SUPPLEMENTAL TABLES:

Table S1: Lymphodepletion chemotherapy regimens.

Lymphodepletion Regimen	Number of Patients
Cyclophosphamide 2 g/m^2 Day 1; Etoposide 100 mg/m^2 Days 1-3	1
Cyclophosphamide 4 g/m^2 Day 1; Etoposide 200 mg/m^2 Days 2-4	1
Cyclophosphamide 2 g/m^2 Day 1	7
Cyclophosphamide 3 g/m^2 Day 1	1
Cyclophosphamide 4 g/m^2 Day 1	1
Cyclophosphamide 30 mg/kg Day 1; Fludarabine 25 mg/m^2 Days 2-4	2
Cyclophosphamide 60 mg/kg Day 1; Fludarabine 25 mg/m^2 Days 2-4	24
Cyclophosphamide 60 mg/kg Day 1; Fludarabine 25 mg/m^2 Days 2-6	5
Cyclophosphamide 300 mg/m^2 and Fludarabine 30 mg/m^2 Days 1-3	11
Total	**53**

Table S2: Sites of extramedullary disease.

Extramedullary site	No. of patients [a]
Any extramedullary disease	18
Soft tissue	7
Bone lesions	5
Renal	2
Lymph nodes	4
Central nervous system	5

[a] some patients had > 1 extramedullary site with leukemic involvement

Table S3: Univariate analyses of factors associated with achievement of MRD-negative CR.

Pre-treatment characteristics	Odds Ratio	*P* value [a]
Age (in years)	0.99	.982
Sex (Female)	1143	.993
ECOG performance status (0-2)	1.12	.871
High risk cytogenetics (Y)	0.90	.894
Prior regimens (no.)	0.94	.742
Prior allogeneic hematopoietic cell transplant (Y)	0.40	.247
Prior blinatumomab (Y)	1.75	.621
Marrow disease burden by flow cytometry (%)	0.99	.828
Extramedullary disease (Y)	0.45	.306
CNS leukemic involvement (Y)	**0.08**	**.013**
Time from leukapheresis to lymphodepletion (days)	4.48	.412
Bridging systemic therapy (Y)	1.09	.922
Therapy-related and CAR-T cell kinetics		
Dose level ($2x10^6$ vs $2x10^5$ CAR-T cells/kg)	5.11	.142
Fludarabine containing lymphodepletion (Y)	0.50	.539
Peak CAR-T cells by qPCR (log_{10} transgene copies/μg DNA)	**7.47**	**.001**
Peak $CD8^+$ CAR-T cell count (log_{10} cells/μL)[b]	**5.55**	**.001**
Peak $CD4^+$ CAR-T cell count (log_{10} cells/μL)[b]	**6.06**	**.003**
AUC28 CD4+ CAR-T cells (log_{10} cells/μL)[b]	**20.57**	**.004**
AUC28 CD8+ CAR-T cells (log_{10} cells/μL)[b]	**9.19**	**.005**
AUC28 CAR-T cells by qPCR (log_{10} transgene copies/μg DNA)	**17.41**	**.012**
Any grade of CRS (Y)	**7.71**	**.014**
Peak CRS grade (0-4)	**2.85**	**.021**
Any grade of neurotoxicity (Y)	1.96	.129
Biomarkers		
Serum soluble TNFRp55 (fold change, pre-lymphodepletion to day 0)	**4662.49**	**.014**
Serum soluble TNFRp75 (log_{10} pg/mL, pre-lymphodepletion)	**0.17**	**.027**
Serum IL-18 (log_{10} pg/mL, pre-lymphodepletion)	**0.11**	**.034**
Serum TNF-α (log_{10} pg/mL, day 0)	**2.57**	**.044**
Serum IL-15 (log_{10} pg/mL, pre-lymphodepletion)	5.51	.061
Serum IL-15 (log_{10} pg/mL, day 0)	11.44	.070
Serum IL-22 (log_{10} pg/mL, AUC28)	4.55	.083
Serum IFN-γ (log_{10} pg/mL AUC28)	236.65	.095
Serum soluble TIM-3 (log_{10} pg/mL AUC28)	2.13	.092
Serum IL-22 (log_{10} pg/mL, day 0)	21.29	.096
Serum MCP-1 (log_{10} pg/mL, day 0)	5.37	.100

[a] *P* value from logistic regression without multiplicity adjustment
[b] absolute counts by flow cytometry
ECOG, Eastern Cooperative Oncology Group; CNS, central nervous system; CRS, cytokine release syndrome; AUC28, calculated area under the curve from day 0 to 28.

