Cancer Immunology, Immunotherapy (2025) 74:149
https://doi.org/10.1007/s00262-025-03998-1

RESEARCH

Infectious complications distribution following CLL1 CAR-T cell therapy for acute myeloid leukemiass

Jianmei Xu[1] · Huan Zhang[1] · Yifan Zhao[1] · Xiaomei Zhang[3] · Shujing Guo[1] · Xiaoxue Shi[1] · Xia Xiao[2] · Hairong Lyu[2] · Yu Zhang[2] · Xiaoyuan He[2] · Mingfeng Zhao[2]

Received: 6 April 2024 / Accepted: 24 February 2025 / Published online: 15 March 2025

Abstract

The CLL1-targeted chimeric antigen receptor T (CAR-T) cell therapy offers a novel therapeutic approach for refractory or relapsed acute myeloid leukemia (AML).The targeted elimination of tumor cells by CLL1 CAR-T therapy also induces cytotoxic effects on neutrophils, leading to a severe granulocytopenia, thereby significantly increasing the risk of infectious complications during CAR-T therapy. However, the infectious complications associated with this strategy have not been comprehensively investigated. The objective of this study was to evaluate the incidence rate of infectious complications within a 28-day period in a cohort of 51 patients who underwent CLL1 CAR-T cell infusion. Meanwhile, the univariate and multivariate analyses were employed to access the risk factors of infectious complications during CLL1 CAR-T therapy. The study observed a total of 46 infection events in 32 out of 51 patients (63%), with the median onset of infection occurring at 9 days following CAR-T cell infusion. The cumulative incidence of infection events within 28 days was 56.9% (95%CI: 50.4–61.3%), with bacterial and fungal infections being the most prevalent early infection events. The results of multivariate analysis revealed that a lower neutrophil counts prior to lymphodepletion chemotherapy (OR = 3.875, P = 0.041) and more severe complications of cytokine release syndrome (OR = 4.141, P = 0.037) were identified as independent risk factors associated with an increased likelihood of early infection events. This study examined the distribution of early infection events and identified potential risk factors, with the goal of offering guidance to physicians on implementing more effective intervention strategies to decrease treatment-related mortality rates and improve patient prognosis. This study has been registered in the Chinese Clinical Trial Registry (Trial registration number: ChiCTR2000041054).

Keywords Infection · Prevention · CLL1 CAR-T · Cytokine release syndrome · Acute myeloid leukemia

Abbreviations

AML	Acute myeloid leukemia
ASTCT	American Society for Transplantation and Cell Therapy
allo-HSCT	Allogeneic hematopoietic stem cell transplantation
ANC	Antinuclear neutrophil cell
ALC	Antinuclear lymphocyte
B-ALL	B-acute lymphoblastic leukemia
CAR-T	Chimeric antigen receptor T cell
CLL1	C-type lectin-like molecule 1
CRS	Cytokine release syndrome
CR	Complete remission
Cri	CR with incomplete hematologic response
CLL	Chronic lymphocytic leukemia
FC	Fludarabine and cyclophosphamide
ICANS	Immune effector cell associated neurotoxicity syndrome
IQR	Interquartile range
MM	Multiple myeloma
MDS	Myelodysplastic syndrome
MPN	Myeloproliferative neoplasm
MLFS	Leukemia-free state
NCCN	National comprehensive cancer network

Jianmei Xu, Huan Zhang and Yifan Zhao contributed equally as co-first authors.

✉ Mingfeng Zhao
mingfengzhao@sina.com

[1] The First Central Clinical College of Tianjin Medical University, Tianjin 300380, China

[2] Department of Hematology, Tianjin First Central Hospital, No.2 Baoshanxi Rd, Xiqing District, 300380, Tianjin, China

[3] Nankai University School of Medicine, Tianjin 300380, China

NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NHL	Non-Hodgkin's lymphoma
NR	No response
ORR	Objective response rate
R/R	Refractory and relapsed
SD	Standard deviation

Introduction

Acute myeloid leukemia (AML) is the most common hematological malignancy in adult patients. The current treatment methods mainly include chemotherapy, targeted drug therapy and hematopoietic stem cell therapy, but the overall treatment effect is limited, and safer and effective treatment methods are urgently needed. Chimeric antigen receptor T cell (CAR-T) therapy is a new type of adoptive immunotherapy, which has achieved ideal therapeutic effects in hematological tumors such as B-acute lymphoblastic leukemia (B-ALL) and multiple myeloma (MM) [1]. However, the application of CAR-T cell therapy in AML is limited by the scarcity of highly specific and uniformly expressed target antigens. Antigens such as CD33, CD123, Lewis Y, etc. have not been further utilized in clinical practice because of insufficient efficacy or significant side effects.

The C-type lectin-like molecule 1 (CLL1) is a type II transmembrane glycoprotein that exhibits widespread expression in AML blasts, while remaining absent in normal hematopoietic stem cells and other normal tissues [2]. In 2018, Wang et al. first constructed CAR-T targeting CLL1, and demonstrated their potent cytotoxicity against AML cell lines and primary cells in vitro as well as in mouse models, while preserving the integrity of hematopoietic stem cells [3]. The recruitment and implementation of several clinical trials for AML involving CLL1 CAR-T therapy are currently underway. The results of phase I clinical study of Mingfeng Zhao et al. showed that the effectiveness of CLL1 CAR-T in the treatment of refractory or relapsed (R/R) AML was about 70%. Moreover, most patients achieved long-term remission after hematopoietic stem cell transplantation [4]. Hui Zhang et al. claimed that among eight patients with Relapsed and refractory acute myeloid leukemia (R/R AML), six patients achieved remission after treatment with CLL1 CAR-T cells [5]. Xiaowen Tang et al. also successfully achieved remission through CLL1 CAR-T in two patients who relapsed with CD38 CAR-T cells [6]. The above studies have proved that CLL1 CAR-T can effectively improve the prognosis of AML patients, which is expected to provide a new treatment option for AML patients.

