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Real-world experience of commercial relmacabtagene autoleucel (relma-cel) for relapsed/refractory central nervous system lymphoma: a multicenter retrospective analysis of patients in China

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ABSTRACT

Background Relapsed/refractory (R/R) central nervous system lymphomas (CNSLs) are associated with a poor prognosis. Relmacabtagene autoleucel (relma-cel), expressing the same chimeric antigen receptor (CAR) as lisocabtagene maraleucel, with an optimized commercial-ready process developed in China, demonstrated remarkable efficacy and manageable safety in the pivotal RELIANCE study. However, no published data are available on the "real-world" use of relma-cel, especially for patients with CNS involvement.

Patients and methods Retrospective analyses were conducted for commercial relma-cel used in patients with R/R CNSL at 12 clinics. The primary endpoint was to evaluate the proportion of patients who achieved complete response (CR) at 3 months. Secondary endpoints included best complete response (BCR), progression-free survival (PFS), duration of response (DOR), overall survival (OS), and the incidence of adverse events.

Results Among the 22 CNSL patients (12 primary CNSLs; 10 secondary CNSLs), the best overall response rate was 90.9% and the BCR rate was 68.2%. With median follow-up of 316 days (range, 55–618 days), the estimated 1-year PFS rate, DOR, and OS rate were 64.4%, 71.5%, and 79.2%, respectively. Significant clinical benefits were observed in patients who were in durable CR or partial response to the most recent prior therapy preleukapheresis and received relma-cel as consolidation therapy (n=8), with 1-year PFS rate of 100.0% versus 41.7% (p=0.02). In addition, in terms of primary endpoint, non-CR at 3 months postinfusion seemed to be predictive of a worse prognosis, with an estimated 1-year PFS of 83.3% versus 37.0% (p=0.03), respectively. CRS occurred in 72.9% of patients (grade 3: 4.5%) and immune effector cell-associated neurotoxicity syndrome in 36.4% of patients (grade 3: 4.5%). With the add-on agent PD-1 inhibitor (tislelizumab) to the ongoing BTKi, significant re-expansions of CAR T-cell were detected by quantitative PCR or flow cytometry after a median of 2 weeks (range, 12–32 days).

Conclusions This study was the first and largest real-world study of commercial relma-cel for R/R CNSL, demonstrating promising efficacy and acceptable safety. We reaffirmed the benefit of immuno-agents such as BTKi or PD-1 inhibitor on CAR T-cell re-expansion and hypothesized a dual-agent CAR-T related combinatorial therapies, which warrants further validation. Most importantly, we highlighted the earlier use of CAR T-cell therapy as a consolidative therapy for patients sensitive to salvage therapy, which provided an impetus and inspired-future strategy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of relapsed/refractory (R/R) large B-cell lymphoma. However, its clinical application in central nervous system lymphoma (CNSL) has been limited, especially in Asian patients. Moreover, relapse or progression shortly after CAR-T therapy for CNSL remains a major challenge to be addressed.

INTRODUCTION

The prognosis of relapsed/refractory (R/R) central nervous system lymphoma (CNSL) has been extremely poor, with a median overall survival (OS) of only 4 months.[1 2] Over the past decades, despite improvement of clinical outcomes for R/R CNSL patients by high-dose methotrexate (HD-MTX)-based chemotherapy, small-molecule inhibitors, radiation therapy, and salvage high-dose chemotherapy with autologous stem cell transplantation (ASCT),[3] poor prognosis of patients at high-risk remained to be challenging,[4 5] highlighting the need for new therapeutic strategies and agents. Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of R/R large B-cell lymphoma (R/R LBCL).[6 7] However, its

WHAT THIS STUDY ADDS

⇒ To our best knowledge, this is the first and largest retrospective real-world study to investigate the clinical outcomes and prognosis analyses of commercial relmacabtagene autoleucel in patients with R/R CNSL. Our study reaffirmed the beneficial effects of immunoagents such as BTKi or PD-1 inhibitor on CAR T-cell re-expansion and further improvement of the response. We hypothesized a CAR T-cell combination-based approach with dual-agents incorporating PD-1 blockade and BTKi for potential synergistic effects. More importantly, this study is the first to highlight the unique management strategy for the subset of patients sensitive to prior salvage therapy, and it suggests the earlier use of CAR T-cells as a consolidative approach in R/R CNSLs, aiming to optimize long-term outcomes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings could contribute to be part of the global studies in this area and serve as regional references for Asian working groups. Further investigation is warranted to understand the mechanism of the synergistic action of dual agents, as well as to directly compare the clinical prognosis of dual-agent with single-agent combinatorial therapy post-CART infusion. Prospective clinical trials are eagerly awaited to compare CAR T-cell therapy with autologous stem cell transplantation for R/R CNSL patients sensitive to salvage treatment, aiming to clarify the role of CAR T-cell therapy as a consolidation strategy.

clinical application in CNSL has been limited, especially in Asian patients. Early clinical proof of the concept was highlighted by describing feasible antitumor effect and acceptable side effect profile in small retrospective case series and post hoc analyses of prospective clinical trials.[8 9]

Relma-cel, also known as Carteyva and JWCAR029, is a CD19-targeted, second-generation CAR T-cell product manufactured in China. It expresses the same CAR as lisocabtagene maraleucel with a 4-1BB costimulatory domain. In the registration RELIANCE trial (NCT04089215),[10] relma-cel demonstrated high rates of durable disease responses and a manageable safety profile. Based on these results, relma-cel has been approved by the Chinese National Medical Products Administration and commercialized in China for the treatment of R/R LBCL patients. In this study, we present our multicenter real-world experiences of relma-cel for R/R CNSL. We hope our findings will contribute to be part of the global studies in this area and serve as regional references for Asian working groups.

MATERIALS AND METHODS

Patients and therapy procedures

This was a real-world study in patients with R/R aggressive B-NHL with CNS involvement, who received relma-cel at 12 clinics in China. Retrospective analyses were conducted based on existing data, which consisted of patients' medical records during hospitalization and returning visit examinations, as well as further data collected by telephone and mail after their discharge. Final data analysis was performed on June 30, 2023. (In our study, data of patients lost to follow-up were recognized as censored data.)

