ORIGINAL ARTICLE

Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma

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ABSTRACT

BACKGROUND

Patients with diffuse large B-cell lymphoma that is refractory to primary and second-line therapies or that has relapsed after stem-cell transplantation have a poor prognosis. The chimeric antigen receptor (CAR) T-cell therapy tisagenlecleucel targets and eliminates CD19-expressing B cells and showed efficacy against B-cell lymphomas in a single-center, phase 2a study.

METHODS

We conducted an international, phase 2, pivotal study of centrally manufactured tisagenlecleucel involving adult patients with relapsed or refractory diffuse large B-cell lymphoma who were ineligible for or had disease progression after autologous hematopoietic stem-cell transplantation. The primary end point was the best overall response rate (i.e., the percentage of patients who had a complete or partial response), as judged by an independent review committee.

RESULTS

A total of 93 patients received an infusion and were included in the evaluation of efficacy. The median time from infusion to data cutoff was 14 months (range, 0.1 to 26). The best overall response rate was 52% (95% confidence interval, 41 to 62); 40% of the patients had complete responses, and 12% had partial responses. Response rates were consistent across prognostic subgroups. At 12 months after the initial response, the rate of relapse-free survival was estimated to be 65% (79% among patients with a complete response). The most common grade 3 or 4 adverse events of special interest included cytokine release syndrome (22%), neurologic events (12%), cytopenias lasting more than 28 days (32%), infections (20%), and febrile neutropenia (14%). Three patients died from disease progression within 30 days after infusion. No deaths were attributed to tisagenlecleucel, cytokine release syndrome, or cerebral edema. No differences between response groups in tumor expression of CD19 or immune checkpoint–related proteins were found.

CONCLUSIONS

In this international study of CAR T-cell therapy in relapsed or refractory diffuse large B-cell lymphoma in adults, high rates of durable responses were produced with the use of tisagenlecleucel. (Funded by Novartis; JULIET ClinicalTrials.gov number, NCT02445248.)

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*A complete list of the JULIET investigators is provided in the Supplementary Appendix, available at NEJM.org.

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Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma.[1] Although in the majority of patients the condition responds well to first-line immunochemotherapy combinations containing rituximab, 10 to 15% have primary refractory disease within 3 months after treatment initiation, and another 20 to 35% have a relapse.[2]

Approximately 40 to 60% of patients with relapsed or refractory DLBCL have a response to second-line chemotherapy; 50% of these patients proceed to undergo autologous hematopoietic stem-cell transplantation, and of these, approximately 30 to 40% remain progression-free 3 years after transplantation.[3-8] For patients who are unable to proceed to high-dose chemotherapy and hematopoietic stem-cell transplantation as second-line therapy, the prognosis is poor, with a median overall survival of 4.4 months and 1-year and 2-year overall survival rates of 23% and 16%, respectively.[8] For a small, highly select group of chemotherapy-sensitive patients who have a relapse after autologous transplantation, allogeneic hematopoietic stem-cell transplantation is possible if the patient has a response to chemotherapy and a donor is available; however, the procedure has a high associated risk of therapy-related complications, and the associated rate of death unrelated to disease relapse is 23% at 1 year.[9-13]

A recent retrospective study reviewed the outcomes of 636 patients with primary refractory DLBCL or a relapse of DLBCL less than 12 months after autologous transplantation.[5] The rate of response to the next line of therapy was 26%, with a complete response rate of 7%; the median overall survival was 6.2 months. These poor outcomes reinforce the need for new therapeutic options for patients with relapsed or refractory DLBCL.

The anti-CD19 chimeric antigen receptor (CAR) T-cell therapy tisagenlecleucel (formerly CTL019) has been shown to have high levels of efficacy with a serious but largely reversible toxic-effects profile in children and young adults with relapsed or refractory acute lymphoblastic leukemia.[14,15] After years of preclinical work and clinical development,[14,15] tisagenlecleucel was approved by the Food and Drug Administration for such patients.[16] High response rates have also been observed among adult patients with relapsed or refractory DLBCL in a phase 2a, single-center study: the response rate was 50% at 3 months, with 43% of the patients having a complete response at 6 months; no patients with a complete response at 6 months had had a relapse by the median follow-up of 28.6 months.[17]

On the basis of these studies, a pivotal phase 2 study was initiated to evaluate the safety and efficacy of tisagenlecleucel in adult patients with relapsed or refractory DLBCL. JULIET is an international study conducted at 27 sites in 10 countries across North America, Europe, Australia, and Asia and involving cryopreserved leukapheresis material, centralized manufacturing, and a global supply chain.

Methods

Study Design

We conducted a single-group, open-label, multicenter, international phase 2 study of tisagenlecleucel in adults with relapsed or refractory DLBCL. To be eligible for enrollment, patients had to be 18 years of age or older and to have previously received at least two lines of therapy, including rituximab and an anthracycline. Patients had either had a relapse after or were ineligible for autologous transplantation. We also included patients who had DLBCL that had transformed from follicular lymphoma, as well as patients who had high-grade B-cell lymphoma with *MYC* rearrangement plus rearrangement of *BCL2*, *BCL6*, or both genes (i.e., double- or triple-hit lymphoma). Patients were excluded if they had previously received CD19-directed therapy, had primary mediastinal DLBCL, had previously received an allogeneic transplant, or had active central nervous system involvement of their DLBCL.

