original reports

Coadministration of CD19- and CD22-Directed Chimeric Antigen Receptor T-Cell Therapy in Childhood B-Cell Acute Lymphoblastic Leukemia: A Single-Arm, Multicenter, Phase II Trial

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abstract

PURPOSE We determined the safety and efficacy of coadministration of CD19- and CD22-chimeric antigen receptor (CAR) T cells in patients with refractory disease or high-risk hematologic or isolated extramedullary relapse of B-acute lymphoblastic leukemia.

PATIENTS AND METHODS This phase II trial enrolled 225 evaluable patients age ≤ 20 years between September 17, 2019, and December 31, 2021. We first conducted a safety run-in stage to determine the recommended dose. After interim analysis of the first 30 patients treated (27 at the recommended dose) showing that the treatment was safe and effective, the study enrolled additional patients according to the study design.

RESULTS Complete remission was achieved in 99.0% of the 194 patients with refractory leukemia or hematologic relapse, all negative for minimal residual disease. Their overall 12-month event-free survival (EFS) was 73.5% (95% CI, 67.3 to 80.3). Relapse occurred in 43 patients (24 with $CD19^+/CD22^+$ relapse, 16 $CD19^-/CD22^+$, one $CD19^-/CD22^-$, and two unknown). Consolidative transplantation and persistent B-cell aplasia at 6 months were associated with favorable outcomes. The 12-month EFS was 85.0% (95% CI, 77.2 to 93.6) for the 78 patients treated with transplantation and 69.2% (95% CI, 60.8 to 78.8) for the 116 nontransplanted patients ($P = .03$, time-dependent covariate Cox model). All 25 patients with persistent B-cell aplasia at 6 months remained in remission at 12 months. The 12-month EFS for the 20 patients with isolated testicular relapse was 95.0% (95% CI, 85.9 to 100), and for the 10 patients with isolated CNS relapse, it was 68.6% (95% CI, 44.5 to 100). Cytokine release syndrome developed in 198 (88.0%) patients, and CAR T-cell neurotoxicity in 47 (20.9%), resulting in three deaths.

CONCLUSION CD19-/CD22-CAR T-cell therapy achieved relatively durable remission in children with relapsed or refractory B-acute lymphoblastic leukemia, including those with isolated or combined extramedullary relapse.

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ASSOCIATED CONTENT

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INTRODUCTION

Autologous CD19-directed chimeric antigen receptor (CAR) T-cell therapy has revolutionized the management of relapsed or refractory pediatric acute lymphoblastic leukemia (ALL).[1-6] Registry data show that tisagenlecleucel induces complete remission in 85.5% of cases and results in a 12-month event-free survival (EFS) of 52.4% in children treated for relapsed or refractory B-ALL.[7] Approximately 50% of patients experienced relapse within 1 year,[3-5,7] owing to loss of CAR T-cell persistence or loss of CD19 antigen because of splice variants, acquired genetic mutations, or lineage switch.[8,9] Although CD22-targeted CAR T-cell therapy induces complete remission in 70%-80% of the patients in whom CD19-targeted CAR T-cell therapy failed, most experience relapse again.[10-12] These observations led some investigators to use CAR T-cell therapy as a bridge to allogeneic transplantation,[13] whereas others developed dual CD19-/CD22-targeted treatment to overcome antigen escape relapse.[14-19]

CONTEXT

Key Objective

Does coadministration of CD19- and CD22-chimeric antigen receptor T-cell therapy result in durable event-free survival in children with refractory disease or high-risk hematologic or extramedullary relapse of B-acute lymphoblastic leukemia?

Knowledge Generated

In this clinical trial that included 225 children, the 12-month event-free survival was 69.2% in patients treated for hematologic relapse without consolidative allogeneic hematopoietic cell transplantation, 95% for isolated testicular relapse and 68.6% for isolated CNS relapse.

Relevance *(S. Bhatia)*

Coadministration of CD19- and CD22-chimeric antigen receptor T-cell therapy may be a promising therapeutic strategy for patients with relapsed or refractory B-acute lymphoblastic leukemia. However, longer follow-up is needed to determine the durability of the response.*

*Relevance section written by *JCO* Associate Editor Smita Bhatia, MD, MPH.

Three recent studies showed the safety and feasibility of dual CD19-/CD22-targeted CAR T-cell therapy, but the results were not superior to those of the CD19-CAR T-cell therapy.[14-16] Three other studies tested sequential administration of CD19-CAR T cells and CD22-CAR T cells, which yielded complete remission rates of 96%, 100%, and 85% and a 1-year leukemia-free survival of 52.9%, 79.5%, and 67.5%, respectively.[17-19] Although this approach was associated with low rates of antigen-escape relapse, the limited CAR T-cell persistence raised concern of impending antigen-positive relapse.[17-19] Preclinical studies have shown that CD19-targeting CAR T cells can downregulate CD22 expression in a subset of tumor cell line models.[20] Therefore, we hypothesized that coadministration of CD19- and CD22-targeted CAR T cells would improve efficacy on the basis of the fundamental treatment principle for ALL that combination therapy forestalls the development of drug resistance and a preclinical model showing that simultaneous targeting may reduce the risk of antigen loss.[21] Moreover, coadministration would avoid repeated lymphodepleting chemotherapy that eradicates CD19-CAR T cells. Here, we report the results of our clinical trial using this treatment approach.

PATIENTS AND METHODS

Study Design and Patient Population

This study (Chinese Clinical Trial Registry: ChiCTR2000032211), an open-label phase II, multicenter clinical trial, enrolled patients between September 17, 2019, and December 31, 2021. The study protocol and detailed eligibility criteria for three study cohorts are provided in the Protocol (online only). The first cohort for the safety run-in stage enrolled patients with refractory leukemia and hematologic relapse who did not achieve remission after ≥ 2 courses of remission induction or were ineligible for allogeneic transplantation. The second cohort for the phase II trial enrolled patients with refractory disease or hematologic relapse with unfavorable genotype, persistent disease after ≥ 2 treatment regimens for relapse, prior CD19-CAR T therapy, or allogeneic transplantation. The third cohort consisted of patients with isolated extramedullary relapse and negative minimal residual disease (MRD) defined as $<0.01\%$ of leukemia cells in bone marrow by flow cytometry. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review boards. Written informed consent was obtained from the parents, guardians, or patients, as appropriate.

