REGULAR ARTICLE

blood advances

Infectious complications of CAR T-cell therapy across novel antigen targets in the first 30 days

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Key Points

- Infectious complications in the first 30 days occurred in 32.7% of patients across a host of 5 different phase 1 CAR T-cell trials.
- Greater lines of prior therapy and recent prior infection (within 100 days of CAR infusion) increased risk of infection postCAR infusion.

Infections are a known complication of chimeric antigen receptor (CAR) T-cell therapy with data largely emerging from CD19 CAR T-cell targeting. As CAR T-cell therapy continues to evolve, infection risks and management thereof will become increasingly important to optimize outcomes across the spectrum of antigens and disease targeted. We retrospectively characterized infectious complications occurring in 162 children and adults treated among 5 phase 1 CAR T-cell clinical trials. Trials included targeting of CD19, CD22, disialoganglioside (GD2) or B-cell maturation antigen (BCMA). Fifty-three patients (32.7%) had 76 infections between lymphocyte depleting (LD) chemotherapy and day 30 (D30); with the majority of infections (61, 80.3%) occurring between day 0 (D0) and D30. By trial, the highest proportion of infections was seen with CD22 CAR T cells (n = 23/53; 43.4%), followed by BCMA CAR T cells (n = 9/24; 37.5%). By disease, patients with multiple myeloma had the highest proportion of infections (9/24; 37.5%) followed by acute lymphoblastic leukemia (36/102; 35.3%). Grade 4 infections were rare (n = 4; 2.5%). Between D0 and D30, bacteremia and bacterial site infections were the most common infection type. In univariate analysis, increasing prior lines of therapy, recent infection within 100 days of LD chemotherapy, corticosteroid or tocilizumab use, and fever and neutropenia were associated with a higher risk of infection. In a multivariable analysis, only prior lines of therapy and recent infection were associated with higher risk of infection. In conclusion, we provide a broad overview of infection risk within the first 30 days post infusion across a host of multiple targets and diseases, elucidating both unique characteristics and commonalities highlighting aspects important to improving patient outcomes.

Introduction

Chimeric antigen receptor (CAR) T-cell therapies are reshaping the therapeutic landscape in pediatric and adult malignancies with FDA-approved products available for acute lymphoblastic leukemia (ALL), B-cell non-Hodgkin lymphomas (NHL), and more recently in multiple myeloma (MM), with promising investigational

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approaches in solid tumors. As these therapies have become more widely administered as standard of care or in clinical trials, a greater understanding of side effects has led to the development of management algorithms for cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).[1-6]

CRS and ICANS describe a state of immune dysregulation that occurs when CAR T-cells recognize and bind to their cognate antigens and initiate a cascade of immune activation, leading to the mobilization of bystander immune cells, production of cytokines, and enhanced vascular permeability.[7] The impact and interplay of CAR T-cell induced CRS on host mechanisms to fight foreign pathogens is unknown. Studies looking at rates and types of infections that occur in patients treated with lymphocyte-depleting (LD) chemotherapy and CAR T cells have identified several parameters that may correlate to an increased infection risk for patients: prior antitumor therapy, CAR T-cell dose, CRS grade, use of immunosuppression to treat CRS, and degree of neutropenia.[8-12] However, as most studies have focused only on therapy with CD19 CAR T cells in adults and infectious disease guidance with CD19 targeting,[7] there is no uniform method to assess infection risk in patients with different underlying malignancies treated with distinct CAR T cell products. Additionally, there is limited data on infectious disease outcomes in pediatric populations and populations treated with CAR T cells targeting alternative antigens.[10,12,14]

To identify infections and explore possible risk factors associated with infections with novel CAR T-cell products targeting multiple antigens across the age spectrum, we conducted a retrospective analysis of patients treated on 1 of 5 phase 1 CAR T-cell trials at the National Cancer Institute (NCI). The primary objective of this study was to establish the incidence of infections occurring between LD chemotherapy through day 30 (D30) after cell infusion. Secondary objectives included identifying potential risk factors for infections and describing the relationship between CRS, neutropenia, and infections.

Methods

Patients and clinical trial protocols

This was a single-center, retrospective study conducted at the NCI, which included subjects infused between 2012 and 2018. Data were extracted from patients treated on 1 of 5 phase 1, dose-escalation trials targeting CD19 (2 separate studies), CD22, B-cell maturation antigen (BCMA), or disialoganglioside (GD2). The manufacture of certain products is previously described (supplemental Appendix).[15-21] Two adult protocols (ages 18-73 years) treated either MM patients with an anti-BCMA CAR (BCMA CAR, NCT02215967) or NHL patients with a fully-human anti-CD19 CAR (FHCD19 CAR, NCT02659943). Three pediatric and young adult protocols (age 3-30 years) treated either ALL or NHL patients with an anti-CD19 CAR (pCD19 CAR, NCT01593696) or an anti-CD22 CAR (CD22 CAR, NCT02315612) or treated patients with neuroblastoma (NB) or osteosarcoma (OS) with an anti-GD2 CAR (GD2 CAR, NCT02107963). The NCI and Institutional Review Board (IRB) both approved all prospective treatment studies and this retrospective analysis (Clinicaltrials.gov NCT 03827343).

Data collection

Basic demographics, disease status, clinical course, and pre-and post-infusion factors that could be associated with infections were collected from enrollment to D30 (supplemental Appendix). Patients were censored either at death, progressive disease (PD), with pursuit of alternative therapies, or if lost to follow-up. For patients receiving multiple infusions on a study, the clinical course of the infusion associated with CRS and clinical response was captured.

An infection was defined as a distinct clinicopathological entity with clinical signs or symptoms along with laboratory, radiographic, microbiologic, or histopathologic results. Detailed definitions that were used to identify an infection are described in the supplemental Appendix. Infections were categorized based on organism and site of infection: bacteremia, site-specific bacterial, fungal, and viral infections. *Clostridioides difficile* (*C. difficile*) infections were categorized as a separate entity than site-specific bacterial infections due to the differing risk-factor profiles. Infections were resolved as of the last date of symptoms or last day of therapeutic intervention.