Table S4: Multivariable model applied to patients who achieved MRD-negative CR and were evaluable by high-throughput sequencing (HTS).

Variable (in patients who achieved MRD-negative CR who were evaluable by HTS, n=28)	Multivariable HR (95% CI)	*P* value
LDH pre-lymphodepletion (per 100 U/L increment)	1.45 (1.12-1.88)	.005
Platelets pre-lymphodepletion (per 50,000/μL increment)	0.47 (0.27-0.80)	.005
Fludarabine added to lymphodepletion (Y)	0.27 (0.09-0.82)	.021
HTS-positive on re-staging (Y)	5.56 (1.75-17.67)	.004

HR, hazard ratio

Table S5: Characteristics of patients in MRD-negative CR after CAR-T cell therapy who did or did not proceed to allogeneic hematopoietic cell transplantation (HCT).

Characteristics prior to CAR-T cell therapy	Allogeneic HCT in MRD-negative CR	No HCT in MRD-negative CR	All patients
Patients Evaluable, n	18	27	45
Age, years			
Median [IQR]	35 [26, 48]	44 [32, 58]	39.0 [28, 53]
Range	22, 73	20, 76	20, 76
Sex, n (%)			
Female	7 (39)	16 (59)	23 (51)
Male	11 (61)	11 (41)	22 (49)
ECOG performance status, n (%)			
0	11 (61)	11 (41)	22 (49)
1	6 (33)	16 (59)	22 (49)
2	1 (6)	0 (0)	1 (2)
Philadelphia chromosome-positive, n (%)			
Yes	4 (22)	6 (22)	10 (22)
No	14 (78)	21 (78)	35 (78)
High-risk cytogenetics, n (%)			
Yes	7 (39)	20 (74)	27 (60)
No	11 (61)	7 (26)	18 (40)
No. of prior regimens[a]			
Median [IQR]	2.5 [1.5, 4]	4 [3, 5]	3 [3, 4]
Range	1, 6	2, 11	1, 11
Prior allogeneic HCT, n (%)			
Yes	2 (11)	16 (59)	18 (40)
No	16 (89)	11 (41)	27 (60)
Prior blinatumomab therapy, n (%)			
Yes	4 (22)	5 (18)	9 (20)
No	14 (78)	22 (82)	36 (80)
Marrow disease burden by flow cytometry, %			
Median [IQR]	11.3 [0.2, 27.2]	46.0 [3.9, 84.8]	28.0 [0.6, 74.7]
Range	0.0, 80.0	0.0, 97.6	0.0, 97.6
Extramedullary disease, n (%)			
Yes	1 (94)	13 (48)	14 (31)
No	17 (6)	14 (52)	31 (69)
CNS leukemic involvement, n (%)			
Yes	1 (6)	1 (4)	2 (4)
No	17 (94)	26 (96)	43 (96)
Pre-lymphodepletion LDH concentration (U/L)			
Median [IQR]	177 [133, 218]	214 [176, 267]	201 [152, 245]
Range	116, 334	107, 1027	107, 1027
Pre-lymphodepletion platelet count (1000/μL)			
Median [IQR]	171 [116, 218]	58 [38, 146]	114 [50, 200]
Range	48, 339	3, 217	3, 339
Lymphodepletion, n (%)			
Cyclophosphamide-based with fludarabine	14 (78)	21 (78)	35 (78)
Cyclophosphamide-based without fludarabine	4 (22)	6 (22)	10 (22)
CAR-T cell dose, n (%)			
2 x 10^5 CAR-T cells/kg	8 (44)	18 (67)	26 (58)
2 x 10^6 CAR-T cells/kg	10 (56)	9 (33)	19 (42)

Multivariable model risk group, n (%)			
High risk	8 (44)	22 (82)	30 (67)
Low risk	10 (56)	5 (18)	15 (33)

IQR, interquartile range

SUPPLEMENTAL FIGURES:

