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Late Events after Treatment with CD19-Targeted Chimeric Antigen Receptor Modified T Cells

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CD19-targeted chimeric antigen receptor-modified T cell (CAR-T cell) therapy has shown excellent antitumor activity in patients with relapsed/refractory B cell malignancies, with very encouraging response rates and outcomes. However, the late effects following this therapy remain unknown. Here we report late adverse events—defined as starting or persisting beyond 90 days after CAR-T cell infusion—in patients who survived at least 1 year after therapy. The median duration of follow-up was 28.1 months (range, 12.5 to 62.6 months). At last follow-up, 73% of patients were still alive and 24% were in ongoing complete remission (CR). The most common late adverse event was hypogammaglobulinemia (IgG <400 mg/dL or i.v immunoglobulinm (IVIG) replacement, observed in 67% of the patients with available data. Infection density was .55 infection/100 days at risk (2.08 per patient-year). The majority (80%) of the infections were treated in the outpatient setting, and 5% necessitated admission to the intensive care unit (ICU). Subsequent malignancies occurred in 15% of patients, including 5% with myelodysplastic syndrome (MDS). Among patients with ongoing CR and with no MDS, 16% experienced prolonged cytopenia requiring transfusions or growth factor support. Graft-versus-host disease occurred in 3 of 15 patients (20%) who had undergone previous allogeneic hematopoietic cell transplantation. Most of the late events observed in this cohort were not severe, and many could be related to previous or subsequent therapies, suggesting a safe long-term profile of CD19-targeted CAR-T cell immunotherapy.

INTRODUCTION

CD19-targeted chimeric antigen receptor-modified T cell (CAR-T cell) therapy has shown excellent antitumor activity in patients with relapsed/refractory acute lymphoblastic leukemia (ALL) [1] and non-Hodgkin lymphoma (NHL) [2,3], which has led to the approval of tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta) by the US Food and Drug Administration (FDA).

At the Fred Hutchinson Cancer Research Center (FHCRC), a phase I/II clinical trial using CD19 CAR-T cells demonstrated high response rates in patients with relapsed/refractory ALL, NHL, and chronic lymphocytic leukemia (CLL). Patients were treated with lymphodepletion chemotherapy, followed by infusion of autologous T cells modified to express a second-generation CD19-targeting chimeric antigen receptor (CAR) incorporating a single-chain variable fragment (scFv) derived from the murine IgG1 anti-CD19 monoclonal antibody, FMC63, and a 4-1BB costimulatory domain [4,5]. The CD19 CAR-T cells were manufactured from defined T cell subsets and administered at a 1:1 $CD4^+$:$CD8^+$CAR-T cell ratio. Optimal results were achieved with cyclophosphamide and fludarabine (Cy/Flu) lymphodepletion before CAR-T cell infusion [6]. The trial evaluated 3 dose levels of 2×10^5, 2×10^6, and 2×10^7 CAR-T cells/kg, as described previously [7]. Across all dose levels, 85% of the patients with ALL achieved minimal residual disease (MRD)-negative complete remission (CR) as determined by high-resolution flow cytometry [8]. The overall response rate was 51% with 40% CR in the patients with NHL [9] and 74% with 21% CR in patients with CLL [10].

CD19 CAR-T cells can cause unique early toxicities, such as cytokine release syndrome (CRS) [11,12] and acute neurotoxicity [13]. However, potential long-term adverse events remain unknown [14-17]. The objective of the present study was to describe late events after treatment with CD19 CAR-T cells in 1-year survivors who either achieved or did not achieve CR after treatment.

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Table 1
Definitions of Late Events and Patient Population Evaluated for Each Event

Event	Definition	Censoring Criteria		Denominator
		Subsequent Systemic Therapy for Underlying Disease	Subsequent Transplantation	
Late significant cytopenias	Cytopenias requiring RBC or platelet transfusion or growth factor support Occurring and/or persisting beyond 90 days after first CAR-T cell infusion	Yes	Yes	N = 19; patients with ongoing CR without diagnosis of MDS
Late hypogammaglobulinemia	IgG levels <400 mg/dL and/or intravenous immunoglobulin (IVIG) replacement Occurring and/or persisting beyond 90 days after first CAR-T cell infusion	Yes	Yes	N = 42; patients with available IgG data >90 days after CAR-T cell infusion
Late infections	Any infection documented in medical records (with or without microbiologic or radiologic evidence) Occurring and/or persisting beyond 90 days after first CAR-T cell infusion We considered 2 different infection events if (1) occurred at different time points, (2) different pathogen groups (viral, bacterial, fungal, parasite) were identified (even if documented at the same time point), or (3) different nonadjacent organs were affected (even if documented at the same time point).	Yes	Yes	N = 54; patients with available infection data >90 days after CAR-T cell infusion
Subsequent malignancies	All malignancies with pathological confirmation diagnosed after CAR-T cell administration	No	Yes	N = 86; entire cohort
Immune-related events	Any possible immune-related condition (except infections), even without a formal diagnostic confirmation Occurring and/or persisting beyond 90 days after first CAR-T cell infusion	No	Yes	N = 86; entire cohort
Psychiatric and neurologic disorders	Occurring and/or persisting beyond 90 days after first CAR-T cell infusion	No	No	N = 86; entire cohort
GVHD	GVHD requiring systemic therapy	No	Yes	N = 15; patients with history of allogeneic HCT before first CAR-T cell infusion

