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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Toxicity and response after CD19-specific CAR T-cell therapy in pediatric/young adult relapsed/refractory B-ALL

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KEY POINTS

- **We present results of a clinical trial that show the safety of CD19-specific CAR T-cell therapy for R/R B-ALL.**
- **Conditioning chemotherapy dose intensity and minimal pretreatment disease burden positively impact response without increase in toxicity.**

Chimeric antigen receptor (CAR) T cells have demonstrated clinical benefit in patients with relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL). We undertook a multicenter clinical trial to determine toxicity, feasibility, and response for this therapy. A total of 25 pediatric/young adult patients (age, 1-22.5 years) with R/R B-ALL were treated with 19-28z CAR T cells. Conditioning chemotherapy included high-dose (3 g/m²) cyclophosphamide (HD-Cy) for 17 patients and low-dose (≤1.5 g/m²) cyclophosphamide (LD-Cy) for 8 patients. Fifteen patients had pretreatment minimal residual disease (MRD; <5% blasts in bone marrow), and 10 patients had pretreatment morphologic evidence of disease (≥5% blasts in bone marrow). All toxicities were reversible, including severe cytokine release syndrome in 16% (4 of 25) and severe neurotoxicity in 28% (7 of 25) of patients. Treated patients were assessed for response, and, among the evaluable patients (n = 24), response and peak CAR T-cell expansion were superior in the HD-Cy/MRD cohorts, as compared with the LD-Cy/morphologic cohorts without an increase in toxicity. Our data support the safety of CD19-specific CAR T-cell therapy for R/R B-ALL. Our data also suggest that dose intensity of conditioning chemotherapy and minimal pretreatment disease burden have a positive impact on response without a negative effect on toxicity. This trial was registered at www.clinicaltrials.gov as #NCT01860937. (*Blood*. 2019;134(26):2361-2368)

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy occurring in children.[1] The implementation of risk-adapted multiagent chemotherapy has increased survival for pediatric patients with B-cell ALL (B-ALL) to ≥90%.[2,3] Despite this achievement, the need for prolonged treatment and the development of short- and long-term side effects complicate current therapy.[3] However, the outcome for pediatric/young adult patients with relapsed or refractory (R/R) B-ALL remains dismal.[4-6] Most notable is the poor prognosis of patients who relapse after an allogeneic hematopoietic stem cell transplant (allo-HSCT), experience an early bone marrow (BM) relapse (<18 months from time of initial complete remission), have ≥2 BM relapses, or respond poorly after reinduction chemotherapy.[4-6] Improved therapy for patients with R/R B-ALL is an unmet need and requires the investigation of novel therapies to increase current survival rates.

Chimeric antigen receptors (CARs) combine antigen recognition (typically through a single-chain variable fragment of a monoclonal antibody) coupled to an intracellular activation signal domain(s) of immune effectors such as T cells.[7] The clinical benefit of CD19-specific CAR T cells in both R/R B-ALL and R/R non–Hodgkin lymphoma (NHL) has now been reported by several groups, leading to approval by the US Food and Drug Administration (FDA) of CD19-specific CAR T cells for treatment of these diseases.[8-14] In these reports, factors correlating with response have included postinfusion CAR T-cell expansion and the addition of fludarabine to cyclophosphamide-based conditioning chemotherapy.[9-13] Herein, we report the results of our multicenter clinical trial detailing toxicity, feasibility, and response, using a Memorial Sloan Kettering Cancer Center (MSKCC)–derived, CD28-containing, second-generation, CD19-specific CAR that has shown clinical impact in adult patients with R/R B-ALL, but has not been demonstrated in a cohort of pediatric/young adult patients with R/R B-ALL.[8,13]

Patients and methods

Trial design and oversight

We conducted a phase 1 clinical study of CD19-specific CAR T cells in pediatric/young adult patients with R/R CD19$^+$ B-cell ALL. The study was conducted in the Departments of Pediatrics at MSKCC and the Dana-Farber Cancer Institute (DFCI)/Boston Children's Hospital Cancer and Blood Disorders Center. The protocol was approved by the respective institutional review boards. All clinical investigation was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all study participants or their legal guardians. Patients received CAR T-cell infusion from May 2013 through February 2017. Data cutoff for evaluation of outcome was 1 April 2019. The primary objective of the study was to assess the safety of CD19-specific CAR T cells; the secondary objectives were to assess the persistence of CAR T cells after infusion, including B-cell aplasia. The response was evaluated after infusion, including predictors of response for all patients.

Eligibility for T-cell collection (apheresis) included patients <26 years of age with very-high-risk B-ALL (including National Cancer Institute [NCI] HR-ALL and age ≥13 years at diagnosis, CNS-3 leukemia at diagnosis, day 29/end of induction BM minimal residual disease [MRD] >0.01%, induction failure [M3 BM at day 29 or end of induction], hypodiploidy [n < 44 chromosomes and/or a DNA index <0.81], t(9;22) ALL (Philadelphia chromosome/Ph+ALL) or Ph-like ALL t(17;19), MLL gene rearrangement, IKZF1 deletions, and intrachromosomal amplification of chromosome 21) and first or subsequent marrow relapse or refractory disease. Apheresis products collected at the participating cancer center (DFCI) were transported overnight to MSKCC by a temperature-controlled (4°C) method after addition of HypoThermosol FRS (BioLife Solutions, Bothell, WA) at a 1:1 ratio. After leukapheresis, patients received interim/bridging therapy at the discretion of their treating physician. CD19-specific CAR T cells were transduced, formulated, and released, as previously described.[15,16] We used a CD28-containing, second-generation CAR (termed 19-28z), as described previously.[17]

Patients qualified for infusion of 19-28z CAR T cells if they met 1 of the following criteria: ≥2 relapse, early (<18 months from initial complete response [CR]) BM relapse, intermediate/late relapse (first CR >18 months) with poor response (M2 marrow) after reinduction therapy, refractory disease, or ineligibility for allo-HSCT or additional chemotherapy (futility), per the treating physician. All patients treated had evidence of CD19$^+$ disease by morphology and/or flow cytometry, immediately before conditioning chemotherapy and CAR T cells.

