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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.)

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 2×10⁶ 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.
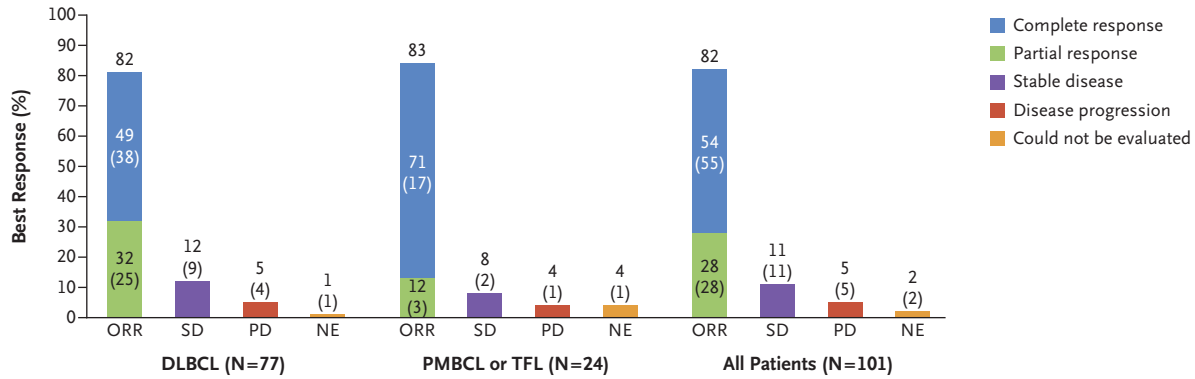
Updated Analysis
To evaluate the durability of response with axi-cel, we performed an updated analysis when the 108 patients in the phase 1 and 2 portions of ZUMA-1 had been followed for a minimum of 1 year. The objective response rate was 82%, including a com-

plete 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

Legend:
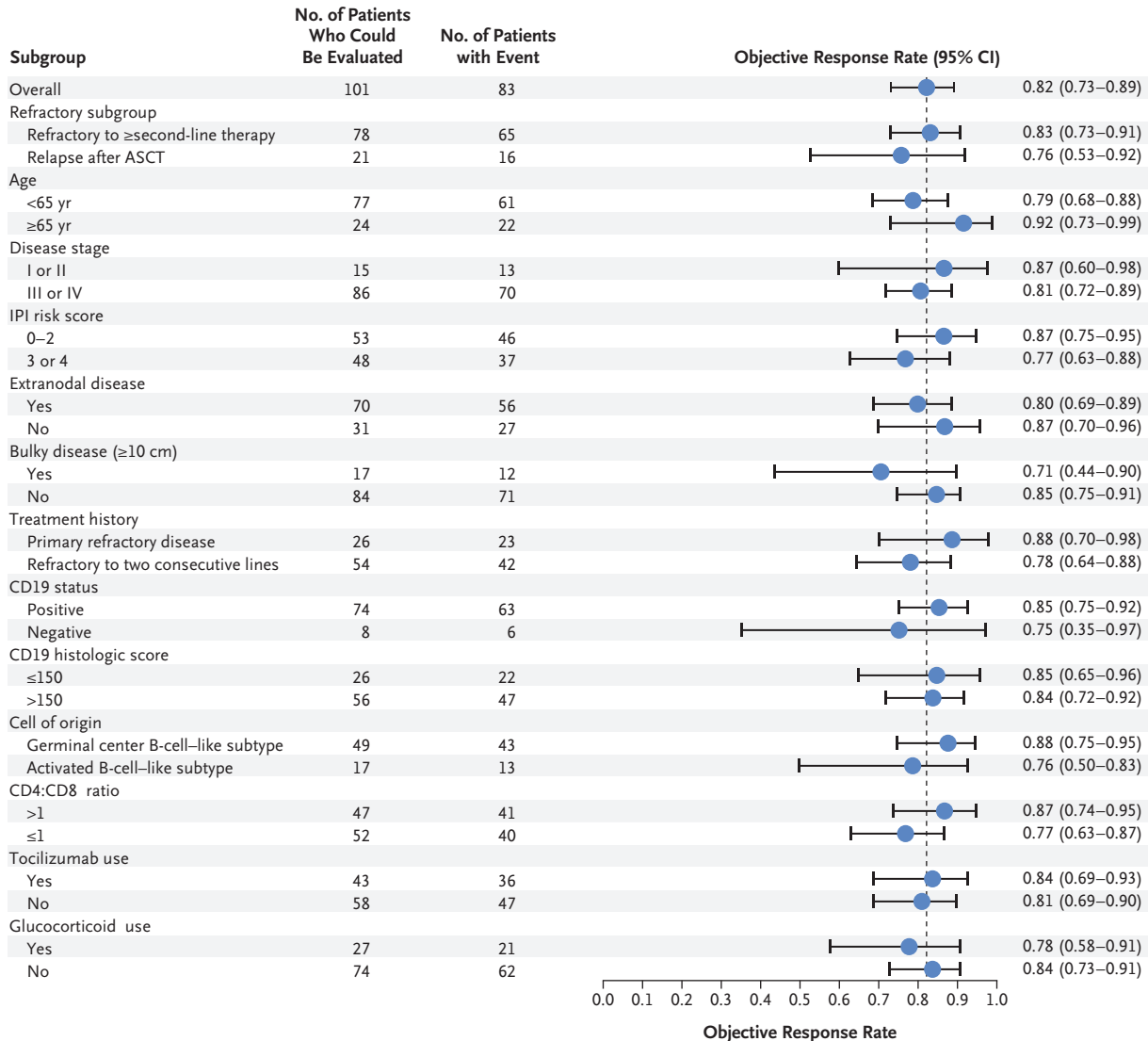- Complete response (blue)
- Partial response (green)
- Stable disease (purple)
- Disease progression (red)
- Could not be evaluated (orange)

