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Humanized CD19-directed CAR-T Cell Therapy in Pediatric Relapsed/Refractory Acute Lymphoblastic Leukemia With CNSL or Neurological Comorbidity

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Summary: Chimeric antigen receptor T cell (CAR-T) therapy has breakthrough potential for relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL). However, because of the risk for neurotoxicity, trials usually exclude patients with central nervous system leukemia (CNSL) or active neurological comorbidities (NC). Here, we evaluated the efficacy and neurotoxicity of humanized CD19-directed CAR-T therapy for R/R ALL with CNSL or NC. Of 12 enrolled patients, 4 had CNSL with bone marrow (BM) or testicular recurrence, 3 had BM relapses with NC, and 5 had BM relapse without NC. Bridging chemotherapy was performed for high tumor burden before CAR-T therapy. Patients with CNSL or BM relapse with NC or without NC experienced 100% complete remission. Tumor burden reduction did not occur in 1 patient with NC, who developed grade 5 neurotoxicity before BM assessment, and one patient with CNSL developed leukoencephalopathy. Severe cytokine release syndrome and neurotoxicity developed in 0% with CNSL, 33.3% with BM relapse and NC, and 0% without NC. CAR-T cells expanded in the cerebrospinal fluid (CSF) of all patients with no difference among CNSL, BM with NC, or no NC (respective median percentages among lymphocyte: 33.7%, 48.2% and 34.5%, $P = 0.899$; respective median concentrations: 0.82, 2.21, and 0.46/μL, $P = 0.719$). Median CSF CAR-T cell duration was 5.5 (3–9) months with CNSL and 3 (2–3) months without CNSL ($P = 0.031$). CAR-T can be given safely and effectively to pediatric patients with R/R ALL with CNSL or NC who have near-normal neurological status. High tumor burden may confer increased risk for severe neurotoxicity.

Key Words: chimeric antigen receptor T cell, central nervous system leukemia, neurotoxicity, neurological comorbidity, relapsed/refractory acute lymphoblastic leukemia

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CD19-specific chimeric antigen receptor T cell (CAR-T) therapy has generated promising results in children with relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (ALL), but its use can be complicated by neurotoxicity and severe cytokine release syndrome (sCRS). Patients with central nervous system (CNS) relapse, CNS stage 3 disease, and/or symptomatic neurological disease have frequently been excluded from pediatric studies of CAR-T therapy out of concern for increased neurotoxicity risks.[1,2] Data are thus limited regarding the antitumor activity of CAR-T against CNS leukemia (CNSL) or ALL with neurological comorbidities (NC).

The CNS is a primary site of relapse in patients with ALL and is often associated with a less favorable response to therapy.[3] Intensive chemotherapy, intrathecal injection (IT), cranial radiation, or even allogeneic hematopoietic stem cell transplantation (allo-HSCT) are the available options for CNS relapse, but these strategies tend to be unsuccessful, especially in patients with multiple relapses.[4] Alternative approaches are urgently required, particularly for CNS relapse after cranial radiation or allo-HSCT. A few recent preliminary clinical reports have revealed the feasibility and safety of CAR-T therapy in treating CNSL or bone marrow (BM) relapse with NC.[5–8]

Neurotoxicity occurs in ~40% of patients, with manifestations ranging from mild delirium to fatal cerebral edema.[9] Reported rates of severe neurotoxicity higher than grade 3 are 0–21% in pediatric studies,[1,2,10] and neurotoxicity shows a significant association with high pretreatment disease burden, higher peak CAR-T cell expansion, and higher elevations of proinflammatory cytokines in blood.[11] CAR-T can effectively eliminate leukemia cells in medullary and extramedullary disease, but a high tumor burden in CNS may cause severe neurotoxicity requiring intense intervention.[7] In a large post hoc analysis of CAR-T use in patients with CNS R/R B-ALL, between the CNS-positive and CNS-negative stratum, the incidence and severity of neurotoxicity did not differ (CNS-positive stratum: CNS3 or persistent CNS2 at relapse or within the 12 months preceding infusion).[12] However, in multivariate analyses of neurotoxicity in that study, a history of CNS R/R disease and a BM burden of M3 were significantly associated with increased risk. Patients with a BM M3 or NC had a significantly higher risk of developing severe neurotoxicity.

Treatment for R/R ALL with CNSL or neurological complications remains challenging, and guidance for these patients is lacking. Tumor burden reduction by IT or bridging chemotherapy and a return to near-normal neurological status before CAR-T infusion may contribute to

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reduced sCRS or neurotoxicity, but the persistence of CAR-T cells has not been confirmed.[5,7,12] In this study, we reported the ability of humanized CD19 CAR-T cells harboring 4-1BB and the CD3 ξ moiety to induce CR in ALL patients with CNS involvement or NC at an appropriately low disease burden.

MATERIALS AND METHODS

Patients and Ethical Review

We carried out a prospective clinical study of CD19-directed CAR-T cell therapy for R/R ALL from December 2019 to June 2021. The study was registered at chictr.org.cn (No. ChiCTR2000030159) and approved by the Shanghai Children's Hospital Institutional Review Board. All participants, with the consent of their legal guardians, volunteered to participate in this trial and they and/or their legal guardians provided written informed consent before enrollment. CNS status was classified as CNS1 [cerebrospinal fluid (CSF) with white blood cell (WBC) <5/μL and no blasts], CNS2 (WBC <5/μL and cytology positive for blasts), and CNS3 (WBC ≥ 5/μL, with the presence of lymphoblasts and/or parenchymal or cranial nerve involvement). The NC-positive subset was defined by an ALL-unrelated neurological complication, including epilepsy, stroke, neurological deficit, and abnormal brain imaging such as posterior reversible encephalopathy syndrome (PRES), leukodystrophy, or encephalatrophy at relapse or within the 12 months preceding CAR-T cell infusion. Patients with NC were controlled for the period of time needed for a return to near-normal neurological status. The patients were followed up to November 30, 2021.

CAR Transduction Procedure

The CAR transgene and CAR-T cell manufacturing were engineered by Shanghai Genbase Biotechnology Co., Ltd. China. Peripheral blood (PB) mononuclear cells were obtained by apheresis. $CD3^+$ T cells were purified by $CD3^+$ microbeads (Miltenyi Biotec, Inc.) and stimulated by anti-CD3/anti-CD28 mAb-coated Human T-Expander beads (cat. no. 11141D; Thermo Fisher Scientific, Inc.) for 24–48 hours. T cells were cultured in T-cell medium X-Vivo 15 (Lonza Group, Ltd.) followed by supplementation with 200 IU/ml rhIL-2 (Proluekin; Novartis International AG). A lentiviral vector encoding CD19 CAR with the humanized signaling domains 4-1BB/CD3ζ (Shanghai Genbase Biotechnology Co., Ltd) was transduced into the activated T cells. The transduction was performed for 48 hours at a multiplicity of infection of 0.3. Transduced T cells were cultured, amplified, and harvested over 5–7 days using the G-Rex system.