After providing written informed consent, all eligible patients underwent leukapheresis; enrollment was complete when the cryopreserved material had been shipped to the manufacturing facility to await manufacturing. Bridging therapy, when needed, was allowed. Before infusion, patients received one cycle of lymphodepleting chemotherapy (not required for patients whose white-cell count was ≤1000 cells per cubic millimeter within 1 week before tisagenlecleucel infusion). For lymphodepletion, patients could receive either fludarabine (25 mg per square meter of body-surface area) and cyclophosphamide (250 mg per square meter) daily for 3 days or bendamustine (90 mg per square meter) daily for 2 days.

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Tisagenlecleucel was manufactured at the Morris Plains facility in New Jersey and at the European Union manufacturing facility, Fraunhofer Institut für Zelltherapie, in Leipzig, Germany. The enrolled patient set included all the patients who completed the screening phase and whose leukapheresis product was received by a manufacturing facility. The full analysis set and safety set were made up of all the patients who received an infusion, including those treated with tisagenlecleucel manufactured in the United States (main cohort) and those treated with tisagenlecleucel manufactured in the European Union (cohort A). The efficacy analysis set consisted of all the patients in the main cohort who had 3 months or more of follow-up before the data cutoff date. All the patients in the main cohort, regardless of their geographic location and participating site, received an infusion of U.S.-manufactured, cryopreserved tisagenlecleucel. Cohort A was evaluated separately from the main cohort to determine the effect of the manufacturing site on clinical outcomes (analysis in progress).

The study was sponsored and designed by Novartis and was approved by the institutional review board at each participating institution. Data were analyzed and interpreted by the sponsor and the authors. All the authors reviewed the manuscript. The authors vouch for the data and analysis and for the adherence of the study to the protocol, which is available with the full text of this article at NEJM.org. Editorial assistance with the preparation of the manuscript for submission was financially supported by Novartis.

END POINTS

The primary end point was the best overall response rate (i.e., the combined percentage of patients who had a complete or partial response), as determined by an independent review committee using the Lugano classification.[18] Secondary end points included response duration, overall survival, safety, and cellular kinetics data for all patients who received an infusion; the evaluation of biomarkers was an exploratory analysis (see the Methods section in the Supplementary Appendix, available at NEJM.org). For the reporting of adverse events, the Medical Dictionary for Regulatory Activities, version 20.1, and Common Terminology Criteria for Adverse Events, version 4.03, were used. The grade of cytokine release syndrome was determined with the use of the University of Pennsylvania grading scale (Table S1 in the Supplementary Appendix), and this toxic effect was managed with the use of a protocol-specific algorithm (Table S2 in the Supplementary Appendix).[19,20]

STATISTICAL ANALYSIS

Interim and primary analyses were planned for the first 50 and first 80 patients, respectively, in the efficacy analysis set to ensure 94% power to reject the null hypothesis of an overall response rate of 20% or less, under the assumption that the underlying response rate was 38%. A P value was planned to be determined at the interim analysis; a P value would be determined at the final analysis only if the interim analysis did not show significance. This was done with the use of a two-look Lan–DeMets group sequential design with an O'Brien–Fleming type boundary and an exact confidence interval at a one-sided cumulative significance level of 0.025. Kaplan–Meier curves were used to examine survival distributions.

RESULTS

PATIENTS

Between July 2015 and the data cutoff date, December 8, 2017, a total of 238 patients were screened and 165 were enrolled (Fig. 1). Of the enrolled patients, 111 (67%) received an infusion: 95 in the main cohort and 16 in cohort A (Fig. 1, and the Methods section in the Supplementary Appendix); 4 patients (2%) were awaiting infusion at the time of analysis. Patients received infusions in either inpatient or outpatient settings. The median time from enrollment to infusion was 54 days (90% of patients received infusions between 30 days and 92 days after enrollment). The median time from infusion to data cutoff was 14 months (range, 0.1 to 26). The baseline characteristics of the enrolled patients and the patients who received an infusion were similar (Table 1, and Table S3 in the Supplementary Appendix); however, the patients who did not receive an infusion tended to have a lower performance status than those who did receive an infusion, and a greater proportion of the patients who did not receive an infusion had DLBCL that was refractory to the last therapy they received before enrollment.