DLBCL (N=77): ORR 82 [49 (38) / 32 (25)], SD 12 (9), PD 5 (4), NE 1 (1)
PMBCL or TFL (N=24): ORR 83 [71 (17) / 12 (3)], SD 8 (2), PD 4 (1), NE 4 (1)
All Patients (N=101): ORR 82 [54 (55) / 28 (28)], SD 11 (11), PD 5 (5), NE 2 (2)

Y-axis: Best Response (%)

B Subgroup Analysis

Subgroup	No. of Patients Who Could Be Evaluated	No. of Patients with Event	Objective Response Rate (95% CI)	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)

X-axis: Objective Response Rate (0.0 to 1.0)

(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 py-

rexia (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
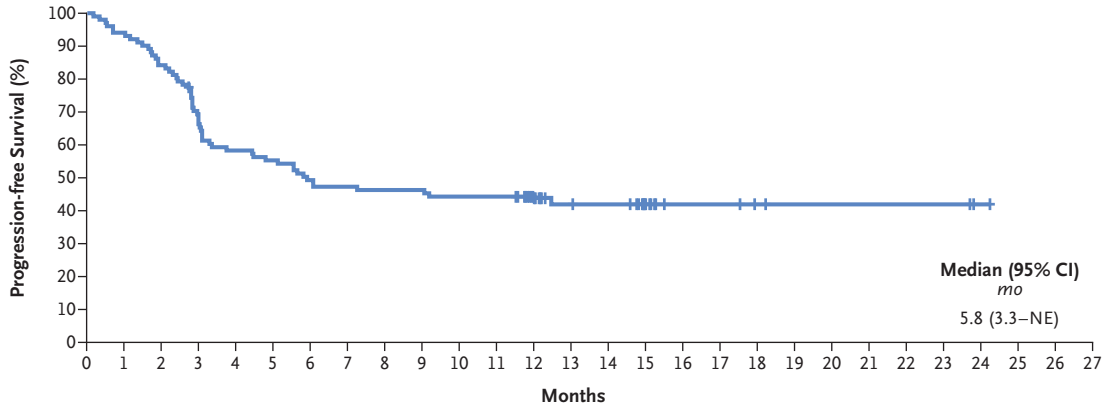
A Duration of Response
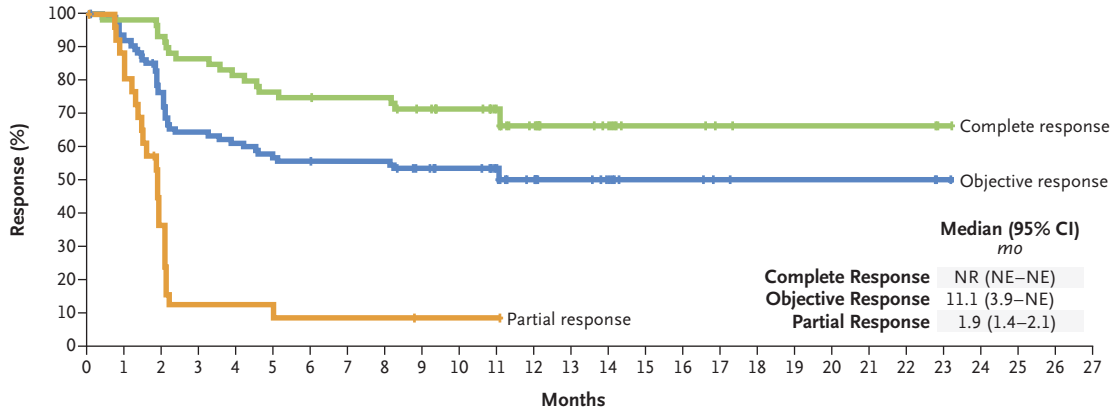
No. at Risk																											
Complete response	63	61	58	53	50	47	46	45	45	41	37	30	19	16	12	6	6	4	3	3	3	3	3	1	0	
Objective response	89	82	67	56	53	49	48	47	47	42	38	31	19	16	12	6	6	4	3	3	3	3	3	1	0	
Partial response	26	21	9	3	3	2	2	2	2	1	1	1	0													

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 | 3 | 1 | 0 |

C Overall Survival
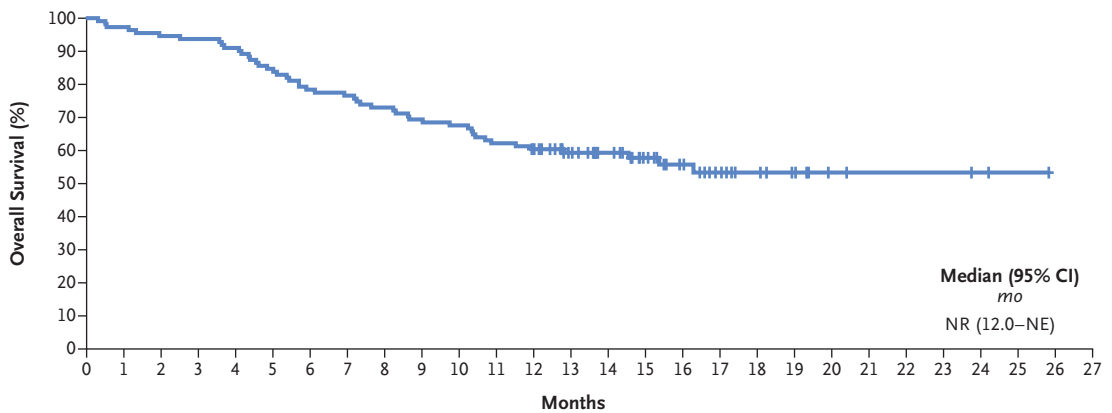
| No. at Risk | 108 | 105 | 102 | 101 | 98 | 91 | 84 | 82 | 78 | 74 | 72 | 66 | 63 | 51 | 40 | 30 | 23 | 16 | 11 | 8 | 4 | 3 | 3 | 3 | 2 | 1 | 0 |

