original reports

Long-Term Follow-Up of CD19-CAR T-Cell Therapy in Children and Young Adults With B-ALL

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abstract

PURPOSE CD19 chimeric antigen receptor (CD19-CAR) T cells induce high response rates in children and young adults (CAYAs) with B-cell acute lymphoblastic leukemia (B-ALL), but relapse rates are high. The role for allogeneic hematopoietic stem-cell transplant (alloHSCT) following CD19-CAR T-cell therapy to improve long-term outcomes in CAYAs has not been examined.

METHODS We conducted a phase I trial of autologous CD19.28ζ-CAR T cells in CAYAs with relapsed or refractory B-ALL. Response and long-term clinical outcomes were assessed in relation to disease and treatment variables.

RESULTS Fifty CAYAs with B-ALL were treated (median age, 13.5 years; range, 4.3-30.4). Thirty-one (62.0%) patients achieved a complete remission (CR), 28 (90.3%) of whom were minimal residual disease−negative by flow cytometry. Utilization of fludarabine/cyclophosphamide–based lymphodepletion was associated with improved CR rates (29/42, 69%) compared with non–fludarabine/cyclophosphamide–based lymphodepletion (2/8, 25%; $P = .041$). With median follow-up of 4.8 years, median overall survival was 10.5 months (95% CI, 6.3 to 29.2 months). Twenty-one of 28 (75.0%) patients achieving a minimal residual disease−negative CR proceeded to alloHSCT. For those proceeding to alloHSCT, median overall survival was 70.2 months (95% CI, 10.4 months to not estimable). The cumulative incidence of relapse after alloHSCT was 9.5% (95% CI, 1.5 to 26.8) at 24 months; 5-year EFS following alloHSCT was 61.9% (95% CI, 38.1 to 78.8).

CONCLUSION We provide the longest follow-up in CAYAs with B-ALL after CD19-CAR T-cell therapy reported to date and demonstrate that sequential therapy with CD19.28ζ-CAR T cells followed by alloHSCT can mediate durable disease control in a sizable fraction of CAYAs with relapsed or refractory B-ALL (ClinicalTrials.gov identifier: NCT01593696).

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INTRODUCTION

CD19 chimeric antigen receptor (CAR) T cells (CD19-CAR) have demonstrated impressive early response rates in B-cell acute lymphoblastic leukemia (B-ALL),[1-6] but limited long-term follow-up data are available. In a global phase II trial of tisagenlecleucel in children and young adults (CAYAs) with relapsed or refractory B-ALL, a complete remission (CR) rate of 82% was achieved by day 28 postinfusion. However, with limited median follow-up of 13.1 months, only 59% of these patients remained in remission, the majority relapsing with CD19-negative disease.[2,7] Allogeneic hematopoietic stem-cell transplantation (alloHSCT) improves long-term disease-free survival in patients with relapsed or refractory B-ALL rendered into remission with chemotherapy,[8,9] but the role for consolidative alloHSCT in patients following CD19-CAR remains unclear[1,3,10] and clinical practice varies.

Following our initial report of a phase I dose-escalation trial of autologous CD19.28ζ-CAR T cells in 20 CAYAs with relapsed or refractory B-ALL,[1] we treated 30 additional patients in an expansion phase, which included treatment of active CNS disease and incorporated intensified lymphodepletion strategies to test the hypothesis that alternative chemotherapy regimens could more effectively reduce disease burden, thereby reducing cytokine release syndrome (CRS) severity, and potentially improve response to CD19-CAR. With a median follow-up of 4.8 years, we report long-term outcomes of 50 CAYAs with B-ALL who received autologous CD19.28ζ-CAR T cells. Additionally, a sizable fraction of patients received alloHSCT following CD19-CAR, providing an opportunity to evaluate the role of alloHSCT in CAYAs following CD19-CAR.

METHODS

Patients and Objectives

This was a single-center, phase I dose-escalation study of CD19.28ζ-CAR T cells for CAYAs (age 3-30 years) conducted in the Pediatric Oncology Branch of the National Cancer Institute (NCI). The Protocol

ASSOCIATED CONTENT

Data Supplement
Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

What is the role of consolidative hematopoietic stem-cell transplant (HSCT) following CD19-targeted CAR T cells for long-term remission in children and young adults (CAYAs) with relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL)?

Knowledge Generated

Our article reports long-term outcomes of 50 CAYAs treated with CD19-CAR T cells for B-ALL. We demonstrate that CAYAs receiving post-CAR HSCT were associated with long-term disease-free survival and a low risk of post-HSCT relapse. Additionally, we provide data supporting the superiority of fludarabine/cyclophosphamide for lymphodepletion over alternative regimens. We also show the feasibility and efficacy of CD19-CAR T cells in the treatment of active CNS disease.

Relevance

We demonstrate durable relapse-free survival in those who underwent post-CAR HSCT, providing evidence that HSCT is important for maintaining remissions in CAYAs receiving CD19-CAR T cells for B-ALL. Our data additionally support treatment of active CNS disease, expanding the therapeutic index for CAR T cells.

