Prolonged hematological toxicity in patients receiving BCMA/CD19 CAR-T-cell therapy for relapsed or refractory multiple myeloma

Hujun Li[1†], Lina Zhao[1,2†], Zengtian Sun[1†], Yue Yao[3†], Li Li[4], Jiaojiao Wang[1], Tian Hua[1], Shengwei Ji[1], Shiyuan Wang[1], Hai Cheng[1], Ming Shi[5], Zhenyu Li[1], Lingyu Zeng[6], Qingyun Wu[6], Jianlin Qiao[6], Chong Chen[6], Junnian Zheng[5*], Jiang Cao[1*] and Kailin Xu[1*]

[1]Department of Hematology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China, [2]Department of Hematology, the First People's Hospital of Lianyungang, Lianyungang, China, [3]The First Clinical Medical College, Xuzhou Medical University, Xuzhou, China, [4]Department of Gastroenterology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China, [5]Cancer Institute, Xuzhou Medical University, Xuzhou, China, [6]Jiangsu Bone Marrow Stem Cell Institute, Xuzhou Medical University, Xuzhou, China

Although chimeric antigen receptor T (CAR-T) cell therapy has been indicated to be effective in treating relapsed or refractory multiple myeloma (R/R MM), severe hematological toxicity (HT) remains an intractable issue. This study enrolled 54 patients with R/R MM following combined infusion of anti-CD19 and anti-BCMA CAR-T cells. The results showed that the rates of severe cytopenia were high, including severe neutropenia (28/54, 52%), severe anemia (15/54, 28%), and severe thrombocytopenia (18/54, 33%). Moreover, the incidence of prolonged HT (PHT) on Day 28 post-infusion was 52% (28/54), including 46% for severe neutropenia, 30% for severe anemia, and 31% for severe thrombocytopenia. Patients with PHT had a poorer median progression-free survival (PFS) and overall survival (OS) than patients without PHT ($P=0.011$; $P=0.007$). Furthermore, Cox regression analyses showed that PHT was an independent risk factor for PFS and OS. Univariate analyses showed that IFNγ (OR: 1.046; 95% CI: 1.002-1.093, $P=0.042$) and severe HT after lymphodepletion chemotherapy (OR: 0.082; 95% CI: 0.017-0.404; $P=0.002$) were independent risk factors for PHT. In conclusion, these results indicated that PHT was associated with poor outcomes following CAR-T-cell therapy in MM patients. Early detection and management of PHT would be beneficial for the prevention of life-threatening complications and improvement in the survival of patients after CAR-T-cell therapy.

Clinical trial registration: This trial was registered on 1 May 2017 at http://www.chictr.org.cn as ChiCTR-OIC-17011272.

KEYWORDS

chimeric antigen receptor T cell, hematological toxicity, BCMA, CD19, multiple myeloma

1. Introduction

Multiple myeloma (MM) is a hematological malignancy caused by the malignant proliferation of plasma cells in the bone marrow (1). It is now clinically treated with chemotherapy, autologous hematopoietic stem cell transplantation (Auto-HSCT), proteasome inhibitors, immunomodulatory drugs, and monoclonal antibodies, but it will eventually relapse. The treatment of relapsed and refractory (R/R) MM remains a challenge (2–5). In recent years, with the development of CAR-T-cell technology, targeted BCMA CAR-T-cell therapy as a new method for the treatment of R/R MM has achieved high response rates and has shown curative effects in clinical trials (6–8). In R/R MM patients, the complete response (CR) rate of BCMA-targeted CAR-T-cell therapy is as high as 70-95% (9–11). However, various adverse effects (AEs) remain unresolved, limiting the wide application of CAR-T-cell therapy (12).

To date, cytokine release syndrome (CRS) and neurotoxicity are the two most common toxicities after CAR-T-cell infusion (13). With supportive approaches, including tocilizumab, corticosteroids and anakinra, symptoms are resolved in most patients. Hematological toxicity (HT) is another common AE with an incidence of higher than 90% and is associated with dismal outcomes (14–16). For instance, Sarah and Wang et al. reported that prolonged HT (PHT) is associated with a shorter 1-year OS in patients with R/R diffuse large B-cell lymphoma and acute lymphoblastic leukemia (17, 18). However, there are relatively few large-sample studies on post-CAR-T-cell therapy PHT among patients with R/R MM, and studies on the correlation between PHT and prognosis are lacking.

Our previous studies confirmed that the combined infusion of humanized anti-CD19 and anti-BCMA CAR-T cells is feasible and that the majority of patients with R/R MM achieved high response rates (19, 20). Here, we systematically analyzed the correlation between PHT and prognosis among patients with R/R MM participating in a phase 1/2 clinical trial of CAR-T-cell therapy. Moreover, the risk factors affecting PHT were evaluated.