Eligible patients were at least 18 years old with histologically confirmed CD19+R/R CNSL, and previously received systemic treatment, including chemoimmunotherapy containing anti-CD20 and HD-MTX-based therapy. The diagnosis of CNSL and LBCL subtype was conducted at each clinic but followed the common guideline of the WHO 2016 classification criteria.[11] The manufacturing and preparation process of relma-cel has been previously described.[12]

For patients who received a single infusion at the assigned dose of 100×10^6 CAR+T cells, lymphodepleting chemotherapy was administered over 3 days, including fludarabine (25 mg/m^2 intravenous daily) and cyclophosphamide (250 mg/m^2 intravenous daily). For patients who received ASCT combined with CAR-T regimen (ASCT+CART), taking the date of CAR-T infusion as day 0, ASCT was performed on the median time of day −3 (range, −6 to −2) with an optional TEAM (Thiotepa; VP-16; Ara-C; and Melphalan) or BEAM (BCNU; VP-16; Ara-C; and Melphalan)-based conditioning regimen. Postinfusion combination therapy, including PD-1 inhibitor, BTKi, or lenalidomide, were administrated at the physician's discretion.

Efficacy and laboratory assessments

Evaluations were performed at each clinic using positron-emission tomography/CT (PET/CT) and/or brain MRI prior to leukapheresis (within 4 weeks) and/or post bridging therapy, and also at regular intervals post infusion per institutional practices, according to International PCNSL Collaborative Group Response Criteria and Lugano Criteria.[13 14] The primary endpoint was to evaluate the proportion of patients who achieved complete response (CR) at 3 months, as assessed by investigators. Secondary endpoints included best complete response (BCR), progression-free survival (PFS), duration of response (DOR), OS, and the incidence of adverse events (AEs).

Pharmacokinetics (PKs) were investigated in the peripheral blood (PB) and/or cerebrospinal fluid (CSF), by analyzing the transgene copy number per microgram DNA (by quantitative PCR (qPCR)) or the frequency of CD3+CAR+ cell (by flow cytometry (FCM)). Fold changes from baseline for serum cytokines and biomarkers (C reactive protein (CRP) and ferritin) were also recorded. Detailed methodology regarding patients' eligibility, endpoints, and toxicity management is provided in online supplemental methods.

Statistical analysis

Descriptive statistics were used to summarize the baseline characteristics, including demographic and disease characteristics. The Kaplan-Meier method was used to estimate survival probabilities, such as PFS, DOR, and OS. The log-rank test was used to determine significant survival differences between groups of individuals. Cox

proportional hazards regression models were applied for univariable analyses, including covariates, to identify prognostic factors for short-term and long-term outcomes. All p values were two-sided, and p values <0.05 were regarded statistically significant. All statistical analyses were performed using SPSS V.23.0 and GraphPad Prism V.8.0.

RESULTS

Patient demographic and clinical features

This study included a total of 22 patients with R/R primary or secondary CNS diffuse large B-cell lymphoma (DLBCL) (12 primary CNSL, 10 secondary CNSL (SCNSL)) who received relma-cel from October 2021 to February 2023. Among the 10 SCNSL patients, 2 (patients #4 and #15) had CNS involvement at the initial diagnosis, while the remaining 8 experienced CNS involvement during the course of treatment, including 3 out of those 8 patients with isolated CNS involvement. As shown in table 1, the median age of the patients was 56 years (range, 29–70), and 45.5% were over 60 years old. Additionally, half of the patients had a Karnofsky Performance Status (KPS) score of 60 or lower. Among the germinal center B-cell (GCB) subtypes, three patients (3/13, 23.1%) were classified as "double hit" lymphoma (DHL) or "triple hit" lymphoma, while only one non-GCB patient (1/9, 11.1%) was classified as DHL. The median number of prior therapies was 2 (range 1–5).

Notably, of the 22 patients who received relma-cel, 8 (36.4%) received it as consolidation strategy. These patients had durable CR or partial response (PR) prior to leukapheresis but were still deemed as high-risk by physicians, including primary refractory disease in four patients, heavily previously treatment in two patients, triple-hit DLBCL or ineligible for ASCT in one patient, respectively. All patients but two received bridging therapy, resulting in disease control in most of them, that is, CR in 5/22 (22.7%) patients, PR in 5/22 (22.7%), stable disease (SD) in 9/22 (40.9%) and progressive disease (PD) in 3/22 (13.7%) patients prior to day 0 infusion. Among the 17/22 patients with measurable disease, most had parenchymal involvement, including two with spinal cord localization alone, one with isolated leptomeningeal disease confirmed by MRI and CSF. (Baseline characteristics and treatment course of each subject are outlined in online supplemental tables S1 and S2.)

Therapeutic outcome

The median time from leukapheresis to infusion was 32 days (range, 28–37). 13 (59.1%) patients underwent a single infusion of CAR T-cell, while 9 patients received the ASCT combined with CAR T-cell infusion. 18 (81.8%) patients also received combination therapy at a median of 31 days postinfusion (range, 3–42 days) for a median duration of 180 days (range, 21–570 days). The combination therapy involved the use of single or dual targeted

Table 1 Demographic and baseline characteristics prior to infusion

Characteristics		No. of patients (%)
Age (years)	Median (range)	56 (29–70)
	≤60	12 (54.5)
	>60	10 (45.5)
Sex	Male	14 (63.6)
	Female	8 (36.4)
ECOG performance status	Median (range)	2 (1-4)
KPS score	70–90	11 (50.0)
	50–60	4 (18.2)
	20–40	7 (31.8)
CNSL	PCNSL	12 (54.5)
	SCNSL	10 (45.5
Histological type	DLBCL GCB	13 (59.1)
	DLBCL non-GCB	9 (40.9)
	DHL/THL	4 (18.2)
Active CNS or systemic lymphoma at infusion	Isolated CNS disease	11 (50.0)
	Isolated systemic disease	0 (0)
	Both CNS and systemic disease	6 (27.3)
Non-active CNS or systemic lymphoma at infusion		5 (22.7)
Bulky disease*		1 (4.5)
CSF involvement		2 (9.1)
Bone marrow involvement		1 (4.5)
Prior lines of therapy	1–2	13 (59.1)
	3–4	8 (36.4)
	≥5	1 (4.5)
Primary refractory†		16 (72.7)
Disease status relative to most recent prior therapy (status preleukapheresis)‡	Refractory	12 (54.5)
	Relapse	2 (9.1)
	CR/PR	8 (36.4)
Previous type of therapy	Rituximb	22 (100.0)
	High-dose MTX	22 (100.0)
	Temozolomide	10 (45.5)
	Auto-HCT	1 (4.5)
	Radiotherapy	7 (31.8)
	Other immunotherapy§	14 (63.6)
Bridging therapy	Yes	20 (90.9)