Transduction efficiency was defined as the ratio of CAR-T to CD3+ T cells, determined by flow cytometry (FCM) with anti-CD19 CAR-T cell–specific detection reagent. The mean CD19 CAR transduction efficiency was 54.1% ± 5.8%. The products included both CD4+ (60.5% ± 14.9%) and CD8+ T cells (33.2% ± 16.8%) and did not contain any CD19+ B cells. The corresponding subpopulations of naive T cells (CD45RA+CCR7+), central memory T cells (CD45RA−CCR7+), effector memory T cells (CD45RA−CCR7−), and effector T cells (CD45RA+CCR7−) were 57.0% ± 17.0%, 20.5% ± 7.1%, 10.2% ± 6.5%, and 12.3% ± 6.6%, respectively.

Bridging Chemotherapy and Lymphodepletion Conditioning

For patients with a tumor burden of marrow blasts ≥ 40%, the bridging regimen included vincristine 1.5 mg/m^2 on day 1 and day 8, daunorubicin 30 mg/m^2 on day 1 or on day 8 in addition, perasparaginase 2000 IU/m^2 on day 3, and dexamethasone 8 mg/m^2 on days 1 to 14, with a 7-day taper. Patients with a high CNS burden of (≥ 5/μL blasts in CSF or solid mass) received IT with cytarabine, methotrexate, and dexamethasone every other day. IT was performed every week or until blasts were <5/μL. The aim was to control CSF blasts <5/μL or deplete parenchymal infiltration with no detection of epileptic waves on electroencephalogram.

Lymphodepletion conditioning was performed with fludarabine 40 mg/m^2/day for days −5 to −3 and cyclophosphamide 500 mg/m^2/day for days −5 to −4. The day of infusion was defined as day 0, and patients were infused at a single dose of 1×10^6 cells/kg of CAR-T cells.

Response Evaluation

Residual blasts were evaluated based on morphology and minimal residual disease (MRD) by FCM and reverse transcription-polymerase chain reaction (if fusion genes were present) in CSF and BM on day 28 after CAR-T infusion. The response for extramedullary invasion was judged by computed tomography (CT), positron emission tomography (PET)-CT, magnetic resonance imaging (MRI), or B ultrasound. Clinical responses were defined as complete remission (CR), CR with incomplete count recovery, partial remission, or no remission (NR) according to the National Comprehensive Cancer Network guidelines. Negativity for MRD was defined as <0.01%, as determined by FCM.

Grading and Management of CRS and Neurotoxicity

CRS and neurotoxicity were graded according to the American Society of Transplantation and Cellular Therapy consensus guidelines.[13] Other adverse events were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. sCRS was defined as grade 3 or higher, and severe neurotoxicity was defined as any seizure or as grade 3 or higher.

Levetiracetam (10 mg/kg, twice daily) was given as anti-epileptic prophylaxis to patients with a history of seizure from the day of CAR-T infusion until 1 month after infusion. Patients already on anti-epileptics continued their home regimen during CAR-T therapy. Seizure prophylaxis with levetiracetam was initiated when patients developed sCRS or new neurological symptoms. Management of immune effector cell–associated neurotoxicity syndrome (ICANS) and CRS was performed based on Neelapu et al,[14] with some adaptations. Active seizures were managed with benzodiazepines (eg, diazepam or midazolam) for patients who developed status epilepticus. Mannitol (2.5–5 mL/kg/dose) and furosemide (1-2 mg/kg/dose) were used to control intracranial hypertension. Details of the management of CRS and ICANS are given in the Supplemental Methods, Supplemental Digital Content 1, http://links.lww.com/JIT/A684.

CAR-T Cell Detection

CAR-T cells in PB were measured on the expansion days, on day 14, and monthly from 1 month to the month when CAR-T became undetectable. CAR-T cells in CSF were detected on the peek CRS day or at acute neurotoxicity

TABLE 1. Patient and Disease Characteristics with CNSL or Neurological Comorbidities

Pt.	Age at CAR-T Infusion (mo)	Sex	Cytogenetic/ Molecular Genetic Alterations	Relapses (n)	Recurrent Sites	Leukemia Burden Reduction	BM Blasts Before CAR-T	CNS Infiltration/ Neurological Comorbidities	EEG	CAR-T Type	Subsequent Therapy
CNSL1	194	Male	E2A/PBX1, PHF6	1	BM, CSF, leptomeninges, spleen, bone, muscle	VDDL I/T	MRD−	CNS3, PET-CT abnormal signal	Abnormal	Auto	MUD
CNSL2	66	Female	None	1	BM, CSF, cerebral cortex	I/T	MRD+	CNS3, MRI/ PET-CT abnormal signal	Abnormal	Auto	Followed up
CNSL3	57	Male	MLL/AF9	2	BM, CSF	VDDL, I/T	MRD−	CNS3, allo-HSCT	Normal	Donor	Sibling
CNSL4	78	Male	MLL/AF10, NRAS	2	Testis, CSF	VDDL, I/T	MRD−	CNS3, testis infiltration, cranial radiation at 1st testis relapse	Normal	Auto	Followed up
NC1	91	Female	Hyperdiploid, PAX5, IKZF1	1	BM	VDDL	MRD+	Generalized seizure with PRES on MRI	Normal	Auto	Sibling
NC2	99	Male	TEL/AML1, TP53, NRAS	3	BM	VDDL, RTX	84.5	Generalized seizure with PRES on MRI, epilepsy, slight encephalatrophy, allo-HSCT	Abnormal	Donor	Death from neurotoxicity
NC3	77	Male	TEL/AML,−7	/	Primary refractory	No	MRD+	Incomplete myelination, abnormal oligoclonal bands	Normal	Auto	Haploid
BM1	131	Female	FLT3/TKD,	3	BM	VDDL	2.5	Negative	Normal	Auto	MUD
BM2	73	Male	ASXL1	1	BM	VDDL	MRD+	Negative	Normal	Auto	Haploid
BM3	143	Male	PTPN11, RHOA, FLT3/TKD, t(1;6)(q31;p21)	/	Primary refractory	No	MRD+	Negative	Normal	Auto	Haploid
BM4	145	Female	Ph+ p190	2	BM	No	20	Negative	Normal	Auto	Death from relapse
BM5	190	Male	NRAS	2	BM	No	6.5	Negative	Normal	Auto	Haploid

auto indicates autologous-derived; BM, bone marrow; CAR-T, chimeric antigen receptor T cell; CNS, central nervous system; CSF, cerebrospinal fluid; donor, donor-derived; EEG, electroencephalogram; FCM, flow cytometry; haploid, haploidentical transplantation; IT, intrathecal injection; mo, months; MRD, minimal residual disease; MRI, magnetic resonance imaging; MUD, matched unrelated donor; Ph+, Philadelphia chromosome; PRES, posterior reversible encephalopathy syndrome; RTX, rituximab; VDDL, vincristine, daunorubicin, dexamethasone, and perasparaginase.

onset, on day 14, monthly at 1 month to 3 months, and then every 2 or 3 months until CAR-T became undetectable. Cells were incubated for 1 hour with B5338 (anti-CD19 CAR-T cell–specific detection antibody for single-chain Fvs, Genbase Biotechnology, Shanghai, China) as the first antibody, and for 0.5 hours with APC-anti mouse immunoglobulin (Ig) G (BioLegend, San Diego, CA) as the second antibody. The proportion of the CD3 and B5338 double-positive population was distinguished as CAR-T cells. CAR-T cell counts were determined using a FACSCalibur flow cytometer (BD Biosciences) and reported as the fraction of live lymphocyte cells. The absolute number of CAR-T cells was defined as the number per microliter. Details of the detection procedure are given in the Supplemental file p2, Supplemental Digital Content 1, http://links.lww.com/JIT/A684.

