock J, Wiezorek J, Go WY. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. Mol Ther 2017; 25(1): 285–295
21. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL. Current concepts in the diagnosis and management of cytokine release syndrome. Blood 2014; 124(2): 188–195

22. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, Maus MV, Park JH, Mead E, Pavletic S, Go WY, Eldjerou L, Gardner RA, Frey N, Curran KJ, Peggs K, Pasquini M, DiPersio JF, van den Brink MRM, Komanduri KV, Grupp SA, Neelapu SS. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant 2019; 25(4): 625–638
23. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, Komanduri KV, Lin Y, Jain N, Daver N, Westin J, Gulbis AM, Loghin ME, de Groot JF, Adkins S, Davis SE, Rezvani K, Hwu P, Shpall EJ. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. Nat Rev Clin Oncol 2018; 15(1): 47–62
24. Hay KA. Cytokine release syndrome and neurotoxicity after CD19 chimeric antigen receptor-modified (CAR-) T cell therapy. Br J Haematol 2018; 183(3): 364–374
25. Jin Z, Xiang R, Qing K, Li X, Zhang Y, Wang L, Zhu H, Mao Y, Xu Z, Li J. The severe cytokine release syndrome in phase I trials of CD19-CAR-T cell therapy: a systematic review. Ann Hematol 2018; 97(8): 1327–1335
26. Curran KJ, Pegram HJ, Brentjens RJ. Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions. J Gene Med 2012; 14(6): 405–415
27. Murthy H, Iqbal M, Chavez JC, Kharfan-Dabaja MA. Cytokine release syndrome: current perspectives. ImmunoTargets Ther 2019; 8: 43–52
28. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Jäger U, Jaglowski S, Andreadis C, Westin JR, Fleury I, Bachanova V, Foley SR, Ho PJ, Mielke S, Magenau JM, Holte H, Pantano S, Pacaud LB, Awasthi R, Chu J, Anak Ö, Salles G, Maziarz RT; JULIET Investigators. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 2019; 380(1): 45–56
29. Gust J, Taraseviciute A, Turtle CJ. Neurotoxicity associated with CD19-targeted CAR-T cell therapies. CNS Drugs 2018; 32(12): 1091–1101