Fever and neutropenia (F&N) events were defined as fever (defined as $>38.3^{\circ}C \times 1$ or persistent 38.0-38.2°C documented at least twice in less than 1 hour) occurring when the absolute neutrophil count (ANC) of a patient was <500 cells/mm^3; resolution of F&N was determined by the date the patient was afebrile for at least 48 hours. CRS was defined per Lee et al grading.[2] Supportive care, including antimicrobial prophylaxis, and monitoring differed for patients based on protocol. Details are outlined in the supplemental Appendix.

Statistical methods

The primary objective of this study was to determine the incidence of infections from LD to D30; secondary objectives included identification and evaluation of factors associated with infections the day of infusion (D0) to D30 and establishing which factors were significant on multivariable analysis.

Cumulative incidence curves were used to describe the time to an event such as the development of any infection within D0-D30, starting at D0 and using a standard approach such as that described by Gooley et al.[22] Death was a competing risk to consider for infections within the first 30 days from D0.

Factors that were known either before D0 or identified within the period from D0-D30 were considered as potential factors for association with infections between D0 and D30. Those factors which were known at or before D0 were considered eligible for multivariable analysis of infections between D0 and D30.

Factors were identified for association and prediction of infections from D0 to D30 as follows: factors reported as continuous parameters were compared between two groups, with and without infection, using a Wilcoxon rank sum test; ordered categorical parameters were compared between the two groups using a Cochran-Armitage test for trend; dichotomous parameters were compared between the two groups using Fisher's exact test; and unordered categorical parameters were compared between 2 groups using Mehta's modification to Fisher's exact test.[23,24]

Following this initial screening by univariate methods, for those parameters for which $P < .10$, univariate logistic regression followed by multivariable logistic regression analysis using both backward and stepwise selection was used to identify a set of factors that could jointly impact the dichotomous infection parameter.

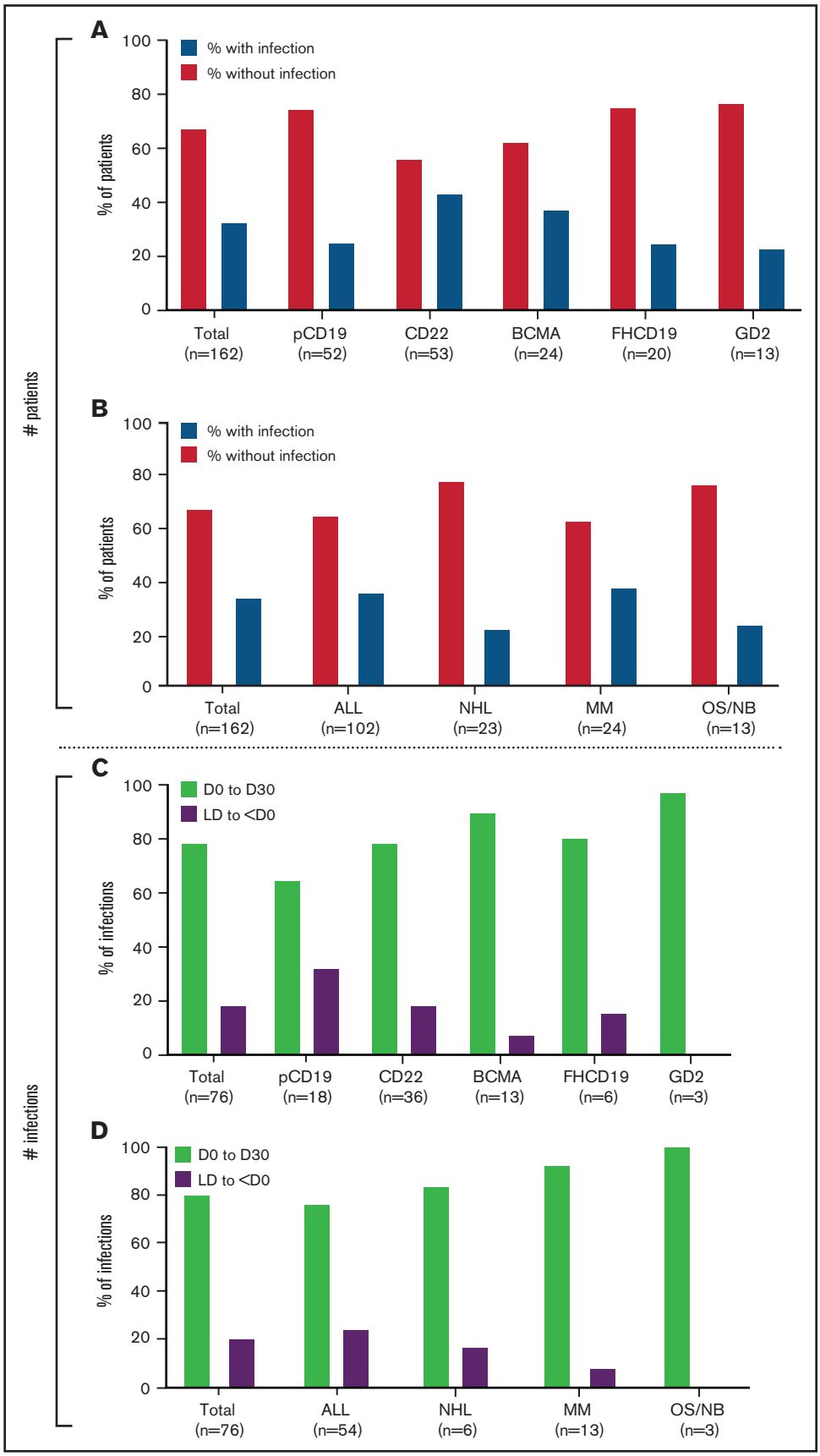

Figure 1. Incidence and frequency of infections across trial and disease cohorts. (A) Percent of patients with and without infections by protocol between LD and D30. (B) Percent of patients with and without infections by disease type between LD and D30. (C) Percent of infections before and after D0 by protocol. (D) Percent of infections occurring before and after D0 by disease type.