Conditioning chemotherapy was administered 2 to 7 days before 19-28z CAR infusion. For the conduct of this study, we defined high-dose cyclophosphamide (HD-Cy) as a total dose of 3 g/m^2 (given as a single dose or split over 2 days) and low-dose cyclophosphamide (LD-Cy) as a total dose of ≤1.5 g/m^2 (given on a single day), which was adopted from MSKCC cyclophosphamide guidelines. After conditioning chemotherapy, all patients received the protocol-specified dose of 19-28z CAR T cells based on pretreatment disease burden, as previously described.[13]

Toxicity assessment

Cytokine release syndrome (CRS) was graded according to the NCI consensus CRS grading system, as previously described,[18] and according to the American Society for Transplantation and Cellular Therapy (ASTCT) consensus CRS grading.[19] Severe CRS was defined as grade 3 CRS or higher. Neurotoxicity was assessed according to NCI Common Terminology Criteria for Adverse Events (CTCAE), v4.03. Severe neurotoxicity was defined as any seizure or as grade 3 or higher toxicity of the nervous system. Adverse events were captured for all treated patients until disease relapse, administration of alternative therapy, or death.

Response assessment

Complete remission (CR) was defined as ≤5% BM blasts by morphology in the setting of a neutrophil count of ≥0.5 K/μL, platelet count of ≥75 000/μL, and no evidence of extramedullary disease. Complete remission with incomplete count recovery (CRi) was defined as CR in the setting of a neutrophil count of <0.5 K/μL or a platelet count of <75 000/μL without evidence of extramedullary disease. A negative status for MRD from BM samples was defined as <0.01% abnormal B cells (aberrant immunophenotypes) assessed by multiparameter flow cytometry performed at MSKCC for all study participants.[20] Relapse disease was defined as patients who met the above criteria for CR/CRi with subsequent development of recurrent morphologic BM or extramedullary disease.

Assessment of 19-28z CAR T-cell expansion

The presence of 19-28z CAR T cells was detected by polymerase chain reaction from peripheral blood, as previously described.[8,16] In brief, DNA was extracted from whole blood, and 200 ng was used in duplicate to amplify the vector and albumin gene. The results were converted to vector copy number (VCN) per milliliter, based on white blood cell counts.

Assessment of cytokine production and C-reactive protein

Measurement of serum cytokines was performed using the Luminex IS100 system and serum C-reactive protein level, as previously described.[8]

Statistical analysis

Fisher's exact test was used to evaluate the association between the end points (complete response, CRS, and neurologic toxicity) and discrete clinical factors: cyclophosphamide dose, fludarabine treatment, pretreatment disease burden, and target dose of CAR T cells. For the continuous clinical factors (total T-cell dose and ex vivo expansion), the Wald test from the logistic regression model was applied when the end point was complete response, and the Wald test from the ordinal regression model was used when the end points were CRS and neurologic toxicity. The Wilcoxon rank sum statistic was used to evaluate all other comparisons. All analyses were considered exploratory, and no adjustment for multiple comparisons was used.

Results

Patients

Between 27 May 2013 and 1 January 2017, 49 pediatric/young adult patients with relapse/ B-ALL were enrolled in the protocol, and 23 patients with R/R B-ALL were treated with 19-28z