2. Methods

2.1 Patient selection

A total of 54 patients with R/R MM who underwent CAR-T-cell therapy between July 2017 and August 2020 were retrospectively reviewed. All patients were enrolled in phase 1/2 open-label single-center clinical trials of CAR-T-cell therapy targeting BCMA and CD19 (Chictr.org.cn ChiCTR-OIC-17011272). This study was conducted in full compliance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou University. Eligible patients had histologically or cytologically confirmed MM and met the International Myeloma Working Group (IMWG) diagnostic criteria for R/R MM (21). Patients were aged 18-69 years, with a life expectancy of 12 weeks or more and adequate organ function. Patients with uncontrollable infections, mental or psychological illnesses, severe allergies, or a history of severe allergies were excluded.

2.2 CAR-T-cell manufacturing and clinical protocol

Peripheral blood mononuclear cells of the enrolled patients were collected for CAR-T-cell production. The protocol for CAR-T-cell manufacturing in our center has been described previously (19, 20). Each individual was administered a single cycle of fludarabine (30 mg/m^2 on Day -5 to -3)- and cyclophosphamide (750 mg/m^2 on Day -5)-based conditioning treatment, followed by CAR-T-cell infusion. The established hospitalization observation time was 1 month, but it could change depending on the severity and the recovery of toxicity. The patients' vital signs were monitored daily. Routine blood tests were conducted, and the levels of serum cytokines were determined by cytometric bead array (CBA) at least three times a week. Detailed information is provided in the Supplementary material.

2.3 Definitions of HT and hematologic recovery

The criteria for cytopenia and recovery were defined as per the Center for International Blood and Marrow Transplant Research (CIBMTR) (22) reporting guidelines. Neutropenia and severe neutropenia were defined as absolute neutrophil counts (ANC) lower than $1.5×10^9$/L and $0.5×10^9$/L, respectively. Anemia was defined as a hemoglobin concentration lower than 120 g/L in men and 110 g/L in women; levels lower than 60 g/L were considered severe anemia. Thrombocytopenia and severe thrombocytopenia were defined as platelet counts $< 100×10^9$/L and $< 20×10^9$/L, respectively. Neutrophil recovery was defined as an ANC > $0.5 ×10^9$/L for three consecutive days, irrespective of growth factor administration. Hemoglobin recovery was defined as a hemoglobin concentration > 60 g/L without the support of

erythrocyte transfusion. Platelet recovery was defined as platelet counts $> 20\times10^9$/L for three consecutive days in the absence of platelet transfusion. PHT was defined as the presence of severe neutropenia, severe anemia, or severe thrombocytopenia on Day 28 post-infusion.

2.4 CRS and neurotoxicity

CRS effects were graded and managed according to the recommendations of Lee et al. (23). Grade 1-2 CRS was classified as "mild," while grade 3-4 CRS was classified as "severe." The assessment of neurotoxicity was based on the Common Terminology Criteria for Adverse Events 5.0 (CTCAE 5.0) (24). CRS and neurotoxicity, along with other factors, including clinical symptoms, vital signs, and levels of serum cytokines, were assessed by three experienced clinicians. Inconsistencies were further discussed.

2.5 Response to CAR T-cell therapy

The entire cohort was evaluated to assess the response to CAR-T-cell therapy. The response to treatment was evaluated on Day +90 using the International Myeloma Working Group criteria (21), including a stringent complete response (sCR), a complete response (CR), a very good partial response (VGPR), a partial response (PR), a minimal response (MR), stable disease (SD) and progressive disease (PD). The overall response rate (ORR) was defined as patients who achieved a PR or better. Overall survival (OS) was defined as the time from CAR-T-cell infusion censored on the date of the last follow-up or death from any cause. Progression-free survival (PFS) was calculated from the date of CAR-T-cell infusion to the date of disease progression (imaging or biopsy) or death from any cause.

2.6 Statistical analysis

Descriptive statistics were used to describe the patients' baseline characteristics and the temporal profiles of severe cytopenia. Univariate and multivariate logistic regression models were applied to assess whether several variables were contributing factors to PHT. Categorical variables were analyzed using the chi-square test and Fisher's exact test. Descriptive and survival analyses were performed using the Kaplan−Meier methodology. A log-rank test was utilized to compare OS and PFS between patient groups. Multivariate Cox regression models were used for the analysis of factors related to survival. A p value <0.05 was considered significant.

Analysis was performed using GraphPad Prism software version 8.0.

3. Results

3.1 Patient characteristics

A total of 54 R/R MM patients treated with CAR-T-cell therapy between July 2017 and August 2020 at the Affiliated Hospital of Xuzhou Medical University were followed. Patients were divided into a PHT group (28 patients, 52%) and a non-PHT group (26 patients, 48%) based on the occurrence of PHT at 28 days after CAR-T-cell infusion. In the PHT group, the median age was 58 years (range, 30 to 67), the median time since diagnosis was 39 months (range, 8 to 167), and 9 patients (32%) with the high tumor burden at CAR T-cell infusion. Thirteen patients (46%) had International Staging System stage III disease, and 8 patients (29%) had extramedullary disease. High-risk cytogenetic abnormalities were detected in 8 patients (29%). Before the lymphodepletion regimen, the patients received a median of 4 (range, 2 to 17) previous therapy lines, and 10 patients (36%) had received autologous hematopoietic stem cell transplantation (ASCT). In the non-PHT group, the median age was 57 (range, 43 to 67), the median time of diagnosis was 40 months (range, 8 to 113) and 4 patients (15%) with the high tumor burden. Eleven patients (42%) had International Staging System stage III disease, and 7 patients (31%) had extramedullary lesions. Seven patients (27%) had high-risk cytogenetic abnormalities. The patients received a median of four chemotherapy cycles (range, 2 to 7), and 5 patients (19%) received ASCT before CAR T-cell infusion (Table 1).

