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RE: National Coverage Analysis (NCA) Tracking Sheet for Chimeric Antigen Receptor (CAR) T-cell Therapy for Cancers (CAG-00451N)

Appendix A

Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 Results of ZUMA-1: A Multicenter Study of KTE-C19 Anti-CD19 CAR T Cell Therapy in Refractory Aggressive Lymphoma. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 Results of ZUMA-1: A Multicenter Study of KTE-C19 Anti-CD19 CAR T Cell Therapy in Refractory Aggressive Lymphoma. *Molecular Therapy*. 2017;25(1):285-295

- ZUMA-1 was the first multicenter study of anti-CD19 CAR T cell therapy in patients with non-Hodgkin's lymphoma (NHL).
- The results of this phase 1 portion of ZUMA-1, the first multicenter study of anti-CD19 CAR T cell therapy in aggressive NHL, demonstrated that the KTE-C19 regimen was tolerable and safe for further study.

Kochenderfer JN, Somerville RPT, Lu T, et al. Lymphoma Remissions Caused by Anti-CD19 Chimeric Antigen Receptor T Cells Are Associated With High Serum Interleukin-15 Levels. *Journal of Clinical Oncology*. 2017;35(16):1803-1813. doi:10.1200/JCO.2016.71.3024.

- In a study at the National Cancer Institute (NCI), 22 patients were treated with CD19 CAR T-cells. 19 of these patients had one of the various types of diffuse large B-cell lymphoma (DLBCL) and two patients had follicular lymphoma. For all 22 patients treated, there was a 73% remission rate with 55% complete remissions and 18% partial remissions.

Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *The New England Journal of Medicine*. 2018;378(5);439-448.

- In this global study of CAR T-cell therapy, a single infusion of tisagenlecleucel provided durable remission with long-term persistence in pediatric and young adult patients with relapsed or refractory B-cell ALL, with transient high-grade toxic effects.

Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *New England Journal of Medicine*. 2017; 377:2531-2544.

- A phase 1 multicenter study (ZUMA-1) involving 7 patients with refractory large B-cell lymphoma showed that axi-cel could be centrally manufactured and safely administered.
- In a large, international, retrospective research study involving patients with non-Hodgkin's lymphoma (SCHOLAR-1), investigators found an objective response rate of 26%, a complete response rate of 7%, and a median overall survival of 6.3 months with existing

therapies among patients who had aggressive B-cell lymphoma that was resistant to chemotherapy or who had a relapse within 12 months after autologous stem-cell transplantation.

- Phase 2 of ZUMA-1, 82% of the 101 patients with refractory large B-cell lymphoma who were treated had an objective response, and 54% had a complete response
    - This compared favorably with the results of the SCHOLAR-1 study of existing therapies for this disease, which showed an objective response rate of 26% and a complete response rate of 7%

Molecular Therapy
Original Article

Phase 1 Results of ZUMA-1: A Multicenter Study of KTE-C19 Anti-CD19 CAR T Cell Therapy in Refractory Aggressive Lymphoma

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Outcomes for patients with refractory diffuse large B cell lymphoma (DLBCL) are poor. In the multicenter ZUMA-1 phase 1 study, we evaluated KTE-C19, an autologous CD3ζ/CD28-based chimeric antigen receptor (CAR) T cell therapy, in patients with refractory DLBCL. Patients received low-dose conditioning chemotherapy with concurrent cyclophosphamide (500 mg/m^2) and fludarabine (30 mg/m^2) for 3 days followed by KTE-C19 at a target dose of 2 × 10^6 CAR T cells/kg. The incidence of dose-limiting toxicity (DLT) was the primary endpoint. Seven patients were treated with KTE-C19 and one patient experienced a DLT of grade 4 cytokine release syndrome (CRS) and neurotoxicity. Grade ≥3 CRS and neurotoxicity were observed in 14% (n = 1/7) and 57% (n = 4/7) of patients, respectively. All other KTE-C19-related grade ≥3 events resolved within 1 month. The overall response rate was 71% (n = 5/7) and complete response (CR) rate was 57% (n = 4/7). Three patients have ongoing CR (all at 12+ months). CAR T cells demonstrated peak expansion within 2 weeks and continued to be detectable at 12+ months in patients with ongoing CR. This regimen of KTE-C19 was safe for further study in phase 2 and induced durable remissions in patients with refractory DLBCL.

INTRODUCTION

Diffuse large B cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL) in the United States, accounting for approximately 30%–40% of all cases of NHL.[1–3] Studies examining outcomes in patients with relapsed/refractory DLBCL show that the response rates to subsequent therapy varies from 14% to 63%.[4–8] However, relapsed/refractory DLBCL is broadly defined and consists of a heterogeneous patient population. Outcomes are particularly poor in those patients with truly refractory DLBCL, defined as no response to last line of chemotherapy or relapse within 1 year of autologous stem cell transplant (ASCT).[6–9] A large patient-level meta-analysis of patients with refractory DLBCL (Retrospective Non-Hodgkin Lymphoma Research, SCHOLAR-1) found that outcomes in this homogeneous population are significantly worse, with a complete response (CR) rate of 8%, a partial response (PR) rate of 18%, and median overall survival (OS) of 6.6 months,[10] indicating a major unmet need for effective therapies for these patients.

Adoptive cell therapy with T cells genetically engineered to express chimeric antigen receptor (CAR) targeting CD19 is a promising approach for treatment of B cell malignancies. A recent single-institution study conducted at the National Cancer Institute (NCI) demonstrated high response rates with an overall response rate of 73% and a CR rate of 55% with anti-CD19 CAR T cells containing CD3ζ/CD28 signaling domains administered in conjunction with low-dose cyclophosphamide conditioning regimen in patients with relapsed/refractory B cell lymphomas.[11] KTE-C19 is an autologous CD3ζ/CD28-based anti-CD19 CAR T cell product that uses the same CAR construct as in the NCI study but is manufactured in a centralized, closed, and streamlined process of approximately 8 days. ZUMA-1 is the first multicenter study evaluating the safety and efficacy of anti-CD19 CAR T cells in patients with refractory NHL (NCT02348216). We report here the safety, efficacy, and correlative studies of apheresis product, KTE-C19, and in vivo effects from the phase 1 portion of ZUMA-1.

RESULTS

Demographics and Baseline Characteristics

As of August 24, 2016, the median follow-up time was 9 months. Nine patients were enrolled in the study. Two patients experienced adverse

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Table 1. Baseline Characteristics

	Patient						
Characteristic	1	2	3	4	5	6	7
Age (years)	59	68	69	67	34	40	29
Sex	male	male	male	male	female	male	female
ECOG PS	0	1	0	1	0	0	1
Prior therapies	(1) R-CHOP	(1) R-CHOP	(1) R-CHOP	(1) R-CHOP	(1) R-CHOP	(1) R-EPOCH	(1) R-CHOP
	(2) R-ICE	(2) R-ICE	(2) R-CEOP	(2) R-ESHAP	(2) R-ICE	(2) R-BEAM-ASCT	(2) R-GDP
	(3) R-BEAM-ASCT		(3) R-ICE	(3) R-BEAM-ASCT	(3) R-AZA/ SAHA/ GEMBUM-ASCT	(3) Ipilimumab+ Lenalidomide	(3) ICE
			(4) R-GEMOX + lenalidomide	(4) R-GEMOX			
Prior lines of therapy	3	2	4	4	3	3	3
Relapsed/refractory status	relapsed post-ASCT within 12 months	refractory second or higher line of therapy	refractory second or higher line of therapy	relapsed post-ASCT within 12 months	relapsed post-ASCT within 12 months	relapsed post-ASCT within 12 months	refractory second or higher line of therapy
Primary diagnosis/ sub-type	DLBCL/non-GCB	DLBCL/GCB	DLBCL/non-GCB	DLBCL/GCB	DLBCL/non-GCB	DLBCL/GCB	DLBCL/non-GCB
Disease stage	IV	II	III	I	IV	I	III

ASCT, autologous stem cell transplant; AZA, azacitidine; BEAM, carmustine, etoposide, cytarabine, melphalan; CEOP, cyclophosphamide, etoposide, vincristine, prednisone; CHOP, cyclophosphamide, adriamycin, vincristine, prednisone; DLBCL, diffuse large B cell lymphoma; ECOG PS, Eastern Cooperative Oncology Group performance status; EPOCH, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; ESHAP, etoposide, methylprednisolone, high-dose cytarabine, cisplatin; GCB, germinal center B cell; GDP, gemcitabine, dexamethasone, cisplatin; GEMBUM, gemcitabine, busulfan, melphalan; GEMOX, gemcitabine, oxaliplatin; ICE, ifosfamide, carboplatin, etoposide; R, rituximab; SAHA, vorinostat.

events (AEs) due to disease progression, discontinued the study, and never received KTE-C19 (one prior to leukapheresis and one prior to conditioning chemotherapy). KTE-C19 manufacturing was successful for all eight leukapheresed patients (Figure S1). Seven patients received conditioning chemotherapy and KTE-C19. One additional patient was added to have the pre-specified dose-limiting toxicity (DLT) set of six patients treated at a target of 2×10^6 CAR^+ T cells/kg (see Materials and Methods). Patients ranged from 29 to 69 years of age and had received two to four prior lines of therapy. Three were refractory to second-line or later lines of therapy, and four patients had relapsed post-ASCT within 1 year (Table 1). Two of the four that relapsed post-ASCT received another regimen before enrollment: patient 4 had stable disease after three cycles of R-GEMOX and patient 6 had progressive disease after two cycles of ipilimumab and lenalidomide therapy, meeting the definition of refractoriness as defined in the protocol. Median absolute lymphocyte count at time of enrollment was 900 lymphocytes/μL (range, 100–1,400 lymphocytes/μL). Four patients were non-germinal center B cell (GCB) subtype, and three were GCB as per the Hans algorithm.[12]

Safety

All AEs occurring within 30 days of KTE-C19 infusion were graded and reported for the seven (100%) treated patients, with maximum grade 3, 4, and 5 events reported in three (43%), three (43%), and one (14%) patient(s), respectively (Table 2). All patients experienced KTE-C19-related AEs of any grade, with grade 3 and 4 events reported in four (57%) and one (14%) patient(s), respectively. No patient had a grade 5 KTE-C19-related event. B cell aplasia and hypogammaglobulinemia was observed in patients 4, 5, and 6 (data not shown).

One patient (14%) experienced a DLT. Patient 7 was a 29-year-old female with DLBCL with an Eastern Cooperative Oncology Group (ECOG) performance status of 1 and refractory to three lines of combination immuno-chemotherapy (Table 1). She experienced grade 3 hypotension, grade 3 metabolic acidosis, and grade 4 encephalopathy on day 0 and required intubation on day 1; grade 3 acute systolic heart failure on day 2; and grade 4 cytokine release syndrome (CRS) comprising grade 4 acute kidney injury on day 6. The grade 4 KTE-C19-related events in patient 7 initially improved with tocilizumab, corticosteroids, and supportive care including dialysis. However, the patient's condition subsequently worsened with grade 4 pseudomonal sepsis, grade 4 thrombocytopenia, grade 4 neutropenia, and subsequent grade 5 intracranial hemorrhage. Death was deemed unrelated to KTE-C19 per the investigator, and on the day of death, the patient was on ongoing heparin for deep vein thrombosis (DVT) prophylaxis in the setting of grade 4 thrombocytopenia and pseudomonas sepsis. Retrospective biomarker analysis revealed that immediately prior to initiation of fludarabine and cyclophosphamide, the patient had a C-reactive protein (CRP) of 655 mg/L (normal range, 0–10 mg/L), which was approximately 100-fold higher than the baseline values of the other patients (median, 7 mg/L; range 4–34 mg/L), indicating an elevated baseline inflammatory state. With the exception of the patient experiencing a DLT, all grade ≥ 3 KTE-C19-related toxicities resolved within 1 month.

Table 2. Grade 3 or Higher Treatment-Emergent Adverse Events

Event	Any, n (%)	Worst Grade 3, n (%)	Worst Grade 4, n (%)	Worst Grade 5, n (%)
Any grade 3 or higher AE within 30 days of KTE-C19 infusion	7 (100)	3 (43)	3 (43)	1 (14)
Febrile neutropenia	4 (57)	3 (43)	1 (14)	0
Encephalopathy	3 (43)	2 (29)	1 (14)	0
Neutropenia	3 (43)	0	3 (43)	0
Anemia	2 (29)	2 (29)	0	0
Hypoxia	2 (29)	2 (29)	0	0
Somnolence	2 (29)	2 (29)	0	0
Thrombocytopenia	2 (29)	0	2 (29)	0
Acute kidney injury	1 (14)	0	1 (14)	0
Agitation	1 (14)	1 (14)	0	0
Ascites	1 (14)	1 (14)	0	0
Aspartate aminotransferase increased	1 (14)	1 (14)	0	0
Cardiac failure	1 (14)	1 (14)	0	0
Delirium	1 (14)	1 (14)	0	0
Fatigue	1 (14)	1 (14)	0	0
Hemorrhage intracranial	1 (14)	0	0	1 (14)
Hypocalcemia	1 (14)	1 (14)	0	0
Hyponatremia	1 (14)	1 (14)	0	0
Hypophosphatemia	1 (14)	1 (14)	0	0
Hypotension	1 (14)	1 (14)	0	0
Metabolic acidosis	1 (14)	1 (14)	0	0
Oral herpes	1 (14)	1 (14)	0	0
Pseudomonal sepsis	1 (14)	0	1 (14)	0
Pyrexia	1 (14)	1 (14)	0	0
Restlessness	1 (14)	1 (14)	0	0
Tremor	1 (14)	1 (14)	0	0
Urinary tract infection	1 (14)	1 (14)	0	0

Table 3. Cytokine Release Syndrome and Neurotoxicity

Event	Any, n (%)	Worst Grade 3, n (%)	Worst Grade 4, n (%)
CRS, any[a]	6 (86)	0	1 (14)
CRS, Specific Symptoms[b]			
Pyrexia	5 (71)	1 (14)	0
Hypotension	3 (43)	1 (14)[c]	0
Tachycardia	2 (29)	0	0
Acute kidney injury	1 (14)	0	1 (14)[c]
Cardiac failure	1 (14)	1 (14)[c]	0
Headache	1 (14)	0	0
Hypoxia	1 (14)	1 (14)	0
Metabolic acidosis	1 (14)	1 (14)[c]	0
Neurotoxicity, any	6 (86)	3 (43)	1 (14)
Neurotoxicity, Specific Symptoms			
Encephalopathy	5 (71)	2 (29)	1 (14)[c]
Tremor	4 (57)	1 (14)	0
Somnolence	2 (29)	1 (14)	0
Agitation	1 (14)	1 (14)	0
Aphasia	1 (14)	0	0
Delirium	1 (14)	1 (14)	0
Dizziness	1 (14)	0	0
Dyskinesia	1 (14)	0	0
Hallucination	1 (14)	0	0
Restlessness	1 (14)	1 (14)	0

[a]CRS was graded per a modified grading system proposed by Lee et al.[13]
[b]Individual symptoms of CRS are graded per CTCAE, version 4.03.
[c]Events occurred in patient 7.

Cytokine release syndrome was reported in six (86%) patients treated with KTE-C19. Five (71%) patients experienced grade $\leq$2 CRS and one (14%) patient experienced grade 4 CRS (occurring in the patient with a DLT; Table 3). The most common CRS-related symptoms were pyrexia (71%), hypotension (43%), and tachycardia (29%). All grade 3 and 4 CRS-related events, except for one grade 3 pyrexia and one grade 3 hypoxia, occurred in the same patient experiencing the DLT. Six of seven (86%) patients (patients 1, 2, 3, 4, 5, and 7) received tocilizumab and four of seven (57%; patients 1, 3, 4, and 7) received corticosteroids for management of CRS and/or neurotoxicity symptoms as described by Lee et al.;[13] four patients (patients 1, 3, 4, and 7) received both tocilizumab and corticosteroids. All evaluable patients experienced at least one neurotoxicity event of any grade (Table 3), with three (43%) having maximum grade 3, and one (14%) having a maximum grade 4 event (occurring in the patient with the DLT). The median time to development of CRS and neurotoxicity were 1 day (range: 0–3 days; n = 6) and 4 days (1–4 days; n = 6), respectively, with a median duration of 7 days (range: 3–17 days; n = 6) and 8 days (range: 2–20 days; n = 6), respectively. Except for the patient who experienced a DLT, CRS and neurotoxicity were found to be self-limiting and reversible. Four of the seven patients have died. With the exception of the patient who experienced the DLT, all deaths were due to progressive disease. Based on the safety profile of the six evaluable DLT patients in phase 1, this regimen was deemed safe for study in phase 2.

Efficacy

Five of seven (71%) patients achieved an objective response within 1 month of KTE-C19 infusion, with four of seven (57%) achieving a CR. Three patients are in ongoing CR at 12 months post-KTE-C19 infusion. All three patients with ongoing CR had previously relapsed within 5.8 months of ASCT (Table S1; Figure 1A). Of the three patients with ongoing CR at 12 months, one received tocilizumab, corticosteroids, and supportive care for the management of both CRS and neurotoxicity, one received only tocilizumab and

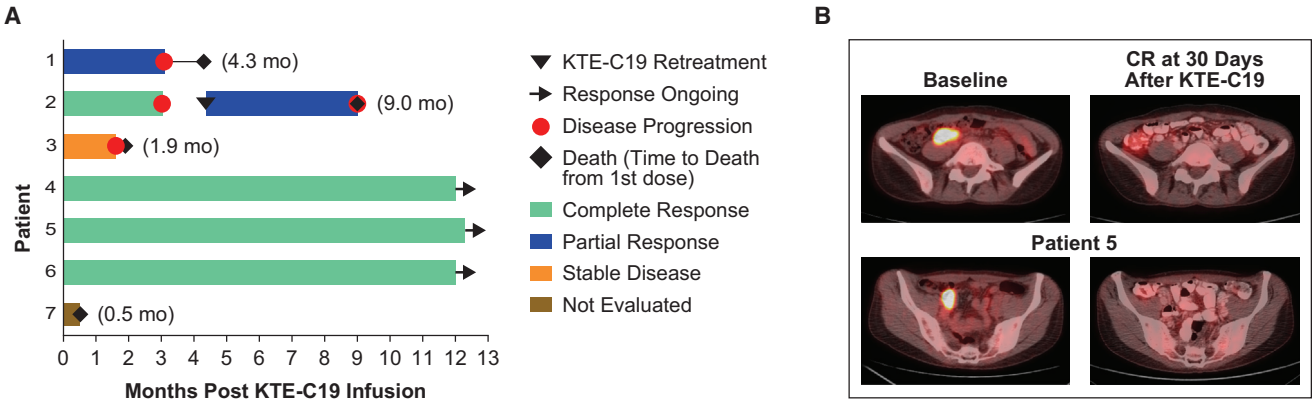

Figure 1. Clinical Efficacy after KTE-C19 Infusion

(A) Duration of response and survival post-infusion with KTE-C19. (B) CR at 30 days post KTE-C19 infusion in patient 5. Representative PET-CT scans at baseline and 30 days post KTE-C19 infusion in a patient with DLBCL relapsing after prior therapy with R-CHOP, R-ICE, and ASCT with Rituximab-gemcitabine-busulfan-melphalan+azacitidine-vorinostat.

supportive care for the treatment of both CRS and neurotoxicity during the first week post-treatment, and one received supportive care only. Figure 1B shows a representative positron emission tomography-computed tomography (PET-CT) with induction of CR 30 days after KTE-C19 infusion in patient 5. The response is ongoing at 12+ months. Patient 5 was previously treated with R-CHOP and R-ICE followed by ASCT conditioned with rituximab-gemcitabine-busulfan-melphalan+azacitidine-vorinostat and relapsed with DLBCL in the terminal ileum, ileocolic, and retroperitoneal lymph nodes 3.2 months after ASCT (Table 1).

Patient 2 achieved CR at 1 month after KTE-C19; however, progressive disease was noted at 3-month restaging and was subsequently proven as $CD19^+$ relapse by biopsy. Per protocol, patient 2 was re-treated with exactly the same low-dose conditioning chemotherapy followed by the same target dose of KTE-C19. After the second infusion, KTE-C19 expansion was detected and the patient achieved a PR at 1 month. This patient progressed 3 months after the second infusion of KTE-C19 and died at 9 months following the initial KTE-C19 infusion due to rapidly progressive bulky lymphoma (Figure 1A). After the second KTE-C19 treatment, the patient developed myelodysplastic syndrome (MDS) with a complex karyotype and nonsynonymous *DNMT3A* and *TP53* (double) mutations. Retrospective analysis demonstrated that these mutations were present in peripheral blood prior to study enrollment, indicating a pre-existing smoldering MDS.

Immunophenotyping and Biomarker Analysis

Detailed phenotypic characterization of the patients' apheresis material, KTE-C19, and subsequent biomarker samples were conducted as outlined in Materials and Methods. The initial apheresis material (Table S2) and subsequent KTE-C19 product had similar intrapatient CD4/CD8 ratios (data not shown). Unfractionated CD4 and CD8 T cells were effectively transduced and showed ex vivo reactivity against $CD19^+$ target cells (Table 4). T cells within the apheresis product typically showed more differentiated phenotypes with higher proportions of cells with effector memory (Tem [$CCR7^-$, $CD45RA^-$]) and effector (Teff [$CCR7^-$, $CD45RA^+$]) phenotypic profiles (Figure 2). The post-manufacture KTE-C19 CAR T cell product showed a less differentiated phenotype (Figure 2) based on CCR7 and CD45RA expression, with lower proportions of cells with Tem and Teff phenotypes compared to apheresis T cells and higher proportions of T cells with central memory (Tcm [$CCR7^+$, $CD45RA^-$]) and naive (TN [$CCR7^+$, $CD45RA^+$]) phenotypes (Figure S2).

Peak expansion of CAR T cells occurred within the first 7–14 days post-infusion (Figure 3A) and were detectable at low levels by qPCR analysis for up to 12 months in the peripheral blood of all three patients with ongoing CRs. Expansion of KTE-C19 was mirrored by sequential induction, elevation, and general clearance of a range of homeostatic (IL-15), inflammatory and immune modulating cytokines, chemokines (such as IP-10), and T cell effector proteins (Figures 3B, 3C, and S3). Some of these cytokines and markers, notably IL-15 (median fold change from baseline, 9.9; range, 7.6–17.8), were initially elevated by the cyclophosphamide and fludarabine conditioning chemotherapy, paralleled by reduction of perforin and endogenous lymphocyte numbers. No antibodies for the scFv portion of KTE-C19 were detected in any of the patients during the course of the study (data not shown).