Before infusion, 92% of the patients received bridging therapy, including combinations of rituximab (54%), gemcitabine (40%), etoposide (26%),

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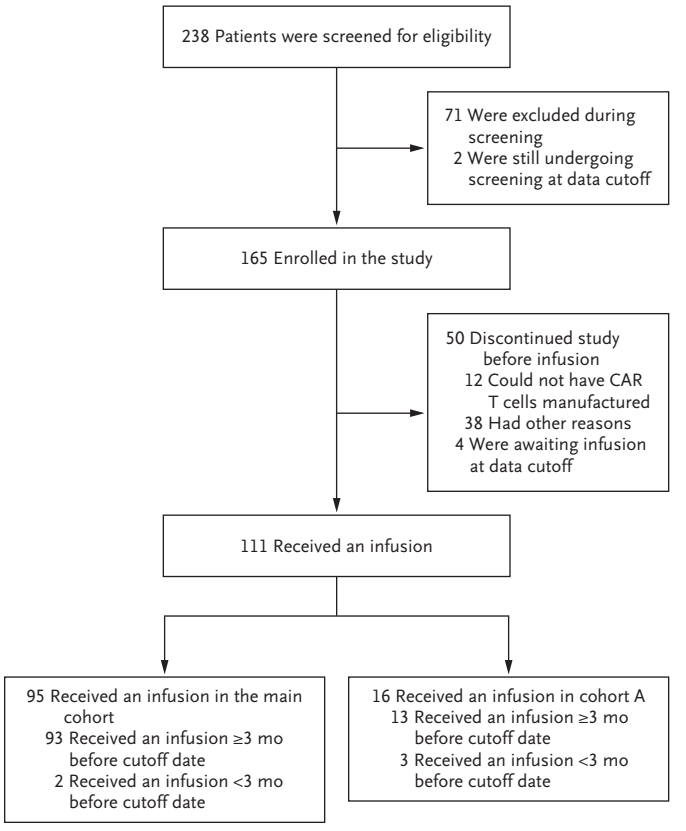

Figure 1. Screening, Enrollment, and Treatment.

Among the 71 patients who were excluded from the study during screening, 55 did not meet all clinical eligibility criteria, 8 decided not to participate, 4 did not complete the screening process, 2 had an adverse event, 1 died, and 1 had the treating physician decide against participation. Among the 38 enrolled patients who had other reasons for discontinuing participation in the study, 16 died before infusion, 16 had their treating physician decide against further participation, 3 had an adverse event, 2 decided against further participation, and 1 had a protocol deviation. Reasons for discontinuation such as death, physician's decision, and patient's decision were mainly related to disease progression as reported by the investigator. The full analysis set and safety set were made up of all the patients who received an infusion, including those treated with tisagenlecleucel manufactured in the United States (main cohort) and those treated with tisagenlecleucel manufactured in the European Union (cohort A).

dexamethasone (25%), cisplatin (19%), and cytarabine (19%), as well as newer agents such as ibrutinib (9%) and lenalidomide (7%). A total of 103 patients (93%) received lymphodepleting chemotherapy (73% received combination fludarabine–cyclophosphamide, and 20% received bendamustine). All 111 patients received a single infusion of tisagenlecleucel (median dose, 3.0×10^8 CAR-positive viable T cells; range, 0.1×10^8 to 6.0×10^8) (Table S4 in the Supplementary Appendix).

EFFICACY

The null hypothesis with regard to the primary end point (i.e., that the best overall response rate would be ≤20%) was rejected in the interim analysis ($P<0.001$).[22,23] Among the 93 patients in the efficacy analysis set who had 3 months or more of follow-up or had discontinued participation in the study before 3 months, the best overall response rate was 52% (95% confidence interval [CI], 41 to 62): 40% of patients had a complete response, and 12% of patients had a partial response (Table S5 in the Supplementary Appendix). The rates of overall and complete response were 38% and 32%, respectively, at month 3 and were 33% and 29% at month 6. A high concordance (85%) was found between local and central assessments of response. Response rates did not differ substantially according to the type of lymphodepleting therapy received (Table S6 in the Supplementary Appendix), and univariate analyses showed a homogeneous and consistent treatment effect across major demographic and prognostic subgroups, including the subgroup based on disease response to previous therapy (Fig. 2, and Fig. S1 in the Supplementary Appendix).

Of the 37 patients who had a complete response, 16 had either stable disease (4 patients) or a partial response (12 patients) 1 month after infusion that improved to a complete response in a median of 2 months (range, 1 to 17). A conversion from a partial to a complete response occurred in 54% of the patients (13 of 24), including in 2 patients who were confirmed to have a complete response by positron-emission–tomography scanning performed 15 to 17 months after their initial response. Among the 35 patients who were in remission at month 3, the estimated probability of remaining in remission at month 12 was 81% (95% CI, 63 to 91). In an intention-to-treat analysis that included all 165 enrolled patients, including patients who discontinued participation before tisagenlecleucel infusion (mostly as a result of disease progression and death), the overall response rate was 34% (95% CI, 27 to 42).

The median response duration has not been reached (95% CI, 10 months to not reached); however, 79% (95% CI, 60 to 89) of patients who had a complete response and 65% (95% CI, 49 to 78) of all patients who had a response are projected

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to remain relapse-free at 12 months after having a response (Fig. 3A, and Fig. S1A in the Supplementary Appendix). Durable responses were observed for up to 18.4 months after infusion. No patient proceeded to undergo transplantation while having a response. Six patients who did not have a response proceeded to undergo hematopoietic stem-cell transplantation (five underwent allogeneic transplantation, and one underwent autologous transplantation followed by allogeneic transplantation).