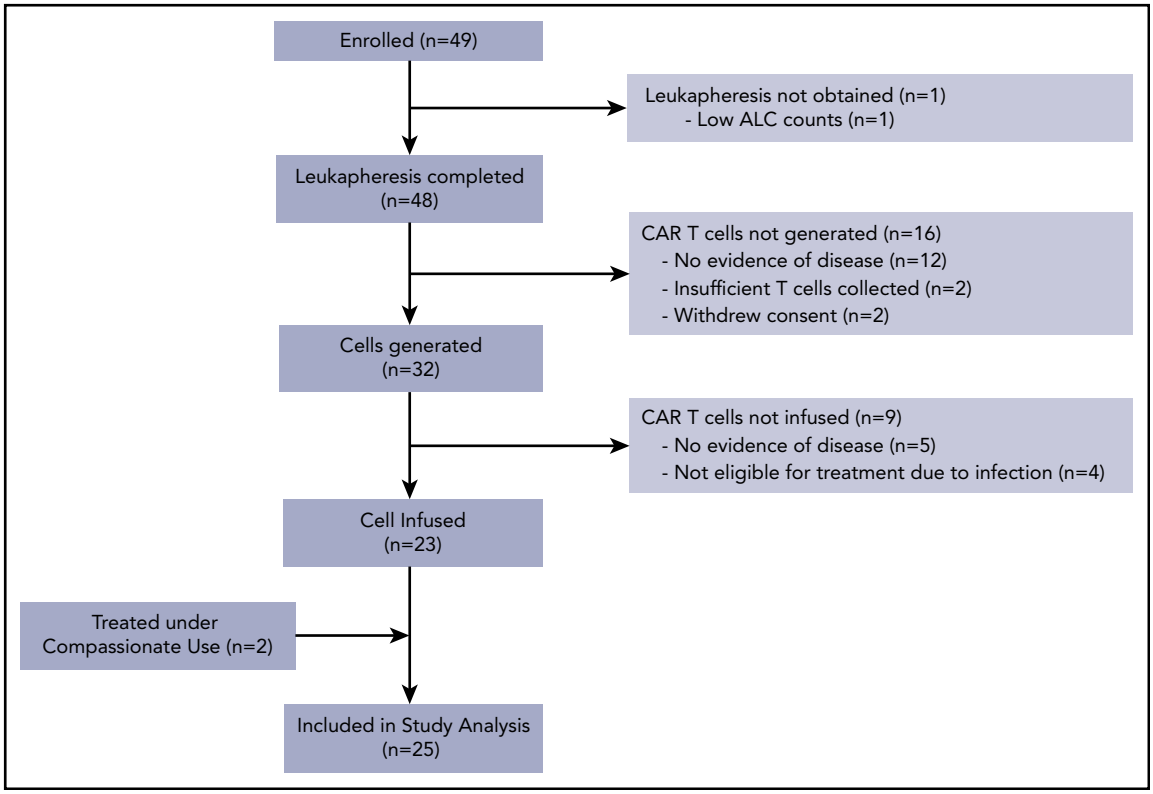

Figure 1. Study flow. Study course for participants from the time of enrollment to treatment.

CAR T cells. DFCI enrolled 11 patients and treated 4. Before April 2014, patients with R/R B-ALL after allo-HSCT were excluded from enrollment in the protocol. After that date, the protocol was amended to allow patients with relapse after allo-HSCT. Two patients (included in this analysis) with relapsed B-ALL after allo-HSCT were treated on a compassionate basis with FDA approval before this protocol amendment. Figure 1 shows a detailed flow diagram of all participants' study course, and supplemental Figure 1 (available on the *Blood* Web site) shows the detailed evolution of this trial with respect to cyclophosphamide dosing and CAR T-cell dosing.

Baseline characteristics of patients who received treatment with CD19-specific CAR T cells are shown in Table 1. High-risk features were found in 76% (19 of 25) of patients treated. Treatment with CAR T cells occurred for 47% (23 of 49) of the enrolled patients. One patient received CD19-targeted immunotherapy (denintuzumab mafodotin) before collection and treatment with CAR T cells. As noted, patients received interim/bridging therapy after leukapheresis at the discretion of their treating physician. Treatment with CAR T cells was not offered to any patient who achieved MRD^- CR after bridging chemotherapy (chemotherapy-sensitive disease; n = 17) including those patients with manufactured CAR T cells (n = 5). Bridging therapy for those who received CAR T cells included high-intensity chemotherapy (n = 21; clofarabine-based [n = 5], high-dose cytarabine-based [n = 7], multiagent induction/consolidation [n = 7], cyclophosphamide/etoposide [n = 2]) or low-intensity chemotherapy (n = 4; vincristine/prednisone/PEG-asparaginase [n = 2], vincristine/prednisone/etoposide [n = 1], or single-agent etoposide [n = 1]). All patients had detectable BM disease before initiation of treatment (conditioning chemotherapy and CAR T cells).

Seventeen patients received HD-Cy (3 g/m^2), and 8 patients received LD-Cy (≤1.5 g/m^2). Fludarabine (25 mg/m^2 per day for 3 days) was also used in 6 patients (3 in the LD-Cy arm and 3 in the HD-Cy arm). Dose intensification of preconditioning chemotherapy using cyclophosphamide (HD-Cy) was used in all patients in the study after review of superior response rates in

Table 1. Baseline characteristics

Characteristic	Patients (n = 25)
Age, median (range), y	13.5 (1-22.5)
Risk category, n (%)	
Relapse following allo-HSCT	5 (20)
Early BM relapse (<18 mo)	7 (28)
Intermediate BM relapse (18-36 mo)	2 (8)
Late BM relapse (> 36 mo)	4 (16)
≥2 BM relapse	1 (4)
Refractory	2 (8)
Poor response, with salvage chemotherapy	4 (16)
BM blasts, median (range), %	4 (1-98)
Distribution	
<5% (pretreatment MRD cohort)	15 (60%)
≥5% (pretreatment morphologic cohort)	10 (40%)
Dose intensity of conditioning chemotherapy, n (%)	
HD-Cy	17 (68)
LD-Cy	8 (32)

Characteristics of patients who underwent treatment with CD19-specific CAR T cells.

Table 2. CAR T-cell–associated adverse events

Event	Any, n (%)	Severe (grade 3/4) n (%)
CRS	20 (80)	4 (16)
Tachycardia	15 (60)	0
Hypertension	5 (20)	1 (4)
Cardiac dysfunction/arrhythmia	1 (4)	1 (4)
Cytopenia		
Neutropenia	3 (12)	3 (12)
Thrombocytopenia	4 (16)	4 (16)
Infection	9 (36)	6 (24)
CAR T-cell–associated neurotoxicity	18 (72)	7 (28)
Headache	5 (20)	1 (8)
Tremor or hyperreflexia/clonus	7 (28)	0
Altered mental status*	12 (48)	5 (20)
Seizure (clinical or abnormal EEG)†	5 (20)	5 (20)
Involuntary movements	1 (8)	0
Ataxia	1 (8)	0

Adverse events were captured for all treated patients (n = 25) until disease relapse, application of alternative therapy, or death. CRS was graded according to the NCI consensus CRS grading system. CRS symptoms include fever, which occurred in 68% (17/25), hypotension in 44% (11/25), and hypoxia in 12% (3/25) of patients. ASTCT CRS consensus grading (fever a required element) occurred in 68% (17/25) of patients, including a sCRS rate of 16% (4/25) of patients. CAR T-cell–associated neurotoxicity included any neurologic sequalae following the infusion of CAR T cells. EEG, electroencephalogram.