DISCUSSION

To our knowledge, this is the first multicenter study of anti-CD19 CAR T cell therapy in patients with NHL. Our results demonstrated that (1) the conditioning regimen of cyclophosphamide at 500 mg/m^2 (days −5, −4, and −3) and fludarabine at 30 mg/m^2 (days −5, −4, and −3) followed by KTE-C19 (day 0) at a dose of 1–2 × 10^6 CAR T cells/kg is safe for further study and the toxicity is manageable; (2) a centralized, ~8-day, closed manufacturing process of CAR T cells is feasible for a multicenter trial; and (3) therapy with

Table 4. Characteristics of KTE-C19 Products

	Patient No.						
	1	2	3	4	5	6	7
CAR T cells/kg × 10^6	2.0	2.0	2.0	1.1	2.0	1.9	1.2
CD4 T cells (%)	18	73	30	34	51	30	68
CD8 T cells (%)	82	27	70	66	49	70	32
CD8/CD4 T cell ratio	4.6	0.4	2.3	1.9	1	2.3	0.5
IFN-γ production in co-culture (pg/mL)[a]	20,930	8,589	3,356	7,598	6,948	2,278	816
Manufacturing time (days)	8	8	8	8	8	9	8

[a]Co-culture experiments were performed using Toledo cells mixed in a 1:1 ratio with KTE-C19 product cells. IFN-γ was measured in cell culture media 24 hr post-incubation using a qualified ELISA.

cryopreserved KTE-C19 product is associated with robust CAR T cell expansion and durable clinical responses that are similar to those observed with fresh anti-CD19 CAR T cell therapy in studies conducted at single institutions.[14] The results of this phase 1 study led to the initiation of the pivotal ZUMA-1 phase 2 registration trial.

The conditioning chemotherapy regimen doses utilized in ZUMA-1 were selected based on the results of the NCI clinical trial demonstrating that lower doses of chemotherapy afforded clinical efficacy with an attenuated toxicity profile versus higher doses of conditioning chemotherapy.[15] Although the fludarabine dose was comparable, the dose of cyclophosphamide utilized here is significantly lower than that used in other trials of CAR T cell therapy.[16–18] The lower doses of the conditioning chemotherapy with KTE-C19 were also associated with an acceptable toxicity profile. CRS and neurotoxicity were the two primary categories of KTE-C19-related AEs. Grade ≥3 CRS and neurotoxicity were observed in 14% (n = 1/7) and 57% (n = 4/7) of patients, respectively. One of seven patients (14%) experienced a DLT of grade 4 CRS and neurotoxicity, and none of the patient deaths was attributed to KTE-C19.

Close examination of the patient who experienced a DLT was revealing. The patient experienced cyclical fevers prior to cell infusion on day 0 attributed to rapidly progressive bulky disease with B symptoms. Although blood cultures remained negative, the patient had mucositis with underlying active HSV-1 that was being treated through KTE-C19 infusion. Biomarker analysis of this patient revealed a baseline CRP of 655 mg/L (normal range, 0–10 mg/L) in conjunction with the elevation of other inflammatory markers (IL-6, TNF-α). This timing and the nature of the cytokine profile were in stark contrast to the other patients in this trial (Figures 3B and 3C), indicating an extremely high level baseline inflammatory state. Following this DLT, the protocol was amended for safety so that patients having an active infection needing treatment or worsening of their end organ function at the time of planned initiation of conditioning chemotherapy would be ineligible to proceed with conditioning chemotherapy or infusion of KTE-C19. Except for the patient who experienced a DLT, all CRS and neurotoxicity AEs were self-limiting, and resolved within 1 month at a similar incidence with those observed on single institutional anti-CD19 CAR T cell trials for B cell malignancies.[19,20] Consistent with the "on-target, off-tumor" effect of KTE-C19, B cell aplasia and hypogammaglobulinemia were observed in subjects with ongoing complete response and persistent CAR T cells at 12 months post-infusion.

Despite the small numbers in this study, the overall and CR rates were high and durable relative to historical controls. Durable efficacy of the KTE-C19 regimen was observed in patients with rigorously defined chemotherapy refractory disease who had no viable treatment options. Rapid CRs were demonstrated after only 1 month of follow-up in only those four (57%) patients who relapsed after prior ASCT, and responses are ongoing at 12+ months in three of seven (43%) patients. In these three patients, the duration of response with KTE-C19 markedly exceeded the time to relapse after their prior ASCT. This is remarkable, as the expected CR rate in this chemotherapy refractory patient population is 8%, and median OS is 6.6 months with conventional therapies.[10] Notably, responses in ZUMA-1 were observed without the use of any bridging chemotherapy: all patients proceeded directly from apheresis to conditioning chemotherapy and KTE-C19 infusion.

Comprehensive characterization of the apheresis product, KTE-C19, and blood/serum samples post-infusion provided insights into the possible mechanisms of efficacy and toxicity observed in these patients. The closed, approximately 8-day, centralized manufacture of a cryopreserved product consistently yielded active CAR T cells in all patients, across a broad range irrespective of lymphocyte counts at time of enrollment (median, 900 lymphocytes/μL; range, 100–1,400/μL). The product T cells showed a less differentiated phenotype based on CCR7 and CD45RA expression, considered desirable for adoptive T cell therapies (Figure S2) as naive and central memory T cells have increased proliferative capacity.[21–23] Adequate $CD4^+$ and $CD8^+$ CAR T cells in the final product were obtained without pre-selection of T cell subsets.

The low-dose cyclophosphamide and fludarabine conditioning chemotherapy alone was capable of enhancing several homeostatic cytokines and chemokines, most notably IL-15, a critical T cell proliferative cytokine.[24] Conditioning chemotherapy followed by KTE-C19 infusion resulted in a rapid and sequential induction,

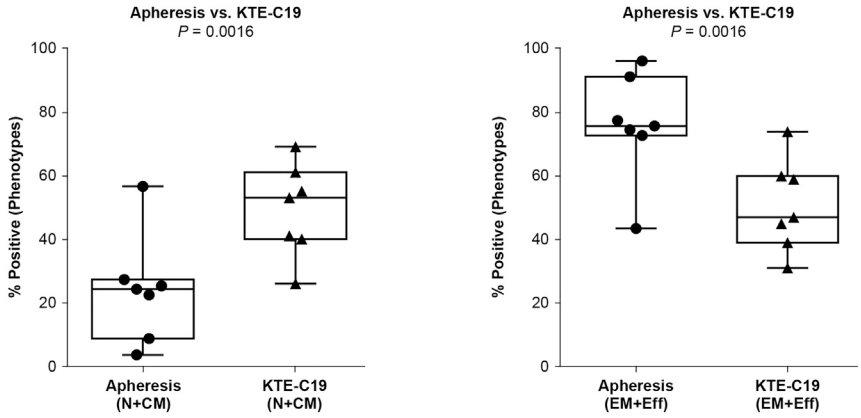

Figure 2. Apheresis and Product Phenotype as Determined by Flow Cytometry Using CD45RA and CCR7 Cell Surface Markers

N, naive; CM, central memory; EM, effector memory; Eff, effector. The bars and boxes show the minimum, maximum, median, and interquartile range.

elevation, and general clearance of an array of cytokines, chemokines, and immune effector proteins (Figure 3C). More specifically, markers, including CRP, IL-6, TNF-α, IFN-γ, and IL-15, increased with peak values occurring 2–3 days post-infusion, and typically resolved to baseline within the first 28 days (Figure S3). Levels of Granzyme B, a key effector of T cell-mediated cytotoxicity, peaked later, 3–7 days post-infusion, providing evidence of when immune-mediated tumor cell killing may occur. This sequence of events paralleled CAR T cell expansion within 1–2 weeks after T cell infusion (Figure 3).

The timing of the elevations and general clearance of these systemic cytokines, chemokines, and immune effectors are similar in timing to the onset and resolution of CRS and neurotoxicity. Although the etiology of CRS is better understood, the pathophysiology of neurotoxicity that manifests as toxic encephalopathy remains unclear. The neurotoxicity reported here has been observed across other anti-CD19 CAR T cell studies and with blinatumomab, a bispecific T cell engager to CD19.[18,19,25–27] Given that there is no direct evidence of CD19 expression in the CNS, two hypotheses that may not be mutually exclusive arise as the cause of neurotoxicity: either passive diffusion into the CNS of systemically generated cytokines; or activated CAR T cells migrating through the blood-brain barrier with subsequent local production of cytokines within the CNS.[28,29] Regardless, the neurotoxicity is generally reversible resolving in a similar temporal fashion to the abatement of peak CAR T cell and cytokines levels.

The agents effectively used for management of CRS and neurotoxicity, tocilizumab and/or systemic corticosteroids, did not appear to ablate CAR T cell expansion nor alter the CAR T cell-related elevation of cytokines, chemokines, and immune effector molecules. Importantly, durable responses were observed in patients who received tocilizumab and/or corticosteroids for toxicity management as well as those who did not. Overall, this analysis suggests the CAR T infusion in concert with chemotherapy conditioning creates an environment that promotes CAR T cell expansion, trafficking, anti-tumor activity, and persistence for an interval of time sufficient to mediate clinical activity.

In conclusion, the results of this phase 1 portion of ZUMA-1, the first multicenter study of anti-CD19 CAR T cell therapy in aggressive NHL, demonstrated that the KTE-C19 regimen was tolerable and safe for further study. It also validated that centralized manufacturing is feasible and established the logistics for transportation of patient-specific product door to door within approximately 2 weeks. More importantly, it showed that a single infusion of cryopreserved KTE-C19 cells could provide durable clinical responses in refractory DLBCL patients including those whose disease has failed to respond to ASCT. Together, these results fulfilled a prerequisite to broaden clinical applicability of this patient-specific adoptive T cell therapy and have led to the initiation of the pivotal multicenter phase 2 portion of ZUMA-1 for patients with refractory aggressive B cell NHL with an unmet clinical need.

MATERIALS AND METHODS

Patient Population

The ZUMA-1 study is a phase 1/2, single-arm, open-label study evaluating the safety and efficacy of anti-CD19 CAR T cells (KTE-C19) in patients with refractory aggressive NHL. Eligible patients must have had all of the following: (1) histologically confirmed B cell NHL, including DLBCL not otherwise specified, primary mediastinal large B cell lymphoma, or transformed follicular lymphoma (TFL) as defined by the World Health Organization 2008 criteria; (2) chemotherapy refractory disease (as defined in the SCHOLAR-1 study:[10] progressive disease or stable disease lasting $\leq$6 months, as best response to most recent chemotherapy regimen; or disease progression or recurrence $\leq$12 months after prior ASCT); (3) prior therapy must have included an anti-CD20 monoclonal antibody-containing regimen and an anthracycline-containing chemotherapy regimen; for patients with TFL, prior chemotherapy for follicular lymphoma and subsequent refractory disease after transformation to DLBCL; (4) at least one measurable lesion according to revised International Working Group (IWG) Response criteria;[30] (5) no evidence of CNS lymphoma by magnetic resonance imaging; and (6) $\geq$2 weeks since prior radiation therapy or systemic therapy at the time of leukapheresis. Eligible patients were also aged $\geq$18 years, with ECOG performance status of 0 or 1, absolute neutrophil count of $\geq$1,000/μL, and platelet count of $\geq$50,000/μL. Patients must have had adequate renal, hepatic, and cardiac function defined as serum creatinine of $\leq$1.5 mg/dL, serum alanine aminotransferase/aspartate aminotransferase of $\leq$2.5 times the upper limit of normal, total bilirubin of $\leq$1.5 mg/dL (except in patients with Gilbert's syndrome), cardiac ejection fraction of $\geq$50%, and no evidence of pericardial effusion, as determined by an

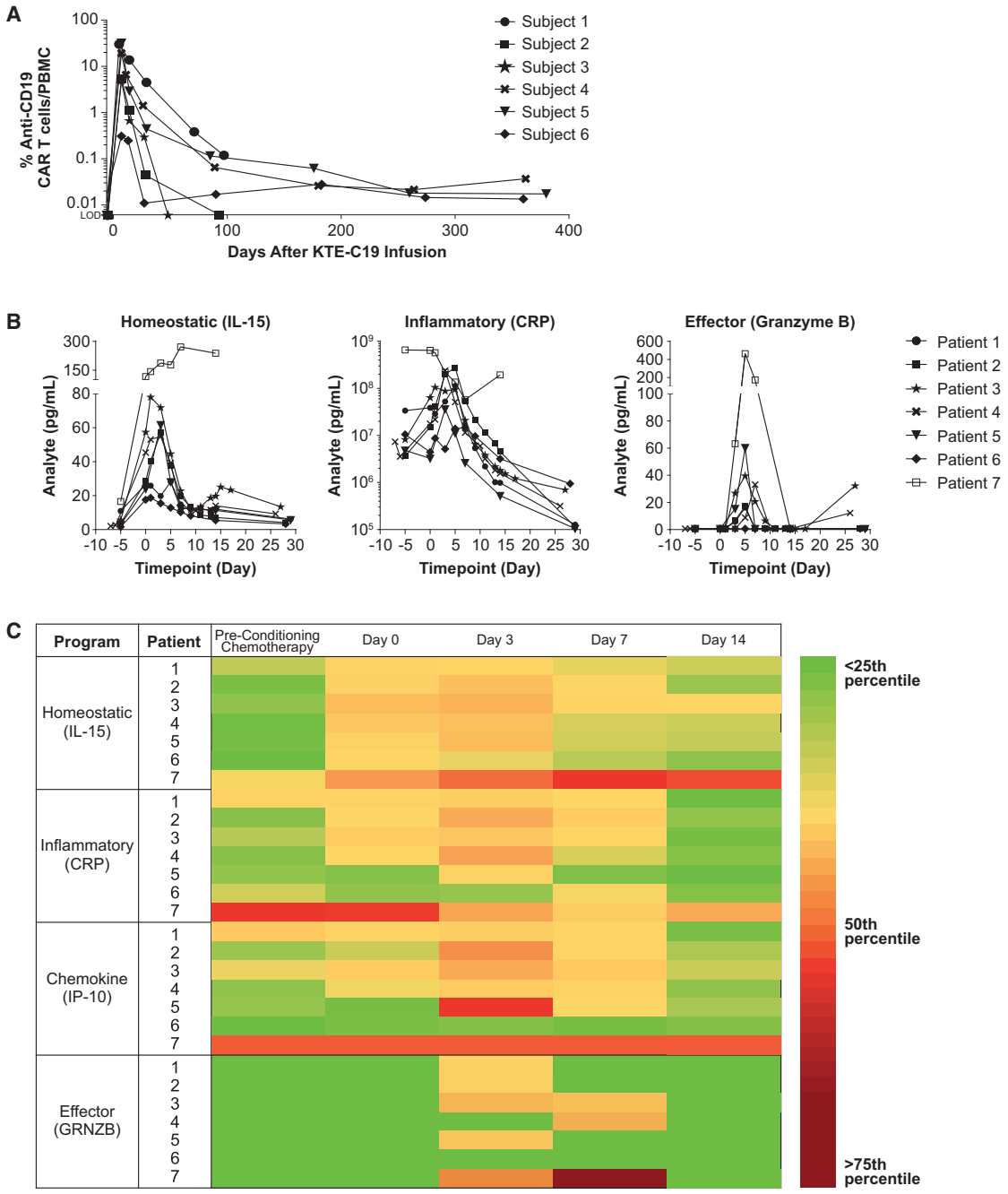

Figure 3. Kinetics of Peripheral Blood CAR T Cells and Serum Biomarkers

(A) PCR data demonstrates exponential expansion and persistence of CD19 CAR T cells in blood. Expansion occurs rapidly with peak levels achieved within the first 7–14 days post-KTE-C19 infusion (note: patient 7 was not tested). Persisting CD19 CAR T cells were detectable in six of six (100%) patients at week 4 and in four of five (80%) patients with samples available for testing at month 3. Three patients with ongoing CR had detectable CAR T cells at 12 months. Limit of detection of the qPCR assay is 0.001% or 1×10^{-5}. (B) Analysis of patient serum reveals a biomarker profile composed of specific cytokines, chemokines, and effector proteins associated with KTE-C19 treatment. The expansion of CD19 CAR T cells (Figure 3A) was mirrored by induction and elevation of a range of cytokines that regulate proliferation, activation, and effector function. Induction of IL-15 occurs during conditioning chemotherapy and levels continue to rise post-infusion, promoting anti-CD19 CAR T cell expansion. CRP levels parallel CRS and generally resolve within the first 28 days. Granzyme B levels peak 3–7 days post-infusion, during peak anti-CD19 CAR T cell expansion, and provide evidence of effector function and tumor killing. (C) Heat map of serum biomarkers demonstrates sequential induction and gradual resolution within the first 2 weeks after KTE-C19 infusion of key cytokines, chemokines, and effector proteins. Patients 1–6 demonstrated similar baseline levels and post-infusion kinetics for induction of IL-15

(legend continued on next page)

echocardiogram. Key exclusion criteria included history of malignancy other than non-melanoma skin carcinoma in situ, ASCT within 6 weeks of informed consent, history of allogeneic hematopoietic stem cell transplant, prior CD19-targeted therapy, and prior CAR T cell therapy (except KTE-C19). All patients provided written, informed consent. The Institutional Review Board/Independent Ethics Committee of each study site approved the protocol. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Phase 1 Study Design and Toxicity Evaluation

In the phase 1 portion of ZUMA-1, the primary endpoint was incidence of DLTs. The study began with cohort A1. Progression to phase 2 would occur if the patient incidence of DLT was less than or equal to one of six DLT-evaluable patients in cohort A1. Dose-limiting toxicities were defined as KTE-C19-related events with onset within 30 days of infusion and included grade 4 neutropenia or thrombocytopenia lasting longer than 21 or 35 days, respectively; any KTE-C19-related AE requiring intubation; all other grade 3 toxicities lasting more than 3 days; and any grade 4 toxicities. Exceptions to this definition, not counting as DLT, included the following: aphasia/dysphasia or confusion/cognitive disturbance resolving to grade ≤ 1 within 2 weeks and baseline within 4 weeks; grade 3 fever or myelosuppression; or grade 3 immediate hypersensitivity reaction within 2 hr reversible to grade ≤ 2 within 24 hr; and grade 3 or 4 hypogammaglobulinemia.

The investigators were responsible for monitoring and reporting of all AEs through 3 months post-infusion. Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. For CRS, a revised grading system created by Lee et al.[13] was used. After 3 months, investigators monitored and reported on a targeted subset of AEs, including neurological and hematological AEs, infections, autoimmune disorders, and secondary malignancies for 24 months (or until disease progression, whichever occurred first). Secondary endpoints included objective response rate per revised IWG Response Criteria for Malignant Lymphoma per Cheson et al.,[30] duration of response, progression-free survival, OS, incidence of AEs and clinically significant changes in laboratory values, incidences of anti-KTE-C19 antibodies, and levels of anti-CD19 CAR^+ T cells and cytokines in blood and serum. Exploratory endpoints included objective response rate (ORR) and duration of second response among patients retreated with KTE-C19, and biomarker development based on assessment of blood and tumor cells and KTE-C19.

Study Procedures and Treatment

Patients were enrolled following screening and confirmation of eligibility and underwent leukapheresis within 5 days of enrollment to obtain peripheral blood mononuclear cells (PBMCs) to manufacture KTE-C19. Approximately 5 to 10×10^9 mononuclear cells from 12 to 15 L of apheresis material were shipped overnight to the central cell processing facility and enriched for the T cell-containing PBMC fraction. T cells were then stimulated to expand with anti-CD3 and IL-2 and transduced with a retroviral vector containing the CAR gene construct.[20] Following expansion, the final KTE-C19 product was washed, cryopreserved, and tested for identity, potency, sterility, and adventitious agents. After meeting acceptance criteria, the KTE-C19 product was shipped back to the clinical sites using a validated cryo-shipper within approximately 2 weeks.[31]

Before receiving CAR T cell infusion, per cohort A1 parameters, patients received a non-myeloablative low-dose conditioning chemotherapy regimen of fludarabine at 30 mg/m^2/day and cyclophosphamide at 500 mg/m^2/day on day −5, day −4, and day −3. On day 0, hospitalized patients received a single intravenous infusion of KTE-C19 at a target dose of 2×10^6 CAR^+ T cells/kg (minimum of 1×10^6 CAR^+ T cells/kg) and remained hospitalized to recovery through day 7 or until all KTE-C19-related non-hematological toxicities returned to grade ≤ 1 or baseline.

Patients were followed in the post-treatment assessment period and returned to the clinic at week 2, week 4 (±3 days), month 2 (±1 week), and month 3 (±1 week). All patients completing the month 3 visit were followed in the long-term follow-up period for survival and disease status every 3 months (±2 weeks) through month 18, every 6 months (±1 month) between months 24 and 60; beginning with year 6 (month 72 ± 3 months), patients will return to the clinic once annually for up to 15 years. Patients achieving a CR or PR could have received a second course of conditioning chemotherapy and KTE-C19 if their disease progressed (not due to $CD19^-$ malignant cells) as part of an exploratory analysis. Each patient was allowed a maximum of one retreatment course.

Biomarker Analysis

Multiparametric flow cytometry was performed from apheresis through manufacturing, and final product, with in vivo characterization until end of study. Biomarker analyses were performed on blood and tumor samples to evaluate predictive and pharmacodynamic markers for KTE-C19. The presence, expansion, and persistence of transduced anti-CD19 CAR^+ T cells in the blood were monitored by PCR. Levels of 44 serum cytokines, chemokines, circulating angiogenic factors, immune effector molecules, and markers of macrophage activating syndrome (MAS) were also assessed. Archived tumor tissue was collected for central pathology review.