The median progression-free survival has not been reached for patients who had a complete response (Fig. 3B); the estimated rate of progression-free survival at 12 months was 83% among patients who had a complete or partial response at 3 months (Fig. 3C). The median overall survival among patients who received an infusion was 12 months (95% CI, 7 months to not reached) (Fig. 3D, and Fig. S1B in the Supplementary Appendix). The estimated probability of survival at month 12 was 49% (95% CI, 39 to 59) among all patients and 90% (95% CI, 74 to 96) among patients with a complete response. In an intention-to-treat analysis that included all 165 enrolled patients, the median overall survival from the time of enrollment was 8.3 months (95% CI, 5.8 to 11.7) and the estimated probability of survival at month 12 was 40% (95% CI, 32 to 49) (Fig. S2 in the Supplementary Appendix).

TISAGENLECLEUCEL EXPANSION AND PERSISTENCE

Similar mean in vivo expansion and concentration–time profiles of tisagenlecleucel, measured as transgene level, median time to maximum transgene level, and mean area under the concentration–time curve from day 0 to day 28 (AUC_{0-28d}), were observed in patients who had a response and those who did not (Table S7 in the Supplementary Appendix). Thus, no apparent effect of exposure on clinical outcome was observed. Persistent CAR transgene levels were observed for up to 2 years after infusion in patients with durable responses (Fig. S3 in the Supplementary Appendix). No relationship between dose and maximal in vivo expansion was apparent, and clinical responses were observed across a wide range of doses.[24]

SAFETY

The most common adverse events of any grade were cytokine release syndrome (58%), anemia

Table 1. Demographic and Clinical Characteristics of the Patients in the Full Analysis Set at Baseline.*

Characteristic	Patients (N = 111)
Median age (range) — yr	56 (22–76)
Age ≥65 yr — no. (%)	25 (23)
ECOG performance status — no. (%)†	
0	61 (55)
1	50 (45)
Disease stage at study entry — no. (%)‡	
Stage I	8 (7)
Stage II	19 (17)
Stage III	22 (20)
Stage IV	62 (56)
Bone marrow involvement at study entry — no. (%)	8 (7)
Diagnosis on central histologic review — no. (%)	
Diffuse large B-cell lymphoma, not otherwise specified	88 (79)
Transformed follicular lymphoma	21 (19)
Other	2 (2)
Double- or triple-hit rearrangement: *MYC* plus *BCL2*, *BCL6*, or both — no./total no. (%)§	19/70 (27)
Cell of origin of cancer — no. (%)	
Germinal center B-cell type	63 (57)
Non–germinal center B-cell type	45 (41)
Missing data	3 (3)
No. of previous lines of antineoplastic therapy — no. (%)¶	
1	5 (5)
2	49 (44)
3	34 (31)
4–6	23 (21)
Relapse after last therapy — no. (%)‖	50 (45)
Refractory diffuse large B-cell lymphoma — no. (%)**	61 (55)
Previous autologous hematopoietic stem-cell transplantation — no. (%)	54 (49)

* The full analysis set includes all the patients who received tisagenlecleucel. Percentages may not total 100 because of rounding.

† Eastern Cooperative Oncology Group (ECOG) performance status values range from 0 to 5, with higher scores indicating greater disability.

‡ Disease stage was defined according to the modified Ann Arbor staging system, in which higher stage numbers indicate greater dissemination of cancer through the body.[21]

§ A total of 38 patients did not have an assessment as a result of less than 40% MYC immunohistochemical staining, and 3 patients had missing data for rearrangement (double or triple hit) of *MYC* plus *BCL2*, *BCL6*, or both genes.

¶ Patients received rituximab- and anthracycline-containing treatment and, if eligible, hematopoietic stem-cell transplantation, before diffuse large B-cell lymphoma transformation. The numbers of lines of treatments given here were given after transformation.

‖ Relapse after last therapy indicates a partial or complete response to the last line of therapy and subsequent progression of lymphoma before enrollment in the current study.

** Refractory disease indicates either progressive or stable disease as the best response to the last therapy before enrollment or an unknown response status.