(online only) was approved by the NCI Institutional Review Board and registered at ClinicalTrials.gov identifier: NCT01593696. This report incorporates data from all patients with B-ALL who received CD19.28ζ-CAR T cells on-study from July 2012 through March 2016 with a data cutoff of October 1, 2019. See the Data Supplement, online only for additional details.

The primary objective of the phase I trial was to define the maximum tolerated dose of CD19-CAR T cells, to describe the toxicity of the regimen, and to determine the feasibility of generating CD19-CAR T cells in this population, which has been previously reported.[1] The trial was subsequently amended to add additional objectives, which specifically included determining the safety of administering cells in two groups of patients with CD19+ disease, stratified by high- and low-burden disease, and to evaluate the impact of alternative lymphodepleting regimens on toxicity and outcomes. Secondary objectives sought to define response rate; to measure expansion and persistence of CD19-CAR T cells in the peripheral blood, bone marrow, and CSF; to describe the toxicity of administration of CD19-CAR T cells in those with CNS disease; and to identify biological correlates of clinical outcomes and toxicity including cytokine values. The impact of alloHSCT on survival was retrospectively analyzed on a separate NCI Institutional Review Board–approved protocol evaluating CAR T-cell–related outcomes (ClinicalTrials.gov identifier: NCT03827343).

Study Design

CAR T-cell manufacturing was done as previously described (Data Supplement).[1] Two dose levels were utilized: 1×10^6 transduced CAR T-cells/kg once on day 0 (DL1), and 3×10^6 transduced CAR T-cells/kg once on day 0 (DL2). For the initial 21 patients, pre-CAR T-cell lymphodepletion consisted of standard low-dose fludarabine (25 mg/m^2 × 3 days) and cyclophosphamide (900 mg/m^2 × 1 day) administered on days −4 to −2 (LD-Flu/Cy). From patient 22 onward, with a goal of reducing disease burden prior to proceeding with CAR T-cell infusion (day 0), those with high-burden disease (defined by ≥ 25% bone marrow blasts, circulating peripheral blasts, or lymphomatous disease) received one of the three alternative regimens used: (1) HD-Flu/Cy, high-dose fludarabine (30 mg/m^2 once a day × 4 days) and cyclophosphamide (1,200 mg/m^2 once a day × 2 days) from days −5 to −2; (2) FLAG, fludarabine (25 mg/m^2 × 5 days) and cytarabine (2,000 mg/m^2/day × 5 days) (days −6 to −2) with filgrastim (5 μg/kg/dose daily) starting on day −7; and (3) Ifos/Etop, ifosfamide (1,800 mg/m^2 once a day × 5 days; with MESNA) and etoposide (100 mg/m^2 once a day × 5 days) (days −6 to −2).

Toxicity and Efficacy Evaluations

Adverse events were captured using Common Terminology Criteria for Adverse Events (version 4.0) through 30 days of post-CAR infusion or resolution. CRS was prospectively graded using the Lee scale following its development (with initial patients retrospectively graded),[11] and American Society of Transplantation and Cellular Therapy CRS consensus grading[12] was retrospectively assigned for CRS and neurotoxicity.

Baseline bone marrow for pre-CAR disease assessment was performed prior to lymphodepletion. Initial response evaluations were performed at day 28 ± 4 days post-CAR T-cell infusion with routine surveillance thereafter. Minimal residual disease (MRD) negativity was defined as < 0.01% detectable leukemic blasts among mononuclear cells by flow cytometry (FC).[13,14] Patients with sufficient samples available had retrospective next-generation sequencing (NGS) analysis using the recently US Food and Drug Administration–approved clonoSEQ assay.[15]

Correlative Studies

FC was used to quantitate CD19-CAR T cells in blood, marrow, and CSF using the anti-idiotype mAb 136.20.1 as described,[16] and circulating CAR T-cell numbers were

TABLE 1. Demographics and Disease Characteristics of Patients With B-ALL

Patient Demographics	n = 50
Median age, n (range), years	13.5 (4.3-30.4)
Male, n (%)	40 (80)
Median prior regimens, n (range)	4 (1-16)
Primary refractory, n (%)	11 (22)
Prior HSCT, n (%)	22 (44)
Prior CD19-targeted therapy,[a] n (%)	7 (14)
≥ 5% marrow blasts, n (%)	32 (64)
Extramedullary disease (non-CNS), n (%)	4 (8)
CNS[b] disease, n (%)	13 (26)
CNS3	3 (6)
CNS2	2 (4)
CNS1[b]	8 (16)

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; CNS3 disease, > 5 WBC/μL in CSF and cytospin-positive for blasts; CNS1, absence of blasts in CSF on cytospin, regardless of the number of WBC; CNS2 disease, < 5 WBC/μL in CSF and cytospin-positive for blasts; HSCT, hematopoietic stem-cell transplantation.

[a]Two patients had prior CD19-CAR; five had blinatumomab.

[b]Inclusive of flow cytometric disease detection.

calculated on the basis of absolute lymphocyte counts. Cytokine profiling and T-cell subsets are described in the Data Supplement.

