original reports

Durable Responses and Low Toxicity After Fast Off-Rate CD19 Chimeric Antigen Receptor-T Therapy in Adults With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia

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abstract

PURPOSE Prognosis for adult B-cell acute lymphoblastic leukemia (B-ALL) is poor, and there are currently no licensed CD19 chimeric antigen receptor (CAR) therapeutics. We developed a novel second-generation CD19-CAR (CAT19-41BB-Z) with a fast off rate, designed for more physiologic T-cell activation to reduce toxicity and improve engraftment. We describe the multicenter phase I ALLCAR19 (NCT02935257) study of autologous CAT19-41BB-Z CAR T cells (AUTO1) in relapsed or refractory (r/r) adult B-ALL.

METHODS Patients age ≥ 16 years with r/r B-ALL were eligible. Primary outcomes were toxicity and manufacturing feasibility. Secondary outcomes were depth of response at 1 and 3 months, persistence of CAR-T, incidence and duration of hypogammaglobulinemia and B-cell aplasia, and event-free survival and overall survival at 1 and 2 years.

RESULTS Twenty-five patients were leukapheresed, 24 products were manufactured, and 20 patients were infused with AUTO1. The median age was 41.5 years; 25% had prior blinatumomab, 50% prior inotuzumab ozogamicin, and 65% prior allogeneic stem-cell transplantation. At the time of preconditioning, 45% had ≥ 50% bone marrow blasts. No patients experienced ≥ grade 3 cytokine release syndrome; 3 of 20 (15%) experienced grade 3 neurotoxicity that resolved to ≤ grade 1 within 72 hours with steroids. Seventeen of 20 (85%) achieved minimal residual disease–negative complete response at month 1, and 3 of 17 underwent allogeneic stem-cell transplantation while in remission. The event-free survival at 6 and 12 months was 68.3% (42.4-84.4) and 48.3% (23.1%-69.7%), respectively. High-level expansion (Cmax 127,152 copies/μg genomic DNA) and durable CAR-T persistence were observed with B-cell aplasia ongoing in 15 of 20 patients at last follow-up.

CONCLUSION AUTO1 demonstrates a tolerable safety profile, high remission rates, and excellent persistence in r/r adult B-ALL. Preliminary data support further development of AUTO1 as a stand-alone treatment for r/r adult B-ALL.

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ASSOCIATED CONTENT

Data Supplement

Protocol

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

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INTRODUCTION

CD19-directed chimeric antigen receptor (CAR)-T has induced sustained disease responses in relapsed or refractory (r/r) pediatric B-cell acute lymphoblastic leukemia (B-ALL),[1] leading to US Food and Drug Administration approval of Tisagenlecleucel. The role of CD19 CAR-T in adult B-ALL is less clear. Adult CD19 CAR-T trials have shown significant immune-mediated toxicity, relatively short duration engraftment and remission, and a requirement for consolidation with allogeneic stem-cell transplantation (allo-SCT).[2-4] There is currently no licensed CD19 CAR-T for adult B-ALL. Here, we describe our experience with CAT19-41BB-Z, a novel CD19 CAR, in adults with r/r B-ALL.

The CD19-targeting single-chain variable fragment (scFv) in CAT19-41BB-Z has a lower affinity for CD19 than FMC63, the scFv used in all currently licensed CD19 CAR-T products.[5] The affinity of an scFv binding its target is determined by its binding on and off rates. The reduced affinity of CAT19-41BB-Z to CD19 is due to a fast off rate, which imparts rapid dissociation from CD19. We hypothesized that the subsequent shorter cell-cell contact may be advantageous by reducing cytokine release and thereby reducing toxicity, as well

CONTEXT

Key Objective

The role of CD19CAR-T in relapsed or refractory (r/r) adult B-cell acute lymphoblastic leukemia (B-ALL) is not established, with clinical studies showing significant toxicity and frequent requirement for allogeneic stem-cell transplantation consolidation. We tested a novel CD19CAR (AUTO1) with a rapid binding off rate for CD19, designed to reduce toxicity and improve persistence in a 20-patient study of r/r adult B-ALL (ALLCAR19).

Knowledge Generated

Seventeen of 20 (85%) patients achieved minimal residual disease–negative complete response following AUTO1 infusion, and toxicity was tolerable despite high baseline B-ALL burden. No ≥ grade 3 cytokine release syndrome was observed, and only 3 out of 20 patients devloped (rapidly reversible) ≥ grade 3 immune effector cell–associated neurotoxicity syndrome. Persistence was demonstrated in 15 of 20 patients at last follow-up. The event-free survival and overall survival for the whole cohort at 12 months was 48.3% and 63.8%, respectively.

Relevance

This manuscript adds to growing experience with CD19CAR-T therapy in adult r/r B-ALL and points to a possible path forward for chimeric antigen receptor-T therapy as a stand-alone treatment.

as reducing T-cell exhaustion, which may enhance CAR T-cell persistence.[6] These features would address major limitations of CAR-T therapy in B-ALL, namely, toxicity and lack of durable responses.

We have previously tested our hypothesis in the pediatric B-ALL setting. Autologous CAT19-41BB-Z CAR-T cells (abbreviated henceforth as AUTO1) demonstrated high efficacy, tolerable safety profile, and durable persistence in a clinical study in pediatric B-ALL (CARPALL; NCT02443831), comparing favorably with outcomes reported in the ELIANA trial of Tisagenlecleucel in the same patient cohort.[6] The present study, ALLCAR19 (NCT02935257), was designed to investigate whether the safety profile, manufacturing feasibility, and preliminary efficacy outcomes of AUTO1 could be reproduced in adults. Given the vulnerability of adult patients with B-ALL to immunotoxicity, we used a fractionated CAR-T dose titrated to disease burden. Furthermore, we explored a semiautomated CAR-T manufacturing system.[7] The results demonstrate encouraging response and safety characteristics coupled to excellent early AUTO1 expansion and persistence, supporting further development as a stand-alone therapy for r/r adult B-ALL.