During the safety run-in stage, one of the three patients treated at the initial dose of 1×10^7 CAR T cells/kg developed a grade 4 neurotoxicity. None of the subsequent three patients experienced grade ≥ 3 toxicity at a de-escalated dose of 5.0×10^6 CAR T cells/kg, which was determined as the recommended dose for patients with hematologic relapse. A dose between 5×10^6 and 1×10^7 CAR T cells/kg was used to treat isolated extramedullary relapse to enhance CAR T-cell proliferation in the setting of low antigen stimulation. After an interim analysis of the first 30 patients showed that the treatment was safe (Data Supplement, online only) and their EFS was superior to that of 46 historical patients treated with CD19-CAR T cells (Data Supplement), the study continued as planned. Consolidative transplantation was planned only for patients with *KMT2A*- or *ZNF384*-rearranged B-ALL to avoid myeloid lineage switch.[22,23]

Treatment

Generally, within 3 days of eligibility confirmation, $CD3^+$ T lymphocytes were collected from peripheral blood (1-2 mL/kg) and CAR T cells were manufactured at the Shanghai Children's Medical Center. Briefly, after Ficoll-Hypaque gradient centrifugation and anti-CD3 Microbeads sorting, T cells were stimulated by anti-CD3/CD28 beads for 24-48 hours and were transduced with CD19-specific or CD22-specific CAR lentiviral vectors with 4-1BB costimulatory and CD3 zeta signaling domains. CD19- and

CD22-specific CAR T cells were cultured separately. After 5-7 days in culture, CD19- and CD22-CAR T cells were pooled together at a ratio of 1:1, washed, resuspended in saline solution with 2.5% human serum albumin, and transported to the participating medical center (Data Supplement) where the patient received the infusion on day 0. The coordination of the timing of CAR T-cell production and lymphodepleting chemotherapy with fludarabine and cyclophosphamide are shown in the Data Supplement.

Outcomes

The primary end points included the recommended phase II dose of combined CD19- and CD22-CAR T cells, CAR T-cell infusion–related adverse effects, complete remission rate at day 28 postinfusion, and EFS and overall survival (OS) at 12 months with or without consolidative transplantation. Exploratory analyses were performed on the effect of sustained B-cell aplasia (as defined by the detection of $< 1\%$ $CD19^+$ lymphocytes in peripheral blood or bone marrow) on treatment outcomes and the safety and outcomes of patients treated for isolated extramedullary relapse. Quantification of CAR T-cell persistence in peripheral blood and cytokine profiling are provided. Cytokine release syndrome and neurotoxicity related to CAR T-cell therapy were graded per the American Society of Transplant and Cellular Therapy criteria[24]; other adverse events were captured using the Common Terminology Criteria for Adverse Events (version 4.03). Complications were managed per the consensus statement of Mahadeo et al[25] with minor modifications.

Statistical Analysis

EFS and OS were estimated using the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards regression model was used for univariate and multivariate analyses of prognostic factors. Transplant was regarded as a time-dependent covariate in the Cox regression model for comparisons between patients who did or did not receive transplant, and display of survival curves was generated according to the method by Bernasconi et al.[26] All analyses were preplanned as described in the protocol. Additional details are provided in the Data Supplement. Outcome data were updated on May 31, 2022.

RESULTS

Trial Population and Treatment

We enrolled 232 patients in the study, of whom 225 were evaluable, including 194 with refractory disease or hematologic relapse and 31 with isolated extramedullary relapse (Fig 1). The baseline characteristics are summarized in the Data Supplement. The median age at the time of enrollment for patients treated for refractory disease or hematologic relapse was 7.6 years (interquartile range [IQR], 4.8-10.8; range, 0.8-19.6 years). The median time from enrollment to infusion was 7 days (range, 6-12 days). The median dose of combined CD19- and CD22-CAR T cells was 5.6×10^6/kg (IQR, 4.1-7.6 $\times$ 10^6; range, 1.3-13.0 $\times$ 10^6). The median dose of CD19-CAR T cells was 2.7×10^6/kg (IQR, 1.9-3.7 $\times$ 10^6), and that of CD22-CAR T cells was 2.8×10^6/kg (IQR, 2.1-4.0 $\times$ 10^6). The median ratio of CD19-CAR T-cell dose to that of CD22-CAR T-cell dose was 0.94 (IQR, 0.78-1.19).

Primary Outcome

Complete remission was achieved in 192 of the 194 patients (99.0% [95% CI, 97.5 to 100]); one patient died of neurotoxicity, and the other of cytokine release syndrome after treatment at the recommended dose. All 192 patients attained negative MRD status. With a median follow-up of 11.0 months after the infusion (IQR, 6.2-18.0 months; range, 0.1-32.4 months), relapse occurred in 43 patients (24 with $CD19^+/CD22^+$ relapse, 16 $CD19^-/CD22^+$, 1 $CD19^-/CD22^-$, and 2 unknown) with a cumulative risk of 22.2% (95% CI, 16.0 to 28.4). The 12-month EFS was 73.5% (95% CI, 67.3 to 80.3) and 69.2% (95% CI, 60.8 to 78.8) after censoring 78 patients for consolidative transplantation (Fig 2A), and the 12-month OS was 87.7% (95% CI, 82.9 to 92.9; Fig 2B).

Consolidative transplantation was performed in 24 of the 37 patients with *KMT2A*-rearranged or *ZNF384*-rearranged ALL and in 54 patients because of parental request. Clinical and biologic characteristics of patients who did or did not undergo consolidative transplantation did not differ significantly, except that none who received transplantation had B-cell aplasia for ≥ 6 months after infusion ($P < .001$, Table 1). Patients who received transplantation had better 12-month EFS than did those who did not ($P = .03$, time-dependent covariate Cox model): 85.0% (95% CI, 77.2 to 93.6) versus 69.2% (95% CI, 60.8 to 78.8; Fig 2C). There was no significant difference in 12-month OS between patients who did or did not receive transplantation ($P = .40$, time-dependent covariate Cox model): 91.3% (95% CI, 84.8 to 98.3) versus 85.0% (95% CI, 78.1 to 92.6; Fig 2D). Transplantation was associated with better EFS for many categories of patients (Table 1).