Measurement of Serum Cytokines

Patient serum was harvested at the following time points: day −5 (prior to conditioning), day 0 (prior to KTE-C19 administration),

(T cell proliferation), CRP (marker of inflammation), granzyme B (evidence of effector function), and IP-10 (chemokine that promotes CAR T cell homing). Early induction of IL-15, CRP, and IP-10 was observed 1–3 days post-infusion and effector function occurred around days 3–7 for these patients. In contrast, patient 7 demonstrated a dysregulated profile relative to the other six patients both at baseline (IL-15, CRP, and IP-10) and after KTE-C19 administration (all markers), indicative of an inflammatory state prior to KTE-C19.

every other day during hospitalization, day 14 and day 28. Serum samples were processed locally using standard serum separator tubes (BD Biosciences). Serum samples were held at −80°C for subsequent analysis by Luminex (EMD Millipore) or Meso Scale Discovery (MSD). Prior to processing, serum samples were briefly thawed on ice and aliquoted into 96-well U-bottom plates (BD Biosciences). Samples were analyzed using the following MSD kits: MSD V-PLEX Plus Angiogenesis Panel 1 (Human) Kit (bFGF, Flt-1/VEGFR-1, PlGF, Tie-2, VEGF-A, VEGF-C, VEGF-D), MSD V-PLEX Plus Chemokine Panel 1 (Human) Kit (Eotaxin, Eotaxin-3, IL-8 [HA], IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC), MSD V-PLEX Plus Cytokine Panel 1 (Human) Kit (GM-CSF, IL-12/IL-23p40, IL-15, IL-16, IL-17A, IL-1α, IL-5, IL-7, TNF-β), MSD V-PLEX Plus Proinflammatory Panel 1 (Human) Kit (IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α), MSD V-PLEX Plus Vascular Injury Panel 2 (Human) Kit (CRP, ICAM-1, SAA, VCAM-1), and Luminex HCD8MAG-15K-04 (Granzyme A, Granzyme B, sFASL, and Perforin). All assays were carried out according to the manufacturer's specifications. Quality and assay standard controls were included for independent runs per the manufacturer's protocol.

All MSD assays were read using a MESO QuickPlex SQ 120 and analysis was performed using DISCOVERY WORKBENCH 4.0 (MSD). Luminex assays were read using a Luminex 200 system, and analysis was performed using Bio-Plex Data Pro software (Bio-Rad). Analyte values were reported as picograms per milliliter of serum.

Flow Cytometry Analysis of Product, Apheresis Material, and CAR T Cells after Co-culture with Target Cells

As part of standard release criteria, KTE-C19 products were evaluated by flow cytometry for anti-CD19 CAR surface expression, percentage of CD4 and CD8 T cells, and also memory phenotypes (Naive, Tcm, Tem,, and Teff as defined by CCR7 and CD45RA). Testing was performed by Progenitor Cell Therapy, using validated protocols.

Cryopreserved apheresis samples were rapidly thawed in a 37°C water bath, washed twice with calcium-free/magnesium-free PBS (VWR Scientific) by gentle centrifugation, and resuspended in FACS Stain Buffer (BD Biosciences). Sample viability and cell density were measured using a Vi-cell as described. Cell densities were adjusted to 1.0×10^6 viable cells/mL in FACS stain buffer. Cells were aliquoted into 5-mL FACS tubes (BD Biosciences) at a final density of 1.0×10^6 viable cells/test. Cells were stained for 30 min on wet ice with the following commercially available fluorochrome-conjugated antibodies from BioLegend, CD3 fluorescein isothiocyante (FITC), CCR7 Brilliant Violet 650 (BV650), CD45RA allophycocyanin (APC), and CD4 Alexa Fluor 700 (AF700).

Cells from co-cultures of CAR T cells and target cell lines CD19-K562 or NGFR-K562 were pooled for analysis by flow cytometry. Sample viability and cell density were measured using a Vi-cell as previously described. Cell densities were adjusted to 1.0×10^6 viable cells/mL. Cells were aliquoted into 5-mL polystyrene FACS tubes (BD Biosciences) at a density of 1.0×10^6 viable cells. Staining was performed for 30 min on wet ice with the following commercially available fluorochrome-conjugated antibodies: (1) from BD Biosciences, PD1 phycoerytherin cyanine 7 (PE-Cy7) and CD57 Brilliant Violet 421 (BV421); (2) from BioLegend, CD3 (FITC), CCR7 (BV650), CD45RA (APC), CD4 (AF700), CD69 (BV510), CD137 (BV421), and CD107a (AF700); (3) from the Surgery Branch of the NCI, anti-CD19 CAR PE. All antibodies used in this study were titrated before use.

Prior to flow-cytometric analysis, samples were stained with propidium iodide (BD Biosciences) to exclude dead and apoptotic cells. Flow cytometry was performed using a FACSCanto II (BD Biosciences). For phenotypic markers, data were reported as percent positive (% pos) relative to the appropriate fluorescence minus one (FMO) control. For activation markers, data were reported relative to the CAR T cell products not subjected to co-culture. The analysis employed a cell-gating strategy that selected viable $CD3^+$ and anti-CD19 $scFV^+$ cells and excluded dead/apoptotic cells. A notable exception to the gating strategy in co-cultured CAR T cell products was a shifting of the gating strategy to viable $CD3^+$ cells (and not anti-CD19 $scFV^+$ cells) due to downregulation of surface CAR on T cells following engagement of the target antigen. Where feasible, data from a minimum of 1×10^4 viable cells were acquired. Data analysis was performed using FlowJo software, version 10 (FLOWJO) using standardized gating and compensation strategies.

Measurement of Anti-CD19 CAR T Cell Presence, Expansion, and Persistence

A qPCR assay, previously described,[14,15,32] was optimized and validated by the University of Rochester Medical Center Central Lab Services (URMC CLS) for monitoring of anti-CD19 CAR T cell expansion and persistence. Sensitivity of the optimized method is 0.001% or 1×10^{-5}. Testing was performed by URMC CLS using cryopreserved PBMC at baseline, prior to conditioning chemotherapy and KTE-C19 administration, at days 7, 14, and 28, and months 3, 6, 9, and 12.

Measurement of Anti-KTE-C19 Antibodies

Presence of anti-KTE-C19 antibodies were monitored by Intertek Laboratories using a qualified bridge ELISA designed to specifically detect anti-FMC63 antibodies in patient serum. Testing was performed at baseline, prior to conditioning chemotherapy and KTE-C19 administration, and at months 1 and 3.

Confirmatory Diagnosis of DLBCL in Archival Tumor Samples

Archived tumor tissue was collected for central pathology review at NeoGenomics Laboratories.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Materials and Methods, three figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.ymthe.2016.10.020.

AUTHOR CONTRIBUTIONS

F.L.L. (Moffitt Cancer Center) and S.S.N. (MD Anderson Cancer Center) participated in the study as investigators, reviewed and interpreted the data, and approved all drafts of the manuscript. F.L.L., S.S.N., N.L.B., T.S., J.C.C., C.M.H., A.G., L.E.B., A.B., J.M.R., Y.J., A.X.X., M.E., J.A., J.W., and W.Y.G. had access to and reviewed the study data, and carefully reviewed and approved all drafts of the manuscript including the final version.

CONFLICTS OF INTEREST

F.L.L. has served as a scientific advisory board attendee for Kite Pharma. S.S.N. has received research funding and honoraria from and served as a consultant and Scientific Board Member for Kite Pharma. N.L.B. served in a consultancy or advisory role for Gilead. T.S. has served on speakers' bureau for Pharmacyclics and Seattle Genetics and has received travel and lodging support from Kite Pharma. J.C.C., C.M.H., A.G., and L.E.B. declare no conflicts of interest. A.B., J.M.R., Y.J., A.X.X., M.E., J.A., J.W., and W.Y.G. are employed by and have equity ownership in Kite Pharma.

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ORIGINAL REPORT

Lymphoma Remissions Caused by Anti-CD19 Chimeric Antigen Receptor T Cells Are Associated With High Serum Interleukin-15 Levels

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ABSTRACT

Purpose

T cells genetically modified to express chimeric antigen receptors (CARs) targeting CD19 (CAR-19) have potent activity against acute lymphoblastic leukemia, but fewer results supporting treatment of lymphoma with CAR-19 T cells have been published. Patients with lymphoma that is chemotherapy refractory or relapsed after autologous stem-cell transplantation have a grim prognosis, and new treatments for these patients are clearly needed. Chemotherapy administered before adoptive T-cell transfer has been shown to enhance the antimalignancy activity of adoptively transferred T cells.

Patients and Methods

We treated 22 patients with advanced-stage lymphoma in a clinical trial of CAR-19 T cells preceded by low-dose chemotherapy. Nineteen patients had diffuse large B-cell lymphoma, two patients had follicular lymphoma, and one patient had mantle cell lymphoma. Patients received a single dose of CAR-19 T cells 2 days after a low-dose chemotherapy conditioning regimen of cyclophosphamide plus fludarabine.

Results

The overall remission rate was 73% with 55% complete remissions and 18% partial remissions. Eleven of 12 complete remissions are ongoing. Fifty-five percent of patients had grade 3 or 4 neurologic toxicities that completely resolved. The low-dose chemotherapy conditioning regimen depleted blood lymphocytes and increased serum interleukin-15 (IL-15). Patients who achieved a remission had a median peak blood CAR^+ cell level of 98/μL and those who did not achieve a remission had a median peak blood CAR^+ cell level of 15/μL ($P = .027$). High serum IL-15 levels were associated with high peak blood CAR^+ cell levels ($P = .001$) and remissions of lymphoma ($P < .001$).

Conclusion

CAR-19 T cells preceded by low-dose chemotherapy induced remission of advanced-stage lymphoma, and high serum IL-15 levels were associated with the effectiveness of this treatment regimen. CAR-19 T cells will likely become an important treatment for patients with relapsed lymphoma.

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INTRODUCTION

Chimeric antigen receptors (CARs) are artificial proteins that incorporate an antigen recognition domain and T-cell signaling domains.[1-8] T cells genetically modified to express a CAR recognize and kill malignant cells expressing the antigen targeted by the CAR.[9-11] T cells expressing CARs that target the B-cell antigen CD19 (CAR-19) have potent activity against acute lymphoid leukemia (ALL).[12-16] Lymphoma is much more common than ALL,[17,18] but compared with ALL, fewer cases of effective treatment of lymphoma with CAR-19 T cells have been published.[19-22] Diffuse large B-cell lymphoma (DLBCL) that is either refractory to chemotherapy[17,18,23-25] or relapsed after autologous stem-cell transplantation (ASCT)[26,27] carries a grim prognosis. New therapies are needed for advanced-stage B-cell lymphomas. CAR T cells cause adverse events including but not limited to hypotension, cardiac toxicity, and neurologic

ASSOCIATED CONTENT

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toxicities. These toxicities are thought to be caused by cytokines directly released by CAR T cells or released by other cells in response to cytokines produced by CAR T cells.[12,13,15,21,28]

Recipient leukocyte depletion by radiation therapy or chemotherapy enhanced the antitumor activity of adoptively transferred T cells in multiple murine models,[29-31] and most clinical trials of adoptive T-cell therapy include chemotherapy before T-cell infusions.[12,14-16,20,21] In mice, one way that recipient lymphocyte depletion enhances the antitumor activity of adoptively transferred T cells is by increasing serum levels of cytokines such as interleukin-15 (IL-15).[30] IL-15 is a glycoprotein in the 4-α-helix bundle family of cytokines.[32-34] IL-15 is primarily produced by dendritic cells, monocytes, and macrophages,[32,34] and it induces T-cell proliferation and enhances T-cell function.[32-34]

We previously treated B-cell malignancies with CAR-19 T cells preceded by treatment with fludarabine and high-dose cyclophosphamide.[21] This high-dose chemotherapy regimen combined with an infusion of CAR-19 T cells resulted in many long-term remissions of B-cell malignancies, but it also caused significant toxicity.[20,21] We hypothesized that administering a low-dose chemotherapy regimen before infusion of CAR-19 T cells would promote their antilymphoma activity and would cause less hematologic toxicity than high-dose chemotherapy. Use of low-dose chemotherapy would also allow a clear determination of the antilymphoma activity of CAR-19 T cells because the low-dose chemotherapy has limited direct antilymphoma activity.

PATIENTS AND METHODS

Clinical Trial and Patient Information

All enrolled patients gave informed consent. The protocol was approved by the Institutional Review Board of the National Cancer Institute. CD19 expression by malignancies was confirmed by either flow cytometry or immunohistochemistry.

Preparation of Anti-CD19 CAR T Cells and Ex Vivo Assays

The CAR used in this work was encoded by a gamma-retroviral vector and contained an anti-CD19 single-chain variable fragment derived from a murine monoclonal antibody, hinge and transmembrane regions from human CD28, the CD28 costimulatory domain, and the CD3ζ T-cell activation domain.[35] Anti-CD19 CAR T cells were cultured for 6 to 10 days by adding the anti-CD3 monoclonal antibody OKT3 directly to whole (unsorted) peripheral blood mononuclear cells suspended in culture medium containing IL-2 and transducing the cells as described in the Data Supplement.[20,36] CAR T-cell doses were administered as $CD3^+CAR^+$ cells per kilogram of body weight (Table 1). The percentage of CAR^+ T cells was determined by flow cytometry and was used to calculate the number of cells to infuse. Flow cytometry, immunohistochemistry, cytokine assays, and quantitative polymerase chain reaction are described in the Data Supplement.[11,20,35]

Anti-CD19 CAR Treatment Plan

A fludarabine and cyclophosphamide chemotherapy regimen[37] was administered before CAR-19 T-cell infusions to enhance the activity of adoptively transferred T cells.[29-31] Both chemotherapy agents were given intravenously once per day for 3 days on the same days (Fig 1A). Treatment responses were defined according to standard international criteria.[38]

RESULTS

Patient Characteristics

Nineteen of the 22 treated patients had one of the various types of DLBCL, two patients had follicular lymphoma, and one patient had mantle cell lymphoma (Table 1). Eleven of 19 patients with DLBCL had chemotherapy-refractory lymphoma. Five other patients with DLBCL had lymphoma that had relapsed 10 months or less after ASCT as their last treatment prior to protocol enrollment. Eleven patients with DLBCL were high risk by second-line, age-adjusted International Prognostic Index.[39] The median number of unique lymphoma therapies received before protocol enrollment was four (range, one to seven). Therapies received before protocol enrollment and lymphoma chromosome rearrangements are provided in the Data Supplement.

Chemotherapy Regimen Depleted Lymphocytes and Modulated Multiple Serum Proteins

The chemotherapy depleted recipient lymphocytes (Fig 1B; Data Supplement). We compared levels of 41 serum proteins before and after the low-dose chemotherapy regimen (Data Supplement). Serum IL-15 levels increased in all 22 patients after the chemotherapy regimen (Figure 1C). Serum perforin decreased after chemotherapy administration.

Infused CAR-19 T-Cell Characteristics

A median of 97.6% (range, 90.6% to 99.5%) of the infused cells were $CD3^+$; a median of 74.4% (range, 39.4% to 93.6%) of the infused $CD3^+$ cells expressed CAR-19. A median of 43.4% of $CD3^+CAR^+$ cells expressed CD4 (range, 1.9% to 82.1%), and 54% of $CD3^+CAR^+$ cells expressed CD8 (range, 13.4% to 92.1%). C-C chemokine receptor-7 (CCR7) and CD45RA were used to divide T cells into four different subsets (Fig 1D). Naïve and central memory T cells have greater proliferative capacity than effector memory and effector memory-RA T cells.[40] Infused CAR-19 T cells included T cells with the phenotypes of all four of these T-cell subsets, including a substantial fraction of cells with the phenotype of central memory T cells.[40] The CAR T cells secreted a variety of proteins in an antigen-specific manner (Fig 1E-H; Data Supplement).

CAR-19 T Cells Induced Remissions of Lymphoma

For all 22 patients treated, there was a 73% remission rate with 55% complete remissions (CRs) and 18% partial remissions (PRs). Among patients with DLBCL, the overall remission rate was 68% with 47% CRs and 21% PRs. The duration of responses currently ranges from 7 months to 24 months (Fig 2A). Twelve of 22 patients achieved CRs, 11 of 12 CRs are ongoing, and the current median duration of all CRs is 12.5 months (Table 1). No patient with an ongoing remission received any further antilymphoma therapy after CAR T-cell infusion. In contrast to CRs, no PRs or outcomes of stable disease (SD) are ongoing. Patient 40 underwent allogeneic hematopoietic stem-cell transplantation after achieving a PR (Table 1). The 12-month progression-free survival of all patients was 63.3% (Fig 2B), and overall survival is

Table 1. Patient Characteristics

Patient No.[a]	Age (years)	Lymphoma Type	Lymphoma Status[b]	No. of Prior Lines of Treatment	sAAIPI[c]	CAR+ T-Cell Dose (per kg)	Best Response	Duration of Response (months)[d,e]
22	66	DLBCL, NOS	Relapse after ASCT	3	High	1×10^6	PR	7
23	63	Follicular	Neither	6	NA	1×10^6	CR	19[f]
24	65	DLBCL, transformed from follicular	Relapse after ASCT	4	High	1×10^6	PR	14
25	47	DLBCL, NOS	Chemotherapy-refractory	2	High	1×10^6	PR	1
26	26	DLBCL, NOS	Chemotherapy-refractory	7	High	1×10^6	PD	NA
27	62	DLBCL, transformed from follicular	Neither	7	Intermediate	1×10^6	CR	24+
28	54	DLBCL, NOS	Chemotherapy-refractory	3	High	1×10^6	PD	NA
29	28	DLBCL, NOS	Chemotherapy-refractory	2	High	2×10^6	SD	2
30	29	PMBCL[g]	Chemotherapy-refractory	3	Low	2×10^6	SD	3
31	65	DLBCL, transformed from follicular	Relapse after ASCT	5	Intermediate	2×10^6	CR	18+
32	40	PMBCL	Chemotherapy-refractory	2	Intermediate	6×10^6	PD	NA
33	67	DLBCL, transformed from CLL	Neither	3	High	2×10^6	CR	15+
34[h]	50	Mantle cell	Neither	1	NA	2×10^6	CR	17+
35[h]	53	DLBCL, NOS	Chemotherapy-refractory	4	Intermediate	2×10^6	CR	13+
36[h]	66	Follicular	Relapse after ASCT	3	NA	2×10^6	CR	11+
37[h]	51	DLBCL, NOS	Relapse after ASCT	3	Intermediate	2×10^6	CR	12+
38	51	DLBCL, NOS	Chemotherapy-refractory	5	High	2×10^6	CR	13+
39	62	DLBCL, NOS	Relapse after ASCT	6	Intermediate	2×10^6	CR	12+
40	39	DLBCL, NOS	Chemotherapy-refractory	4	High	2×10^6	PR	3[i]
41	67	DLBCL, NOS	Neither	4	Intermediate	2×10^6	CR	7+
42	64	DLBCL, NOS	Chemotherapy-refractory	4	High	2×10^6	CR	11+
43	51	DLBCL, NOS	Chemotherapy-refractory	5	High	2×10^6	PD	NA

Abbreviations: ASCT, autologous stem-cell transplantation; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; NA, not applicable; NOS, not otherwise specified; PD, progressive disease; PMBCL, primary mediastinal B-cell lymphoma; PR, partial remission; sAAIPI, second-line age-adjusted International Prognostic Index; SD, stable disease.

[a]Patients 22 through 43 were sequentially enrolled on the protocol from August 2013 to September 2015.

[b]Chemotherapy refractory was defined as failure to achieve PR or CR after the most recent chemotherapy. Relapse after ASCT is listed if the lymphoma had relapsed after ASCT, and ASCT was the last chemotherapy-containing treatment that the patient received before enrollment on the CAR targeting CD19 (CAR-19) trial, so the lymphoma was not proven to be chemotherapy-refractory at the time of protocol enrollment. "Neither" means that the lymphoma was not chemotherapy refractory and that the patient did not have an ASCT as the last chemotherapy-containing treatment before CAR-19 protocol enrollment.

[c]This incorporates lactate dehydrogenase, Karnofsky performance score, and lymphoma stage to separate patients with DLBCL receiving second-line therapy into low-, intermediate-, and high-risk groups. NA means not applicable because these patients did not have DLBCL.

[d](+) Indicates an ongoing response.

[e]Duration of response is the time elapsed from first documentation of response until progressive disease. Some patients with CR had an initial response of PR that evolved into a CR over time as positron emission tomography and computed tomography scans normalized.[f]Patient 23 developed myelodysplastic syndrome and went off-study to receive treatment for this disorder after a remission duration of 19 months. Also, after 19 months of remission, scattered CD10-expressing B cells were detected on bone marrow immunohistochemistry, which was consistent with recurrence of follicular lymphoma.

[g]PMBCL is a type of DLBCL.

[h]These patients received cyclophosphamide at 500 mg/m^2; all other patients received 300 mg/m^2.

[i]Patient 40 proceeded to allogeneic hematopoietic stem-cell transplantation while in PR.

provided in the Data Supplement. Figure 2C-D shows two examples of positron emission tomography/computed tomography scans from patients who achieved CR of chemotherapy-refractory DLBCL.

Toxicities of CAR-19 T Cells Preceded by Low-Dose Chemotherapy

All toxicities greater than grade 1 are listed in Table 2. The most prominent toxicities in this trial were neurologic; 55% of patients had grade 3 or 4 neurologic toxicities. In contrast, only four (18%) of 22 patients had grade 3 or 4 hypotension; three patients required brief courses of vasopressor drugs to treat it. One patient required mechanical ventilation for severe dyspnea; another patient required mechanical ventilation for airway control during severe neurologic toxicity. Common grade 3 and 4 neurologic toxicities included dysphasia, confusion, and tremor. All acute toxicities resolved completely, and no patients died as a result of toxicity. Three patients received specific immunosuppressive drugs: patient 32 received the corticosteroid dexamethasone for severe neurologic toxicity, and patients 36 and 40 received the IL-6 receptor antagonist tocilizumab. Aside from those three patients, all other patients received only supportive treatment without any immunosuppressive drugs, so most patients on this trial had acute CAR T-cell toxicities that resolved spontaneously within a limited period of time.