METHODS

Study Design

This multicenter, nonrandomized, open-label phase I study was conducted in three centers, treating 20 adult patients with r/r B-ALL. Inclusion and exclusion criteria are detailed in the Data Supplement (online only) and Protocol (online only). Following leukapheresis and CAR-T manufacture, patients received lymphodepletion (LD) with intravenous fludarabine (30 mg/m^2, 3 doses over 3 days) and cyclophosphamide (60 mg/kg, single dose). CAR-T was administered in a split-dose schedule, titrated to pre-LD bone marrow (BM) disease burden. On day 0, patients with blasts > 20% received 10×10^6 CAR-T and patients with blasts ≤ 20% received 100×10^6 CAR-T. At an interval of 9 days (in the absence of grade 3-4 cytokine release syndrome (CRS) or immune effector cell–associated neurotoxicity syndrome [ICANS] or grade 1-2 ICANS not fully resolved), dose 2 was administered, to a total dose of 410×10^6 CAR-T cells (Fig 1A).

Primary end points were safety and feasibility of CAR-T manufacture. Secondary end points included efficacy, CAR-T persistence, incidence and duration of B-cell aplasia and hypogammaglobulinemia, and event-free survival (EFS) and overall survival (OS) at 1 and 2 years. Study end points are listed in the Data Supplement.

The study was approved by the UK Medicines and Healthcare products Regulatory Agency (clinical trial authorization No. 20363/0375/001), GTAC Research Ethics Committee (REC ref no.17/LO/0117), and the research and development department of each participating National Health Service trust. The study was managed by the Cancer Research UK and University College London Cancer Trials Centre. Written informed consent was obtained from patients before study entry in accordance with the Declaration of Helsinki. This report incorporates data from all participants who received AUTO1 on study before November 12, 2020. Data were locked as of February 26, 2021.

Toxicity Assessment

Adverse events over the first 28 days post–CAR-T infusion were graded according to Common Terminology Criteria for

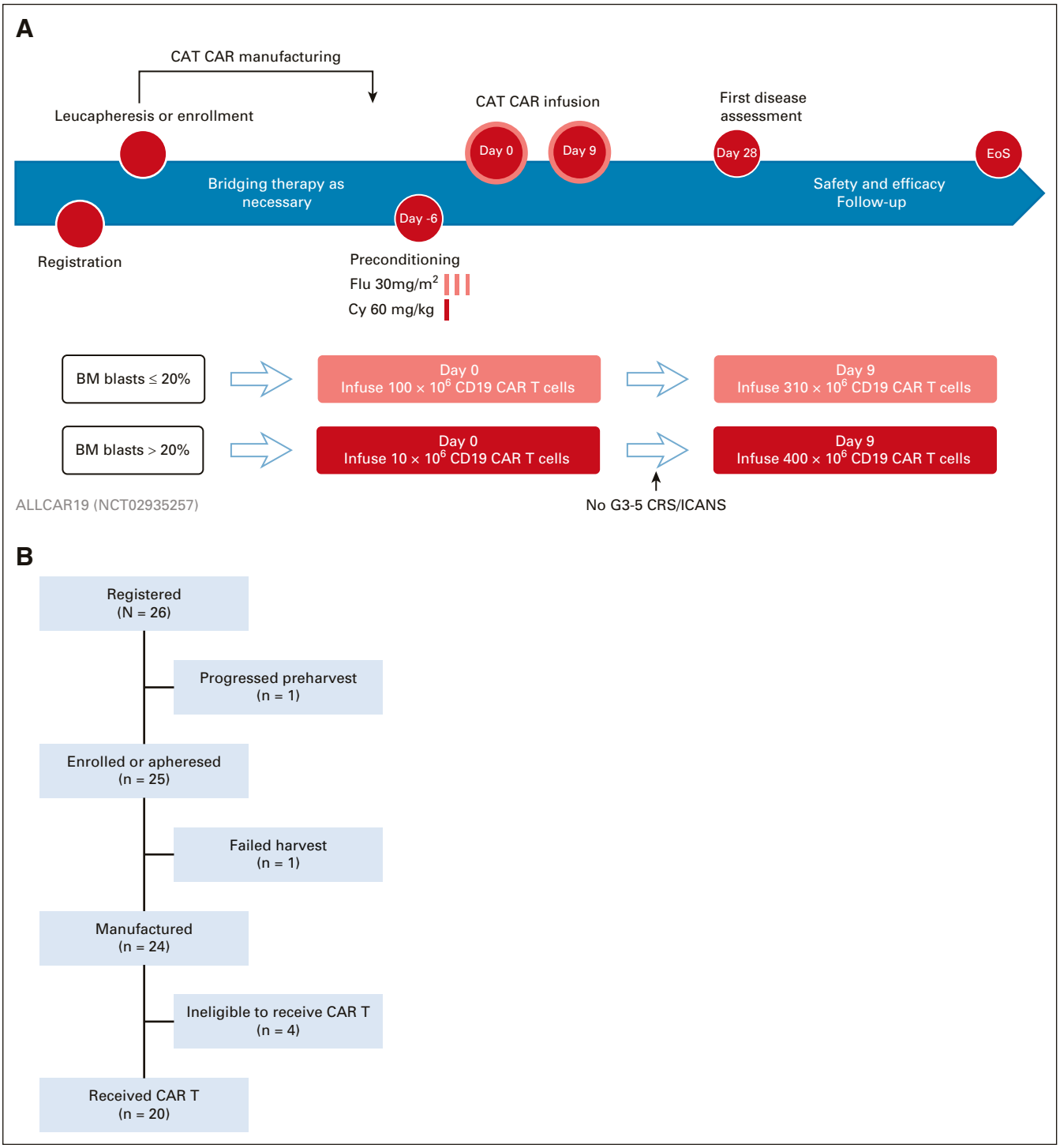

FIG 1. ALLCAR19 study design and recruitment. (A) ALLCAR19 trial schema. Red circles with a pink rim symbolize patients with both ≤ 20% and > 20% blasts infused with CAR-T on day 0 and again on day 9. The small pink rectangles indicate the number of fludarabine doses administered (3 doses; 30 mg/m^2) and the small red rectangle indicates a single dose of cyclophosphamide (60 mg/kg) was administered. (B) Flow diagram of patients on ALLCAR19. BM, bone marrow; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; EoS, end of study; ICANS, immune effector cell–associated neurotoxicity syndrome.