TABLE 2. Toxicity and Response (N = 50)

	Lee Scale No. (%)	ASTCT Scale No. (%)
CRS grade		
0	15 (30)	15 (30)
1	13 (26)	15 (30)
2	13 (26)	9 (18)
3	6 (12)	8 (16)
4	3 (6)	3 (6)
CRS intervention		
Tocilizumab	7 (14)	
Steroids	4 (8)	
Neurotoxicity	10 (20)	
Severe	4 (8)	
Mild	6 (12)	
Response		
MRD-negative CR	28 (56)	
MRD-positive CR	3 (6)	
SD or PD	19 (38)	

NOTE. Severe neurotoxicity defined by ≥ Common Terminology Criteria for Adverse Events grade 3 event or seizure.

Abbreviations: ASTCT, American Society of Transplantation and Cellular Therapy; CR, complete remission; CRS, cytokine release syndrome; MRD, minimal residual disease; PD, progressive disease; SD, stable disease.

Statistical Analysis

Descriptive statistics were computed to summarize patient and disease characteristics. Kaplan-Meier survival curves[17] were used to show event-free survival (EFS) and overall survival (OS) in B-ALL. In addition, we evaluated the EFS and OS for patients who proceeded to alloHSCT, starting at the day of alloHSCT (HSCT day 0). The cumulative incidence of relapse after alloHSCT was calculated on the basis of a competing risk of death from any cause before a relapse could be identified[18] (Data Supplement).

RESULTS

Patients

Among 53 enrolled patients, 51 had B-ALL and two had diffuse large B-cell lymphoma (Data Supplement; Table 1). One patient with B-ALL developed invasive fungal disease that precluded infusion. All subsequent analyses are based on the infused population with B-ALL (n = 50). The median age at infusion was 13.5 years (range, 4.3-30.4 years). Thirty-three patients (66.0%) had ≥ M2 marrow (≥ 5% marrow blasts), and 22 (44%) had undergone at least one prior alloHSCT. Two patients had received a prior CD19-CAR T-cell therapy and five had prior blinatumomab. Thirteen patients had CNS involvement at infusion (CNS1 with flow cytometric disease only [n = 8], CNS2 disease [n = 2], and CNS3 [n = 3]), which included a patient with extensive leptomeningeal involvement. Five patients were treated at DL2, and 45 patients were treated at DL1, including two patients who did not meet the target dose.

Toxicity

CRS occurred in 35 (70.0%) patients; nine patients (18.0%) had CRS grade 3-4 (Table 2). Seven patients received tocilizumab and four also received steroids. The median time to CRS onset was 5 days (range, 1-12 days). Neurotoxicity occurred in 10 (20.0%) patients, with four patients experiencing severe neurotoxicity (seizure, n = 2; grade 3 dysphasia, n = 2). All had resolution of symptoms to baseline, including patients with CNS3 disease. One patient had cardiac arrest during CRS with full recovery and has been previously reported.[1,11] Disease burden was associated with CRS severity. Specifically, among responders receiving Flu/Cy (standard or low-dose, n = 29), one of 15 (6.67%) patients with an M1 marrow had grade 3-4 CRS versus eight of 14 (57.1%) patients with a ≥ M2 marrow had grade 3-4 CRS (P = .005). Among the nine patients with grade 3-4 CRS, all but one patient had ≥ M2 marrow. Neurotoxicity was also associated with high-grade CRS and high-disease burden. Specifically, seven of 10 patients with neurotoxicity had grade 3-4 CRS and eight of 10 had ≥ M2 marrow.

Response

Thirty-one of 50 treated patients with B-ALL (62.0%) achieved a CR, among whom 28 patients (90.3%) were MRD-negative by FC (56.0% MRD-negative CR rate