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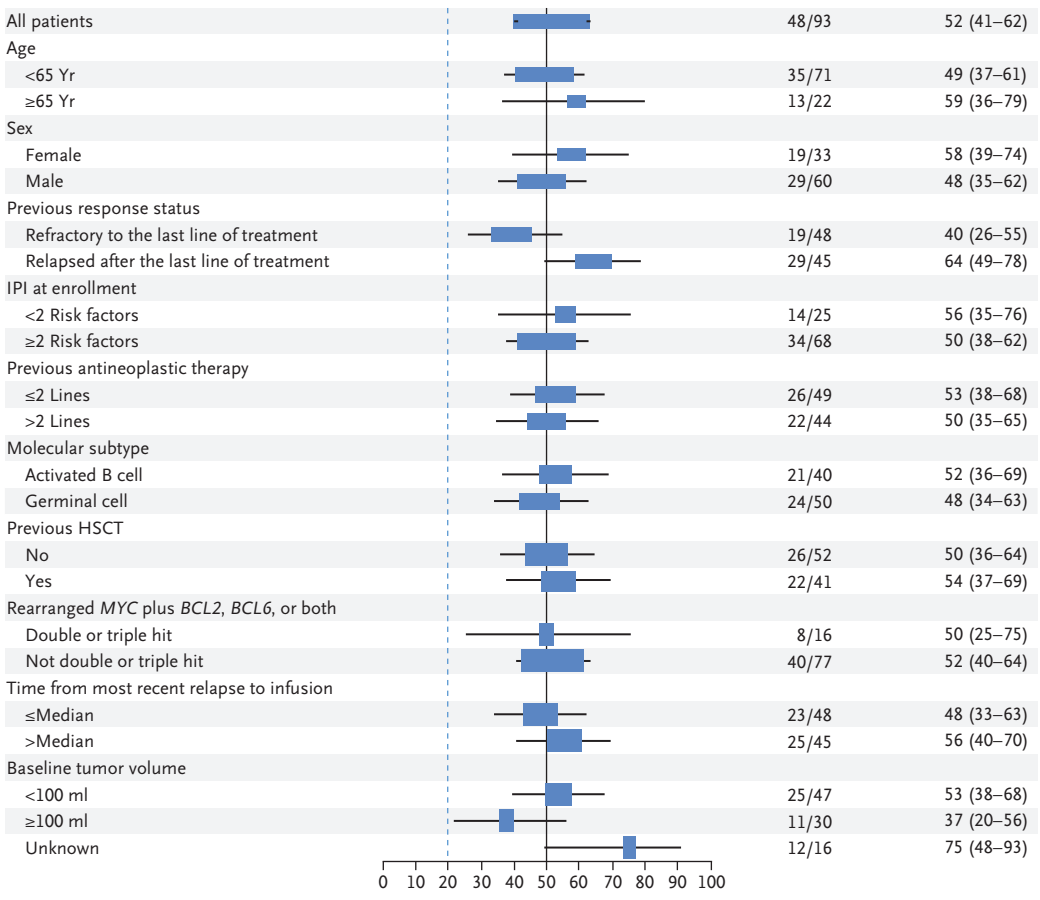

Figure 2. Best Overall Response Rate According to Subgroup.

The best overall response rate was the combined percentage of patients who had a complete or partial response. The dashed vertical line indicates a rate of 20% (the null hypothesis was that the best overall response rate would be 20% or less). IPI denotes International Prognostic Index; an IPI score of less than 2 (i.e., fewer than two risk factors) indicates a low risk, a score of 2 a low–intermediate risk, a score of 3 a high–intermediate risk, and a score of 4 or 5 a high risk of death within 5 years.

(48%), pyrexia (35%), decreased neutrophil count (34%), decreased platelet count (33%), decreased white-cell count (33%), and diarrhea (32%) (Table S8 in the Supplementary Appendix). Grade 3 or 4 adverse events of special interest within the first 8 weeks after infusion (Table 2) included cytokine release syndrome (22% of the patients, according to the University of Pennsylvania grading scale[19]), cytopenias not resolved by day 28 (32%) (see Table S9 in the Supplementary Appendix for information on the different types of prolonged cytopenias), infections (20%), neurologic events (12%), and febrile neutropenia (15%).

The median time from infusion to the onset of symptoms of cytokine release syndrome was 3 days (all patients except one had onset within 9 days), and the median duration was 7 days (range, 2 to 30). The median time to the onset of grade 3 or 4 cytokine release syndrome was 4 days (range, 2 to 8); 97% of cases had resolved by data cutoff. Overall, 14% of the patients received tocilizumab, and 10% received both tocilizumab and glucocorticoids. No patient received more than two doses of tocilizumab (5% received one dose and 9% received two doses). Patients with cytokine release syndrome received supportive

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care, including oxygen supplementation (24%), endotracheal intubation (7%), high-dose vasopressors[19] (6%), and dialysis (5%); 24% were admitted to the intensive care unit. Infections concurrent with cytokine release syndrome occurred in 6% of the patients.

Neurologic events of any grade occurred in 21% of the patients within 8 weeks after infusion; the median time to onset was 6 days (range, 1 to 17), and the median duration was 14 days. Headaches (not classified as a nervous system disorder) occurred in 20% of the patients 8 weeks or less after infusion. A total of 13 patients (12%) had grade 3 or 4 events, the majority of which had resolved by data cutoff with supportive treatment in accordance with local guidelines (e.g., glucocorticoids). Nine patients with grade 3 or 4 neurologic events had concurrent cytokine release syndrome. No fatal cerebral edema was observed.

Only one patient had normal CD19+ B-cell counts in peripheral blood before tisagenlecleucel infusion (normal range, 80 to 616 per cubic millimeter); the majority had CD19+ B-cell counts below the lower limit of quantitation (0.2 per cubic millimeter) (Table S10 in the Supplementary Appendix). After infusion, six patients with ongoing complete response had CD19+ B-cell counts return to the normal range (five patients at >6 months after infusion and one patient at month 3). Intravenous immune globulin was administered at the local investigator's discretion; 30% of patients who received a tisagenlecleucel infusion were treated with intravenous immune globulin after the infusion.

Three patients died within 30 days after infusion, all from lymphoma progression. No deaths after infusion were attributed to tisagenlecleucel by the investigators.