Figure S1

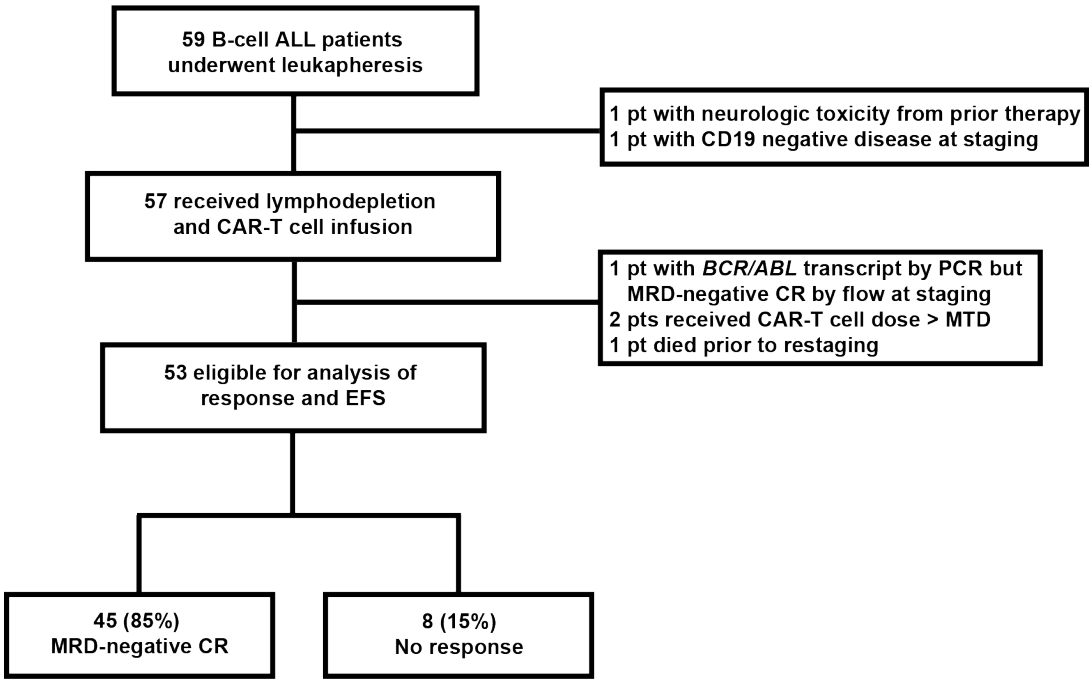

Figure S1: Flow chart of patient enrollment and eligibility for response and event-free survival (EFS) analysis. MTD, maximum tolerated dose; MRD-negative CR, minimal residual disease-negative complete response.