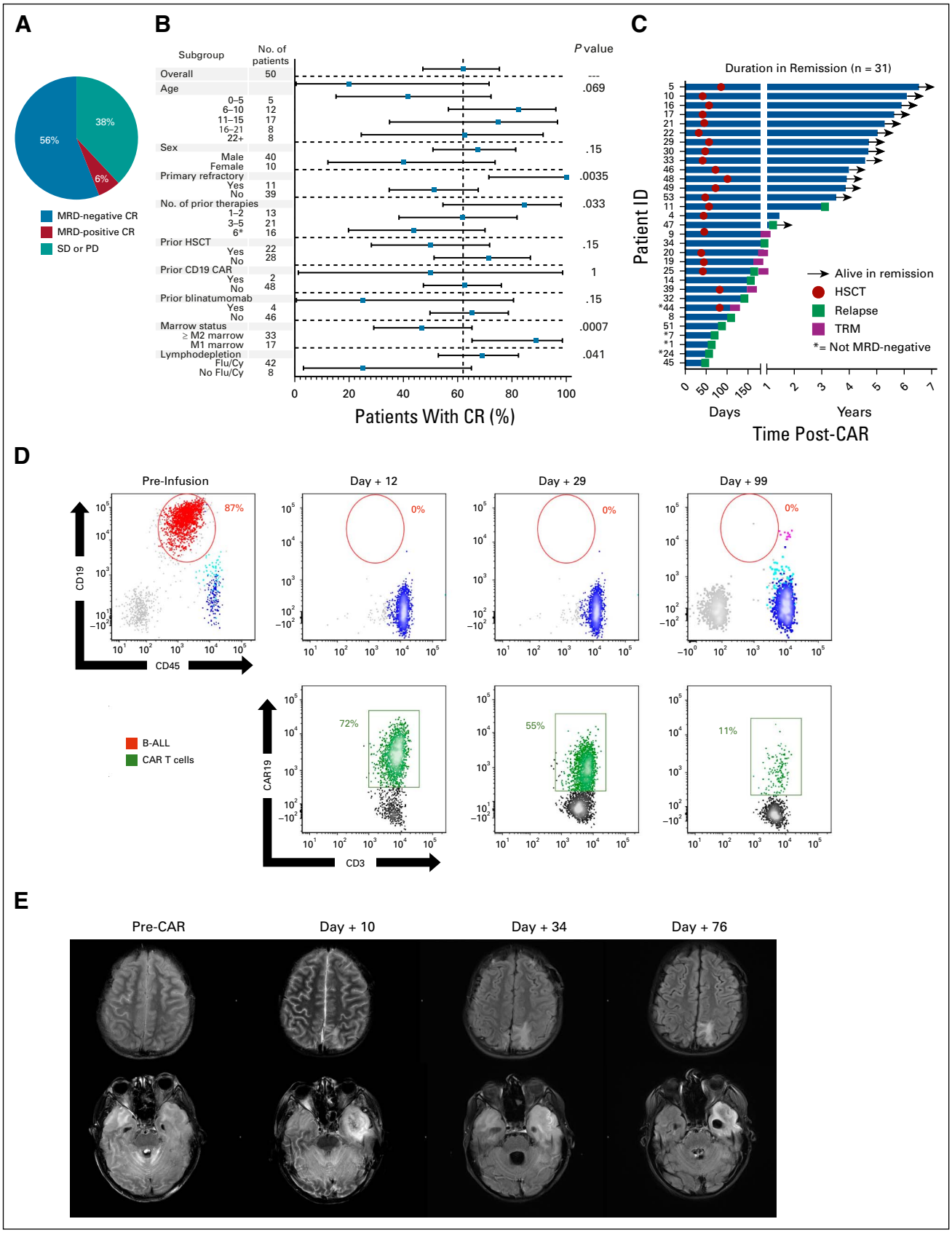

FIG 1. Response to CD19.28ζ CAR T-cell therapy. (A) Rate of those with MRD-positive or MRD-negative CR and those who had SD or PD among those with acute lymphoblastic leukemia. (B) Fraction of CR (MRD-positive and MRD-negative) according to patient (continued on following page)

overall) (Fig 1A). Among 12 patients with B-ALL who were MRD-negative by FC and had concurrent NGS MRD testing, five were NGS-positive at the day-28 assessment, with MRD levels ranging from 1×10^1 to 1×10^3 cells/million. CR rates were higher in patients who were primary refractory ($P = .0035$), had fewer prior lines of therapy ($P = .033$), had an M1 marrow (MRD-positive, $< 5\%$ blasts) ($P = .0007$), or received Flu/Cy lymphodepletion ($P = .041$) (Fig 1B). Specifically, patients with low-burden disease (M1 marrow) had higher rates of CR (16/17, 94.1%) than those with high-burden disease (≥M2 marrow; 15/33, 45.5%) ($P = .0007$). Duration in remission is shown in Figure 1C and was affected by alloHSCT as further described.

CNS B-ALL involvement was effectively treated in all who had CRS and a concurrent marrow response, with one patient having residual low-level CNS flow cytometric disease (CNS1). There was resolution of leptomeningeal enhancement when present and evidence of CAR T-cell trafficking to the CNS in the majority of patients (Figs 1D and 1E). Notably, three of 13 patients with CNS involvement also had evidence of non-CNS extramedullary (EM) disease, including one patient who had an M1 marrow with extensive EM involvement, including renal masses. Two of the three were rendered into an MRD-negative CR with resolution of both CNS and non-CNS EM disease at 1 month.

CAR T-Cell Expansion and Cytokine Profiling

CR was associated with higher CAR T-cell expansion and grade 3-4 CRS (Figs 2A-2C; Data Supplement). Twenty-nine patients had evidence of CAR T-cell trafficking to the CNS with 0.3 to 81% of T cells in the CSF as CAR T-cell–positive. We observed no difference in the response rate associated with expression of the T-cell exhaustion markers PD1, TIM3, or LAG3 on infused CAR T cells (Fig 2D). Extended immunophenotyping of the CAR T-cell product revealed that CD4+ and CD8+ CAR T-cell populations were balanced between central and effector immunophenotypes (Figs 2E and 2F). Peak levels for the majority of evaluated cytokines were differentially elevated between those with low- and high-grade CRS (Figs 2G-2I, Data Supplement).

Lymphodepleting Regimen

Among 50 patients with B-ALL, 35 patients received LD-Flu/Cy and 15 patients were treated with alternative regimens, including HD-Flu/Cy (n = 7), FLAG (n = 6), or Ifos/Etop (n = 2). The CR rate was higher among those who received any Flu/Cy regimen ($P = .041$) with CR in four of seven (57.1%) patients receiving HD-Flu/Cy and in 25 of 35 (71.4%) receiving LD-Flu/Cy. Cumulatively, 29 of 42 (69.0%) patients receiving any Flu/Cy regimen achieved a CR. Responses using alternative lymphodepleting regimens were suboptimal with zero of two (0.0%) patients receiving Ifos/Etop and two of six (33.3%) patients receiving FLAG chemotherapy achieving CR.