BIOMARKERS

Quantitative immunofluorescence analysis was performed on preinfusion tumor tissues to measure the expression of CD19; CD3, PD-1, and PD-L1; and CD3, TIM3, and LAG3. Samples were obtained 1 month to 1 year (in 60 of 82 patients) or more than 1 year (in 22 of 82 patients) before tisagenlecleucel infusion (Table S11 in the Supplementary Appendix). Responses to tisagenlecleucel were observed in patients with tumor samples that had unequivocal CD19 expression (best overall response rate, 49%; 95% CI, 34 to 64) and low or negative CD19 expression (best overall response rate, 50%; 95% CI, 29 to 71) (Fig. S4A and Table S12 in the Supplementary Appendix).

We found no apparent differences between the best-overall-response groups in the median or mean PD-1–PD-L1 interaction scores (see the Methods section in the Supplementary Appendix) or in the percentage of cells expressing immune checkpoint–related proteins (percentage of total cells positive for PD-L1, PD-1, LAG3, or TIM3 and percentage of total CD3 T cells expressing PD-1, LAG3, or TIM3) at baseline (Fig. S4B through S4E in the Supplementary Appendix and data not shown). However, the 5 patients with the highest PD-1–PD-L1 interaction scores either did not have a response to tisagenlecleucel (4 patients) or had a relapse by month 3 (1 patient). Similarly, the 11 patients with the highest percentages of LAG3+ T cells (among total T cells) did not have a response to tisagenlecleucel (7 patients) or had a relapse within 3 to 6 months (4 patients, 2 after a complete response and 2 after a partial response) (see the Methods section and Table S13 in the Supplementary Appendix). A clear absence of response or an early relapse was not observed in patients with the highest percentages of PD-1+ T cells or TIM3+ T cells (among total T cells).

DISCUSSION

This study showed a high rate and duration of response to tisagenlecleucel therapy among heavily pretreated adult patients with relapsed or refractory DLBCL. The results were significant with regard to the primary end point, with a best overall response rate of 52%.[23] Four patients who had stable disease and 12 patients who had a partial response at 1 month had improvement to a complete response in a median of 2 months. At 3 months, the rates of complete and partial response were 32% and 5%, respectively, and were sustained through 6 months, which suggests that responses at 3 months are usually durable. For patients with double-hit lymphoma, the response rate was 50% and the complete response rate was 25%. Long-term persistence of tisagenlecleucel was shown for up to 2 years. Patients who had a response had longer persistence of CAR transgene levels than did patients who did not have a response; however, no evidence suggested a dose–response or exposure–response (with exposure measured as the AUC_{0-28d} or peak cell expansion) relationship. The retrospective SCHOLAR-1 study

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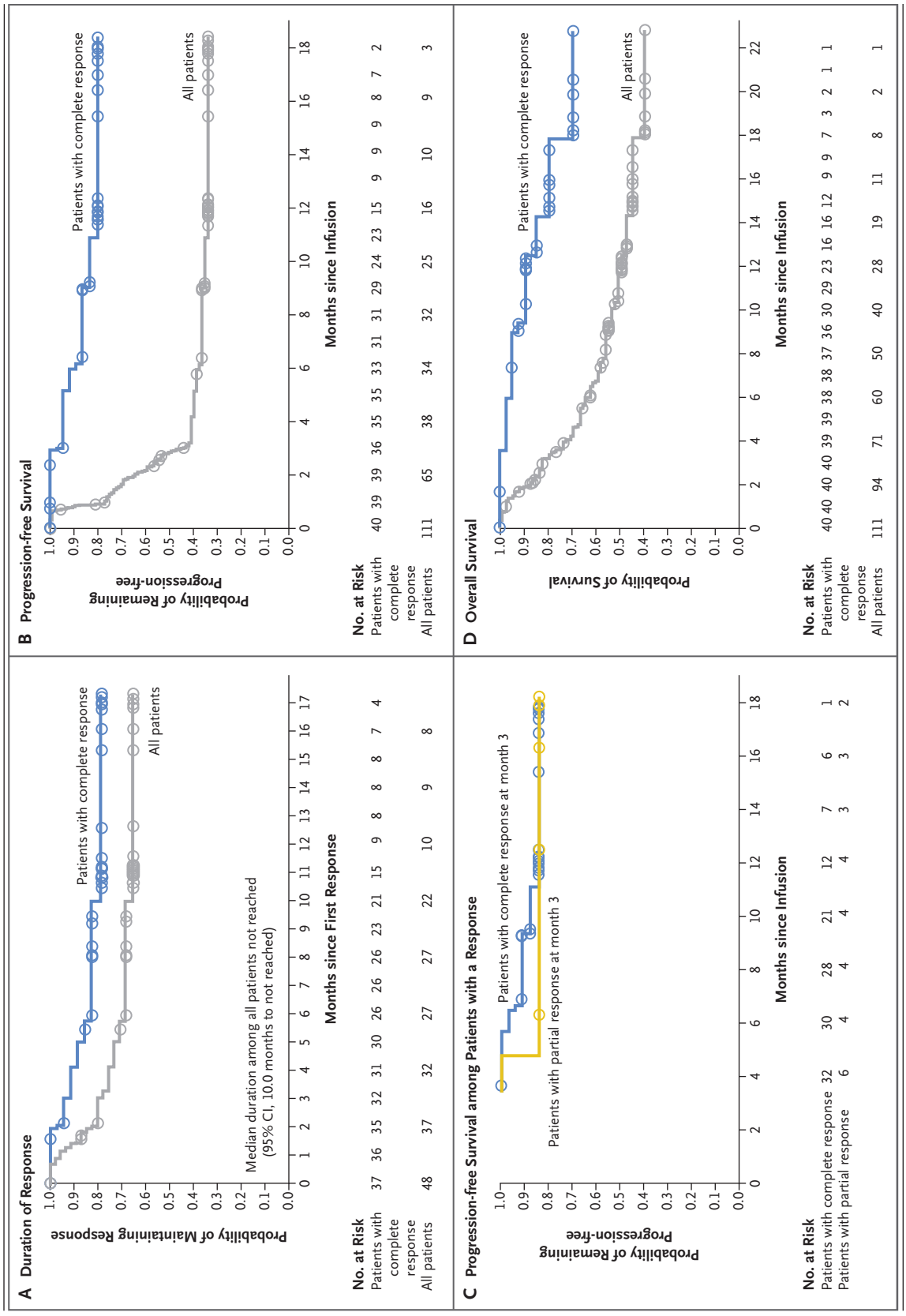