Cytokine Detection

Serum and CSF concentrations of target cytokines [interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and IFN-α] were measured using a multiple microspheres flow immunofluorescence assay (12 cytokine kits, Raisecare, China) according to the manufacturer's instructions. Serum cytokines were detected within 7 days before CAR-T infusion, on the days of CRS or ICANS onset, and on days 14 and 28. Cytokines in CSF were assessed within 7 days before CAR-T infusion, on the day of peak CRS or ICANS onset, and on days 14 and 28.

B Lymphocytes Detection

PB lymphocyte subsets (CD3+T, CD4+T, CD8+T, NKT, B cells) by percentage and cell count were analyzed with FCM and with Cell Quest software. Antibodies to CD19 (BD Biosciences) were used for B-cell quantification. B-cell recovery was defined as $>3\%$ B cells/lymphocytes (the lower limit of normal for blood B-cell levels).[15]

Statistical Analysis

Non-normally distributed data are presented as medians and full ranges. Comparisons between 2 groups were made using the Mann–Whitney *U* test or Wilcoxon matched pairs test. Comparisons among 3 or more groups were made using one-way analysis of variance and the Kruskal-Wallis test. Comparisons of two or more factors were made using two-way one-way analysis of variance. Correlations were analyzed by the Spearman correlation, and correlation coefficients were calculated. Data for patients who died without evidence of disease before their first post-infusion assessment were not evaluated. All

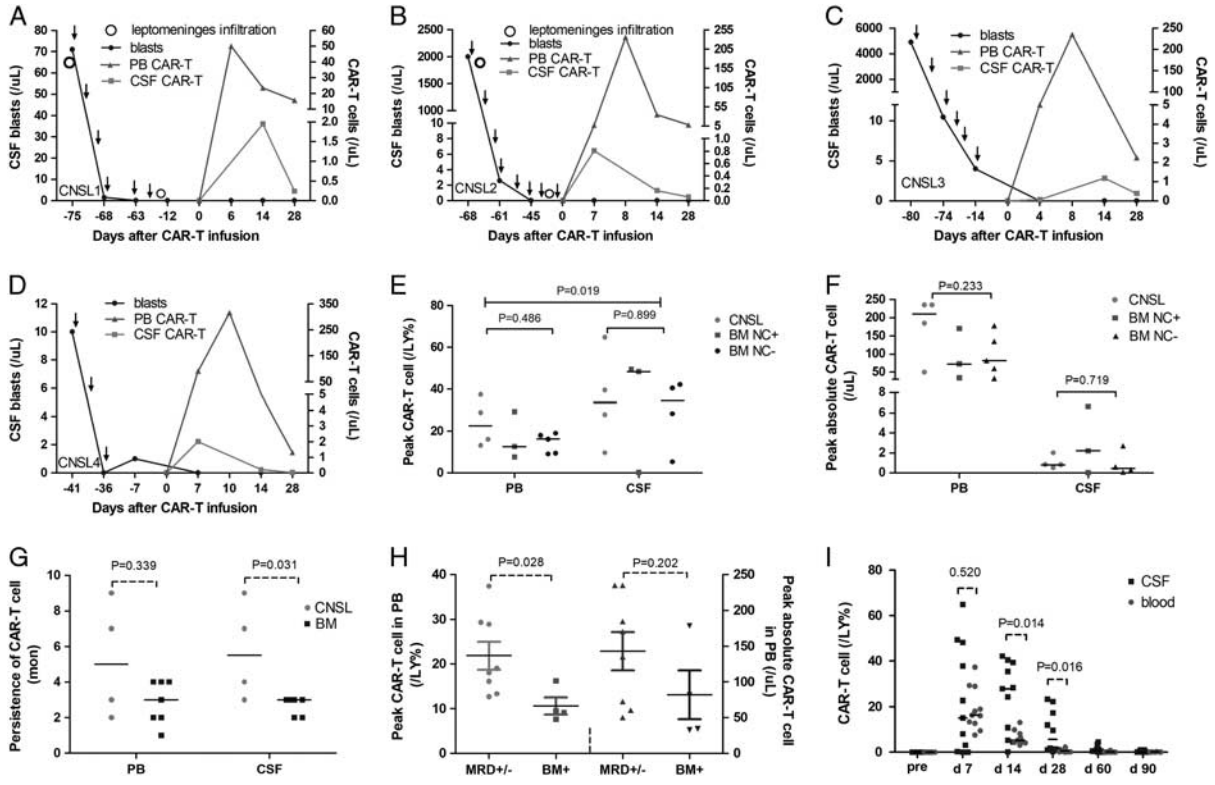

FIGURE 1. Fluctuation of CAR-T cells in CSF and PB after CD19 CAR-T therapy by flow cytometry. A–D, Monitoring of CAR-T cells in CSF and PB, and blasts in CSF. Black arrows indicate triple intrathecal chemotherapy. E, Peak CAR-T cell values among lymphocytes in CSF were higher than in PB. CAR-T cells were at similar levels in CSF and PB in patient with CNSL, with BM relapse with NC, and without NC. F, Peak absolute CAR-T cell values in CSF and PB were comparable among patients with CNSL, BM relapse and NC, and without NC. G, CAR-T cells in CSF persisted longer in patient with CNSL than in those with BM relapses. The duration of CAR-T cells in PB was similar between the 2 groups. H, Peak fraction of CAR-T cells in PB was higher in BM MRD+/– patients than in those with residual blasts. I, Peak CAR-T cell count in CSF was likely to occur later than in PB. *P*-values with ⌐¬ indicate a comparison among the 3 patient subsets; *P*-values with ⌐ - - ¬ indicates a comparison between the 2 indicated subsets. *P*-values with ⊢⊣ indicate a comparison between patient subsets with PB and CSF. BM indicates bone marrow relapse; CSF, cerebrospinal fluid; CNSL, central nervous system leukemia; PB, peripheral blood; LY, lymphocytes; MRD, minimal residual disease; NC, neurological comorbidity.

statistical analyses were performed with GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA) and SPSS 19.0 (SPSS Inc., Chicago, IL) software. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Patients

A total of 12 pediatric R/R ALL patients were enrolled [4 girls, 8 boys; median age, 7 (range 4–16) years], 2 with primary refractory disease and 10 with a median of 2 (range 1–3) relapses. Four patients had CNSL, 3 had BM relapse with NC, and 5 had BM relapse without NC. Of the 4 patients with CNSL, 3 experienced BM relapses and 1 had bilateral testis relapses. Of the 3 patients with NC, 1 case was complicated with PRES, 1 patient had PRES and epilepsy, and 1 had incomplete myelination accompanied by intrathecal synthesis of IgG.

Two patients who relapsed after allo-HSCT received donor-derived CAR-T. Seven patients received bridging chemotherapy, which failed for 1 of them. All patients with CNSL received IT preceding CAR-T infusion and had CSF blasts <5/μL or obvious depletion of parenchymal infiltration (Fig. 1A–D). One patient with NC and 1 with CNSL received anti-epileptic prophylaxis with levetiracetam (see Table 1 for details).