Long-Term Outcomes

With a median follow-up of 4.8 years (range, 3.5-7.2 years), the median OS for the entire cohort was 10.5 months (95% CI, 6.3 to 29.2 months). Median EFS was 3.1 months (95% CI, 0.9 to 7.7 months), with 3-month EFS of 52.0% (95% CI, 37.4 to 64.7) and 6-month EFS of 38% (95% CI, 24.8 to 51.1) (Fig 3A).

Receipt of any Flu/Cy regimen was associated with an improved EFS and OS (Figs 3C and 3D). Median EFS was not reached in patients treated with an M1 marrow, whereas median EFS was 0.9 months (95% CI, 0.9 to 2.0 months) in patients treated with ≥ M2 marrow ($P \leq .0001$) (Figs 3E and 3F). Importantly, only eight of 15 patients achieving CR proceeded to HSCT among this high-burden disease cohort.

Role of Consolidative HSCT

Twenty-one of 28 (75.0%) patients achieving MRD-negative CR proceeded to a consolidative alloHSCT. The median time to alloHSCT was 54 days from CAR infusion (range, 42-97 days). In four patients, this represented a second alloHSCT. All 17 patients for whom this was a first HSCT underwent myeloablative conditioning (Data Supplement). The median OS from HSCT day 0 was 70.2 months (95% CI, 10.4 months to not estimable), and median EFS was not reached (Fig 3F). The 5-year EFS following alloHSCT was 61.9% (95% CI, 38.1 to 78.8). Eight patients died between 0.8 and 71 months following alloHSCT, causes of which included transplant-related

FIG 1. (Continued). demographics, disease characteristics, and treatment course. Squares represent the observed CR rate with the lines representing the 95% CIs for the difference of proportions from the reference category. The forest plots are based on the fraction and a Clopper-Pearson exact two-tailed 95% CI. (C) Duration in continuous remission among those who achieved a CR, indicating those who remain alive in remission, when HSCT was performed, the time of relapse, and those who experienced TRM. (D) Flow cytometric analysis of the CSF in patient 45 showing progressive CAR T-cell expansion (green) along with leukemia regression (red) in a patient with extensive CNS disease. (E) Brain magnetic resonance imaging findings from before, during, and after CAR T-cell infusion corresponding with patient 45, whose concurrent flow cytometry samples are shown in (D). Top panel (postcontrast axial fluid-attenuated inversion recovery) shows the evolution of intraparenchymal edema related to the leptomeningeal involvement to encephalomalacia. Bottom panel shows the evolution of widespread patchy white matter injury or edema involving the cerebrum and cerebellum with extensive involvement of the left temporal lobe (pretreatment). At day +10, neurologic symptoms included encephalopathy and right gaze preference. Necrosis had developed in the portion of the left temporal lobe that was most edematous. Diffusion-weighted images (not shown) demonstrated restricted diffusion corresponding to the area that is dark on fluid-attenuated inversion recovery. By day +76, the necrosis in the left temporal lobe had evolved to encephalomalacia (not ex vacuo expansion of the left temporal horn). B-ALL, B-cell acute lymphoblastic leukemia; CR, complete remission; HSCT, hematopoietic stem-cell transplant; MRD, minimal residual disease; PD, progressive disease; SD, stable disease; TRM, transplant-related mortality.