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Figure 3 (facing page). Duration of Response, Progression-free Survival, and Overall Survival.

Panel A shows the duration of response (time from the date of first documented disease response [complete response or partial response] to the date of first documented progression or death due to diffuse large B-cell lymphoma) among the 48 patients in the main cohort who had a complete or partial response. Data from 26 patients were censored because at data cutoff the patients continued to not have an event, with a duration of response between 181 and 527 days. Data from 7 other patients were censored, 5 because the patient received new cancer therapy as deemed necessary by the treating investigator (for disease progression [3 patients], stable disease [1], or partial response [1] by local assessment), 1 because of withdrawn consent, and 1 because adequate radiologic assessment was no longer available. Panel B shows progression-free survival (time from the date of tisagenlecleucel infusion to the date of disease progression or death from any cause) for all 111 patients who received an infusion. Panel C shows progression-free survival among patients with a response, according to response status at month 3. Panel D shows overall survival (time from the date of tisagenlecleucel infusion to the date of death from any cause) for the 111 patients in the full analysis set (gray line) and for patients who had a complete response (blue line).

showed an integrated complete response rate of 7% and a median overall survival of 6.2 months with standard-of-care therapy.[5] Thus, our findings suggest that tisagenlecleucel has the potential to improve outcomes in patients with relapsed or refractory DLBCL.

Although cytokine release syndrome occurred in 58% of patients, grade 3 or 4 events (defined on the basis of the therapeutic interventions used to manage symptoms or hemodynamic complications [or both] of cytokine release assessed on the University of Pennsylvania grading scale[19]) occurred in 22% of the patients and responded to tocilizumab in most cases. By monitoring the patients for fever, which is the first symptom of cytokine release syndrome, and ensuring that the syndrome was managed by appropriately trained site personnel using a protocol-specific algorithm,[20] this serious toxic effect was controlled without fatal events. No deaths were attributed to tisagenlecleucel, cytokine release syndrome, or cerebral edema.

Before infusion, most patients had B-cell depletion resulting from previous treatment with rituximab. The majority of the patients with measurable preinfusion rituximab levels had B-cell counts below the limit of quantification (0.2 per cubic millimeter) (Table S10 in the Supplementary Appendix). Rituximab causes depletion of circulating and tissue-based B cells for up to 9 months in 83% of patients with lymphoma.[25] Immunoglobulin levels were monitored throughout the study at protocol-specified time points. Most of the patients (96%) had a history of rituximab-based therapies before entering into the study and, as expected, IgG, IgA, and IgM levels were decreased in 74%, 49%, and 63% of patients, respectively, before infusion. There was no analysis conducted relating infections to immunoglobulin levels. Although tisagenlecleucel therapy has been reported to cause persistent B-cell depletion in pediatric patients with acute lymphoblastic leukemia,[17,26,27] sustained reappearance of B cells with improvement in immunoglobulin levels over time in patients with a complete response after tisagenlecleucel treatment has been reported in adult patients with lymphoma with longer follow-up.[17] Further follow-up would be needed to assess B-cell and immunoglobulin recovery in adult patients in this study.

Our study design mirrored a real-world scenario for CAR T-cell therapy candidates. Leukapheresis with cryopreservation and the use of bridging therapy allowed flexibility in scheduling and maintaining disease control, and the use of centralized manufacturing and a global supply chain allowed for international distribution of tisagenlecleucel to patients.

A total of 30% of enrolled patients discontinued participation in the study without receiving an infusion, mostly as a result of disease progression and death; 7% of enrolled patients did not receive an infusion because of manufacturing failure (mainly due to low cell growth). Discontinuations before infusion were driven primarily by limited manufacturing capacity at the start of this study, which is a reversible logistic factor.

