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ORIGINAL REPORT

Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor–Modified T Cells After Failure of Ibrutinib

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ABSTRACT

Purpose

We evaluated the safety and feasibility of anti-CD19 chimeric antigen receptor–modified T (CAR-T) cell therapy in patients with chronic lymphocytic leukemia (CLL) who had previously received ibrutinib.

Methods

Twenty-four patients with CLL received lymphodepleting chemotherapy and anti-CD19 CAR-T cells at one of three dose levels (2×10^5, 2×10^6, or 2×10^7 CAR-T cells/kg). Nineteen patients experienced disease progression while receiving ibrutinib, three were ibrutinib intolerant, and two did not experience progression while receiving ibrutinib. Six patients were venetoclax refractory, and 23 had a complex karyotype and/or 17p deletion.

Results

Four weeks after CAR-T cell infusion, the overall response rate (complete response [CR] and/or partial response [PR]) by International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria was 71% (17 of 24). Twenty patients (83%) developed cytokine release syndrome, and eight (33%) developed neurotoxicity, which was reversible in all but one patient with a fatal outcome. Twenty of 24 patients received cyclophosphamide and fludarabine lymphodepletion and CD19 CAR-T cells at or below the maximum tolerated dose ($\leq 2 \times 10^6$ CAR-T cells/kg). In 19 of these patients who were restaged, the overall response rate by IWCLL imaging criteria 4 weeks after infusion was 74% (CR, 4/19, 21%; PR, 10/19, 53%), and 15/17 patients (88%) with marrow disease before CAR-T cells had no disease by flow cytometry after CAR-T cells. Twelve of these patients underwent deep IGH sequencing, and seven (58%) had no malignant IGH sequences detected in marrow. Absence of the malignant IGH clone in marrow of patients with CLL who responded by IWCLL criteria was associated with 100% progression-free survival and overall survival (median 6.6 months follow-up) after CAR-T cell immunotherapy. The progression-free survival was similar in patients with lymph node PR or CR by IWCLL criteria.

Conclusion

CD19 CAR-T cells are highly effective in high-risk patients with CLL after they experience treatment failure with ibrutinib therapy.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia. Patients with high-risk disease manifest by del17(p13.1), p53 mutation, complex karyotype, or unmutated immunoglobulin variable regions require earlier therapy and have shorter survival.[1-3] For patients able to tolerate aggressive therapy, chemo-immunotherapy has been the preferred approach[4]; however, recently, the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, was approved, initially for relapsed and refractory disease and subsequently for first-line therapy.[5,6] Although the overall response rate (ORR) to ibrutinib is high, the complete response (CR) rate is low, and survival of patients who experienced progression while receiving ibrutinib is short, with one study reporting median overall survival (OS) of only 3 months.[7,8] The BCL2 inhibitor, venetoclax, has shown activity in some patients who experienced treatment failure with ibrutinib therapy, but CR is rare and durability not reported.[9]

ASSOCIATED CONTENT

Appendix
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Lymphodepletion chemotherapy followed by CD19-specific chimeric antigen receptor-modified T (CAR-T) cell infusion has produced high response rates in patients with refractory B-cell acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL).[10-16] In a small study, CD19 CAR-T cells induced durable remissions in a subset of patients with CLL, few of whom had previously received ibrutinib.[14,17] Here, we report a high rate of elimination of marrow disease and molecular CR in patients with high-risk ibrutinib-refractory CLL after lymphodepletion and CD19-targeted CAR-T cell therapy.

METHODS

Study Design and Patient Selection

We performed a phase I/II open-label clinical trial with the primary objective of evaluating the feasibility and safety of infusing a defined composition of $CD4^+$ and $CD8^+$ CD19-specific CAR-T cells after lymphodepletion chemotherapy in patients with relapsed or refractory $CD19^+$ B-cell malignancies (Appendix, online only). CAR-T cells were administered at dose level (DL) 1 (2×10^5 CAR-T cells/kg), DL2 (2×10^6 CAR-T cells/kg), or DL3 (2×10^7 CAR-T cells/kg), and a 3 + 3 design was used to establish a maximum tolerated dose of CAR-T cells in each disease cohort. The study was conducted with informed consent and approval of the Fred Hutchinson Cancer Research Center institutional review board. Patients with CLL were eligible if they had experienced treatment failure after receiving an anti-CD20 antibody and fludarabine (Flu) or bendamustine. This article reports the outcome of patients with CLL, all of whom had previously received ibrutinib, treated in the study before September 2016.

Lymphodepletion Chemotherapy and CAR-T Cell Manufacturing and Infusion

Peripheral blood mononuclear cells were collected by leukapheresis for manufacturing CAR-T cells as described.[15,16] Autologous $CD4^+$ and either bulk or central memory (T_{CM})-enriched $CD8^+$ T cells were immunomagnetically selected and then modified with a lentivirus encoding a chimeric antigen receptor comprising a CD19-specific scFv, IgG4-hinge, CD28 transmembrane domain, and 4-1BB and CD3ζ signaling domains. The chimeric antigen receptor was separated by a ribosomal skip sequence from a truncated human epidermal growth factor receptor (EGFRt), which enabled CAR-T cell enumeration by flow cytometry and formulation of a 1:1 $CD4^+$:$CD8^+$ CAR-T cell ratio for infusion. CAR-T cells were administered after lymphodepletion chemotherapy consisting of cyclophosphamide (Cy), Flu, or Cy plus Flu.

Clinical Response Assessment

Patients underwent whole-body imaging with a diagnostic-quality computed tomography (CT) scan before and 4 weeks after CAR-T cell administration. Nodal responses are reported by International Workshop on Chronic Lymphocytic Leukemia (IWCLL; 2008) criteria, and when positron emission tomography (PET) imaging was available, by Cheson 2014 criteria (Appendix).[18,19] Marrow biopsies were obtained before lymphodepletion and 4 weeks after administration of CAR-T cells. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.03), with the exception of cytokine release syndrome (CRS), which was graded as described.[20] Neurotoxicity did not contribute to organ toxicity in CRS grading.

Cytokine Assay

Serum cytokine concentrations were evaluated by Luminex assay (Luminex Corporation, Austin, TX) according to the manufacturer's instructions.

BTK and PLCG2 Mutation Analyses

Peripheral blood mononuclear cells or marrow samples collected before lymphodepletion were sequenced without B cell selection to detect BTK or PLCG2 mutations, as described.[21,22]

Statistical Analysis

The statistical analyses are described in the Appendix.

RESULTS

Patient Characteristics

Thirty patients underwent screening and leukapheresis (Table 1). Five patients were responding to or had not received ibrutinib and did not proceed to lymphodepletion and CAR-T cell infusion, and one ibrutinib-refractory patient became ineligible for study treatment. Twenty-four patients (median age, 61 years; range, 40-73 years) who had received a median of five previous therapies (range, 3-9) received lymphodepletion followed by CD19 CAR-T cell infusion. All patients had high-risk disease, with 96% having high-risk cytogenetics. The median percentage of marrow abnormal B cells before lymphodepletion chemotherapy was 61.6% (range, 0%-96%). Twenty-three patients had nodal disease, and two had active CNS disease. Fourteen of 15 patients who had measurable disease by CT and underwent PET imaging had [^{18}F]fluorodeoxyglucose (FDG)-avid disease.

Twenty-three of 24 patients were refractory to or had relapsed after a regimen containing Flu and rituximab; one patient who received bendamustine plus rituximab experienced treatment failure. All patients had received ibrutinib (median duration, 13 months; range, 0.75-39 months). Nineteen patients experienced disease progression while receiving ibrutinib, and three were ibrutinib intolerant. Ibrutinib was discontinued in all patients before lymphodepletion, including two who had not experienced disease progression while receiving ibrutinib because the safety of concurrent CAR-T cells and ibrutinib had not been established. Mutations associated with ibrutinib resistance were detected in nine of 19 patients (47%; BTK, n = 7; PLCG2, n = 2). Six patients had received venetoclax, and all were refractory. During the 3 weeks between leukapheresis and lymphodepletion, six patients required high-dose corticosteroids to control progressive disease, and two others required treatment of tumor-associated hypercalcemia.