*Altered mental status included somnolence, depressed level of consciousness, confusion, slurred speech, cognitive disturbance, delirium, and/or personality change.

†Seizure included clinically apparent seizure or electroencephalogram findings consistent with seizure.

a concurrent study of adult patients with R/R B-ALL (www.clinicaltrails.gov #NCT01044069) who received HD-Cy and 19-28z CAR T cells compared with the suboptimal response rates in the initial 8 patients treated with LD-Cy (supplemental Figure 1).[8] Fifteen patients had pretreatment MRD (<5% blasts in BM), and 10 patients had pretreatment morphologic evidence of disease (≥5% blasts in BM), as assessed by BM morphology or flow cytometry before conditioning chemotherapy.

CAR T-cell manufacturing

Eligibility criteria for the study did not exclude patients based on absolute lymphocyte count (ALC), circulating blasts, or predefined T-cell proliferation assay during CAR T-cell manufacturing. Median ALC or peripheral blood $CD3^+$ count at the time of leukapheresis was 0.9 K/μL (range, 0.2–2.7 K/μL; n = 42). A sufficient number of T cells was not collected from 2 patients (precollection ALC, 1 K/μL and 0.2 K/μL; 1.4×10^6 and 10.4×10^6 total T cells collected, respectively), and they were removed from the study, as CAR T-cell manufacturing was not feasible with this starting number of T cells (Figure 1). Production was not initiated for an additional 14 patients (12 achieved MRD^- CR after reinduction chemotherapy, and 2 patients withdrew consent; Figure 1). One patient's apheresis was delayed by the treating physician's preference; this patient ultimately achieved a CR after chemotherapy, and collection was permanently suspended.

In this heavily pretreated patient population, the protocol-specified CAR T-cell dose was successfully produced in 100% (32 of 32) of patients who underwent production, including all patients with cells collected and manufactured at DFCI (n = 4). Mean γ-retroviral 19-28z CAR gene transfer efficiency was 24.1% (range, 7.9%-61.4%; n = 32) for all products manufactured.

Toxicity

The most common treatment-related adverse events were CRS and neurotoxicity, consistent with previously reported findings.[8-11,14,21] Grade 3/4 toxicity occurred in 32% (8 of 25) of patients. CRS most commonly clinically manifested as high persistent fever, tachycardia, hypotension, respiratory distress, and hypoxia. Neurological adverse events included confusion, disorientation, word-finding difficulties, depressed level of consciousness, encephalopathy, and seizure. CAR T-cell–associated adverse events are listed in Table 2 and supplemental Table 1.

CRS was graded according to the NCI consensus grading system.[18] CRS of any grade occurred in 80% of patients (20 of 25) with severe CRS (sCRS; grades 3 and 4) occurring in 16% (4 of 25) of patients treated (Table 2). sCRS occurred in both HD-Cy (12%; 2 of 17) and LD-Cy (25%; 2 of 8) cohorts; in pretreatment morphologic (30%; 3 of 10) and MRD (7%; 1 of 15) cohorts; with severe neurotoxicity (43%; 3 of 7) and without (6%, 1 of 18). An alternate CRS grading system (ASTCT CRS consensus grading[19]) which requires fever to be present, demonstrated an overall CRS rate of 68% (17 of 25) and an sCRS rate of 16% (4 of 25). Disease response (CR/CRi) was seen in 3 of 3 evaluable patients with sCRS. Dose intensity of cyclophosphamide, pretreatment disease burden, and several other predictors, including total T cells, target CAR T cells, and fold expansion of CAR T cells during manufacturing, did not correlate with CRS (supplemental Table 2). One patient with sCRS experienced

Table 3. Response to CD19-specific CAR T cells

Characteristic	Patients, % (n = 24)
Complete response/complete response with incomplete count recovery (CR/CRi) (n)	75 (18 of 24)
MRD^- (in the CR cohort)	89 (16 of 18)
Response/conditioning chemotherapy (n)	
Cyclophosphamide	
HD-Cy	94 (15 of 16)
LD-Cy	38 (3 of 8)
Fludarabine	
With fludarabine	83 (5 of 6)
Without fludarabine	72 (13 of 18)
Response/pretreatment disease burden	
Morphologic disease (≥5% BM blasts)	50 (5 of 10)
MRD (<5% BM blasts)	93 (13 of 14)

Disease response for all patient treated and surviving more than 28 days after infusion (n = 24), MRD absence (MRD^-) in patients who achieved response (n = 18), cohort response based on dose intensity of conditioning chemotherapy (HD-Cy vs LD-Cy; $P = .01$), and cohort response based on pretreatment disease burden (morphologic disease vs MRD; $P = .05$).