METHODS

The study cohort comprised 86 patients with relapsed/refractory ALL, NHL, or CLL treated with CD19 CAR-T cells on a phase I/II clinical trial (ClinicalTrials.gov identifier NCT01865617) between July 2013 and February 2017 who survived at least 12 months after treatment. The study was approved by the FHCRC Institutional Review Board, and all patients provided informed consent for treatment and for long-term follow-up.

Here we report late events in the entire study cohort, as well as in patients with ongoing CR, defined as CR achieved at first evaluation (approximately 4 weeks after CAR-T cell administration) and continued until last follow-up with no additional therapy. CR was defined according to the diagnosis: NHL, based on the Lugano criteria [4]; CLL, based on International Workshop on Chronic Lymphocytic Leukemia (IWCLL; 2008) criteria [10]; and ALL, based on disease detection in bone marrow sample by morphology, flow cytometry, and molecular testing. For the latter, CR was defined as <5% blasts by morphology, and minimal residual disease (MRD) was defined as <5% blasts by morphology but with evidence of disease by flow cytometry or molecular testing. Patients who achieved CR after CAR-T cell administration and received consolidation allogeneic hematopoietic cell transplantation (HCT) were not included in the ongoing CR group.

We retrospectively reviewed patients' medical records for evaluation of late events after CAR-T cell therapy. Late events were defined as events that presented and/or persisted beyond 90 days after CAR-T cell administration. The following event categories were identified: (1) significant cytopenias, (2) hypogammaglobulinemia, (3) infections, (4) subsequent malignancies, (5) immune-related events, (6) graft-versus-host disease (GVHD) in previous allogeneic HCT recipients, and (7) neurologic and psychiatric events.

Owing to the different nature of the events, there is variability in the patient population that was included for evaluation of each event. Table 1 describes in detail the late events and the specific patient population evaluated for each event. In brief, significant cytopenias were defined as cytopenias that required RBC or platelet transfusions or growth factor support. For analysis of cytopenias, we included only patients with ongoing CR who were not diagnosed with myelodysplastic syndrome (MDS) after CAR-T cell therapy. Late hypogammaglobulinemia was defined as IgG level <400 mg/dL and/or documentation in the medical record of at least one-time i.v. immunoglobulin (IVIG) replacement beyond day 90 after CAR-T cell infusion. Evaluation for hypogammaglobulinemia was censored at the time of receipt of any systemic therapy for the underlying disease. For analysis of late infections, we considered infection events reported in the medical records between day 90 after CAR-T cell infusion and death or initiation of any systemic therapy for treatment of the underlying disease. All newly diagnosed malignancies after CD19 CAR-T cell therapy were reported. All possible immune-related events, neurologic events, and psychiatric conditions necessitating medical intervention (ie, referral to specialist or pharmacologic therapy) that presented or persisted beyond day 90 after CAR-T cell administration are reported. All late events observed are reported without taking the level of attribution to CAR-T cell therapy into account.

Statistical Methods

This is a retrospective observational study. To evaluate the association between CR status and late events. *P* values were calculated using Wilcoxon rank-sum test for continuous characteristics and Fisher's exact test for categorical characteristics. Infection density was reported as the mean number of infections per 100 days at risk and was calculated as the total number of infection events in the entire cohort divided by the number of days at risk and multiplied by 100. Owing to our limited sample size, multivariate analyses were not performed. Reported *P* values are 2-sided without multiplicity adjustment.

RESULTS

Patient Characteristics and Outcomes

Of 163 patients treated on NCT01865617 through the end of February 2017, 86 patients (53%) survived for at least 1 year