Figure S2

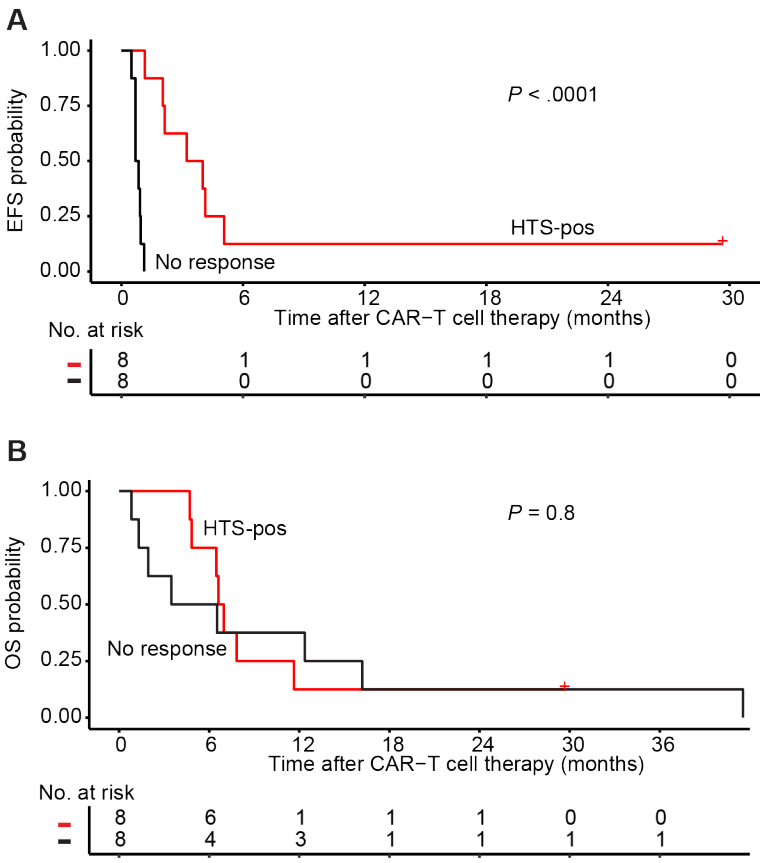

Figure S2: Event-free (EFS) and overall survival (OS) in B-cell ALL patients after CD19 CAR-T cell therapy. (A-B) Kaplan-Meier analyses demonstrating EFS and OS in patients who achieve MRD-negative CR by high-resolution flow cytometry but had a persistence clone on high-throughput sequencing (HTS-pos, red line, n=8) compared to those with no response (black line, n=8).

