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RESEARCH ARTICLE

# C-CAR066, a novel fully human anti-CD20 CAR-T therapy for relapsed or refractory large B-cell lymphoma after failure of anti-CD19 CAR-T therapy: A phase I clinical study

Ping Li[1] | Wei Liu[2,3] | Lili Zhou[1] | Shiguang Ye[1] | Dan Zhu[4] | Jiaqi Huang[4] | Jing Li[4] | Chengxiao Zheng[4] | Shigui Zhu[4] | Xin Yao[4] | Kevin Zhu[5] | Yihong Yao[4] | Lugui Qiu[2,3] | Aibin Liang[1] | Dehui Zou[2,3]

[1]Tongji Hospital of Tongji University, Shanghai, China

[2]State Key Laboratory of Experimental Hematology, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

[3]Tianjin Institutes of Health Science, Tianjin, China

[4]Shanghai AbelZeta Ltd., Shanghai, China

[5]University of Maryland School of Medicine, Baltimore, Maryland, USA

**Correspondence**
Dehui Zou, State Key Laboratory of Experimental Hematology, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 288 Nanjing Road, Heping District, Tianjin, China.
Email: zoudehui@ihcams.ac.cn

Aibin Liang, Tongji Hospital of Tongji University, No. 389, Xincun Road, Shanghai, China.
Email: lab7182@tongji.edu.cn

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## Abstract

Managing large B-cell lymphoma (LBCL) that is refractory to or relapsed after chimeric antigen receptor (CAR)-T therapy remains a significant challenge. Here we aimed to investigate the safety and efficacy of C-CAR066, an autologous fully human anti-CD20 specific CAR-T, for relapsed/refractory LBCL after failure of anti-CD19 CAR-T therapy. This first-in-human, single-arm, phase 1 study was conducted at two sites in China. Eligible patients had to be histologically confirmed with CD20-positive LBCL and must have received prior anti-CD19 CAR-T therapy. Patients received a single intravenous infusion of C-CAR066 at a target dose of $2.0 \times 10^6$ or $3.0 \times 10^6$ CAR-T cells/kg. The primary endpoint was the incidence of adverse events (AEs). As of October 10, 2023, 14 patients had received C-CAR066. The most common AEs of Grade 3 or higher were hematological toxicities. Cytokine release syndrome occurred in 12 (85.7%) patients, with only one was Grade 4 event. No patient experienced immune effector cell-associated neurotoxicity syndrome events. The overall response rate was 92.9% with a complete response rate of 57.1%. With a median follow-up of 27.7 months (range, 3.3–40.9), the median progression-free survival and overall survival were 9.4 months (95% CI, 2.0 to NA) and 34.8 months (95% CI, 7.5 to NA), respectively. C-CAR066 demonstrated a manageable safety profile and promising efficacy in patients in whom prior anti-CD19 CAR-T therapies had failed, providing a promising treatment option for those patients. This trial was registered with ClinicalTrials.gov, NCT04316624 and NCT04036019.

Ping Li and Wei Liu contributed equally to this work.

## 1 | INTRODUCTION

Since 2017, several chimeric antigen receptor (CAR)-T therapies targeting CD19 have been approved, including tisagenlecleucel, axicabtagene ciloleucel (axi-cel), lisocabtagene maraleucel (liso-cel) and relmacabtagene autoleucel, marking important milestones for the treatment of large B-cell lymphoma (LBCL). Although remarkable response rates have been achieved at 53%–80% in patients with relapsed/refractory (r/r) LBCL, nearly 60% of patients experience progression at 2 years after CAR-T infusion,[1–4] and clinical outcomes in those patients are poor. Overall response rate (ORR) and complete response (CR) rate of salvage therapies, including bispecific antibodies and antibody–drug conjugates (ADCs), were 8%–54.1% and 3.8%–35%, respectively, while median progression-free survival (PFS) and overall survival (OS) were 1.4–3.8 months and 3.8–9.3 months.[5–12] Therefore, novel and effective treatments for r/r LBCL after failure of anti-CD19 CAR-T are urgently needed.