Lymphodepletion and CD19 CAR-T Cell Infusion

Lymphodepletion regimens are listed in Table 2. CD19 CAR-T cells were formulated for infusion by combining $CD4^+$ CAR-T cells with CAR-T cells manufactured from the $CD8^+$ T_{CM} subset (n = 7) or bulk $CD8^+$ T cells (n = 17). A CAR-T cell product was manufactured for all patients, and 22 of 24 patients received $CD4^+$ and $CD8^+$ CAR-T cells in the prescribed 1:1 ratio; two patients received less than the target $CD8^+$ CAR-T cell dose (58.5% and 56.3%). Four patients received DL1, 19 received DL2, and one received DL3 (Table 2). Six patients with persistent or relapsed disease after initial restaging received a second cycle of lymphodepletion and CAR-T cell infusion.

Table 1. Patient Characteristics

No.	Histology	Age (years)	Prior Therapies (No.)	Progression on Ibrutinib	Intolerant to Ibrutinib	Time on Ibrutinib (months)	Venetoclax	Complex Karyotype	Del 17p	Marrow Abnormal B Cells (% leukocytes)	Blood Abnormal B Cells (% leukocytes)	Absolute Lymphocyte Count (× 1,000 cells/μL)	Tumor Cross-sectional area (mm^2)	Maximum SUV
1	CLL/Richter's	65	9	Yes	No	12	No	No	Yes	0.0	0.0	0.70	NE	12.9
2	CLL/PLL	54	3	No	No	0.75	No	Yes	Yes	21.9	10.6	0.88	1,223	3.4
3	CLL/Richter's	64	9	Yes	No	10	No	No	Yes	77.0	29.0	6.59	2,018	NA
4	CLL	59	7	No	Yes	1	No	Yes	No	78.8	75.1	0.41	4,276	NA
5	CLL	55	7	Yes	No	17	Refractory	Yes	No	89.8	23.0	0.81	20,406	9.1
6	CLL	61	6	Yes	No	11	No	No	Yes	77.7	92.0	66.63	NE	NA
7	CLL	63	7	No	No	3	Refractory	No	Yes	32.2	31.2	6.24	1,140	NA
8	CLL	62	5	Yes	No	14	No	Yes	Yes	66.4	39.7	8.93	3,867	NA
9	CLL	53	5	Yes	No	13	No	Yes	Yes	79.3	22.3	0.62	2,909	4.3
10	CLL/Richter's	68	4	Yes	No	16	No	Yes	No	3.5	0.2	0.66	1,683	27.5
11	CLL	53	5	Yes	No	34	No	Yes	Yes	64.5	26.1	1.04	4,753	10.9
12	CLL	70	5	No	Yes	5	No	No	Yes	55.4	62	5.08	1,490	NA
13	CLL/Richter's	47	3	Yes	No	13	No	No	Yes	6.7	0.1	1.28	11,057	10.1
14	CLL/IPCs	40	4	Yes	No	14	No	Yes	Yes	84.2	67.0	30.11	5,833	4.9
15	CLL	73	3	Yes	No	4	No	No	Yes	0.4	0.0	1.11	3,229	3.7
16	CLL	61	4	No	Yes	0.75	No	Yes	No	31.8	2.3	1.13	8,223	NA
17	SLL/Richter's	70	6	Yes	No	8	No	No	No	0.0	0.0	0.88	546	17.8
18	CLL	58	7	Yes	No	26	Refractory	Yes	No	96.0	84.9	10.68	2,482	NA
19	CLL	50	6	Yes	No	22	Refractory	Yes	Yes	90.0	79.0	22.62	3,223	NA
20	CLL	64	5	Yes	No	19	No	Yes	No	78.0	28.9	3.19	4,349	NA
21	CLL/IPCs	53	5	Yes	No	39	No	Yes	No	41.1	21.7	3.29	1,235	5.1
22	CLL	62	7	Yes	No	9	Refractory	Yes	No	40.0	0.03	1.02	3,093	11.5
23	CLL	66	4	Yes	No	26	No	Yes	No	58.6	13.1	2.49	2,400	3.8
24	CLL	58	7	Yes	No	19	Refractory	Yes	Yes	81.0	90.1	31.79	6,071	5.0

Abbreviations: CLL, chronic lymphocytic leukemia; IPCs, increased proliferation centers; NA, not applicable; NE, not evaluated (pretherapy imaging did not permit high-resolution tumor measurement); PLL, prolymphocytic leukemia; Richter's, Richter's transformation; SLL, small lymphocytic lymphoma; SUV, standardized uptake value.

Table 2. Treatment and Response

		Lymphodepletion and CD19 CAR-T Cell Infusion			Restaging After CD19 CAR-T Cell Therapy				Toxicity			
No.	CD8+ T Cell Isolation Method	Lymphodepletion	$CD4^+/EGFRt^+$ Dose (cells/kg)	$CD8^+/EGFRt^+$ Dose (cells/kg)	Marrow Abnormal B Cells by Flow Cytometry (% leukocytes)	Marrow IGH Deep Sequencing (malignant sequence copies/1×10^6 BMMC)	PET Scan (Deauville)	CT Scan (IWCLL)	CRS Grade	Neurotoxicity (CTCAE v4.03)	Tocilizumab (4-8 mg/kg IV; No. of doses)	Dexamethasone (10 mg IV; No. of doses)
1	CD8TCM	Cy 1 g/m^2 + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	ND	2	CR	0	0	No	No
2	CD8TCM	Cy 2 g/m^2	1×10^6	1×10^6	0	17	5	PD	2	0	No	No
3	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	8.7×10^5	5.9×10^5	0	0	4	PR	2	3	No	No
4	CD8	Flu 25 mg/m^2 x 3	1×10^6	1×10^6	61.1	ND	ND	PR	1	0	No	No
5	CD8TCM	Cy 60 mg/kg + Flu 25 mg/m^2 x 5	1×10^7	1×10^7	0	86	ND	PR	4	3	2	14
6	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^5	1×10^5	0	0	ND	CR	0	0	No	No
7	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^5	1×10^5	0	38	ND	PR	1	0	No	No
8	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	9.9×10^4	9.9×10^4	0	7*	ND*	PR	2	0	No	No
9	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	0	2	PR	2	3	2	2
10	CD8TCM	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1.4×10^6	5.6×10^5	0	52*	5*	PD	0	0	No	No
11	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	64	4	PR	1	0	No	No
12	CD8	Cy 30 mg/kg + Flu 25 mg/m^2 × 3	1×10^5	1×10^5	0†	ND	ND	PR	1	2	No	No
13	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	ND	ND	PD	1	0	No	No
14	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	9.9×10^5	ND‡	ND	ND	PD	1	0	No	No
15	CD8TCM	Cy 30 mg/kg + Flu 25 mg/m^2 ×3	1×10^6	9.9×10^5	0	ND	2	PR	1	0	No	No
16	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	9.9×10^5	0	0	2	PR	1	0	No	No
17	CD8	Cy 30 mg/kg + Flu 25 mg/m^2 × 3	1×10^6§	1×10^6§	ND	ND	2	CR	0	0	No	No
18	CD8	Flu 25 mg/m^2 × 3	1×10^6	1×10^6	ND‡	ND	ND	SD	2	0	No	No
19	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	44.8	ND	5	PD	2	0	No	No
20	CD8TCM	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	0	ND	PR	2	3	1	1
21	CD8	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	13	2	CR	2	0	No	No
22	CD8TCM	Cy 60 mg/kg + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	ND‖	ND‖	ND‖	ND‖	5	5	3¶	13
23	CD8	Cy 500 mg/m^2 × 3 + Flu 25mg/m^2 × 3	1×10^6	1×10^6	0	0	5#	SD#	2	2	1	3
24	CD8	Cy 500 mg/m^2 x 3 + Flu 25 mg/m^2 × 3	1×10^6	1×10^6	0	0	1	PR	2	3	2	6