Figure S3

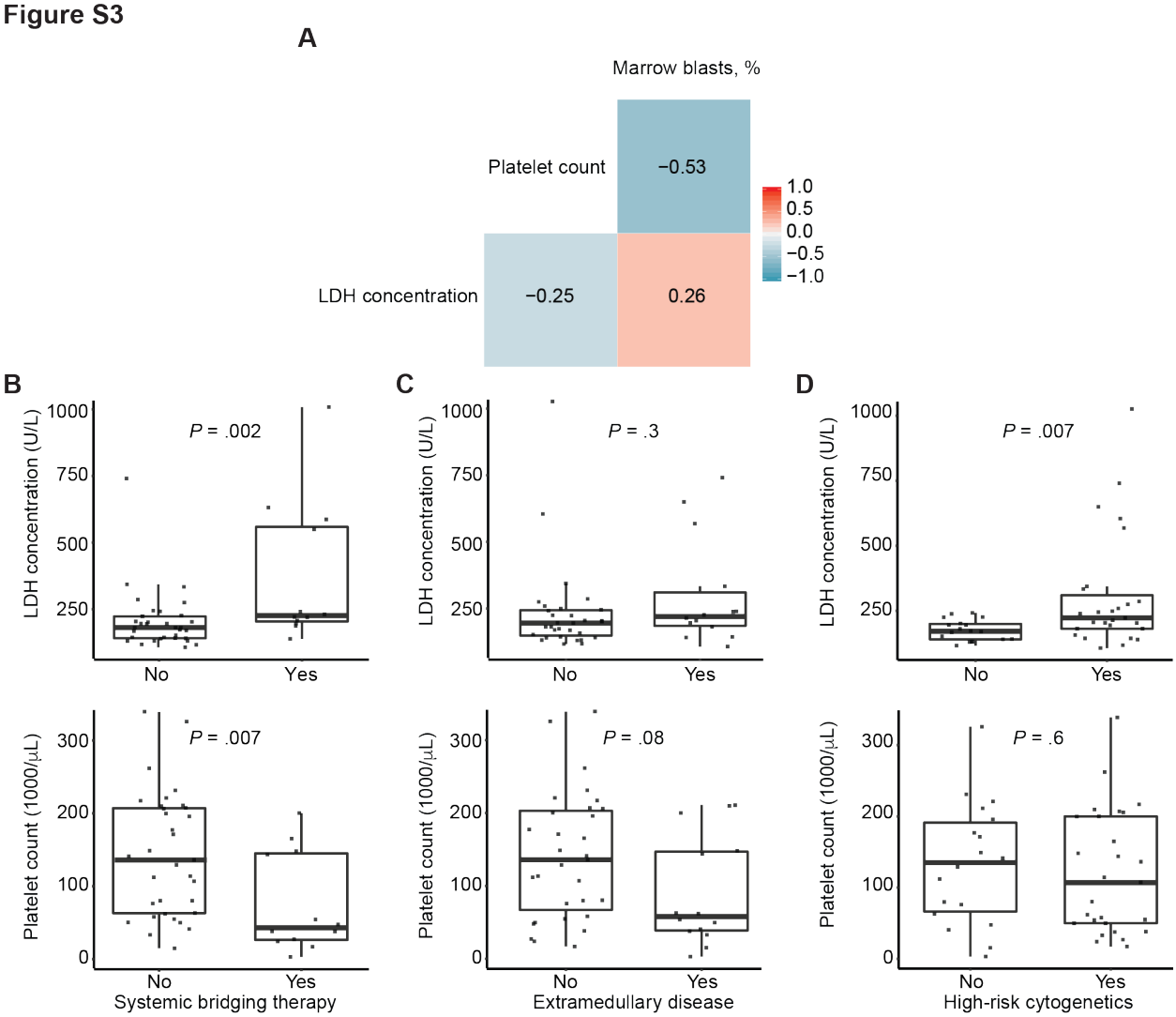

Figure S3: Correlations between variables significant in univariate analysis of event-free survival. (A) Correlations (R value) between pre-lymphodepletion LDH concentration, platelet count, and marrow blasts in the bone marrow by flow cytometry. **(B-D)** Boxplots demonstrating the associations of pre-lymphodepletion LDH concentration and platelet count with **(B)** systemic bridging therapy between leukapheresis and lymphodepletion, **(C)** presence of extramedullary disease, and **(D)** high-risk cytogenetics.

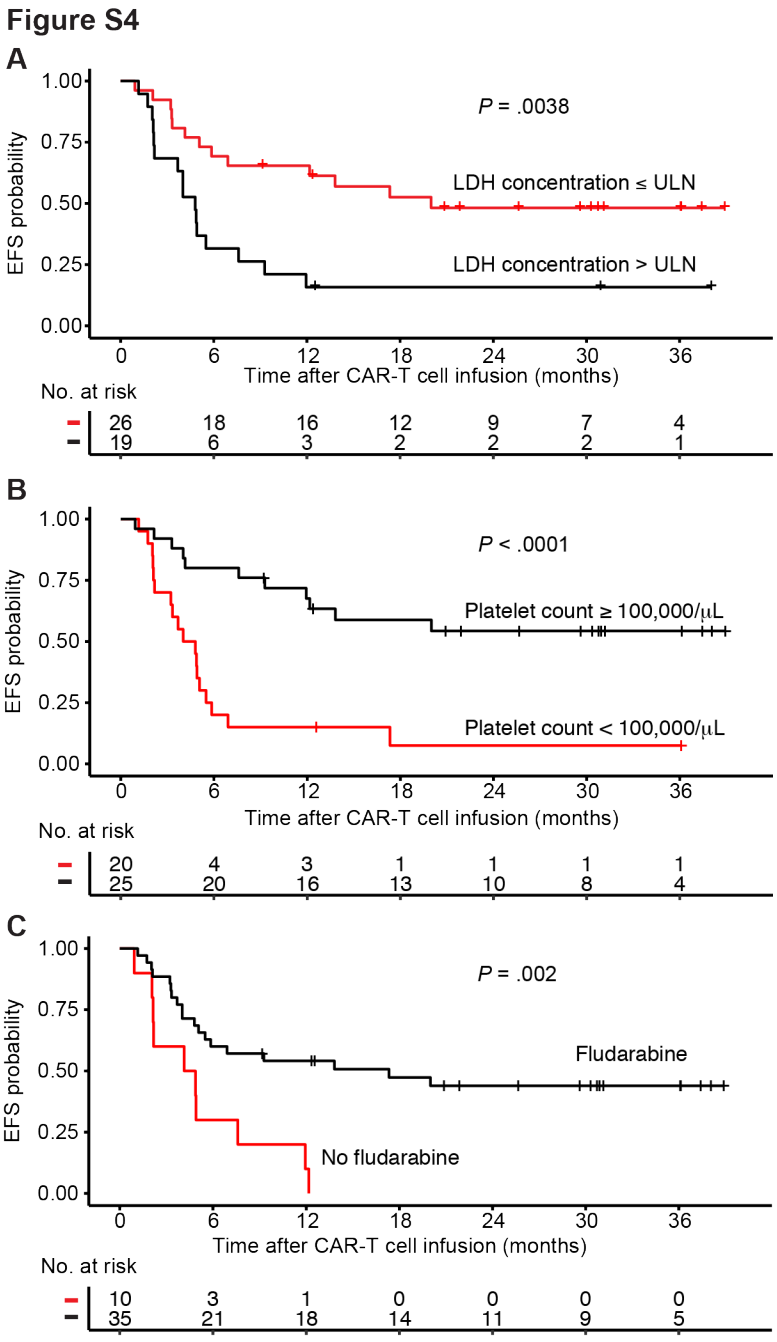

Figure S4: Effect of factors associated with better event-free survival (EFS) in the multivariable model. (A-C) Kaplan-Meier analysis demonstrating better EFS in patients with pre-lymphodepletion LDH ≤ upper limit of normal (ULN, *P*=.0038), platelet count ≥100,000/μL (*P*<.0001), or who received fludarabine containing lymphodepletion (*P*=.002, Log-rank test).