Clinical Responses

Eleven patients had a response-evaluable assessment on day 28. The rate of CR was 100%, so that the rate of NR was 0%. One patient (NC2) who died of severe ICANS and sCRS on day 18 had no data available for BM assessment. All 4 patients with CNSL achieved CR (100%) with blast-negative CSF and BM by FCM, with CAR-T cell expansion in CSF and PB (Fig. 1A–D). In 2 patients (CNSL1, CNSL2), MRI or PET-CT was negative for parenchymal infiltration after CAR-T therapy (Fig. 2A–D). The 2 CNSL patients (CNSL1, CNSL4) had other sites of extramedullary infiltration that had become negative on imaging. CR/incomplete count recovery was also 100% among patients with BM relapses, with or without NC, and they all were FCM-MRD negative on day 28.

One patient (NC2) died during CAR-T therapy. He had a CRS of grade 4 and a seizure accompanied with cerebellar edema. This patient ultimately died of severe ICANS on day 18 after CAR-T therapy. Mortality related to CAR-T therapy was 8.3%.

Circulating CAR-T Cells in CSF and PB

CAR-T cells in CSF were measured in 11 of 12 patients (a CSF sample from patient BM1 was unavailable). Circulating CAR-T cells in CSF were found in 9 patients (81.8%) on day 7 (± 2) and in all specimens (100%) on day 14. The proportion of CAR-T cells among lymphocytes in the CSF peaked at 39.5% (range 0.2–64.8%; Fig. 3), with peak expansion on day 14 (7–28). The peak CSF CAR-T expansion was higher than in PB, at 39.5% versus 16.2% ($P = 0.019$). CSF CAR-T cells in patients with CNSL and those with BM relapses with and without NC had similar peaks ($P = 0.899$; Fig. 1E); median values were 33.7% (range 9.6–64.8%) with CNSL, 48.2% (range 0.2–49%) with BM relapse and NC, and 34.5% (range 5.3–42.2%) with BM relapse without NC. The peak absolute CAR-T cell count in CSF occurred around day 14 (7–28) with a median of 0.809/μL (range 0.027–6.62/μL). The peak absolute number of CSF CAR-T cells was 2.207 (0.027–6.62)/μL with BM relapse and NC, 0.82 (0.5–2.005)/μL with CNSL, and 0.458 (0.098–2.7)/μL with BM relapse and no NC ($P = 0.719$; Fig. 1F). The median duration of CSF CAR-T cells in patients with CNSL was 5.5 (range 3–9) months, which differed significantly from the 3 (range 2–3) months for patients with isolated BM relapses ($P = 0.031$; Fig. 1G).

The peak fraction of CAR-T cells in PB was similar with CNSL, BM relapse with NC, and BM relapse without NC on peak day 8 (5–11) ($P = 0.486$; Fig. 1E, Supplemental Fig. 1, Supplemental Digital Content 2, http://links.lww.com/JIT/A685). Median values were 209.7 (50–184.4)/μL with CNSL, 72.5 (34.2–170.0)/μL with BM relapse and NC, and 71 (32.8–134.9)/μL with BM relapse and no NC ($P = 0.233$; Fig. 1F). The duration of PB CAR-T cells was similar between CNSL and isolated BM relapses ($P = 0.339$; Fig. 1G). The peak day of CAR-T cells in CSF occurred later than in blood ($P = 0.005$, day 14 vs. day 8), whereas CAR-T cells in CSF remained at a higher fraction than in blood on day 14 ($P = 0.014$) and on day 28 ($P = 0.016$; Fig. 1I). The peak for PB CAR-T cells in fractional and absolute counts was higher in patients with MRD+/− than for BM with residual blasts ($P = 0.028$, $P = 0.202$; Fig. 1H). The persistence of CAR-T cells was similar between 2 groups with medians of 3 (2–9) months with MRD+/– and 4 (1–4) months with BM with residual blasts ($P = 0.917$).

Neurotoxicity developed in 2 patients. Patient NC2 had grade 5 ICANS, and patient CNSL2 had chronic neurotoxicity with white matter lesions at 1 month after CAR-T infusion. The 2 patients who experienced neurotoxicity did not have greater peak CAR-T cell values in CSF (0.2% and 9.6%, and 0.027 and 0.809 cells/μL, respectively) compared with those without neurotoxicity (40.5%, range 5.3–64.8%; 0.839 cells/μL, range 0.098–6.62 cells/μL).

Neurotoxicity in CNSL and Neurological Comorbidities

Initial MRI or CT/PET-CT imaging of CNS infiltration was obtained before lymphodepletion chemotherapy. Patients CNSL1 and CNSL2 had abnormal PET-CT brain imaging (Fig. 2A, B). CNSL2 had extensive leptomeninges infiltration with hyperintensities on enhanced T2-FLAIR images (Fig. 2C) at 2 months before CAR-T cell infusion. Patient NC2 had a history of epilepsy and PRES, and T1-weighted images at 1 month before CAR-T cell infusion showed slight encephalatrophy and ventriculomegaly (Fig. 2G). Electroencephalogram monitoring showed abnormal waveforms in all 3 of these patients (CNSL1, CNSL2, and NC2), 2 of whom (CNSL2 and NC2) developed neurotoxicity. Patient CNSL2 did not have neurological symptoms at CRS peak, but the brain MRI showed leukodystrophy on day 30 (Fig. 2E). Routine CSF testing was normal in this patient, with WBC 0/μL, glucose 2.7 mmol/L, and protein 200 mg/L. IgM and IgG for toxoplasma, rubella virus, cytomegalovirus, and herpes simplex virus (HSV) in CSF were negative. The DNA of HHV-6, HSV-1, HSV-2, and JC virus in CSF was not detectable. The patient received IgG 1 g/kg for 2 days and gradually recovered until 1 year post CAR-T infusion (Fig. 2F). Patient NC2 had a seizure with a decreased level of consciousness and required invasive ventilatory support on day 13. Brain CT showed acute cerebellar edema and subsequently cerebral hemorrhage coincident with thrombocytopenia (Fig. 2H). This patient ultimately died of ICANS 5. None of the 5 patients without NC developed neurotoxicity (Fig. 2I).