CD20 is specifically expressed in more than 95% of normal and cancerous B cells, which makes it an ideal target for immunotherapy of B-cell malignancies.[13] Anti-CD20 monoclonal antibody has been used in a variety of malignancies. Recently, CAR-T therapies targeting CD20 or CD19/CD20 have shown promising efficacy in the treatment of refractory/relapsed B-cell lymphoma.[14–19] However, there have been few studies using anti-CD20 CAR-T to treat patients with r/r LBCL in whom prior anti-CD19 CAR-T therapies had failed.

C-CAR066 is a fully human CD20-specific autologous CAR-T product developed by Shanghai AbelZeta Ltd. We conducted an open-label, single-arm phase 1 trial to explore the safety and efficacy of C-CAR066 in r/r LBCL after failure of anti-CD19 CAR-T therapy. Here we present the primary results of patients treated with C-CAR066 in this phase 1 trial.

## 2 | METHODS

### 2.1 | Patients and study design

This first-in-human (FIH) study is a single-arm, phase 1 study conducted at two sites in China (ClinicalTrials.gov identifier: NCT04316624 and NCT04036019). Eligible patients had to be at least 18 years old and have histologically confirmed CD20 positive diffuse large B-cell lymphoma (DLBCL) (including de novo and transformed from follicular lymphoma), or primary mediastinal large B cell lymphoma (PMBCL) by 2016 revision of World Health Organization (WHO) classification. Patients must have received prior anti-CD19 CAR-T therapy and have adequate organ and bone marrow function. Patients with central nervous system (CNS) involvement at screening were ineligible.

Patients underwent leukapheresis followed by lymphodepleting chemotherapy (fludarabine and cyclophosphamide) on days 5 through 3 and received a single intravenous infusion of C-CAR066 on Day 0. The dosage of cyclophosphamide is fixed at 300 mg/m$^2$/day, but the dosages of fludarabine and C-CAR066 were determined by different institutions. Patients enrolled at the Institute of Hematology and Blood Diseases Hospital received fludarabine of 30 mg/m$^2$/day and C-CAR066 of $2.0 \times 10^6$ cells/kg. Patients enrolled at Tongji Hospital received fludarabine of 25 mg/m$^2$/day and C-CAR066 of $3.0 \times 10^6$ cells/kg. Hospital admission was required for observation and monitoring after C-CAR066 infusion.

All patients provided written informed consent. The study was approved by the institutional review board at each study site and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization guidelines for Good Clinical Practice.

### 2.2 | Endpoints and assessments

The primary endpoint was the incidence of adverse events (AEs). Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were graded according to American Society for Transplantation and Cellular Therapy criteria (ASTCT) 2019,[20] other AEs were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. Secondary endpoints included ORR, CR rate, duration of response (DOR), PFS, and OS as assessed by the investigators according to Lugano 2014 criteria.

### 2.3 | Statistical analyses

The safety analysis set included all patients who received C-CAR066. The efficacy analysis set included all patients who had confirmed active disease before lymphodepletion and received C-CAR066.

Descriptive statistics were used to summarize means with standard deviations or medians with minimum and maximum for continuous variables and counts and percentages for categorical variables. Linear trapezoid method was used for AUC calculation. Two-sided 95% confidence intervals (95% CIs) were calculated using the Clopper–Pearson exact method for each response category. The median of DOR, PFS and OS were estimated by using the Kaplan–Meier method, and the corresponding 95% CI were estimated using the Kaplan–Meier combined Brookmeyer–Crowley method. DOR was defined as the time from the first documented response to the first documented progressive disease (PD) or death from any cause. In patients who did not show disease progression or death on the analysis day, censoring was performed, and their response were noted as ongoing, except for those who were censored due to being lost to follow-up, withdrawal of consent, or initiating subsequent therapy. PFS was defined as the time from C-CAR066 infusion to first documented PD or death from any cause. Patients not meeting the criteria for progression by the analysis cutoff date were censored. OS was defined as the time from C-CAR066 infusion to death from any cause. Patients who had not died by the analysis cutoff date were censored at the last date known alive.