*Bulky disease defined as size of any single node/nodal mass ≥7 cm in diameter.
†Primary refractory disease is defined as no response or relapse within 6 months after the ending of the first-line treatment.
‡Relapsed indicates best response of complete or partial remission to most recent prior therapy but progressive afterwards, and refractory indicates best response of stable or progressive disease to most recent prior therapy.
§Other immunotherapy included agents such as BTK inhibitor, lenalidomide, PD-1 inhibitor, selinexor, etc.
auto-SCT, autologous stem-cell transplantation; CNSL, central nervous system lymphoma; CR, complete response; CSF, cerebrospinal fluid; DHL/THL, high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; GCB, germinal-center B-cell-like; HCT, hematopoietic cell transplantation; KPS, Karnofsky Performance Status; MXT, methotrexate; PCNSL, primary CNSL; PR, partial response; SCNSL, secondary CNSL.

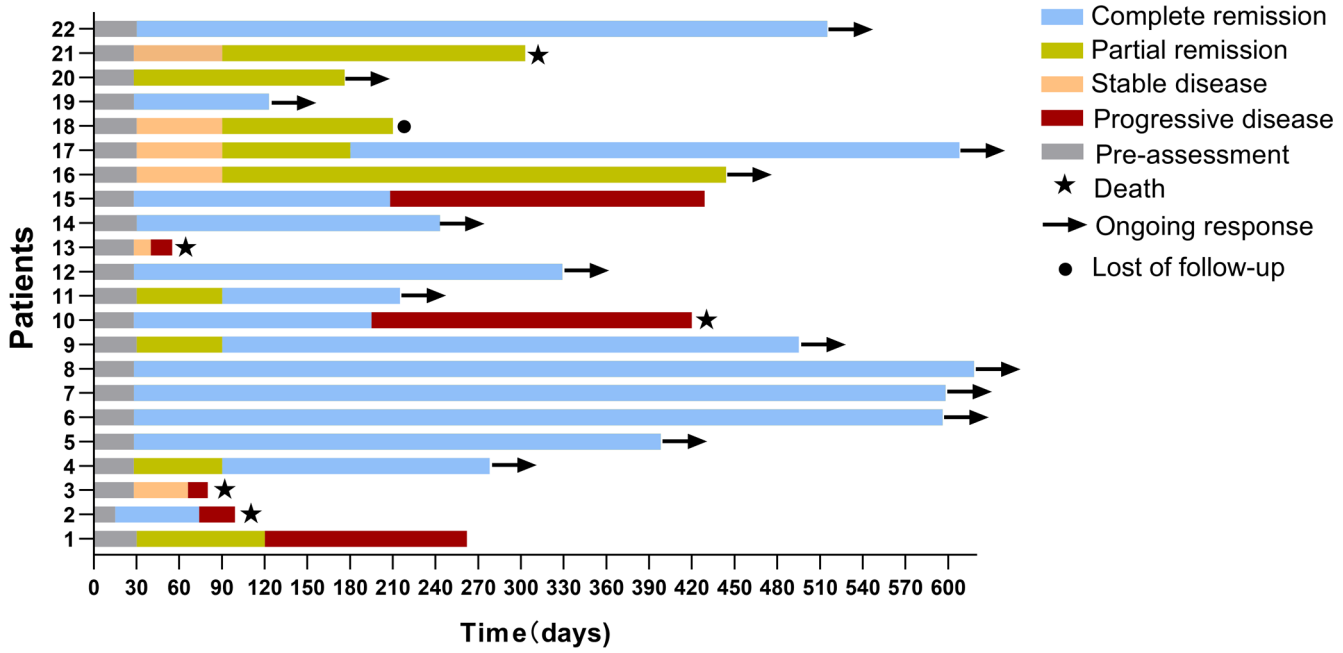

Figure 1 Response characteristics and outcomes over time in 22 patients.

agents, including BTKi (n=15), PD-1 inhibitors (n=5), or lenalidomide (n=4) (online supplemental table S2).

The best overall response rate was 90.9%, and the best CR rate was 68.2%. Three patients (patients #4, #9, and #11) achieved early PR at 1 month and then improved to CR by 3 months; four patients (patients #16, #17, #18, and #21) initially achieved SD, which improved to PR by 3 months; remarkably, one patient (patient #17) further improved to CR by 6 months. All 22 patients (100%) achieved CNS responses, including 16 (72.7%) patients achieved CNS BCR. The median time to response was within 1 month (range, 15–90 days).

With a median follow-up of 316 days (range, 55–618 days), among the 16 patients achieving CNS CR, 13 (81.3%) were still alive with a sustained response, including 8 (50.0%) who had a CNS CR lasting over 1 year. Overall, six (27.3%) patients had radiologically confirmed PD, including four who progressed within 6 months (one isolated systemic PD, three CNS PD, two concurrent systemic and CNS PD), and three patients died within 2 weeks due to rapid progression. The estimated probability of PFS, DOR and OS rate at 1 year was 64.4%, 71.5%, and 79.2%, respectively. The median PFS, DOR, and OS were not reached. (Individual responses over time are shown in figure 1.)

Prognostic analysis

As shown in online supplemental table S3 and figure S1, clinically meaningful activities were noted across populations including variables such as age, sex, cell-of-origin subtype, disease nature of CNSL (PCNSL vs SCNSL), high-risk genotype, and number of previous lines of therapy. Systemic involvement was a predictive factor for PFS (p=0.02) Likewise, disease status at infusion (CR vs PR vs SD/PD) seemed to be associated with prognosis. All patients with non-active diseases preinfusion sustained their CR at 3 months with a survival superiority reaching 1-year PFS of 100%, while patients with SD/PD were identified to have a higher risk of failure, reaching 46.9% at 1 year. Additionally, no significant difference was noted according to infusion method (ASCT+CART vs single CAR-T infusion). ASCT+CART group demonstrated a slightly inferior clinical outcomes including BCR (55.6% vs 76.9%, p=0.28) and estimated 1-year PFS (62.2% vs 67.3%, p=0.51). In terms of primary endpoint, non-CR at 3 months postinfusion seemed to be predictive of a worse prognosis, with an estimated 1-year PFS of 83.3% versus 37.0% (p=0.03), respectively.