Abbreviations: BMMC, bone marrow mononuclear cells; CAR-T, chimeric antigen receptor-modified T; CD8, $CD8^+$ T cells; CD8TCM, $CD4^+/CD14^+/CD45RA^+$-depleted and $CD62L^+$-selected ($CD8^+$ T_{CM}-enriched) T cells; CR, complete remission; CRS, cytokine release syndrome; CT, computed tomography; CTCAE, Common Terminology Criteria for Adverse Events (version 4.03); Cy, cyclophosphamide; $EGFRt^+$, truncated human epidermal growth factor receptor; Flu, fludarabine; IV, intravenous; IWCLL, International Workshop on Chronic Lymphocytic Leukemia; ND: Not done; PD, progressive disease; PET, positron emission tomography; PR, partial remission; SD, stable disease.

*Deauville score of 2 and IGH deep sequencing of marrow negative after a second cycle of lymphodepletion chemotherapy and infusion of CAR-T cells at 10-fold the dose used in the first cycle.

†Preexisting CD19-negative abnormal plasma cells detected.

‡Peripheral blood disease detected by flow cytometry.

§Received a second CAR-T cell infusion at the same dose (without lymphodepletion) 14 days after the first CAR-T cell infusion.

‖Died of toxicity before restaging.

¶Also received siltuximab 11 mg/kg IV.

#Restaging 8 weeks later without additional antitumor therapy showed no FDG-avid disease and PR (maximum node 16 mm) on CT.

CD19 CAR-T Cell Expansion and Persistence

We detected CAR-T cells by flow cytometry in blood obtained after infusion in all patients. We observed a direct correlation between peak in vivo CAR-T cell frequency and the absolute abnormal B cell count in blood, the percentage of marrow abnormal B cells, and the tumor cross-sectional area before lymphodepletion (Figs 1A, 1B, and 1C) and an inverse correlation between CAR-T cell expansion and the maximum standardized uptake value in those with FDG-avid disease on pretreatment PET scans (Fig 1D). CAR-T cells were detected by quantitative polymerase chain reaction in blood at ≥ 6 months in all patients (n = 11) who were evaluated and did not undergo subsequent allogeneic hematopoietic stem cell transplantation.

Toxicity Assessment Following CD19 CAR-T Cell Therapy in Patients With CLL

A majority of patients developed toxicities expected with lymphodepletion chemotherapy. A total of 20 of 24 had CRS. Eighteen of 24 had grade 1-2 CRS. One of 24 had grade 4 CRS. One of 24 had grade 5 CRS. However, clinical symptoms were sufficiently severe to require tocilizumab and corticosteroid treatment in only six of 24 patients (25%; Table 2; Appendix). A total of eight of 24 had neurotoxicity. Two of 24 had grade 1-2 neurotoxicity. Five of 24 had grade 3 neurotoxicity. One of 24 had grade 5 neurotoxicity. All patients with neurotoxicity also had CRS. Two patients required intensive care unit management, and one of these developed fatal neurotoxicity after infusion of 2×10^6 CAR-T cells/kg. Neurotoxicity was reversible in all other patients. One patient who had previously received alemtuzumab, Flu, and rituximab developed late John Cunningham (JC) virus–positive progressive multifocal leukoencephalopathy, which was considered unrelated to CAR-T cell therapy. The median duration of all-cause hospitalization was 9 days (range, 0-49 days). We previously reported that the infusion of 2×10^7 CAR-T cells/kg after Cy plus Flu lymphodepletion was excessively toxic in patients with ALL and NHL[15,16]; therefore, after one patient with CLL developed grade 4 CRS and grade 3 neurotoxicity after receiving 2×10^7 CAR-T cells/kg, we selected a maximum dose of 2×10^6 CAR-T cells/kg for subsequent patients with CLL (Appendix).

Factors Correlating With Cytokine Release Syndrome and Neurotoxicity

CRS is initiated by activation and proliferation of CAR-T cells after $CD19^+$ target cell recognition. The percentage of leukemic B cells in marrow before therapy was higher in patients who developed CRS and neurotoxicity compared with those who did not (CRS grade 0 *v* grade 1-5; median, 1.8% *v* 65.5%; $P = .035$; neurotoxicity, grade 0 *v* grade 1-5; 36.7% *v* 77.5%; $P = .13$). Peak $CD4^+$ and $CD8^+$ CAR-T cell numbers in blood after infusion were higher in patients with grade 1 to 3 CRS and grade 1 to 3 neurotoxicity, compared with those without CRS or neurotoxicity, but were not higher in patients with grade 4 to 5 toxicity, potentially a result of high-dose corticosteroids administered to treat serious toxicity (Figs 2A and 2B).

Peak serum concentrations of distinct cytokines, ferritin, and C-reactive protein after CAR-T cell infusion differed in patients

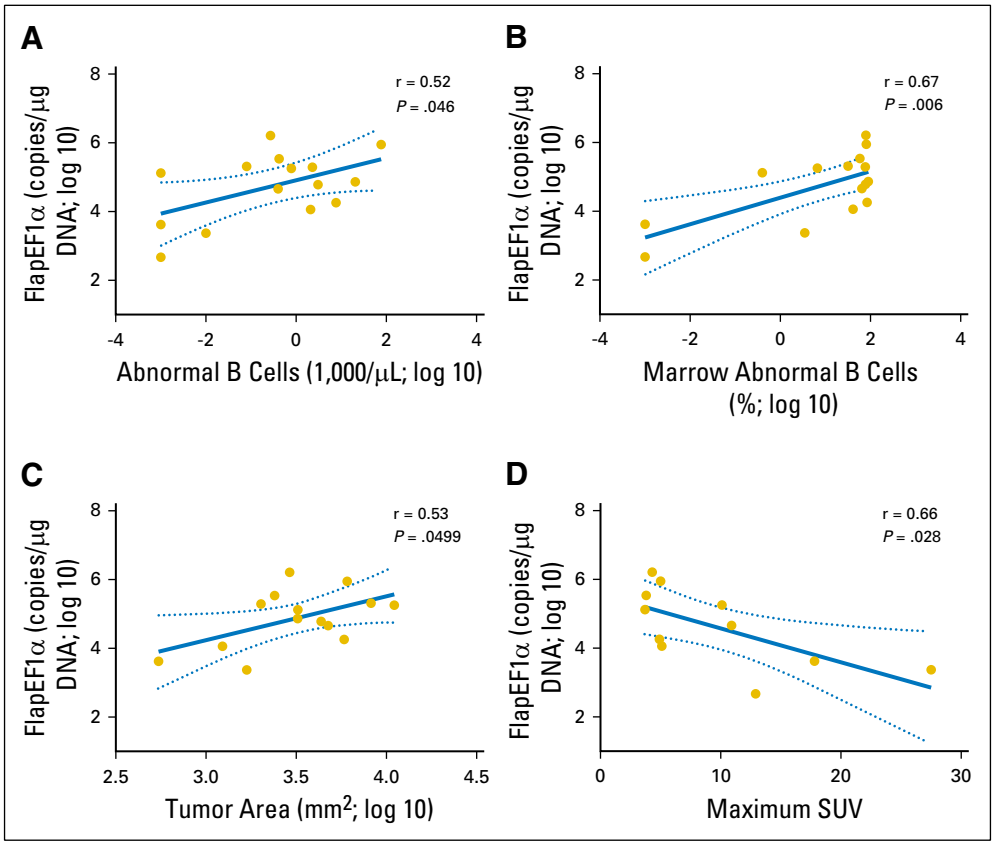

Fig 1. The peak of chimeric antigen receptor-modified T (CAR-T) cells in blood correlates with tumor burden before commencing lymphodepletion chemotherapy. The peak of CAR-T cells in blood (vector copies/μg DNA) is plotted against (A) the absolute abnormal B cell count in blood; (B) the percentage of abnormal B cells in bone marrow; (C) the tumor cross-sectional area; and (D) the maximum standardized uptake value (SUV) on positron emission tomography imaging. Graphs depict data from all patients treated with cyclophosphamide plus fludarabine and 2×10^6 CAR-T cells/kg, with the exception of one patient who died before the peak of CAR-T cell expansion and is not shown. FlapEF1α, flap elongation factor-1 alpha.