Pharmacokinetic analyses used PKNCA R Package version 0.9.5, and all other statistical analyses were done with SAS version 9.4.

## 3 | RESULTS

### 3.1 | Preclinical

The structure of CAR in C-CAR066 has been optimized with a 4-1BB co-stimulatory domain and a long IgG4 hinge. A fully human single-chain variable fragment (scFv) was used, and two mutations (L235E/N297Q) were introduced in the IgG4 hinge to abolish the binding of FcgRI/II (Figure S1A). We included two CARs constructed with Leu16, another CD20-specific scFv, for comparison in preclinical studies. One of the Leu16 CARs has the same structure as C-CAR066, and the other one is a third-generation CAR with both the 4-1BB and CD28 costimulatory domains. Our results suggest that C-CAR066 has superior in vitro and in vivo anti-tumor activity compared with CAR-Ts derived from Leu16 scFv (Figure S1B,C).

### 3.2 | Patients

As of October 10, 2023, 20 patients were apheresed in the study. Of the 14 patients who successfully received C-CAR066 infusion manufactured with CliniMACS Prodigy system, 7 received $2.0 \times 10^6$ cells/kg, 7 received $3.0 \times 10^6$ cells/kg. Of six patients who were not dosed, one was due to manufacturing failure, three were due to disease progression and the other two were due to AE after bridging including heart failure and pneumonia (Figure 1).

Among the 14 patients in the safety and efficacy analysis set, the median age was 54.5 years (range, 37–67) and 10 (71.4%) patients had DLBCL. None of them had CNS involvement at screening or in their medical history. Most patients (85.7%) had stage III or IV disease. Half of the patients had c-MYC/BCL-2 double-expressor lymphoma, and 64.3% patients had refractory disease. All patients were heavily pretreated, with a median five lines of previous systemic therapy, and all patients received anti-CD19 CAR-T therapy. Median duration of response to prior CAR-T therapy was 1.9 months (range, 0.4–6.1) and median time from prior anti-CD19 CAR T-cell therapy to C-CAR066 was 5.5 months (range, 3.4–14.2). Two patients (14.3%) underwent previous autologous stem cell transplant (ASCT). Bridging therapy was administered in 7 (50.0%) patients at the investigator's discretion. The median time from apheresis to receive C-CAR066 was 23 days (range, 17–91) (Tables 1 and S1).

### 3.3 | Safety

The safety analysis set included all 14 patients who received an infusion of C-CAR066. Adverse events were collected up to 2 years after C-CAR066 infusion. All patients had at least one adverse event. As shown in Table 2, the most common adverse events of Grade 3 or higher were all hematological adverse events, including lymphopenia (92.9%), neutropenia (92.9%), leukopenia (78.6%), anemia (50%) and thrombocytopenia (28.6%). Prolonged cytopenias, defined as Grade 3 or 4 cytopenias not resolved by Day 30, occurred in 4 (28.6%) patients (Table S2), which were resolved by Day 90 in two patients and by Day 180 in one patient. The other patient with prolonged cytopenias withdrew from the study due to disease progression at 2 months and received other treatments. Twelve (85.7%) patients had CRS, eight were Grade 1, three were Grade 2 and one patient dosed at $3.0 \times 10^6$ cells/kg experienced Grade 4 CRS. CRS was managed with tocilizumab, corticosteroids, or both in 2 (14.3%) patients. The median time to onset of CRS was 5.5 days (range, 2–15), and the median time to resolution was 4.0 days (range, 1–15). No patient had experienced ICANS. No patient was admitted to the ICU. Eight (57.1%) patients had infections after C-CAR066 infusion, mostly were Grades 1 and 2. Pneumonia and urinary tract infection were the most common infections (Table S3). Four patients had five events of SAEs after C-CAR066 infusion, and only two SAEs in one patient were related to C-CAR066, which were CRS and myelosuppression (Table S4). As of cut-off date, seven deaths occurred. All were due to disease progression. No second primary malignancy and new safety signals were observed with longer follow-up.