Two patients had notable delayed adverse events. Patient 23 developed myelodysplastic syndrome (MDS) 20 months after CAR T-cell infusion, but the CAR-19 gene was not detectable in the patient's bone marrow at the time of MDS diagnosis. MDS is

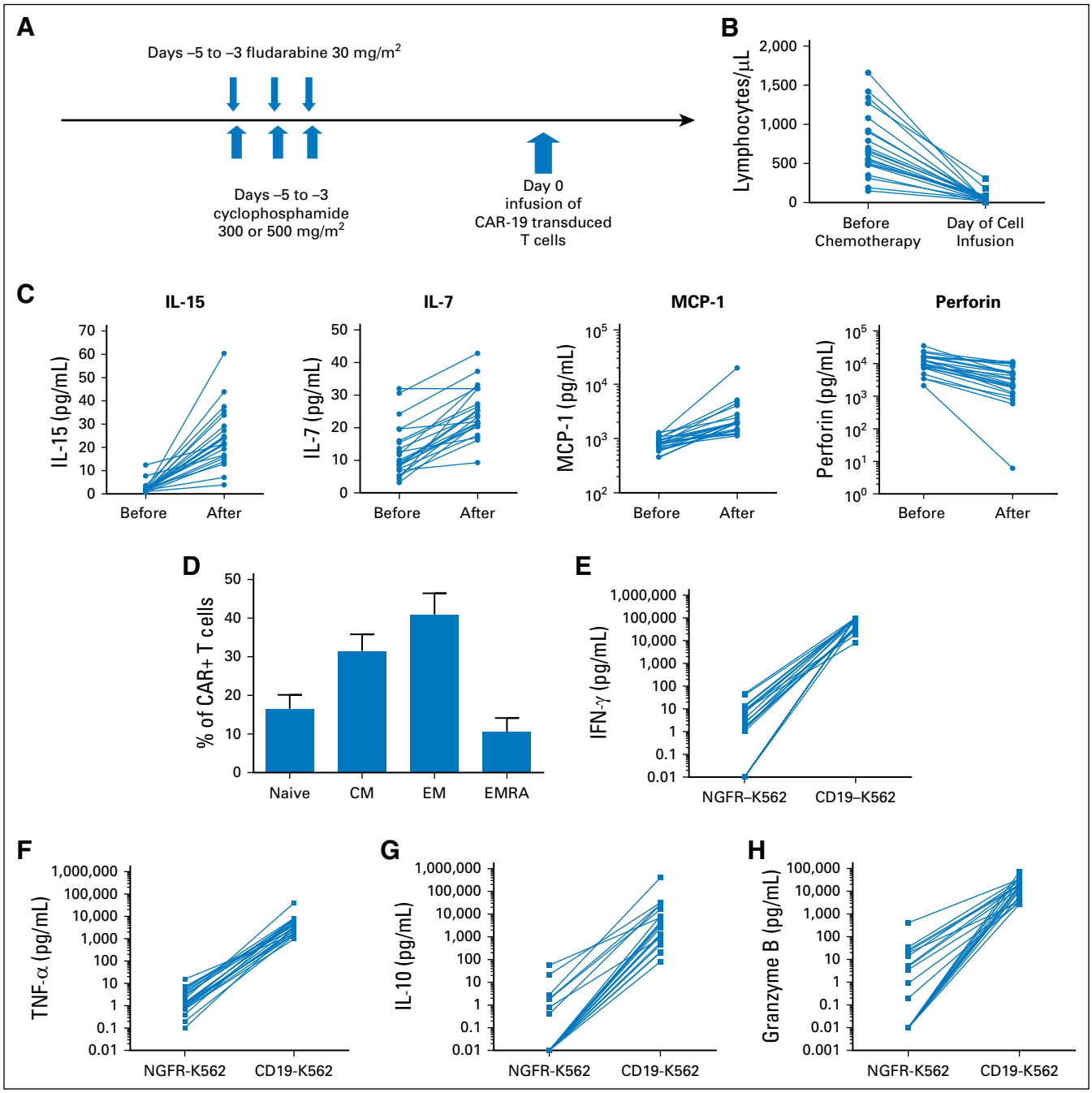

Fig 1. Low-dose chemotherapy depletes lymphocytes and modulates serum proteins. (A) Schematic of the clinical protocol. Fludarabine and cyclophosphamide were administered on days –5 to –3. Fludarabine dose was 30 mg/m^2 per day for all patients. Cyclophosphamide dose was 300 mg/m^2 for 18 patients and 500 mg/m^2 for four patients. A single dose of chimeric antigen receptor (CAR) targeting CD19 (CAR-19) T cells was administered on day 0. (B) The blood lymphocyte counts for each patient are shown before and after chemotherapy. Each patient's lymphocyte counts are connected by a line ($P < .001$ for the paired comparison of before and after chemotherapy). The after time point was the day of CAR T-cell infusion. The median blood lymphocyte count just before the start of chemotherapy was 635/μL (range, 150 to 1,660/μL). The median blood lymphocyte count on the day of CAR T-cell infusion was 40/μL (range, 0 to 310/μL). The normal range for blood lymphocytes was 1,320 to 3,570/μL. (C) Interleukin-15 (IL-15), IL-7, and monocyte chemoattractant protein-1 (MCP-1) all increased after chemotherapy, and perforin decreased. The protein levels before and after chemotherapy for each patient are connected by a line. Chemotherapy samples for the after time point were all drawn on the day of CAR T-cell infusion. For all four proteins, $P < .001$ when paired before and after chemotherapy levels for each patient were compared. (D) CAR$^+$ T cells from the time of infusion were stained for C-C chemokine receptor-7 (CCR7) and CD45RA to identify T cells with phenotypes of these subsets: naïve (CCR7$^+$CD45RA$^+$), central memory (CM; CCR7$^+$CD45RA$^-$), effector memory (EM; CCR7$^-$CD45RA$^-$), and effector memory RA subsets (EMRA; CCR7$^-$CD45RA$^+$). Plots were gated on CD3$^+$CAR$^+$ lymphocytes. Means and standard error of the mean are shown. (E-H) Anti-CD19 CAR T cells secreted a variety of proteins in an antigen-specific manner. Graphs show the levels of proteins in culture supernatants after CAR-19 T cells from the time of infusion were cultured overnight with either CD19$^-$NGFR-K562 cells or CD19$^+$CD19-K562 cells. Results for each patient are connected by a line. CD19-specific production of interferon-γ (IFN-γ), granzyme B, IL-10, and tumor necrosis factor alpha (TNF-α) occurred.

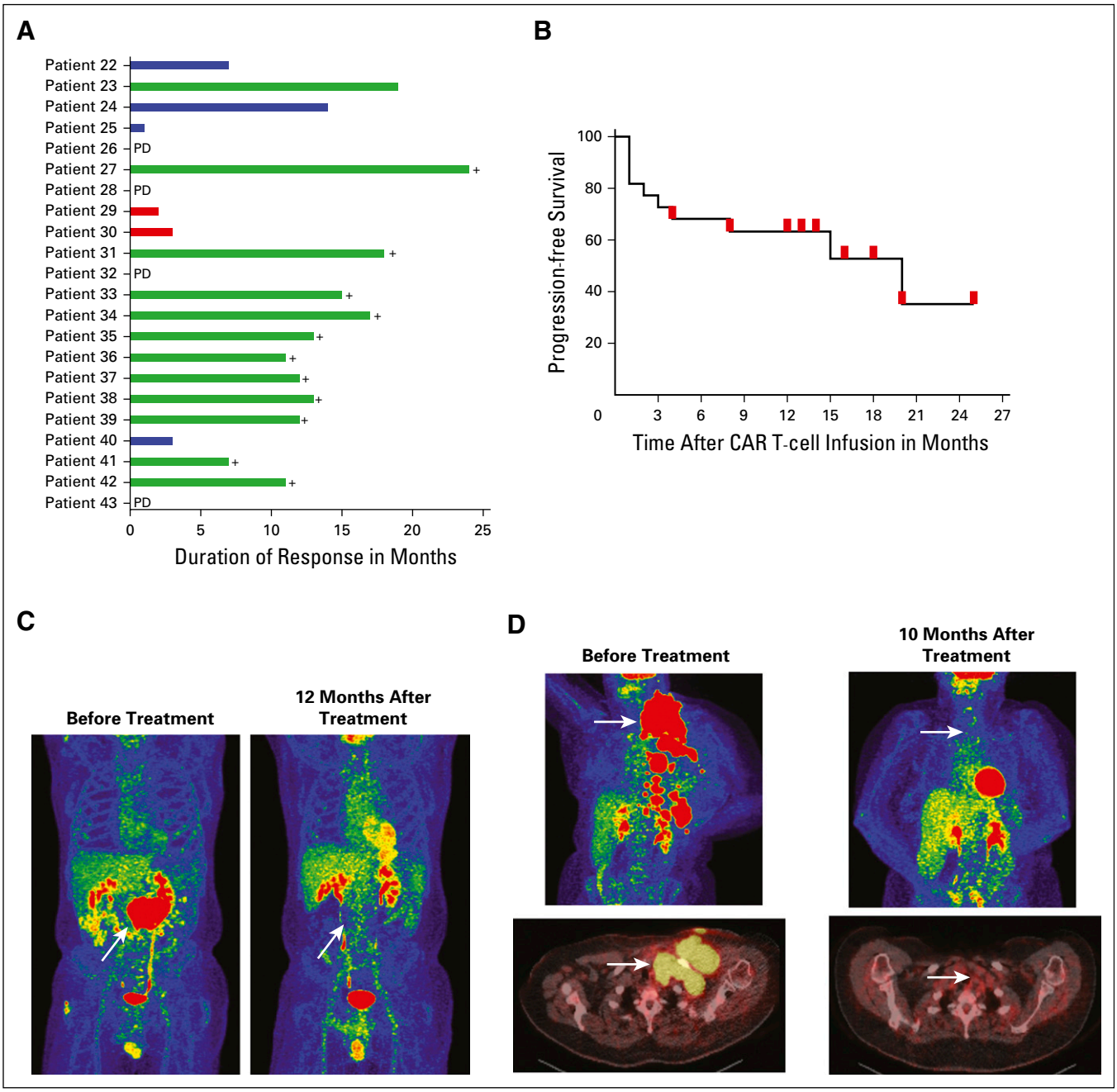

Fig 2. Chimeric antigen receptor (CAR) targeting CD19 (CAR-19) T cells eradicated large masses of chemotherapy-refractory lymphoma. (A) Graphical representation of the types of antilymphoma responses and the durations of responses. (B) Progression-free survival starting at the day of cell infusion and ending at the day of disease progression is shown for all patients. Red marks indicate censored patients with ongoing complete remissions (CRs) at the time of last follow-up with one exception: the red mark at 4 months after CAR T-cell infusion indicates the time point when patient 40 underwent allogeneic stem-cell transplantation while in partial remission. Two patients were censored at the 13-month time point and three patients were censored at the 14-month time point, but there is only one red mark on the graph for each of these time points. (C) Patient 35 had received four types of lymphoma therapy, and his diffuse large B-cell lymphoma was chemotherapy refractory at the time of protocol enrollment. After CAR-19 T-cell infusion, his lymphoma entered an ongoing CR. (D) At the time of protocol enrollment, patient 38 had received five types of prior lymphoma therapy, and her diffuse large B-cell lymphoma was refractory to chemotherapy. After CAR-19 T-cell infusion, the lymphoma entered an ongoing CR. For (C) and (D), the white arrows indicate sites of lymphoma. Residual red-colored areas in the after-treatment images are normal findings in the brain, heart, kidneys, and bladder. PD, progressive disease.

common in lymphoma patients with histories of chemotherapy and ASCT, so the extensive treatment received by patient 23 before protocol enrollment likely contributed to his MDS.[41] Patient 27 developed vision loss 3 months after CAR T-cell infusion. Although the etiology of this vision loss is not definitively known, the clinical course and electroretinography were consistent with fludarabine toxicity.[42]

Depletion of normal B cells is an expected toxicity of anti-CD19 CAR T cells.[8,12,19,20] Because of extensive prior treatment, B-cell counts were already low in most patients at

Table 2. All Grade 2, 3, and 4 Adverse Events in the First Month After Anti-CD19 CAR T-Cell Infusion

	Adverse Events		
Patient No.	Grade 2	Grade 3	Grade 4*
22	None	Platelets, neutrophils, and hemoglobin decreased, febrile neutropenia, hypotension	Leukocytes decreased
23†	Fever	Febrile neutropenia, encephalopathy, neutrophils decreased	None
24	Dysphasia	Hemoglobin decreased, febrile neutropenia	Neutrophils and leukocytes decreased
25	None	Leukocytes and neutrophils decreased, hyponatremia	None
26	Sensory neuropathy (numbness)	Hyponatremia	Neutrophils and leukocytes decreased
27‡	Fever	ALT and AST increased, leukocytes and neutrophils decreased§	None
28	Fever	Hemoglobin decreased, febrile neutropenia, partial thromboplastin time increased	Neutrophils decreased
29	Ataxia, motor neuropathy (gait disturbance), dysphasia, tremor, fever	Neutrophils and hemoglobin decreased	None
30	Fever	Neutrophils decreased	None
31	Dysphasia, fever, hypotension	Syncope	None
32	Fever, dyspnea, hypoxia	Hemoglobin decreased	Confusion
33	Fever, sinus tachycardia	ALT, AST, and bilirubin increased, hemoglobin and neutrophils decreased, confusion	Dysphasia
34	Sinus tachycardia, hypoxia, sensory neuropathy (numbness), pain (headache)	Fever, ataxia, agitation, motor neuropathy (gait disturbance, arm weakness), dysphasia, tremor	Confusion
35	Laryngeal nerve dysfunction, atrial fibrillation	Hemoglobin and platelets decreased, fever, confusion, dysphagia	Hypotension, neutrophils decreased, dysphasia
36	Hypotension	Febrile neutropenia, confusion, somnolence, dysphasia, hemoglobin decreased, cardiac left ventricular systolic dysfunction, urinary tract infection	Platelets and neutrophils decreased, dysphasia, dyspnea, hypoxia
37	Motor neuropathy (gait disturbance)	Febrile neutropenia, somnolence, confusion, psychosis (hallucinations/delusions)	Neutrophils and hemoglobin decreased, dysphasia,
38	Tremor	Hemoglobin decreased, fever, confusion, febrile neutropenia, somnolence	Neutrophils decreased, dysphasia
39	Dysphasia	Hemoglobin decreased, febrile neutropenia	Neutrophils decreased
40	None	Fever, hemoglobin and neutrophils decreased, somnolence, seizure, psychosis (hallucinations/delusions)	Hypotension, dysphasia, supraventricular tachycardia
41	Tremor	Febrile neutropenia, neutrophils and hemoglobin decreased, somnolence, confusion, urinary incontinence, generalized muscle weakness, motor neuropathy (gait disturbance), dysphasia, dysphagia, urinary tract infection	Cognitive disturbance
42	None	Hemoglobin decreased, cognitive disturbance, febrile neutropenia, agitation, dysphasia, catheter-related infection, somnolence	Hypotension, platelets, and neutrophils decreased
43	Dysphasia, psychosis (hallucinations/delusions)	Hemoglobin and neutrophils decreased, febrile neutropenia,	None

Abbreviation: CAR, chimeric antigen receptor.

*All grade 2, 3, and 4 adverse events within the first month after CAR T-cell infusion are listed regardless of timing with the exception of grade 4 lymphocytes decreased. All patients had grade 4 lymphocytes decreased as expected with cyclophosphamide and fludarabine, so it is not repetitively listed for each patient. The highest grade of each individual toxicity is listed; for example, if a toxicity occurred at both grade 2 and grade 3, only grade 3 is listed.

†Patient 23 developed myelodysplastic syndrome (MDS) 20 months after CAR T-cell infusion. The CAR gene was not detectable in the bone marrow of patient 23 at the time of diagnosis of MDS.

‡Three months after CAR that targets the B-cell antigen CD19 (CAR-19) T-cell infusion, patient 27 developed vision loss that was consistent with prior reports of fludarabine toxicity.

§All patients had fevers of some grade.

the time of protocol enrollment. The median blood B-cell count at enrollment was 1/μL (range, 0 to 123/μL). Only patient 23 had a normal pretreatment B-cell count. Six patients in CR have recovered normal blood B-cell counts, and five remain in CR (Data Supplement). This demonstrates that patients can remain in CR after recovery of normal B-cell counts.

Higher Numbers of Blood CAR$^+$ Cells Were Present in Patients With Lymphoma Remission

The number of blood CAR$^+$ cells peaked at a median of 8.5 days (range, 6 to 35 days) after infusion (Fig 3A-B). CAR$^+$ cell numbers dropped rapidly after peaking. Blood CAR$^+$ cell numbers decreased to 0 or 1/μL by 3 months after infusion in all patients. Peak blood CAR$^+$ cell numbers were higher in patients who

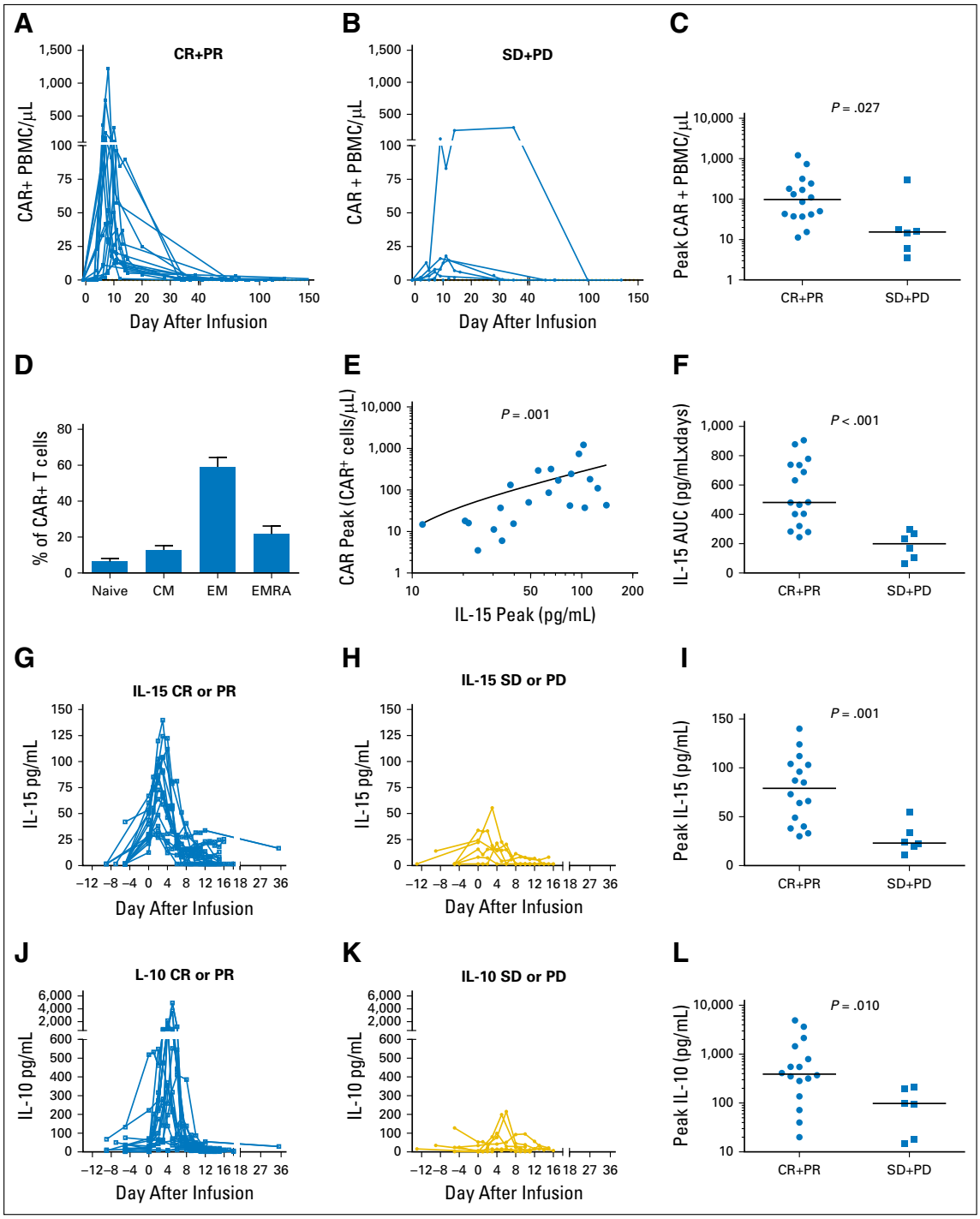

Fig 3. Clinical remissions of lymphoma were associated with high peak blood levels of chimeric antigen receptor–positive (CAR^+) cells and interleukin-15 (IL-15). The absolute number of CAR^+ peripheral blood mononuclear cells (PBMCs) in patients who achieved clinical antilymphoma responses of either (A) complete remission (CR) or partial remission (PR) or (B) stable disease (SD) or progressive disease (PD) were quantified by polymerase chain reaction. (C) Patients who achieved remission (CR + PR) had higher levels of CAR^+ PBMCs than patients who did not (SD + PD; $P = .027$). Horizontal lines represent the medians in panels C, F, I, and L. (D) CAR^+ T cells from patient blood samples collected between 4 and 15 days after CAR T-cell infusion were stained for C-C chemokine receptor-7 (CCR7) and CD45RA. The graph shows the fraction of T cells with phenotypes of four different T-cell subsets: naïve ($CCR7^+CD45RA^+$), central memory (CM; $CCR7^+CD45RA^-$), effector memory (EM; $CCR7^-CD45RA^-$), and effector memory-RA (EMRA; $CCR7^-CD45RA^+$) subsets. Plots were gated on $CD3^+CAR^+$ cells. For each T-cell subset, mean and standard error of the mean are shown. (E) The peak level of blood CAR^+ cells correlated with the peak level of IL-15 (Spearman correlation $r = 0.7$; $P = .001$). (F) Patients who achieved remission of lymphoma (CR + PR) had higher serum IL-15 area-under-the-curve (AUC) levels from day –5 to day 14 than patients who did not (SD + PD; $P < .001$). Serum levels of IL-15 for (G) all patients who achieved remission (CR + PR) or (H) patients who did not (SD + PD) are shown. Day 0 is the day of CAR T-cell infusion. (I) Patients who achieved remission (CR + PR) after CAR T-cell infusion had higher peak serum IL-15 levels than patients who did not ($P = .001$). The serum levels of IL-10 for (J) all patients who achieved remission (CR + PR) or (K) patients who did not (SD + PD) are shown. (L) Patients who achieved remission (CR + PR) had higher peak serum levels of IL-10 than patients who did not achieve remission (SD + PD; $P = .010$). A total of 41 serum proteins were assessed (panels I and L), and the results of the two proteins with the most impressive differences are shown. Results for all 41 serum proteins are provided in the Data Supplement. Statistical correction for multiple comparisons was not performed.

achieved lymphoma responses of CR or PR compared with those who achieved SD or progressive disease (Fig 3A-C). There was no statistically significant difference in the peak number of blood CAR$^+$ T cells in patients who received infusions of CAR-19 T cells at 1×10^6/kg versus 2×10^6/kg. CAR$^+$ T cells collected from the blood of patients after infusion primarily had phenotypes of effector memory or effector memory-RA T cells (Fig 3D); in comparison, CAR-19 T cells at the time of infusion included more T cells with phenotypes of naïve and central memory T cells (Fig 1D). There was an association between the percentage of central memory T cells among the infused CAR T cells and peak blood CAR T-cell levels ($P < .001$; Spearman $r = 0.7$; Data Supplement).

Peak Blood CAR$^+$ Cell Numbers and Lymphoma Remission Were Associated With High Serum IL-15 Levels

The low-dose chemotherapy conditioning regimen administered before CAR-19 T-cell infusions caused an increase in serum IL-15 (Fig 1C). Because IL-15 is known to induce T-cell proliferation,[32-34] we reasoned that IL-15 might be associated with peak blood levels of CAR-19 T cells after infusion. We measured serum IL-15 levels at several time points before and after CAR-19 T-cell infusion, and the median day of peak IL-15 levels was day 2 after CAR T-cell infusion. We found a correlation between peak serum IL-15 levels and peak blood CAR$^+$ cell numbers (Fig 3E).