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

A CAR T-Cell Expansion

B Association between CAR T-Cell Expansion and Clinical Outcomes

C Serum Biomarkers Associated with Neurologic Events
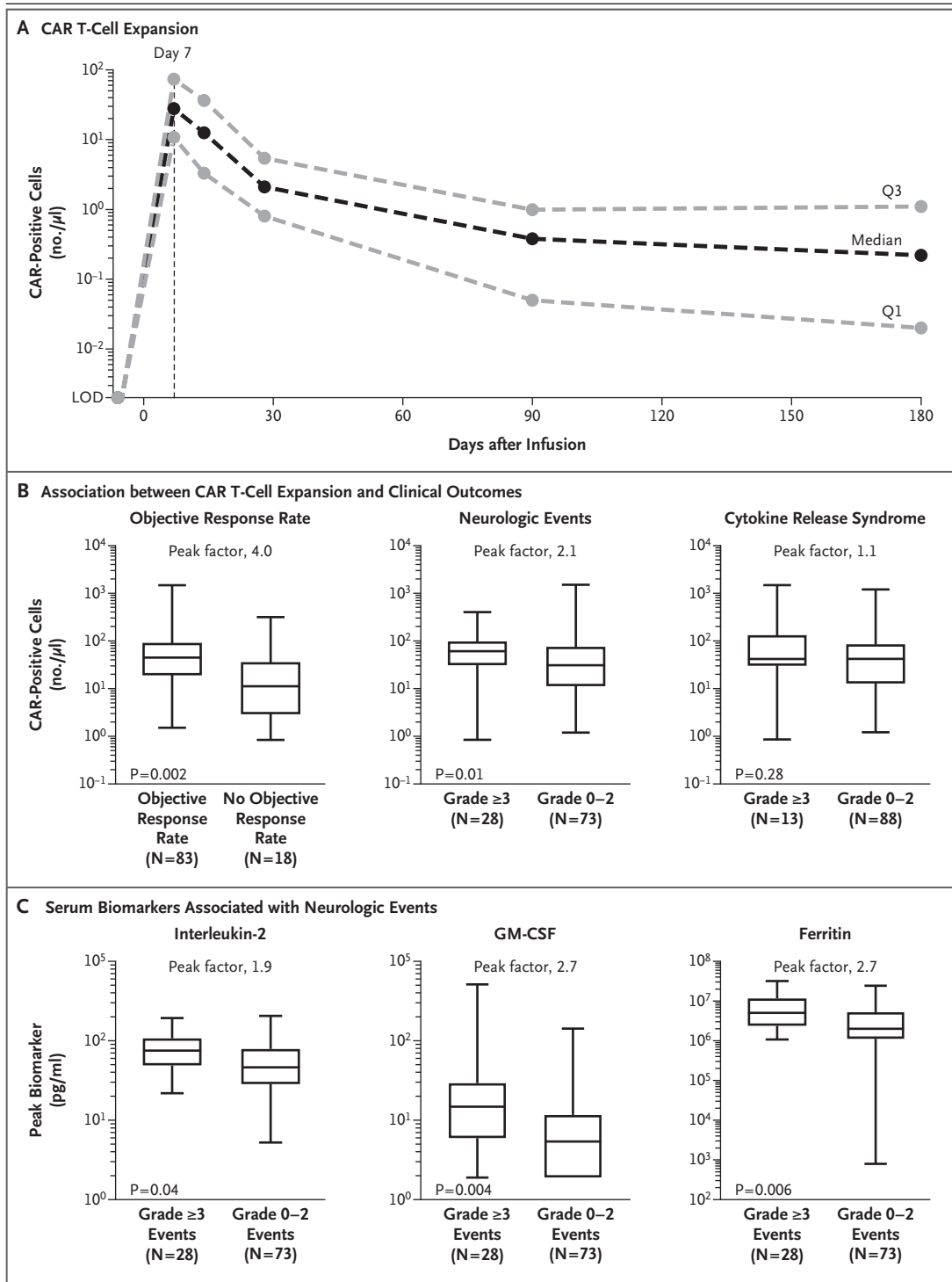
biomarker of the cytokine release syndrome or neurologic events may assist clinicians in determining when to intervene and optimize the management of toxic effects while preserving efficacy.

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.

APPENDIX

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