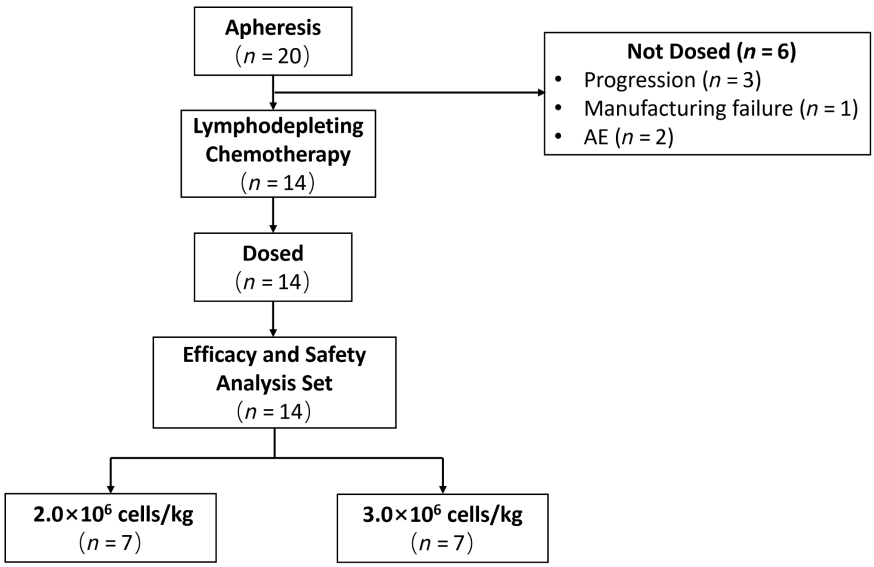


**FIGURE 1** Patients disposition.
*A manufacturing deviation occurred for one subject in the $3.0 \times 10^6$ cell/kg cohort who received $4.8 \times 10^6$ cell/kg C-CAR066. Data for this subject was included in the $3.0 \times 10^6$ cell/kg cohort. Two patients were not dosed due to AE after bridging including heart failure and pneumonia.

**TABLE 1** Baseline characteristics of patients who received C-CAR066.

| Baseline characteristics | All patients ($n = 14$) |
|---|---|
| Median age, years (range) | 54.5 (37–67) |
| ≥65 years, *n* (%) | 2 (14.3) |
| Male, *n* (%) | 5 (35.7) |
| Lymphoma subtype, *n* (%) | |
| Diffuse large B-cell lymphoma | 10 (71.4) |
| Transformed follicular lymphoma | 4 (28.6) |
| Ann Arbor Stage III/IV, *n* (%) | 12 (85.7) |
| ECOG performance status, *n* (%) | |
| 0 | 5 (35.7) |
| 1 | 9 (64.3) |
| International prognostic index score 3/4, *n* (%) | 8 (57.1) |
| Cell of origin, *n* (%) | |
| Germinal center B-cell type | 9 (64.3) |
| Non-germinal center B-cell type | 5 (35.7) |
| SPD, *N* (%) | |
| ≥4000 $mm^2$ | 6 (42.9) |
| ≤4000 $mm^2$ | 8 (57.1) |
| Double-expresser lymphoma, *n* (%) | 7 (50.0) |
| Refractory lymphoma,[a] *n* (%) | 9 (64.3) |
| Median number of prior lines of therapy (range) | 5.0 (2–7) |
| ≥4, *n* (%) | 12 (85.7) |
| Prior autologous stem cell transplant | 2 (14.3) |
| Best response to prior CAR-T | |
| CR | 2 (14.3) |
| PR | 10 (71.4) |
| SD | 1 (7.1) |
| PD | 1 (7.1) |
| Median duration of response of prior CAR-T therapy, months (range) | 1.9 (0.4–6.1) |
| Prior CAR-T Target | |
| CD19 | 12 (85.7) |
| CD19 and CD79b | 1 (7.1) |
| CD19 and CD22 | 1 (7.1) |
| Median time from prior CAR-T to C-CAR066, months (range) | 5.5 (3.4–14.2) |
| Received bridging therapy, *n* (%) | 7 (50.0) |
| Median time from leukapheresis to infusion, days (range) | 23 (17–91) |