Secondary Outcomes

B-cell aplasia occurred in the peripheral blood or bone marrow of all 181 patients analyzed by day 28 postinfusion. The median time to normal B-cell recovery ($\geq 1\%$) in blood and/or bone marrow was 74.0 days (IQR, 47.8-97.8 days; range, 27-371 days). The cumulative incidence of loss of B-cell aplasia by 6 months postinfusion was 59.8% (95% CI, 50.4 to 69.2; Fig 2E). There was a steady improvement in EFS for patients who had persistent B-cell aplasia at 2 months after infusion and beyond: 77.0% (95% CI, 68.2 to 87.0), 88.7% (95% CI, 81.1 to 97.1), 97.4% (95% CI, 92.6 to 100), and 100% at 2, 3, 4, and $\geq$6 months, respectively (Fig 2F). Among the 116 patients who received only coadministration of CD19- and CD22-CAR T cells and did not undergo consolidative transplantation, MRD before CAR T-cell treatment $< 15\%$ (70.7% [95% CI, 60.6 to

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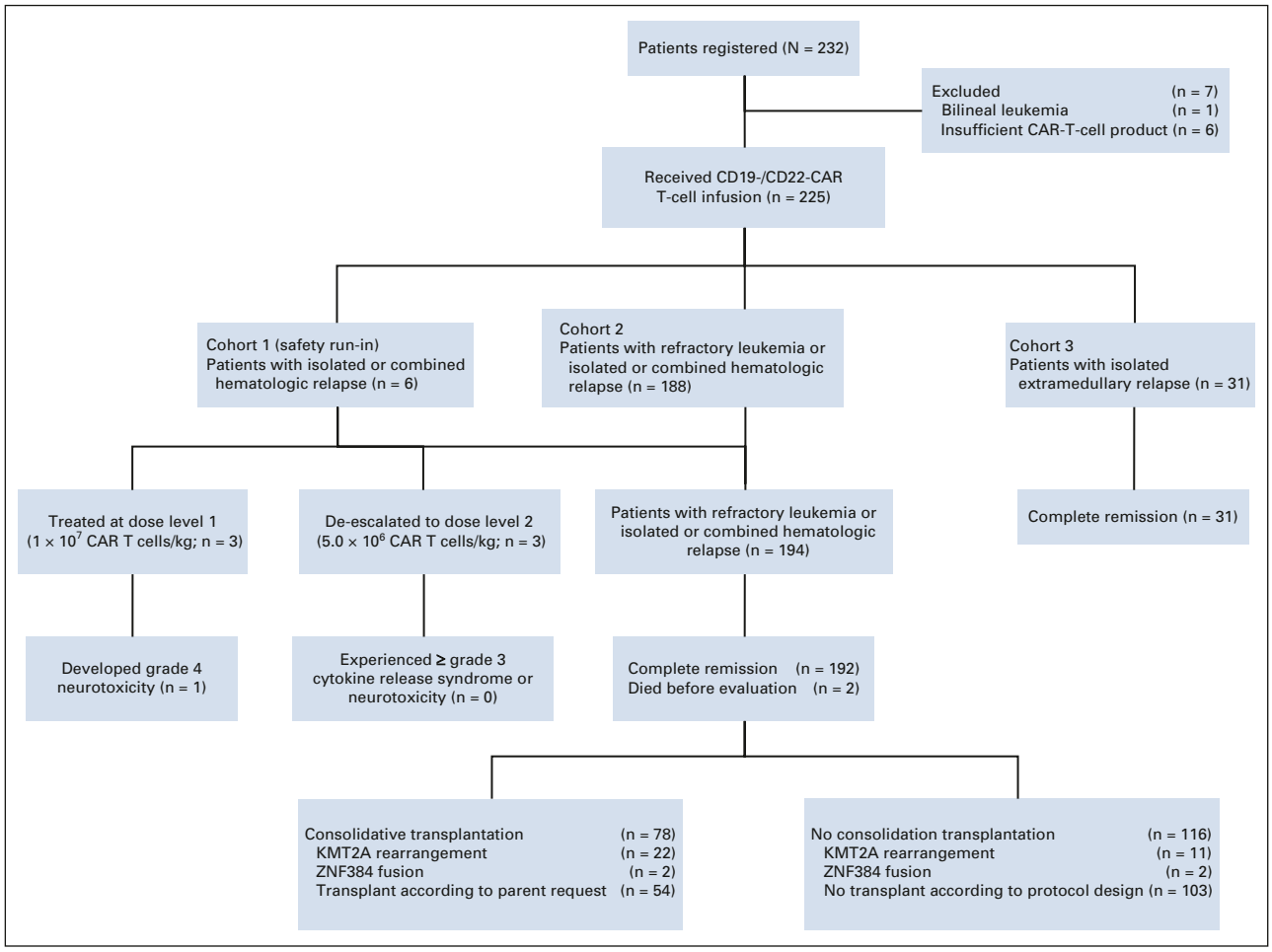

FIG 1. CONSORT diagram. Seven patients were excluded from the study because of the diagnosis of acute bilineal leukemia or insufficient CAR T-cell production ($< 1 \times 10^6$ CAR T cells/kg). The first six patients with hematologic relapse (cohort 1) were treated in the safety run-in stage. Subsequent 188 patients with refractory leukemia or hematologic relapse (cohort 2) were treated with recommended phase II dose. Among the total 194 patients in cohort 1 and cohort 2, 192 achieved complete remission, of whom 78 received consolidative transplantation. All 31 patients with isolated extramedullary disease (cohort 3) achieved complete remission. CAR, chimeric antigen receptor.

82.5] *v* 54.6% [95% CI, 39.9 to 74.7], $P = .04$), M1 bone marrow status (76.3% [95% CI, 64.5 to 90.1] *v* 58.3% [95% CI, 46.8 to 72.5], $P = .05$), and persistent B-cell aplasia for ≥ 6 months were significantly associated with favorable 12-month EFS (100% *v* 47.2% [95% CI, 34.8 to 64.0], $P < .001$; Data Supplement).

In the multivariate analysis, factors associated with better EFS included consolidative transplantation (hazard ratio, 0.24 [95% CI, 0.10 to 1.22], $P = .07$) and persistence of B-cell aplasia for ≥ 6 months postinfusion (100% event-free; hazard ratio, 1.88×10^{-9} [95% CI, 7.40×10^{-10} to 3.16×10^{-9}], $P < .001$; Table 2).

Quantification of CAR T-Cell Persistence

By using quantitative polymerase chain reaction to detect the CAR transgene, we found that expansion occurred earlier for CD19-CAR T cells than for CD22-CAR T cells (peaked at mean ± SE: 7.3 ± 0.5 days *v* 10.9 ± 0.9 days, $P = .0013$) in 76 patients tested. CD19-CAR T cells had more robust expansion for longer duration than CD22-CAR T cells (Fig 3). Among the 21 relapsed patients tested, all 11 with $CD19^+/CD22^+$ relapse had lost CD19- and CD22-CAR T-cell persistence at relapse. Of the nine patients with $CD19^-/CD22^+$ relapse tested, four lost CD19-CAR T-cell persistence, but all nine lost CD22-CAR T cells at relapse. The patient with $CD19^-/CD22^-$ relapse did not lose CD19- but lost CD22-CAR T-cell persistence at relapse.