Remarkably, patient's responses to the most recent prior therapy had significant impact on both their short and long-term outcomes. Patients with durable CR or PR preleukapheresis (n=8) showed significant favorable responses over refractory/relapsed patients (3-month CRR: p=0.01; BCR: p=0.02). All of eight patients maintained CR until the last follow-up visit, five of them (patients #6, #7, #8, #9, and #22) remained progression-free for over 12 months. Conversely, refractoriness or relapse to the latest prior therapy exerted significantly adverse effect on PFS and DOR (1-year PFS: 100.0% vs 41.7%, p=0.02, 1-year DOR: 100.0% vs 50%, p=0.03, figure 2A,B), which was also revealed as the marginally significant adverse factor in terms of OS (1-year OS: 100.0% vs 65.5%, p=0.05, figure 2C).

Safety and tolerability for CAR T-cell infusion in CNSL

As shown in table 2, five deaths (22.7%) were reported, with three (13.6%) deaths attributed to disease progression, and two (9.1%) deaths due to non-relapse-related reasons (COVID-19). There were no CAR-T-related deaths. Cytokine release syndrome (CRS) of any grade

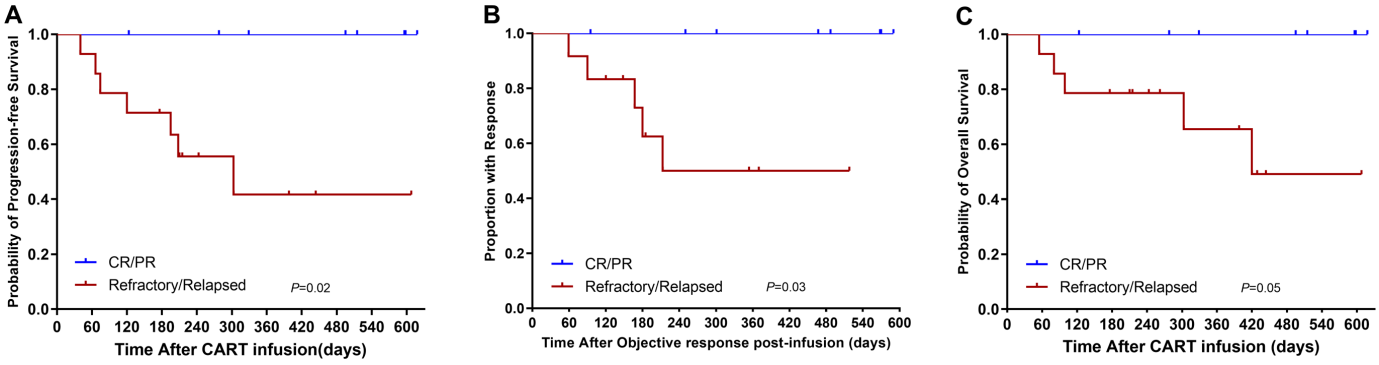

Figure 2 Kaplan-Meier curves of progression-free survival (PFS), duration of response (DOR), and overall survival (OS) for patients based on the response to most recent prior therapy (A, B, C). (A) Curves of PFS. (B) Curves of DOR. (C) Curves of OS. CART, chimeric antigen receptor T-cells; CR, complete response; PR, partial response.

occurred in 16/22 (72.7%) patients, most of which were as mild as grade 1 or 2 (15/16, 93.7%). Immune effector cell-associated neurotoxicity syndrome (ICANS) of any grade reported in eight patients, including one case (4.5%) of grade 3. All cases of neurotoxicity (NT) were reported concurrently with CRS. No patient suffered grades 4–5 CRS or NT. Representative cases with high-grade CRS or ICANS are detailed in online supplemental results. To alleviate the symptoms of the toxicities, tocilizumab was administered to 10/22 (45.4%) patients, and 13.6% of them received two doses. Steroids were administered to 9/22 (40.9%) patients.

Regarding other important AEs, prolonged cytopenia ≥grade 3 or higher that were present at or after 28 days postinfusion occurred in two patients. Patient #7 experienced prolonged grade 3 neutropenia, which lasted for 3 months, but no evidences showing disease progression in bone marrow. Infection complications were reported in eight patients, including upper respiratory tract infection in five patients and severe pneumonia due to COVID-19 in three patients, which directly led to two deaths. It is worth noting that patient #16 experienced transient hallucination 1 year after infusion, with sustained PR per PET/MRI scans.

CAR T-cell expansion and CSF trafficking

PK testing showed a marked expansion in PB of relma-cel during the first 28 days after infusion in all 21 evaluable patients (online supplemental table S4), which was monitored by FCM in 9/21 cases (patients #2, #3, #5–#7, #9–#12) and qPCR in 17/21 cases (patients #1, #5–#9, #11, #13–#22), respectively. As shown in figure 3A,B, the median peak numbers of CD19 CAR T cells were 79,646.5 (range, 55,128–138,305) copies/µg DNA by qPCR and 139.8 (range, 112–422) CD3+CAR+ cells/µL by FCM, with a median time to achieve peak levels of 1.43 (range: 0.85–2.0) weeks. Relma-cel was identified in CSF among all eight evaluable patients (figure 3C,D). The available expansion data indicated that CAR T cells could traffic to the CSF, regardless of the absence of systemic lymphoma or non-measurable disease.

PK of relma-cel combined with PD-1 and BTK inhibitor

The persistence of CAR T-cell in blood decreased over time. A re-expansion of circulating CAR T cells could be observed in evaluable patients treated with BTK inhibitors alone (such as patients #2, #6, #20) or PD-1 inhibitor alone (patient #3).