with grade 2 to 5 CRS or neurotoxicity compared with those with grade 0 to 1 CRS or neurotoxicity (Figs 2C and 2D). We evaluated serum biomarkers in the first 48 hours after CAR-T cell infusion to determine whether patients at highest risk for more severe neurotoxicity might be identified for early intervention. In univariable analyses, we found higher interferon-gamma and interleukin-10 in the first 48 hours after CAR-T cell infusion in patients who subsequently developed grade $\geq$ 2 neurotoxicity (Fig 2E). Interferon-gamma ($P = .034$) and interleukin-10 ($P = .045$) remained associated in stepwise multivariable regression analysis.

High Lymph Node Response Rate in High-Risk CLL

Twenty-four patients received lymphodepletion and CAR-T cells. The ORR by IWCLL criteria 4 weeks after CAR-T cell infusion was 71% (17 of 24 patients). One patient developed fatal neurotoxicity and did not undergo response assessment. Among the 23 restaged patients, the ORR at 4 weeks after CAR-T cell infusion by IWCLL lymph node criteria was 70% (16 of 23 patients). One additional patient achieved a late response. During the course of this study, data in ALL and NHL demonstrated superior response rates in patients who received CAR-T cells after Cy/Flu compared with Flu lymphodepletion.[15,16] Three patients with CLL did not receive Cy plus Flu lymphodepletion, and only one patient cleared marrow disease, one had a partial response (PR; IWCLL imaging criteria), and all developed progressive disease. Although the study was not powered to determine the optimal regimen, our preferred lymphodepletion regimen contains both Cy and Flu (Appendix).

Twenty patients received Cy plus Flu lymphodepletion and a single CD19 CAR-T cell infusion at or below the maximum tolerated dose ($\leq 2 \times 10^6$ CAR-T cells/kg). In 19 restaged patients, we identified a lymph node response by IWCLL criteria in 14 of 19 patients, 74%, 95% CI, 49% to 91%; CR, four of 19, 21%; PR, 10 of 19, 53%. Response rates were similar in the 16 ibrutinib-refractory patients included in this subgroup treated with Cy plus Flu and $\leq 2 \times 10^6$ CAR-T cells/kg with an ORR in 11 of 16 patients, 69%, 95% CI, 41% to 89%; and CR in four of 16 patients, 25%. A lymph node response (CR or PR by IWCLL) was associated with longer progression-free survival (PFS) and OS compared with those who experienced treatment failure (stable disease or progressive disease), and patients who achieved PR by IWCLL criteria did not have inferior PFS and OS compared with those who achieved CR (Figs 3A and 3B). The PFS and OS for all patients with CLL are shown in Appendix Figure A1 (online only). No responding patients underwent hematopoietic stem cell transplantation after receiving CAR-T cells.

Because study participants had highly aggressive disease, where feasible, we also assessed the lymph node response by PET imaging 4 weeks after CAR-T cell infusion. The CR rate using PET-CT restaging observed in seven of 11 patients (64%, 95% CI, 31% to 89%; Deauville score, 1-2) was higher than that observed after restaging by IWCLL (four of 19 patients, 21%, 95% CI, 6% to 46%). Four of five patients (80%) classified as PR by IWCLL had no FDG-avid disease by PET imaging after CAR-T cell immunotherapy, and an additional patient with stable disease on PET-CT at 4 weeks subsequently achieved CR on follow-up PET-CT 8 weeks later.

High Rate of Elimination of Malignant IGH Sequences From Marrow

Twenty-two of 24 patients had marrow disease before treatment, and 21 patients had a bone marrow evaluation 4 weeks after CAR-T cells. Seventeen of 21 patients (81%) had no marrow disease detected by high-resolution flow cytometry. Fifteen of 17 patients (88%, 95% CI, 64% to 99%) with marrow involvement before therapy and who received Cy plus Flu lymphodepletion and $\leq 2 \times 10^6$ CAR-T cells/kg eliminated CLL from marrow by flow cytometry.[18] The flow-negative marrow response rate in the subset of ibrutinib-refractory patients who received the same treatment regimen was similar (12 of 14, 86%, 95% CI, 57% to 98%). Fluorescent in situ hybridization and conventional karyotyping did not identify residual CLL in patients without detectable disease by flow cytometry; however, two patients had preexisting abnormalities considered to be due to the effects of prior chemotherapy on the myeloid lineage, and one had a persistent constitutional translocation. Twelve patients who cleared marrow by flow cytometry after Cy plus Flu and $\leq 2 \times 10^6$ CAR-T cells/kg also had an identified clonal malignant IGH sequence in CLL cells, and seven of these patients (58%) had no detectable malignant IGH sequences in marrow obtained 4 weeks after CAR-T cell infusion.

Complete Remissions After a Second Cycle of Lymphodepletion and CAR-T Cells

Six patients with persistent or relapsed disease after 1 cycle of lymphodepletion and CAR-T cell infusion had a second cycle of lymphodepletion and CAR-T cells at the same (n = 1) or a 10-fold higher dose (n = 5). Four of six patients developed CRS (two grade $\geq$ 3), and one developed reversible neurologic toxicity (grade 3) after the second CAR-T cell infusion. Two patients achieved CR (by PET-CT criteria), and residual CLL was not detected by bone marrow flow cytometry and IGH sequencing.

Factors Correlating With the Clinical Response to CAR-T Cells

We investigated the factors associated with antitumor efficacy of CAR-T cell therapy. Peak $CD4^+/EGFRt^+$ and, most notably, $CD8^+/EGFRt^+$ CAR-T cell counts were higher in patients who cleared marrow by flow cytometry compared with those who failed to eliminate CLL from marrow (Fig 3C) and were higher in those who achieved CR by flow and had no malignant IGH sequences detected in marrow compared with those who had CR by flow with residual malignant IGH sequences (Fig 3D). Probability curves demonstrated a window where peak $CD4^+/EGFRt^+$ and $CD8^+/EGFRt^+$ CAR-T cell numbers that correlate with a high probability of marrow clearance were associated with an acceptable risk of grade $\geq$ 2 neurotoxicity (Fig 3E-F).