However, it is worth noting that serious infections may occur during the process of CAR-T cell eradication. Currently, there were five studies (four in adults and one in children) specifically investigating the incidence of infection among patients undergoing CAR-T therapy [7–11]. The early infection rate (< 30 days) ranged from 17 to 42% across these five cohorts, while the late infection rate (30–120 days) varied between 14 and 31%. Most reported infections exhibit mild to moderate severity, with bacterial infections being more prevalent during the initial stages and viral infections becoming more common after the first month. The incidence of invasive fungal infections is infrequently reported, with rates of up to 8% and 3% in the early and late stages, respectively. The literature on infectious complications of CLL1 CAR-T cell therapy in AML is limited. The targeted elimination of tumor cells by CLL1 CAR-T therapy also induces cytotoxic effects on neutrophils, resulting in a severe grade 4 granulocyte deficiency that can only be alleviated through hematopoietic stem cell transplantation [12].Theoretically, the incidence and severity of concurrent infections during CLL1 CAR-T therapy may be elevated. Furthermore, a range of side effects associated with cytokine inflammatory storm, such as cytokine release syndrome (CRS), immune effector cell associated neurotoxicity syndrome (ICANS) and their corresponding interventions, could potentially exacerbate infections during CLL1 CAR-T therapy.

The present study aims to conduct a comprehensive analysis of infectious complications before and after therapy, encompassing the site of infection, distribution of pathogenic microorganisms, and identification of potential risk factors for infection. Our research endeavors to offer guidance in predicting and managing infectious complications in patients with R/R AML following CLL1 CAR-T cell therapy, thereby enhancing patient prognosis and reducing mortality associated with infections.

Materials and methods

Patients and data collection

From January 2021 to December 2023, a total of 56 patients with R/R AML were enrolled and received infusion of CLL1 CAR-T cells. The enrolled patients in this study were definitively diagnosed with acute myeloid leukemia based on the diagnostic criteria outlined by the National Comprehensive Cancer Network (NCCN) for AML and exhibited refractoriness or relapse following multiple lines of treatment[13].The present study has obtained approval from the institutional review board of Tianjin First Central Hospital and has been officially registered in the Chinese Clinical Trial Registry (Clinical trial No. ChiCTR2000041054).

All participants enrolled in this retrospective analysis provided informed consent in accordance with the principles outlined in the Declaration of Helsinki. The general characteristics of patients, disease information at initial diagnosis,

previous treatment history, and relevant laboratory and imaging examinations before and after infusion of CLL1 CAR-T cells were collected. The infections that occurred within the 30-day period prior to and 28-day period following CAR-T cell infusion were accurately documented, while patient data was analyzed up until 28 days after CAR-T cell infusion or until follow-up failure, disease recurrence, or death. All patients were followed up through outpatient examination and telephone interview.

CLL1 CAR-T cells manufacturing process and lymphodepletion chemotherapy regimens

Excluding the five patients who withdrew from the experimental group due to failed CAR-T manufacturing, a total of 51 patients were successfully infused with CLL1 CAR-T cells. The CAR-T cell preparation process was conducted at the laboratory of Department of Hematology at Tianjin First Central Hospital. This process included white blood cell separation and enrichment, T cell magnetic bead sorting and stimulation, lentivirus-mediated T cell transfection, CAR-T cell culture, quality control prior to infusion, and amplification detection of CAR-T levels after infusion. All patients received meticulous inpatient care throughout the treatment period. Additionally, all patients underwent lymphodepletion chemotherapy using the fludarabine and cyclophosphamide (FC) regimen, which included fludarabine and cyclophosphamide. The patients with a substantial tumor burden were additionally administered decitabine or cytarabine.

Supportive care and adverse events management

Acyclovir prophylaxis for herpes simplex virus and varicella-zoster virus was initiated prior to FC regimen application until 3–6 months post CAR-T cell infusion. Compound sulfamethoxazole was administered for Pneumocystis carinii prevention until 6 months after CAR-T cell infusion. Posaconazole was given for fungal prophylaxis until neutrophil count exceeded 0.5×10^9/L on day 28 after CAR-T cell infusion if neutrophil count was $< 0.5 \times 10^9$/L or tocilizumab or steroids were required for CRS or ICANS. No prophylactic antibiotics were provided in the absence of bacterial infection. Empirical intravenous antibiotics were administered to patients presenting with neutropenia and fever, followed by tailored antibiotic treatment recommendations based on etiological evidence. The serum levels of immunoglobulin G (IgG) were assessed prior to the infusion of CLL1 CAR-T cells and at 14- and 28-days post-administration. Intravenous immunoglobulin supplementation is recommended for individuals with serum IgG levels below 400 mg/dl.

The grading of CRS and ICANS was primarily assessed based on the American Society for Transplantation and Cell Therapy (ASTCT) grading system [14], with grade 3–4 being defined as severe CRS/ICANS. The administration of tocilizumab and corticosteroids was considered for patients presenting with a CRS or ICANS grade exceeding 2. The remaining adverse events related to CLL1 CAR-T cell infusion were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5.0.

Definition of infection and laboratory tests

Infection onset was defined as the day of the first clinical diagnosis or microbiologic return, regardless of mode of onset. The infection discussed in our study comprised infections that were microbiologically certified and clinically evaluated, which were further classified into bacterial, viral, fungal, or parasitic types [15, 16].The severity of infection was categorized as mild, moderate, severe, and life-threatening [17, 18]. The infection is classified as mild if no treatment is necessary, moderate if only oral treatment or minimal supportive care is required, and severe if intravenous antibiotics are needed or it is associated with other severe clinical conditions. Life-threatening infections are characterized by symptoms that can be fatal.

The routine infection-related tests included: a minimum of two blood cultures, urine and stool cultures, testing for Clostridium difficile toxin, procalcitonin, C-reactive protein, ferritin levels, G test or GM test, as well as virus screening for influenza A/B virus, rhinovirus, respiratory syncytial virus, adenovirus, novel coronavirus, and BK virus prior to administering the first dose of FC regimen. If the patient presents with symptoms of fever, it is crucial to determine whether it is caused by an infection or CRS, as identified by three experienced clinicians. The microbiological cultures, histopathological smears, or pathogen DNA sequencing were repeated in case the patient presented with infectious clinical symptoms such as coughing, production of sputum, diarrhea, dysuria, abscess formation, or skin ulceration.

Statistical analysis

SPSS 26.0 software was utilized for conducting statistical analysis. The data were presented as mean ± standard deviation (SD) for normally distributed data, while median and interquartile range (IQR) were used for non-normally distributed data. Intergroup comparisons were performed using either t-test or nonparametric test, depending on the appropriateness of the method. In order to identify risk factors associated with infection following CAR-T cell infusion, both univariate and multivariate logistic regression analyses were conducted, considering $p \leq 0.05$ as statistically significant.