Adverse Events (version 4.03). CRS and ICANS were graded by the American Society for Transplantation and Cellular Therapy criteria.[8]

Response Assessment

Morphologic complete response (CR) was defined as ≤ 5% BM blasts. Minimal residual disease (MRD) was defined as

negative by 1-2 markers with a quantitative assay range of 1×10^{-4}. Where a molecular marker was not available, MRD-negative CR was defined as BM blasts ≤ 0.01% by multiparametric flow cytometry.

Statistical Analysis

Details of statistical analysis are described in the Data Supplement.

RESULTS

Patient and Disease Characteristics

Of 26 patients registered, 25 were enrolled. One registered patient did not proceed to enrollment because of rapid disease progression. Of the 25 enrolled patients, 24 had apheresate with adequate CD3+ T cells for CAR-T manufacture and generated an autologous CAR-T product, which met predetermined release criteria. Four of 24 patients were not infused. One of four developed CD19-negative relapse following blinatumomab bridging; one of four developed graft-versus-host disease (GVHD), and two of four died from infection in the context of progressive refractory B-ALL (Fig 1B).

Patient demographics for the 20 infused patients are summarized in Table 1. The median age was 41.5 years (range, 18-62 years). 75% of patients had an abnormal disease karyotype, and 30% had Ph+ disease. Patients had received a median of three previous lines (range, 2-6) including blinatumomab in 25%, inotuzumab ozogamicin (IO) in 50%, and allo-SCT in 65%. No patients had previous CAR-T therapy, and no patients had CNS involvement. All Ph+ patients received ≥ 2 tyrosine kinase inhibitors before LD (imatinib 6/6, ponatinib 6/6, and dasatinib 1/6; Data Supplement) and were either relapsed or refractory (4 of 6) or intolerant (2 of 6) to last-line tyrosine kinase inhibitor. The median observed follow-up on all 20 patients from first infusion was 21.7 months (range, 0.6-33.9 months). Detailed demographic information for all registered patients is given in the Data Supplement.

CAR T-Cell Manufacturing

Two processes were used for CAR-T manufacture (details are given in the Data Supplement), and all products met release criteria (Data Supplement). Six products were manufactured using process A, and all were infused. Eighteen were manufactured using process B, and 14 of 18 were infused. A target cell dose of 410×10^6 CAR-T cells was achieved in 22 of 24 products (5 of 6, process A; 17 of 18, process B). The median CD3 viability was 99.6% (range, 80.9%-99.9%) at cryopreservation for all manufactured products. The mean CD4/8 ratio was 3.56. The mean transduction efficiency was 66% with a mean stem-cell memory or naive T-cell population of 13.4% and a central memory T-cell compartment of 28.7%. Individual product details are outlined in the Data Supplement.

Baseline Disease Burden and Bridging Treatment

The median disease burden for all infused patients was 43% blasts at registration (range MRD level disease to 98% blasts), and 18 of 20 (90%) received bridging therapy (Data Supplement). Thirteen of 20 received vincristine/corticosteroid; 3 of 20 received ponatinib; 3 of 20

TABLE 1. Patient Demographics on the ALLCAR19 Study

Baseline Characteristic	n = 20 (%)
Sex, No. (%)	
Female	7 (35)
Male	13 (65)
Median age in years (range)	41.5 (18-62)
Chromosomal or molecular status, No. (%)	
Ph+ (bcr-abl)	6 (30)
MLL	1 (5)
Others	8 (40)
Normal	4 (20)
Failed	1 (5)
Previous treatment	
Median previous lines (range)	3 (2-6)
Inotuzumab ozogamicin exposure, No. (%)	10 (50)
Blinatumomab exposure, No. (%)	5 (25)
Previous allo-SCT, No. (%)	13 (65)
Sibling donor, No. (%)	4 (20)
Matched-unrelated donor, No. (%)	8 (40)
Haploidentical donor, No. (%)	1 (5)
T-cell chimerism at enrollment (n = 13), No. (%)	
≥ 95% donor	8 of 13
< 95% donor	5 of 13
Marrow burden before lymphodepletion, No. (%)	
≤ 5% blasts	7 (35)
5%-49% blasts	4 (20)
≥ 50% blasts	9 (45)
CNS disease status at registration, No. (%)	
I	0 (0)
II-III	0 (0)
Other extra-nodal sites,[a] No. (%)	3 (15)
Karnofsky performance status, No. (%)	
100	4 (20)
90	4 (20)
80	5 (25)
70	6 (30)
60	1 (5)

Abbreviations: allo-SCT; allogeneic stem-cell transplantation; MLL, mixed-lineage leukemia.

[a]Extranodal sites include liver, lymph nodes, and spleen.

received IO; 1 of 20 received FLAG-IDA; and 1 of 20 received UKALL14 reinduction therapy. Repeat BM assessment before LD revealed that 45% of patients had ≥ 50% blasts; 20% had 5%-49% blasts, and 35% had ≤ 5% blasts. Of the 18 of 20 patients who received bridging, only 4 had a significant reduction in disease burden to ≤ 5% blasts: two patients with IO, one with UKALL14, and one with vincristine/dexamethasone.

Toxicity and Fractionated Dosing

CRS. Eight of 20 patients (40%) developed grade 2 CRS; 3 of 20 (15%) developed grade 1 CRS, but no patients experienced ≥ grade 3 CRS, despite 65% having > 5% blasts pre-LD. Ten of 11 patients with CRS had > 5% blasts pre-LD (median, 70%; range, 4%-90%). The median onset of CRS was at 6 days postinfusion (range, 2-31 days), and the median duration was 4.5 days (range, 1-27 days).

Tocilizumab was administered in 7 of 20 (35%) cases, all in patients who developed grade 2 CRS. One of eight patients with grade 2 CRS recovered from their blood pressure with fluid boluses and did not require tocilizumab. No patients received corticosteroids for the management of CRS. Using the split-dosing schedule, 15 of 20 patients (75%) received the full protocol-defined dose of 410×10^6 AUTO1. Five of 20 patients (25%) received a single dose (10×10^6 AUTO1) because of ongoing grade 1 or 2 CRS at day 9 in 3 of 5 cases and because of infection in 2 of 5 cases.

