Supplementary Files

METHODS

Additional supportive care and monitoring details

Patients who had undergone prior allogeneic hematopoietic cell transplant (HCT) continued their prescribed prophylactic antimicrobial regimen according to standard guidelines after CD19-targeted chimeric antigen receptor-modified T (CAR-T) cell therapy. This consists of acyclovir 800 mg or valacyclovir 500 mg twice a day for herpes simplex or varicella zoster virus seropositive individuals for at least one year after HCT; levofloxacin 750 mg daily during neutropenia; fluconazole 400 mg daily through day 75 after HCT; and trimethoprim 160 mg/sulfamethoxazole 800 mg twice a day for two days a week starting after neutrophil recovery until at least 6 months after HCT.

Patients with temperature ≥38.5°C and neutropenia were typically treated with intravenous ceftazidime 2 grams every 8 hours with or without intravenous vancomycin 15 mg/kg every 12 hours. Blood cultures were performed when patients had temperatures ≥38°C. Patients with upper respiratory symptoms had a nasal wash performed, and patients with lower respiratory tract disease were evaluated by bronchoalveolar lavage (BAL) as clinically indicated. Respiratory viral PCR or direct fluorescent antibody (DFA) staining and culture were performed on all nasal wash, BAL, lung biopsy, and autopsy specimens. All BAL, biopsy, and autopsy specimens were also submitted for routine bacterial, fungal, and acid-fast bacilli cultures; DFA staining and culture were performed for *Legionella* species. The Bio-Rad Platelia Assay was used to determine the galactomannan index (GMI) to test for *Aspergillus* in all BAL samples. Serum was also tested if there was clinical suspicion for *Aspergillus* infection. A GMI of ≥0.5 with a confirmatory index processed separately on the same sample was considered positive for both BAL fluid and serum samples.

Preferred lymphodepletion chemotherapy and CAR-T cell dosing regimens

Our previous studies of CD19 CAR-T cell therapy for patients with acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL) or chronic lymphocytic leukemia (CLL) established that the risk of severe cytokine release syndrome (CRS) was associated with higher infused CAR-T cell dose and (in ALL patients) the percentage of bone marrow blasts before lymphodepletion chemotherapy. We also previously demonstrated that CAR-T cell persistence could be limited in a subset of patients by an immune response directed against the CAR transgene and showed that addition of fludarabine to cyclophosphamide-based lymphodepletion mitigated anti-CAR transgene immune responses and enhanced clinical outcomes. We therefore established a preferred treatment regimen, in which lymphodepletion includes both cyclophosphamide and fludarabine, and the CAR-T cell dose is determined by the disease type and tumor burden ($2x10^5$ CAR-T cells/kg for B-ALL with >5% marrow blasts; $\leq 2x10^6$ CAR-T cells/kg for B-ALL with ≤5% marrow blasts and for patients with NHL or CLL).[1–3] The optimal cyclophosphamide and fludarabine-containing lymphodepletion regimen has not been determined.

RESULTS

Incidence of bacterial, viral, and fungal infections between 29 and 90 days after CAR-T cell infusion

The most common characteristics among patients with late infections (n=23 infections) were persistent disease (48%) and neutropenia (22%). No late infections were caused by encapsulated bacterial pathogens. All 4 bacteremias occurred in patients with an ANC <500 cells/mm^3, persistent disease, and indwelling central venous catheters. Among 5 non-bacteremia infection events categorized as severe or life-threatening, 1 occurred in the setting of persistent disease and neutropenia (*Aspergillus fumigatus* invasive sinusitis), 1 occurred in

the setting of relapsed disease (multi-drug resistant *Escherichia coli* urinary tract infection), 1 occurred in a patient not taking prescribed trimethoprim/sulfamethoxazole prophylaxis (*Pneunomocystis jiroveci* pneumonia), and 2 occurred in a patient without clear risk factors at the time of diagnosis. Among the additional 14 late infections, 5 occurred in the context of persistent disease, 1 occurred in the setting of an indwelling Foley catheter, and 8 had no clear risk factors. We were not able to identify a relationship between the incidence of late infections and hypogammaglobulinemia in part due to intravenous immunoglobulin replacement therapy.

References

1. Turtle CJ, Hanafi L, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. *Sci. Transl. Med.* 2016;8(355):355ra116.

2. Turtle CJ, Hanafi L-A, Berger C, et al. CD19 CAR–T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J. Clin. Invest.* 2016;126(6):2123–2138.