Abbreviations: LDH, lactate dehydrogenase; PD, progressive disease; SD, stable disease.
[a]Refractory was defined as progressive disease or stable disease as best response to last therapy (≥2 cycles) or relapsed at ≤12 months from autologous stem cell transplantation.

## 3.4 | Efficacy

Among the 14 patients included in the efficacy analysis set, 13 (92.9%) patients displayed a response to C-CAR066, with a CR rate of 57.1% (Figure 2D,E, Table S1). By the cut-off date, 4 (28.6%) patients remain in CR for more than 30 months (Figure 2D,E). The median time to response (TTR) was 1.0 month (range, 0.9–2.8), and among the patients who achieved CR, the median time to CR was 2.5 months (range, 1.0–2.8). At a median follow-up of 27.7 months (range, 3.3–40.9), the median duration of response was 8.4 months (95% CI, 1.1 to NA) (Figure 2A). Median progression-free survival was 9.4 months (95% CI, 2.0 to NA). Median overall survival was 34.8 months (95% CI, 7.5 to NA). Among those who had a complete response, the median PFS and OS were not reached (Figure 2B,C). A total of 9 patients experienced disease recurrence, of whom 8 had post relapse biopsies tested. Three patients showed CD20 negative expression at relapse.

**TABLE 2** Adverse events occurring in >20% patients.

| | Any grade ($n = 14$) | Grade ≥3 ($n = 14$) |
|---|---|---|
| Neutropenia | 14 (100) | 13 (92.9) |
| Leukopenia | 14 (100) | 11 (78.6) |
| Anemia | 14 (100) | 7 (50.0) |
| Lymphopenia | 13 (92.9) | 13 (92.9) |
| Pyrexia | 13 (92.9) | 2 (14.3) |
| Cytokine release syndrome | 12 (85.7) | 1 (7.1) |
| Thrombocytopenia | 9 (64.3) | 4 (28.6) |
| Hypogammaglobulinemia | 9 (64.3) | 2 (14.3) |
| Hypoalbuminemia | 9 (64.3) | 1 (7.1) |
| Hypokalemia | 8 (57.1) | 0 |
| Hypertriglyceridemia | 7 (50.0) | 0 |
| Blood fibrinogen decreased | 6 (42.9) | 0 |
| Hypotension | 5 (35.7) | 0 |
| Hyponatremia | 5 (35.7) | 0 |
| Hypocalcemia | 5 (35.7) | 0 |
| Constipation | 5 (35.7) | 0 |
| Aspartate aminotransferase increased | 5 (35.7) | 1 (7.1) |
| Alanine aminotransferase increased | 5 (35.7) | 0 |
| Hyperuricemia | 4 (28.6) | 0 |
| Hypomagnesemia | 4 (28.6) | 0 |
| Rash | 4 (28.6) | 0 |
| Sinus tachycardia | 3 (21.4) | 0 |
| Hypophosphatemia | 3 (21.4) | 1 (7.1) |
| Nausea | 3 (21.4) | 0 |
| *Infections* | *8 (57.1)* | *2 (14.3)* |