Isolated Extramedullary Relapse

Thirty-one patients were treated for isolated extramedullary relapse (Data Supplement). Their median age was 7.6 years (IQR, 6.0-10.3; range, 1.4-15.5 years), the median time from enrollment to infusion was 7 days (range, 6-11 days), and the median dose of combined CD19- and

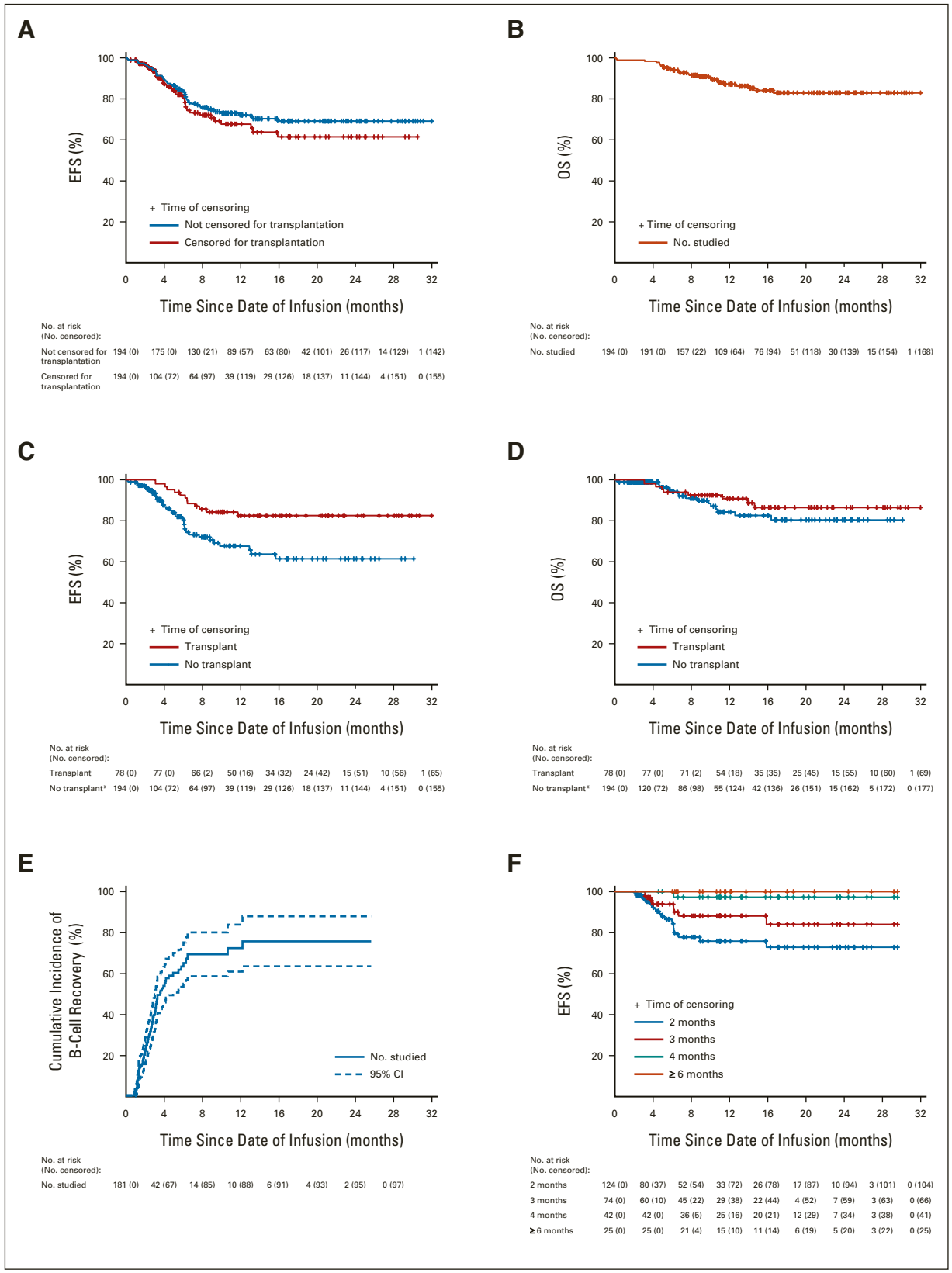

FIG 2. (A) The EFS for patients treated for hematologic relapse or refractory leukemia with or without censoring consolidative allogeneic hematopoietic cell transplantation. (B) The OS for patients treated for hematologic relapse or refractory leukemia with or without transplantation. (C) Comparisons of EFS and (D) OS between patients who did or did not receive consolidative allogeneic transplantation after CAR T-cell therapy. Transplant was regarded as a time-dependent factor; consequently, the initial total number (continued on following page)

FIG 2. (Continued). at risk in the no-transplant group equaled the full sample size (indicated by an asterisk). (E) Cumulative incidence of B-cell recovery as defined by the detection of ≥ 1% $CD19^+$ lymphocytes in bone marrow and/or peripheral blood samples by flow cytometry. Dashed lines denote 95% CI. (F) Landmark EFS analyses for patients with persistent B-cell aplasia reaching 2 months, 3 months, 4 months, and ≥ 6 months. Tick marks indicate the time of censoring. CAR, chimeric antigen receptor; EFS, event-free survival; OS, overall survival.

CD22-CAR T cells was 7.0×10^6/kg (IQR, 5.3-8.9 $\times 10^6$; range, 1.4-14.0 $\times 10^6$). The median dose of CD19-CAR T cells was 3.0×10^6/kg (IQR, 2.2-4.1 $\times 10^6$), and that of CD22-CAR T cells was 3.4×10^6/kg (IQR, 2.7-4.8 $\times 10^6$). The median ratio of CD19-CAR T-cell dose to CD22-CAR T-cell dose was 0.87 (IQR, 0.77-1.01). Sixteen patients had one or more high-risk factors, including second or third relapse, prior allogeneic transplantation or CD19-CAR T-cell therapy, on-therapy relapse, or unfavorable genotypes. All patients experienced complete remission without local irradiation. With a median follow-up of 13.3 months, three of the 10 patients treated for CNS relapse had adverse events (two CNS relapses and one fatal neurotoxicity) and one of the 20 patients treated for testicular relapse developed hematologic relapse, resulting in a 12-month EFS of 68.6% (95% CI, 44.5 to 100) and 95.0% (95% CI, 85.9 to 100), respectively (Data Supplement). The patient with combined testicular and CNS relapse remained in complete remission for 14.4 months.