Results

Patient characteristics

The study enrolled a total of 51 patients with R/R AML, and their baseline characteristics are shown in Table 1. The median age of the patients was 35 years (range: 10–73), with 24 females and 27 males. Additionally, a prior history of myelodysplastic syndrome or myeloproliferative neoplasm was observed in nine patients, accounting for approximately 17.6% of the cohort. 33.3% patients presented with primary refractory AML, while 66.7% patients experienced relapse after prior remission. All patients had a median of 4 (range: 1–13) previous lines of therapy, and 25.5% patients had undergone allogeneic hematopoietic stem cell transplantation. The study included 23.5% patients with a Karnofsky performance status (KPS) score of 60–80 and 76.5% patients with a KPS score of 80–100. Prior to CAR-T cell therapy, 45.1% patients had extramedullary infiltration, with 25.5% of them having central nervous system involvement.

Table 1 Clinical characteristics of patients enrolled in this study

Baseline characteristics	$N=51$, (%)
Age	
<40, *n* (%)	30 (58.8%)
≥ 40, *n* (%)	21 (41.2%)
Gender	
Female, *n* (%)	24 (47.1%)
Male, *n* (%)	27 (52.9%)
Karnofsky performance score	
60–80, *n* (%)	12 (23.5%)
80–100, *n* (%)	39 (76.5%)
History of MDS/MPN	
Yes, *n* (%)	9 (17.6%)
No, *n* (%)	42 (82.4%)
Prior lines of anti-tumor treatment	
1–2, *n* (%)	19 (37.3%)
≥ 3, *n* (%)	32 (62.7%)
Bone marrow malignant cell percentage	
$<50\%$, *n* (%)	33 (64.7%)
$\geq 50\%$, *n* (%)	18 (35.3%)
Prior allogeneic HSCT history	
Yes, *n* (%)	13 (25.5%)
No, *n* (%)	38 (74.5%)
ANC cells at pre-lymphodepletion	
<500, *n* (%)	22 (43.1%)
≥ 500, *n* (%)	29 (56.9%)
ALC cells at pre-lymphodepletion	
<200, *n* (%)	35 (68.6%)
≥ 200, *n* (%)	16 (31.4%)
Level of IgG at pre-lymphodepletion	
<400 mg/dL, n (%)	19 (37.3%)
≥ 400 mg/dL, n (%)	32 (62.7%)
CLL-1 positivity in AML blasts	
$<80\%$, *n* (%)	20 (39.2%)
$\geq 80\%$, *n* (%)	31 (60.8%)
CLL1 CAR-T cell doses	
$0.5–1.5\times10^6$ cells/kg, *n* (%)	28 (54.9%)
$1.6–3\times10^6$ cells/kg, *n* (%)	23 (45.1%)

MDS; myelodysplastic syndromes, MPN; myeloproliferative neoplasm, HSCT; hematopoietic stem cell transplantation, CLL-1; C-type lectin-like molecule 1, ANC; antinuclear neutrophil cell, ALC; antinuclear lymphocyte, CAR-T; chimeric antigen receptor T

The median bone marrow tumor burden detected by flow cytometry was 27.1% (2.3–93.9%), and the positive expression rate of CLL1 in AML cells was 88.1% (55.3–99.5%). The median dose of infused CLL1 CAR-T cells was 1.25 $(0.20–3.00)\times10^6$/kg. In this cohort of 51 patients, the median duration of neutropenia was 14 days (range: 5–21) and 23 individuals exhibited neutropenia prior to CAR-T cell infusion. Further analysis of the recovery time of the remaining 28 patients' granulocyte showed a median recovery time of 12 days (range: 7–21). The pre-lymphodepletion median neutrophil count was 0.72 $(0.00–7.15)\times10^9$/L, lymphocyte count was 0.70 $(0.04–10.14)\times10^9$/L, C-reactive protein (CRP) was 49.3 (3.5–156.0) mg/L and IgG level was 492.0 (179.0–1020.0) mg/dl.

Infections and antimicrobial prophylaxis prior to CAR-T cell infusion

Out of 51 patients with R/R AML, 51.0% (26/51) had a history of infection within the 30 days preceding lymphodepletion. Among these, bacterial infections were observed in 61.5% cases, fungal infections in 19.2% cases, and viral infections in 15.4% cases. The organs primarily involved in infection are the respiratory tract, urinary tract, skin and soft tissue, and bloodstream. The infection status is shown in Fig. 1. The patients were all administered adequate anti-infection treatment, and the lymphodepletion chemotherapy was conducted subsequent to successful eradication of the infection. All patients enrolled in this study received prophylactic acyclovir and compound sulfamethoxazole during CAR-T therapy, while posaconazole tablets were administered to 22 patients with neutropenia for the prevention of fungal infections. The remaining patients, excluding those with evidence of bacterial infection, did not receive prophylactic antibacterial treatment.

Evaluation of efficacy after CAR-T cell infusion

The therapeutic response of all patients was assessed on day 14 following CAR-T cell infusion, in accordance with the guidelines formulated by NCCN. Among them, three patients were excluded from evaluation due to complications or discontinuation of treatment. Out of the 48 evaluable

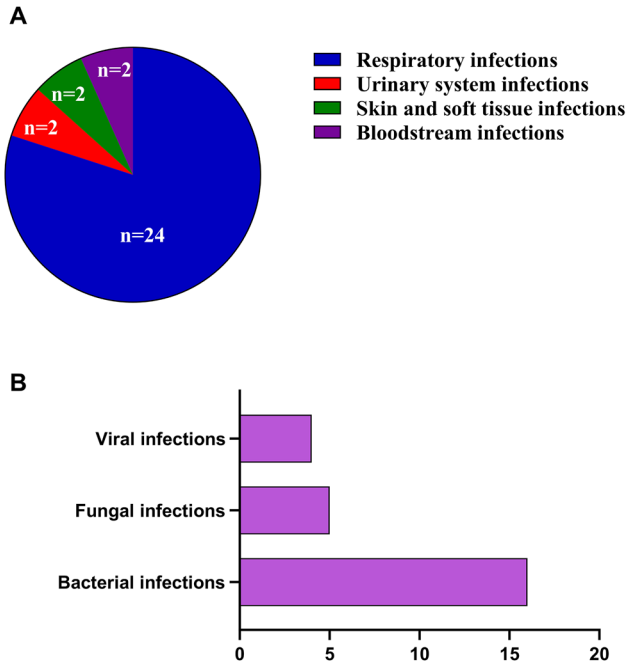

Fig. 1 Infections occurring in the 30 days prior to lymphodepletion. The patients included in this study evaluated the infections that occurred within the 30-day period preceding lymphodepletion. **A** The distribution of infected organs prior to lymphodepletion, **B** the detected microorganism species in patients who had experienced infection prior to lymphodepletion

patients, 35.4% (17/48) achieved complete remission (CR), 18.8% (9/48) achieved CR with incomplete hematologic response (CRi), and 14.6%(7/48) achieved morphologic leukemia-free state (MLFS).