## 3.5 | Pharmacokinetics and pharmacodynamics

Of 14 patients, 13 had assessable pharmacokinetic and pharmacodynamics data (one patient dropped before Day 28). The median $T_{max}$ was 11 days (range, 10–23). The median $C_{max}$ was 398 996 copies/μg gDNA (range, 51 667–1 286 932). The median of $AUC_{0-28d}$ was

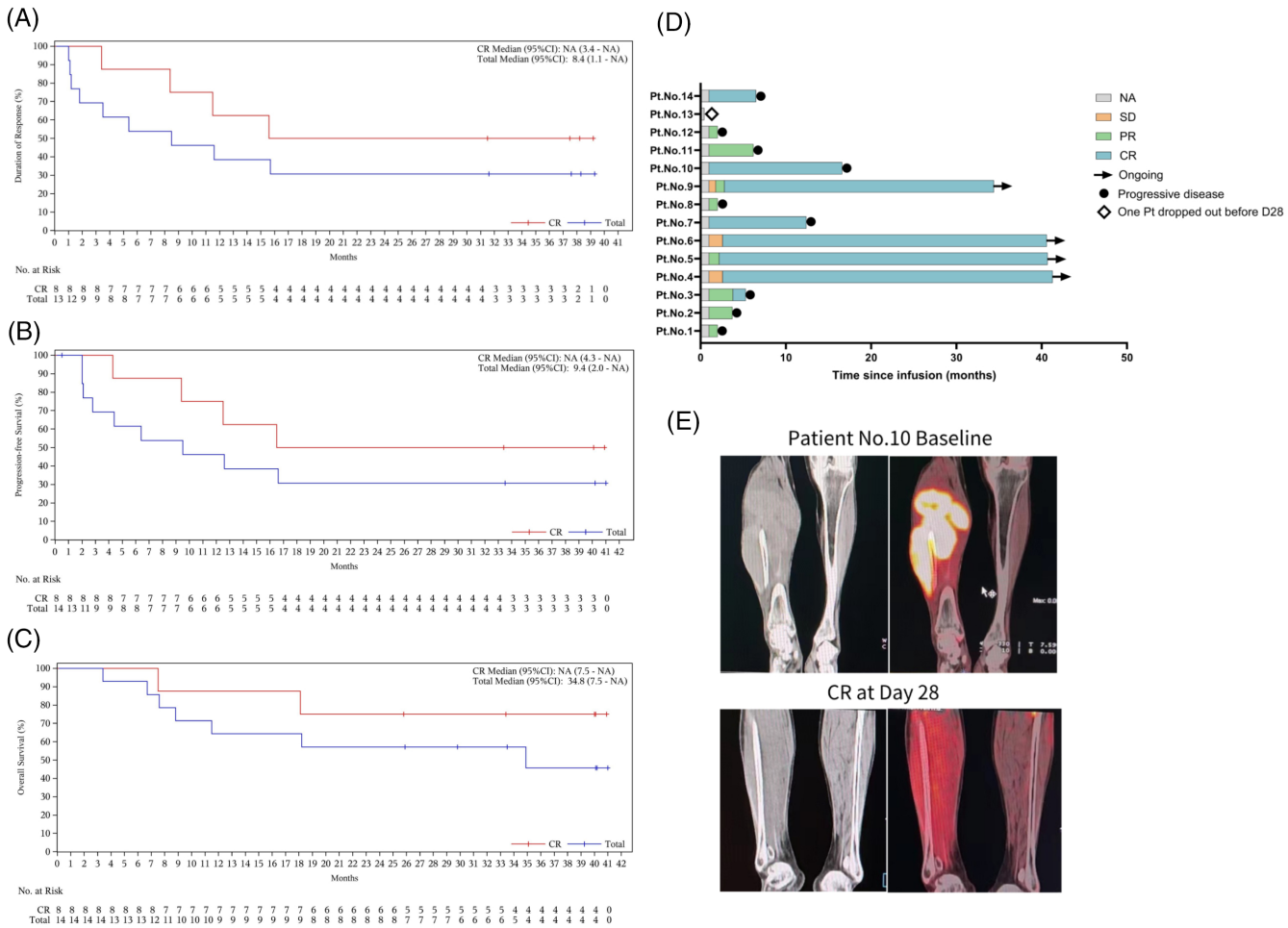


**FIGURE 2** Clinical outcomes of treatment with C-CAR066. (A) Kaplan–Meier curves of duration of response. (B) Kaplan–Meier curves of progression-free survival. (C) Kaplan–Meier curves of overall survival. (D) Treatment responses in patients following C-CAR066 infusion. (E) Representative PET scans of complete response.

4 437 474 day × copies/μg gDNA (range, 431 534–17 842 217), and the median $T_{last}$ was 59 days (range, 21–571+). CAR-T cell persistence was detected for >6 months in three patients, and two patients still had detectable CAR-T cells after 18 months (Figure S2A). Four (30.8%) patients had detectable levels of CD45+ CD19+ and CD45+ CD20+ B cells at baseline. After C-CAR066 infusion, the proportion of CD45+ CD19+ and CD45+ CD20+ B cells in peripheral blood decreased gradually and correlated with the expansion of the C-CAR066 CAR-T cells. Half of the patients showed B-cell recovery during 2-year follow-up (Figure S2B).

## 4 | DISCUSSION

Our study indicated that C-CAR066 can produce a deep and durable response in patients with r/r LBCL in whom prior anti-CD19 CAR-T therapy has failed. The ORR was 92.9% with a CR rate of 57.1%. With a median follow-up of 27.7 months, the median PFS and OS were 9.4 and 34.8 months, respectively.