Antitumor activity was seen in a subset of patients with large lymph node burdens (Fig 3G), including those with Richter's transformation; however, patients with higher lymph node bulk were less likely to respond to CAR-T cells (CR *v* PR *v* no response [NR] by IWCLL; $P = .098$), as were those with fewer prior therapies (CR and/or PR *v* NR by IWCLL; median, 5.5 *v* four; $P = .04$). This suggests that bulky and aggressive nodal disease might be less amenable to CAR-T cell therapy. Although the relationship

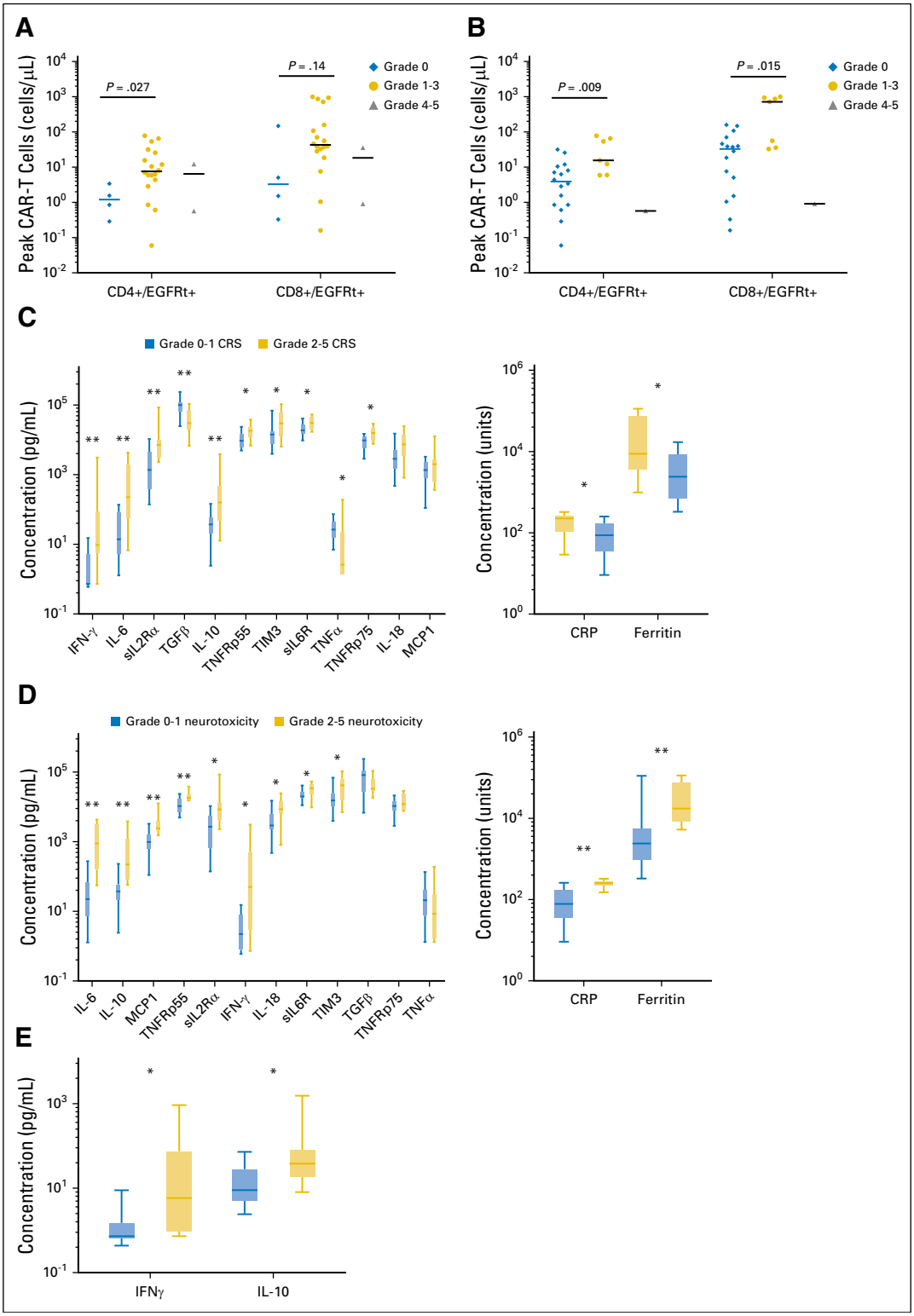

Fig 2. Factors correlating with cytokine release syndrome (CRS) and neurotoxicity. (A) Peak CD4$^+$/truncated human epidermal growth factor receptor (EGFRt$^+$) and CD8$^+$/EGFRt$^+$ chimeric antigen receptor-modified T (CAR-T) cells (cells/μL) in blood after CAR-T cell infusion are shown in patients with grade 0 versus 1 to 3 versus 4 to 5 CRS. (B) Peak CD4$^+$/EGFRt$^+$ and CD8$^+$/EGFRt$^+$ CAR-T cells (cells/μL) in blood after CAR-T cell infusion are shown in patients with grade 0 versus 1 to 3 versus 4 to 5 neurotoxicity. (C) Peak serum cytokine, C-reactive protein (CRP) and ferritin concentrations in patients with grade 0 to 1 compared with grade 2 to 5 CRS. (D) Peak serum cytokine, CRP, and ferritin concentrations in patients with grade 0 to 1 compared with grade 2 to 5 neurotoxicity. (E) Serum cytokine concentrations 0 to 48 hours after CAR-T cell infusion in patients who subsequently developed grade 0 to 1 compared with grade 2 to 5 neurotoxicity. CRP units are mg/L. Ferritin units are ng/mL. FlapEF1α, flap elongation factor-1 alpha; IFNγ, interferon-gamma; IL, interleukin; MCP1, monocyte chemotactic protein-1; sIL6R, soluble IL-6 receptor; sIL2Rα, soluble IL-2 receptor alpha; TGFβ, transforming growth factor-beta; TIM3, T cell immunoglobulin and mucin domain containing 3; TNFα, tumor necrosis factor-alpha; TNFβ, tumor necrosis factor-beta; TNFRp55, tumor necrosis factor receptor p55; TNFRp75, tumor necrosis factor receptor p75. (*) $P < .05$, (**) $P < .01$, Wilcoxon two-sample test.