After receiving CLL1 CAR-T therapy, a total of 52.9% (27/51) patients underwent bridging allogeneic hematopoietic stem cell transplantation (allo-HSCT) based on their disease response and transplantation intention. Among them, 81.5% (22/27) patients achieved CR, CRi, or MLFS after CAR-T therapy, while the remaining 18.5% (5/27) patients did not respond to CAR-T therapy. The median interval from CAR-T therapy to bridging transplantation was 23 days (range: 16–58 days). The five patients who showed no response to CLL1 CAR-T therapy all achieved CR after transplantation.

Adverse events and corresponding management

The study primarily focused on adverse events associated with CAR-T therapy occurring within a 28-day period. Among them, 94.1% experienced CRS, with 54.9% (28/51) having grade 1–2 CRS and 39.2% (20/51) experiencing grade ≥ 3 CRS. 21.6% (11/51) of the patients demonstrated ICANS, with grade 1–2 being the most prevalent ($n = 8$, 15.7%), while three patients experienced grade ≥ 3 ICANS, as shown in Table 2. Among the cohort of 48 patients presenting with CRS and/or ICANS, tocilizumab was administered to 41.7% (20/48) of individuals, low-dose corticosteroids (≥ 10 mg/day dexamethasone equivalent) were prescribed for 45.8% (22/48), high-dose corticosteroids (≥ 500 mg/day methylprednisolone equivalent) were given to only a small proportion of patients at 4.2% (2/48), and plasma exchange was performed on a subset of individuals accounting for 10.4% (5/48).

Hematologic toxicity was frequently observed following CLL1 CAR-T therapy, with leukopenia being experienced by all patients, of whom 96.1% had a grade ≥ 3 leukopenia. By day 28 post-CAR-T infusion, only five patients achieved normal leukocyte counts while the remaining 46 patients continued to exhibit agranulocytosis. However, it is noteworthy that 43.1% (22/51) of the patients were in agranulocytosis due to non-remission of the disease prior to lymphodepletion.

The term hypogammaglobulinemia is defined as IgG levels below 400 mg/dL, which were observed in 61% ($n = 31$) of recipients who underwent CLL1 CAR-T cell therapy, with a median IgG level of 373.0 mg/dL (range: 51.0–3210.0). Additionally, patients diagnosed with hypogammaglobulinemia received a median of 3three (range: 1–8) doses of intravenous immunoglobulin treatment over the course of 28 days. The adverse events shown in Table 2 are related to the study, and no fatal adverse events were reported.

Infection episodes and factors after CAR-T cell therapy that were associated with increased risk for infection

The cumulative incidence curves and infection severity of any, bacterial, viral, and fungal infections within 28 days after CLL1 CAR-T therapy are shown in Fig. 2. Within the period of 0 to 28 days following CART cell infusion, a total of 46 infection events were observed in 63% (32/51) of patients, all of whom experienced concurrent CRS.

Of the 32 patients with early infection, 65.6% experienced a single infection event, while 34.4% experienced multiple infection events (2 events, $n = 8$;3 events, $n = 3$), with a median infection time of 9 days (range: 2–24), which was delayed by 4 days compared to the median time for CRS response. In the absence of mild infections, moderate infections accounted for 41% ($n = 13$) of the early infection events, severe infections accounted for 44% ($n = 14$), and life-threatening infections accounted for 15% ($n = 5$). Two patients required admission to the intensive care unit and four patients succumbed to extremely severe infections.

The most frequently observed infection sites included respiratory tract infections ($n = 21$), bloodstream infections ($n = 6$), and skin and soft tissue infections ($n = 3$). One patient presented with a urinary tract infection, while

Table 2 Adverse events after CLL1 CAR-T cells infusion

Adverse events ($N=51$)	All grades	Grade 1–2	Grade 3	Grade 4
CRS, *n* (%)	48 (94.1%)	28 (54.9%)	20 (39.2%)	0 (0%)
ICANS, *n* (%)	11 (21.6%)	8 (15.7%)	3 (5.9%)	0 (0%)
Hematological				
Leukopenia, *n* (%)	51 (100.0%)	2 (3.9%)	9 (17.6%)	40 (78.4%)
Anemia, *n* (%)	44 (86.3%)	7 (13.7%)	18 (35.3%)	19 (37.3%)
Thrombocytopenia, *n* (%)	47 (92.2%)	6 (11.8%)	33 (64.7%)	8 (15.7%)
Increased aminotransferase, *n* (%)	15 (29.4%)	12 (23.5%)	2 (3.9%)	1 (2.0%)
Increased creatinine, n (%)	6 (11.8%)	5 (9.8%)	1 (2.0%)	0 (0%)
Cardiovascular events				
Heart failure, *n* (%)	6 (11.8%)	5 (9.8%)	1 (2.0%)	0 (0.0%)
Malignant arrhythmia, *n* (%)	4 (7.8%)	4 (7.8%)	0 (0.0%)	0 (0.0%)
Gastrointestinal				
Nausea, *n* (%)	24 (47.1%)	21 (41.2%)	3 (5.9%)	0 (0%)
Decreased appetite, n (%)	31 (60.8%)	25 (49.0%)	4 (7.8%)	2 (3.9%)
Constipation, *n* (%)	7 (13.8%)	6 (11.8%)	1 (2.0%)	0 (0%)
Coagulopathy				
APTT prolonged, *n* (%)	21 (41.2%)	19 (37.3%)	2 (3.9%)	0 (0%)
PT prolonged, *n* (%)	17 (33.3%)	15 (29.4%)	2 (3.9%)	0 (0%)
D-dimer elevated, *n* (%)	31 (60.8%)	24 (47.1%)	4 (7.8%)	3 (5.9%)
Electrolyte disorder				
Hypokalemia, *n* (%)	15 (29.4%)	13 (25.5%)	2 (3.9%)	0 (0%)
Hyponatremia, n (%)	10 (19.6%)	9 (17.6%)	1 (2.0%)	0 (0%)
Hypocalcemia, n (%)	14 (27.5%)	9 (17.6%)	4 (7.8%)	1 (2%)
Hypoalbuminemia, n (%)	9 (17.6%)	7 (13.8%)	2 (3.9%)	0 (0%)