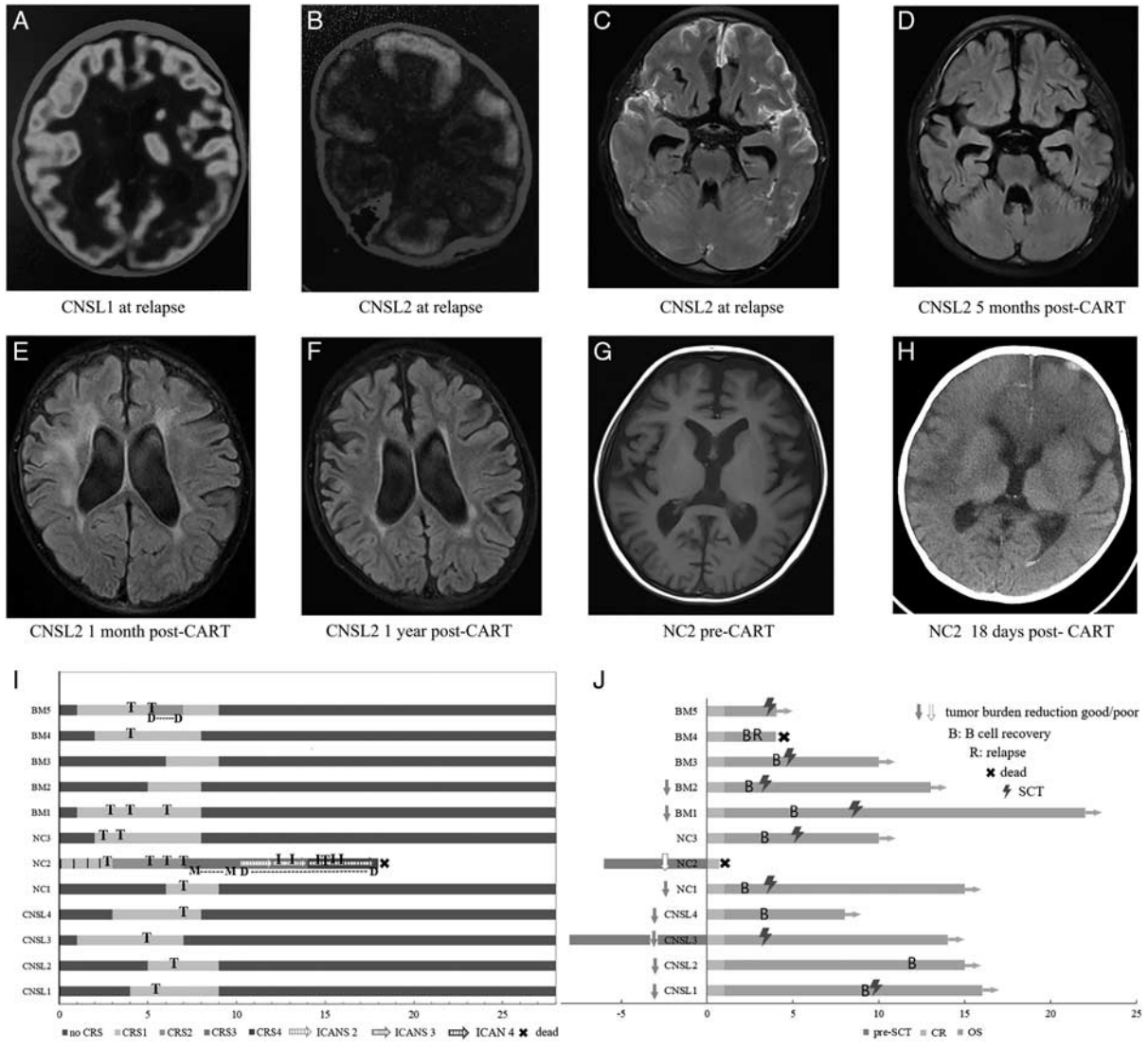

FIGURE 2. Imaging findings for patients with CNSL or neurological comorbidity and overall survival after CAR-T therapy. A, 18F-FDG metabolism on PET-CT was obviously increased in the left lateral fissure meninges in patient CNSL1 at relapse. B, Diffuse uneven FDG metabolism was abnormally elevated in the left cerebral cortex of patient CNSL2 at relapse. C, Extensive leptomeninges infiltration with hyperintensity and encephalomalacia with hypointensity in the frontal lobe on enhanced T2-FLAIR images in patient CNSL2 at relapse. D, Leptomeninges lesions disappeared after CAR-T therapy on enhanced T2-FLAIR images in patient CNSL2. E, Extensive white matter hyperintensities on T2-FLAIR imaging in patient CNSL2 at 1 month after CAR-T infusion. F, The near complete resolution of white matter hyperintensities after 1 year post CAR-T. G, Slight encephalatrophy and ventriculomegaly on T1-weighted images in patient NC2 before CAR-T. H, Severe cerebellar edema and cerebral hemorrhage in the frontal lobe on CT in patient NC2 on day 18 post CAR-T. I, CRS grade of and neurotoxicity graded by ASTCT consensus guidelines. Severity of CRS is represented by color codes and neurotoxicity grade is represented by color arrows. Striped rows represent patients with fever before CAR-T cell administration that did not reflect CRS precisely. Day 0 is the day of cell administration. J, Overall survival after CAR-T therapy.18F-FDG indicates 18F-fluorodeoxyglucose; BMI, bone marrow relapse; CNSL, central nervous system leukemia; CR, complete remission; CRS, cytokine release syndrome; CT, computed tomography; FDG, fluorodeoxyglucose; ICANS, immune effector cell-associated neurotoxicity syndrome; NC, neurological comorbidity; Pre-SCT, previous stem cell transplantation; OS, overall survival.

Cytokines in CNSL and Neurological Comorbidities

CSF Cytokines

In the CSF, IL-6, IL-8, and IL-10 had risen significantly by the peak of CRS or ICANS onset ($P = 0.009$, 0.005, 0.001, respectively), from a median of 6.1 (2–42.4) pg/mL to 124.2 (15.4–5618) pg/mL for IL-6, 100 (21.9–205.8) pg/mL to 342.3 (70.4–1755) pg/mL for IL-8, and 2 (1.6–2) pg/mL to 17.3 (2–56.1) pg/mL for IL-10 in the whole cohort. Other cytokines, including IL-1β, IL-2, IL-5, and IFN-γ, showed mild but significant elevations ($P = 0.016$, 0.004, 0.031, 0.028, respectively), although median values remained in the normal range (see Table 2 for details). At the acute phase of CRS, CSF IL-6, IL-8, and IL-10 showed extreme increase in patients with NC than those with CNSL and no NC (for comparison of each biomarker among the 3 groups: IL-6, $P = 0.041$; IL-8, $P = 0.016$; IL-10, $P = 0.177$;