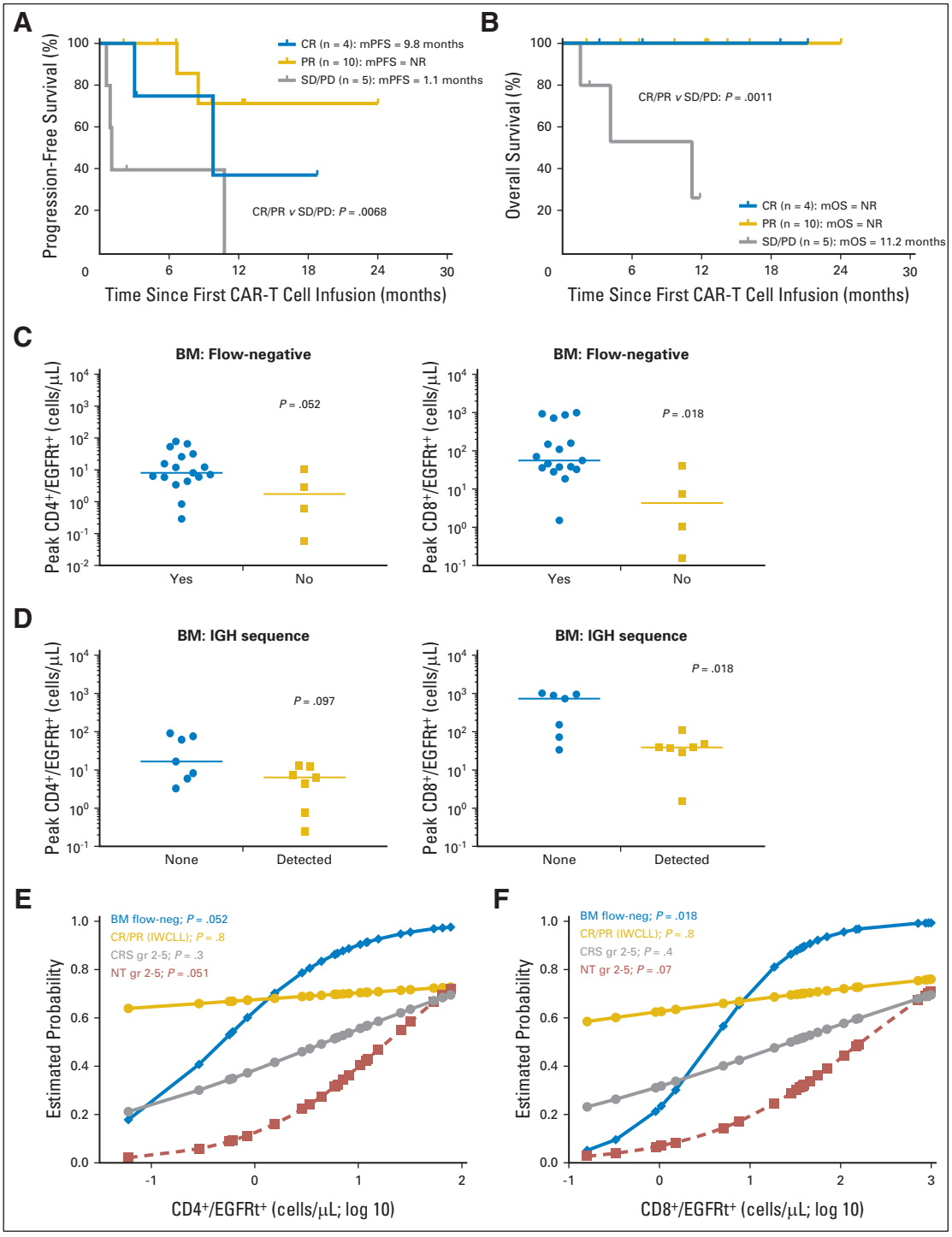

Fig 3. Factors correlating with the response to chimeric antigen receptor-modified T (CAR-T) cell therapy. (A) progression-free survival (PFS) and (B) overall survival (OS) in patients with complete remission (CR), partial remission (PR), or no response (stable disease [SD] and/or progressive disease [PD]) by International Workshop on Chronic Lymphocytic Leukemia (IWCLL; 2008) after cyclophosphamide plus fludarabine lymphodepletion and CAR-T cell infusion at or below the maximum tolerated dose (dose level 1 or dose level 2). The median PFS and OS follow-up for patients in CR/PR was 12.3 and 12.4 months, respectively. (C) The peak $CD4^+$/truncated human epidermal growth factor receptor ($EGFRt^+$; left) and $CD8^+/EGFRt^+$ (right) CAR-T cell counts in blood are shown in patients who did or did not clear disease from the bone marrow (BM) by high-resolution flow cytometry. (D) The peak $CD4^+/EGFRt^+$ (left) and $CD8^+/EGFRt^+$ (right) CAR-T cell counts in blood are shown in patients who cleared disease from BM by high-resolution flow cytometry and did or did not have detectable malignant IGH sequences in marrow. Curves depict the probability estimated by logistic regression of clinical outcomes associated with (E) peak $CD4^+/EGFRt^+$ and (F) $CD8^+/EGFRt^+$ CAR-T cell counts in blood. (G) Waterfall plot showing the change in cross-sectional area of the six largest lymph nodes on computed tomography scan by IWCLL (2008) imaging criteria at best response in high-risk patients with CLL after CAR-T cell immunotherapy. Four patients (two CR, one SD, one died) without high-resolution imaging to enable tumor measurement are not shown. (H) CD19-negative progression in a patient with robust CAR-T cell expansion in blood. BM, bone marrow; CRS, cytokine release syndrome; flow-neg, flow-negative; gr, grade; mOS, median OS; mPFS, median PFS; NR, not reached; NT, neurotoxicity.

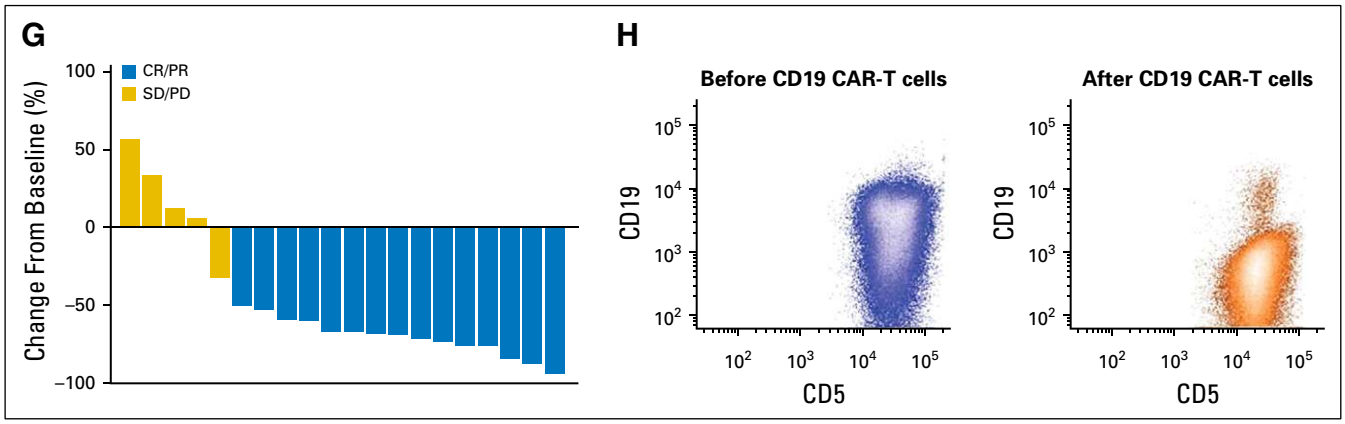

Fig 3. (Continued).

between the peak CAR-T cell count in blood and the probability of a response in lymph nodes was less robust than that noted for marrow (Fig 3E-F), higher peak $CD3^+/EGFRt^+$ CAR-T cell counts in blood were associated with reduced risk of disease progression and death in high-risk patients with CLL (hazard ratio, 0.56; 95% CI, 0.34 to 0.93; $P = .025$). One patient with robust CAR-T expansion experienced failure to clear marrow because of the outgrowth of CD19-negative disease (Fig 3H). No other patients developed CD19-negative disease.

IGH Sequencing Identifies Patients With Durable PFS and OS

A subset of patients who achieved PR by IWCLL at initial restaging had no FDG-avid disease by PET-CT criteria (four of five) and/or had no detectable malignant IGH sequence in marrow (four of six). Further evidence that IWCLL criteria might underestimate the depth of response achieved with CAR-T cells was suggested by the equivalent PFS in patients who achieved PR or CR and ongoing tumor regression after initial restaging in one patient. We analyzed the survival of patients who cleared marrow by flow cytometry in relation to the presence or absence of malignant IGH sequences in marrow 4 weeks after CAR-T cell infusion. Independent of the IWCLL response, patients who were negative for malignant IGH sequences had better PFS compared with those with persistent malignant IGH sequences (Fig 4A and 4B). Median OS was not reached in either group. The positive effect of marrow clearance by IGH sequencing on outcome was also observed when the analysis was restricted to PFS in patients who responded (CR or PR) by IWCLL criteria ($P = .063$; median PFS for detectable malignant IGH copies, 8.5 months; median PFS for no detectable malignant IGH copies, not reached).