ICANS. ICANS was observed in 4 of 20 (20%) patients of which 3 (15%) were grade 3 but resolved within 24-72 hours to ≤ grade 1 with corticosteroids. The median onset of ICANS was at 22 days postinfusion (range, 14-41 days), and the median duration was 1.5 days (range, 1-8 days). All cases were investigated using computed tomography and/or magnetic resonance imaging of brain, EEG, and CSF analysis. Findings of magnetic resonance imaging of brain were variable between the 3 grade 3 ICANS patients: 1 of 3 showed leukoencephalopathy (from previous therapy), 1 of 3 was normal, and 1 of 3 showed ill-defined white matter lesions. EEG detected diffuse slowing in all cases and nonconvulsive status in one case, which terminated with lorazepam. CSF analysis was performed at day 28 in 12 patients, including all cases that developed ICANS. CAR-T cells were readily detected in all CSF samples (Fig 2A), and the proportion of CD3+ CAR-T cells was increased in patients with ICANS (Fig 2B). CSF cytokines were low at day 28 (Data Supplement), which is commensurate with findings in the peripheral blood (PB) at the same timepoint (Fig 2C).

Cytopenias. Three of 20 (15%) patients developed maximum grade 1-2 thrombocytopenia, 3 of 20 (15%) grade 3 thrombocytopenia, and 14 of 20 (70%) grade 4 thrombocytopenia, which did not resolve to < grade 4 by day 28 in 4 of 14 (22%) cases. Twenty of 20 (100%) patients experienced maximum grade 4 neutropenia, which did not resolve to < grade 4 by day 28 in 7 of 20. The median time to recovery to ≤ grade 2 neutropenia was 28 days (range, 28-121 days). Pre-existing cytopenias were common on ALLCAR19, reflecting intensive previous treatment and ≥ grade 3 neutropenia predated CAR-T in 8 of 20 (40%) patients.

Infection. There were 33 early (≤ 30 days postinfusion) infectious events: 12 bacterial, 17 viral, and four fungal. The incidence of late (30-90 days postinfusion) infection was lower, with 15 infectious events of which five were bacterial, seven viral, and three fungal (Data Supplement). Late infectious events were more common in the allo-SCT cohort (15 infectious events) compared with the non–allo-SCT cohort (two infectious events; Data Supplement). Infectious deaths occurred in two patients before day 28, from multidrug-resistant bacterial sepsis and fungal pneumonia. Both had primary refractory B-ALL, and both were neutropenic and lymphopenic for ≥ 1 year. One patient died at month 6 on study while in molecular remission, from a peripherally inserted central catheter line–associated multidrug-resistant *Pseudomonas aeruginosa* septicaemia in the context of ongoing pancytopenia.

Hypogammaglobulinemia. Intravenous immunoglobulin (IVIG) was initiated for serum immunoglobulin G levels ≤ 4 g/L in the context of ≥ 2 non-severe bacterial infections or one severe infection requiring hospital admission. Two of 20 patients were on IVIG before study registration. Of the remaining patients, 13 of 18 had immunoglobulin G levels ≤ 4 g/L at a median of 2 months post–CAR-T, and 5 of 13 commenced IVIG for recurrent infections (Fig 3D).

GVHD. Despite 63% of patients having previous allo-SCT, no GVHD was observed on study.

Toxicity is summarized in Table 2; immunotoxicity is detailed in the Data Supplement, and all other adverse events are listed in the Data Supplement.

Biologic Correlates of Toxicity

Cytokines were measured between day -6 and day 28 in all patients, and levels were low across the study (Figs 2C and 2D). We observed a statistically significant difference in peak interleukin-6 (P = .0007) and serum ferritin (P = .0047) between patients with ≥ 20% blasts compared with those with < 20% blasts (Figs 2E), but there was no association with ≥grade 2 CRS/ICANS.

CAR Expansion and Persistence

The mean peak CAR-T concentration (Cmax) by quantitative polymerase chain reaction (qPCR) was 127,152 copies/μg genomic DNA (gDNA; range, 15,201-672,711) at a median of 13 days (range, 7-21 days) post-AUTO1. At peak qPCR expansion, the median PB CAR+ T-cell fraction by flow cytometry was 83.7% (range, 5.71%-96.6%), with a median absolute CAR T-cell number (CARs/mL) of 468 (range, 88-8,627). Pharmacokinetic