Previous studies have demonstrated that patients with disease progression after CAR19 therapy have a poor prognosis, despite the use of salvage treatment options including bispecific antibodies, antibody-drug conjugates, checkpoint inhibitors, even a second anti-CD19 CAR-T infusion.

In a recent DESCAR-T analysis of 238 r/r LBCL pts who experienced progression or relapse after CAR T-cell, the median PFS and OS are 2.8 and 5.2 months respectively.[9] Studies suggested that antibody–drug conjugates including loncastuximab, an anti-CD19 ADC, and polatuzumab vedotin (pola), an anti-CD79b ADC, to be options for patients in whom prior anti-CD19 CAR-T therapy has failed, with a median PFS of 1.4 months and a median OS of 8.2 months for loncastuximab and a median PFS of 10 weeks for pola.[12,21] Pembrolizumab, an anti-PD-1 drug, showed a best ORR of 25% for B-cell lymphomas relapsing after or refractory to CD19-directed CAR-T therapy.[22] In addition, bispecific antibodies (BsAbs), an emerging therapeutic modality, have demonstrated a good efficacy in patients with r/r LBCL who had previous CAR T-cell therapy, with CR rates from 24% to 35%,[5–7] but long-term follow-up results are needed. Radiation and allogeneic hematopoietic cell

transplantation were also considered as salvage treatments, but only small minority of specific patients might benefit from them.[8,9,23,24] Compared with these salvage treatments, C-CAR066 might result in a better clinical benefit, with higher response rates and longer survival.