DISCUSSION

Patients with relapsed and/or refractory CLL with complex cytogenetics and/or del17(p13.1) after prior ibrutinib therapy have a short expected survival.[1-3,7,8] In this study, we treated 24 such patients with CLL with lymphodepletion chemotherapy and CD19-targeted CAR-T cells. We observed a high rate of elimination of CLL from marrow and lymph node response after CAR-T cell therapy. Approximately half of the patients who were evaluated by IGH sequencing to detect residual tumor in marrow lacked

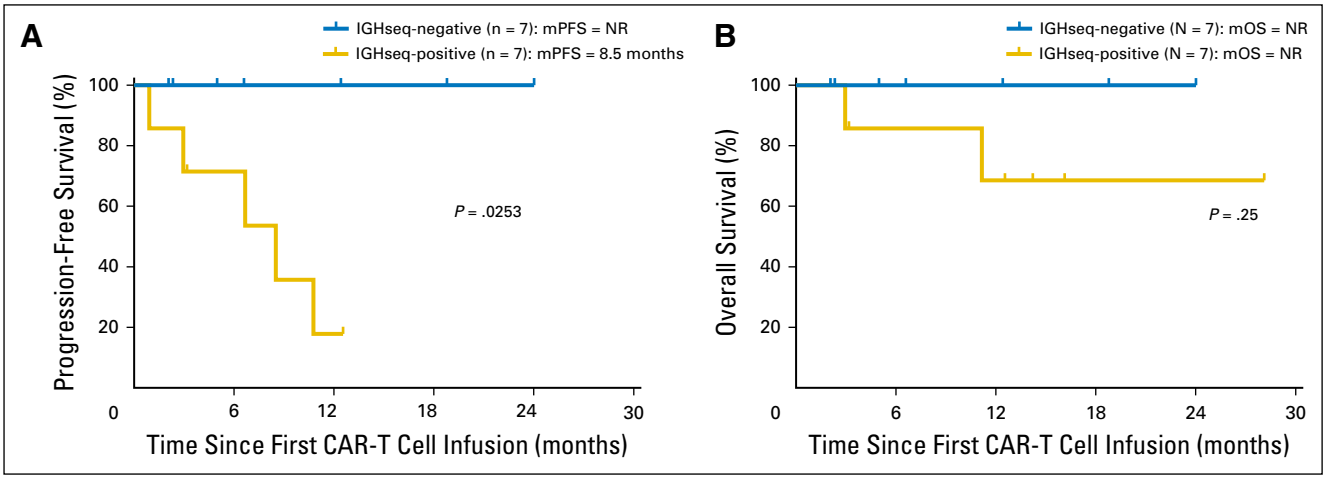

Fig 4. (A) Progression-free survival and (B) overall survival in patients who cleared disease from bone marrow 4 weeks after CAR-T cell infusion by flow cytometry and had no detectable malignant IGH copies (IGHseq-negative) compared with those who had detectable malignant IGH copies (IGHseq-positive). mOS, median OS; mPFS, median PFS; NR, not reached.

detectable malignant IGH copies. Absence of detectable malignant IGH sequences after conventional therapies for CLL is associated with a lower risk of relapse, but is infrequent.[23] Achieving a malignant IGH-negative status after CAR-T cells in our study correlated better with superior PFS than CR by IWCLL CT criteria, which requires that all lymph nodes be ≤ 15 mm. These data indicate that early restaging by tumor size criteria alone, 4 weeks after CAR-T cell administration, may not be the optimal determinant of prognosis, as suggested after immune checkpoint blockade in other malignancies.[24,25] Additional studies will be required to determine whether strategies such as IGH sequencing, PET imaging, or delayed restaging after CAR-T cell immunotherapy of CLL can identify patients who might benefit from additional CAR-T cell infusions to improve outcomes. In this study, two of six patients who received a second CAR-T cell infusion achieved CR by PET imaging and IGH deep sequencing.

Although bone marrow disease was highly responsive to CAR-T cells, complete elimination of bulky nodal disease was less common, suggesting the malignant lymph node environment may impair CAR-T cell infiltration and/or function. The nodal and molecular CR rate in advanced CLL might be improved if CAR-T cell immunotherapy is delivered when ibrutinib-induced mobilization of lymph node disease into blood and/or marrow is still effective and before development of bulky lymphadenopathy. Such a strategy might be used by monitoring patients receiving ibrutinib for increasing prevalence of ibrutinib-resistance mutations. We detected BTK and PLCG2 mutations in 47% of patients who experienced disease progression receiving ibrutinib, which is lower than results reported by Woyach et al,[26] likely due to the inclusion in our study of a higher proportion of patients who had experienced early treatment failure while receiving ibrutinib and those with Richter's transformation, and the absence of B cell selection in the ibrutinib-resistance mutation assay.[26]

The incidence of serious toxicity after CAR-T cell administration was low. One patient died with CRS and neurotoxicity, illustrating the importance of understanding the pathogenesis of neurotoxicity and evaluating intervention strategies. Our previous report in which CD19 CAR-T cells were administered to patients with ALL found that tumor burden is associated with robust CAR-T cell expansion, CRS, and neurotoxicity, and similar associations were observed in this study in patients with CLL. Thus, modifying CAR-T cell dose in relation to tumor burden may further improve outcomes in CLL. Furthermore, our identification of cytokines whose levels early after CAR-T cell infusion predicted severe toxicity will facilitate the study of pre-emptive therapy to mitigate toxicity.

In conclusion, CD19 CAR-T cells are highly effective with manageable toxicity in patients with high-risk CLL, including those who are ibrutinib refractory. This approach can achieve sustained molecular remissions and improve the poor prognosis of ibrutinib-refractory CLL. Future studies in patients who are likely to become refractory to ibrutinib on the basis of high-risk cytogenetics or early detection of mutations before relapse that confer ibrutinib resistance are warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor–Modified T Cells After Failure of Ibrutinib

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Appendix

Clinical Trial of CD19 Chimeric Antigen Receptor-Modified T Cells in B Cell Malignancies

We conducted a phase I/II clinical trial of lymphodepletion chemotherapy followed by infusion of CD19 chimeric antigen receptor-modified T (CAR-T) cells for patients with CD19+ B cell malignancies. In this article, we report all patients with chronic lymphocytic leukemia (CLL) who were treated in the trial before September 2016. The following section describes the study and evaluation of the patients with CLL.

Primary study objective. The primary objective was evaluation of the safety and feasibility of infusing a defined composition of $CD4^+$ and $CD8^+$ CD19-specific CAR-T cells after lymphodepletion chemotherapy in patients with relapsed or refractory $CD19^+$ acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), or CLL.

Secondary study objective. A secondary objective was to establish evidence of antitumor efficacy at restaging 4 weeks after lymphodepletion chemotherapy and CAR-T cell infusion.

Eligibility criteria for patients with CLL. Patients with CLL were eligible if they were beyond first remission and had experienced treatment failure with combination chemoimmunotherapy with an anti-CD20 antibody and fludarabine (Flu) or bendamustine. There were no exclusion criteria on the basis of the presence of high-risk cytogenetics or disease histology, minimum or maximum absolute lymphocyte count, lymph node tumor burden, or history of prior allogeneic hematopoietic stem-cell transplantation. This article reports all patients with CLL treated in the study before September 2016, all of whom had previously received ibrutinib.

Toxicity assessment. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.03), with the exception of cytokine release syndrome (CRS), which was graded as described.[20] Neurotoxicity did not contribute to organ toxicity in CRS grading.

Antitumor efficacy assessment. Marrow response was assessed by bone marrow aspirate and biopsy obtained 4 weeks after CAR-T cell infusion in those with marrow disease before lymphodepletion chemotherapy. Morphology analysis and high-resolution flow cytometry were performed on the marrow, with conventional karyotyping and fluorescent in situ hybridization in patients with an identified cytogenetic abnormality. We performed IGH deep sequencing (Adaptive Biotechnologies, Seattle, WA) on marrow from patients who had no detectable marrow disease by flow cytometry 4 weeks after CAR-T cell infusion and had an identified malignant clonal sequence before lymphodepletion. High-resolution flow cytometry was performed on blood 2 weeks and 1, 2, 3, 6, and 12 months after CAR-T cell infusion.