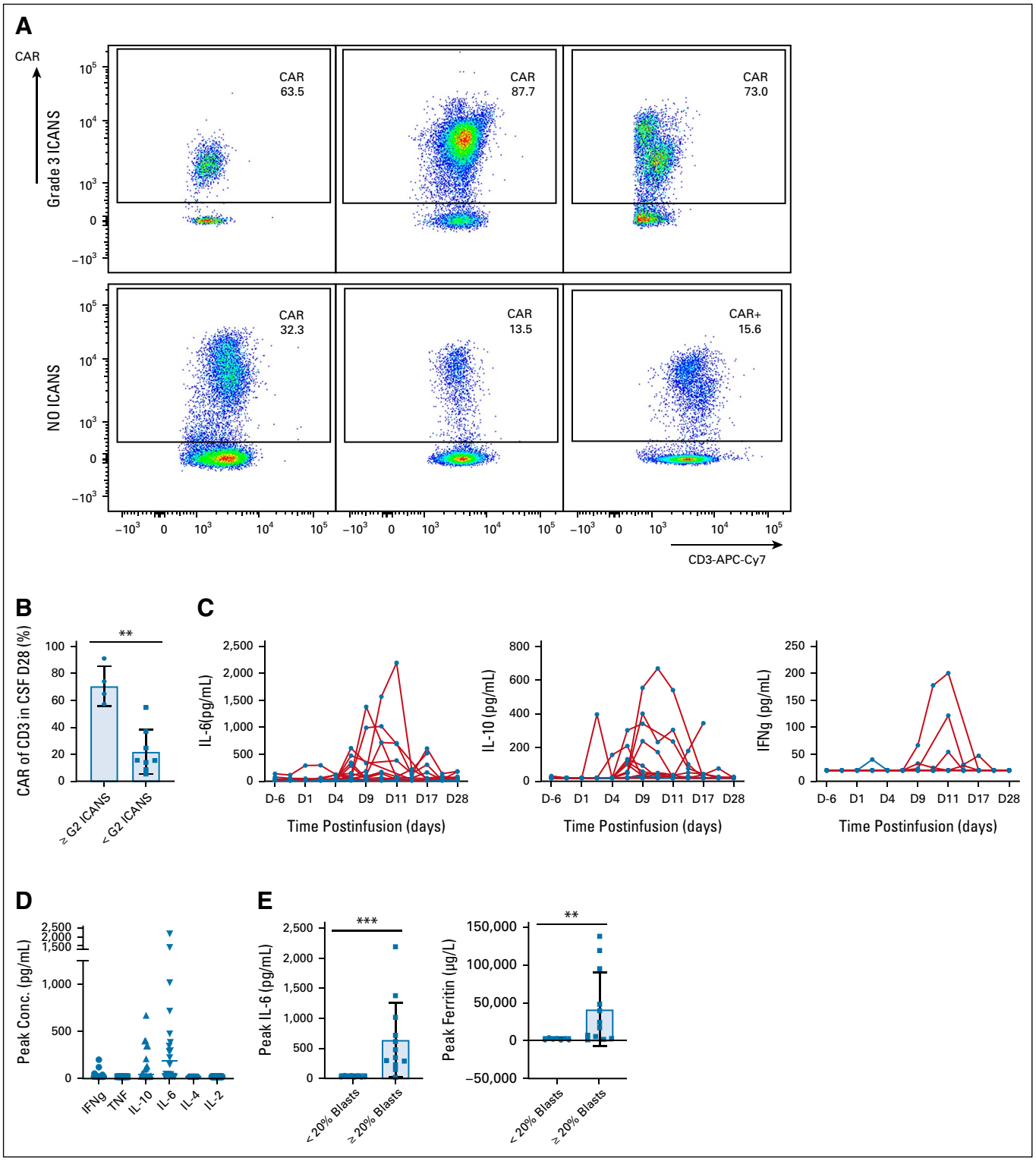

FIG 2. Toxicity and cytokine secretion on ALLCAR19. (A) Example flow plots of CAR T cells in the CSF at day 28 in patients with and without a history of grade 3 ICANS; (B) %CAR of CD3 in the CSF at day 28 is significantly higher in patients with ≥ grade 2 ICANS than in those without (Mann-Whitney; $^{**}P = .004$); (C) cytokine analysis on ALLCAR19 was assessed by cytometric bead array during the first 28 days after AUTO1 infusion in all 20 patients; PB IL-6, IL-10, and IFNg concentrations for individual patients are shown over time to day 28. The y-axis denotes serum level in pg/mL (lower limit of detection, 20 pg/mL). The red lines indicate patients with ≥ 20% blasts pre-LD, and the black lines indicate patients with < 20% blasts. This clearly shows that although the overall cytokine secretion on ALLCAR19 is low, detectable cytokine production is almost exclusively in the context of ≥ 20% blasts. (D) Panel of peak PB cytokine concentration for all patients, illustrating the low cytokine secretion observed in this study. (E) Correlation between peak IL-6 (Mann-Whitney; $^{***}P = .0007$) and peak ferritin (Mann-Whitney; $^{**}P = .0047$) with high disease burden (≥ 20% BM blasts) compared with low disease burden (< 20% BM blasts). BM, bone marrow; CAR, chimeric antigen receptor; D, day; ICANS, immune effector cell–associated neurotoxicity syndrome; IFN, interferon; IL, interleukin; LD, lymphodepletion; PB, peripheral blood; TNF, tumor necrosis factor.

analysis for all treated patients (Data Supplement) showed a mean area under the curve (AUC D0-28) of 1,251,802 copies/μg gDNA. Cmax and AUC D0-28 are higher for AUTO1 (all patients) than those reported for adult B-ALL Tisagenlecleucel responders (Cmax = 54,248.9 and AUC 0-28 = 485,033 copies/μg gDNA, respectively).[9]

Peak expansion by qPCR was not correlated with total CAR-T dose but was strongly associated with both disease burden (patients with ≥ 20% blasts had significantly higher expansion than those with < 20% blasts [$P = .0001$] and with grade 2 CRS [$P = .0011$] but not with ≥ grade 2 ICANS; Fig 3F).

PB CAR-T persistence was evident in 15 of 20 patients at a median of 166.5 days (range, 16-735 days). Four of 20 patients have a follow-up duration > 2 years: 3 of 4 have ongoing CAR persistence and 1 of 4 lost CAR post–allo-SCT. Figure 3A illustrates CAR-T cells in the PB by flow cytometry over a 15-month follow-up in an exemplar patient. Figures 3B and 3C illustrate CAR-T persistence in all infused patients by qPCR and flow cytometry. Assay details are given in the Data Supplement.

As a further surrogate for functional CAR-T persistence, B-cell aplasia was ongoing in 15 of 20 patients at last follow-up (Fig 3D).[9] In four patients with B-cell reconstitution, there was contemporaneous loss of CAR by qPCR. In one case, B-cell recovery occurred with low-level CAR T-cell persistence. The median follow-up for B-cell aplasia was 14.5 months (range, 0.6-29.4 months), and the probability of ongoing B-cell aplasia at 6 and 12 months was 88.2% (60.2%-96.9%) and 73.5% (43.3%-89.3%), respectively (Fig 3E).

Response Rates and Survival

Seventeen of 20 (85%; 95% CI, 62.1 to 96.7) patients achieved MRD-negative CR at month 1 (Fig 4A), and 14 of 20 (70%; 95% CI, 45.7 to 88.1) were in ongoing MRD-negative CR at month 3.

Only one evaluable patient did not respond at month 1. Of note, this patient had rapidly progressive B-ALL, with a white cell count of 12.58×10^9/L on day 7, rising to 69×10^9/L on day 6 and to 104×10^9/L on day 5 with massive (22 cm) splenomegaly. No other patients displayed such a rapid disease progression on admission.