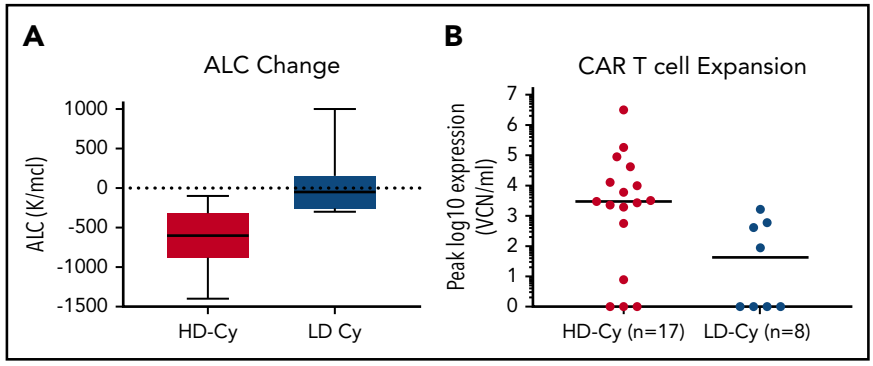

Figure 2. Dose intensity of cyclophosphamide impacts lymphodepletion and CAR T-cell expansion. (A) ALC change before and after HD-Cy and LD-Cy before treatment with CAR T cells (n = 23) demonstrates more significant lymphodepletion in the HD-Cy cohort ($P < .001$). (B) In vivo CAR T-cell expansion (peak CAR T-cell VCN per milliliter) in peripheral blood was greater in the HD-Cy cohort as compared with the LD-Cy cohort ($P = .01$).

reversible grade 4 cardiac dysfunction and arrhythmia, which has been reported in another CAR T-cell clinical trial.[10] As previously reported, several biomarkers correlated with severity of CRS, including serum C-reactive protein and serum interleukin-6 (IL-6) levels; however, peripheral blood in vivo CAR T-cell expansion (peak CAR T-cell VCN per milliliter) did not correlate with severe CRS (supplemental Figure 2).[8-10]

CRS was managed according to protocol-specific guidelines, as previously published.[13] Most patients with CRS (70%; 14 of 20) were given supportive care alone (antipyretics, intravenous fluid, and low-flow supplementary oxygen via nasal canula). Two patients required vasopressors to manage hypotension, 4 patients required the anti-IL-6 receptor blocking antibody tocilizumab following development of end-organ dysfunction (hypoxia/respiratory distress and cardiac dysfunction), and 1 required systemic corticosteroids for end-organ dysfunction not responsive to IL-6 receptor blockade (global ventricular dysfunction/decreased ventricular ejection fraction). One patient died of refractory *Stenotrophomonas* septic shock on day +17 of treatment after grade 4 CRS and neurotoxicity that was clinically improving. CRS was reversible in the remaining cases, and, in those patients treated with immunosuppressive agents, response was rapid.

Neurologic symptoms of any grade occurred in 72% (18 of 25) of subjects, with severe neurotoxicity (grades 3 and 4 or seizure) occurring in 28% (7 of 25) of subjects (Table 2). Severe neurotoxicity occurred in both the HD-Cy (29%; 5 of 17) and LD-Cy (25%; 2 of 8) cohorts, in the pretreatment morphologic (50%; 5 of 10) and MRD (13%; 2 of 15) cohorts, and those with (75%, 3/4) and without (19%, 4 of 21) sCRS. Disease response (CR/CRi) was seen in 6 of 6 evaluable patients with severe neurotoxicity. One patient (HD-Cy/MRD cohort) with severe neurotoxicity had radiographic evidence of diffuse cerebral edema after presenting with fever (day +3; grade 1 CRS), right-side hemiparesis, and seizure (day +5; on prophylactic levetiracetam). Intensive care unit management included high-dose corticosteroids (dexamethasone 10 mg IV, every 6 hours; increased levetiracetam dosage; and addition of valproic acid), hypertonic saline (serum sodium goal, >140 mEq/L), and rapid cerebral spinal fluid diversion by lumbar puncture (opening pressure, 30-35 cm H_2O). Neurologic toxicity improved with these interventions, with clinical improvement by day +7, near normalization of magnetic resonance imaging and discontinuation of corticosteroids by day +9, and discharge to outpatient management by day +14. Cyclophosphamide dose, pretreatment disease burden, and peripheral blood in vivo peak CAR T-cell expansion did not significantly correlate with neurotoxicity (supplemental Table 2; supplemental Figure 2).

CAR T-cell–associated neurotoxicity was managed according to protocol-specific guidelines, as previously published.[13] The majority of patients with neurotoxicity (78%; 14 of 18) were given supportive care alone (observation and frequent neurologic examinations) including prophylactic levetiracetam in 92% (23 of 25) of the patients. Four patients required systemic corticosteroids for severe neurologic toxicity (seizure). Neurotoxicity was reversible in all patients, without evidence of long-term neurologic deficits and rapidly responded to immunosuppressive agents when required.