CRS; cytokine release syndrome, ICANS; immune effector cell associated neurotoxic syndrome, PT; prothrombin time, APTT; activated partial thromboplastin time

another had a digestive system infection. Three patients were clinically diagnosed with pneumonia based on imaging criteria; however, it was inconclusive whether the cause was bacterial or viral. The specific locations of the infections and corresponding pathogens are shown in Fig. 3. Bacterial infections accounted for the highest proportion among all infectious pathogens, with a cumulative incidence of 56.9% (95% CI: 50.4–61.3%) within a 28-day period. Among the identified pathogens, there were 25 cases of Gram-negative bacteria (including Acinetobacter baumannii [$n=6$], Enterobacter cloacae [$n=1$], Pseudomonas aeruginosa [$n=4$], Klebsiella pneumoniae [$n=5$], Proteus mirabilis [$n=2$], Escherichia coli [$n=2$], and Smmaltophilia [$n=5$]), while Gram-positive bacteria (Enterococcus faecium) were detected in four cases. Fungal infection ranked as the second most prevalent early infectious event, exhibiting a 28-day cumulative incidence of 15.6% (95%CI: 11.7–19.1%), encompassing two instances of potential invasive pulmonary aspergillosis. Among the confirmed cases of fungal infection, there were two cases of Rhizobium microsporum, one case of Aspergillus, two cases of mucor, and three cases of Candida albicans. The incidence rate of viral infection was relatively low, with a cumulative incidence rate of 11.7% (95%CI: 9.3–14.8%) within a period of 28 days. The viral infections mainly included leptospirosis virus ($n=1$), Herpesvirus ($n=2$), Cytomegalovirus ($n=2$), and Epstein-barr virus ($n=1$). The occurrence of end-organ disease associated with these viruses was not observed in patients with viral infection. Among them, 11 patients had concurrent infections with multiple pathogens, six patients had co-infection of bacteria and fungi, four patients had bacterial infection combined with viral infection, and one patient had simultaneous bacterial, fungal, and viral infections.

The study further examined the high-risk factors for infection in CLL1 CAR-T cell therapy. Univariate analysis was conducted to identify potential factors contributing to infection, revealing that the number of previous treatment lines ($P=0.027$), infection within 30 days after CAR-T infusion ($P=0.045$), pre-lymphodepletion neutrophil level ($P=0.046$), incidence of CRS ($P=0.011$), and IgG level ($P=0.035$) were associated with an increased risk of early infection following CLL CAR-T infusion. Logistic multivariate analysis confirmed that pre-lymphodepletion neutrophil level (OR = 3.785, 95%CI: 0.8605–20.34) and incidence

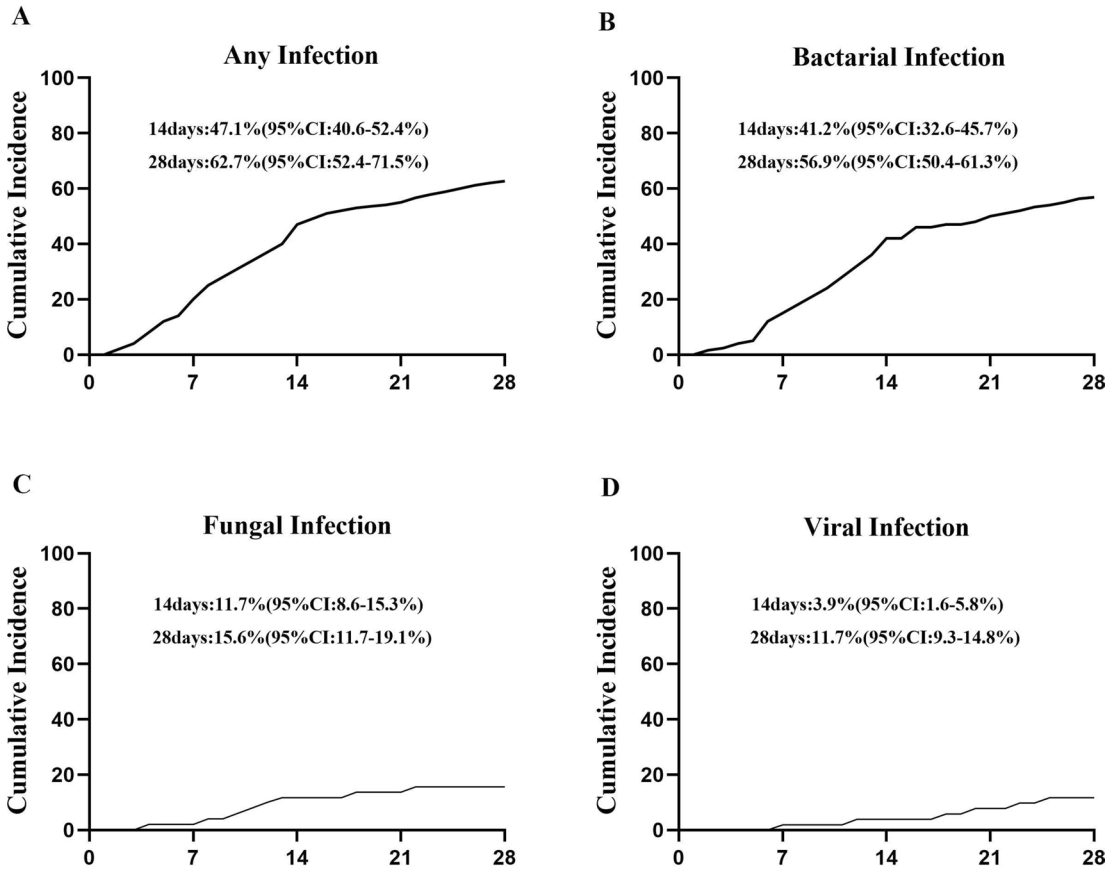

Fig. 2 Cumulative incidence and severity of infection after 28 days following CAR-T cell infusion The cumulative incidence rate of infections occurred in patients during the 14-day and 28-day periods following infusion of CLL1 CAR-T cells. **A**–**D** The cumulative incidence of any categories, including bacterial, viral, and fungal infections, was observed at 14 days and 28 days following the infusion of CAR-T cells

of CRS (OR = 4.141, 95%CI: 0.8840–24.99) independently served as prognostic factors for heightened infection risk, as shown in Table 3.