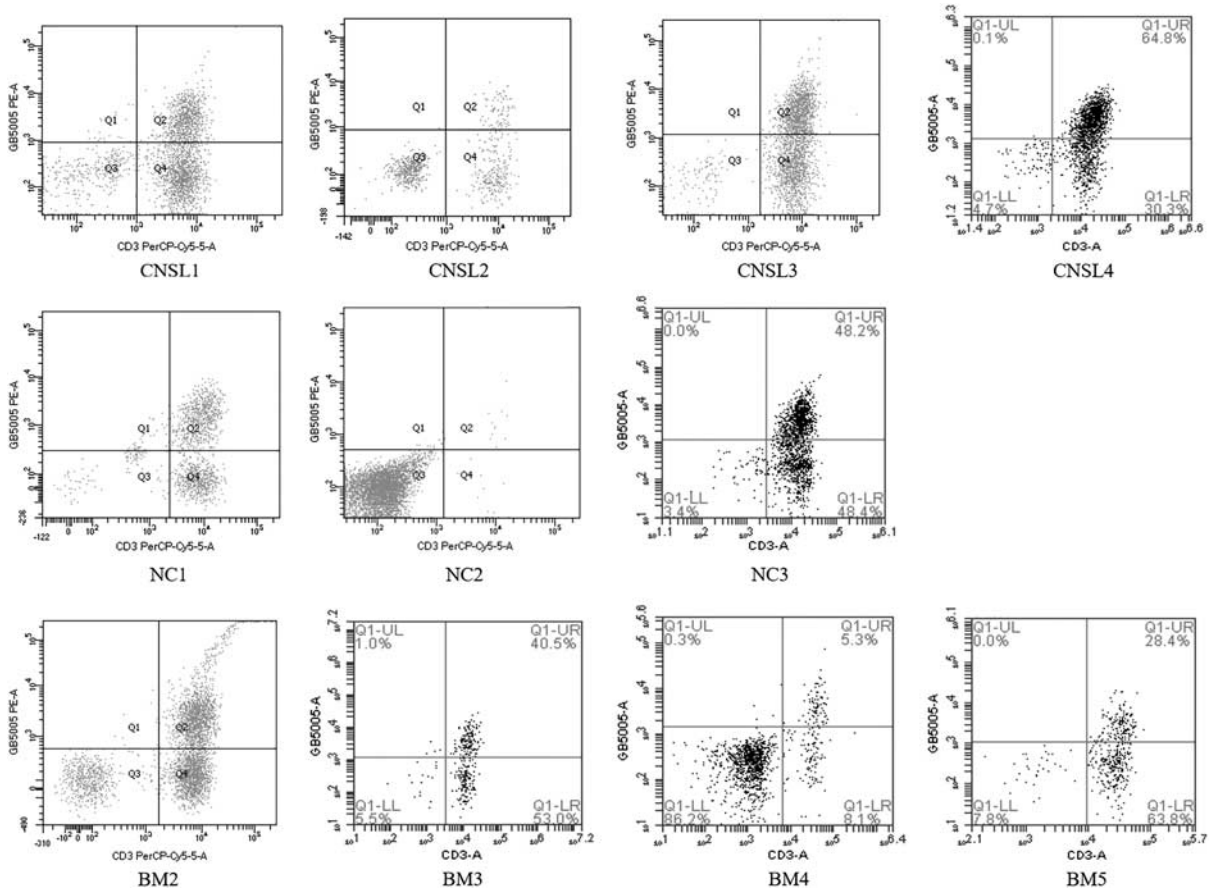

FIGURE 3. Fluorescence activated cell sorting plots of peak CSF CAR-T cells among lymphocytes in 11 patients after CD19 CAR-T therapy by flow cytometry. GB5005 indicates CAR-T cells expressing humanized anti-CD19 single-chain Fvs. CAR-T indicates chimeric antigen receptor; CSF, cerebrospinal fluid.

Fig. 4A–C). IL-2 was only mildly elevated overall, whereas IL-2 increased significantly in patients with NC ($P = 0.010$; Fig. 4G). Results for other biomarkers are shown in Supplemental Fig. 2, Supplemental Digital Content 3, http://links.lww.com/JIT/A686.

Serum Cytokines

In serum, IL-6, IL-2, IL-10, IFN-γ, IL-5, and IL-1β had increased significantly by the peak phase ($P = 0.001$, 0.003, 0.001, 0.001, 0.006, 0.008, respectively; Table 2 for details). IL-8 and IL-4 rose slightly but remained within normal range ($P = 0.005$, 0.047, respectively; Table 2). IL-12P70, IL-17, IFN-α, and TNF-α did not increase ($P = 0.063$, 0.063, 0.063, 0.078, respectively). Cytokine release in serum after CAR-T therapy was seen in Supplemental Fig. 3, Supplemental Digital Content 4, http://links.lww.com/JIT/A687.

Cytokine Comparisons between Compartments

In comparing biomarker changes between compartments, we found that among patients with NC, in the paired samples, IL-6 levels were higher in CSF (median 847.85 pg/mL) than in serum (66.6 pg/mL) (Fig. 4D). In patients with CNSL or no NC, IL-6 was lower in CSF than in the serum (CNSL: median 19.8 pg/mL CSF vs. 33.6 pg/mL serum; without NC: 102.9 pg/mL CSF vs. 493.9 pg/mL serum; Fig. 4D). IL-8 levels were higher in CSF than in PB before CAR-T and at peak CRS ($P = 0.027$, 0.0002, respectively; Fig. 4E), but IL-10 did not differ in the paired samples at peak CRS ($P = 0.547$; Fig. 4F).

Cytokines and Neurotoxicity

When we examined biomarkers in each compartment in association with neurotoxicity, we found that IL-6, IL-8, and IL-2 in CSF and IL-6, IL-8, IL-2, IL-5, IL-10, and IFN-γ in PB were markedly elevated (over 10 times normal levels) during acute neurotoxicity (patient NC2). IL-1β and IL-5 in CSF and IL-1β in PB were only mildly elevated; however, IL-10 and IFN-γ remained at normal levels in CSF. IL-4, IL-12P70, IL-17, IFN-α, and TNF-α in PB and CSF remained normal during acute neurotoxicity. Further analysis showed no correlation between IL-6 elevation and CSF CAR-T cell expansion ($P = 0.126$).

WBCs and Proteins in the CSF

CSF WBCs had increased by the CRS peak from a median of 1 (0–7) cells/μL to 10 (1–46) cells/μL ($P = 0.001$; Fig. 4H) in the whole group, and from 3 (0–7) cells/μL to 8 (3–20) cells/μL with CNSL ($P = 0.097$), from 1 (0-1) cells/μL to 19 (2–46) cells/μL with NC ($P = 0.250$), and from 1 (0–2) cells/μL to 12 (1–23) cells/μL without NC ($P = 0.098$). CSF WBCs did not differ among the 3 patient subsets ($P = 0.752$). CSF WBCs in the patient with ICANS remained normal at 2 cells/μL. CSF protein rose from a

TABLE 2. CSF and Serum Cytokines from Baseline to Peak CRS After CAR-T Therapy

Tissue	Cytokine	IL-6	IL-8	IL-10	IFN-γ	IL-1β	IL-2	IL-4	IL-5
CSF (pg/mL) Median (range)	Baseline	6.1 (2.0–42.4)	100.0 (21.9–205.8)	2.0 (1.6–2.0)	2.0 (2.0–30.2)	2.5 (2.0–10.3)	2.0 (1.6–5.3)	2.0 (2.0-3.3)	2.0 (2.0–3.67)
	Peak	124.2 (15.4–5618)	342.3 (70.4–1755)	17.3 (2.0–56.1)	5.5 (2.0–46.2)	7.4 (2.0–27.5)	3.9 (2.0–36.7)	2.0 (2.0-4.9)	2.6 (2.0–13.6)
	P	0.009	0.005	0.001	0.028	0.016	0.004	0.375	0.031
Serum (pg/mL) Median (range)	Baseline	3.9 (2.0–209.7)	2.0 (2.0–272.5)	2.0 (2.0–10.9)	12.4 (2.0–33.9)	2.0 (2.0–29.6)	2.0 (2.0–9.1)	2.0 (1.0–3.2)	2.0 (2.0–7.8)
	Peak	213.6 (17–8673.7)	18.5 (3.8–1682.7)	19.5 (4.5–214.8)	46.6 (10.8–5448.5)	14.8 (2.0–63.6)	16.7 (2.0–79.8)	2.6 (2.0–5.0)	5.4 (2.0–501.6)
	P	0.001	0.005	0.001	0.001	0.008	0.003	0.047	0.006

P-value indicates the comparison with peak and baseline.
CAR-T indicates chimeric antigen receptor T cell; CRS, cytokine release syndrome; CSF, cerebrospinal fluid; IFN, interferon; IL, interleukin.

median of 235 (range 210–940) mg/L to 330 (range 240–1070) mg/L in the whole group ($P = 0.060$) and from 230 (210–250) mg/L to 380 (260–910) mg/L in those with BM relapses ($P = 0.008$; Fig. 4I). CSF protein in patients with CNSL decreased from 315 (210–940) mg/L to 235 (220–780) mg/L ($P = 0.250$) and increased most significantly in the patient with ICANS, from 210 mg/L to 910 mg/L.