Adverse Events

Toxicities that occurred within 4 weeks of infusion are shown in Table 3. Cytokine release syndrome developed in 198 (88.0%) patients, was grade ≥ 3 in 64 (28.4%) patients, and was fatal in one patient. Neurotoxicity occurred in 47 (20.9%) patients, was grade ≥ 3 in nine (4.0%) patients, and was fatal in two patients who received 12.0×10^6 and 5.6×10^6 CAR-T cells/kg, respectively. Grade 3 or 4 seizure developed in 14.2% of the patients and was more common in those presenting with isolated or combined CNS leukemia as compared with the other patients (10 of 42 *v* 22 of 183 patients). Grade 3 or 4 hypotension occurred in 40.9% of the patients. Tocilizumab was given to 167 (74.2%) patients, and corticosteroids to 79 (35.1%). The peak levels of interleukin-6 and interferon-gamma were significantly higher among patients with grade 3-4 cytokine release syndrome than in those with grade 0-2 ($P < .001$; Data Supplement).

DISCUSSION

To our knowledge, in this largest prospective CAR T-cell trial for childhood ALL to date, CD19-/CD22-CAR T cells induced complete remission with negative MRD in 99.0% of the patients. Their 12-month EFS was 69.2% and 73.5% with or without censoring on consolidative transplantation, respectively, and their 12-month OS was 87.7%. These results appeared to be better than those of real-world experience with tisagenlecleucel.[5,7,27] We attributed our favorable results partly to the simultaneous administration of two different CAR T cells to enhance early eradication of leukemia clones, thereby impeding the development of resistance. Compared with two large CD19 CAR-T studies,[5,27] this trial yielded a higher complete remission rate (99% *v* 88% and 93.5%, respectively) and a lower relapse rate (22.2% *v* 36% and 31.5%, respectively), suggesting additional immune pressure via CD22 CAR-T cells. Our rapid manufacturing of the CAR T cells enabled infusion of fresh CAR T cells within approximately 1 week, which may also contribute to improved outcomes. Compared with cryopreserved CAR T cells, fresh CAR T cells are more functional and effective.[28] The rapid and robust proliferation of our CAR T cells is suggested by the median onset of cytokine release syndrome of only 1 day and the median time to first tocilizumab treatment of 2 days.

The rapid production of CAR T cells in 7 days without leukapheresis also allowed us to optimize the timing of infusion on the basis of patient's clinical condition and total B-cell and blast counts, decreasing the need of bridging chemotherapy for patients with progressing disease during the waiting period. Disease burden, too low or too high, before CAR T-cell infusion was associated with disease recurrence.[4,27,29-32] Decreased CAR T-cell persistence because of a lack of antigen stimulation has been associated with early loss of B-cell aplasia and $CD19^+$ leukemia relapse in patients with low disease burden.[4,30,31] High disease burden has been associated with $CD19^-$ relapse, a finding attributed to the development of resistance during leukemia proliferation or pre-existing minor population of $CD19^-$ disease, which was undetectable by standard flow cytometry but emerged after clearance of $CD19^+$ disease.[27,31,33] In this regard, our patients with MRD ≥ 15% have poorer EFS than those with levels < 15%.

In a bicistronic CD19-/CD22-targeted CAR T-cell study, five of 10 patients with progressive leukemia had negative or low CD19 expression but preserved CD22 expression.[14] Similarly, three of the eight marrow relapses in another bicistronic CD19-/CD22-targeted study were $CD19^-$, but only one was $CD22^-$.[15] A recent study of tandem CD19.22.BB.zeta CAR-T cells also demonstrated suboptimal CD22-targeting activity.[16] Of our 43 relapsed patients, 17 lost CD19 expression, but only one lost CD22 expression in leukemic cells at relapse. Collectively, these data suggest relatively stronger CD19-specific immune pressure and inadequate CD22-CAR T activity, regardless of dual-targeting approach. Among our 21 relapsed patients tested, all lost CD22-CAR T cells, but six retained CD19-CAR T cells. By using quantitative polymerase chain reaction to detect the CAR transgene, we found that CD19-CAR T-cell expansion occurred earlier and for longer duration than CD22-CAR T-cell expansion, and CD19-CAR T cells had more robust expansion than CD22-CAR T cells. The lack of expansion and persistence of CD22-CAR T cells can be explained by lower CD22 versus CD19 antigen

TABLE 1. Clinical and Biologic Features of the 194 Patients With Refractory Leukemia or Hematologic Relapse and the Comparison of EFS Between the 78 Patients Who Received Consolidation Hematopoietic Cell Transplantation and the 116 Who Did Not According to Various Features