Discussion

The occurrence of infection is frequently observed in CAR-T cell therapy. Food and Drug Administration-approved CAR-T cell products have been reported to induce infection in 45–72% of patients, with severe infections occurring in 12% to 48% of patients. These rates may vary depending on the specific CAR targets and indications for the same target [19, 20]. The infection profile of CD19 CAR-T cell therapy in 133 patients with Non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), and Acute lymphoblastic leukemia (ALL) was described by Hill et al. Within the initial 28 days post-treatment, a total of 43 infection events were observed in 30 (23%) patients, with an incidence rate of any infection being 29.8% for ALL patients, 20.8% for CLL patients, and 17.7% for NHL patients. The majority of infections (50%) exhibited mild to moderate severity, while two deaths were attributed to infections [7]. The infection rate in pediatric patients appears to be comparable to that observed in adults, with bacterial pathogens being responsible for the majority of severe or life-threatening infections [8].

Patients receiving CAR-T cell therapy are at an elevated risk of infection due to prior immunosuppression, lymphodepletion chemotherapy, tocilizumab and/or steroid hormone toxicity, target effects of hypogammaglobulinemia, and prolonged cytopenia [9]. The use of prophylactic antibiotics before CAR-T cell therapy is a current research focus for mitigating the risk of infection associated with this treatment modality [9]. The role of antimicrobial prophylaxis prior to lymphodepletion remains uncertain, and certain institutions opt for fluoroquinolone prophylaxis during severe neutropenia ($ANC < 0.5 \times 10^9/L$) [7]. Most institutions recommend the prophylactic administration of acyclovir or valacyclovir until 6 months post CAR-T cell therapy before lymphodepletion, emphasizing that preventive treatment may reduce the risk of herpes virus, Epstein-Barr virus, and

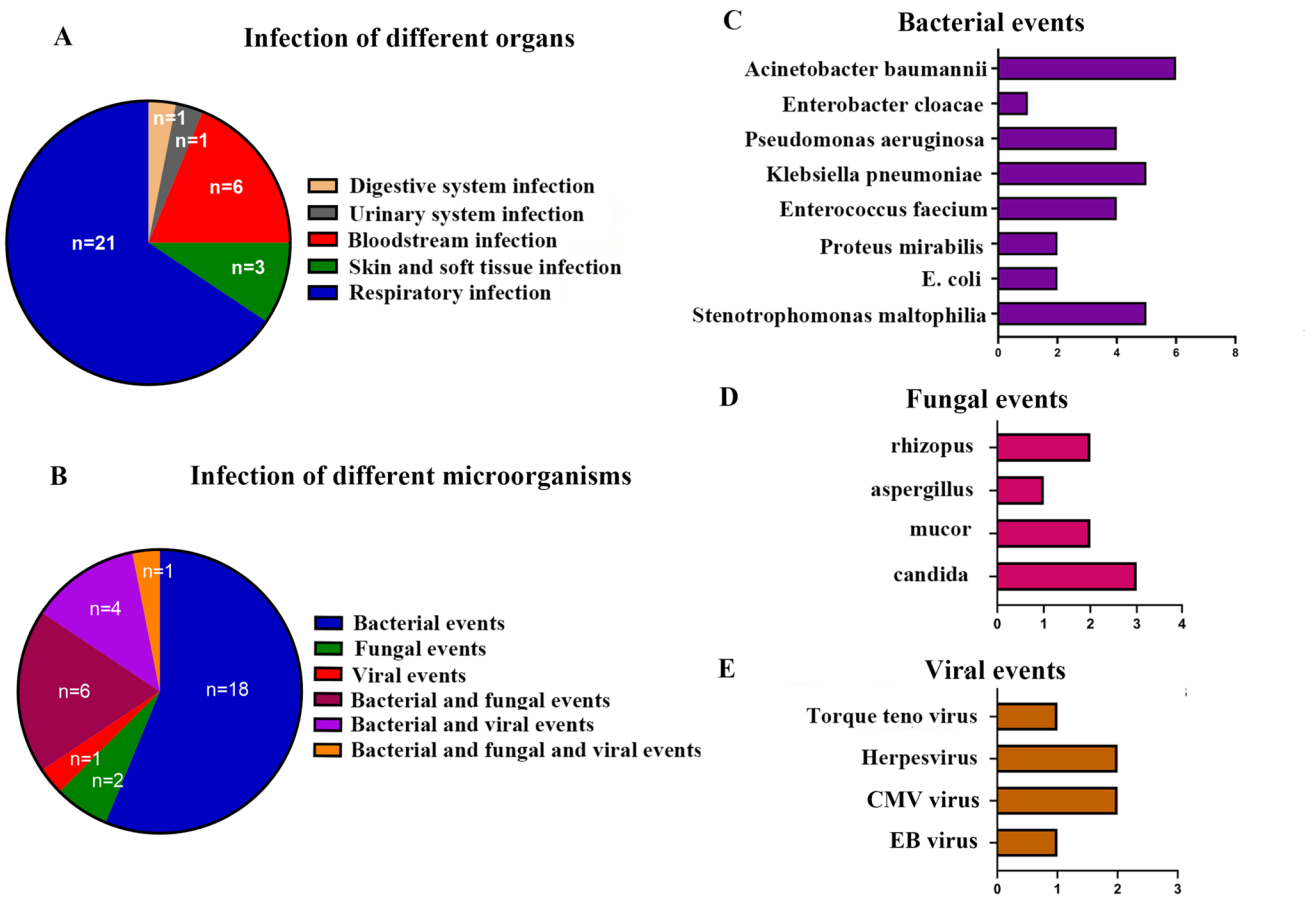

Fig. 3 Infections complications observed during CLL1 CAR-T cell therapy within the 28-day period. The occurrence of infectious microorganisms and organs within 28 days after CLL1 CAR-T therapy were meticulously presented. **A** The involved organs of patients experienced infections following CAR-T cells infusion, **B** the investigated microorganism's species caused infections in patients, **C–E** the respective numbers of specific pathogens causing bacterial, viral, and fungal infections in patients

cytomegalovirus infections [7]. Some studies also suggest employing fluconazole or micafungin as a means to prevent antifungal infections during severe neutropenia until neutrophil recovery [9].