The EFS at 6, 12, and 24 months was 68.3% (95% CI, 42.4 to 84.4), 48.3% (95% CI, 23.1 to 69.7), and 48.3% (95% CI, 23.1 to 69.7), respectively, by morphologic relapse criteria (≥ 5% blasts) and 68.7% (95% CI, 42.9 to 84.6), 43.6% (95% CI, 20.0 to 65.2), and 43.6% (95% CI, 20.0 to 65.2) by MRD relapse criteria (Fig 4B). The OS at 6, 12, and 24 months was 69.1% (95% CI, 43.6 to 84.8), 63.8% (95% CI, 38.6 to 80.8), and 58% (95% CI, 33.1 to 76.4), respectively (Fig 4C). OS and EFS were not significantly different between patients with and without previous allo-SCT. A tabulated summary of outcomes is given in the Data Supplement. Three of 17 patients had allo-SCT while in morphologic remission (1 of 3 had MRD level CD19-negative relapse) at a median of 9 months post–CAR-T (range, 3-9 months).

Four of 20 (20%) patients experienced CD19-negative relapse (with no emergent myeloid features) at a median of 4.5 months post–CAR-T, with ongoing CAR-T persistence. Two of 20 (10%) developed CD19-positive relapse at month 6 and month 9, with CAR-T loss and B-cell recovery. Three of 20 patients had B-cell recovery without relapse. One patient lost CAR-T at 3 months and proceeded directly to allo-SCT but relapsed 9 months later. A second patient recovered B cells at month 9, but with low-level CAR-T persistence by qPCR. A third patient lost CAR following allo-SCT at month 3 post–CAR-T while in remission.

Impact of Previous Allo-SCT on Deliverability, Toxicity, and Outcomes

Deliverability, toxicity, and outcomes were compared between enrolled patients treated with previous allo-SCT (18 of 25) and those who were allo-SCT–naïve (7 of 25). Acknowledging the small numbers, 7 of 7 allo-SCT–naïve patients successfully reached CAR-T infusion compared with 13 of 18 post–allo-SCT patients. EFS, OS, and toxicity were not significantly different between the groups. Of the four patients developing ICANS (1 × grade 2 and 3 × grade 3), 2 were post–allo-SCT and 2 of 4 were allo-SCT–naïve.

DISCUSSION

Although CD19 CAR-T has an established role in pediatric r/r B-ALL,[1,10,11] its role in adult B-ALL is not well-established. Toxicities have been more prohibitive in older patients, and response duration more limited with frequent requirement for consolidation with allo-SCT. Immunotoxicity is a particular challenge in adults. Park et al[2] observed severe CRS or ICANS in 14 of 53 patients and 22 of 53 patients, respectively, using a CD28-Z CAR. Frey et al[4] used a 41BB-Z CAR and reported severe CRS in 25 of 35 patients and severe ICANS in 2 patients. Finally, Turtle et al[12] described severe CRS in 7 of 25 CAR T-cell recipients and severe ICANS in 15 of 30.

We developed CAT19-41BB-Z, a CD19 CAR with a rapid binding off rate for CD19,[6] designed to reduce the magnitude of T-cell activation per target cell encounter and hence reduce immunotoxicity and improve engraftment. Toxicity was low in pediatric r/r B-ALL with CAR-T persistence for ≥ 1 year in most patients, although the majority had low disease burden before CAR-T. Given the potential for toxicity in adults and consideration of treating patients with higher disease burden, we incorporated both risk-adaptive and split-dosing into the ALLCAR19 study design. Dose titration to marrow disease burden can reduce toxicity,[12] whereas split-dosing permits delay or discontinuation of the second dose in the event of onset of