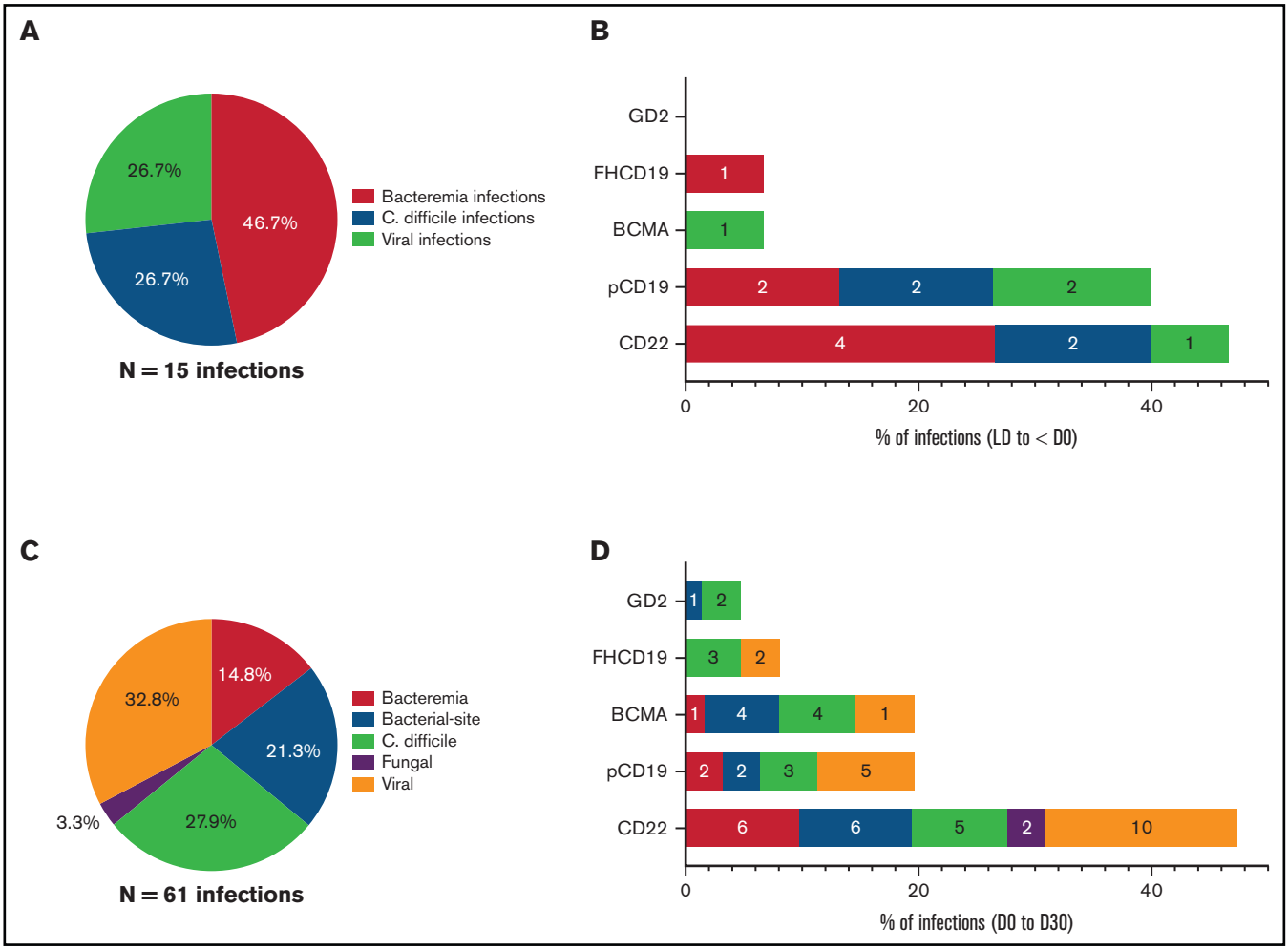

Figure 2. Incidence and frequency of infections by time point and across trials. (A) Infection subtype between LD and <D0. (B) Infection subtype by protocol between LD and < D0. (C) Infection subtype between D0 and D30. (D) Infection subtype by protocol between D0 to D30.

Results

Patient and treatment characteristics

Data were collected from 162 patients treated on 5 protocols: 102 (63%) with ALL, 23 (14.2%) with NHL, 24 (14.8%) with MM, and 13 (8%) with OS/NB (Table 1). Of 162 patients, 52 pediatric patients (32.1%) received CD19 CAR, 20 adult patients (12.3%) received FHCD19 CAR, 53 pediatric patients (32.7%) received CD22 CAR, 24 adult patients (14.8%) received BCMA CAR, and 13 pediatric patients (8.0%) received GD2 CAR. The median age of treated patients was 19 (range 4-69). Five patients were censored prior to D30 due to progressive disease, death from toxicity, and loss of follow-up (supplemental Appendix).

The majority of patients had multiply relapsed/refractory disease: 70/162 patients (43.2%) received 3 to 5 prior antimalignancy therapies, and 67/162 patients (41.4%) received more than 5 prior therapies. Twenty-nine of 162 patients (17.9%) with MM, NHL, or OS/NB had prior autologous transplants (auto-SCT). Forty-seven of 162 (29.0%) patients had at least one prior allogeneic transplant (allo-SCT)(ALL, n = 46), and 14/162 ALL patients (8.6%) had two prior allo-SCT. Thirty-eight of 53 (71.6%) patients on the CD22 CAR protocol had prior allo-SCT. Notably, 31 (58.4%) patients treated with CD22 CAR and 2 (3.8%) patients treated with CD19 CAR had received a prior CAR T-cell therapy with a distinct CAR T-cell product.

Clinical course of patients. Full descriptions of the clinical course of patients treated in each trial are outlined separately.[15-17,20,21,25] The majority of patients, 134/162 (82.7%) received standard-dose LD chemotherapy with fludarabine 30 mg/m^2 and cyclophosphamide 300 mg/m^2 for 3 days (or 900 mg/m2 × 1 day) while 28/162 (17.3%) received alternative, high-dose regimens which are outlined in Table 1.

Thirty-three patients of 162 (20.4%) had no evidence of CRS, 107/162 patients (66%) had grade 1-2 CRS, and 22/162 patients (13.6%) had grade 3 CRS or higher. Thirty-nine of 162 patients (24.1%) had evidence of neurological toxicity. The objective response rate (ORR) for all patients at D30 was 58%. Toxicity management and response details are outlined in Table 1.

Prior infections and baseline cytopenias. Seventy-two patients of 162 (44.4%) had a prior infection within 100 days of LD chemotherapy; of whom 29/72 patients (40.3%) had a documented bacterial infection.