We assessed serum levels of 41 different proteins in patients who achieved a remission (CR or PR) and patients who did not achieve a remission (SD or progressive disease; Data Supplement). IL-15 was the cytokine associated most closely with remissions. Patients who achieved a remission of lymphoma had higher IL-15 area-under-the-curve levels from day –5 to day 14 and higher peak serum IL-15 levels than patients who did not achieve a remission (Fig 3F-I). On the day of CAR T-cell infusion, the median serum IL-15 level of patients who achieved a remission was higher than that of patients who did not achieve a remission (30 *v* 16 pg/mL; $P = .010$; Data Supplement). This suggests that the environment that CAR T cells enter upon infusion is a determinant of treatment outcomes.

IL-10 is a cytokine that is known to have immunosuppressive properties[43]; however, IL-10 can also promote T-cell antitumor activity.[44,45] Peak serum IL-10 levels were significantly higher among patients who achieved a remission of lymphoma compared with those who did not achieve a remission (Fig 3J-L). Aside from IL-15, IL-10 was the cytokine with the clearest difference in peak serum levels when comparing patients who did or did not achieve a remission.

Grade 3 and 4 Neurologic Toxicities Were Associated With High Blood CAR$^+$ Cell Levels, and CAR-19 T Cells Were Detected in Cerebrospinal Fluid

Patients who experienced grade 3 or 4 neurologic toxicity had higher levels of blood CAR$^+$ cells compared with patients who had only < grade 3 neurologic toxicities (Fig 4A). We performed lumbar punctures to collect cerebrospinal fluid (CSF) from 11 patients with neurologic toxicity. The median number of white blood cells in the CSF of these patients was 22/μL (range, 1 to 215/μL). Flow cytometry was performed on the CSF of nine of the 11 patients who underwent CSF collection, and CAR-19 T cells were detected in the CSF of all nine patients (Fig 4B; Data Supplement).

Serum Proteins Are Associated With Neurologic Toxicities

We assessed serum levels of 41 proteins in patients with grade 3 or 4 neurologic toxicities and patients with only < grade 3 neurologic toxicities (Data Supplement). Peak serum granzyme B levels were higher in patients with grade 3 or 4 neurologic toxicities compared those who had only < grade 3 neurologic toxicities (Fig 4C-E). In addition, peak levels of serum IL-10 and IL-15 were higher in patients with grade 3 or 4 neurologic toxicities compared with those who had only < grade 3 neurologic toxicities (Fig 4F-G).

DISCUSSION

An infusion of CAR-19 T cells preceded by a low-dose conditioning chemotherapy regimen of cyclophosphamide and fludarabine induced remissions of advanced-stage lymphoma. The low-dose chemotherapy regimen used in this study could not directly induce the remissions of lymphoma reported here because of the documented resistance of these lymphomas to antilymphoma chemotherapy regimens (Data Supplement). Importantly, our CAR T-cell production process was simple and robust. No patient failed to receive treatment because of cell production issues.

We recently treated patients who had had an allogeneic hematopoietic stem-cell transplantation with human leukocyte antigen–matched allogeneic T cells expressing the same CAR-19 used with autologous T cells in this study.[36,46] We did not administer chemotherapy before infusions of the allogeneic CAR-19 T cells. We administered allogeneic CAR$^+$ T cells at up to 8.2×10^6/kg with acceptable levels of toxicity.[46] In stark contrast to this report of patients treated with autologous CAR-19 T cells at 1 to 2×10^6/kg, neurologic toxicity in the trial of allogeneic anti-CD19 CAR T cells without chemotherapy was rare and mild.[36,46] These results suggest that lowering or eliminating the conditioning chemotherapy administered before CAR T-cell infusions might lessen the severity of toxicity; however, lowering chemotherapy intensity might also lead to lower remission rates.[46]

We compared hematologic toxicities in patients who received CAR-19 T cells after our current low-dose chemotherapy regimen and patients who received our previously reported high-dose chemotherapy regimen, which included a total dose of cyclophosphamide of 60 to 120 mg/kg and a total dose of fludarabine of 125 mg/m^2.[21] Two of 22 patients treated with the new low-dose chemotherapy required platelet transfusions; 10 of 15 patients receiving high-dose chemotherapy required platelet transfusions ($P < .001$). The median length of time that patients had severe neutropenia with an absolute neutrophil count of less than 500/μL was 0 days (range, 0 to 6 days) with the low-dose chemotherapy versus 5 days (range, 0 to 16 days) with the

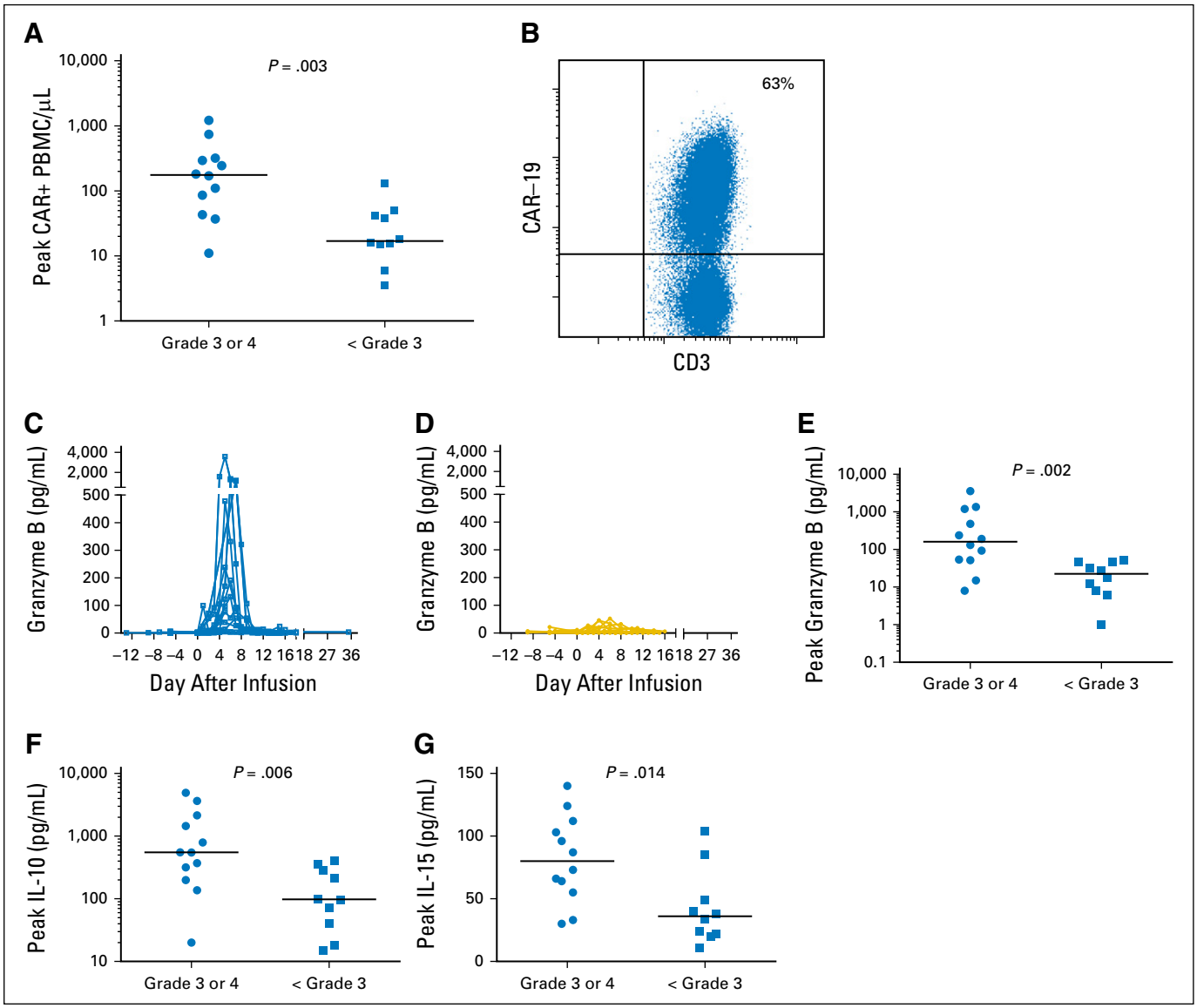

Fig 4. Neurologic toxicity was associated with high peak blood levels of chimeric antigen receptor–positive (CAR$^+$) cells and increased levels of certain serum proteins. (A) The number of blood CAR$^+$ cells in patients with grade 3 or 4 neurologic toxicities was higher than that in patients with only < grade 3 neurologic toxicities ($P = .003$). (B) Flow cytometry revealed CAR targeting CD19 (CAR-19) T cells in the cerebrospinal fluid (CSF) in all assessed patients with neurologic toxicity. The flow cytometry result of CSF from patient 31 from 9 days after CAR-19 T-cell infusion is shown as a representative example. The plot is gated on CSF mononuclear cells. Granzyme B serum levels of (C) patients with grade 3 or 4 neurologic toxicities and (D) those with only < grade 3 neurologic toxicities are shown. Patients with grade 3 or 4 neurologic toxicities had higher peak serum levels of (E) granzyme B ($P = .002$), (F) interleukin-10 (IL-10; $P = .006$), and (G) IL-15 ($P = 0.014$) than patients with only < grade 3 neurologic toxicities. A total of 41 serum proteins were assessed (panels E, F, and G), and the results of the three proteins with the most impressive differences are shown. Results for all 41 serum proteins are provided in the Data Supplement. Statistical correction for multiple comparisons was not performed.

previously used high-dose chemotherapy ($P < .001$). In this study, we increased the daily cyclophosphamide dose from 300 mg/m^2 to 500 mg/m^2 in four patients in an attempt to increase the CR rate. Because all four of these patients experienced substantial toxicity (Table 2), we reduced the cyclophosphamide dose back to 300 mg/m^2; however, because we had a small number of patients treated with the 500 mg/m^2 dose of cyclophosphamide, we cannot draw firm conclusions.

Our data indicate that IL-15 is associated with the efficacy of CAR-19 T cells. In agreement with prior work,[47] IL-15 was one of the serum proteins most prominently increased after the low-dose chemotherapy regimen. Serum IL-15 levels were strongly associated with peak levels of CAR$^+$ cells, and blood IL-15 levels were higher in patients who achieved a remission of lymphoma than in patients who did not achieve a remission. IL-15 causes T-cell (including CAR T-cell) proliferation and activation,[32-34,48-50] so the mechanism of IL-15 improving lymphoma treatment outcomes by increasing activated CAR-19 T-cell levels is quite plausible. Other investigators have performed preclinical experiments that demonstrated the ability of IL-15 to enhance adoptive T-cell therapies in mice.[9,48,50-53] To the best of our knowledge, our work is the first to show an association in humans between serum IL-15 levels and remission of lymphoma after adoptive T-cell therapy. Clinical trials that evaluate the ability of IL-15 agonists to enhance CAR T-cell

antimalignancy activity should be considered to formally assess the role of IL-15 in T-cell therapy.[32,33] It must be kept in mind that IL-15 might worsen toxicity in clinical trials combining CAR T cells and IL-15.

We undertook experiments to better understand the important problem of neurologic toxicity with CAR-19 T-cell therapies.[12,14-16,21] Neurologic toxicity was closely associated with high peak blood CAR^+ cell levels, and by using flow cytometry, we found CAR T cells in the CSF of every patient with neurologic toxicity. Interestingly, peak serum granzyme B levels were closely associated with neurologic toxicity (Fig 4C-E). High granzyme B levels were associated with grade 3 or 4 neurologic toxicity but not with a remission of lymphoma (Data Supplement). Granzyme B has been previously linked to neuronal toxicity.[54] Notably, high serum IL-10 and IL-15 levels were associated with both remission of lymphoma and neurologic toxicity. Other investigators have previously shown an association of interferon-γ and IL-6 with neurologic toxicity.[16] We also found that serum levels of interferon-γ were higher in patients with grade 3 or 4 neurologic toxicities than in those with only < grade 3 neurologic toxicities ($P = .04$; Data Supplement), but this difference was not as consistent as the differences for granzyme B, IL-10, and IL-15. We observed a trend that was not statistically significant for higher peak serum IL-6 levels in patients with grade 3 or 4 neurologic toxicity (Data Supplement). Development of new approaches to prevent or treat neurologic toxicity caused by CAR T cells is an important need.

Patients with DLBCL that is refractory to chemotherapy or relapsed less than 12 months after ASCT generally survive less than 9 months.[23,25-27] Our results from treating advanced-stage lymphoma with CAR-19 T cells compare favorably to established treatment regimens.[17,18,23-25] Induction of CRs in patients with large masses of chemotherapy-refractory lymphoma is especially notable (Fig 2). Our results should encourage further research aimed at developing CAR T-cell therapies with less toxicity and higher remission rates.

AUTHORS DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Lymphoma Remissions Caused by Anti-CD19 Chimeric Antigen Receptor T Cells Are Associated With High Serum Interleukin-15 Levels

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ORIGINAL ARTICLE

Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia

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ABSTRACT

BACKGROUND

In a single-center phase 1–2a study, the anti-CD19 chimeric antigen receptor (CAR) T-cell therapy tisagenlecleucel produced high rates of complete remission and was associated with serious but mainly reversible toxic effects in children and young adults with relapsed or refractory B-cell acute lymphoblastic leukemia (ALL).

METHODS

We conducted a phase 2, single-cohort, 25-center, global study of tisagenlecleucel in pediatric and young adult patients with CD19+ relapsed or refractory B-cell ALL. The primary end point was the overall remission rate (the rate of complete remission or complete remission with incomplete hematologic recovery) within 3 months.

RESULTS

For this planned analysis, 75 patients received an infusion of tisagenlecleucel and could be evaluated for efficacy. The overall remission rate within 3 months was 81%, with all patients who had a response to treatment found to be negative for minimal residual disease, as assessed by means of flow cytometry. The rates of event-free survival and overall survival were 73% (95% confidence interval [CI], 60 to 82) and 90% (95% CI, 81 to 95), respectively, at 6 months and 50% (95% CI, 35 to 64) and 76% (95% CI, 63 to 86) at 12 months. The median duration of remission was not reached. Persistence of tisagenlecleucel in the blood was observed for as long as 20 months. Grade 3 or 4 adverse events that were suspected to be related to tisagenlecleucel occurred in 73% of patients. The cytokine release syndrome occurred in 77% of patients, 48% of whom received tocilizumab. Neurologic events occurred in 40% of patients and were managed with supportive care, and no cerebral edema was reported.

CONCLUSIONS

In this global study of CAR T-cell therapy, a single infusion of tisagenlecleucel provided durable remission with long-term persistence in pediatric and young adult patients with relapsed or refractory B-cell ALL, with transient high-grade toxic effects. (Funded by Novartis Pharmaceuticals; ClinicalTrials.gov number, NCT02435849.)

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Tisagenlecleucel (formerly CTL019), an anti-CD19 chimeric antigen receptor (CAR) T-cell therapy, is under investigation in patients with relapsed or refractory B-cell cancers, including B-cell acute lymphoblastic leukemia (ALL). Results from a single-center phase 1–2a study of tisagenlecleucel involving 60 children and young adults with relapsed or refractory B-cell ALL that was conducted at the Children's Hospital of Philadelphia and the University of Pennsylvania showed a rate of complete remission of 93%.[1] The cytokine release syndrome, a common adverse event associated with CAR T-cell therapies, occurred in 88% of patients and was effectively managed with supportive measures and anticytokine therapy, including the interleukin-6 receptor antagonist tocilizumab.[1] Long-term disease control without additional therapy and with persistence of tisagenlecleucel for up to 4 years has been observed.[1,2]

On the basis of these results, a phase 2 pivotal, multisite study of tisagenlecleucel was initiated. In this nonrandomized study of CAR T-cell therapy, we used a global supply chain and included 25 study sites in 11 countries across North America, Europe, Asia, and Australia. Here we report the results of a planned analysis of data from the study, including analyses of the efficacy, safety, and cellular kinetics of tisagenlecleucel in 75 patients with at least 3 months of follow-up.

Methods

Study Design

We conducted a single-cohort, phase 2, multicenter, global study of tisagenlecleucel in children and young adults with relapsed or refractory B-cell ALL. To be eligible for participation in the study, patients had to be at least 3 years of age at screening and no older than 21 years of age at diagnosis and to have at least 5% lymphoblasts in bone marrow at screening. Patients who had previously received anti-CD19 therapy were excluded (see the Methods section of the Supplementary Appendix, available with the full text of this article at NEJM.org).

Tisagenlecleucel was generated ex vivo with the use of autologous T cells transduced with a lentiviral vector to express a CAR containing a CD3-zeta domain to provide a T-cell activation signal and a 4-1BB (CD137) domain to provide a costimulatory signal.[3]

The study was sponsored and designed by Novartis Pharmaceuticals and was approved by the institutional review board at each participating institution. Patients or their guardians provided written informed consent or assent. Data were analyzed and interpreted by the sponsor in collaboration with the authors, and all the authors reviewed the manuscript and vouch for accuracy and completeness of the data and analyses and for adherence of the study to the protocol, available at NEJM.org. The first author wrote the first draft of the manuscript in conjunction with authors from Novartis. All the authors contributed to the writing of the manuscript and approved the final version for submission. Medical editorial assistance was provided by editors whose work was financially supported by Novartis.

End Points

The primary end point was an overall remission rate higher than 20% (the null hypothesis). The overall remission rate was defined as the rate of a best overall response of either complete remission or complete remission with incomplete hematologic recovery within 3 months, as assessed by an independent review committee on the basis of the results of laboratory testing of blood, bone marrow, and cerebrospinal fluid (CSF), as well as physical examination. Responses were required to be maintained for at least 28 days (see the Methods section in the Supplementary Appendix). Secondary end points included the rate of complete remission or complete remission with incomplete hematologic recovery with undetectable minimal residual disease (<0.01%) assessed by means of central multiparameter flow cytometry, the duration of remission, event-free survival (i.e., the time from infusion to the earliest of the following events: no response, relapse before response was maintained for at least 28 days, or relapse after having complete remission or complete remission with incomplete hematologic recovery), overall survival, cellular kinetics, and safety. Additional details regarding the secondary end points are provided in the Supplementary Appendix.

Statistical Analysis

The primary end point was evaluated in the full analysis set. We determined that a sample of 76 patients receiving a tisagenlecleucel infusion would provide more than 95% power to reject

the null hypothesis of an overall remission rate of 20% against the alternative hypothesis of an overall remission rate of 45% or higher at an overall one-sided significance level of 2.5%.

An interim analysis was planned after the first 50 patients who received a tisagenlecleucel infusion had completed 3 months of follow-up or discontinued participation in the study. The results with regard to the primary end point were considered to be significant in the interim analysis if the one-sided P value was lower than 0.0057. Key secondary end points were tested sequentially (after the primary end point was significant) to control the overall alpha.

The results with regard to overall remission rate, response duration, event-free survival, overall survival, cellular kinetics, and safety that are presented in this report are from an updated analysis that included 75 patients who received tisagenlecleucel and had completed 3 months of follow-up or discontinued the study at an earlier point. For the time-to-event analyses, Kaplan–Meier curves were used to estimate survival distributions after infusion. All statistical tests were performed with the use of SAS software, version 9.4 (SAS Institute). Additional details regarding the statistical analysis are provided in the Supplementary Appendix.

RESULTS

PATIENTS

Between April 8, 2015, and the data cutoff on April 25, 2017, a total of 107 patients were screened, and 92 were enrolled (Fig. 1). A total of 75 patients received an infusion of tisagenlecleucel, with a median time from enrollment to infusion of 45 days (range, 30 to 105). The median duration of follow-up among patients who received a tisagenlecleucel infusion was 13.1 months. At enrollment, patients who received tisagenlecleucel had a median age of 11 years (range, 3 to 23), a median of 3 previous therapies (range, 1 to 8), and a median marrow blast percentage of 74% (range, 5 to 99); 46 patients (61%) had undergone previous allogeneic hematopoietic stem-cell transplantation (Table S1 in the Supplementary Appendix).

Before tisagenlecleucel infusion, 72 of 75 patients (96%) received lymphodepleting chemo-

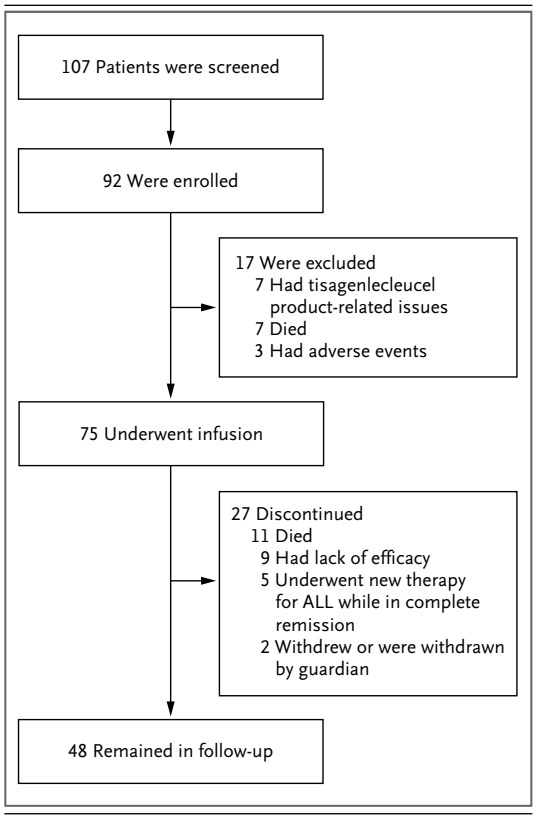

Figure 1. Screening, Enrollment, Treatment, and Follow-up.