Table 2
Patient Characteristics

Characteristic	Entire cohort (N = 86)	NHL* (N = 43)	CLL (N = 17)	ALL (N = 26)	Ongoing CR (N = 21)	Non-ongoing CR (N = 65)	*P* Value†
Sex, n (%)							
Male	63 (73)	32 (74)	12 (71)	19 (73)	12 (57)	51 (78)	.09
Female	23 (27)	11 (26)	5 (29)	7 (27)	9 (43)	14 (22)	
Age, yr, median (range)	57 (23-75)	59 (34-70)	63 (41-73)	40 (23-75)	58 (22-73)	56 (23-74)	.92
Previous lines of therapy, median (range)	4 (1-8)	4 (1-7)	5 (2-8)	2 (1-7)	4 (2-8)	3 (1-7)	.51
Previous HCT, n (%)	35 (41)	26 (60)	3 (18)	6 (23)	13 (62)	22 (34)	.04
Autologous HCT before CAR-T cells	24 (28)	24 (56)	0	0	8 (38)	16 (25)	.27
Allogeneic HCT before CAR-T cells	15 (17)	6 (14)	3 (18)	6 (23)‡	8 (38)	7 (11)	.008
Time from previous allogeneic HCT to first CAR-T cell infusion, mo, median (range)	23.5 (3.2-143.6)	32.1 (6.7-55.2)	88.4 (26.5-143.6)	16.8 (3.2-76.5)	18 (3-144)	24 (16-88)	.46
Lymphodepletion chemotherapy, n (%)							
Cy/Flu§	74 (86)	35 (81)	16 (94)	23 (88)	20 (95)	54 (83)	.28
Other§	12 (14)	8 (19)	1 (6)	3 (12)	1 (5)	11 (17)	
Dose of CAR T-cells/kg, n (%)							
Level 1 (2×10^5)	20 (23%)	2 (4)	4 (23)	14 (54)	1 (5)	19 (29)	.04
Level 2 (2×10^6)	60 (70)	36 (84)	13 (77)	11 (42)	18 (85)	42 (65)	
Level 3 (2×10^7)	6 (7)	5 (12)	0	1 (4)	2 (10)	4 (6)	

* NHL: diffuse large B-cell lymphoma (n = 21); follicular lymphoma (n = 13); mantle cell lymphoma (n = 6); marginal zone lymphoma (n = 2), Burkitt lymphoma (n = 1).
† Ongoing CR versus non-ongoing CR. *P* values calculated using the Wilcoxon rank-sum test for continuous characteristics and Fisher's exact test for categorical characteristics.
‡ One patient underwent 2 allogeneic HCTs before CAR-T cell infusion.
§ Patients received one of several lymphodepleting regimens: cyclophosphamide (Cy), 2 to 4 g/m^2 i.v. on day 1; Cy, 2 to 4 g/m^2 i.v. on day 1 plus etoposide (E), 100 to 200 mg/m^2/day i.v. on days 1 to 3 (Cy/E); Cy, 60 mg/kg i.v. on day 1 plus fludarabine (Flu) 25 mg/m^2/day i.v. on either days 2 to 4 or days 2 to 6, Cy 300 mg/m^2/day for 3 days plus Flu 30 mg/m^2/day for 3 days, or Cy 500 mg/m^2/day for 3 days plus Flu 30 mg/m^2/day for 3 days (Cy/Flu); bendamustine 90 mg/m^2/day (1 patient).

following CD19 CAR-T cell infusion as of March 2018 and were included in this study, including 43 with NHL, 26 with ALL, and 17 with CLL. The median age of the study cohort was 57 years (range, 23 to 75 years), and 73% were males. The median number of previous lines of therapy was 4 (range, 1 to 8). Twenty-four patients with NHL had undergone previous autologous HCT, accounting for 56% of the patients with NHL and 28% of the entire cohort. Fifteen patients (17%) had undergone previous allogeneic HCT, including 23% of those with ALL, 18% of those with CLL, and 14% of those with NHL. Characteristics of the cohort are summarized in Table 2.

Patients were followed from first CAR-T cell infusion until last contact or death. The median duration of follow-up was 28.1 months (range, 12.5 to 62.6 months). Fifteen patients (17%) received a second CAR-T cell infusion, and 2 patients (2%) received a third infusion. Thirty-five patients (41%) underwent allogeneic HCT after CAR-T cell administration, including 12 patients (14%) who underwent allogeneic HCT as consolidation therapy after achieving CR with CAR-T cell administration (11 patients with ALL and MRD-negative CR and 1 patient with NHL).

Seventeen patients (20%) died of disease progression, at a median of 19 months (range, 12.5 to 39 months) after first CAR-T cell infusion. Six patients died of non-relapse-related causes (7%), including 5 of complications after allogeneic HCT and 1 of complications of multiple myeloma (MM). Sixty-three patients (73%) were alive at last follow-up. Twenty-one patients (24%) were in ongoing CR (14 with NHL, 4 with CLL, and 3 with ALL) at a median follow-up of 34 months (range, 18 to 44 months).

Late Events after CAR-T Cell Therapy

Significant prolonged cytopenias

Three of 19 patients (16%) with ongoing CR and no diagnosis of MDS experienced prolonged significant cytopenias that lasted for 15.2 to 21.7 months after CAR-T cell infusion. Supplementary Table 1 describes these cytopenia events in detail.

Hypogammaglobulinemia

Thirty-four patients (40%) had hypogammaglobulinemia (documented IgG level < 400 mg/dL and/or IVIG replacement) before lymphodepletion. Data on IgG levels or IVIG replacement beyond day 90 after CAR-T cell infusion and before subsequent systemic therapy for underlying disease were available for 42 patients (19 with ongoing CR and 23 with non-ongoing CR). Twenty-eight patients (67%) had evidence of hypogammaglobulinemia beyond day 90 and before initiation of subsequent therapy, of whom 18 already had hypogammaglobulinemia before CAR-T cell administration. In the ongoing CR group, 14 patients (74%) had documented hypogammaglobulinemia beyond day 90 after CAR-T cell infusion (Table 3).