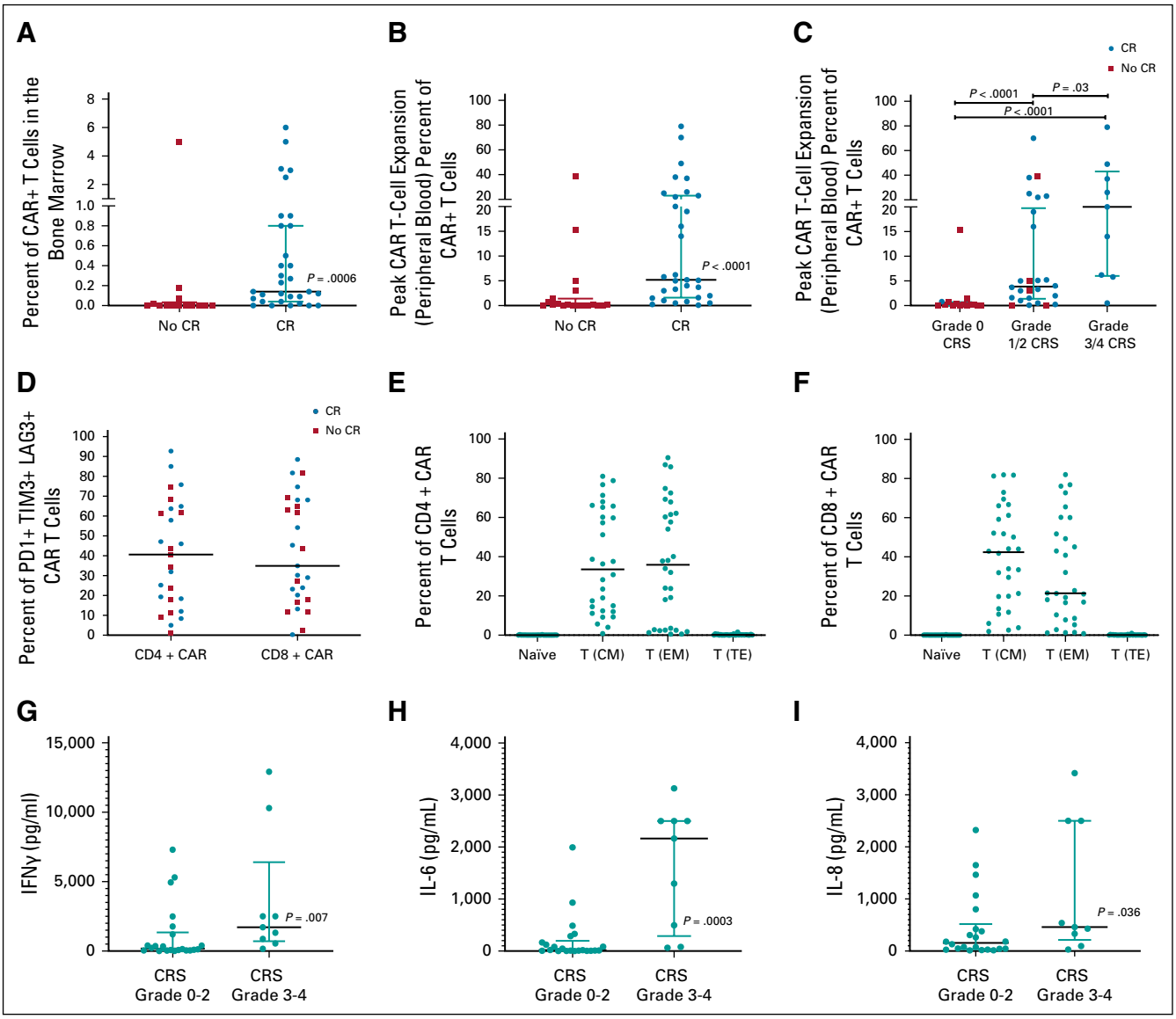

FIG 2. (A) Median and interquartile range of CAR T-cell expansion (as a percentage of T cells that were CAR T-cell–positive) in the bone marrow at day 28 (± 4 days) post-CAR T-cell infusion, stratified by those who achieved a CR and those who did not (lower limit of CAR T-cell detection by flow cytometry is 0.05% of CD3+ T cells). (B) Median and interquartile range of peak CAR T-cell expansion (as a percentage of T cells that were CAR T-cell–positive) in the peripheral blood within the first 28 days (± 4 days) post-CAR T-cell infusion, stratified by those who achieved a CR and those who did not. (C) Median and interquartile range of peak CAR T-cell expansion (as a percentage of T cells that were CAR T-cell–positive) in the peripheral blood within the first 28 days (± 4 days) post-CAR T-cell infusion, stratified by those who had grade 0, grade 1/2, and grade 3/4 CRS. (D) Percentage of CAR T cells positive for PD1, TIM3, or LAG3 as stratified by CD4 and CD8 populations with red indicating those who achieved a CR and blue indicating those who did not. (E) Proportion of the CD4+ CAR T-cell product that are naïve T cells; T (CM), T (EM), and T (TE). (F) Proportion of the CD8+ CAR T-cell product that are naïve T cells; T (CM), T (EM), and T (TE). (G) Peak IFNγ values stratified by CRS grade 0-2 versus CRS grade 3-4 in those who achieved a CR. (H) Peak IL-6 values stratified by CRS grade 0-2 versus CRS grade 3-4 in those who achieved a CR. (I) Peak IL-8 values stratified by CRS grade 0-2 versus CRS grade 3-4 in those who achieved a CR. CR, complete remission; CRS, cytokine release syndrome; EM, extramedullary; IFN, interferon; IL, interleukin; PD, progressive disease; T (CM), central memory T cells; T (EM), effector memory or effector T cells; T (TE), terminal effector T cells.

complications and/or graft-versus-host disease or infection (n = 6), complications of second malignancy 3 years post-HSCT (n = 1)[19], and relapsed disease (n = 1). A total of two patients relapsed after alloHSCT, and the cumulative incidence of relapse after alloHSCT, with death as a competing risk, was 4.8% (95% CI, 0.3 to 20.3) at 12 months and 9.5% (95% CI, 1.5 to 26.8) at 24 months.