Response rates

Twenty-four of 25 patients survived for more than 28 days after CAR T-cell infusion and were evaluable for response assessment by BM evaluations (Table 3). Data from a patient who died of refractory *Stenotrophomonas* septic shock were not included in the efficacy analysis but were assessed for all other parts of the

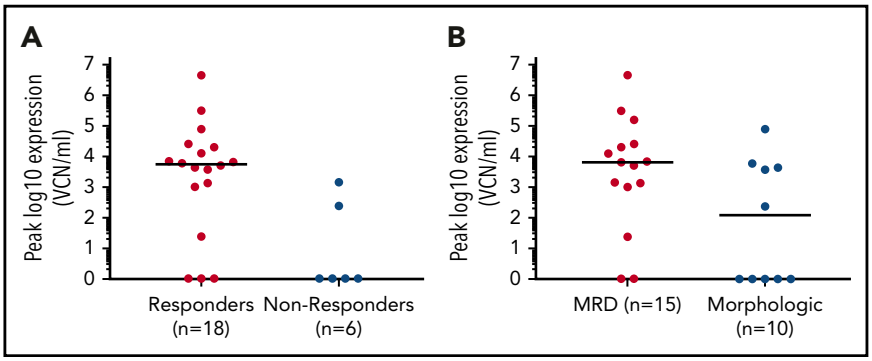

Figure 3. Disease response and pretreatment disease burden impact CAR T-cell expansion. In vivo CAR T-cell expansion (peak CAR T-cell VCN per milliliter) in peripheral blood was also higher in patients with disease response (responders), compared with nonresponders ($P = .01$) (A), and in patients with MRD, compared with morphologic pretreatment disease burden ($P = .05$) (B).

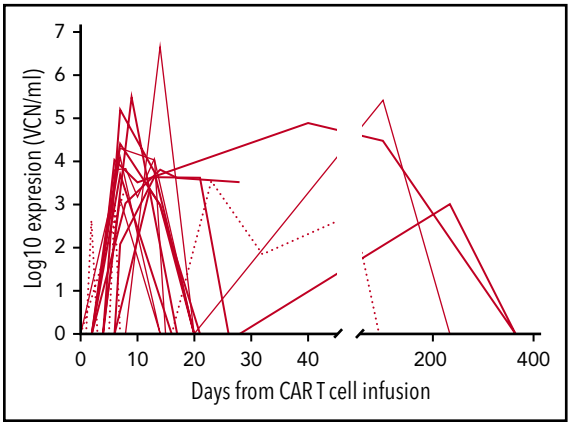

Figure 4. CAR T-cell detection. CAR T-cell detection in peripheral blood by PCR (VCN per milliliter) including the HD-Cy cohort (solid line; 14/17 patients detected for at least 1 time point measured) and LD-Cy (dotted line, 4 of 8 patients detected for at least 1 time point measured). Detection of CAR T cells following allo-HSCT was not standardized. However, 14 patients had samples taken after allo-HSCT with 4 patients demonstrating 1 positive sample each (median, 121 days after allo-HSCT; range, 44-195 days) without subsequent positive test.

study. The postmortem analysis of this patient did not demonstrate evidence of leukemia. Of the 24 patients evaluable for response assessment, overall CR/CR with incomplete count recovery (CRi) rate was 75% (18 of 24) with subset CR/CRi rates of 94% (15 of 16) and 38% (3 of 8) in the HD-Cy and LD-Cy cohorts, respectively, and 93% (13/14) and 50% (5/10) in the pretreatment MRD and morphologic cohorts, respectively (Table 3). Combined response for HD-Cy/MRD was 100% (12 of 12), HD-Cy/morphologic 75% (3 of 4), LD-Cy/MRD 50% (1 of 2), and LD-Cy/morphologic 33% (2 of 6). Several predictors did not correlate with response, including total T cells, target CAR T cells, fold expansion of CAR T cells during manufacturing, and in vitro cytotoxicity (supplemental table 3). In comparison, response rates for all patients with manufactured products (n = 32) and all patient with manufactured products who were eligible for infusion (chemotherapy refractory disease; n = 27) demonstrated a CR/CRi rate of 56% (18 of 32) and 67% (18 of 28), respectively.

The absence of MRD (MRD$^-$) was assessed by multiparameter flow cytometry for all responders and was achieved by 89% of responding patients (16 of 18; Table 3). Posttreatment MRD$^-$ status in responding patients included 93% (14 of 16) in the HD-Cy cohort, 66% (2 of 3) in the LD-Cy cohort, 92% (12 of 13) in the pretreatment MRD cohort, and 80% (4 of 5) in the pretreatment morphologic cohort.

Biomarkers of response

ALC before and after cyclophosphamide (ALC change) was available for 23 patients. Peripheral blood lymphodepletion (ALC change) was significantly greater in the HD-Cy cohort than in the LD-Cy cohort ($P < .001$; Figure 2). Mean absolute ALC was 0.2 K/μL (range, 0-0.5 K/μL) and 0.35 K/μL (range, 0-1.8 K/ μL) in the HD-Cy and LD-Cy cohorts, respectively. Differences in the in vivo CAR T-cell expansion (peak CAR T-cell VCN per milliliter) in peripheral blood were also significantly higher in the HD-Cy cohort, as compared with the LD-Cy cohort ($P = .01$; Figure 2). CAR T-cell expansion in vivo was also higher in responders ($P = .01$) and pretreatment MRD ($P = .05$) cohorts, than in the nonresponder and pretreatment morphologic cohorts, respectively (Figure 3). The median duration of CAR T-cell detection for the entire treated cohort was 7 days (range, 0-234; Figure 4). Median CAR T-cell detection was 13 days (range, 0-234) vs 1 day (range, 0-23) in the HD-Cy and LD-Cy cohorts, respectively. Median CAR T-cell detection was 8 days (range, 0-234) vs 2 days (range, 0-101) in the pretreatment MRD and morphologic cohorts, respectively. Twenty-three patients had available serum cytokines on day 0 (pre-CAR T-cell infusion) and a comparison between the HD-Cy and LD-Cy cohorts demonstrated significantly higher levels of EGF (median, 394 pg/mL vs 74 pg/mL; $P = .01$), Flt-3L (median, 62 pg/mL vs 3 pg/mL; $P = .01$), sCD40L (median, 6338 pg/mL vs 632 pg/mL; $P = .01$), and IL-3 (2 pg/mL vs 0 pg/mL; $P = .02$), whereas IL-10 (median, 11 pg/mL vs 46 pg/mL; $P = .005$) was lower.