Late infections

Data on late infections were available for 54 patients, 33 of whom (61%) had at least 1 infection, for a total of 153 infection events. The infection density was .55 infection/100 days at risk (2.08 per patient-year). The most common infections were upper respiratory tract infections (48%), followed by lower respiratory infections (23%). Eighty percent of the infections were treated in the outpatient setting, and 20% necessitated admission, including 5% requiring intensive care (Figure 1A). Thirty-seven of 153 (24%) recorded infection events had a microbiological etiology, of which 60% were bacterial infections, 31% were viral infections (mostly respiratory viruses), and 9% were fungal infections (2 with *Aspergillus*, 1 with *Candida*, and 1 with *Coccidioides*) (Figure 1B). Infection events are described in detail in Supplementary Table 2, and the identified organism are listed in Supplementary Table 3.

Table 3
Hypogammaglobulinemia

Type	NHL (N = 43)	CLL (N = 17)	ALL (N = 26)	Entire Cohort (N = 86)	Ongoing CR (N = 21)	Non-Ongoing CR (N = 65)	*P* Value*
Hypogammaglobulinemia (IgG <400 mg/dL or IVIG) before lymphodepletion, n (%)†	13 (30)	13 (76)	8 (31)	34 (40)	9 (43)	25 (38)	.80
Late hypogammaglobulinemia, n/N (% of patients with available data)‡	13/23 (56)	10/13 (77)	5/6 (83)	28/42 (67)	14/19 (74)	14/23 (61)	.51

* *P* values are for ongoing CR versus non-ongoing CR.
† Last IgG level/IVIG data before lymphodepletion (within 60 days).
‡ Documentation in medical records of IgG level <400 mg/dL and/or IVIG replacement at any time beyond 90 days after CAR-T cell infusion and before subsequent systemic therapy for the underlying disease.

Subsequent malignancies

Thirteen patients (15%) in the entire cohort developed subsequent malignancies, including 6 (7%) with non-melanoma skin cancer, 4 (5%) with MDS, 1 (1%) with melanoma, 1 (1%) with noninvasive bladder cancer, and 1 (1%) with MM. Among the 21 patients with ongoing CR, 6 (29%) developed subsequent malignancies, including 2 with MDS (N= 2 with non-melanoma skin cancer (N= 1 with melanoma (N= and 1 with MM.

Among the 13 patients diagnosed with subsequent malignancies, the median number of previous lines of therapy was 4 (range, 1 to 7), and 8 patients (62%) had undergone previous autologous or allogeneic HCT. Importantly, 2 of the 4 patients with a subsequent diagnosis of MDS had a cytogenetic abnormalities before receiving CAR-T cell therapy, and the patient who developed subsequent MM had a diagnosis of monoclonal gammopathy of undetermined significance (MGUS) before receiving CAR-T cell therapy.

The median time from first CAR-T cell infusion to diagnosis of non-melanoma skin cancer was 16 months (range, 1 to 35 months) and to diagnosis of MDS was 6 months (range, 4 to 17 months). The cases of bladder cancer, MM, and melanoma were diagnosed at 2, 6, and 8 months after CAR-T cell infusion, respectively. No replication-competent lentivirus was detected in any of the CAR-T cell products before infusion or in any blood samples tested after CAR-T cell infusion.

All patients with skin cancer and the patient with bladder cancer were treated with resection, with or without topical treatment. Among the patients with MDS, 1 patient died from MDS and active NHL, 1 patient underwent allogeneic HCT for MDS and CLL, and 2 patients with ongoing CR for NHL received hypomethylation treatment for MDS. The patient with MM died from that disease. The subsequent malignancies are described in detail in Supplementary Table 4.

Immune-related events

Among the 86 patients in the cohort, we identified 7 patients (8%) with new possible immune-related events, including lymphocytic alveolitis (associated with elevated ferritin level), persistent skin rash (with biopsy findings consistent with spongiosis and psoriasiform dermatitis, and with CAR-T cells detected in skin biopsy tissue by quantitative PCR), eosinophilic pneumonia, pneumonitis not otherwise specified (NOS), granulomatous disease NOS, persistent flu-like syndrome (malaise, fatigue, arthralgia, myalgia) for several months with negative infectious workup, and collagenous colitis. The median time of symptom onset was 234 days after CAR-T cell infusion (range, 67 to 1099 days). The eosinophilic pneumonia occurred in the setting of relapsed NHL, and the pneumonitis NOS occurred in the setting of ibrutinib therapy. Two events occurred in patients with ongoing CR: lymphocytic alveolitis and flu-like syndrome. No reexpansion of CAR-T cell counts in blood was seen around the time of clinical events, and no association could be made between CAR-T cells and the presumed immune-related events. The immune-related events are described in Supplementary Table 5.