3. Turtle CJ, Hay KA, Hanafi L-A, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor–Modified T Cells After Failure of Ibrutinib. *J. Clin. Oncol.* 2017;JCO.2017.72.851.

Table S1. Infection densities for any infection and for infection categories after CAR-T cell infusion

Infection type	ALL (n = 47)	CLL (n = 24)	NHL (n = 62)	Total (N = 133)
Day 0-28				
Any	1.66	1.22	0.83	1.19
Bacterial	1.03	0.61	0.41	0.66
Bacteremia	0.63	0.31	0.12	0.33
Viral	0.47	0.46	0.24	0.36
Respiratory virus	0.47	0.15	0.18	0.28
Fungal	0.16	0.15	0.18	0.17
Day 29-90				
Any	0.76	1.27	0.40	0.67
Bacterial	0.28	0.63	0.07	0.23
Bacteremia	0.14	0.42	0.00	0.12
Viral	0.41	0.42	0.33	0.38
Respiratory virus	0.35	0.00	0.33	0.29
Fungal	0.07	0.21	0.00	0.06

Table S2. IgG concentration in serum after CAR-T cell infusion

Days post-CAR-T cell infusion	Median, mg/dL	Range, mg/dL	<400 mg/dL, n (%)
15-30 (n=65)	449	94-894	23 (35)
31-60 (n=33)	491	178-776	9 (27)
61-90 (n=37)	420	155-771	17 (46)

n, number of patients with available data during each time period.

Table S3. Incidence of specific infections between day 29 and day 90 after CAR-T cell infusion

Type of Infection	ALL (n = 43)		CLL (n = 22)		NHL (n = 54)		Total (N = 119)[a]	
	Events	No. patients	Events	No. patients	Events	No. patients	Events	No. patients
Any infection	11	9 (20.9)	6	4 (18.2)	6	4 (7.4)	23	17 (14.3)
Bacterial infections	4	4 (9.3)	3	2 (9.1)	1	1 (1.9)	8	7 (5.9)
Bacteremia[b]	2	2 (4.7)	2	1 (4.5)	0	0 (0.0)	4	3 (2.5)
Bacterial site infections[c]	2	2 (4.7)	1	1 (4.5)	1	1 (1.9)	4	4 (3.4)
Viral infections	6	5[d] (11.6)	2	2 (9.1)	5	4 (7.4)	13	11 (9.2)
Respiratory virus[e]	5	5 (11.6)	0	0 (0.0)	5	4 (7.4)	10	9 (7.6)
Other virus[f]	1	1 (2.3)	2	2 (8.7)	0	0 (0.0)	3	3 (2.5)
Fungal infections[g]	1	1 (2.3)	1	1 (4.5)	0	0 (0.0)	2	2 (1.7)
Non-mold[h]	1	1 (2.3)	0	0 (0.0)	0	0 (0.0)	1	1 (0.8)
Mold[i]	0	0 (0.0)	1	1 (4.3)	0	0 (0.0)	1	1 (0.8)

Data are presented as No. (%). 'Events' can include multiple entries per patient; the 'No. patients' columns include patients only once per category.

[a]The total number of patients decreased by n=14 due to censoring at time of new anti-tumor therapy, death, or last clinical contact at our center prior to day 29.

[b]Gram positive, n=2 (coagulase-negative *Staphylococcus aureus*, n=1; *Enterococcus faecium*, n=1); gram negative, n=2 (*Stenotrophomonas maltophilia*, n=1; *Fusobacterium naviforme*, n=1).

[c]Lower respiratory tract, n=2; urinary tract, n=2.

[d]One patient had an infection in both the 'respiratory virus and 'other' virus categories.

[e]Upper respiratory tract infection, n=9 (rhinovirus, n=4; parainfluenza virus 3, n=2; influenza A, n=1; metapneumovirus, n=1; coronavirus, n=1); lower respiratory tract infection, n=1 (influenza B).

[f]CMV pneumonia, n=1; CMV viremia, n=1; BK virus viruria with cystitis, n=1.

[g]Lower respiratory tract disease in 1/2 events.

[h]*Pneumocystis jiroveci* pneumonia, n=1.

[i]*Aspergillus fumigatus* invasive sinusitis, n=1.