The first patient's first visit occurred on April 8, 2015. The median time from tisagenlecleucel infusion to data cutoff was 13.1 months. The reasons for patients not enrolling in the study after screening included not meeting the inclusion criteria or meeting the exclusion criteria (11 patients, including <5% blasts in the bone marrow in 8 patients), death before acceptance of the apheresis sample at the manufacturing facility (2 patients; 1 who died from pulmonary hemorrhage and 1 who died from multiorgan failure), physician decision (1), and apheresis-related issue (1). All patients who completed screening and whose apheresis product was received and accepted by the manufacturing facility were enrolled in the study. Of the 75 patients who received an infusion, 65 (87%) received bridging chemotherapy between enrollment and infusion, and 72 (96%) received lymphodepleting chemotherapy (fludarabine–cyclophosphamide [71 patients] or cytarabine–etoposide [1]). Seventeen enrolled patients did not receive a tisagenlecleucel infusion because of product-related issues (7 patients), death (7 patients; 4 from disease progression and 1 each from sepsis, respiratory failure, and fungemia), and adverse events (3 patients; 1 each from graft-versus-host disease, systemic mycosis, and fungal pneumonia). Tisagenlecleucel product-related issues included an inability to manufacture as a result of poor cell growth for 6 patients and a technical issue unrelated to cell growth for 1 patient. Patients who received the infusion but discontinued follow-up were followed for survival. At the time of data cutoff, 27 patients had discontinued follow-up owing to death (11 patients; 7 from disease progression and 1 each from encephalitis, cerebral hemorrhage, systemic mycosis, and hepatobiliary disorders related to allogeneic hematopoietic stem-cell transplantation), lack of efficacy (9 patients; nonresponse or relapse), new therapy while in complete remission (5), and patient or guardian decision (2); 48 patients remained in follow-up. ALL denotes acute lymphoblastic leukemia.

therapy, which was not given at investigator discretion if a patient had leukopenia. Patients received a median weight-adjusted dose of 3.1×10^6 transduced viable T cells per kilogram of body weight (range, 0.2×10^6 to 5.4×10^6 cells per kilogram); the median total dose of transduced viable T cells was 1.0×10^8 (range, 0.03×10^8 to 2.6×10^8 cells) (Table S2 in the Supplementary Appendix).

EFFICACY

In the interim analysis, which included 50 patients, the primary end point was met, with an overall remission rate of 82% (95% confidence interval [CI], 69 to 91; P<0.001); the results with regard to all key secondary end points were also significant.[4] In this updated analysis involving 75 patients who received a tisagenlecleucel infusion and had at least 3 months of follow-up, the overall remission rate was 81% (95% CI, 71 to 89); 45 patients (60%) had complete remission, and 16 (21%) had complete remission with incomplete hematologic recovery. All patients who had a best overall response of complete remission with or without complete hematologic recovery were negative for minimal residual disease; 95% (58 of 61) of these patients were negative by day 28. In an intention-to-treat analysis of the full enrolled population (92 patients), which included patients who discontinued participation in the study before tisagenlecleucel infusion, the overall remission rate was 66% (95% CI, 56 to 76) (Table S3 in the Supplementary Appendix). In subgroup analyses that included patients with or without previous transplantation, with high-risk genomic lesions, or with Down's syndrome, the overall remission rate ranged from 79% to 83% (Fig. S1 in the Supplementary Appendix).

Among the 61 patients with complete remission with or without complete hematologic recovery, the median response duration was not reached (Fig. 2A). The rate of relapse-free survival among patients with a response to treatment was 80% (95% CI, 65 to 89) at 6 months and 59% (95% CI, 41 to 73) at 12 months. Among patients with complete remission, 17 had a relapse before receiving additional anticancer therapy. Relapse also occurred in 3 patients who proceeded to receive new cancer therapy for the emergence of minimal residual disease or loss of tisagenlecleucel persistence and in 2 patients who had already been classified as not having a response to treatment because remission was not maintained for at least 28 days. No patients were found to have relapses in the central nervous system (CNS) during primary follow-up; 1 CNS relapse was reported after new anticancer therapy. Characterization of CD19 status at the time of relapse showed that 1 patient had a CD19+ recurrence and 15 patients had CD19− (3 with concomitant CD19+ blasts); 6 patients had unknown CD19 status.

The rate of event-free survival was 73% (95% CI, 60 to 82) at 6 months and 50% (95% CI, 35 to 64) at 12 months (Fig. 2B); median event-free survival was not reached. Eight patients underwent allogeneic hematopoietic stem-cell transplantation while in remission, including 2 patients with minimal residual disease–positive bone marrow and 2 with B-cell recovery within 6 months after infusion. All 8 patients were alive at the time of manuscript submission — 4 with no relapse and 4 with unknown disease status. The rate of overall survival among the 75 patients who received tisagenlecleucel was 90% (95% CI, 81 to 95) at 6 months after infusion and 76% (95% CI, 63 to 86) at 12 months after infusion (Fig. 2B, and Fig. S2 in the Supplementary Appendix).

TISAGENLECLEUCEL EXPANSION AND PERSISTENCE

Tisagenlecleucel transgene was detected in peripheral blood by means of qualitative polymerase chain reaction.[5] Among the 60 patients with a response at day 28 who could be evaluated for cellular kinetics, the median time to maximum expansion (T_{max}) was 10 days (range, 5.7 to 28), whereas 6 patients with no response had a T_{max} of 20 days (range, 13 to 63) (Table S4 in the Supplementary Appendix). Nine patients who could not be evaluated for response were not included in the analysis. Expansion, measured as the geometric mean of the area under the concentration–time curve in peripheral blood from time 0 to day 28 (expressed as copies per microgram of DNA times days), was 315,000 in patients with a response and 301,000 in patients without a response (Table S4 in the Supplementary Appendix). The median duration of persistence of tisagenlecleucel in blood was 168 days (range, 20 to 617 days; 60 patients) at data cutoff. Across the wide range of doses infused, no relationship between dose and expansion was observed ($r^2<0.001$) (Fig. S3 in the Supplementary Appendix), and clinical responses were observed across the entire dose range.

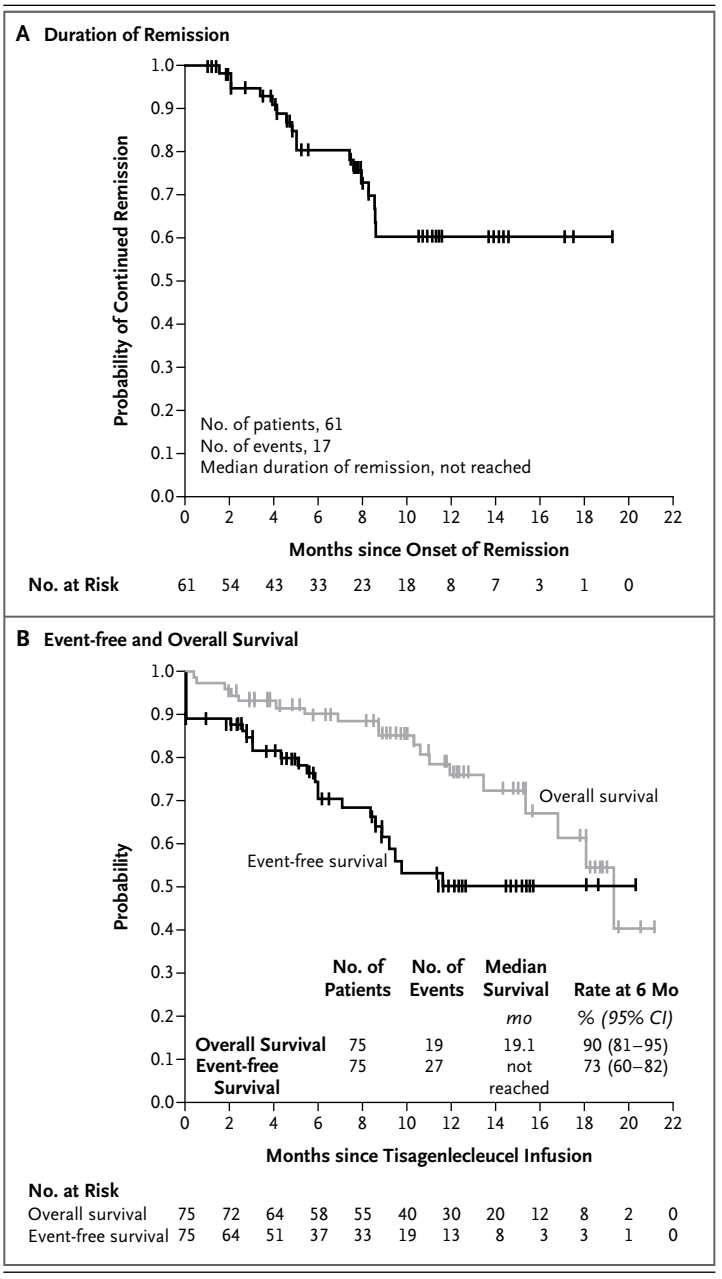

Figure 2. Duration of Remission, Event-free Survival, and Overall Survival.

Panel A shows the duration of remission, defined as the time to relapse after the onset of remission, in the 61 patients who had a best overall response of either complete remission or complete remission with incomplete hematologic recovery. Panel B shows event-free survival among the 75 patients who received an infusion, defined as the time from tisagenlecleucel infusion to the earliest of the following events: no response (8 patients), relapse before response was maintained for at least 28 days (2), or relapse after having complete remission or complete remission with incomplete hematologic recovery (17). A total of 32 patients had still not had an event at the time of data cutoff. Data for 16 more patients were censored for event-free survival — 8 patients for allogeneic stem-cell transplantation during remission, 7 patients for new cancer therapy other than stem-cell transplantation during remission (4 received humanized anti-CD19 CAR T cells, 1 received ponatinib, 1 received vincristine sulfate and blinatumomab, and 1 received antithymocyte globulin), and 1 patient for lack of adequate assessment. Ten patients were followed for relapse after new therapy, 4 of whom had a relapse or died. Panel B also shows overall survival among the 75 patients who received an infusion from the date of tisagenlecleucel infusion to the date of death from any cause. Nineteen patients died after tisagenlecleucel infusion, and 56 patients had their data censored at the time of the last follow-up. Tick marks indicate the time of censoring.

B-CELL APLASIA

All patients with a response to treatment had B-cell aplasia, and most patients in the study received immunoglobulin replacement in accordance with local practice. The median time to B-cell recovery was not reached (Fig. S4 in the Supplementary Appendix). The probability of maintenance of B-cell aplasia at 6 months after infusion was 83% (95% CI, 69 to 91).

CYTOKINE RESPONSE

Among the 75 patients who received tisagenlecleucel, transient increases in serum interleukin-6, interferon gamma, and ferritin levels occurred during the cytokine release syndrome after infusion; these increases tended to be more pronounced in patients with grade 4 cytokine release syndrome than in patients with lower grades (Fig. S5 in the Supplementary Appendix). Similar trends were observed in the levels of other cytokines, including interleukin-10, interleukin-12p70, interleukin-1β, interleukin-2, interleukin-4, interleukin-8, and tumor necrosis factor α. A transient increase in the C-reactive protein level was observed in most patients, but with large variability.

SAFETY

The safety analysis set included all 75 patients who received an infusion of tisagenlecleucel; the median time from infusion to data cutoff was 13.1 months (range, 2.1 to 23.5). Eighteen patients (24%) received their infusions in an outpatient setting. All patients had at least one adverse event during the study; 71 of 75 patients (95%)

Table 1. Overall Safety of Tisagenlecleucel.

Event	Any Time (N=75)	≤8 Wk after Infusion (N=75)	>8 Wk to 1 Yr after Infusion (N=70)
	number of patients (percent)		
Adverse event of any grade	75 (100)	74 (99)	65 (93)
Suspected to be related to tisagenlecleucel	71 (95)	69 (92)	30 (43)
Grade 3 or 4 adverse event	66 (88)	62 (83)	31 (44)
Suspected to be related to tisagenlecleucel	55 (73)	52 (69)	12 (17)

had an adverse event that was suspected by the investigators to be related to tisagenlecleucel (Table 1). The most common nonhematologic adverse events of any grade at any time after infusion were the cytokine release syndrome (77%), pyrexia (40%), decreased appetite (39%), febrile neutropenia (36%), and headache (36%) (Tables S6 and S7 in the Supplementary Appendix). Within 8 weeks after infusion, febrile neutropenia occurred in 35% of the patients, and grade 3 or 4 neutropenia with a body temperature higher than 38.3°C occurred in 46 of 75 patients (61%). Fever, neutropenia, and the cytokine release syndrome often occurred concurrently after lymphodepleting chemotherapy and tisagenlecleucel infusion; differences in reporting may reflect differential attribution of fever to the cytokine release syndrome rather than to neutropenia.

A total of 66 of 75 patients (88%) had a grade 3 or 4 adverse event; 55 of 75 patients (73%) had a grade 3 or 4 tisagenlecleucel-related adverse event (Table 1). Within 8 weeks after infusion, 52 of 75 patients (69%) had a grade 3 or 4 tisagenlecleucel-related adverse event; during the period from 8 weeks to 1 year after infusion, the incidence decreased to 12 of the 70 patients for whom follow-up data were available (17%) (Table 2). Adverse events of special interest included the cytokine release syndrome, cytopenias not resolved by day 28, infections, neurologic events, and the tumor lysis syndrome; 67 of 75 patients (89%) had an adverse event of special interest within 8 weeks after infusion (Table 3). The cytokine release syndrome occurred in 58 of 75 patients (77%); the median time to onset was 3 days (range, 1 to 22), and the median duration was 8 days (range, 1 to 36). A total of 35 of 75 patients (47%) were admitted to the intensive care unit (ICU) for management of the cytokine release syndrome, with a median stay of 7 days (range, 1 to 34). Nineteen patients (25%) were treated with high-dose vasopressors, 33 (44%) received oxygen supplementation, 10 (13%) received mechanical ventilation, 7 (9%) underwent dialysis, and 28 (37%) received tocilizumab for management of the cytokine release syndrome.[6]

Neurologic events occurred in 30 of 75 patients (40%) within 8 weeks after infusion. Ten patients (13%) had grade 3 neurologic events; no grade 4 events or cerebral edema were reported. The most common neurologic events of any grade were encephalopathy (11%), confusional state (9%), delirium (9%), tremor (8%), agitation (7%), and somnolence (7%); 1 patient had a seizure (grade 3). The majority of neurologic events occurred during the cytokine release syndrome or shortly after its resolution. Severe neurologic events occurred more frequently in patients with higher-grade cytokine release syndrome (Table S7 in the Supplementary Appendix); grade 3 neurologic events occurred more frequently in patients with grade 4 cytokine release syndrome than among those with grade 0 through 3 (32% [6 of 19] vs. 7% [4 of 56]; 95% CI for the difference, –1 to 50 percentage points). Among grade 3 neurologic episodes that resolved, 50% resolved within 10 days, and 75% resolved within 18 days. Four grade 3 neurologic episodes were unresolved in 3 patients at the time of discontinuation for no response (1 patient) or at the time of death (1 death due to leukemia progression and 1 due to encephalitis), 2 of which were thought to be related to tisagenlecleucel (1 each of encephalopathy and delirium). Neurologic events were managed with supportive care after ruling out other potential causes of the symptoms.

Table 2. Grade 3 or 4 Adverse Events Suspected to Be Related to Tisagenlecleucel That Occurred in at Least 5% of Patients.

Event	≤8 Wk after Infusion (N=75)		>8 Wk to 1 Yr after Infusion (N=70)	
	Grade 3	Grade 4	Grade 3	Grade 4
	number of patients (percent)			
Any grade 3 or 4 adverse event	19 (25)	33 (44)	8 (11)	4 (6)
Cytokine release syndrome	16 (21)	19 (25)	—	—
Hypotension	7 (9)	6 (8)	—	—
Decrease in lymphocyte count	5 (7)	4 (5)	1 (1)	—
Hypoxia	5 (7)	3 (4)	—	—
Increase in blood bilirubin	8 (11)	—	—	—
Increase in aspartate aminotransferase	5 (7)	2 (3)	—	—
Pyrexia	5 (7)	2 (3)	—	—
Decrease in neutrophil count	1 (1)	6 (8)	1 (1)	1 (1)
Decrease in white-cell count	—	7 (9)	—	—
Decrease in platelet count	3 (4)	4 (5)	—	—
Decrease in appetite	6 (8)	1 (1)	—	—
Acute kidney injury	3 (4)	3 (4)	—	—
Hypophosphatemia	5 (7)	1 (1)	—	—
Hypokalemia	6 (8)	—	—	—
Pulmonary edema	4 (5)	1 (1)	—	—
Thrombocytopenia	1 (1)	4 (5)	—	1 (1)
Encephalopathy	4 (5)	—	—	—
Increase in alanine aminotransferase	4 (5)	—	—	—
Fluid overload	4 (5)	—	—	—

A total of 31 of 75 patients (41%) had grade 3 or 4 decreased platelet counts that had not resolved by day 28. Of those 31 patients, 22 had resolution to grade 2 or lower by the last assessment, and 9 did not. By month 3, the Kaplan–Meier estimate of the percentage of patients with resolution to grade 2 or lower was 73%. A grade 3 or 4 decreased neutrophil count that had not resolved by day 28 was reported in 40 of 75 patients (53%). Of those 40 patients, 32 had resolution to grade 2 or lower by the last assessment, and 8 did not; the Kaplan–Meier estimate of the percentage of patients who had resolution to grade 2 or lower by month 3 was 66%. Eighteen of these 40 patients (45%) had grade 3 or 4 infections. In rare cases, prolonged grade 3 or 4 neutropenia before and after tisagenlecleucel infusion was associated with infections that were severe (grade 3 human herpesvirus 6 [HHV-6] encephalitis) or fatal (encephalitis and systemic mycosis).

Table 3. Adverse Events of Special Interest within 8 Weeks after Infusion, Regardless of Relationship to Tisagenlecleucel.*

Type of Event	Any Grade (N=75)	Grade 3 (N=75)	Grade 4 (N=75)
	number of patients (percent)		
Any adverse event of special interest	67 (89)	26 (35)	30 (40)
Cytokine release syndrome	58 (77)	16 (21)	19 (25)
Neurologic event	30 (40)	10 (13)	0
Infection	32 (43)	16 (21)	2 (3)
Febrile neutropenia	26 (35)	24 (32)	2 (3)
Cytopenia not resolved by day 28	28 (37)	12 (16)	12 (16)
Tumor lysis syndrome	3 (4)	3 (4)	0

* The criteria for defining adverse events of special interest were based on experience from ongoing clinical studies. The cytokine release syndrome includes the Medical Dictionary for Regulatory Activities preferred terms "cytokine release syndrome," "cytokine storm," "shock," "macrophage activation," and "hemophagocytic lymphohistiocytosis." Neurologic events include the standardized Medical Dictionary for Regulatory Activities query terms "noninfectious encephalopathy" and "delirium."

Nineteen deaths occurred after tisagenlecleucel infusion. Within 30 days after infusion, 1 patient died from cerebral hemorrhage in the context of coagulopathy and resolving cytokine release syndrome (15 days after infusion), and 1 patient died from progressive B-cell ALL. More than 30 days after infusion, 17 patients died; the causes of death were B-cell ALL relapse or progression (12 patients), HHV-6–positive encephalitis in association with prolonged neutropenia and lymphopenia (1), systemic mycosis in association with prolonged neutropenia (1), and unknown causes (1); in 2 patients, death occurred after new therapies for B-cell ALL (1 from pneumonia and 1 from hepatobiliary disease).

DISCUSSION

In this global, multicenter, pivotal study of CAR T-cell therapy, high response rates were shown in children and young adults with relapsed or refractory B-cell ALL, 61% of whom had had a relapse after allogeneic hematopoietic stem-cell transplantation. Effective distribution of tisagenlecleucel across four continents with the use of a global supply chain was shown to be feasible and resulted in efficacy and safety similar to those observed in the previous, single-center study.[1]

This updated analysis showed an overall remission rate of 81% among 75 patients with at least 3 months of follow-up after a single infusion of tisagenlecleucel. The remissions were durable, with a 6-month relapse-free survival rate of 80%. The durability of the clinical response was associated with persistence of tisagenlecleucel in peripheral blood and with persistent B-cell aplasia.

The treatment of patients who have relapsed or refractory B-cell ALL after failure of two regimens is challenging. The rate of minimal residual disease–negative overall remission of 81% and the 6-month overall survival rate of 90% found in this study of tisagenlecleucel compare favorably with the rates achieved with Food and Drug Administration–approved agents for relapsed B-cell ALL. A pivotal phase 2 study of clofarabine involving 61 pediatric patients with relapsed or refractory ALL showed a response rate of 20%, a median response duration of 29 weeks (range, 1 to 48), and a median overall survival of 13 weeks (range, 1 to 89).[7] In a study of the CD19–CD3 bispecific antibody blinatumomab, complete remission occurred in 27 of the 70 pediatric patients (39%) with relapsed or refractory B-cell ALL within the first two cycles of blinatumomab treatment, with a rate of negativity for minimal residual disease of 20% and a median overall survival of 7.5 months.[8]

High rates of complete remission have also been found in pediatric and adult patients with relapsed or refractory ALL treated with other anti-CD19 CAR T-cell therapies, and U.S. multicenter phase 1–2 studies of the CAR T-cell therapy KTE-C19 have been initiated in pediatric and adult patients with relapsed or refractory ALL.[9-14] One key difference between CAR designs is the costimulatory domain; tisagenlecleucel contains a 4-1BB domain, which has been suggested to improve the persistence of CAR T cells through amelioration of T-cell exhaustion.[15] In a phase 1 study of KTE-C19, which contains a CD28 domain, involving 21 children and young adults, CAR T cells were not detected beyond 68 days; therefore, KTE-C19 has been used as a bridge to allogeneic transplantation for most patients who receive it.[13] In an updated analysis involving 38 patients, all but 1 patient in sustained remission proceeded to undergo allogeneic transplantation, and median leukemia-free survival was 17.7 months.[16] The anti-CD19 CAR T-cell therapy JCAR017, which contains a 4-1BB costimulatory domain, was recently shown to result in a median expected duration of B-cell aplasia of 3 months in a cohort of 42 pediatric and young adult patients with ALL and was detected at 6 months in patients with relapsed or refractory non-Hodgkin's lymphoma who had a response to treatment.[17,18] In an analysis involving 29 adult patients with ALL who were treated with JCAR017, of whom 27 had complete remission, 8 of the 13 in ongoing remission underwent subsequent allogeneic transplantation.[19] In the present study, the median persistence of tisagenlecleucel was 168 days at data cutoff, with ongoing persistence for as long as 20 months and relapse-free survival rates of 80% at 6 months and 59% at 12 months, with only 9% of patients proceeding to undergo allogeneic transplantation.