GVHD

Fifteen patients had undergone previous allogeneic HCT at a median of 37 months (range, 3.2 to 143.6 months) before CAR-T cell infusion and were at risk of developing GVHD. None

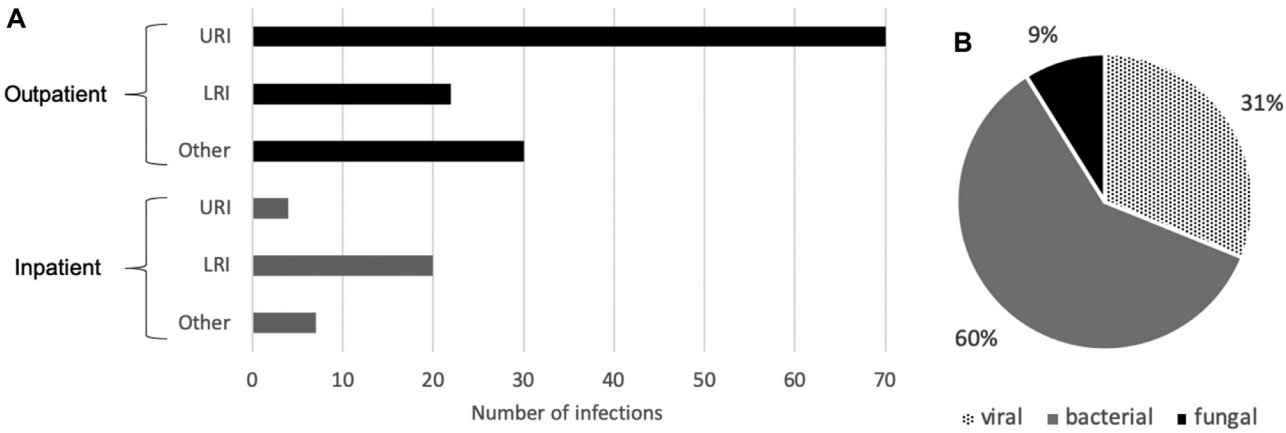

Figure 1. Late infections. (A) Late infections managed in the outpatient (n = 122) or inpatient (n = 31) setting, including infections requiring intensive care (n = 7; all LRI). URI indicates upper respiratory infection; LRI, lower respiratory infection; other, other infections (n = 37): bacteremia (n = 1), febrile neutropenia (n = 1), conjunctivitis (n = 2), oral infections (including herpes simplex virus [HSV] and *Candida*) (n = 4), genitourinary tract infections (n = 4), gastrointestinal infections (n = 5), osteomyelitis (n = 1), and skin infections (including cellulitis, human papillomavirus, HSV, zoster, and tinea) (n =19). (B) Infections with microbiologic evidence (n = 37).

had GVHD at the time of CAR-T cell infusion. We identified 3 patients (20%) who developed GVHD requiring systemic therapy after CAR-T cells; all were in ongoing CR for their baseline disease. One patient with no previous history of GVHD developed late acute GVHD after CAR-T cell administration that affected the gastrointestinal tract, liver, and skin and experienced complete resolution after treatment with prednisone. Two patients, including 1 with a previous history of acute GVHD after transplantation, developed chronic GVHD after CAR-T cell administration, requiring multiple lines of therapy. GVHD developed at 1.9 to 3.2 months after CAR-T cell infusion, and all patients had low-level CAR-T cell persistence at the time of initial GVHD manifestations. The GVHD events are described in detail in Supplementary Table 6.

Neurologic and psychiatric events

Among the 86 patients in the cohort, 9 patients (10%) had 11 new neurologic findings, including 3 cerebrovascular accident events and 1 transient ischemic attack. Two events occurred in 2 patients (13%) with ongoing CR: Alzheimer's dementia and peripheral neuropathy.

We identified 8 patients (9%) with psychiatric events requiring intervention, including 4 with a newly diagnosed mood disorder and 4 with exacerbation of a previous mood disorder (depression and anxiety). Two events occurred in patients with ongoing CR; 1 patient had overlap depression and dementia, and 1 patient had exacerbation of depression, including a suicide attempt. The neuropsychiatric events are described in Supplementary Table 7.

Late Events in Patients with Ongoing CR

Three of 19 patients (16%) with ongoing CR and without a subsequent diagnosis of MDS were found to have prolonged cytopenia lasting for 15.2 to 21.7 months after CAR-T cell infusion. Seventy-four percent of the patients with available data had prolonged hypogammaglobulinemia, and the infection density in this group was .56/100 days at risk. Six patients (29%) developed subsequent malignancies (2 with MDS, 2 with nonmelanoma skin cancer, 1 with melanoma, and 1 with MM), and 2 patients (10%) experienced possible immune-related events. Figure 2 shows the late events in this group, and Table 4 summarizes the late events in this group and in the entire cohort. Although the numbers are small, our data suggest no significant differences in late events between patients with ongoing CR and patients without ongoing CR (Table 4).

DISCUSSION

CD19-targeted CAR-T cell therapy has revolutionized the treatment of relapsed/refractory B cell malignancies, with unprecedented response rates in heavily pretreated patient populations. However, data on long-term events after this novel therapy remain scarce. In this study, we evaluated late events in 86 patients with NHL, CLL, and ALL who had at least 1 year of follow-up after treatment with CD19-targeted CAR-T cells on a dose-finding phase I/II clinical trial.