Dual immuno-agents, PD-1 inhibitor, and BTKi were concurrently administrated to four patients (patients #7, #8, #9, and #17) for over 1 year. As shown in figure 3E,F, with the add-on agent tislelizumab to the ongoing BTKi, significant re-expansions of CAR T-cell were detected by qPCR or FCM after a median of 2 weeks post-injection of tislelizumab (range, 12–32 days). Details of the PKs for representative cases were shown in online supplemental figure S2.

Overall, all four patients maintained durable responses over 15.5 months, including further improvement of efficacy in one patient (patient #17, 1-month SD→3-month PR→6-month CR). Circulating relma-cel could be detected in all these patients beyond 3 months. Despite the level of CAR T-cell detected by FCM was relatively low for patients #7 and #9 in both PB (0.8 and 0.55 cells/µL) and CSF (0.035 and 0.021 cells/µL) after 6 months, and even no longer detectable from day 478 and day 375, respectively, the beneficial clinical responses for the two patients were still ongoing. Moreover, the longest persistence of CAR T-cell was observed in patient #8 until day 400, whose CAR transgene level in CSF was much higher than that in blood sample (797 vs 73 copies/µg DNA).

Correlation of CAR T-cell expansion with response and safety

Correlation analyses of serum CAR transgene copy number per microgram DNA with efficacy and CRS/ICANS was carried out. In the evaluable patients who achieved BCR, a trend of longer persistence of relma-cel was observed. This correlation was associated with a higher median AUC_{0-28} of 911,052 (range, 552,357–1,803,393) copies/µg DNA*days compared with patients of non-CR (p=0.06) (figure 4A). In terms of safety, a significantly higher peak expansion of CAR T-cell was noted in patients who experienced any grade neurological events. The median copy

Table 2 Summary of safety events occurring within 1-month post-chimeric antigen receptor T-cell infusion

Events	Any grade, n (%)	Grade 1–2, n (%)	Grade ≥3, n (%)
CRS			
All grades	16 (72.7)	15 (68.2)	1 (4.5)
Time to onset, days (median, range)		4 (1–10)	
Duration, days (median, range)		4 (1–14)	
Subjects with any CRS			
Fever	15 (68.2)	14 (70.0)	1 (5.0)
Hypoxia	4 (18.2)	2 (9.1)	2 (9.1)
Hypotension	2 (9.1)	0 (0.0)	2 (9.1)
Others*	5 (22.7)	5 (22.7)	0 (0.0)
ICANS			
All grades	8 (36.4)	7 (31.8)	1 (4.5)
Time to onset, days (median, range)		8 (6–18)	
Duration, days (median, range)		5 (1–17)	
Subjects with any neurological events			
Encephalopathy†	7 (31.8)	4 (18.2)	3 (13.6)
Seizure	1 (4.5)	0 (0.0)	1 (4.5)
Tremor	1 (4.5)	1 (4.5))	0 (0,0)
Delirium	2 (9.1)	1 (4.5)	1 (4.5)
Dysarthria	3 (13.6)	2 (9.1)	1 (4.5)
Headache/migraine	7 (31.8)	7 (31.8)	0 (0.0)
Clinical management of CRS (n, %)			
Tocilizumab only		6 (27.3)	
Corticosteroids only		4 (18.2)	
Both tocilizumab and corticosteroids		2 (9.1)	
Clinical management of ICANS (n, %)			
Tocilizumab only		2 (9.1)	
Corticosteroids only		3 (13.6)	
Both tocilizumab and corticosteroids		3 (13.6)	
Other AEs			
Leukopenia	20 (90.9)	12 (54.5)	8 (36.4)
Neutropenia	20 (90.9)	7 (31.8)	13 (59.1)
Lymphopenia	14 (63.6)	9 (40.9)	5 (22.7)
Hypogammaglobulinemia	11 (50.0)	9 (40.9)	2 (9.1)
Anemia	9 (40.9)	7 (31.8)	2 (9.1)
Thrombocytopenia	7 (31.8)	6 (27.3)	1 (4.5)
Infection	8 (36.4)	5 (22.7)	3 (13.6)
Nausea	2 (9.1)	2 (9.1)	0 (0.0)
Fatigue	11 (50.0)	10 (45.5)	1 (4.5)
Hepatic dysfunction	3 (13.6)	3 (13.6)	0 (0.0)
Hypokalemia	1 (4.5)	1 (4.5)	0 (0.0)

*Other symptoms of CRS included chills, tachycardia, etc.
†Encephalopathy included encephalopathy, cognitive disorder, confusion state, depressed level of consciousness, disturbed attention, hypersomnia, memory impairment, disorientation, dyscalculia, mental status changes, somnolence, coma.
AEs, adverse events; CRS, cytokine release symptom; ICANS, immune effector cell associated neurotoxicity syndrome.