Previous studies showing promising results with anti-CD19 CAR T-cell therapies in the treatment of relapsed and refractory B-cell ALL were single-center studies, with manufacture occurring on site; therefore, the reproducibility and

feasibility of central manufacture in a global, multicenter setting remained uncertain. The toxicity and efficacy of tisagenlecleucel in this global, multicenter study were consistent with those in the single-center study, and the feasibility of a global supply chain was demonstrated.[1,4] Because this study used cryopreserved leukapheresis product (see the Methods section in the Supplementary Appendix), it did not require fresh product and an open manufacture slot for enrollment. Training in the management of toxic effects and data collection included implementation of a grading scale for the cytokine release syndrome that was developed at the University of Pennsylvania and Children's Hospital of Philadelphia, as well as a defined cytokine release syndrome management algorithm (Table S9 in the Supplementary Appendix).[20] Tisagenlecleucel was administered as a single infusion, and most toxic effects were observed only during the first 8 weeks after infusion. The product could be administered in the outpatient setting. In some cases, centers would initially elect to administer infusions to inpatients and then change to outpatient administration after they had gained more experience. Patients who were treated in the outpatient setting were admitted for fever. The median time from the onset of the cytokine release syndrome to grade 3 or 4 levels was 3 days. Although nearly half the patients received care in an ICU, the criteria for admission to the ICU varied widely across institutions. A total of 25% of patients were treated with high-dose vasopressors, a treatment commonly administered in intensive care settings.

Neurologic adverse events, which have been observed with anti-CD19 CAR T-cell therapies and blinatumomab,[8-10,21] occurred in our study. Most neurologic adverse events were transient, did not include cerebral edema, and appeared to be more frequent in patients with higher-grade cytokine release syndrome.

Ongoing tisagenlecleucel persistence was observed more than 1 year after infusion in patients with a treatment response. Across a 2-log tisagenlecleucel dose range, multi-log expansion occurred, and no relationship between infusion dose and expansion was found. This finding indicates that patients can be effectively treated with tisagenlecleucel across a wide dose range without an apparent effect on expansion and response.

In conclusion, tisagenlecleucel produced high remission rates and durable remissions without additional therapy in high-risk pediatric and young adult patients with relapsed or refractory B-cell ALL. The risks associated with tisagenlecleucel are substantial, leading to ICU-level care in some cases, but were mitigated in most patients with supportive measures and cytokine blockade.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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ORIGINAL ARTICLE

Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma

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ABSTRACT

BACKGROUND

In a phase 1 trial, axicabtagene ciloleucel (axi-cel), an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy, showed efficacy in patients with refractory large B-cell lymphoma after the failure of conventional therapy.

METHODS

In this multicenter, phase 2 trial, we enrolled 111 patients with diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, or transformed follicular lymphoma who had refractory disease despite undergoing recommended prior therapy. Patients received a target dose of 2×10^6 anti-CD19 CAR T cells per kilogram of body weight after receiving a conditioning regimen of low-dose cyclophosphamide and fludarabine. The primary end point was the rate of objective response (calculated as the combined rates of complete response and partial response). Secondary end points included overall survival, safety, and biomarker assessments.

RESULTS

Among the 111 patients who were enrolled, axi-cel was successfully manufactured for 110 (99%) and administered to 101 (91%). The objective response rate was 82%, and the complete response rate was 54%.With a median follow-up of 15.4 months, 42% of the patients continued to have a response, with 40% continuing to have a complete response. The overall rate of survival at 18 months was 52%. The most common adverse events of grade 3 or higher during treatment were neutropenia (in 78% of the patients), anemia (in 43%), and thrombocytopenia (in 38%). Grade 3 or higher cytokine release syndrome and neurologic events occurred in 13% and 28% of the patients, respectively. Three of the patients died during treatment. Higher CAR T-cell levels in blood were associated with response.

CONCLUSIONS

In this multicenter study, patients with refractory large B-cell lymphoma who received CAR T-cell therapy with axi-cel had high levels of durable response, with a safety profile that included myelosuppression, the cytokine release syndrome, and neurologic events. (Funded by Kite Pharma and the Leukemia and Lymphoma Society Therapy Acceleration Program; ZUMA-1 ClinicalTrials.gov number, NCT02348216.)

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Large B-cell lymphomas, including diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, and transformed follicular lymphoma, are treated with combination chemoimmunotherapy at diagnosis.[1-3] Patients who have a relapse with chemotherapy-sensitive disease may be treated with high-dose chemotherapy followed by autologous stem-cell transplantation.[1-3] However, patients who have disease that is resistant to primary or salvage chemoimmunotherapy or who have had a relapse after transplantation have an extremely poor prognosis.[4-13] Recently, in a large, international, retrospective research study involving patients with non-Hodgkin's lymphoma (SCHOLAR-1), investigators found an objective response rate of 26%, a complete response rate of 7%, and a median overall survival of 6.3 months with existing therapies among patients who had aggressive B-cell lymphoma that was resistant to chemotherapy or who had a relapse within 12 months after autologous stem-cell transplantation.[14]

Single-institution studies of anti-CD19 chimeric antigen receptor (CAR) T-cell therapy have shown high response rates in refractory B-cell lymphomas after the failure of conventional therapy.[15-19] Investigators at the National Cancer Institute have found that many responses have been ongoing beyond 4 years, which suggests that this therapy may be potentially curative.[15-17] Axicabtagene ciloleucel (axi-cel, Kite Pharma) is an autologous anti-CD19 CAR T-cell therapy that uses the same CAR construct that was developed at the National Cancer Institute.[15-17,20] It consists of a single-chain variable fragment extracellular domain targeting CD19 proteins with CD3ζ (also called CD247) and CD28 intracellular domains that signal T-cell activation.[20] In this therapy, T cells that have been removed from a patient are genetically engineered to express anti-CD19 CARs and are then injected back into the patient.

A phase 1 multicenter study (ZUMA-1) involving seven patients with refractory large B-cell lymphoma showed that axi-cel could be centrally manufactured and safely administered.[21] An overall response to axi-cel therapy was reported in five patients and a complete response in four patients, with an ongoing complete response in three patients reported at 1 year.[21] Here, we report the results of the primary analysis of phase 2 of ZUMA-1 and an updated analysis with 1 year of follow-up.

Methods

Patients and Study Design

The study was approved by the institutional review board at each study site and was conducted in accordance with the Good Clinical Practice guidelines of the International Conference on Harmonisation. All the patients provided written informed consent. The study was designed by employees of Kite Pharma, which also paid for medical-writing support. All the authors discussed and interpreted the results and vouch for the completeness and accuracy of the data and analyses and for the adherence of the study to the protocol, available with the full text of this article at NEJM.org. All the authors contributed to the conduct of the study, data analyses, and writing of the manuscript.

The phase 2 treatment portion of the study ran from November 2015 through September 2016 at 22 study centers (21 in the United States and 1 in Israel). (A complete list of study sites is provided in the Supplementary Appendix, available at NEJM.org.) Follow-up to evaluate the duration of response, survival, and late adverse events is ongoing.

All the patients had histologically confirmed large B-cell lymphoma, including diffuse large B-cell lymphoma (cohort 1) and primary mediastinal B-cell lymphoma or transformed follicular lymphoma (cohort 2), on the basis of the 2008 World Health Organization guidelines.[22] Central confirmation of the diagnosis was performed retrospectively. Patients had refractory disease, which was defined as progressive or stable disease as the best response to the most recent chemotherapy regimen or disease progression or relapse within 12 months after autologous stem-cell transplantation. Eligibility criteria and therapy were similar to those in the phase 1 study (see the Methods section in the Supplementary Appendix).[21]

After leukapheresis and axi-cel manufacturing, patients received fixed low-dose conditioning chemotherapy consisting of fludarabine (at a dose of 30 mg per square meter of body-surface area per day) and cyclophosphamide (at a dose of 500 mg per square meter per day) on days −5, −4, and −3 before the administration of a single intravenous infusion of axi-cel at a target dose of 2×10^6 CAR T cells per kilogram of body weight (on day 0).[21] Systemic bridging chemotherapy was

not allowed after leukapheresis and before the administration of axi-cel. Patients who had an initial response and then had disease progression at least 3 months after the first dose of axi-cel could be retreated.

END POINTS AND ASSESSMENTS

The primary end point was the rate of objective response (calculated as the combined rates of complete response and partial response), as assessed by the investigators according to the International Working Group Response Criteria for Malignant Lymphoma.[23] Secondary end points included the duration of response, progression-free survival, overall survival, incidence of adverse events, and blood levels of CAR T cells and serum cytokines. The cytokine release syndrome was graded according to the criteria of Lee et al.[24] We used the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03, to grade symptoms of the cytokine release syndrome and neurologic events along with other adverse events. CAR T-cell expansion and serum cytokines, and their associations with clinical outcomes, were analyzed as described previously.[21,25] The cell-of-origin subtype was assessed centrally by means of the NanoString Lymphoma Subtyping Test.[26] Details regarding the response criteria, grading of the cytokine release syndrome, and calculation of the CD19 histologic score are provided in the Methods section in the Supplementary Appendix.

STATISTICAL ANALYSIS

The primary analysis was conducted at the point when 92 patients could be evaluated 6 months after the axi-cel infusion. Efficacy and safety analyses were reported in the modified intention-to-treat population of all the patients who had received axi-cel. We also performed an updated analysis of all the patients who had been treated in phase 1[21] and phase 2 of ZUMA-1.

To analyze the response rate, we used a single-group design in which we compared the response of patients with a prespecified rate of response of 20% on the basis of historical values for refractory diffuse large B-cell lymphoma.[4-12] Efficacy testing had a power of at least 90% to distinguish between an active therapy with a 40% true response rate and a therapy with a response rate of 20% or less with the use of a one-sided alpha level of 0.025. The primary end point was tested with an exact binomial test. We used the Wilcoxon rank-sum test to measure the associations between outcomes and levels of CAR T cells and cytokines, with P values adjusted using Holm's procedure. Confidence intervals were calculated with the use of the Clopper–Pearson method.

RESULTS

PATIENTS

A total of 111 patients were enrolled in the study. Axi-cel was manufactured for 110 patients (99%) and administered to 101 patients (91%); the latter population was included in the modified intention-to-treat analysis. Patients included 77 with diffuse large B-cell lymphoma and 24 with primary mediastinal B-cell lymphoma or transformed follicular lymphoma (Table 1, and Fig. S1 in the Supplementary Appendix). The date of data cutoff for the primary analysis was January 27, 2017; the median follow-up was 8.7 months. The cutoff date for the updated analysis was August 11, 2017, which resulted in a median follow-up of 15.4 months.

The median time from leukapheresis to delivery of axi-cel to the treatment facility was 17 days. Of the 10 patients who did not receive axi-cel, 1 had unsuccessful manufacture of the CAR T-cell product, 4 had adverse events, 1 died from disease progression, and 2 had nonmeasurable disease before conditioning chemotherapy. After conditioning chemotherapy but before axi-cel infusion, 1 patient had sepsis and 1 died from multiple factors with laboratory abnormalities suggestive of the tumor lysis syndrome, gastrointestinal bleeding and perforation, and disease progression.

Among the patients who were treated with axi-cel, the median age was 58 years (range, 23 to 76). Most of the patients (85%) had stage III or IV disease; 77% had disease that was resistant to second-line or later therapies, 21% had disease relapse after transplantation, 69% had received at least three previous therapies, and 26% had a history of primary refractory disease (Table 1).

EFFICACY

Primary Analysis

At a minimum of 6 months of follow-up, the objective response rate among the protocol-specified 92 patients was 82% (95% confidence interval [CI], 72 to 89; P<0.001 for the comparison with a 20% historical control rate); among these patients, the complete response rate was 52% (Table S1 in the

Supplementary Appendix). An additional 9 patients were enrolled and awaiting treatment at the time that the 92nd patient received the axi-cel infusion. Among the 101 patients who received axi-cel, the objective response rate was 82% (95% CI, 73 to 89), with a 54% complete response rate (Fig. 1A, and Fig. S2 in the Supplementary Appendix).

The median time to response was rapid (1.0 month; range, 0.8 to 6.0). The median duration of response was 8.1 months (95% CI, 3.3 to could not be estimated). Response rates were consistent across key covariates, including age, disease stage, International Prognostic Index score at enrollment, presence or absence of bulky disease, cell-of-origin subtype, and use of tocilizumab or gluco-

Table 1. Treatment Disposition and Baseline Characteristics of the Patients.*

Variable	Patients with DLBCL	Patients with PMBCL or TFL	All Patients
Treatment disposition			
No. of patients enrolled	81	30	111
Treatment with axi-cel — no. (%)			
Yes	77 (95)	24 (80)	101 (91)
No	4 (5)	6 (20)	10 (9)
Death before treatment†	1 (1)	2 (7)	3 (3)
Adverse event‡	3 (4)	2 (7)	5 (5)
Other§	0	2 (7)	2 (2)
Characteristics at baseline			
No. of patients	77	24	101
Disease type — no. (%)			
DLBCL	77 (100)	0	77 (76)
PMBCL	0	8 (33)	8 (8)
TFL	0	16 (67)	16 (16)
Age			
Median (range) — yr	58 (25–76)	57 (23–76)	58 (23–76)
≥65 yr — no. (%)	17 (22)	7 (29)	24 (24)
Male sex — no. (%)	50 (65)	18 (75)	68 (67)
ECOG performance-status score of 1 — no. (%)	49 (64)	10 (42)	59 (58)
Disease stage — no. (%)			
I or II	10 (13)	5 (21)	15 (15)
III or IV	67 (87)	19 (79)	86 (85)
International Prognostic Index score — no. (%)¶			
0–2	40 (52)	13 (54)	53 (52)
3 or 4	37 (48)	11 (46)	48 (48)
CD-19 status — no./total no. (%)‖			
Negative	7/63 (11)	1/19 (5)	8/82 (10)
Positive	56/63 (89)	18/19 (95)	74/82 (90)
Prior therapies — no. (%)			
≥Three prior lines of therapy	49 (64)	21 (88)	70 (69)
History of primary refractory disease**	23 (30)	3 (12)	26 (26)
History of resistance to two consecutive lines	39 (51)	15 (62)	54 (53)

Table 1. (Continued.)

Variable	Patients with DLBCL	Patients with PMBCL or TFL	All Patients
Refractory subgroup at study entry — no. (%)			
Primary refractory	2 (3)	0	2 (2)
Refractory to second-line or subsequent therapy	59 (77)	19 (79)	78 (77)
Relapse after autologous stem-cell transplantation	16 (21)	5 (21)	21 (21)

* The abbreviation axi-cel denotes axicabtagene ciloleucel, DLBCL diffuse large B-cell lymphoma, ECOG Eastern Cooperative Oncology Group, PMBCL primary mediastinal large B-cell lymphoma, and TFL transformed follicular lymphoma.
† Two patients died from disease progression (one after unsuccessful manufacture of the CAR T-cell product) and one from the tumor lysis syndrome.
‡ The adverse events in the four patients who had undergone leukapheresis but had not received conditioning therapy or axi-cel were small intestine obstruction, hypoxia and pleural effusion, spinal column stenosis, and deep-vein thrombosis. The remaining patient received conditioning therapy but had a skin and wound infection that led to ecthyma and sepsis before axi-cel treatment.
§ The two patients in this category had nonmeasurable disease after leukapheresis.
¶ Scores on the International Prognostic Index include low risk (0 or 1 point), low-intermediate risk (2 points), high-intermediate risk (3 points), and high risk (4 or 5 points).
‖ The CD19 histologic score was assessed in the 82 patients with available samples.
** Patients may have had other therapies after primary refractory disease.

corticoids. Responses were also consistent in 26 patients who had a history of primary refractory disease (response rate, 88%) and in 21 patients who had a history of autologous stem-cell transplantation (response rate, 76%). The response rates did not appear to be influenced by biologic covariates, such as the prevalence and intensity of CD19 expression, or by product characteristics, such as the ratio of CD4 cells to CD8 cells and T-cell phenotypes (Fig. 1B, and Tables S2, S3, and S4 in the Supplementary Appendix).

At the time of the primary analysis, 52 patients had disease progression, 3 patients had died from adverse events during treatment, 1 patient started an alternative therapy before disease progression, 44 remained in remission (of whom 39 had a complete response), and 1 had stable disease. Of the patients who had disease progression after an initial response, 9 were retreated with axi-cel, according to the protocol. Of these patients, 5 had a response (2 complete and 3 partial), and 2 of these patients had an ongoing response.

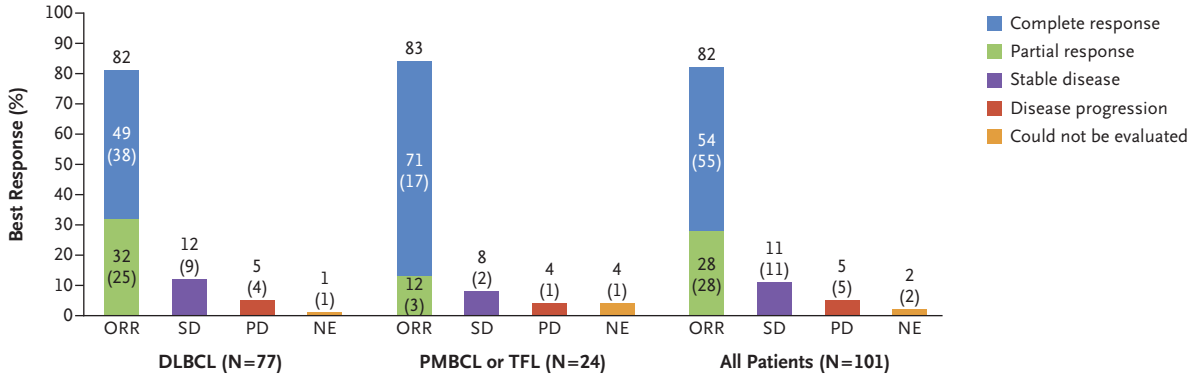
Updated Analysis

To evaluate the durability of response with axi-cel, we performed an updated analysis when the 108 patients in the phase 1 and 2 portions of ZUMA-1 had been followed for a minimum of 1 year. The objective response rate was 82%, including a complete response rate of 58%. Of the patients who did not have a complete response at the time of the first tumor assessment (1 month after the infusion of axi-cel), 23 patients (11 of 35 with a partial response and 12 of 25 with stable disease) subsequently had a complete response in the absence of additional therapies as late as 15 months after treatment. At the data cutoff, 42% remained in response, including 40% with a complete response. Of the 7 patients in phase 1 of the study, 3 had an ongoing complete response at 24 months.

Preliminary analysis of CD19 expression at baseline and at the time of disease progression was ongoing. Of the 11 patients with disease progression who were included in the analysis, 3 (27%) with CD19-positive status at baseline had CD19-negative disease at time of disease progression.

Ongoing response rates were consistent across key covariates, including the use of tocilizumab or glucocorticoids (Fig. S3 in the Supplementary Appendix). The median duration of response was 11.1 months (95% CI, 3.9 to could not be estimated) (Fig. 2A). The median duration of progression-free survival was 5.8 months (95% CI, 3.3 to could not be estimated) (Fig. 2B), with progression-free survival rates of 49% (95% CI, 39 to 58) at 6 months, 44% (95% CI, 34 to 53) at 12 months, and 41% (95% CI, 31 to 50) at 15 months. The median overall survival was not yet reached

A Objective Response Rate



B Subgroup Analysis

Subgroup	No. of Patients Who Could Be Evaluated	No. of Patients with Event	Objective Response Rate (95% CI)
Overall	101	83	0.82 (0.73–0.89)
Refractory subgroup			
Refractory to ≥second-line therapy	78	65	0.83 (0.73–0.91)
Relapse after ASCT	21	16	0.76 (0.53–0.92)
Age			
<65 yr	77	61	0.79 (0.68–0.88)
≥65 yr	24	22	0.92 (0.73–0.99)
Disease stage			
I or II	15	13	0.87 (0.60–0.98)
III or IV	86	70	0.81 (0.72–0.89)
IPI risk score			
0–2	53	46	0.87 (0.75–0.95)
3 or 4	48	37	0.77 (0.63–0.88)
Extranodal disease			
Yes	70	56	0.80 (0.69–0.89)
No	31	27	0.87 (0.70–0.96)
Bulky disease (≥10 cm)			
Yes	17	12	0.71 (0.44–0.90)
No	84	71	0.85 (0.75–0.91)
Treatment history			
Primary refractory disease	26	23	0.88 (0.70–0.98)
Refractory to two consecutive lines	54	42	0.78 (0.64–0.88)
CD19 status			
Positive	74	63	0.85 (0.75–0.92)
Negative	8	6	0.75 (0.35–0.97)
CD19 histologic score			
≤150	26	22	0.85 (0.65–0.96)
>150	56	47	0.84 (0.72–0.92)
Cell of origin			
Germinal center B-cell–like subtype	49	43	0.88 (0.75–0.95)
Activated B-cell–like subtype	17	13	0.76 (0.50–0.83)
CD4:CD8 ratio			
>1	47	41	0.87 (0.74–0.95)
≤1	52	40	0.77 (0.63–0.87)
Tocilizumab use			
Yes	43	36	0.84 (0.69–0.93)
No	58	47	0.81 (0.69–0.90)
Glucocorticoid use			
Yes	27	21	0.78 (0.58–0.91)
No	74	62	0.84 (0.73–0.91)

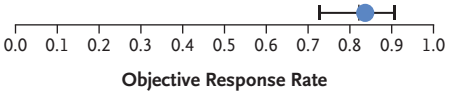


Figure 1 (facing page). Objective Response Rate among the 101 Treated Patients.

Panel A shows the objective response rate (ORR; calculated as complete response [CR] plus partial response [PR]) among the patients who received axicabtagene ciloleucel (axi-cel), an anti-CD19 chimeric antigen receptor T-cell therapy, as well as the response among the patients with stable disease (SD), disease progression (PD), and those who could not be evaluated (NE). The patients in the modified intention-to-treat population were evaluated according to the two main disease cohorts: diffuse large B-cell lymphoma (DLBCL) and either primary mediastinal large B-cell lymphoma (PMBCL) or transformed follicular lymphoma (TLF). The numbers in parentheses indicate the number of patients who had the specified response. On independent central review, the objective response rate was 71%, including a complete response rate of 51% and a partial response rate of 20%. Panel B shows the subgroup analysis of the objective response rate for key baseline and clinical covariates. Scores on the International Prognostic Index (IPI) include low risk (0 or 1 point), low-intermediate risk (2 points), high-intermediate risk (3 points), and high risk (4 or 5 points). The 95% confidence interval (CI) was calculated with the use of the Clopper–Pearson method. ASCT denotes autologous stem-cell transplantation.

(95% CI, 12.0 months to could not be estimated) (Fig. 2C), with overall survival rates of 78% (95% CI, 69 to 85) at 6 months, 59% (95% CI, 49 to 68) at 12 months, and 52% (95% CI, 41 to 62) at 18 months. A total of 56% of patients remained alive at the time of the data cutoff. Two patients who had a response underwent allogeneic stem-cell transplantation.