However, the aforementioned data and recommendations for preventive treatment strategies primarily rely on findings from CD19 CAR-T cell therapy for B-cell malignancies. Considering the efficacy of CLL1 CAR-T cells in treating AML, the clinical significance of utilizing CLL1 CAR-T cells in relapsed or refractory AML has progressively escalated. It is imperative to delineate the incidence of infections during CLL1 CAR-T therapy in AML, thereby furnishing a reference for prophylactic measures aimed at averting infections or implementing preemptive interventions following early infection.

This study presents an analysis of infections occurring within 28 days following CLL1 CAR-T cell therapy in a cohort of patients with R/R AML. After infusion of CAR-T cells, infection events were observed in 63% (32/51) of patients, with 47.1% occurring within the first 14 days and 62.7% within the full 28-day period. Bacterial infections were the most prevalent type, primarily caused by Gram-negative bacteria (86.2%). The respiratory tract was identified as the most common site of infection (41.1%). Additionally, fungal infections were found to have an etiological basis in eight patients, making it the second most common pathogen after bacterial infections; viral infections had the lowest incidence rate. Among these cases, severe infection was observed in 14 patients, five experienced life-threatening infection, four died due to infection-related complications, while remaining patients achieved control over their infections through active anti-infection treatment.

In the analysis of infection complications following CD19 CAR-T therapy, it was observed that the number of prior treatment regimens, the severity of CRS, and recent history of infection before treatment were correlated with an elevated risk of early infection after CAR-T cell infusion [21].In this study, we identified several factors associated with an increased risk of early infection following CLL1 CAR-T infusion, including psrevious treatment regimens,

Table 3 Univariate and multivariate logistic analysis of the risk of infections within 28 days

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age						
≥ 40 versus < 40	1.048	0.295–3.569	0.941			
Sex						
Male versus female	0.625	0.181–2.104	0.447			
Prior antitumor treatment regimens						
< 3 versus ≥ 3	0.200	0.0403–0.757	0.027	0.230	0.039–1.042	0.071
Prior infections						
Yes versus No	8.909	1.522–170.400	0.045	1.013	0.217–4.603	0.986
Extramedullary infiltration						
Yes versus No	1.225	0.358–4.153	0.743			
Tumor burden, percentage						
≥ 50% versus < 50%	1.789	0.524–6.728	0.364			
CAR-T-cell dose, cells per kg						
$0.5–1.5 \times 10^6$ versus $1.6–3 \times 10^6$	3.674	0.838–25.870	0.119			
ALC cells at pre-lymphodepletion						
< 200 versus ≥ 200	0.440	0.124–1.548	0.197			
ANC cells at pre-lymphodepletion						
< 500 vs ≥ 500	3.850	1.087–16.100	0.046	3.785	0.870–20.340	0.041
lgG level at pre-lymphodepletion						
< 400 versus ≥ 400 mg/dL	3.900	1.127–14.550	0.035	2.879	0.695–12.870	0.149
CRS grade						
0 vs 1–2 versus 3–4	5.600	1.477–27.830	0.018	4.141	0.884–24.990	0.037
ICANS grade						
Yes versus No	0.382	0.0422–3.453	0.361			
Tocilizumab application						
Yes versus No	1.200	0.356–4.249	0.770			
Corticosteroid application						
Yes versus No	1.429	0.425–4.904	0.563			

CAR-T; chimeric antigen receptor T, ALC; antinuclear lymphocyte, ANC; antinuclear neutrophil cell, CRS; cytokine release syndrome, ICANS; immune effector cell associated neurotoxic syndrome

infection status within 30 days prior to lymphodepletion, pre-lymphodepletion neutrophil count, incidence of CRS, and IgG level. Multivariate analysis revealed that both pre-lymphodepletion neutrophil count and incidence of CRS were independent prognostic factors for an increased risk of infection. Therefore, preemptive treatment is recommended for patients with low pre-lymphodepletion neutrophil counts and those experiencing CRS during the course of treatment.

In conclusion, CLL1 represents a promising target for CAR-T treatment in AML, exhibiting an impressive efficacy rate of up to 70% and clear advantages in managing R/R AML. Our center has achieved long-term remission in R/R AML patients through the utilization of CLL1 CAR-T therapy followed by bridging allo-HSCT, significantly enhancing patient prognosis. However, it is crucial to acknowledge that compared to CD19 CAR-T cell therapy for B-cell tumors, the additional risk of severe agranulocytosis during CLL1 CAR-T therapy may exacerbate patient susceptibility to infections. In our study, we observed a heightened risk of infection within the initial 28 days following CLL1 CAR-T cell therapy, with bacterial and fungal infections being the predominant events. The pre-lymphodepletion neutrophil level and severity of CRS were identified as independent prognostic factors associated with an increased susceptibility to infection. However, it is important to note that this study was conducted at a single center with a limited sample size; therefore, further large-scale or multi-center studies are warranted to comprehensively evaluate the infectious complications associated with CLL1 CAR-T cell therapy in the future. By summarizing the infection-related complications and identifying high-risk factors during CLL1 CAR-T cell therapy, this study aims to assist clinicians in early identification of patients who may be more susceptible to infections, enabling timely intervention and reducing treatment-related mortality rates. Ultimately, these findings contribute

towards enhancing the safety profile of CLL1 CAR-T therapy for patients with R/R AML.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00262-025-03998-1.

Acknowledgements The authors thank the patients and their families for contributing to this study.