Treatment post-CAR T cells and overall survival

Of the 18 responding patients, 83% (15 of 18) underwent consolidation with allo-HSCT as the standard of care for R/R B-ALL in a primarily allo-HSCT–naive cohort. Median time from CAR T-cell infusion until allo-HSCT infusion was 57 days (range, 30-200). After allo-HSCT, 2 patients died of subsequent CD19$^+$ disease relapse, 2 died after development of multiorgan veno-occlusive disorder, and 1 died of complications of graft-versus-host disease. Two additional patients developed a CD19$^+$ relapse (17.5 and 25.7 months after CAR T cells) of which 1 remains alive and disease free after salvage therapy and 1 remains alive and is undergoing salvage therapy. CD19$^-$ disease relapse was not seen in any patient treated in this cohort. Eight patients remain alive and disease free after infusion of CAR T cells consolidated with allo-HSCT. The 3 remaining responding patients who did not undergo allo-HSCT because of organ dysfunction (n = 1) and detectable disease (MRD$^+$; n = 2) ultimately died. Overall survival of combined dose intensity and pretreatment disease burden is shown in Figure 5 and individual cohorts in supplemental Figure 3.

Discussion

In this multicenter clinical trial of CD19-specific CAR T cells in pediatric/young adult patients with R/R CD19$^+$ B-ALL, we report the results of 25 treated patients with a median follow-up of 7.7 months (range, 0.5-43.7 months) and a median follow-up for responding patients (n = 18) of 28.6 months (range, 1.8-43.7). This analysis has allowed us to determine the toxicity profile, confirm feasibility, evaluate the response to this approach, and provide a direct comparison of the same CD19-specific CAR T-cell product that was previously published in adult patients for the same indication.[8,13]

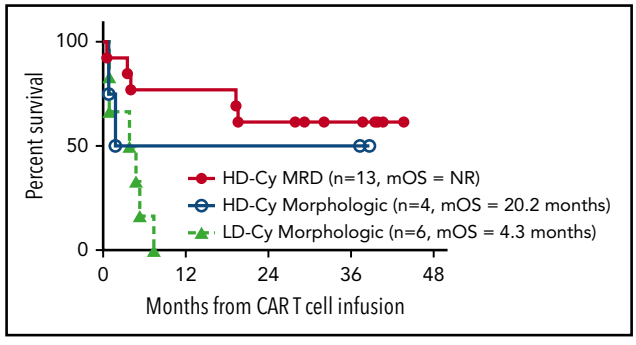

Figure 5. Overall survival of combined dose intensity and pretreatment disease burden. Low-dose Cy/MRD cohort (n = 2) not shown (1 of 2 patients alive). mOS, median overall survival; NR, not reached.

The major clinical limitation to the widespread implementation of CAR T-cell therapy, particularly as part of front-line therapy, is the high rate of toxicity after infusion. Currently, the 2 FDA-approved therapies require specialized treatment centers to adhere to FDA-mandated Risk Evaluation and Mitigation Strategies programs. Rates of severe CRS (grades 3 and 4) have ranged from 23% to 46% in pediatric R/R B-ALL and adult NHL populations, respectively.[9,10,12,22] The low incidence of sCRS (16%) in this study could be attributed to the low tumor burden status of the majority of patients, as pretreatment disease burden has been clearly shown to correlate with sCRS.[8-10] This is in contrast with the most recent published rate of 46% sCRS after the FDA approved tisagenlecleucel (CD19-specific 4-1BB CAR T cells) in pediatric/young adult patients with R/R B-ALL.[22] In the present study, 28% of patients experienced severe neurotoxicity, which is comparable to previous reports but higher than the pivotal tisagenlecleucel study.[8,9,11,12,21,22] The reversible toxicity profile within the present study confirms the tolerability of this approach. with the majority of patients (70% for CRS and 78% for neurotoxicity) only requiring postinfusion supportive care. Importantly, incidence of CAR-associated toxicity did not increase with the use of more dose-intense (HD-Cy) conditioning chemotherapy in this cohort. Further studies should prospectively evaluate any negative impact on the toxicity profile when higher intensity preconditioning chemotherapy is used.

In this cohort, we demonstrated a response rate of 94% (88% MRD^- response) in our HD-Cy cohort, without a negative impact on the toxicity profile. In contrast, the use of LD-Cy (900 mg/m^2) and fludarabine have demonstrated an overall response rate of 70% (60% MRD^- response) in a previous study of pediatric/young adult patients with R/R B-ALL.[10] In comparison, a global multicenter study using the FDA-approved tisagenlecleucel preceded by LD-Cy (500 mg/m^2 $\times$ 2 days) and fludarabine demonstrated a response rate of 81% at 3 months in treated patients.[22] Dose intensification of cyclophosphamide has had mixed results in adult patients with R/R NHL, with 1 study demonstrating unfavorable (increased toxicity; CD19-specific, CD28-containing CAR T cells) or favorable (improved preinfusion cytokine profile/progression-free survival; CD19-specific, BB-containing CAR T cells) outcomes.[23,24] Dose intensification with the addition of fludarabine has also been demonstrated to influence CAR T-cell kinetics (peak expansion/persistence) and efficacy and to reduce cellular-mediated rejection of CAR T cells.[11,12] The impact of fludarabine in this cohort could not be assessed because of the low number of patients (n = 6) receiving this agent.