Table S4. Relationship between CRS grade, CAR T-cell dose level, and neurotoxicity

	CRS grade				
	0	1-3	4-5	Total	*P* value[a]
Number of patients	40	83	10	133	
CAR-T cell dose level					0.002
$2x10^5$ cells/kg	10 (25.0)	25 (30.1)	0 (0.0)	35 (26.3)	
$2x10^6$ cells/kg	27 (67.5)	54 (65.1)	5 (50.0)	86 (64.7)	
$2x10^7$ cells/kg	3 (7.5)	4 (4.8)	5 (50.0)	12 (9.0)	
Neurotoxicity					<0.001
Grade 0	35 (87.5)	45 (54.2)	0 (0.0)	80 (60.2)	
Grade 1-2	5 (12.5)	20 (24.1)	0 (0.0)	25 (18.8)	
Grade 3-5	0 (0.0)	18 (21.7)	10 (100.0)	28 (21.1)	

Data are presented as No. (%).
[a]Two-sided p-values were calculated using Fisher's Exact test.

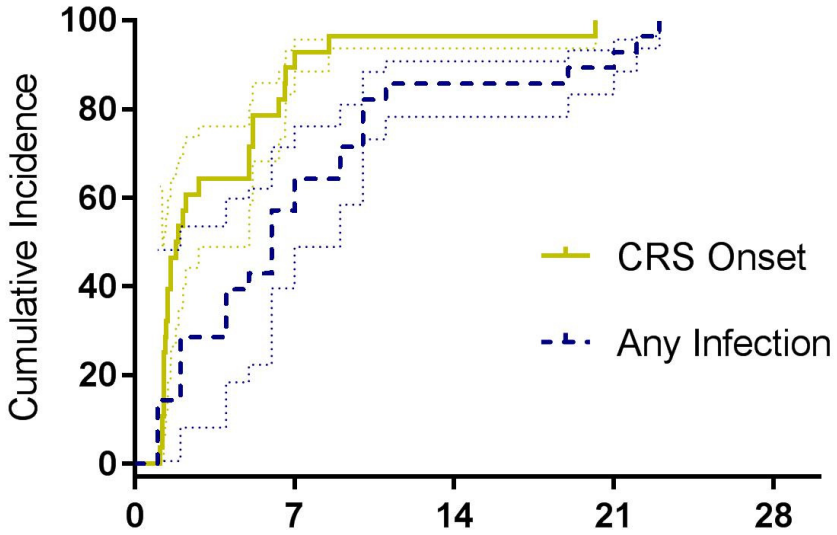

Figure S1. Cumulative incidence curves comparing time-to-cytokine release syndrome (CRS) onset and time-to-first infection.

Cumulative incidence curves of the time-to-CRS onset and the time-to-first infection are shown among 28 patients who developed both CRS and infection after CAR-T cell infusion. The median times to the onsets of CRS and the first infection were 1.9 and 6 days, respectively (p=0.002, Wilcoxon signed-rank test). Dotted lines represent 95% confidence intervals.