Outcome

Eleven of 12 patients survived CAR-T–related toxicity, and 1 patient (NC2) died of ICANS. One patient (BM4) experienced relapse in the second month after CD19 CAR-T and received CD19/22 CAR-T therapy in the third month. The disease did not respond positively to remission, and the patient died in NR (Fig. 2J). Ten patients remained in CR, 8 of whom underwent allo-HSCT and 2 of whom had CNSL and did not undergo HSCT. One-year event-free survival was 90.9% ± 8.7%, with a median of 12 (0–22) months of follow-up.

DISCUSSION

Currently, treatment for R/R ALL remains a difficult challenge, especially with CNS relapse or neurological complications. The most common site of extramedullary relapse is the CNS, representing ~20% of the total.[16,17] For patients with CNSL that is refractory to CNS-directed therapies, few treatment options are available.[18] CAR-T cells can migrate into a leukemia cell sanctuary such as the CSF by overcoming the blood–brain barrier (BBB), which may provide an opportunity for patients whose CNSL is refractory to conventional therapeutic regimens.[19]

Nevertheless, neurotoxicity remains a severe and potentially fatal event with CAR-T therapy. Most clinical trials of CAR-T have excluded patients with CNSL or symptomatic neurological disease because of a potentially increased risk of neurotoxicity.[1,2,20] For the wider application of CAR-T therapy, patients with CNS recurrences have been intermittently enrolled to explore the feasibility of eliminating the blasts in the CNS.[8,21] However, the case numbers are limited for pediatric patients. In this prospective cohort study of children undergoing CD19-directed CAR-T cell treatment, we performed comprehensive CSF analyses, CAR-T cell detection, cytokines quantification, and imaging scans, which together may provide new insights into R/R ALL with CNSL or NC and the use of CAR-T cell therapy for these patients.

Humanized CAR-T cells showed good efficacy in inducing remission in the CNS in our cohort. High rates of detectable CAR-T cells in the CSF offer evidence that CAR-T cells can traffic to extramedullary sites. In our data, CAR-T cells persisted in the CSF of patients with CNSL for a median of 5.5 months, with the longest durability of 9 months. Results from a published clinical trial showed that CAR-T cells with a 4-1BB domain persisted for more than 6 months in most cases.[22] However, the durability of our native CAR-T products in CSF was short in those with BM recurrences, with a median of 3 months. We found that patients with CNSL had a longer duration of CAR-T cells in the CSF than those without CNS infiltration. It should be noted that the patients with CNSL in our cohort had either BM relapse or testis recurrence. He et al[21] found that patients with only CNS recurrence had higher levels of CAR-T cells in the CSF and relatively lower severity of toxic effects than those with BM and CNS recurrence. Li

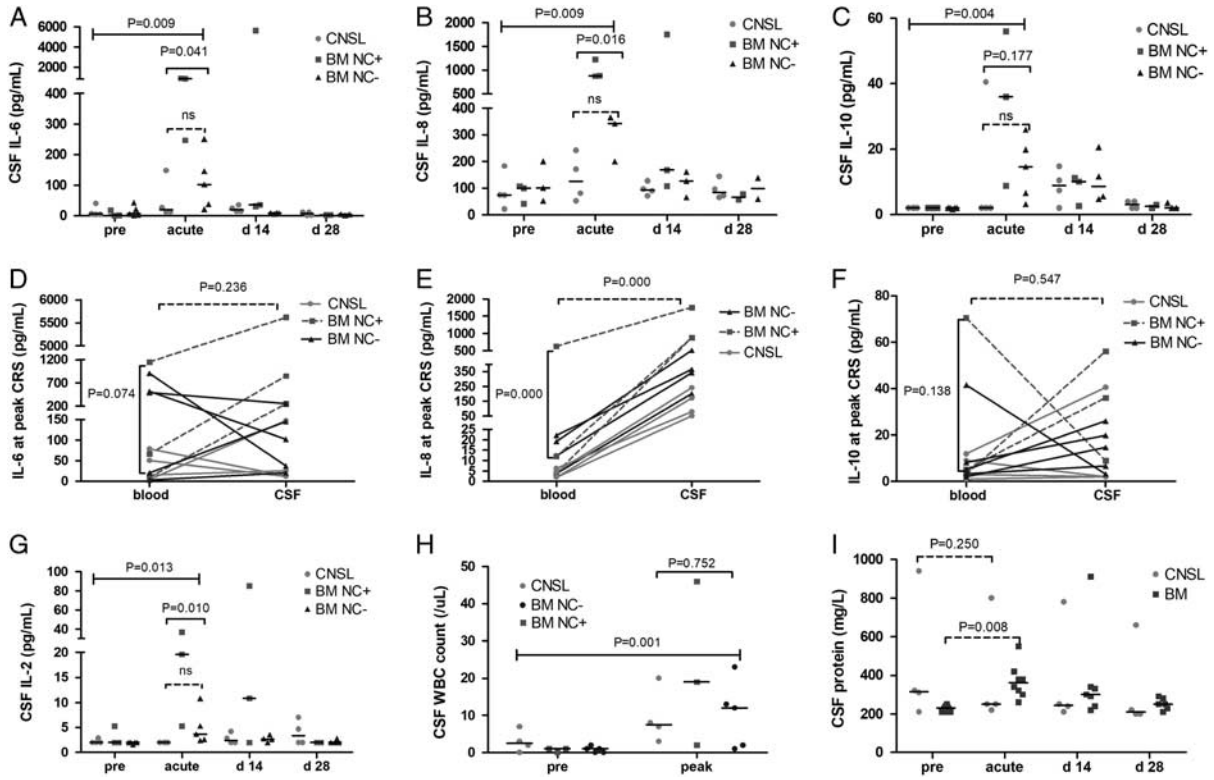

FIGURE 4. Cytokine release in CSF and serum after CAR-T therapy. A–C, CSF IL-6, IL-8, and IL-10 increased significantly at CRS peak. Cytokines in patient with NC increased more than in those with CNSL or without NC. D, IL-6 tended to be higher in CSF than in serum in paired samples at peak CRS in those with NC, whereas IL-6 levels were lower in CSF than in serum in patients with CNSL or without NC. E, IL-8 was higher in CSF than in PB at baseline and CRS peak. F, IL-10 levels were comparable between CSF and PB at CRS peak. G, IL-2 reached higher levels in patients with NC than in those with CNSL or without NC. H, CSF white blood cells increased significantly after CAR-T therapy with no difference among the 3 patient subsets. I, CSF protein decreased in patients with CNSL and increased in those with BM relapses. "Acute" indicates peak level obtained at CRS and/or neurotoxicity (days 1–13); d14, day 14 after treatment; d28, day 28 after treatment; pre, sample obtained before CAR-T infusion (days -7–0); peak, the peak value during CRS and/or neurotoxicity. *P*–values with ⌐¬ indicate a comparison among the three patient subsets; *P*–values with ⌐ - -¬ indicates a comparison between the two indicated subsets. *P*–values with |—| indicate a comparison between patient subsets with "pre" and "acute". BM indicates bone marrow relapse; CAR-T, chimeric antigen receptor T cell; CRS, cytokine release syndrome; CSF, cerebrospinal fluid; IL, interleukin; NC, neurological comorbidities; PB, peripheral blood; ns, not significant at 95% confidence level.

et al[23] observed that the peak fraction of CAR-T cells in the CSF during recurrent CNSL was higher compared with PB, consistent with our results. We further confirmed that the peak fraction of CSF CAR-T cells with isolated BM relapses was also higher in CSF than in PB. We performed bridging chemotherapy in patients with high tumor burden, but a lower tumor burden did not appear to reduce the peak level or persistence of CAR-T cells.