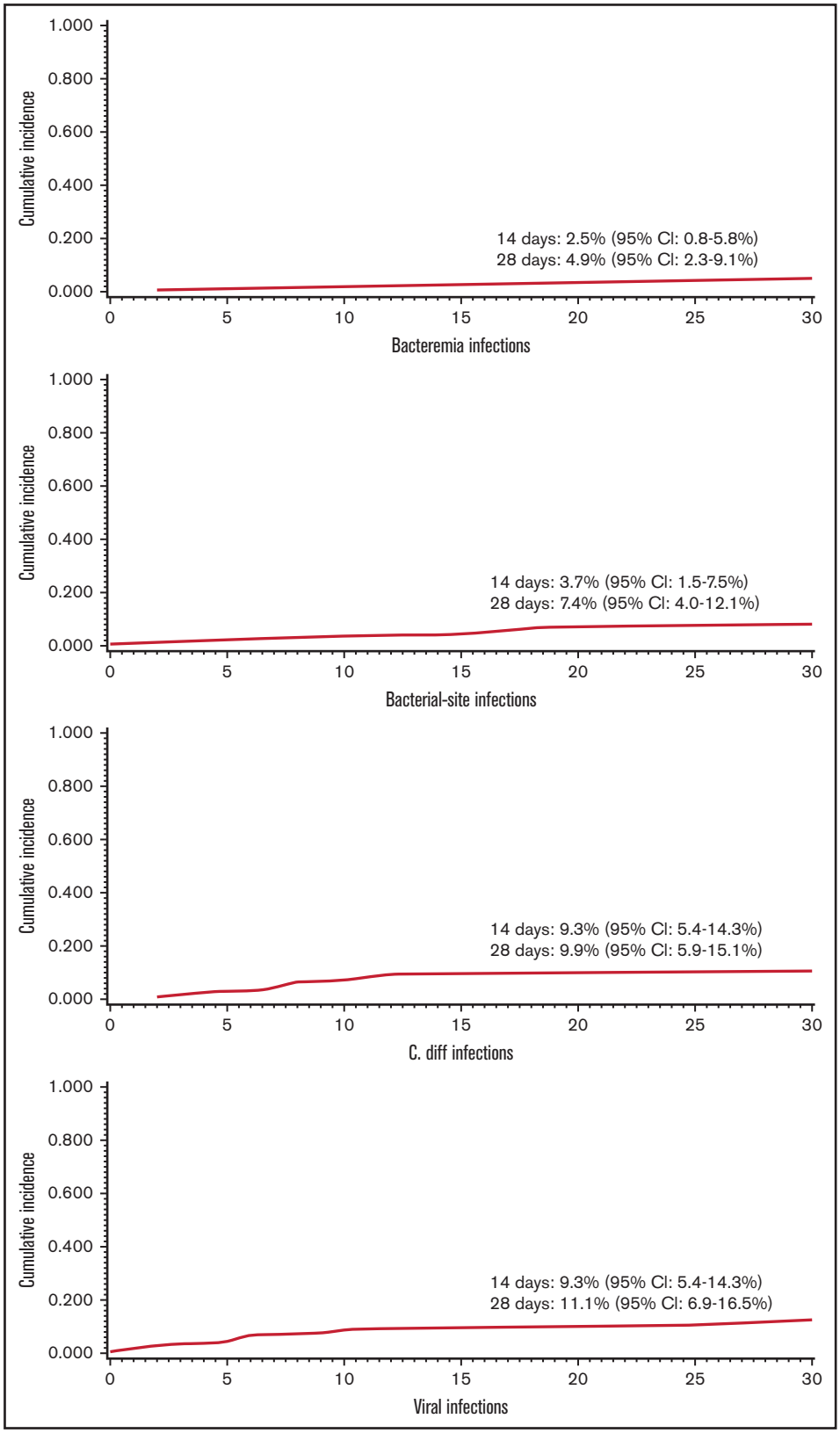

Figure 3. Cumulative incidence curves for bacteremias, bacterial-site infections, *C.* diff infections, and viral infections.

Thirty-three of 162 patients (20.4%), all who had ALL, had an ANC of less than 500 cells/mm^3 14 days prior to LD chemotherapy, while 17/162 patients (10.5%) had an absolute lymphocyte count (ALC) of less than 200 cells/mm^3 during this time period. On D0, the median ANC was 1590 cells/mm^3 (range <200 to 2620 cells/mm^3) and the median ALC was <200 cells/mm^3 (<200 to 1380 cells/mm^3).

Infection incidence

Fifty-three of 162 (32.7%) patients had 76 infections from LD to D30 (Figure 1A). Among 53 patients with infections, 33 (62.3%) had one infection, 17 (32.1%) had two infections, and 3 (5.7%) had 3 infections. The CD22 CAR protocol had the highest proportion of patients with infections (n = 23/53; 43.4%) (Figure 1B-C), followed by the