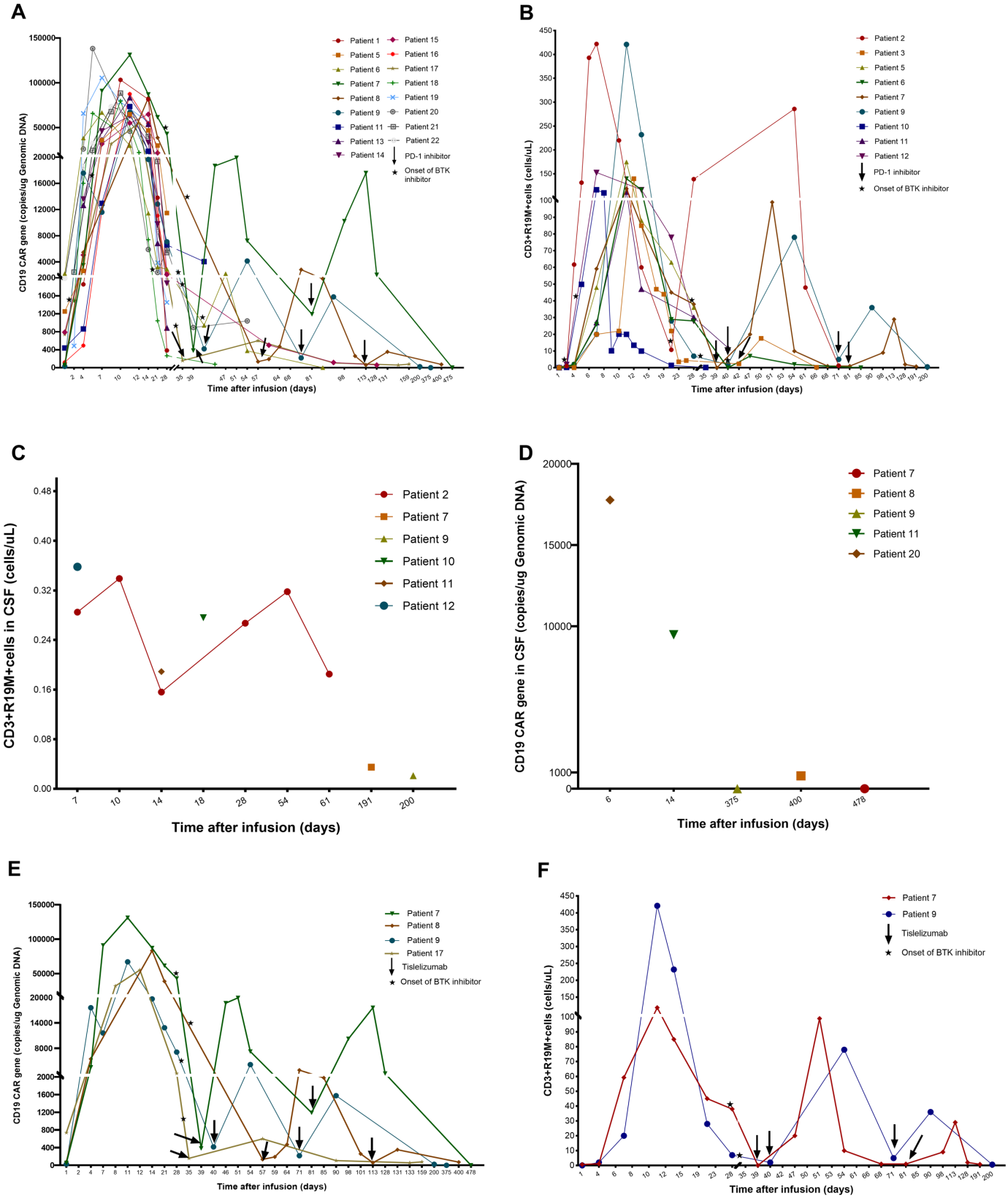

Figure 3 Pharmacokinetic (PK) analysis postinfusion. (A, B) Expansion and persistence of serum relma-cel levels as detected by quantitative PCR (qPCR) (A) and flow cytometry (FCM) (B). (C, D) Expansion of CD19 chimeric antigen receptor (CAR) T-cells in cerebrospinal fluid (CSF) as detected by FCM (C) and qPCR (D). (E, F) PK analysis with combination of PD-1 and BTK inhibitor by qPCR (E, patients #7, #8, #9, #17) and FCM (F, patients #7, #9).

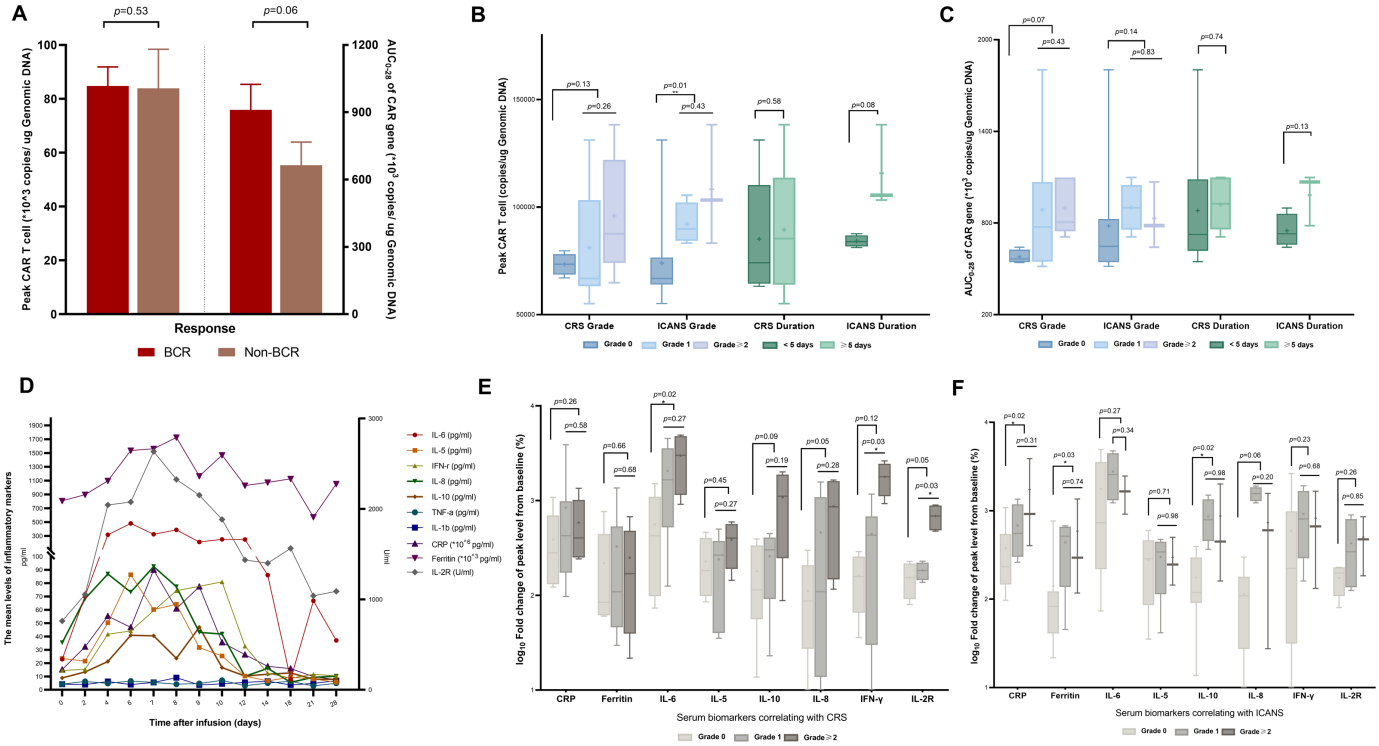

Figure 4 Correlation of PK and serum biomarkers with response or safety. (A) Correlation of peak CAR T cells level and $AUC_{0\text{-}28}$ (defined as accumulation of CAR gene copy number in blood during the first 28 days after relma-cel therapy) by qPCR with the best overall response. (B, C) Correlation of peak CAR T-cells level (B) and $AUC_{0\text{-}28}$ (C) by qPCR with the occurrence/severity and duration of CRS and ICANS. (D) Dynamic changes of mean value of each biomarker during the first 28 days postinfusion. (E, F) Correlation of median fold changes of peak level from baseline (Δpeak) in selected cytokine with the occurrence/severity of CRS (E) and ICANS (F). BCR, best complete response; CAR, chimeric antigen receptor; CRP, C reactive protein; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IFN-γ, interferon gamma; IL, interleukin; TNF-α, tumor necrosis factor alpha.