Overall, our data suggest that CD19 CAR-T cell therapy has acceptable long-term safety. Only 6 patients in our cohort (7%) died of nonrelapse related causes, with 5 deaths due to HCT-related complications.

The most common late event identified was hypogammaglobulinemia, an expected "on-target off-tumor" effect of CD19 CAR-T cell therapy. Among the patients with available data, 67% of the patients had documented hypogammaglobulinemia/IVIG replacement. Locke et al [17] reported 44% IVIG replacement among patients with diffuse large B cell lymphoma with ongoing remission in the ZUMA1 study, and Park et al [16] demonstrated low IgG levels in 83% of ALL patients at least 1 month after achieving CR following treatment with CD19 CAR-T cells on a phase 1 study. Maude et al [1] demonstrated B cell aplasia at 6 months after treatment with tisagenlecleucel in 83% of patients with ALL. Taken together, our data support previous findings demonstrating hypogammaglobulinemia as the most common late event after treatment with CD19 CAR-T cells.

Hill et al [15] studied early infections (up to 90 days after CAR-T cell infusion) in patients treated on the clinical trial reported here and found that the incidence and severity of infections were comparable to those seen after other chemoimmunotherapies. The majority of the late infections identified in our study were mild and managed in the outpatient setting. In most cases that required hospital or ICU admission, there were other factors that contributed to an increased risk of infection. Locke et al [17] reported that 28% of patients on the ZUMA1 trial developed grade $\geq$3 infections, including early infections; however, only 1 serious lung infection was reported beyond 12 months after treatment. Maude et al [2] reported grade $\geq$3 infections in 36% of patients during a median follow-up of 13.1 months, and 3 deaths associated with infections more than 30 days after CAR-T cell infusion. Park et al [16] showed that 10 of 32 patients (31%) had 15 infections between day 31 and day 180 after CAR-T cells, 6 (40%) with documented hypogammaglobulinemia. Similar to our findings, Kochenderfer et al [14] reported mild viral infections, and 1 patient with pneumonia required hospitalization among 4 patients with DLBCL with ongoing CR after CD19 CAR-T cell therapy. Porter et al [18] reported 1 death in a patient in CR at 21 months after CAR-T cell infusion from complications of a *Pseudomonas* wound infection after basal cell carcinoma removal despite IVIG replacement. Comparing our results with other CD19 CAR-T cell studies is challenging, because we only included infections occurring at least 90 days after CAR-T cell infusion in patients who survived at least 1 year after CAR-T cell infusion, whereas others included early infections as well. However, in line with others, our present findings support a low rate of severe late infections after CD19 CAR-T cell therapy.

Cytopenias are expected early after CAR-T cell therapy due to lymphodepletion chemotherapy. Some patients on our study received high-intensity lymphodepletion regimens, which may increase the risk of developing prolonged cytopenia. Three of 19 patients (16%) with ongoing CR and with no MDS had persistent significant cytopenias (requiring RBC or platelet transfusion, or growth factor support) more than 3 months after treatment (2 of whom received a high-intensity lymphodepletion regimen). Similarly, 17% of patients in the ZUMA1 study had grade $\geq$3 cytopenia at 3 months after CAR-T cell infusion or later [17], and 12% and 11% of patients in the ELIANA study had grade $\geq$3 thrombocytopenia and neutropenia, respectively, at a median follow-up of 13.1 months after CAR-T cell therapy [1]. The mechanism of prolonged cytopenia after CD19 CAR-T cell therapy is unclear, and more work is needed to evaluate its etiology. Because all patients who receive CAR-T cell therapy are heavily pretreated, it is important to rule out preexisting treatment-related marrow hypoplasia or MDS as the cause of cytopenia after CD19 CAR-T cell therapy.

The development of a new malignancy is a potential risk of genetically modified cellular therapies. However, to date, there have been no reports of T cell malignancy following CAR-T cell therapy and no reports of replication-competent retrovirus/