ICANS has previously been reported in 40% of children and young adults with ALL (13% severe) and 50% of adults (50% severe).[10,24] Risk predictors for developing neurotoxicity are not clear, but factors that have been considered include higher disease burden, higher CAR-T cell expansion, higher proinflammatory cytokines, and extramedullary disease.[11,25,26] To date, the impact of CNS disease burden on ICANS is not well defined.[11,25] In a study of patients who retained > 5/μL blasts in CSF or had a solid mass before CAR-T cell expansion, 80% developed severe neurotoxicity that featured persistent cerebral edema and seizure,[7] even though reducing a high tumor burden in CNS was expected to decrease neurotoxicity severity. Rubinstein et al[27] reported that 7 pediatric patients with controlled NC or CNSL under CNS2 were infused with tisagenlecleucel and experienced mild to no neurotoxicity. One report described 3 adult patients with CNSL at stage CNS3 (blasts > 5/μL) who had undergone burden reduction (blasts ≤ 5/μL) with chemotherapy before CAR-T cell infusion. They all achieved CR accompanied by mild neurotoxicity.[21] In the post hoc analysis of CAR-T use, CNS2 or CNS3 at infusion was not associated with a higher risk for any or severe neurotoxicity, although patients in the R/R CNSL stratum were more likely to develop any symptoms of neurotoxicity.[12]

In addition, a strong association between BM burden and the risk of CRS and neurotoxicity has been reported.[12,24] Because of the small number of patients in our study, we could not analyze correlations between BM burden and neurotoxicity risk. Neurotoxicity might be mitigated by a low burden in BM disease.[12] Our choice for reducing tumor burden was bridging chemotherapy or enhanced lymphodepletion, which remain controversial among clinicians. We conducted IT and/or intensive chemotherapy to reduce the potential risk of severe neurotoxicity, as well as symptomatic NC controlled before CAR-T infusion. In our cohort, one of 7 patients in the group with high tumor burden did not experience blast reduction to

under 20%, whereas in all patients with CNSL, blasts were controlled to <5/μL, and parenchymal lesions were controlled, as well. CRS of grade 1-2 and no ICANS were seen in patients with well-controlled tumor burden. Life-threatening cerebral edema occurred in a patient who did not experience blast depletion.

The increased permeability of the BBB and increased cytokines caused by the activation of CNS endothelial cells contribute to neurotoxicity in CAR-T therapy.[25] Immune effector cells can pass into the CNS, where they can have a localized inflammatory function. In fact, proinflammatory cytokines in the CSF likely accumulate both from the circulating blood and from CNS production.[11,25] CAR-T cells were present in all CSF specimens from our patients, similar to previously reported findings.[28,29] Not all inflammatory cytokines were significantly elevated with CAR-T cell expansion in the CSF, however, suggesting that infiltrating CAR-T cells do not have a primary role in the development of ICANS. In our cohort, patients received a fixed dose of CAR-T cells, but the cells themselves vary qualitatively among patients, which may influence cytokine production. We speculated that the toxicity is primarily mediated by the inflammatory cytokine surge that accompanies CAR-T cell expansion, rather than by the CAR-T cells themselves. The role of direct infiltration by CAR-T cells as a driver of neurotoxicity is still unclear. Necropsy from a nonhuman primate monkey model showed brain intraparenchymal infiltration of CAR-T cells,[30] but CAR-T cells were not seen on autopsy of a patient who died from cerebral edema.[31] It is possible that preautopsy lysis of lymphocytes followed steroid treatment.

In our data, the key inflammatory cytokines IL-6, IL-8, and IL-10 were significantly elevated in CSF after CAR-T therapy and comparably so between patients with CNSL and BM recurrences. These findings imply that local cytokine production in patients with CNSL with a low disease burden is not a prominent feature of neurotoxicity. Cytokines were enriched in CSF in the patient experiencing acute neurotoxicity, with disproportionately high levels of IL-6, IL-8, and IL-2, and mild elevation in IL-1β and IL-5, even as CSF CAR-T cells remained at low concentrations. Limited CSF data are available from CAR-T recipients, but IL-6 expression has been reported to be related to endothelial activation and dysfunction of the BBB, which is the key mediator of CRS and modulates CNS response to injury.[11,25,32] IL-1 is reported to have a direct role in ICANS in a humanized mouse model,[33] and IL-1α in CSF[11] and in serum[32] has been correlated with ICANS severity. IL-1β also has been identified as a key mediator of CNS injury, inflammation, and neurodegeneration, although its association with ICANS remains indeterminate.[32,33] IL-8 and IL-10 are increased in ICANS, but a causative role for these cytokines has not been established.[11,25,28] IL-2, IL-4, IL-17a, IL-15, IFN-α, and IFN-γ all have been reported to occur at high levels in the CSF with CNS involvement,[11,25,34,35] which is not entirely consistent with our results. This divergence may be explained by different CAR constructs leading to release of different cytokines.

Previous studies have shown that patients with NC, such as seizure, stroke, brain imaging abnormalities, or neurological deficit, may have an increased risk for neurotoxicity under CAR-T therapy.[12,25,28] For the paired specimens of blood and CSF that we evaluated in NC patients, IL-6 increased more in CSF than in blood. CSF IL-6, IL-2, and IL-8 in these patients occurred at much higher levels than in CNSL patients and patients without NC, suggesting increased permeability of the BBB in NC. CSF protein levels increased in our cohort at CRS onset, especially among those with NC. The increased CSF protein might indicate BBB dysfunction after CAR-T infusion.[11] We also observed that CSF protein in patients with CNSL decreased after CAR-T infusion, indicating depletion of blasts in the CNS. Although ICANS is reversible in most patients, cerebral edema remains a severe event in clinical trials.[36] Brain MRI may be normal or show vasogenic cerebral edema, leptomeningeal enhancement or microhemorrhages with neurotoxicity.[37] Long-term toxicity following CAR-T in reports is relatively low.[27] In the current study, 1 patient with CNSL experienced chronic toxicity with white matter lesions that gradually recovered after 1 year, suggesting that cellular therapy approaches might offer a tolerable treatment for this case presentation.

In summary, we describe outcomes with CD19 CAR-T cell therapy in 7 cases of pediatric ALL with CNSL and/or NC, and in 5 cases with BM relapse and no NC. We reduced the tumor burden before the initiation of CAR-T therapy, showing a potentially effective method for preventing life-threatening ICANS. The results demonstrated that CAR-T therapy is a safe and effective treatment option for clearance of extramedullary infiltration and medullary blasts in patients with neurological complications, if the tumor burden or neurological disease can be adequately controlled. Further and larger studies are needed to verify these findings. The clinical features and biological correlates of neurotoxicity need to be deeply explored to mitigate toxicity.

CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

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