		No Transplant		Transplant		
Parameter	**Total, No. (%)**	**Patients, No.**	**12-Month EFS (95% CI)**	**Patients, No.**	**12-Month EFS (95% CI)**	***P*[a]**
Age at infusion, years						
< 10	136 (70.1)	82	74.7 (65.4 to 85.2)	54	80.1 (69.8 to 92.0)	.45
≥ 10	58 (29.9)	34	55.6 (40.0 to 77.4)	24	90.8 (79.3 to 100)	.01
Sex						
Female	66 (34.0)	41	78.1 (66.5 to 91.9)	25	83.5 (69.9 to 99.7)	.55
Male	128 (66.0)	75	65.8 (55.4 to 78.1)	53	83.3 (73.3 to 94.7)	.03
Disease at the time of CAR T-cell therapy						
Primary refractory	22 (11.3)	18	75.7 (57.2 to 100)	4	75.0 (42.6 to 100)	.87
First relapse	136 (70.1)	76	68.1 (57.7 to 80.5)	60	89.5 (81.9 to 97.8)	.009
≥ 2 relapses	36 (18.6)	22	65.8 (47.6 to 91.1)	14	57.7 (35.0 to 95.0)	.81
Prior allogeneic transplantation or CAR T-cell therapy						
Yes	14 (7.2)	11[b]	81.2 (60.2 to 100)	3[b]	33.3 (6.7 to 100)	.06
No	180 (92.8)	105	67.8 (58.8 to 78.1)	75	85.9 (78.2 to 94.4)	.01
MRD before CAR T-cell therapy						
< 1%	62 (32.0)	42	68.1 (54.4 to 85.2)	20	86.1 (69.5 to 100)	.19
1% to < 15%	72 (37.1)	40	77.8 (64.5 to 93.9)	32	85.1 (72.3 to 100)	.21
≥ 15%	60 (30.9)	34	59.9 (45.3 to 79.2)	26	75.9 (60.9 to 94.7)	.20
Marrow status before CAR T-cell therapy						
M1	79 (40.7)	52	78.2 (66.9 to 91.5)	27	84.2 (71.2 to 99.7)	.42
M2 or M3	115 (59.3)	64	62.4 (51.0 to 76.2)	51	83.3 (73.4 to 94.6)	.02
Extramedullary disease						
No	146 (75.3)	86	70.4 (60.9 to 81.5)	60	86.4 (78.1 to 95.6)	.03
Yes	48 (24.7)	30	65.3 (49.1 to 86.9)	18	73.4 (54.1 to 99.7)	.62
High-risk cytogenetics						
No	138 (71.1)	89	71.0 (61.4 to 82.0)	49	86.9 (77.6 to 97.3)	.03
Yes[c]	56 (28.9)	27	62.5 (46.2 to 84.5)	29	78.8 (65.1 to 95.4)	.25
B-cell aplasia by 6 months after CAR T-cell infusion						
Yes	25 (25.8)	25	100	0	—	—
No	79 (74.2)	53	50.8 (38.2 to 67.7)	26	84.0 (70.8 to 99.8)	.009

Abbreviations: CAR, chimeric antigen receptor; EFS, event-free survival; iAMP21; intrachromosomal amplification of chromosome 21; M1, < 5% blasts in bone marrow; M2, 5%-25% blasts in bone marrow; M3, > 25% blasts in bone marrow; MRD, minimal residual disease.

[a]*P* value was based on the coefficient in a time-dependent covariate Cox model.

[b]For transplant patients, there was one patient who received allogeneic transplantation only, one CD19-CAR T cells only, and one both allogeneic transplantation and CD19-CAR T cells. For nontransplant patients, there were five patients who received allogeneic transplantation only, three CD19-CAR T cells only, and three both allogeneic transplantation and CD19-CAR T cells.

[c]*BCR–ABL1*, *TCF–HLF*, *KMT2A* rearrangement, *ZNF384*, *MEF2D*-rearranged, iAMP21.

expression on leukemia blasts in general or by poor CD22-scFV signaling activity. Other explanations for more frequent loss of CD19 may include pre-existing CD19$^-$ leukemia cells being more frequent than CD22$^-$ leukemia cells before CAR T-cell therapy or acquired mutations and alternative splicing being more common with CD19.[14,34-36] Studies are needed to determine whether enhancing CD22-CAR T-cell persistence and activity would improve outcomes, such as increasing the ratio of CD22- to CD19-CAR T-cell dose, repeated infusion of CD22-CAR T cells, and the use of alternative promotor-scFV-signaling domains or naive T cells.[37]

Hitherto, there were no reliable markers to predict relapse after CAR T-cell therapy. Hence, some investigators proposed

TABLE 2. Univariate and Multivariate Analyses of Factors Associated With EFS and OS Among 194 Patients Who Received CD19/CD22-CAR T-Cell Therapy

		EFS			OS		
Parameter	**Patients, No.**	**Events, No.**	**HR[a] (95% CI)**	***P*[a]**	**Events, No.**	**HR[a] (95% CI)**	***P*[a]**
Univariate Analysis							
Consolidative transplantation after CD19/22-CAR T-cell therapy							
No	116	39	1		17	1	
Yes	78	12	0.47 (0.24 to 0.93)	.03	8	0.69 (0.29 to 1.63)	.40
Age at infusion, years							
< 10	136	35	1		17	1	
≥ 10	58	16	1.04 (0.57 to 1.87)	.90	8	1.07 (0.46 to 2.48)	.87
Sex							
Female	66	15	1		6	1	
Male	128	36	1.16 (0.63 to 2.11)	.64	19	1.50 (0.60 to 3.76)	.39
Disease status							
Primary refractory	22	6	1		2	1	
First relapse	136	33	0.98 (0.41 to 2.34)	.96	14	1.24 (0.28 to 5.45)	.78
≥ 2 relapses	36	12	1.38 (0.52 to 3.69)	.52	9	3.31 (0.71 to 15.32)	.13
Prior allogeneic transplantation or CD19-CAR T-cell therapy							
No	180	46	1		22	1	
Yes[b]	14	5	1.52 (0.60 to 3.82)	.38	3	2.14 (0.64 to 7.14)	.22
MRD							
1% to < 15%	72	14	1		4	1	
< 1%	62	15	1.23 (0.59 to 2.55)	.58	10	2.77 (0.87 to 8.85)	.08
≥ 15%	60	22	2.07 (1.06 to 4.05)	.03	11	3.08 (0. 98 to 9.68)	.05
MRD							
1% to < 15%	72	14	1		4	1	
< 1% or ≥ 15%	122	37	1.62 (0.88 to 3.00)	.12	21	2.93 (1.00 to 8.53)	.049
Marrow status							
M1	79	16	1		9	1	
M2 or M3	115	35	1.59 (0.88 to 2.86)	.13	16	1.18 (0.52 to 2.67)	.69
Extramedullary disease							
No	146	37	1		17	1	
Yes[c]	48	14	1.18 (0.64 to 2.18)	.60	8	1.49 (0.64 to 3.46)	.35
High-risk cytogenetics							
No	138	34	1		13	1	
Yes[d]	56	17	1.29 (0.72 to 2.31)	.39	12	2.36 (1.07 to 5.16)	.03

(continued on following page)

TABLE 2. Univariate and Multivariate Analyses of Factors Associated With EFS and OS Among 194 Patients Who Received CD19/CD22-CAR T-Cell Therapy (continued)