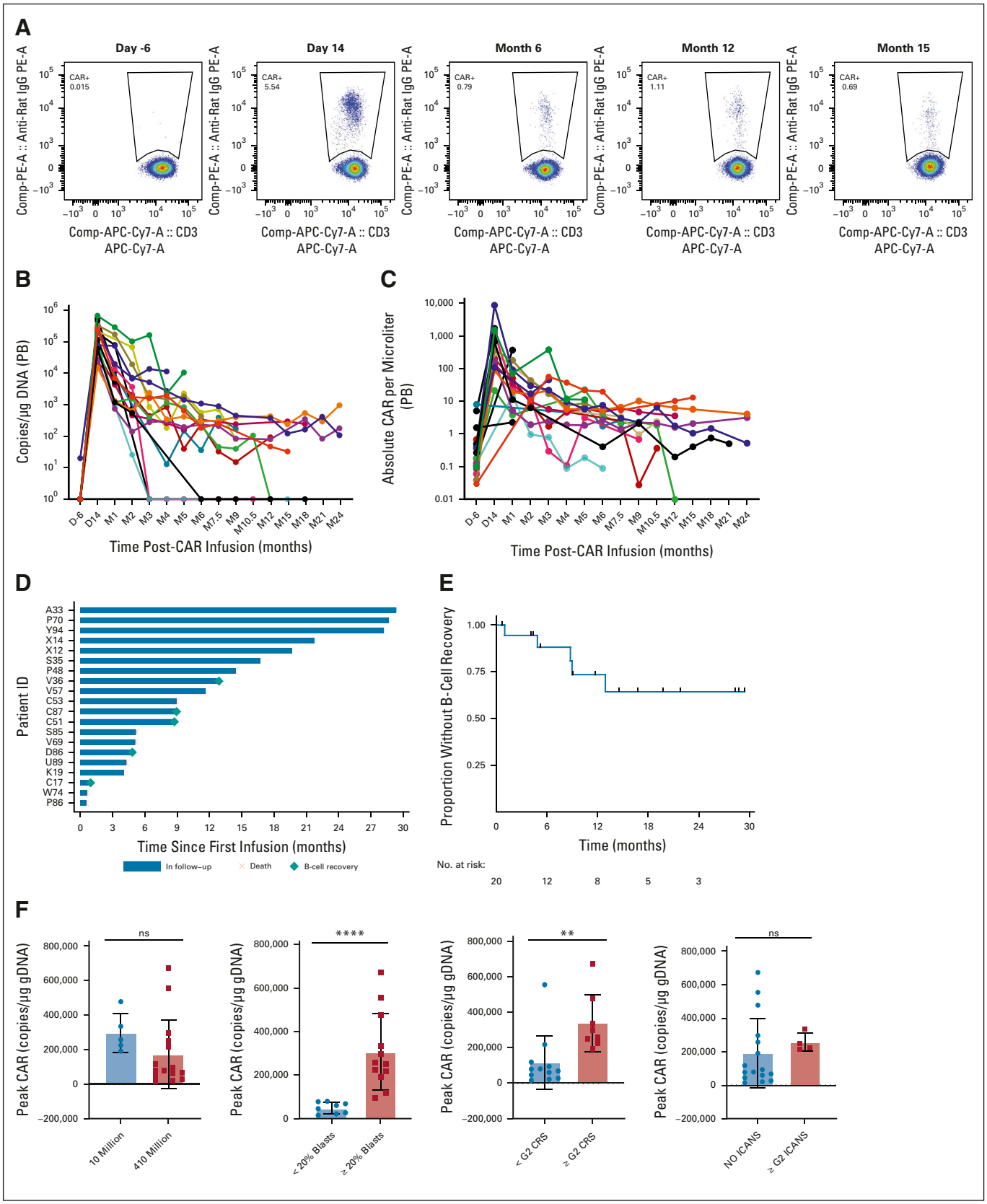

FIG 3. CAR T-cell persistence on ALLCAR19. (A) CAR T cells in the PB by flow cytometry of an exemplary patient over a 9-month follow-up; (B) transgene-specific qPCR analysis of CAT19-41BB-Z in the PB for all patients; (C) flow cytometric analysis of CAR T cells in the PB for all patients; (D) B-cell aplasia on ALLCAR19; (E) Kaplan-Meier analysis of B-cell aplasia; (F) peak expansion by qPCR was not correlated with total AUTO1 dose (continued on following page)

FIG 3. (Continued). but was strongly associated with disease burden (patients with ≥ 20% blasts had significantly higher expansion than those with < 20% blasts [Mann-Whitney; ****$P \leq .0001$] and with ≥ grade 2 CRS [Mann-Whitney; **$P = .011$], but not with ≥ grade 2 ICANS). CAR, chimeric antigen receptor; CRS, cytokine release syndrome; D, day; ICANS, immune effector cell–associated neurotoxicity syndrome; IgG, immunoglobulin G; M, month; ns, not significant; PB, peripheral blood; qPCR, quantitative polymerase chain reaction.

toxicity.[10] Despite high disease burden in many patients, immunotoxicity was relatively limited in our study with no CRS ≥ grade 3 and only three patents developed grade 3 ICANS, which responded swiftly to corticosteroids, resolving to ≤ grade 1 within 72 hours in all cases.

Infection was a significant cause of morbidity and mortality on study. Contributory factors included intensive previous therapies, high frequency of previous allo-SCT, pre-existing cytopenias, and previous colonization with multiresistant organisms. This highlights the need to try to minimize infections during previous therapies, of antimicrobial stewardship, and importance of appropriate specialist input during CAR-T therapy. Fecal microbiota transfer as a decolonization strategy for multiresistant organisms may hold promise for high-risk patients.[13] Late infections were more common in those who underwent previous allo-SCT, underscoring the need for ongoing monitoring and appropriate prophylaxis post–CAR-T.

Response rates were encouraging, particularly considering the favorable toxicity profile. The high levels of CAR-T engraftment and prolonged persistence are likely important to the sustained remissions. MRD-negative remission was achieved in 85% of patients at 1 month, and the 1-year OS and EFS were 63.8% and 48.3%, respectively. Other studies have reported 54%-85% MRD-negative responses in adult cohorts,[2-4] although the median EFS was below 8 months even in those achieving MRD-negative responses.[3,4] We documented only two cases of CD19-positive relapse, both in patients without CAR-T persistence. CD19-negative relapse was more frequent, occurring in four patients, likely reflecting ongoing selective pressure exerted by persisting CAR-T. Dual antigen targeting may help to reduce relapse risk from CD19 escape when CAR-T persistence is durable.[14]

TABLE 2. Summary of Immunotoxicity on ALLCAR19 Of Adverse Events Noted After CAR T-Cell Infusion, by Grade and Type of Toxicity

Maximum Grade Toxicity	No. (%)
Maximum grade CRS (ASTCT criteria)	
CRS (any)	11 of 20 (55)
Grade 2	8 of 20 (40)
≥ Grade 3	0 of 20 (0)
Maximum grade neurotoxicity (ICANS)	
ICANS (any)	4 of 20 (20)
Grade 2	1 of 20 (5)
Grade 3	3 of 20 (15)
Cytopenias at day 28[a]	
≥ Grade 3 neutropenia	9 of 18 (50)
≥ Grade 3 thrombocytopenia	10 of 18 (56)
Maximum grade infections	
All grades	20 of 20 (100)
Grades 1-3	8 of 20 (40)
Grade 4	9 of 20 (45)
Grade 5	3 of 20 (15)

NOTE. Cytopenias were defined as reduced neutrophil or platelet count since lymphodepletion. B-cell aplasia was defined as $< 0.11 \times 10^9$/L B cells assessed from day 28 onward after CAR T-cell infusion.

Abbreviations: ASTCT, American Society for Transplantation and Cellular Therapy; CAR, chimeric antigen receptor; ICANS, immune effector cell–associated neurotoxicity syndrome.

[a]Two patients not evaluable.

It is notable that only 3 of 20 patients underwent allo-SCT consolidation while in morphologic remission. Of these, one died of adenoviremia, one relapsed with CD19-positive disease, and one remains in remission beyond 2 years. Response durability in most patients with detectable CAR-T not receiving allo-SCT supports the use of CATCAR-T as a definitive therapy rather than a bridging strategy. We accept that patients can relapse because of CD19 escape or lack of CAR-T persistence.[15] Indeed, optimal patient selection for allo-SCT consolidation remains an important question for the field. Whether biomarkers (CAR-T marking and B-cell aplasia) can be used to refine such decisions requires further investigation.