number per microgram DNA was 100 167 (range, 83,187–138,305) compared with a median value of 74,019 (range, 55 128–131,141) in patients without any NT (p=0.01). However, no significant differences were found for the severity of ICANS (ICANS grade ≥2vs grade 1, median C_{max}: 108,249 vs 92,085, p=0.43). Additionally, no statistically significant association between $AUC_{0\text{–}28}$ of CAR+T cells and the occurrence or severity of CRS/ICANS were observed. No positive impact was found between peak levels or $AUC_{0\text{–}28}$ of CAR+T cells and the duration of CRS/ICANS (figure 4B,C).

Biomarker analysis

Figure 4D shows dynamic fluctuations in the levels of interleukin (IL)-2R, IL-6, IL-5, IL-8, IL-10, interferon gamma (IFN-γ), CRP, and ferritin. However, the levels of cytokines such as IL-1b and tumor necrosis factor alpha (TNF-α) appeared to be lower and flatter. In terms of ICANS, the median fold changes from baseline in peak levels (Δpeak) of CRP (11.32-fold vs 3.76-fold increase, p=0.02), ferritin (5.02-fold vs 1.42-fold increase, p=0.03), and IL-10 level (8.67-fold vs 1.76-fold increase, p=0.02) appeared to be associated with the occurrence of ICANS, but not relevant with severity. Furthermore, the occurrence of CRS was positively associated with the median Δpeak level of serum IL-6 (24.2-fold vs 5.63-fold increase, p=0.02). Patients with high-grade CRS (≥2) had significantly higher median Δpeak levels of serum IFN-γ (17.82-fold vs 4.45-fold increase, p=0.03) and soluble IL-2 receptor (IL-2R) (6.86-fold vs 1.83-fold increase, p=0.03) compared with those with CRS of grade 1 (figure 4E,F).

DISCUSSION

To the best of our knowledge, this is the first and largest retrospective real-world study to investigate the clinical efficacy, safety and prognosis analyses of commercial relma-cel in patients with R/R CNSL. It is worth noting that our study included a diverse patient population with conventional poor prognostic features: 72.7% of patients with primary refractory disease, a relatively high proportion (45.5%) of elderly (>60 years old), and 50% of patients with a KPS score lower than 60. The demographic baseline in this real-world study was more representative of the realistic features of the patient populations in clinical practices. Compared with historical data from the conventional therapy era, which showed a median response duration of only 2–3 months for R/R CNSL, our off-trial findings provided substantial evidence of the encouraging high rate of response and prolonged survival in heavily treated patients. No significant differences were

observed in terms of severity and incidence of CRS or ICANS for CNSL compared with systemic disease in the RELIANCE trial (G3/4 CRS: 4.5% vs 5.1%, G3/4 ICANS: 4.5% vs 3.4%). In general, the types of AEs in this study were consistent with the established safety profiles among real-world settings and clinical trials. The potential for longer-term adverse effects warrants further monitoring.

As shown in online supplemental table S5, data from other previous studies of CAR-T therapy for CNSLs indicated a CRR and ORR, ranging from 20% to 66.7% and from 40% to 100%, respectively. Compared with previous data, the current cohort appeared to achieve superior short-term or long-term outcomes. Several factors might contribute to this discrepancy. First, based on our univariate analysis, disease status preleukapheresis was identified as the only risk factor for both short-term and long-term outcomes. Given the difference in patients' inclusion criteria, a significantly higher proportion of patients in our cohort (8/22, 36.4%) with durable CR or PR to the most recent salvage regimen received CAR-T consolidation therapy, which could be the major reason for the better outcomes. Second, as previously reported, an unequivocal finding is that disease burden retains significant influence on efficacy and safety despite undergoing CAR T-cell treatment.[15 16] Consistently, in our study, covariates reflecting tumor burden such as systemic involvement and active disease at infusion seemed to be predictive of a worse prognosis. Compared with published data,[17] the current cohort included a lower proportion of active lesion at infusion (77.3% vs nearly 100%) and less systemic involvement (27.3% vs nearly 50%), reflecting a patient population with lower tumor burden. Notably, cross-study comparisons of efficacy and durability of CAR T-cell therapies are difficult in view of the small sample size, baseline characteristics, ethnic or geographic composition, different drug mechanism, treatment strategies, etc.

With the increasing evidences from studies on systemic LBCL such as ZUMA-7,[18] TRANSFORM[19] showing that outcomes may be improved with CAR T-cell therapy earlier in the treatment sequence, some researchers have further proposed that patients with good remission after salvage therapy should be treated with ASCT, with CAR-T therapy then reserved for post-ASCT relapse. As reported by Shadman *et al*,[20] in patients with DLBCL in a CR or PR after salvage therapy, auto-hematopoietic cell transplantation was associated with a lower incidence of relapse and a superior OS compared with CAR-T. In R/R CNSLs, previous CD19 CAR T-cell therapy is commonly administrated as a third-line or later treatment option for patients at relapsed or refractory status. Here, it was the first time to raise the important yet unanswered and highly clinically relevant question regarding the role of CAR T therapy as a consolidation approach for the subset of salvage therapy-sensitive patients.