Table 1. Patient, disease, and treatment characteristics

		CD19 (peds)	CD22	BCMA	CD19 (adult)	GD2	Total
		NCT01593696	NCT02315612	NCT02215967	NCT02659943	NCT02107963	
	Diseases treated	ALL (n = 50), NHL (n = 2)	ALL (n = 52), NHL (n = 1)	MM	NHL	OS (n = 11), NB (n = 2)	
	n	52	53*	24	20	13	162
Demographics	Median age, (range) years	13 (4-30)	17 (4-30)	55 (43-66)	58 (39-69)	19 (10-28)	19 (4-69)
	Male, n (%)	41 (78.8)	34 (64.2)	12 (50)	12 (60)	10 (76.9)	109 (67.3)
	# patients with 1-2 lines of prior therapy, n (%)	15 (28.8)	2 (3.8)	0 (0)	7 (35)	1 (7.7)	25 (15.4)
	# patients with 3-5 lines of prior therapy, n (%)	23 (44.2)	23 (43.4)	6 (25)	9 (45)	9 (69.2)	70 (43.2)
	# patients with >5 lines of prior therapy, n (%)	14 (26.9)	28 (52.8)	18 (75)	4 (20)	3 (23.1)	67 (41.4)
	Prior allogeneic HSCT, n (%)[#]	23 (44.2)	38 (71.6)	0 (0)	0 (0)	0 (0)	61 (37.7)
	Prior autologous HSCT, n (%)	0	0	20 (83.3)	7 (35)	2 (15.4)	29 (17.9)
	Prior distinct CAR T-cell therapy, n (%)	2 (3.8)	31 (58.5)	0 (0)	0 (0)	0 (0)	33 (20.4)
Disease status	% marrow involvement, median (range), n (%)	30% (0-99)	69 (0-95)	15 (0-40)	5 (0-40)	N/A	
	IgG <400 prior to LD chemo, n (%)	5 (9.6)	16 (30.2)	10 (41.7)	6 (30)	0 (0)	37 (22.8)
	ANC <500 14 d prior to LD chemo, n (%)	13 (25)	20 (37.7)	0 (0)	0 (0)	0 (0)	33 (20.4)
	ALC <200 14 d prior to LD chemo, n (%)	3 (5.8)	10 (18.9)	4 (16.7)	0 (0)	0 (0)	17 (10.5)
	Median ANC on day 0, (range)	1590 (<200-6620)	900 (<200-9790)	990 (<200-5230)	2335 (600-5810)	2620 (300-7550)	1590
	Median ALC on day 0, (range)	<200 (<200-1360)	<200 (<200-1250)	<200 (<200 – 970)	<200 (<200-300)	<200 (<200-660)	<200
Treatment characteristics	Low dose LD[†] chemotherapy, n (%)	37 (71.2)	53 (100)	24 (100)	20 (100)	0 (0)	134 (82.7)
	High dose LD[‡] chemotherapy, n (%)	7 (13.5)	0 (0)	0 (0)	0 (0)	13 (100)	28 (17.3)
	Any CRS, n (%)	37 (71.2)	47 (88.7)	18 (75)	17 (85)	10 (76.9)	129 (79.6)

ALC, absolute lymphocyte count (reported as units of cells per mm^3); ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count (reported as units of cells per mm^3); CRS, cytokine release syndrome; HSCT, hematopoietic stem cell transplant; ICU, intensive care unit; LD chemo, lymphocyte depleting chemotherapy; MM, multiple myeloma; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; OS, osteosarcoma; Peds, pediatric population.

*X patients who were retreated after undergoing allogeneic stem cell transplant were treated as newly enrolled patients after transplant.

[†]Standard dose lymphocyte depleting regimen was fludarabine 30 mg/m^2 for D3 and cyclophosphamide 300 mg/m^2 on day −3 to −5 prior to cell infusion.

[‡]High dose LD regimens were either etoposide 100 mg/m^2 on days −5 to −1 and ifosfamide 1800 mg/m^2 on days −5 to −1; or fludarabine 25 mg/m^2 on days −5 to −1 and cytarabine 2000 mg/m^2 on days −5 to −1 with filgrastim 5 μg/kg daily starting on day −6 until ANC >1000 for 2 days; or cyclosphosphamide 1800 mg/m^2 on days −3 to −2.

[§]Many CAR T-cell trials have response rates that improve over time; this ORR reflects only response rates at D30 and not the true ORR of the CAR T-cell trial.

Table 1. (continued)

		CD19 (peds)	CD22	BCMA	CD19 (adult)	GD2	Total
CAR T-cell course and outcome	CRS grade 1-2, n (%)	28 (53.8)	42 (79.2)	12 (50)	15 (75)	10 (76.9)	107 (66)
	CRS ≥grade 3, n (%)	9 (17.3)	5 (9.4)	6 (25)	2 (10)	0 (0)	22 (13.6)
	ICU admission, n (%)	22 (42.3)	20 (37.7)	12 (50)	9 (45)	0 (0)	63 (13.6)
	Tocilizumab administered, n (%)	7 (13.5)	20 (37.7)	5 (20.8)	2 (10)	0 (0)	33 (20.4)
	Corticosteroids administered, n (%)	3 (5.8)	18 (33.9)	4 (16.7)	2 (10)	0 (0)	27 (16.7)
	Objective response rate at D30[§], n (%)	32 (61.5)	38 (71.7)	11 (45.8)	13 (65)	0 (0)	94 (58)

ALC, absolute lymphocyte count (reported as units of cells per mm^3); ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count (reported as units of cells per mm^3); CRS, cytokine release syndrome; HSCT, hematopoietic stem cell transplant; ICU, intensive care unit; LD chemo, lymphocyte depleting chemotherapy; MM, multiple myeloma; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; OS, osteosarcoma; Peds, pediatric population.

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§Many CAR T-cell trials have response rates that improve over time; this ORR reflects only response rates at D30 and not the true ORR of the CAR T-cell trial.

BCMA CAR protocol (Figure 1B-C). Patients with MM had the highest proportion of infections (n = 9/24; 37.5%) followed by ALL patients (n = 36/102; 35.3%) (Figure 1D-E). Four patients (2.5%) had a grade 4, life-threatening infection between LD to D30: 1 pCD19 CAR patient, 2 CD22 CAR patients, and 1 BCMA CAR patient. One of the CD22 CAR patients subsequently died as a result of multi-organ failure as a sequela of bacteremia despite clearance.[20,27]

Of 76 infections recorded, 15 (19.7%) occurred between LD to <D0, while 61 (80.3%) occurred between D0 to D30 (Figure 1F). The breakdown of infections by protocol and disease is presented in Figure 1G-H.[1] Rates of infection subtype by trial and disease type are shown in Figure 2A-B. Bacterial infections and *C. difficile* infections occurred across all trials (Figure 2A).

Infection type and prophylaxis

Infections from initiation of lymphodepletion (LD) chemotherapy to Day <0. A total of 12 patients had 15 infections between LD to D<0 (Figure 2C); 10 had ALL, 1 had MM, and 1 had NHL. One pCD19 CAR patient had 2 bacteremias separated by 18 days (*Streptococcus mitis* [*S. mitis*] and an unknown organism) while a CD22 CAR patient had 2 concurrent bacteremias (*Citrobacter fruendii* and *Pseudomonas* species); among two CD22 CAR patients, one bacteremia with *Pseudomonas aeruginosa* (*P. aeruginosa*) *and Staphylococcus epidermidis* (*S. epidermidis*); one FHCD19 CAR patient had bacteremia with *Bacteroides fragilis.* Two pCD19 patients had rhinovirus/enterovirus and respiratory syncytial virus; a CD22 CAR patient had influenza, and a BCMA CAR patient had coronavirus. Two patients, one in the pCD19 and one in the CD22 CAR trial, had *C. difficile* infections.