Intention to treat in the high-risk adult B-ALL is an important consideration. We manufactured products for all but one patient, demonstrating the robustness of semiautomated manufacturing systems.[7] We lost patients during the manufacturing period to infections and disease progression. In the future, improved logistics and rapid release testing should improve vein to delivery times to < 14 days. Allogeneic approaches have been proposed to expedite patient treatment, but current iterations add considerable toxicity because of the requirement for more profound immunosuppression to prevent rejection and the requirement for subsequent (and in some cases second) allo-SCT because of limited CAR-T persistence.[16]

In summary, ALLCAR19 demonstrates feasibility of manufacturing and administration of AUTO1 to adults with r/r B-ALL. AUTO1 expanded and persisted in most patients with limited immunotoxicity, and remissions were maintained for over 24 months without allo-SCT. ALLCAR19 is a proof of concept that a fast off-rate CD19 CAR can be a stand-alone salvage therapy in adult r/r B-ALL with acceptable toxicity.

These findings are being confirmed in a larger phase II study (NCT04404660) including primarily not only patients with morphologic BM relapse but also a smaller cohort with MRD level or extramedullary disease. Study end points include

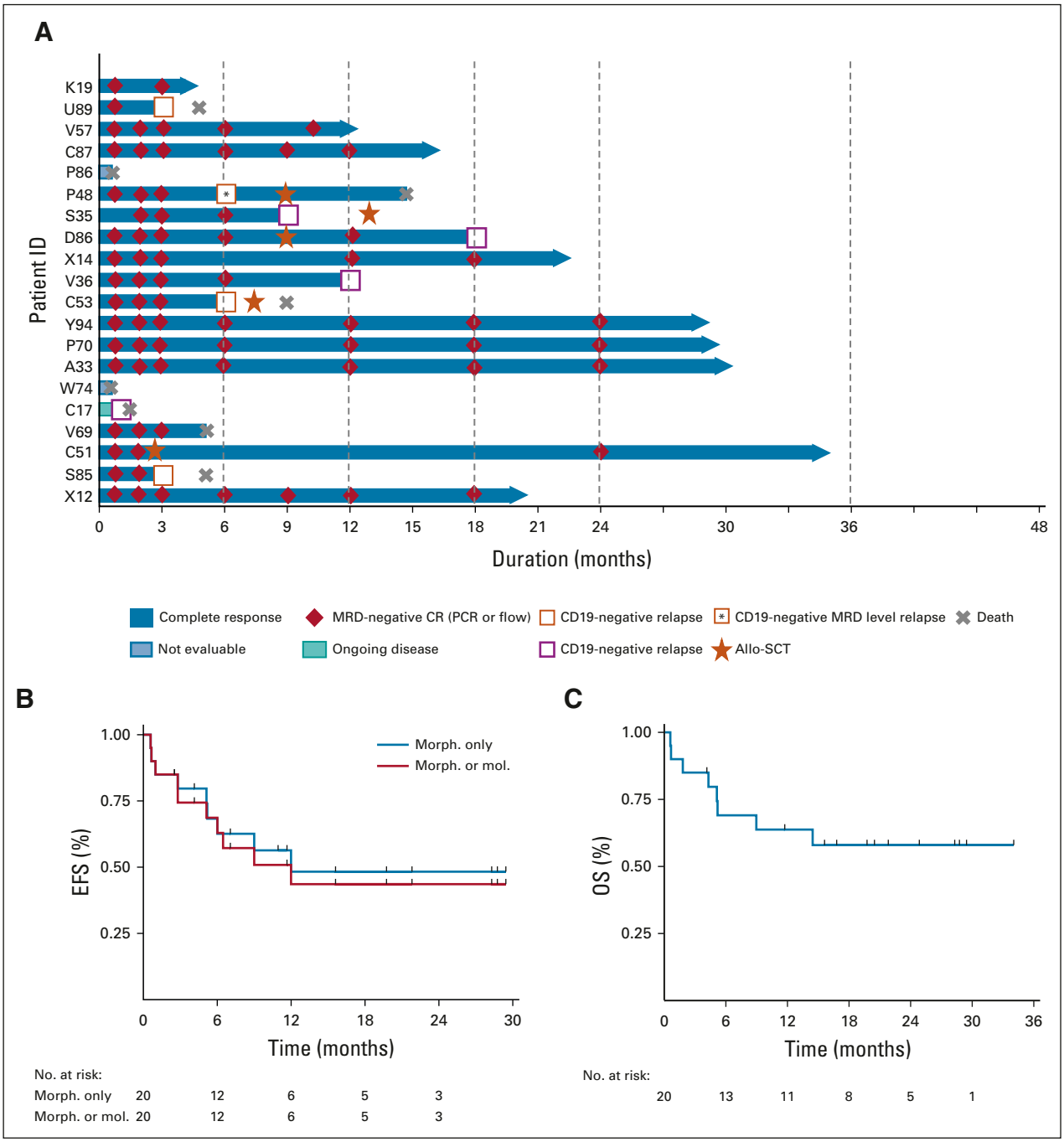

FIG 4. Response rates and survival on ALLCAR19. (A) Swimmers' plot showing responses of individual patients infused with AUTO1, duration of response, and nature of relapse and deaths; the median follow-up was 21.7 months; (B) EFS by morphologic (morph. only) and by morphologic and/or molecular relapse (morph. or mol.), defined as time from date of infusion to date of either morphologic relapse (or molecular relapse) or all-cause mortality, whichever occurred first. Patients were censored at date of last observed follow-up or date of allo-SCT received; (C) Kaplan-Meier plot of OS in all infused patients. For patients still on study and in follow-up, date of data cutoff (February 26, 2021) was used as the date they were last observed for OS and EFS analyses. allo-SCT, allogeneic stem-cell transplantation; CR, complete response; EFS, event-free survival; MRD, minimal residual disease; OS, overall survival; PCR, polymerase chain reaction.

clinical efficacy (overall response rates, progression-free survival, EFS, OS, proportion of patients achieving MRD-negative CR, incidence of CD19-negative relapse, proportion of CR/CRi (CR and incomplete count recovery) without additional therapy, including allo-SCT), in addition to expansion of the safety data set and manufacturing success rates. Parallel translational end points are designed to document expansion and persistence of AUTO1 in the PB and BM with the overall aim of establishing AUTO1 as a definitive stand-alone therapy for patients with relapsed disease.

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CLINICAL TRIAL INFORMATION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Durable Responses and Low Toxicity After Fast Off-Rate CD19 Chimeric Antigen Receptor-T Therapy in Adults With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia

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