According to initial experiences, for R/R CNSL patients sensitive to salvage chemotherapy, subsequent HDC-ASCT which is commonly used for patients with systemic disease, may not be a reasonable option, especially for those at high risk. This is characterized by a significantly shorter median PFS ranging from 4 to 11.6 months and a worse prognosis for ASCT ineligible patients.[2] Moreover, due to the aggressive nature of CNSLs, patients who relapse after ASCT are likely to face delays in receiving CAR-T, potentially precluding access to the treatment, which could have fatal consequences. Therefore, unlike strategies for systemic lymphoma, for CNSLs, rather than exploring the treatment sequence, it is more urgent to identify a "winner" approach for this special patient population. Based on previous research, early intervention may benefit the lymphocyte collection and CAR T-cell function owing to the patient's immune status and may also affect the immune system mobilization. Additionally, a lower disease burden preinfusion was associated with improved safety and efficacy. As aforementioned, our study enrolled a subset of eight patients with durable CR or PR prior to leukapheresis, who then received consolidation of CAR T-cell therapy. All maintained CR postinfusion at the last follow-up, including five patients remained progression-free for over 12 months. Our results showed that this subset of patients favored CAR T-cell therapy as consolidation over standard care, suggesting that CAR T-cell therapy rather than ASCT alone, might be able to eradicate chemo-refractory subclones at the minimal residual level. Consequently, given the reasons described earlier as well as our findings, earlier use of CAR-T therapy as a consolidative approach seemed to be a potentially viable option for R/R high-risk CNSL, particular for ASCT ineligible patients. While the small sample size precludes definitive conclusions, this finding provides preliminary evidence to inform a treatment choice that can afford meaningful clinical benefit for patients sensitive to prior salvage therapy.

Immuno-agents including BTKi, immune modulatory small molecules, and PD-1 inhibitor have shown some activity in R/R CNSLs with high ORR and CR, but without durable responses. Therefore, their combination in R/R CNSLs requires a novel approach to tackle their drug resistance and immune regulations. As previously reported, progression shortly after CAR-T therapy for CNSLs remains a major challenge to be addressed.[8] Emerging evidences from systemic lymphomas identified the effects of BTKi[21] or PD-1 inhibitor[22] on the functionality of CAR T-cell in vitro and in vivo, which might mitigate CAR T-cell dysfunction or refuel exhausted T cells. Limited clinical data have suggested that CD19 CAR-T in combination with such immune-agents can further enhance the antitumor effect, especially for those who failed to achieve an early response.[23] The dual benefits of immuno-agents in terms of disease control in CNSLs and immunomodulatory augmentation of CAR-T function, make these agents a logical consideration for incorporation into CART-related combinatorial therapies for CNSLs. Moreover, previous studies have demonstrated synergistic antitumor effects between ibrutinib and immune checkpoint blockade. BTKi has been reported to

have superior target effects by downregulating exhaustion markers, such as PD-1. Thus, there is a biological plausibility for concurrent application of PD-1 and BTK inhibitors for enhancing CAR-T activity through improving inhibitory TME. It is worthy mentioned that, while re-expansion and persistence of CAR T-cells, as well as the long-term survival benefit, seemed aligned to the concurrent use of dual-agents incorporating PD-1 blockade and BTKi post-infusion in the four patients as reported here, there is no definitive conclusion on whether this new CAR-T combinatorial approach is indeed superior to either agent alone. This may be partly explained by the following factors: (1) three of these four patients were in durable CR or PR preleukapheresis, which likely to confer significant benefit on PFS and DOR as described earlier. (2) The length and degree of persistence necessary for a durable response are debatable based on the available data and could conceivably vary with different CAR constructs. Further investigation is warranted to directly compare prognosis of dual-agent versus single-agent combinatorial therapy post-CART infusion, as well as to explore the mechanism of the synergistic action of these two agents.

Prior studies confirmed that both the qPCR and FCM methods are suitable for monitoring CAR-T cellular kinetics. Simultaneous incorporation of these two approaches can yield a more reliable result for CAR T cell kinetics. In our study, the single-chain variable fragment transgene sequences for qPCR or CD19 CAR detection reagent for FCM are not always available in each hospital laboratory, thus limiting the simultaneous application of two modalities in some patients. Overall, five patients (#5, #6, #7, #9, #11) performed both qPCR and FCM for PK detection, which showed a good correlation in CAR T-cell expansion and persistence. Additionally, our PK analyses reconfirmed the expansion of CAR T-cells in PB and their abilities to traffic to the CNS, even in patients without active lesions. One possible explanation for this observation is that CAR T-cells could expand in response to minimal residual disease that was undetected by regular imaging.

Previously, important laboratory variables and biomarkers, including $C_{max}/T_{max}/AUC_{0\text{-}28}$ of CAR T-cells, were not homogenously reported. Herein, similar to prior studies such as ZUMA-1, we demonstrated a trend toward a better CAR T-cell persistence in responders. A meta-analysis by Grant *et al*[24] concluded that ICANS onset and degree were mostly associated with the elevated CRP and ferritin levels. The most commonly identified cytokines associated with ICANS/CRS were IL-6, IL- 2R, IL-15, and IFN-γ. Notably, certain serum biomarker peak values being statistically correlated to severe NT (grade ≥3) as previously observed, were not found in our study.[25] Apart from that, the severity of ICANS was not associated with largely increases in peak CAR T-cell expansion levels. The median duration of NT was also shorter in our study (5 vs 15 days, respectively).[10] In addition to variations in CAR-T products, it might be partly due to evolving practices of toxicity management, including the early and increased use of tocilizumab, corticosteroids, and supportive cares in real-world settings.

CONCLUSION

Several key insights can be drawn from this study. First, our study demonstrated the clinical meaningful efficacy and acceptable safety of relma-cel for CNSL. Second, it reaffirmed the benefit of immuno-agents such as BTKi or PD-1 inhibitor on CAR T-cell re-expansion and further improvement of the response. We hypothesized a CAR T-cell combination-based approach with dual immuno-agents for potential synergistic effects. Third, our study suggested earlier use of CAR T-cell therapy as a consolidative therapy for patients sensitive to salvage therapies, which provided an impetus and inspired-future strategy.

We acknowledge several limitations of our study, in addition to its retrospective nature. There was heterogeneity in patients' selection, bridging therapy, and maintenance agents across centers, which was at the physicians' discretion. Further research with a larger sample size is warranted to validate our promising findings. More importantly, randomized prospective clinical trials are eagerly awaited to compare CAR T-cell therapy versus ASCT for R/R CNSL patients who respond to salvage treatments, aiming to further clarify the role of CAR T-cell therapy as a consolidation strategy.

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