Infections D0 to D30. Forty-six patients had 61 infections between D0 and D30 (Figure 2D). The cumulative incidence of any infections between D0 and D30 as a function of the day of infection onset is presented in Figure 3A, demonstrating 21.1% cumulative incidence (95% CI: 15.2% to 27.7%) by day 14 and 26.7% (95% CI: 20.1% to 33.7%) by day 28. Most infections occurred early on: of 46 total patients who experienced an infection within the first 30 days, 35 of 46 (76.1%) had infection develop within the first 14 days, while 44 of 46 (95.7%) developed within 28 days. Six patients had no evidence of CRS; these patients had at least 1 infection between D0-D30: 4 PCD19 CAR patients, 1 CD22 CAR patient, and 1 BCMA CAR patient.

Among 61 infections, bacteremias (9; 14.8%) and bacterial-site infections (13; 21.3%) were the most common type of infection (combined 22; 36.1%) followed by viral infections (20; 32.8%) and *C. difficile* infections (17; 27.9%). Fungal infections were rare, occurring in only two patients (3.3%) treated with anti-CD22 CAR T-cells. Bacteremias and bacterial site-specific infections other than *C. difficile* occurred at a steady rate throughout the first 28 days (Figure 3B-C). *C. difficile* infections and viral infections occurred mainly in the first 14 days post-infusion (Figure 3D).

Bacterial infections and prophylaxis. Nine bacteremias occurred in patients treated with pCD19 (n = 2, *P. aeruginosa, Escherichia coli* [*E. coli*]) CAR, CD22 CAR (n = 6, *Klebsiella pneumoniae, S. mitis, Enterobacter clocae, Corynebacterium stratium, S. epidermidis, Weeksella* species) and BCMA CAR (n = 1, *Staphylococcus aureus* [*S. aureus*]). The median ANC at bacteremia onset was 320 cells/mm^3. Thirteen bacterial-site infections occurred in patients on the pCD19 CAR (n = 2), CD22 CAR (n = 6), BCMA CAR (n = 4), and GD2 CAR (n = 1) trials in the following situations: typhlitis (n = 2, unknown organisms), non-*C. difficile* diarrheal illness (n = 2, *Yersinia enterocolitica*, *Salmonella enterica*), urinary tract infection (UTI, n = 3, *Enterococcus faecalis* [*E. faecalis*]), *S. aureus*, unknown organism), wound infections (n = 2, *S. aureus* at line site, unknown organism from bone marrow biopsy site), infections of

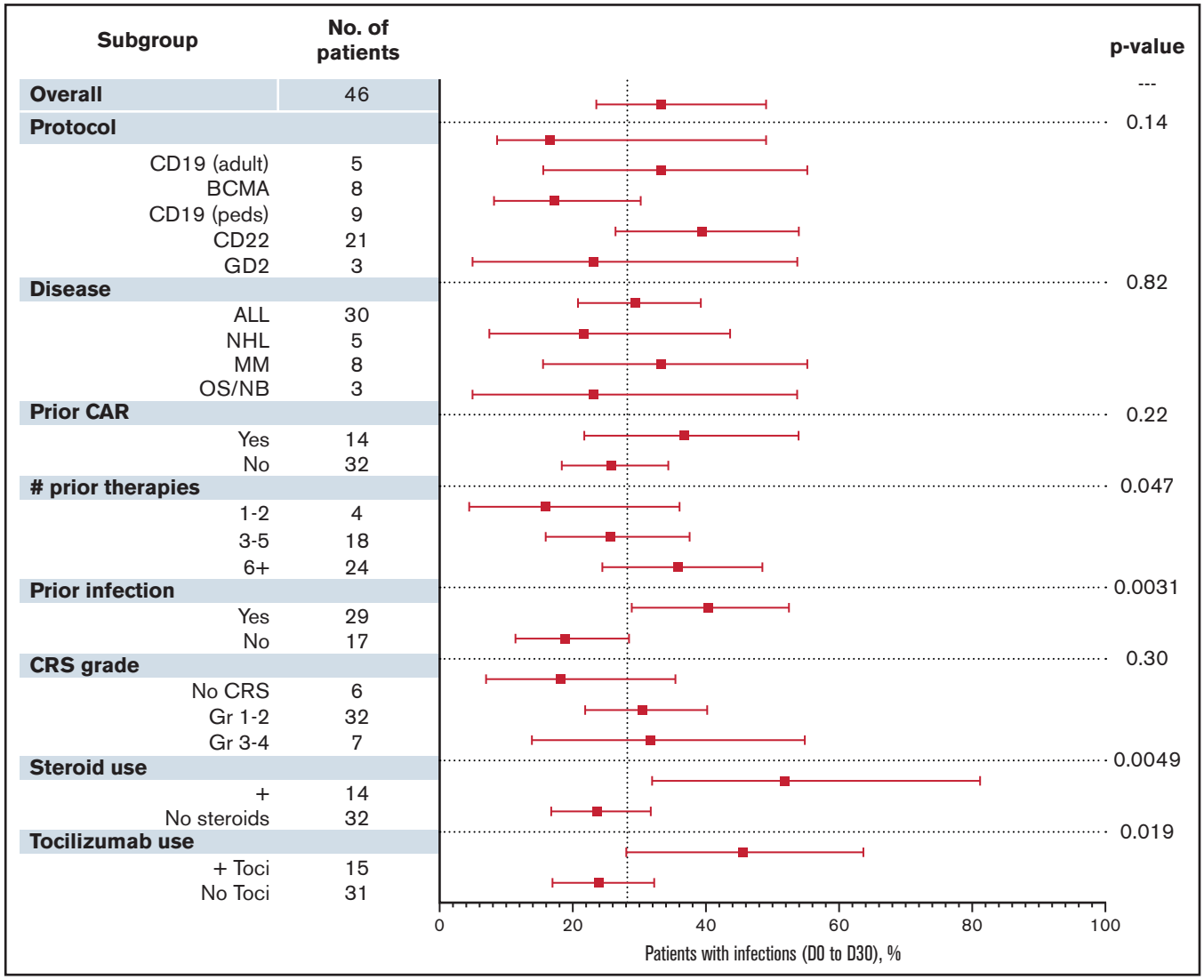

Figure 4. Patient and treatment characteristics associated with infection between D0 to D30.

oropharynx (n = 2, Streptococcus Group C, unknown organism) and lung infections (n = 2, *Stenotrophomonas maltophilia* [*S. maltophilia*], *S. aureus*).