		EFS			OS		
Parameter	**Patients, No.**	**Events, No.**	**HR[a] (95% CI)**	***P*[a]**	**Events, No.**	**HR[a] (95% CI)**	***P*[a]**
B-cell aplasia at 6 months after CAR T-cell infusion							
No	79	33	1		12	1	
Yes[e]	25	0	3.13×10^{-9} (2.54×10^{-9} to 4.06×10^{-9})	< .001	0	3.41×10^{-9} (3.00×10^{-9} to 4.80×10^{-9})	< .001
Multivariate analysis							
Consolidative transplantation after CD19/22-CAR T-cell therapy							
No	78	29	1		9	1	
Yes[e]	26	4	0.24 (0.10 to 1.22)	.07	3	0.58 (0.17 to 2.06×10^{8})	.49
B-cell aplasia at 6 months after CAR T-cell infusion							
No	79	33	1		12	1	
Yes[e]	25	0	1.88×10^{-9} (7.40×10^{-10} to 3.16×10^{-9})	< .001	0	2.75×10^{-9} (8.14×10^{-10} to 5.43×10^{-9})	< .001

Abbreviations: CAR, chimeric antigen receptor; EFS, event-free survival; HR, hazard ratio; iAMP21, intrachromosomal amplification of chromosome 21; M1, < 5% blasts in bone marrow; M2, 5%-25% blasts in bone marrow; M3, > 25% blasts in bone marrow; MRD, minimal residual disease; OS, overall survival.

[a]Except for transplantation, all *P* values and the HR were estimated using the usual Cox model. For transplant, the *P* value was based on the coefficient in a time-dependent covariate Cox model and the HR was estimated from the same model.

[b]Six had prior allogeneic transplantation only, four had CD19-CAR T-cell therapy and allogeneic transplantation, and four had CD19-CAR T-cell therapy only.

[c]Involved extramedullary sites include CNS (n = 26), testes (n = 14), CNS and testes (n = 5), kidney (n = 2), and bone (n = 1); 10 patients (one with bone, three with CNS, and six testes involvement) relapsed in bone marrow alone after CAR T-cell therapy; one patient with CNS involvement relapsed in bone marrow and CNS; one patient with CNS and testes involvement and one patient with kidney involvement died after transplantation. One patient with CNS involvement who did not undergo transplant died of infection.

[d]*BCR–ABL1*, *TCF–HLF*, *KMT2A* rearrangement, *ZNF384*, *MEF2D*-rearranged, iAMP21.

[e]The CI is basic bootstrap 95% CI. The *P* value is obtained by inverting basic bootstrap CIs (Data Supplement). In the multivariate analysis of the EFS, the 90% CI of HR for transplantation after CD19-/CD22-CAR T-cell therapy is 0.11 to 0.87.

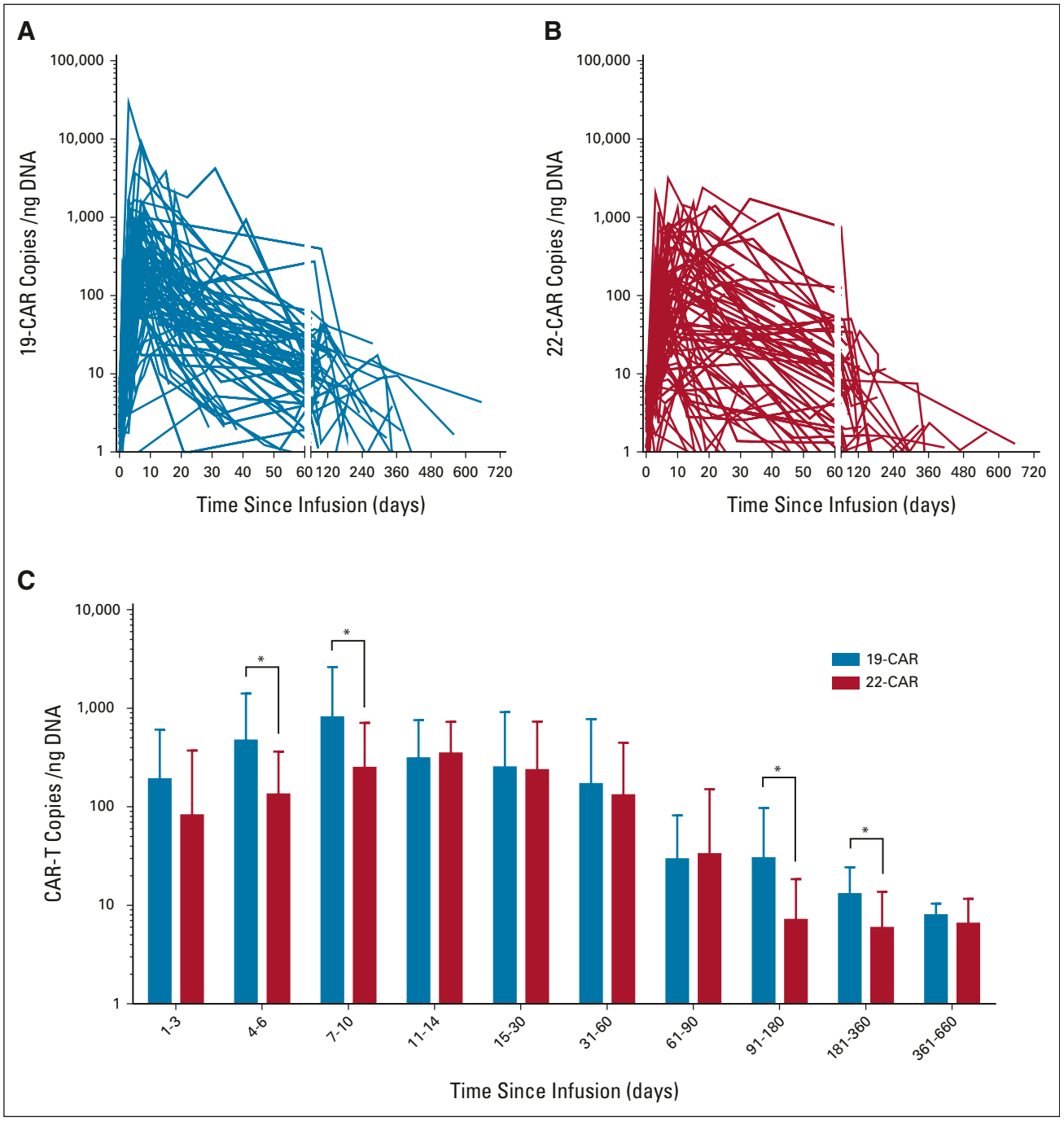

FIG 3. Expansion and persistence of (A) CD19, (B) CD22, and (C) both CD19 and CD22 CAR T cells in blood. The CAR-T copies/ng genome DNA of circulating CD19- and CD22-BB-3ζ CAR T cells as measured by quantitative real-time PCR showing significant differences at initial expansion and subsequent time points, ie, CAR-T copies/ng of genome DNA of CD19- versus CD22-BB-3ζ CAR T cells were 190.7 versus 81.9 at days 1-3 ($P = .138$), 468.8 versus 133.5 at days 4-6 ($P = .028$), 809.7 versus 248.4 at days 7-10 ($P = .021$), 310.1 versus 347.0 at days 11-14 ($P = .721$), 251.6 versus 236.0 at days 15-30 ($P = .895$), 170.4 versus 130.7 at days 31-60 ($P = .679$), 29.4 versus 33.0 at days 61-90 ($P = .869$), 30.1 versus 7.1 at days 91-180 ($P = .039$), 13.0 versus 5.9 at days 181-360 ($P = .021$), and 7.9 versus 6.5 at days 361-660 ($P = .620$). The asterisks in (C) denote significant differences in the CAR T-cell copies/ng DNA. CAR, chimeric antigen receptor.