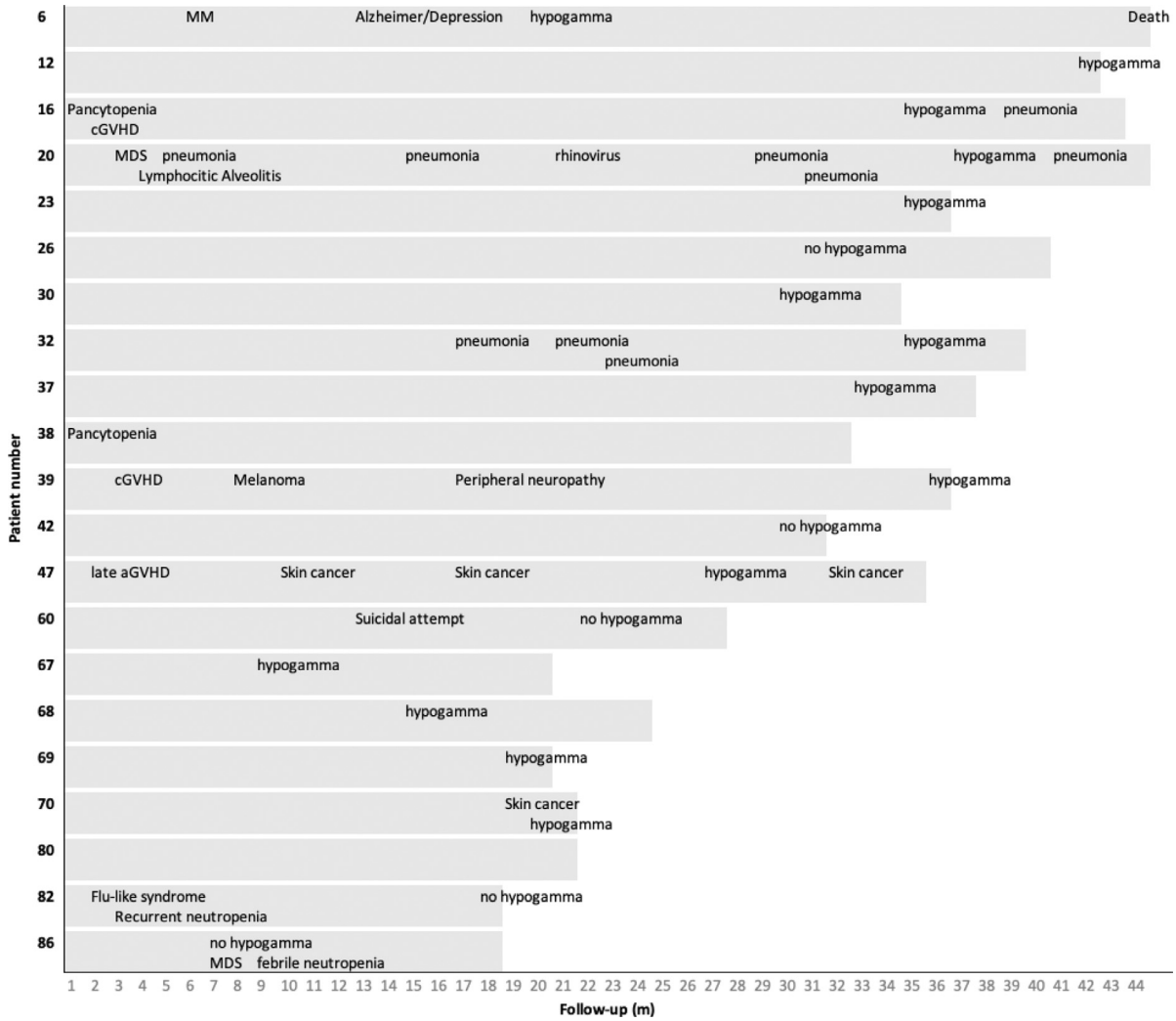

Figure 2. Late events in patients with ongoing CR. MM = multiple myeloma, hypogamma = hypogammaglobulinemia (IgG <400 and/or IVIG replacement); MDS = myelodysplastic syndrome, cGVHD, chronic graft-versus-host disease; aGVHD, acute graft-versus-host disease. Hypogammaglobulinemia data were not available for patients 38 and 80.

Table 4
Summary of Late Adverse Events after CD19-Targeted CAR-T Cell Therapy.

Late Adverse Event	Entire Cohort (N = 86)	Ongoing CR (N = 21)	Non-Ongoing CR (N = 65)	*P* Value*
Cytopenias (evaluated only in patients with ongoing CR and no diagnosis of subsequent MDS), n/N (%)	–	3/19 (16)	–	–
Hypogammaglobulinemia, n/N (% of patients with available data)	28/42 (67)	14/19 (74)	14/ 23 (61)	.51
Infection density; mean number of infections/100 days at risk (number of patients evaluated)	.57 (53)	0.57 (20)	0.58 (33)	.58†
Subsequent malignancies, n (%)				.08
All subsequent malignancies	13 (15)	6 (29)	7 (11)	
MDS	4 (5)	2 (10)	2 (3)	.25
Non-melanoma skin cancer	6 (7)	2 (10)	4 (6)	.63
Immune-related events, n (%)	7 (8)	2 (10)	5 (8)	1.00
Neurologic events, n (%)	9 (10)	2 (10)	7 (11)	1.00
Cerebrovascular accident/transient ischemic attack, n (%)	4 (5)	0 (0)	4 (5)	.57
Psychiatric events, n (%)	8 (9)	2 (10)	6 (9)	1.00
GVHD, n/N (% of patients with previous allogeneic HCT)	3/15 (20)	3/8 (38)	0/7 (0)	.20

* Ongoing CR versus non-ongoing CR; *P* values calculated using Fisher's exact test.
† *P* value calculated using the *t* test from 100 bootstrap samples.