Twenty-seven patients received non-*C. difficile* bacterial prophylaxis at the time of conditioning chemotherapy: 7 pCD19 CAR patients (4 received levofloxacin, 3 received ciprofloxacin), 18 pCD22 CAR patients (levofloxacin), 1 BCMA CAR patient (levofloxacin), and 1 FHCD19 CAR patient (penicillin). Between D0 and D30, 4 infections (in 1 CD19CAR, 3 CD22 CAR patients) were caused by organisms resistant to levofloxacin (*E. coli* and *S. epidermidis* bacteremias, *E. faecalis* UTI, *S. maltophilia* lung infection). Incidence of bacterial infections did not differ between those who received and did not receive antibacterial prophylaxis, specifically 3 of 27 (11.1%) patients receiving prophylaxis and 14 of 135 (10.4%) not receiving prophylaxis developed a bacterial infection. Fluoroquinolone-resistant organisms were identified in 4 patients, only 1 of whom was on fluoroquinolone prophylaxis.

***C. difficile* and prophylaxis.** Seventeen *C. difficile* infections occurred in patients treated on all trials: 3 pCD19 CAR, 5 CD22 CAR, 4 BCMA CAR, 3 FH CD19 CAR, and 2 GD2 CAR patients. All patients who had *C. difficile* infections had evidence of CRS and were tested because of concurrent gastrointestinal symptoms or diarrhea. Although 10/17 (58.8%) patients with *C. difficile* infections had evidence of any prior infection, only 1 patient had a documented history of a *C. difficile* infection; 4 patients had a documented bacterial infection. One patient with *C. difficile* had a history of therapeutic antimicrobials at the time of LD chemotherapy. Of note, patients were not screened for *C. difficile* colonization prior to enrollment or treatment. *C. difficile* prophylaxis with oral vancomycin was effectively used in 1 CD22 CAR patient for history of recurrent *C. difficile* infection.

Viral infections and prophylaxis. Viral infections occurred in patients treated on pCD19 CAR, CD22 CAR, BCMA CAR, and FHCD19 CAR trials and consisted of upper respiratory tract

infections (URI, n = 14; adenovirus, coronavirus, influenza, enterovirus/rhinovirus, parainfluenza virus, respiratory syncytial virus), cytomegalovirus (CMV) viremia (n = 2), CMV pneumonitis (n = 1), and both herpes simplex (n = 2) and herpes zoster infections (n = 1).

Anti-herpetic prophylaxis prior to LD chemotherapy with acyclovir or valacyclovir was used in 111 (68.5%) patients. Two patients developed a herpes simplex infection (n = 1) and herpes zoster (n = 1) infection between D0 and D30 despite prophylaxis. Of 51 patients not receiving anti-herpetic prophylaxis, only one pCD19 CAR patient developed a herpes simplex infection on day 17.

Fungal infections and prophylaxis. Fungal infections were rare events occurring in only 2 patients (3.3%) treated with anti-CD22 CAR T cells. Both infections were presumptive fungal infections of the lungs based on imaging findings without confirmed organisms and were identified on day +1 in a patient on fungal prophylaxis at the time of conditioning chemotherapy and on day +10 in a patient with a history of a fungal infection within 100 days of LD chemotherapy.

Fifty-nine patients (36.4%) received fungal prophylaxis (43 received azole, 16 received micafungin), the majority of whom were patients with ALL (n = 56). Fifteen patients continued therapeutic antifungals that they were on prior to LD chemotherapy; thus, 68.6% of ALL patients received some type of antifungal coverage prior to D0. Only one patient on each of the BCMA CAR, FHCD19 CAR, and GD2 CAR trials received fluconazole as fungal prophylaxis.

One hundred and fifty-seven patients (97%) received *Pneumocystis* pneumonia (PCP) prophylaxis; no patients developed PCP pneumonia at the start of conditioning chemotherapy; for the majority of patients, this was continued through day 30 or until CD4 count was >200.

Relationship between infection and clinical course

Fever and CRS and neutropenia. In patients with an infection after D0, the median time to CRS onset was 6 days (range 0-13), while the median time to infection was 10 days (range 0-30). We sought to explore the relationship between fever and neutropenia and CRS, given the implication of F&N on infection. Among 91 (56.2%) patients with at least one episode of F&N between D0 and D30, 82 (90.1%) had CRS, and 32 (35%) had an infection, the majority of which (19/32, 59.3%) was bacterial. All 4 patients with a grade 4 infection experienced F&N. Severe neutropenia (ANC <500 cells/mm^3), with or without fever, was a common occurrence, occurring in 130 (80.8%) of patients, but did not correlate with infection. Among all 76 infections between LD and D30, 39 (51.3%) started during severe neutropenia; 37 (48.7%) occurred when the patient was not severely neutropenic.

Factors associated with infections D0 to D30. Factors identified by univariate analysis that were associated with occurrence of infections D0 to D30 are shown in Figure 4. Increased prior lines of antimalignancy therapy or having at least 1 infection within 100 days of LD chemotherapy independently increased the risk of infection D0 to D30. Additional variables analyzed are described in the supplemental Appendix.

Incidence of any infection did not vary by CRS grade (30.2% vs 31.8% in patients with Gr 1-2 CRS vs Gr 3-4 CRS). Utilization of immunosuppression with corticosteroids or tocilizumab for CRS management was associated with a higher incidence of infections on univariate analysis. Patients with evidence of F&N at any time between D0 and D30 had higher rates of infections compared with patients without F&N.

Bacterial infections (excluding *C. difficile*) occurred more often in patients who had more lines of prior chemotherapy and in those with an ANC <500 cells/mm^3 or ALC <200 cells/mm^3. Bacterial infections occurred more frequently in patients with an incidence of F&N between D0 and D30 and in those who received tocilizumab or corticosteroids.