Furthermore, a second CAR-T infusion has been explored after failure of anti-CD19 CAR-T in patients with r/r LBCL. In TRANSCEND-NHL-001 trial, retreatment with liso-cel in 16 patients relapsing after the initial complete response resulted in a low response rate of 19%.[1] In ZUMA-1, 13 patients with progressive disease received axi-cel retreatment. A total of 7 (54%) patients achieved response (4 CR, 3 PR), but median DOR for the retreatment was only 81 days.[25] Data from another study of retreatment with a second infusion of anti-CD19 CAR-T in patients in whom a first CAR-T infusion had failed showed a CR rate of 19%.[26] In a case report, three patients with r/r DLBCL were administered axi-cel after treatment with tisagenlecleucel or investigational anti-CD19 CAR-T, showing a response of 2 PD and 1 CR.[27] These indicated that a second anti-CD19 CAR T infusion, with same or different CD19-directed CAR T-cell was feasible, but the efficacy was unsatisfactory. In a phase 1 dose-escalation study of CD22-directed CAR T-cell therapy in LBCL relapsing after anti-CD19 CAR-T, the ORR was 68% and the CR rate was 53%. The median PFS was 3.0 months, and the median OS was 14.1 months.[28] Zhu et al. also reported their findings of anti-CD22 CAR-T cell therapy as a salvage treatment after anti-CD19 CAR-T therapy. Of the seven DLBCL patients, four achieved CR, while two achieved PR and one achieved SD.[29] Our study further confirmed that after failure of anti-CD19 CAR-T treatment, a second infusion of CAR-T targeting a different target may be a valid option.

The safety profile of C-CAR066 was acceptable and manageable. The most common AEs were hematological toxicities and CRS. Of the 12 (85.7%) patients experiencing CRS, only one had Grade 4 CRS on Day 6, which resolved on Day 10 after tocilizumab and steroid treatment. The incidence and grade of CRS were similar to those after treatment with other CAR-T cell therapy.

Our study has some limitations. This is an open-label, single-arm study design, so caution is required when comparing its results with other CAR-T products. The expression of CD20 before treatment was only qualitatively assessed by immunohistochemistry, so the impact of the intensity of CD20 expression on efficacy could not be determined. In addition, the small sample size of this study made it impossible to analyze the factors that influence efficacy. Furthermore, the residual of prior CAR-T was not detected, so the impact of the presence of prior CAR-T on C-CAR066 was not analyzed.

In conclusion, preliminary results of C-CAR066 showed a favorable safety profile and a high and durable response in r/r LBCL after failure of anti-CD19 CAR-T therapy, providing a promising treatment option for patients in whom prior anti-CD19 CAR-T therapy has failed. Further investigation of C-CAR066, including an open-label study (NCT05784441) in relapsed or refractory B-cell non-Hodgkin lymphoma is ongoing.

## AUTHOR CONTRIBUTIONS

Lugui Qiu, Aibin Liang and Dehui Zou contributed to the study design, study conduct, data analysis, and data interpretation. Ping Li, Wei Liu, Lili Zhou and Shiguang Ye contributed to the study conduct, data acquisition, data analysis, and data interpretation. Dan Zhu, Jiaqi Huang, Jing Li, Shigui Zhu, Xin Yao and Yihong Yao contributed to the study design, data analysis, and data interpretation. Chengxiao Zheng contributed to study design and data acquisition. Kevin Zhu contributed to the statistical analyses. Analyses of pharmacokinetics and pharmacodynamics were done by Jiaqi Huang. All authors approved the final version to be published and agree to be accountable for all aspects of the work.

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## CONFLICT OF INTEREST STATEMENT

Dan Zhu, Jiaqi Huang, Jing Li, Chengxiao Zheng, Shigui Zhu, Xin Yao and Yihong Yao are employed by Shanghai AbelZeta Ltd. All other authors declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

Access to study data may be requested from the corresponding authors.

## INFORMED CONSENT

The study was conducted in accordance with Declaration of Helsinki and was approved by the institutional independent ethics committee of Institute of Hematology & Blood Diseases Hospital and Tongji Hospital of Tongji University. All patients provided written informed consent.

## ORCID

*Wei Liu* https://orcid.org/0000-0002-0448-7210
*Kevin Zhu* https://orcid.org/0000-0002-2728-1741
*Dehui Zou* https://orcid.org/0000-0002-8135-4913

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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