The mechanisms by which HD-Cy and minimal pretreatment disease burden contribute to response is not determined in this study and remains speculative. The more profound lymphodepletion (reduction of ALC) was associated with HD-Cy compared with LD-Cy in this study (Figure 2) and is an expected finding after higher total exposure to cyclophosphamide. More profound lymphodepletion could result in a reduction in immune-mediated suppression or cell-mediated rejection of CAR T cells, thereby translating into improved response. Reduced immune-mediated suppression could also account for the higher peak CAR T-cell expansion (Figure 2) seen in this cohort; however, this finding must be further investigated, as risk-adapted CAR T-cell dosing based on pretreatment burden was adopted in this study, as previously published.[13,21] Similar impact on immune-mediated suppression has been demonstrated with addition of fludarabine to preconditioning chemotherapy.[11,12] T-cell differentiation has been implicated in the demise of circulating CAR T cells, and, in the setting of high antigen exposure (high pretreatment disease burden or lower conditioning intensity), T-cell exhaustion could result in less CAR T-cell expansion.[14] Irrespective of mechanism, the contribution of dose-intense cyclophosphamide is evident by improved response and survival in this cohort of patients. Minimal pretreatment disease burden has been shown by our group to impact durability of response and survival in a cohort of adult patients with R/R B-ALL.[13] The cohort in the present study provided additional evidence as to the positive impact of minimal pretreatment disease burden on CAR T-cell response. Prospective studies to discern the optimal intensity of conditioning chemotherapy and pretreatment disease burden are warranted to define maximal efficacy and tolerability of this therapy.

In conclusion, we found a reversible toxicity profile for pediatric/young adult patients with R/R B-ALL treated with CAR T-cell therapy. Dose intensity of preconditioning chemotherapy and minimal pretreatment disease burden have an impact on response without reducing tolerability. Intention to treat was not included in the study design and therefore was not evaluated in this cohort. In addition, the need for consolidative allo-HSCT is not defined in this cohort of primarily allo-HSCT–naive patients, and analysis of overall survival was limited because the majority of responding patients proceeded to consolidation with allo-HSCT (CAR T cells as a bridge to allo-HSCT). In contrast, CAR T cells have been shown to provide durable remission in the absence of consolidative allo-HSCT for a subset of patients (59% relapse-free survival at 12 months for responding patients).[22] The need for consolidative allo-HSCT warrants further investigation and is influenced by prior history of allo-HSCT, available donor options, recovery from post-CAR T-cell toxicity, and persistence of CAR T-cell activity. Within this cohort, the long-term persistence of response is encouraging, and in our primarily transplant-naive patient population, the ability to proceed to allo-HSCT has demonstrated a favorable overall survival, manageable toxicity, and limited incidence of relapse.

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data for this report. Only the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Authorship

Contribution: K.J.C., N.A.K., N.S., R.K., C.J.F., P.S., S.P., F.B., Y.K., B. Spitzer, M.I.C., R.J.O., S.P.M., L.B.S., D.A.W., J.H.P., C.S.S., M.S., and R.J.B. designed the research; K.J.C., N.A.K., N.S., R.K., C.J.F., P.S., S.P., Y.K., F.B., B. Spitzer, M.I.C., R.J.O., S.P.M., L.B.S., D.A.W., A.L.K., V.S., J.H.P., C.S.S., M.S., and R.J.B. performed research (treated patients/collected data); G.H., X.W., B. Senechal, I.R., M.S., J.J.B., R.J.O., and R.J.B. contributed vital new reagents or analytical tools; K.J.C., R.J.B., R.J.O., J.J.B., G.H., X.W., B. Senechal, M.S., I.R., J.H.P., and C.S.S. analyzed the data and made the figures; K.J.C. wrote the manuscript; and all authors critically reviewed the manuscript and approved the content.

Conflict-of-interest disclosure: K.J.C. has received research support from Juno Therapeutics and Novartis and has consulted, served on advisory boards, and participated in educational seminars for Juno Therapeutics, Novartis, Gerson Lehrman Group (GLG), Geurson Medical Group, AXIS Education, and Omnicron Healthcare. S.P.M. has participated on advisory boards for Novartis. R.J.B., M.S., and I.R. are cofounders and receive royalties from Juno Therapeutics. C.S.S. has received research support from Juno Therapeutics and has consulted for or served on advisory boards of Juno Therapeutics, Kite, and Novartis. The remaining authors declare no competing financial interests.

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Footnotes

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The protocol summary, a statistical summary, and informed consent form will be made available on clinicaltrials.gov when required as a condition of Federal awards, other agreements supporting the research, and/or as otherwise required. Requests for deidentified individual participant data can be made beginning 12 months after publication and for up to 36 months after publication. Deidentified individual participant data reported in the manuscript will be shared under the terms of a Data Use Agreement and may only be used for approved proposals. Requests may be made to crdatashare@mskcc.org. Requests should contain adequate information on the type of analysis to be performed and full listing of parties that will have access through this request.

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

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