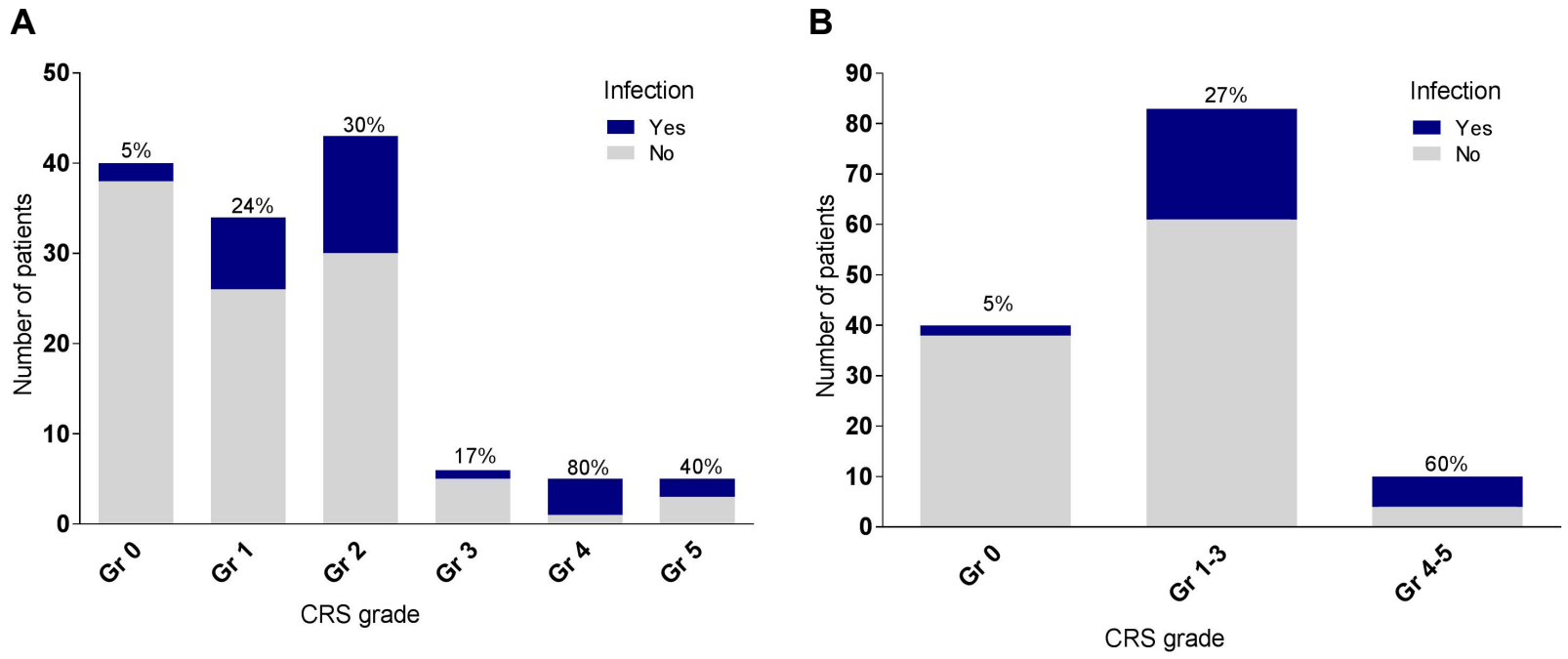

Figure S2. Proportion of patients developing any infection stratified by cytokine release syndrome severity
A-B) The proportion of patients developing any infection after CAR-T cell infusion stratified by each cytokine release syndrome (CRS) grade (A) and severity category (B). Gr, grade.

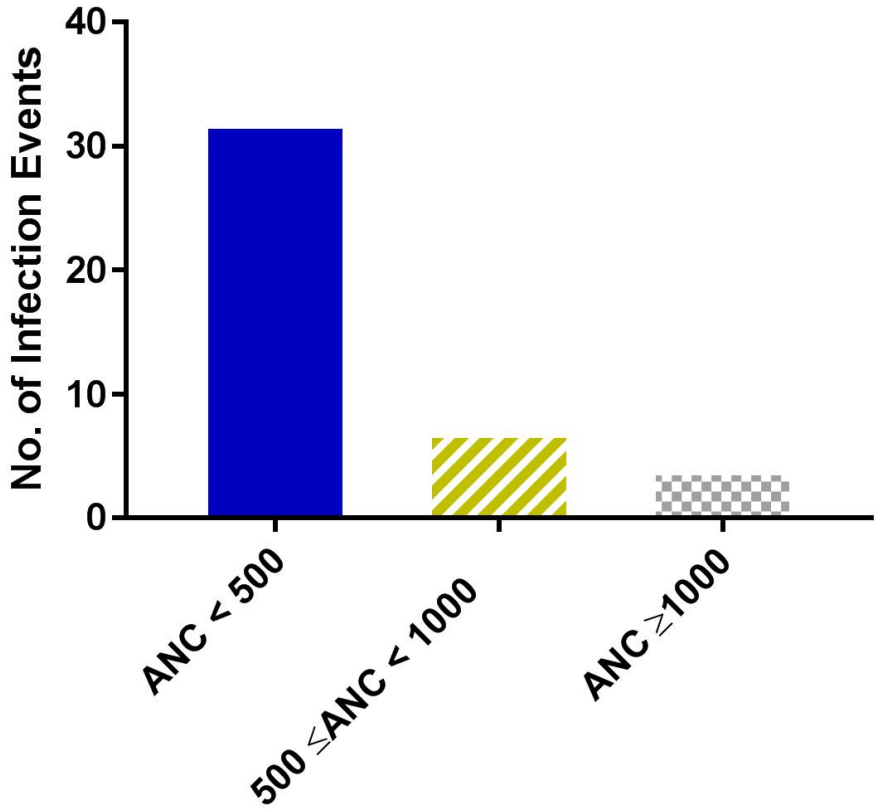

Figure S3. Infection occurrence stratified by absolute neutrophil count
This histogram demonstrates the number of infection events occurring during periods of neutropenia within the first 28 days after CAR-T cell infusion. Thirty-two of 43 (74%) infections occurred in patients with an ANC <500 cells/mm^3, 7 (16%) infections occurred in patients with an ANC ≥500 but <1,000 cells/mm^3, and 4 (9%) infections occurred in patients with an ANC ≥1,000 cells/mm^3.