C. difficile infections were associated with and exclusively occurred in patients with CRS: 0 of 33 patients without CRS had a *C. difficile* infection while 17/129 patients (13.2%) with CRS had a *C. difficile* infection (P = .02). *C. difficile* infections were not associated with cytopenias, immunosuppression, antibacterial prophylaxis, or prior infection within 100 days of LD chemotherapy.

Viral infections were more likely to occur in patients who had an infection within 100 days of LD chemotherapy and in those who received corticosteroids between D0 and D30.

Based on the univariate screening tests performed, (a) number of prior chemotherapy lines, (b) previous infection within 100 days of LD, and (c) extent of bone marrow involvement were identified as potentially associated with infection incidence from D0 to D30. Following a univariate logistic regression analysis, both prior chemotherapy and previous infection were found to remain as candidates for multiple logistic regression. Bone marrow involvement at baseline was less well associated with outcome and only accounted for disease with marrow involvement and thus was excluded from further consideration. Multivariable analysis confirmed only prior lines, and previous infections were associated with infection between D0 and D30.

Discussion

As a quaternary care center focused on the implementation of novel phase 1 CAR T-cell trials targeting unique antigens and across various diseases, we sought to evaluate the rate and type of infections across 5 different trials encompassing 4 antigen targets, a range of ages, and a host of tumor types. With a focus on infections in the first 30 days, the overall rate of infection was 32.7% from LD to D30. Despite the extensive prior therapy this patient population received, 20.4% of whom had received a prior CAR T cell, life-threatening infections were rare, and the overall incidence of infection was comparable to published studies of infection with CD19 CAR.[9-13,26]

We identify infection incidence and risks in patients with MM receiving BCMA CAR T-cell therapy and in those with B-ALL receiving CD22 CAR providing insights into infections with these more novel constructs. In this study, MM patients accounted for the largest proportion of infections, underscoring the fact that MM leads to long-term immunocompromise in patients. Of the CAR constructs studied, the CD22 CAR construct was associated with the highest proportion of infections, consisting mostly of bacterial and viral infections. Notably, this represented a very heavily pretreated population, many who were refractory to all standard and some investigative therapies, with a large majority receiving prior CD19 targeting. In addition, this cohort of patients had a higher incidence of hemophagocytic lymphohistiocytosis (HLH)-like toxicities requiring additional immunosuppression, (https://pubmed.ncbi.nlm.nih.gov/34525183/)[27] which may have compounded infection risk and needs to be monitored for.

In similar to prior studies,[9] we found that prior lines of therapy and history of recent infection (within 100 days of LD chemo) were independent of increased risk of infection. The presence of both factors compounded a patient's infection risk within the first 30 days. With many unknown elements of how novel CAR T cells may pose unique infection risks, these generalizable risk factors may serve to identify patients receiving novel CAR T-cell therapies who may be at increased risk for infectious complications and potentially intervene to reduce risk. Determining the relationship between immunosuppression use with corticosteroids or tocilizumab and infection risk after D0 continues to remain a challenge.[9,12,13,28] Use of both agents, however, was associated with infections in our univariate analysis. As patients with high-grade CRS often require both immunosuppressive agents, it is unclear whether the CRS severity alone or the immunosuppression required to treat it is a greater contributor to infection risk. Interestingly, a study analyzing 391 patients with grade 1 CRS found no difference in rates of infection between patients who received tocilizumab and those who did not.[28] Although 90.1% of patients with F&N had evidence of CRS in the first 30 days, only 35% had an infection during this time period. More work is required to understand the interplay of F&N, CRS, and infection risk and whether the risk of F&N is similar to patients undergoing induction chemotherapy.

Antimicrobial prophylaxis differed between patient populations and trial but appeared to be nearly uniformly used for and effective in preventing PCP. Approximately two-thirds of patients received herpes simplex virus (HSV) prophylaxis with a low occurrence of HSV. Fungal infections were reassuringly rare, with only 2 possible infections occurring in heavily-pretreated ALL patients, the majority of whom received prophylaxis. The data highlight that MM, NHL, and solid-tumor patients without a prior history of or specific risk for fungal infections were not at high risk of fungal infections post CAR. Rates of bacterial infection were comparable between those who did and did not receive levofloxacin prophylaxis, prompting our center to develop guidelines to restrict antibacterial prophylaxis to only those at inherently high risk (https://pubmed.ncbi.nlm.nih.gov/21258094 https://pubmed.ncbi.nlm.nih.gov/30179565/).

Limitations of our study include the fact that this is based on a heterogeneous population with subgroup analysis and is limited by the relatively small numbers of each group. Furthermore, although we identified a relatively high rate of *C. difficile* detection, because of the lack of baseline assessment we cannot be certain if the *C. difficile* represented a true infection or detection of colonization with diarrhea associated with CRS. Prospective surveillance is needed to understand this infection profile. Additionally, assessing for infections beyond day 30 could not be accurately performed as most patients returned to their home institutions following the first assessment with only interval follow-up.

In conclusion, this is one of the largest studies to date looking at the rate of infections occurring in patients with a variety of underlying malignancies and is one of the few that pools both pediatric and adult populations.[13] How rates of infection change in future patient populations as CAR T-cell therapy moves closer to frontline therapy (and patients receive fewer prior lines of therapy) will be important to track, although uncertainties about the impact of novel CAR T-cell strategies and their respective infection risk require vigilance in optimal management, particularly in these highly refractory populations. Through a thoughtful assessment of infection history, prior therapies, and overall infection risk profile, developing tailored management plans to mitigate and prevent infections will be needed to reduce infection risk, particularly as novel strategies are tested.

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Authorship

Contribution: L.M. and N.N.S. wrote the first version of the manuscript and designed the study; L.M., N.N.S., B.Y., S.A.S., J.C.M., and T.J. collected primary data from patient charts; L.M., N.N.S., and S.M.S. did primary data analysis; T.P., D.W.L., R.N.K., C.L.M., T.J.F., J.G.-B., V.N., and J.N.K. provided critical input and contributed to the writing of select sections within the manuscript; L.M., N.N.S., C.L.M., D.W.L., B.Y., and J.N.K. provided patient care and generated, collected, and analyzed data. No nonauthor wrote the first draft or any part of the paper; and all authors contributed to reviewing the final manuscript and have agreed to be coauthors.

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