Lymph node response was assessed by whole-body imaging with a diagnostic quality computed tomography scan before and 4 weeks after CAR-T cell therapy, and reported by IWCLL (2008) criteria.[18,19] Nodal tumor bulk was assessed as the sum of the cross-sectional areas of the six largest index lymph nodes identified on a diagnostic quality computed tomography scan. Whole-body positron emission tomography imaging was not part of the planned response analysis, but was performed in a subset of patients with insurance approval and reported using Cheson 2014 criteria. Additional imaging studies were performed when clinically indicated.

Dose-limiting toxicity criteria.

1. Death within 8 weeks of the CAR-T cell infusion thought to be definitely or probably related to CAR-T cell therapy.
2. Other dose-limiting toxicities are defined as follows:
3. Grade ≥ 3 nonhematologic toxicity in any major organ system that is probably or definitely attributed to T cell infusion and is unresponsive (does not improve to $<$ grade 3 toxicity) to treatment with dexamethasone 10 mg every 12 hours intravenously (IV) for ≥ 7 days (or an equivalent corticosteroid dose) or tocilizumab 8 mg/kg IV for ≥ 3 doses or > 28 days duration. Hematologic toxicity is an expected complication of chemotherapy and, other than B cell depletion, has not been observed in prior trials of CAR-T cell therapy and therefore is not considered for altering T cell dose.
4. Common Terminology Criteria for Adverse Events grades 3 to 5 allergic reactions related to the study cell infusion.
5. Common Terminology Criteria for Adverse Events grades 2 to 5 autoimmune reactions, other than expected B cell depletion.

Rationale for CAR-T cell manufacturing approach. CAR-T cells were manufactured from $CD4^+$ T cells and either bulk $CD8^+$ T cells or $CD8^+$ central memory T cells and formulated in a 1:1 ratio of CD4+:CD8+ CAR-T cells for infusion. This approach was based on preclinical studies that indicated optimal potency was obtained using these formulations. Our preferred approach in the

study was to manufacture CAR-T cells from $CD4^+$ T cells and $CD8^+$ central memory T cells; however, in patients with severe lymphopenia and a low $CD8^+$ central memory T cell count in blood or malignant lymphocytosis, we elected to manufacture CAR-T cells from $CD4^+$ T cells and bulk $CD8^+$ T cells. No difference in clinical outcome was observed between patients who received CAR-T cells manufactured from $CD4^+$ T cells and either bulk $CD8^+$ T cells or $CD8^+$ central memory T cells.

Rationale for selection of lymphodepletion regimens. During the initial stage of the study, patients with B-cell ALL, NHL, and CLL were treated with escalating doses of CAR-T cells without stratification by disease type. In a subset of patients who received cyclophosphamide (Cy)-based lymphodepletion without Flu, we observed a $CD8^+$ T cell–mediated immune response to the CAR transgene.[15,16] To abrogate the effect of immune CAR-T cell rejection, we intensified the lymphodepletion regimen by adding Flu to Cy (Cy/Flu). Many of the patients treated in the study had received multiple previous cycles of chemotherapy, had previously undergone allogeneic transplantation, had poor marrow reserve, and/or had other serious comorbidities. To minimize toxicity in these patients, we modified their lymphodepletion chemotherapy by reducing or omitting the dose of Cy, or administering a regimen including a lower total dose of Cy administered concurrently with Flu.

Identification of a maximum tolerated CD19 CAR-T cell dose. We previously reported that the addition of Flu to Cy lymphodepletion enhanced CAR-T cell proliferation and could result in excessive toxicity in patients with B-cell ALL and NHL who received infusion of 2×10^7 CAR-T cells/kg.[15,16] After one patient with CLL developed grade 4 CRS and grade 3 neurotoxicity after receiving Cy plus Flu and 2×10^7 CAR-T cells/kg, we elected to reduce the CAR-T cell dose by two dose levels to 2×10^5 CAR-T cells/kg and re-escalate in separate disease-specific cohorts of $\geq$ three patients at each dose level. No dose-limiting toxicity and no grade $>$ 2 CRS or neurotoxicity events were seen in patients with CLL who received Cy plus Flu lymphodepletion and 2×10^5 CAR-T cells/kg; therefore, the CAR-T cell dose was escalated to 2×10^6 CAR-T cells/kg. No dose-limiting toxicity events were observed in three patients with CLL treated with Cy plus Flu and 2×10^6 CAR-T cells/kg. Therefore, we selected a maximum tolerated dose of 2×10^6 CAR-T cells/kg after Cy plus Flu lymphodepletion for subsequent patients with CLL.

A total of 15 patients in the study received Cy plus Flu lymphodepletion and 2×10^6 CAR-T cells/kg. One patient developed fatal neurotoxicity. The patient became febrile on day 1, progressing to grade 3 to 4 CRS from day 4 that was refractory to tocilizumab and dexamethasone. On day 9, the patient developed cerebral edema that was refractory to siltuximab and mannitol, and died 11 days after CAR-T cell infusion. There was no history of CNS leukemia, but CSF sampling was not performed. No other patients developed grade $>$ 2 CRS, and only four patients developed grade 3 neurotoxicity.

Criteria for tocilizumab and dexamethasone administration. Tocilizumab (4-8 mg/kg IV) and dexamethasone (10 mg twice a day IV) were administered to patients who either required management in the intensive care unit or were under evaluation for intensive care. Intervention was initiated in patients with grade 2 to 3 cytokine release syndrome[20] that was not responding to intravenous fluids and/or low-dose vasopressor support and grade 2 to 3 neurotoxicity. CRS and neurotoxicity resolved in all patients treated according to these criteria, with the exception of a patient with fatal cerebral edema. Additional clinical studies will be required to determine whether early intervention guided by serum biomarker levels will be more effective in treating or preventing severe CRS and/or neurotoxicity.

Statistical Analyses

Comparisons of continuous variables between two categories were made using the exact Wilcoxon test and of categorical variables between two categories using Fisher's exact test. Univariable logistic regression was used to estimate the probability of CRS, neurotoxicity, and response according to peak CAR-T cells in blood. Multivariable logistic regression was performed to assess predictors for the occurrence of severe neurotoxicity by adjusting for baseline factors, including CAR-T cell dose, lymphodepletion, number of prior therapies, tumor area, and abnormal B-cell count, where log10 values were used to transform data as appropriate. Relationships between continuous variables were analyzed using Spearman rank correlation. For time-to-event analyses, the Kaplan-Meier method was used to estimate survival distributions, and the reverse Kaplan-Meier method was used to estimate median follow-up time; log-rank tests were used to compare between-group differences in survival curves. *P* values reported were two-sided. No adjustments were made for multiple comparisons. Analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC).

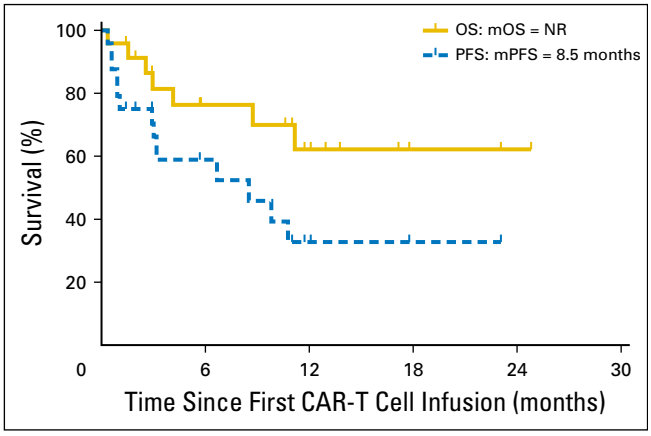

Fig A1. Progression-free survival (PFS) and overall survival (OS) for all patients with CLL. CAR-T, chimeric antigen receptor-modified T; mOS, median OS; mPFS, median PFS; NR, not reached.