Author contributions Conceptualization of the project was done by JMX, HZ, YFZ and MFZ; Development of methodology was done by JMX and YFZ; Manufacturing of CLL1 CAR-T products was done by XMZ and SJG; Management of patients was done by HZ, XMZ, HRL, YZ, XYH and MFZ; Collection, analysis and interpretation of data were done by JMX, HZ, YHZ, XXS and XMZ; Writing articles and revision of the manuscript were done by JMX, HZ, YFZ and MFZ. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding This work was supported by grants from the General Project of the National Natural Science. Foundation of China (81970180 to MFZ), the Science and Technology Project of Tianjin Municipal Health Committee (TJWJ2022QN030 to MFZ), Key projects of Tianjin Applied Basic Research and Multi-Investment Fund (21JCZDJC01240), Science and Technology Project of Tianjin Municipal Health Committee (TJWJ2022XK018 to MFZ), and the Key Science and Technology Support Project of Tianjin Science and Technology Bureau (20YFZCSY00800 to MFZ), as well as Tianjin Key Medical Discipline (Specialty) Construction Project (TJYXZDXK-056B).

Data availability corresponding author at mingfengzhao@sina.com upon reasonable request.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Tianjin First Center Hospital.

References

1. Bupha-Intr O, Haeusler G, Chee L, Thursky K, Slavin M, Teh B (2021) CAR-T cell therapy and infection: a review. Expert Rev Anti Infect Ther 19:749–758. https://doi.org/10.1080/14787210.2021.1855143
2. Bakker AB, van den Oudenrijn S, Bakker AQ et al (2004) C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia. Cancer Res 64:8443–8450. https://doi.org/10.1158/0008-5472.Can-04-1659
3. Wang J, Chen S, Xiao W et al (2018) CAR-T cells targeting CLL-1 as an approach to treat acute myeloid leukemia. J Hematol Oncol 11:7. https://doi.org/10.1186/s13045-017-0553-5
4. Jin X, Zhang M, Sun R et al (2022) First-in-human phase I study of CLL-1 CAR-T cells in adults with relapsed/refractory acute myeloid leukemia. J Hematol Oncol 15:88. https://doi.org/10.1186/s13045-022-01308-1
5. Zhang H, Bu C, Peng Z et al (2022) Characteristics of anti-CLL1 based CAR-T therapy for children with relapsed or refractory acute myeloid leukemia: the multi-center efficacy and safety interim analysis. Leukemia 36:2596–2604. https://doi.org/10.1038/s41375-022-01703-0
6. Ma YJ, Dai HP, Cui QY et al (2022) Successful application of PD-1 knockdown CLL-1 CAR-T therapy in two AML patients with post-transplant relapse and failure of anti-CD38 CAR-T cell treatment. Am J Cancer Res 12:615–621
7. Hill JA, Li D, Hay KA et al (2018) Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. Blood 131:121–130. https://doi.org/10.1182/blood-2017-07-793760
8. Vora SB, Waghmare A, Englund JA, Qu P, Gardner RA, Hill JA (2020) Infectious complications following CD19 chimeric antigen receptor T-cell therapy for children, adolescents, and young adults. Open Forum Infect Dis 7:ofaa21. https://doi.org/10.1093/ofid/ofaa121
9. Park JH, Romero FA, Taur Y, Sadelain M, Brentjens RJ, Hohl TM, Seo SK (2018) Cytokine release syndrome grade as a predictive marker for infections in patients with relapsed or refractory B-cell acute lymphoblastic leukemia treated with chimeric antigen receptor T cells. Clin Infect Dis 67:533–540. https://doi.org/10.1093/cid/ciy152
10. Luo H, Wang N, Huang L et al (2019) Inflammatory signatures for quick diagnosis of life-threatening infection during the CAR T-cell therapy. J Immunother Cancer 7:271. https://doi.org/10.1186/s40425-019-0767-x
11. Wudhikarn K, Palomba ML, Pennisi M et al (2020) Infection during the first year in patients treated with CD19 CAR T cells for diffuse large B cell lymphoma. Blood Cancer J 10:79. https://doi.org/10.1038/s41408-020-00346-7
12. Atilla E, Benabdellah K (2023) The black hole: CAR T cell therapy in AML. Cancers (Basel). https://doi.org/10.3390/cancers15102713
13. Pollyea DA, Altman JK, Assi R et al (2023) Acute myeloid leukemia, version 3.2023, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 21:503–513. https://doi.org/10.6004/jnccn.2023.0025
14. Lee DW, Santomasso BD, Locke FL et al (2019) ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant 25:625–638. https://doi.org/10.1016/j.bbmt.2018.12.758
15. Haeusler GM, Phillips RS, Lehrnbecher T, Thursky KA, Sung L, Ammann RA (2015) Core outcomes and definitions for pediatric fever and neutropenia research: a consensus statement from an international panel. Pediatr Blood Cancer 62:483–489. https://doi.org/10.1002/pbc.25335
16. Donnelly JP, Chen SC, Kauffman CA et al (2020) Revision and update of the consensus definitions of invasive fungal disease from the European organization for research and treatment of cancer and the mycoses study group education and research consortium.

Clin Infect Dis 71:1367–1376. https://doi.org/10.1093/cid/ciz1008

17. van Burik JA, Carter SL, Freifeld AG et al (2007) Higher risk of cytomegalovirus and aspergillus infections in recipients of T cell-depleted unrelated bone marrow: analysis of infectious complications in patients treated with T cell depletion versus immunosuppressive therapy to prevent graft-versus-host disease. Biol Blood Marrow Transplant 13:1487–1498. https://doi.org/10.1016/j.bbmt.2007.08.049
18. Young JH, Logan BR, Wu J et al (2016) Infections after transplantation of bone marrow or peripheral blood stem cells from unrelated donors. Biol Blood Marrow Transplant 22:359–370. https://doi.org/10.1016/j.bbmt.2015.09.013
19. Zhang X, Zhu L, Zhang H, Chen S, Xiao Y (2022) CAR-T cell therapy in hematological malignancies: current opportunities and challenges. Front Immunol 13:927153. https://doi.org/10.3389/fimmu.2022.927153
20. Fusaroli M, Isgrò V, Cutroneo PM, Ferrajolo C, Cirillo V, Del Bufalo F, Raschi E, Poluzzi E, Trifirò G (2022) Post-marketing surveillance of CAR-T-cell therapies: analysis of the FDA adverse event reporting system (FAERS) database. Drug Saf 45:891–908. https://doi.org/10.1007/s40264-022-01194-z
21. Lehrnbecher T, Fisher BT, Phillips B et al (2020) Clinical practice guideline for systemic antifungal prophylaxis in pediatric patients with cancer and hematopoietic stem-cell transplantation recipients. J Clin Oncol 38:3205–3216. https://doi.org/10.1200/jco.20.00158