KTE-C19, an anti-CD19 CAR T-cell agent with a CD28 costimulatory domain, was evaluated as an inpatient therapy in patients with relapsed or refractory DLBCL in ZUMA-1, a pivotal, single-group, phase 2 study.[28] In an interim analysis in that study involving the cohort of 51 patients with DLBCL who had at least 3 months of follow-up, the response rate was 76%, with 92% of responses occurring within the first month and a rate of complete response of 33% at month 3.[29] In the primary analysis (involving 77 patients), the rate

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Table 2. Overall Safety of Tisagenlecleucel.*

Type of Adverse Event	Patients with Any Event (N=111)	Patients with Events Starting ≤8 Wk after Infusion (N=111)	Patients with Events Starting >8 Wk after Infusion (N=96)
		number of patients (percent)	
Any adverse event	111 (100)	111 (100)	69 (72)
Adverse event suspected to be related to study drug	99 (89)	96 (86)	30 (31)
Serious adverse event	72 (65)	55 (50)	30 (31)
Serious adverse event suspected to be related to study drug	52 (47)	46 (41)	9 (9)
Grade 3 or 4 adverse event	99 (89)	94 (85)	47 (49)
Grade 3 or 4 adverse event suspected to be related to study drug	70 (63)	64 (58)	21 (22)
Adverse events of special interest†			
Cytokine release syndrome‡			
Any grade		64 (58)	0
Grade 3		15 (14)	0
Grade 4		9 (8)	0
Infection			
Any grade		38 (34)	37 (39)
Grade 3		20 (18)	13 (14)
Grade 4		2 (2)	4 (4)
Cytopenia not resolved by day 28§			
Any grade		49 (44)	NA
Grade 3		18 (16)	NA
Grade 4		18 (16)	NA
Neurologic event¶			
Any grade		23 (21)	5 (5)
Grade 3		8 (7)	3 (3)
Grade 4		5 (5)	0
Febrile neutropenia			
Any grade		17 (15)	2 (2)
Grade 3		14 (13)	1 (1)
Grade 4		2 (2)	1 (1)
Tumor lysis syndrome			
Any grade		1 (1)	0
Grade 3		1 (1)	0
Grade 4		0	0

* NA denotes not applicable.
† Events are those with two or more reported cases, regardless of their relationship to the study drug.
‡ Cytokine release syndrome was graded with the use of the University of Pennsylvania grading scale and managed by a protocol-specific algorithm.[19]
§ Cytopenias not resolved by day 28 are defined as those that began within the first 4 weeks after infusion. Prolonged cytopenias occurring more than 8 weeks after infusion would have begun more than 8 weeks after infusion.
¶ The neurologic events (percentage of any grade at any time after infusion) that occurred were confusional state (9%), encephalopathy (6%), tremor (5%), dysphagia (4%), aphasia (3%), delirium (3%), disturbance in attention (3%), mental status changes (3%), agitation (2%), dyskinesia (2%), seizure (2%), somnolence (2%), cognitive disorder (1%), dysarthria (1%), irritability (1%), lethargy (1%), loss of consciousness (1%), memory impairment (1%), metabolic encephalopathy (1%), speech disorder (1%), stupor (1%), and abnormal thinking (1%).

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of complete response at month 6 was 31%, with 5% partial responses.[30] Cytokine release syndrome occurred in 93% of the patients (13% with grade ≥3, according to the Lee grading scale).[28,31] Four patients had ongoing cytokine release syndrome events at the time of death.[28] Neurologic toxic effects occurred in 64% of the patients (28% with grade ≥3). CAR T-cell expansion was significantly associated with response.[28] Although differences in patient populations, study designs, and CAR constructs preclude direct comparisons between studies, the results of that study combined with those of our study show that CD19-directed CAR T-cell therapy provides a high rate of durable response with serious, albeit somewhat different, safety profiles.

Low or negative preinfusion expression of CD19 could, in theory, be a cause of tisagenlecleucel failure in some patients who did not have a response. In the exploratory analysis assessing relative CD19 expression in preinfusion biopsies, similar response rates between the subgroup with CD19 expression and those with low or negative CD19 expression were found, with responses observed across all CD19 expression levels; these findings suggested that, at the immunohistochemical level, low or undetectable CD19 expression may be sufficient for tisagenlecleucel therapy to be effective (Fig. S4A in the Supplementary Appendix).

The exploratory biomarker analysis showed that preinfusion expression of inhibitory immune checkpoint proteins by cells in the tumor or the tumor microenvironment in the best-overall-response groups or in patients whose responses improved over time did not differ from those in patients who ultimately had lymphoma progression. However, a small number of patients with the highest PD-1–PD-L1 interaction scores and a subgroup with high proportions of LAG3+ T cells (among total T cells present) either did not have a response to tisagenlecleucel or had responses followed by rapid disease progression within 3 to 6 months. It should be emphasized that these are preliminary observations and bear further investigation.

The high and durable response rates observed with tisagenlecleucel treatment are promising. However, it should be noted that follow-up is short, and the potential for long-term toxic effects requires further analysis. Adverse effects such as cytokine release syndrome can be severe or even life-threatening; however, they were managed in most patients with supportive measures and cytokine blockade. Patients with relapsed or refractory DLBCL who are not eligible for high-dose therapy and hematopoietic-cell transplantation or for whom such therapy was not successful have very few treatment options. For these patients, tisagenlecleucel shows promise that will need to be confirmed through larger studies with longer follow-up.

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APPENDIX

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