lentivirus in T cell products [19]. One case of transduction of leukemic cells associated with resistant ALL has been reported [20]. We identified subsequent malignancies in 13 patients (15%); however, most (7%) were nonmelanoma skin cancer. Four patients (5%) were diagnosed with MDS, including 3 who had undergone previous autologous HCT and 2 who had received a high/intermediate-intensity lymphodepletion regimen, which may increase the risk of subsequent MDS. The 12.5% (3 of 24) rate of treatment-related MDS is within the range of expected risk in a post-autologous HCT population [21]. Other groups also reported cases of MDS after CD19 CAR-T cell therapy, likely related to previous therapies [14,17]. The 11% rate of secondary malignancies among patients with NHL in our cohort is similar to the expected incidence of secondary malignancies after conventional treatment of NHL (4% to 10%) [22-25]. At this time, we have no data on the effect of CAR-T cells on the development or aggressiveness of subsequent malignancies. Additional studies are needed to further assess whether or not CAR-T cell therapy increases the risk for subsequent malignancies.

Autoimmune reactions are another potential concern related to CAR-T cell therapy. In this study, we identified 7 patients (8%) with possible immune-related events. Most of these events were idiopathic and did not meet diagnostic criteria for any autoimmune disease. Although 1 event was associated with elevated serum ferritin level, and in 1 event CAR-T cells were detected in the skin biopsy specimen by quantitative PCR, there was no good evidence indicating that the subsequent events were due to CAR-T cell therapy. Hu et al [26] reported that 0.2% of patients with NHL treated with conventional treatment in a large registry study developed autoimmune diseases at a median interval of 2.6 years after the lymphoma diagnosis. An important difference between the report by Hu et al and our study is that we report any possible immune-related event, whereas Hu et al reported only diseases that met diagnostic criteria of defined autoimmune diseases.

Acute neurotoxicity is a well-documented adverse event after CAR-T cells [13,27]. We identified late neurologic events in 10% of the patients. Although none of the events could be directly attributed to the CAR-T cell therapy, the presence of vascular phenomena may be related to endothelial activation reported during acute CAR-T cell-mediated neurotoxicity [13]. Neuropsychiatric events are very difficult to analyze owing to subjectivity and confounding factors in this heavily pretreated population, and ongoing efforts are in progress to determine whether there are lasting neuropsychiatric sequelae after CAR-T cell therapy.

Patients who receive CAR-T cell therapy after previous allogeneic HCT could be at risk for GVHD exacerbation associated with alloreactive T cell infusion and systemic inflammation during CRS. Fifteen patients in our cohort had previous allogeneic HCT, 3 of whom among them three (20%) developed GVHD requiring systemic immunosuppressive therapy after CAR-T cell therapy. Similarly, Hu et al [28] reported acute GVHD in 2 out of 11 patients (18%) who received CAR-T cells after previous allogeneic HCT. This is not an unexpected rate of GVHD during this period after transplantation [29], suggesting that the risk of exacerbation of GVHD is not markedly increased after CAR-T cell therapy. Although the 3 patients in our cohort who developed GVHD had CAR-T cell persistence at the time of GVHD diagnosis, at this time there are no data to support an association between CAR-T cell persistence and the development of GVHD. Larger studies are needed to further assess the potential association between CAR-T cell persistence and the development of GVHD after CAR-T cell therapy.

This study has several limitations, the most significant being the retrospective data collection and missing data, small sample size, and progressively smaller number of subjects in remission for whom results are reported, as well as the phase I/II dose-finding nature of the clinical trial on which the patients were treated. Several patients in the cohort returned to the care of their primary oncologist outside of our center, and thus data collection was not consistent for all patients. Despite the limitations this study provides useful approximations of the incidences of late adverse events that will be helpful in counseling patients undergoing CD19 CAR-T cell therapy.

CONCLUSION

Our results suggest that CD19 CAR-T cell therapy has acceptable safety, because most of the late events seen in our cohort were mild and likely related to the underlying disease and/or previous or subsequent therapies. Continuing prospective systematic follow-up is needed for a better understanding of the late effects of CAR-T cell therapy and for establishing evidence-based long-term follow-up and treatment guidelines.

DECLARATION OF COMPETING INTEREST

A.C., E.B., A.V.H., M.S., and M.B. have no conflicts of interest to report. J.A.H. has served as a consultant for Nohla Therapeutics and Amplyx and has received research support from Nohla Therapeutics, Karius, and Shire. D.G.M. has received research funding from Kite Pharma, Juno Therapeutics, and Celgene and has served on advisory boards for Kite Pharma, Gilead, Genentech, Novartis, and Eureka Therapeutics. C.J.T. receives research funding from and has patents licensed or pending with Juno Therapeutics and Nektar Therapeutics; has served on advisory boards and has equity in Caribou Biosciences, Eureka Therapeutics, and Precision Biosciences; and has served on advisory boards for Aptevo, Bluebird Bio, Adaptive Biotechnologies, Juno Therapeutics, Kite Pharma, Humanigen, Nektar Therapeutics, Novartis, T-CURX, and Allogene.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.08.003.

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