Figure S5

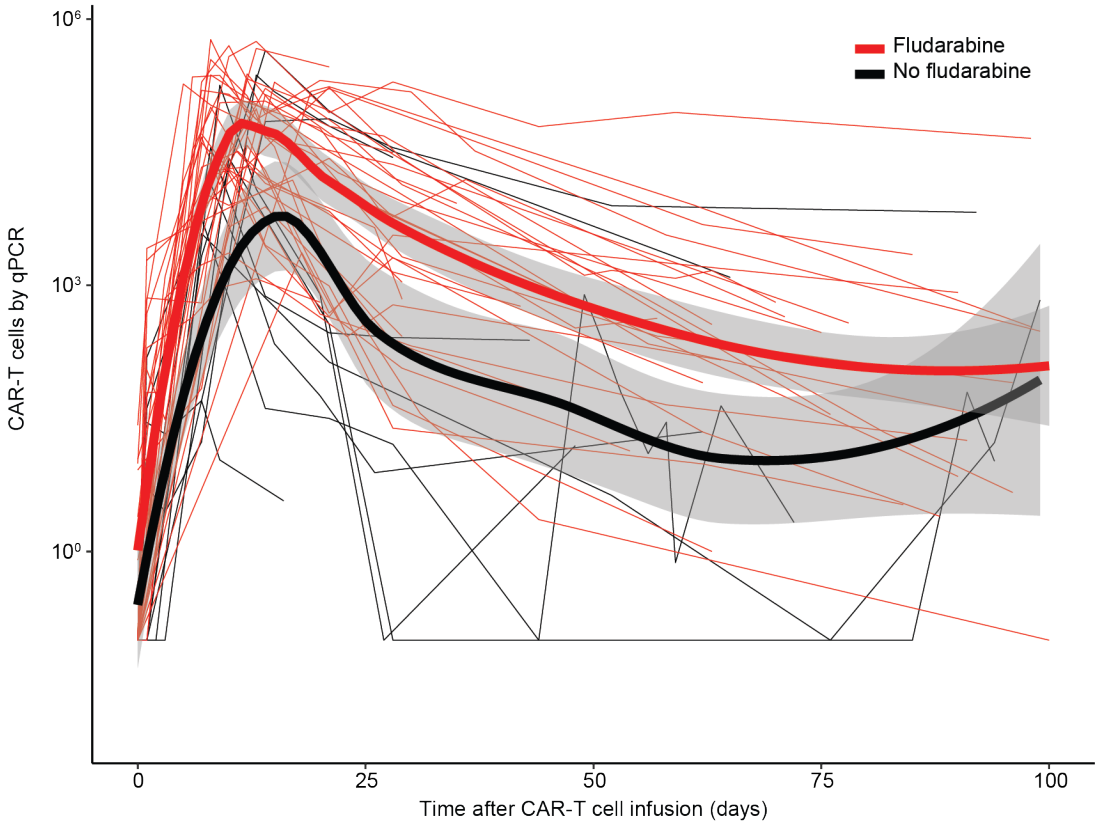

Figure \ CAR-T cell kinetics according to lymphodepletion regimen. Patients who received cyclophosphamide-based lymphodepletion with fludarabine (red lines) had higher CAR-T cell AUC28-90 compared to patients who received cyclophosphamide-based lymphodepletion without fludarabine (black lines, *P*=.03, Welch two sample t-test). CAR-T cell levels in the blood were assessed by quantitative polymerase chain reaction (qPCR) to detect FlapEF1α transgene copies/μg DNA. Each thin line represents a single patient; the bold lines represent the averaged data using LOESS (Local Polynomial Regression) curve fitting approximation with the standard error shown in grey.