to use CAR T-cell therapy as a bridge to consolidative transplantation for all patients. Consolidative transplantation provided long-term durable disease control in one CD19-CAR T-cell trial,[13] but did not improve survival in another sequential CD19- and CD22-CAR T-cell study.[17] In our trial, consolidative transplantation was associated with better EFS, a result not yet translated to better OS because some non-transplanted patients were salvageable, and others were

TABLE 3. Safety Outcomes of 194 Patients Treated for Refractory Leukemia or Hematologic Relapse and 31 for Isolated Extramedullary Disease

Toxicity	Total	Relapsed or Refractory Disease	Isolated Extramedullary Relapse
Cytokine release syndrome, No. (%)			
Any	198 (88.0)	171 (88.1)	27 (87.1)
Grade 3 and 4	64 (28.4)	53 (27.3)	11 (35.5)
Grade 5[a]	1 (0.4)	1 (0.5)	0 (0)
Time to onset of cytokine release syndrome, days			
Median	1	1	1
Range	0-10	0-10	0-10
Duration of cytokine release syndrome, days			
Median	5	5	5
Range	1-18	1-18	1-10
Neurotoxicity, No. (%)			
Any	47 (20.9)	41 (21.1)	6 (19.4)
Grade 3 and 4	9 (4.0)	8 (4.1)	1 (3.2)
Grade 5[b]	2 (0.9)	1 (0.5)	1 (3.2)
Time to onset of neurotoxicity, days			
Median	4	4	4
Range	0-9	0-9	1-5
Seizure, grade 3 and 4, No. (%)	32 (14.2)	30 (15.5)	2 (6.5)
Infection, No. (%)			
Grade 3 and 4	33 (14.7)	31 (16.0)	2 (6.4)
Grade 5	0 (0)	0 (0)	0 (0)
Fever, No. (%)	198 (88.0)	170 (87.6)	28 (90.3)
Hypotension, grade 3 and 4, No. (%)	92 (40.9)	84 (43.3)	8 (25.8)
Hypoxemia, grade 3 and 4, No. (%)	49 (21.8)	45 (23.2)	4 (12.9)
Grade 5 adverse events, No. (%)	3 (1.3)	2 (1.0)	1 (3.2)
Received tocilizumab, No. (%)	167 (74.2)	149 (76.8)	18 (58.1)
Time to first tocilizumab, days, median (range)	2 (0-12)	2 (0-9)	4 (0-12)
Received corticosteroids, No. (%)	79 (35.1)	74 (38.1)	5 (16.1)
Time to first corticosteroids, days, median (range)	4 (0-11)	4 (0-11)	4 (4-5)

[a]One patient with hematologic relapse died of cytokine release syndrome at 7 days after infusion.

[b]One patient with hematologic relapse died of neurotoxicity at 4 days after infusion. One patient with second isolated CNS relapse died of neurotoxicity at 1.8 months after infusion.

still alive with disease. Persistent B-cell aplasia at 6 months and beyond was also an independent favorable prognostic factor in this study and was associated with an excellent 12-month EFS of 100%, suggesting that patients with this feature would not need transplantation. However, in a recent study of tisagenlecleucel, measuring B-cell aplasia was not as predictive of relapse as MRD detection by next-generation sequencing and also $CD19^-$ relapse could occur early and at higher frequency in patients with persistence of B-cell aplasia.[38] Additional studies are needed to establish the clinical utility of measuring B-cell aplasia as a complimentary test.

Encouraged by the ability of CD19-CAR T cells to eradicate leukemic cells in cerebrospinal fluid of patients with relapsed $CD19^+$ B-ALL,[3,5] several studies tested this approach in the treatment of isolated extramedullary relapse.[39-42] In a study of testicular relapse, six of seven patients were alive in remission for 5-23 months.[40] In one study of CNS relapse, four of five patients remained alive in remission for 15-29 months.[39] In an analysis of pooled data of 44 patients with CNS relapse from five studies, the 2-year relapse-free survival was 66%.[41] In another recent consortium study, the 12-month relapse-free survival for the 22 patients with isolated CNS relapse was 66.1% and that for the 13 with combined CNS and hematologic relapse was 49.5%.[42] In this study, all 31 patients with isolated testicular or CNS relapse attained complete remission. The 12-month EFS was 95.0% and 68.6% for

patients treated for isolated testicular and CNS relapse, respectively. Notably, among our 48 patients treated for combined hematologic and extramedullary relapse, only one developed a subsequent extramedullary relapse (Table 2). These preliminary results are encouraging, and CAR T-cell therapy could become a therapeutic option for patients with extramedullary relapse.

We encountered relatively high frequencies of CAR T-cell–related grade 3 or 4 hypotension episodes (41.3%) and seizures (14.2%), which we attributed to rapid and robust CAR T-cell expansion. The seizure rate was especially high among patients with isolated or combined CNS leukemia (23.8% *v* 12.0% in the other patients) for whom anticonvulsant prophylaxis is now implemented.

This study had several limitations. We could not use our historical controls for the comparison of long-term outcomes because of a large proportion of patients in this trial undergoing consolidative transplantation. Another limitation is the lack of measurement of MRD with next-generation sequencing, which improved prediction of relapse beyond the assessment of B-cell aplasia.[38] Longer follow-up is needed to determine if late CD19$^-$ relapse would occur as observed among those treated with tisagenlecleucel.[38]

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Coadministration of CD19- and CD22-Directed Chimeric Antigen Receptor T-Cell Therapy in Childhood B-Cell Acute Lymphoblastic Leukemia: A Single-Arm, Multicenter, Phase II Trial

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