All seven who achieved an MRD-negative CR and did not proceed to a consolidative HSCT experienced relapse at a median of 152 days post-CAR infusion (range, 94-394

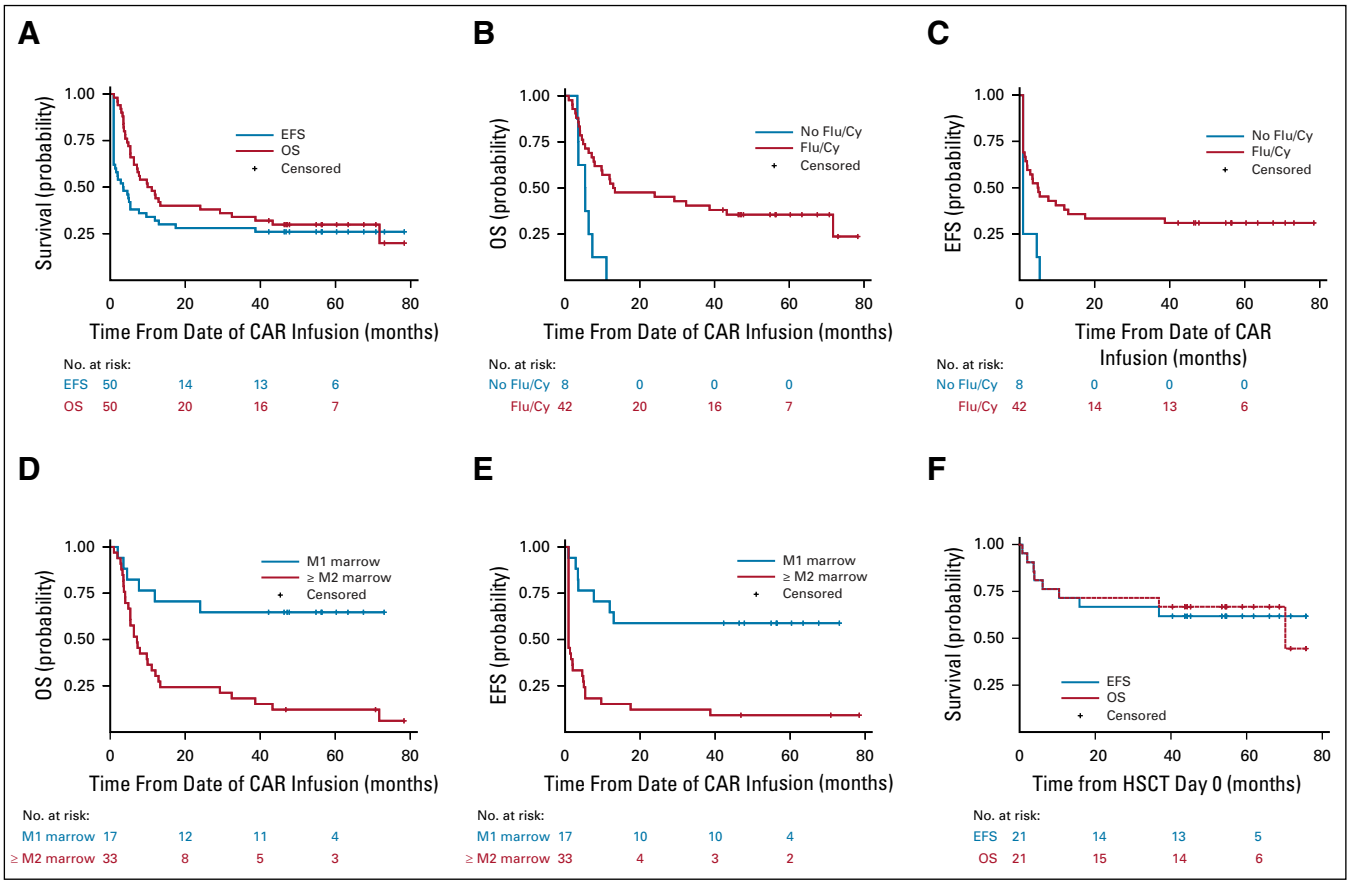

FIG 3. Continuous remission, OS, and EFS. (A) OS and EFS among patients with B-cell acute lymphoblastic leukemia, starting from CAR infusion at day 0. (B) OS, (C) EFS among patients who received Flu/Cy–based pre-CAR T-cell lymphodepletion versus those who did not. (D) OS, (E) EFS among patients who had an M2 marrow (≥ 5% marrow disease) or higher compared with those who had an M1 marrow (< 5%) disease. (F) OS and EFS among patients who proceeded to a consolidative allogeneic HSCT, from HSCT day 0. CAR, chimeric antigen receptor; EFS, event-free survival; Flu/Cy, fludarabine/cyclophosphamide; HSCT, hematopoietic stem-cell transplant; OS, overall survival.

days), highlighting the importance of consolidative alloHSCT following this construct. Six patients did not proceed to HSCT because of concern for second alloHSCT–associated risks. In one patient with underlying trisomy 21, a first HSCT was deferred because of concern for toxicity. No patient achieved a CR following a second CAR T-cell infusion (Data Supplement).

CD19 Antigen Expression and Prior CD19 Targeting

With a limited sample size (n = 42), CD19 antigen site density at enrollment was not significantly different between responders and nonresponders (median CD19 site density 7,603 *v* 8,175, respectively [*P* = .82]). Among those who had received prior CD19-targeted therapy, one of two who had received prior CD19-CAR T cells and four of five who had received prior blinatumomab were nonresponders. CD19 immunophenotype on leukemia blasts at relapse for patients achieving MRD-negative CR and not proceeding to HSCT was CD19+ (n = 3), CD19−/dim (n = 3), and CD19 unknown (n = 1) blasts.