SAFETY

Primary Analysis

During treatment, all 101 patients who had received axi-cel had adverse events, which were grade 3 or higher in 95% (Table 2). The most common adverse events of any grade were pyrexia (in 85% of the patients), neutropenia (in 84%), and anemia (in 66%). The most common adverse events of grade 3 or higher were neutropenia (in 78%), anemia (in 43%), and thrombocytopenia (in 38%). The cytokine release syndrome occurred in 94 patients (93%). Most cases were of low grade (37% of grade 1 and 44% of grade 2), with 13% of grade 3 or higher (9% of grade 3, 3% of grade 4, and 1% of grade 5).

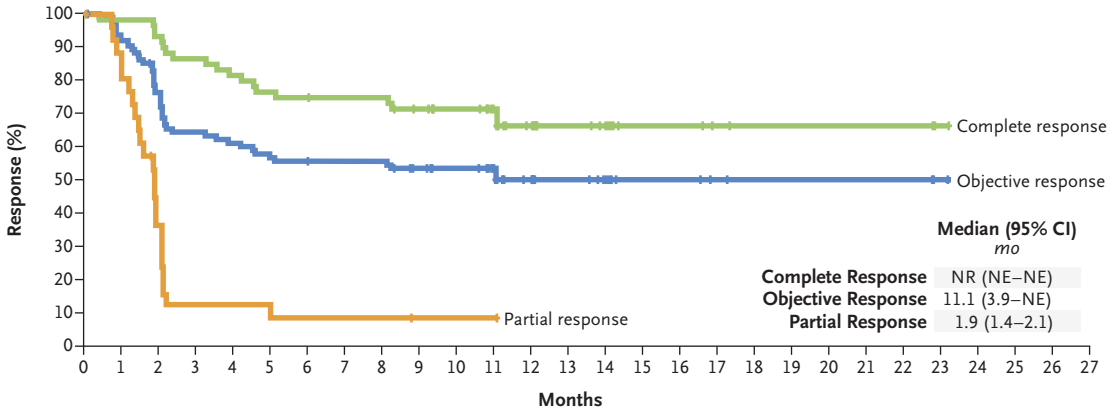
The most common symptoms of the cytokine release syndrome of grade 3 or higher were pyrexia (in 11% of the patients), hypoxia (in 9%), and hypotension (in 9%). Vasopressors were used in 17% of the patients. The median time after infusion until the onset of the cytokine release syndrome was 2 days (range, 1 to 12), and the median time until resolution was 8 days. All the events associated with the cytokine release syndrome resolved except for one event of grade 5 hemophagocytic lymphohistiocytosis. Another event of grade 5 cardiac arrest occurred in a patient with the cytokine release syndrome.

Neurologic events occurred in 65 patients (64%); 28% were grade 3 or higher. The most common neurologic events of grade 3 or higher were encephalopathy (in 21% of the patients), confusional state (in 9%), aphasia (in 7%), and somnolence (in 7%). Early neurologic signs included word-finding difficulties (dysphasia), attention or calculation defects (counting backward by serial 7s), and difficulty executing complex commands (handwriting).[27] The median onset of neurologic events occurred on day 5 (range, 1 to 17), with median resolution on day 17 after infusion. One patient had ongoing grade 1 memory impairment that resolved after the data cutoff for the primary analysis. All the other neurologic events resolved except for four events, which were ongoing at the time of death (two deaths from progressive disease and two from adverse events unrelated to neurologic events). Rates of the cytokine release syndrome and neurologic events decreased over the course of the study (Table S5 in the Supplementary Appendix). Forty-three percent of patients received tocilizumab and 27% received glucocorticoids for the management of the cytokine release syndrome, neurologic events, or both,[24] with no apparent effect on overall or ongoing response rates (Fig. 1B, and Fig. S3 in the Supplementary Appendix).

Updated Analysis

Ten patients had serious adverse events (including nine infections in 8 patients) after the data cutoff for the primary analysis (Table S6 in the Supplementary Appendix). There were no new events associated with the cytokine release syndrome or neurologic events related to axi-cel treatment. Forty-four patients (44%) died from causes that included disease progression (in 37 patients), adverse events (in 3 patients, including 2 with the above-mentioned axi-cel–related events associated

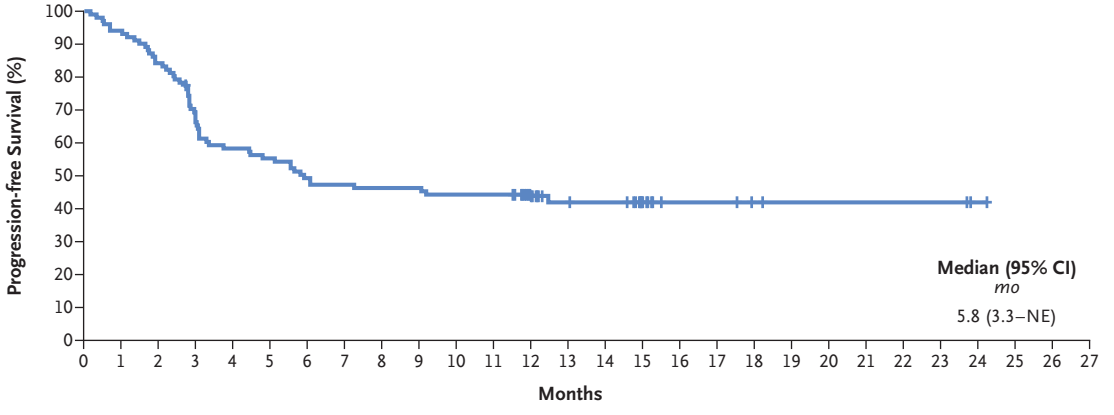
A Duration of Response



No. at Risk

Complete response	63	61	58	53	50	47	46	45	45	41	37	30	19	16	12	6	6	4	3	3	3	3	1	0
Objective response	89	82	67	56	53	49	48	47	47	42	38	31	19	16	12	6	6	4	3	3	3	3	1	0
Partial response	26	21	9	3	3	2	2	2	2	1	1	1	0											

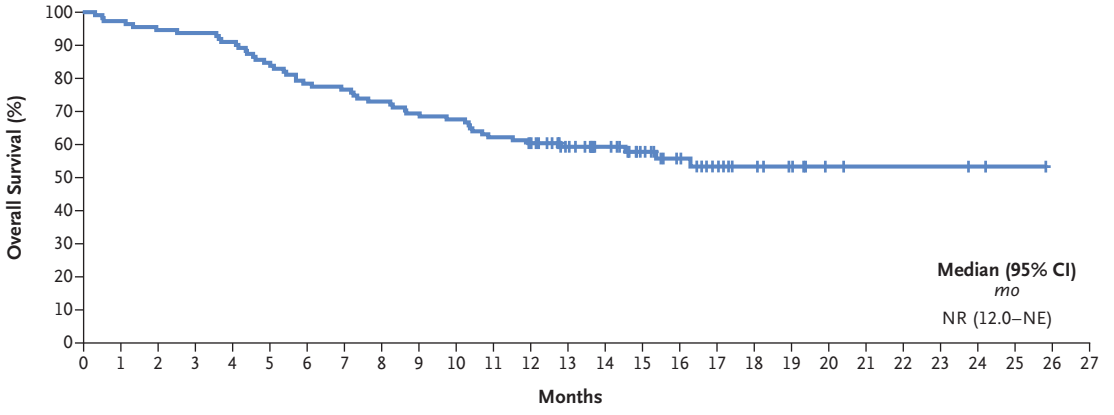
B Progression-free Survival



No. at Risk

	108	101	90	71	61	58	52	50	49	49	47	47	34	21	20	12	6	6	4	3	3	3	3	1	0

C Overall Survival



No. at Risk

	108	105	102	101	98	91	84	82	78	74	72	66	63	51	40	30	23	16	11	8	4	3	3	3	2	1	0

Figure 2 (facing page). Kaplan–Meier Estimates of the Duration of Response, Progression-free Survival, and Overall Survival.

Panel A shows the duration of response, according to investigator assessment, in the 89 study patients who had an objective response, including those with a complete response and those with a partial response. Patients who had a complete response had a longer duration of response than those with an objective or partial response. According to independent central review, the median duration of response was 8.1 months (range, 3.5 to could not be estimated [NE]). Panel B shows the rate of progression-free survival, and Panel C the rate of overall survival in the 108 patients who were treated in the phase 1 and phase 2 studies. Tick marks indicate the time of data censoring at the last follow-up. NR denotes not reached.

with the cytokine release syndrome and 1 with pulmonary embolism that was not related to axi-cel), and other causes after disease progression and subsequent therapies that were not related to axi-cel (in 4). One death that was not associated with axi-cel was previously reported in phase 1 of ZUMA-1.[21] There were no new deaths from adverse events after the primary analysis. No cases of replication-competent retrovirus or axi-cel treatment-related secondary cancers were reported.

BIOMARKERS

CAR T levels peaked in the peripheral blood within 14 days after infusion of axi-cel and were detectable in most patients at 180 days after infusion (Fig. 3A). Three patients with ongoing complete remission at 24 months still had detectable CAR T levels in the blood. Expansion was significantly associated with response ($P<0.001$), with an area under the curve within the first 28 days after treatment that was 5.4 times as high among the patients who had a response as among those who did not have a response. Peak expansion and area under the curve were significantly associated with neurologic events of grade 3 or higher but not with the cytokine release syndrome (Fig. 3B, and Table S7 and Fig. S4 in the Supplementary Appendix). Of 44 serum biomarkers that were examined, several biomarkers, including interleukin-6, -10, -15, and -2Rα and granzyme B, were significantly associated with neurologic events and the cytokine release syndrome of grade 3 or higher (Table S8 in the Supplementary Appendix). Several biomarkers, including interleukin-2, granulocyte–macrophage colony-stimulating factor (GM-CSF), and ferritin, were significantly associated only with neurologic events of grade 3 or higher (Fig. 3C). The induction of anti-CAR antibodies was not observed in any patient.

DISCUSSION

In this multicenter, phase 2 trial of axi-cel therapy, 82% of the 101 patients with refractory large B-cell lymphoma who were treated had an objective response, and 54% had a complete response. These findings compare favorably with the results of the recent SCHOLAR-1 study of existing therapies for this disease, which showed an objective response rate of 26% and a complete response rate of 7%.[14] With a median follow-up of 15.4 months in our study, responses were ongoing in 42% of the patients, including in 40% who had a complete response, with the emergence of a plateau in the duration of the response curve at 6 months. Although most responses occurred in the first month, 23 patients had a complete response as late as 15 months. It would be reasonable to monitor patients who did not have a complete response at the first disease assessment and allow for an opportunity for an improved response, since consolidation with allogeneic stem-cell transplantation comes with a high rate of treatment-related death and would also ablate CAR T cells. The median overall survival had not been reached, with an overall survival rate at 18 months of 52%. Ongoing durable remissions have been observed in patients at 24 months. These results, combined with the observation of ongoing long-term remissions beyond 4 years in the previous National Cancer Institute study,[17] suggest that axi-cel provides substantial clinical benefit for patients with refractory disease.

In our study, the responses to treatment, including ongoing ones, were consistent across key covariates. Similar response rates were observed in the 8 patients with CD19-negative disease as in those with CD19-positive disease at baseline, which suggests the potential limitations in CD19 detection rather than true CD19 negativity. Analyses of product characteristics, including the ratio of CD4 cells to CD8 cells and T-cell phenotypes, also showed similar outcomes, which further

highlights the consistency in treatment effects across clinical and biologic covariates. One limitation of our study is the lack of a planned, detailed analysis of molecular and cytogenetic characteristics. Prospective data are needed on the influence of disease biology, such as double- and triple-hit lymphomas, on outcomes with CAR T-cell therapy.

To be successful, a personalized cell therapy must be delivered in a safe and timely manner. In this study, we confirmed the feasibility and reliability of centralized manufacturing and coordination of leukapheresis procedures and shipping from multiple centers across the country. The product was manufactured for 99% of the enrolled patients and was administered to 91%.

Table 2. Adverse Events, the Cytokine Release Syndrome, and Neurologic Events Associated with Treatment.*

Event	Any Grade	Grade 1 or 2	Grade ≥3
		number of patients (percent)	
Adverse event			
Any	101 (100)	5 (5)	96 (95)
Pyrexia	86 (85)	72 (71)	14 (14)
Neutropenia	85 (84)	6 (6)	79 (78)
Anemia	67 (66)	24 (24)	43 (43)
Hypotension	60 (59)	46 (46)	14 (14)
Thrombocytopenia	59 (58)	21 (21)	38 (38)
Nausea	59 (58)	59 (58)	0
Fatigue	52 (51)	50 (50)	2 (2)
Decreased appetite	50 (50)	48 (48)	2 (2)
Headache	47 (47)	46 (46)	1 (1)
Diarrhea	43 (43)	39 (39)	4 (4)
Hypoalbuminemia	41 (41)	40 (40)	1 (1)
Hypocalcemia	40 (40)	34 (34)	6 (6)
Chills	39 (39)	39 (39)	0
Tachycardia	39 (39)	37 (37)	2 (2)
Febrile neutropenia	35 (35)	4 (4)	31 (31)
Encephalopathy	34 (34)	13 (13)	21 (21)
Thrombocytopenia	59 (58)	21 (21)	38 (38)
Vomiting	34 (34)	33 (33)	1 (1)
Hypokalemia	33 (33)	30 (30)	3 (3)
Hyponatremia	33 (33)	23 (23)	10 (10)
Constipation	31 (31)	31 (31)	0
White-cell count decreased	31 (31)	2 (2)	29 (29)
Cytokine release syndrome			
Any	94 (93)	81 (80)	13 (13)
Pyrexia	77 (76)	66 (65)	11 (11)
Hypotension	41 (41)	32 (32)	9 (9)
Hypoxia	22 (22)	13 (13)	9 (9)
Tachycardia	21 (21)	20 (20)	1 (1)
Chills	20 (20)	20 (20)	0
Sinus tachycardia	8 (8)	8 (8)	0
Headache	5 (5)	5 (5)	0

Table 2. (Continued.)

Event	Any Grade	Grade 1 or 2	Grade ≥3
		number of patients (percent)	
Neurologic event			
Any	65 (64)	37 (37)	28 (28)
Encephalopathy	34 (34)	13 (13)	21 (21)
Confusional state	29 (29)	20 (20)	9 (9)
Tremor	29 (29)	28 (28)	1 (1)
Aphasia	18 (18)	11 (11)	7 (7)
Somnolence	15 (15)	8 (8)	7 (7)
Agitation	9 (9)	5 (5)	4 (4)
Memory impairment	7 (7)	6 (6)	1 (1)
Mental-status change	6 (6)	4 (4)	2 (2)

* Listed are adverse events that occurred in at least 30% of the patients, along with symptoms of the cytokine release syndrome and neurologic events that occurred in at least 5% of the patients. The cytokine release syndrome was categorized according to a modified grading system proposed by Lee et al.[24] Individual symptoms of the cytokine release syndrome and neurologic events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.

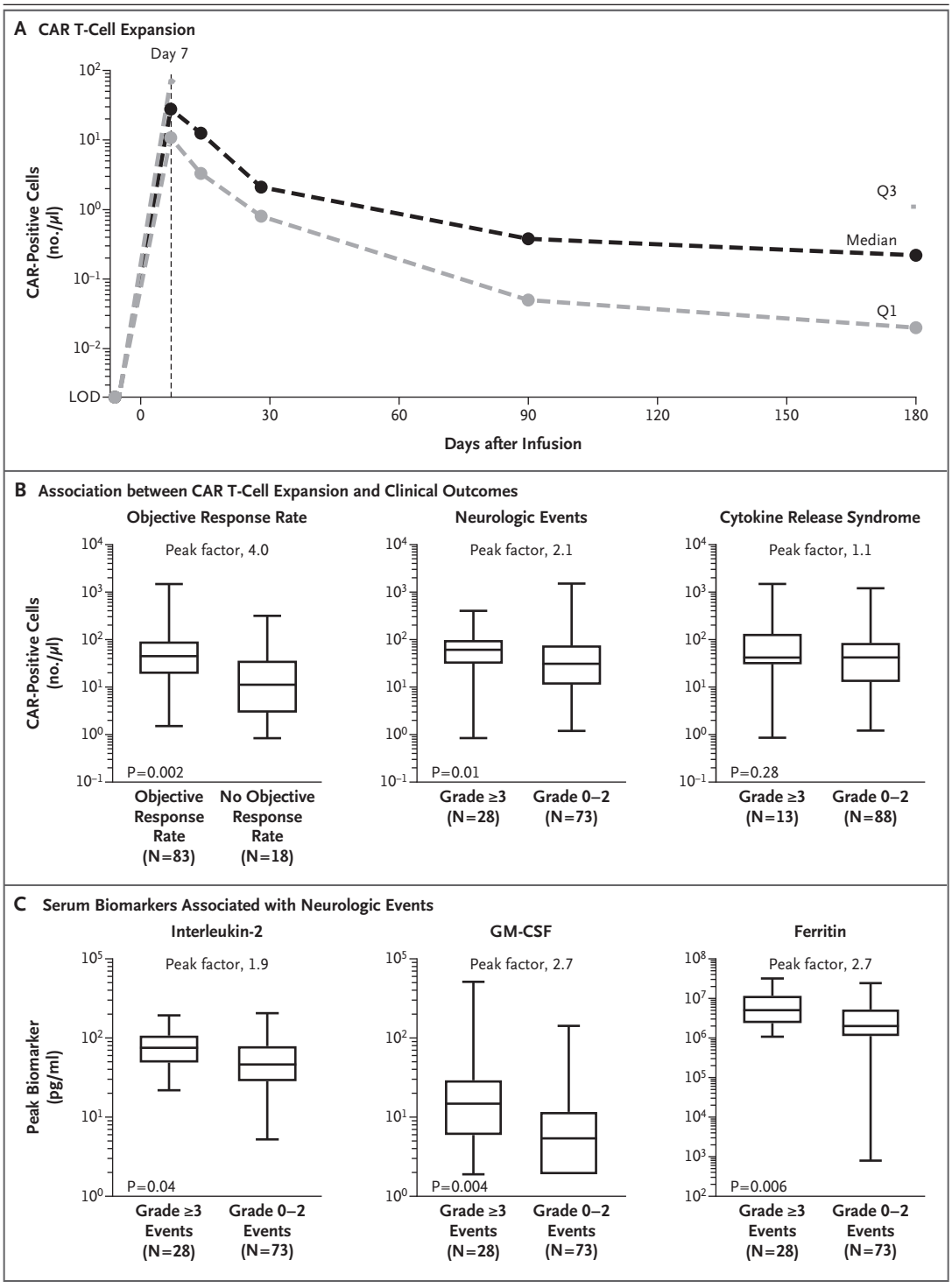
The short 17-day median turnaround time was critical for these patients with refractory large B-cell lymphoma, who generally have rapidly growing disease.

We found that axi-cel could be administered safely at medical facilities that perform transplantation, even if such centers had no experience in CAR T-cell therapy. Algorithms for the management of the cytokine release syndrome and neurologic events were effectively implemented. The incidence of the cytokine release syndrome and neurologic events of grade 3 or higher decreased over the course of the study, possibly because of increased experience at the study centers and a protocol amendment allowing for earlier administration of tocilizumab or glucocorticoids.[27] The cytokine release syndrome and neurologic events were generally reversible with no clinical sequelae. With extended follow-up, there were no new unexpected serious adverse events and no new onset of the cytokine release syndrome or neurologic events related to CAR T cells. Furthermore, the 3% rate of death during treatment compares favorably with rates observed during allogeneic stem-cell transplantation.[28]

CAR T-cell levels over the first 28 days of treatment correlated with an objective response. This finding was consistent with prior single-institution trials of CAR T-cell therapy[19,25] and strengthens the hypothesis that the presence of higher CAR T-cell levels after infusion may further augment efficacy. Recent studies have shown intrinsic differences in CAR T cells that use CD28 rather than other costimulatory molecules, such as 4-1BB,[29,30] but it remains unclear whether either costimulatory domain will confer differences in activity or persistence in patients and whether such responses are dependent on the tumor type.[31] Therefore, optimization of CAR constructs and manufacturing as well as combination strategies with immunomodulatory agents are being explored.

Serum biomarker analysis confirmed associations of the presence of interleukin-6, -15, and -2Rα, as well as other markers, with the cytokine release syndrome of grade 3 or higher[19,32-35] and with neurologic events of grade 3 or higher.[19,34] However, CAR T-cell levels and specific cytokines, including interleukin-2, GM-CSF, and ferritin, were associated only with grade 3 or higher neurologic events, which suggests that distinct mechanisms may underlie the pathogenesis of these adverse events.

Although there is a theoretical concern regarding the use of immunosuppressive agents to manage the cytokine release syndrome or neurologic events, the use of tocilizumab or glucocorticoids did not appear to affect the overall response among the patients in our study. Furthermore, the development of a predictive or prognostic early



biomarker of the cytokine release syndrome or neurologic events may assist clinicians in determining when to intervene and optimize the management of toxic effects while preserving efficacy.

In conclusion, our findings support the use of axi-cel as an effective therapeutic option in adult patients with relapsed or refractory large B-cell lymphoma after at least two prior systemic therapies. Adverse events included myelosuppression,

Figure 3 (facing page). CAR T-Cell Expansion and Correlations with Response and Adverse Events.

Serial blood samples were analyzed for chimeric antigen receptor (CAR) T-cell levels and serum biomarkers in all 101 patients who were treated with axi-cel, as described previously.[21] Panel A shows CAR T-cell expansion and persistence with median values and interquartile ranges (Q1 and Q3). Panel B shows the association between CAR T-cell expansion, which was measured as peak levels of CAR cells per microliter of blood, and the objective response rate, neurologic events, and the cytokine release syndrome. The peak factor change is shown for patients with a response as compared with those without a response, for those with neurologic events of grade 3 or higher, and for those with the cytokine release syndrome of grade 3 or higher. P values were calculated by means of the Wilcoxon rank-sum test. Panel C shows serum biomarkers (interleukin-2, granulocyte–macrophage colony-stimulating factor [GM-CSF], and ferritin) that were associated only with neurologic events and not with the cytokine release syndrome. The peak value is defined as the maximum level of the cytokine after baseline. The peak factor is the value in patients with neurologic events of grade 3 or higher versus those with events of grade 0 to 2. Adjusted P values were calculated with the use of Holm's procedure after multiple testing by means of the Wilcoxon rank-sum test. In Panels B and C, the horizontal line within each box represents the median, and the lower and upper borders of each box represent the 25th and the 75th percentiles, respectively, and the I bars represent the minimum and maximum range.

the cytokine release syndrome, and neurologic events.

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APPENDIX

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