DISCUSSION

Nearly all published reports detailing activity of CD19-CAR in B-ALL have focused on CR rates at day 28, which occur in approximately 60%-100% of patients.[1-4,20-25] Long-term follow-up data from these studies are largely lacking. However, even with limited follow-up, high relapse rates have been observed and a clear plateau in survival has not been demonstrated. Additional confounding data regarding long-term outcomes is the fact that a significant fraction of patients rendered into remission with CD19-CAR receive additional treatment, including alloHSCT. One previous series examined outcomes in adults with a median age of 44 years (range, 23-74) and concluded that post-CAR alloHSCT did not improve survival.[3] However, it remains unknown whether alloHSCT after CAR T-cell therapy affects outcome in the CAYA population.

With a median follow-up of 4.8 years, this report provides the longest duration of follow-up of any series following treatment with CD19-CAR for B-ALL. In CAYA patients with relapsed or refractory B-ALL treated with autologous T cells expressing a

CD19.28ζ CAR, we demonstrate that patients who achieved a CR and proceeded to a consolidative alloHSCT had a relapse rate of < 10% at 24 months post-CAR, a 5-year EFS of 61.9%, and a median OS of 70.2 months from HSCT. All patients who did not undergo post-CAR T-cell alloHSCT experienced relapse. The contrast to outcomes reported by Park et al[3] using a similar CAR T-cell construct, where HSCT did not lead to improved outcomes, and may relate to the higher morbidity and mortality associated with alloHSCT for B-ALL in adults, along with variability in conditioning regimen and graft choice.[26] Importantly, the decision to undergo alloHSCT and the choice of preparative regimen were not dictated by our protocol, and therefore, this study is not capable of providing information regarding the optimal regimen for use in this setting. Nonetheless, the results provide strong evidence that a treatment regimen that incorporates autologous CD19.28ζ CAR T cells to achieve remission followed by a consolidative alloHSCT is associated with a remarkably low relapse rate in a very high-risk patient population. The high relapse rates for those not proceeding to alloHSCT may relate, in part, to the shorter persistence of the CD28 costimulatory domain construct,[1,3,27] supporting a role for utilization of this construct as a bridge to HSCT. However, given that patients who experience long-term persistence of CD19.BBζ-CARs may relapse with CD19-negative B-ALL, consolidative alloHSCT could improve outcomes following alterative CAR T-cell constructs regardless of persistence. Prospective trials that directly incorporate consolidative alloHSCT following CAR T cells are warranted for further evaluation in improving outcomes for these high-risk patients.

This series also provides additional data to support the use of CD19-CAR for the treatment of CNS leukemia. We demonstrate that CNS2 and CNS3 disease can be safely and effectively treated, potentially expanding the therapeutic indications for CAR T cells and raising the prospect that CD19-CAR could provide effective therapy without toxicity from standard radiation and intensified chemotherapy-based approaches. These results support further study of CAR T cells in patients with B-ALL and active CNS involvement.

Additionally, we provide data confirming the importance of Flu/Cy–based lymphodepletion in improving responses to CD19-CAR (Figs 3C and 3D), as previously reported,[25,28] and confirm previous data demonstrating that patients with low-burden or primary refractory disease experience superior long-term outcomes following CD19-CAR therapy compared with heavily pretreated patients with high-burden disease (Figs 3E and 3F).[3]

Given the role of CD19-CAR T cells as a bridge to HSCT, it warrants consideration of alternative approaches for remission induction. Blinatumomab, for instance, is US Food and Drug Administration–approved for children and adults with relapsed or refractory B-ALL. However, despite its more ready availability, which is not dependent on manufacturing time or success thereof, the efficacy of blinatumomab in children is lower than in adults receiving blinatumomab[29] and also lower than remission rates following CD19-CAR T cells, using any construct, particularly for those with high-burden disease.[30,31] Additionally, there is no role for blinatumomab in active CNS disease, and there are limited data for its use in EM disease. Therefore, selection of CAR T cells over blinatumomab may be advantageous in patients with higher-burden disease and EM disease or as a salvage for blinatumomab nonresponders. Although patients with high-burden disease in our study had lower response rates, this may be related to the utilization of non–Flu/Cy–based lymphodepletion, which would not be endorsed in future studies. Of note, our results raise the concern that prior CD19 targeting may affect response to CD19-CAR T cells. Notably, the global registration study for tisagenlecleucel excluded patients who had received prior CD19-targeted therapies,[2] and recent studies suggest that blinatumomab prior to CD19-CAR T cells may negatively affect outcomes.[32]

In summary, we demonstrate that CD19.28ζ CAR T cells followed by a consolidative alloHSCT can provide long-term durable disease control in CAYAs with relapsed or refractory B-ALL. Following alloHSCT, we observed a significant long-term EFS with an apparent plateau and a low relapse rate, providing support for this sequential approach for long-term cure.

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DISCLAIMER

The content of this publication does not necessarily reflect the views of policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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Long-Term Follow-Up of CD19 CAR T-Cell Therapy in Children and Young Adults With B-ALL

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