3.2 Patient outcomes

3.2.1 Response rates

In the PHT group, the ORR to CAR-T-cell therapy was 93% (26/28) 3 months after infusion. The best response to therapy was as follows: 6 patients achieved an sCR, 5 achieved a CR, 7 achieved a VGPR, 8 achieved a PR and 2 achieved SD (Figure 1A). In the non-PHT group, the ORR to CAR-T-cell therapy was 96% (25/26). The best response to therapy was as follows: 11 patients achieved an sCR, 7 achieved a CR, 5 achieved a VGPR, 2 achieved a PR and 1 achieved SD (Figure 1B). We found no significant difference in the ORR between the two groups.

3.2.2 PFS and OS

The deadline of follow-up was April 30, 2022, with a median follow-up of 24.3 months (range, 0.5 to 58.6) after CAR-T-cell

TABLE 1 Demographic and clinical characteristics of the enrolled patients.

	All MM patients (N = 54)	Patients with PHT (N = 28)	Patients without PHT (N = 26)	p
Characteristic				
Age, y, median (range)	58 (30-67)	58 (30-67)	57(43-67)	0.812
Male sex - no. (%)	26 (48)	11 (39)	15 (58)	0.176
ISS stage - no. (%)				0.761
I or II	30 (56)	15 (54)	15 (58)	
III	24 (44)	13 (46)	11 (42)	
High tumor burden - no, (%) ¶	13 (24)	9 (32)	4 (15)	0.262
High-risk cytogenetic #	15 (28)	8 (29)	7 (27)	0.893
Extramedullary disease, no. (%)	15 (28)	8 (29)	7 (27)	0.893
Previous therapy lines, median (range)	4 (2-17)	4 (2-17)	4 (2-7)	0.083
Median time since diagnosis (mos., range)	40 (8-167)	39 (8-167)	40 (8-113)	0.901
Previous ASCT - no. (%)	15 (28)	10 (36)	5 (19)	0.177
Prelymphodepletion				
Median ANC, ×10^9/L	2.11 (0.37-6.87)	1.82 (0.37-6.87)	2.42 (0.97-3.98)	
Median hemoglobin, g/L	99 (47-158)	91 (47-149)	108 (59-158)	
Median platelet count, ×10^9/L	115 (13-329)	81 (13-230)	153 (79-329)	

Percentages may not total to 100 because of rounding

PHT, prolonged hematological toxicity; ISS, International Staging System; ASCT, autologous stem cell transplantation; ANC, absolute neutrophil count; HT, hematological toxicity; ORR, overall response rate; sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease. ¶ High tumor burden was defined as at least 50% clonal plasma cells or bone marrow plasma cells. # The cytogenetic risk profile was reported by investigators on the basis of fluorescence in situ hybridization. A high-risk cytogenetic profile was defined by the presence of the following abnormalities: del(17p), t (4;14), or t (14;16). Two-sided P values were calculated using the Mann–Whitney U test for continuous variables and Pearson's chi-square or Fisher's exact test for categorical variables.

infusion. Based on Kaplan−Meier estimates, the median PFS and OS for 54 patients were 16.4 months (95% CI, 7.8 to 25.0) and 42.8 months (95% CI, 25.2 to 60.4), respectively (Figures 2A, B).

3.2.3 Risk factors for PFS and OS

Subgroup analyses showed that patients' baseline characteristics, including disease stage, extramedullary disease, time since diagnosis and previous therapy lines, were not associated with PFS or OS. Patients with a high baseline tumor burden (HR: 5.117; 95% CI: 2.340-11.451; P=0.001) and high-risk cytogenetic (HR: 2.278; 95% CI: 1.155-4.496; P=0.014) had a lower PFS than those with a low

baseline tumor burden and no high-risk cytogenetic. Moreover, the severity of CRS (HR: 3.027; 95% CI: 1.115-8.214; P=0.022) was significantly associated with OS (Figure S1).

3.2.4 Prognosis

In the PHT group, 21 patients (75%) relapsed, and 15 (54%) died during follow-up. Among these patients, 10 patients (36%) died from disease progression or associated complications. Two patients (7%) died from intracranial hemorrhage, 2 (7%) died from severe infection, and 1 (4%) died from severe CRS within 2 months. In the non-PHT group, 15 patients (58%) relapsed, and
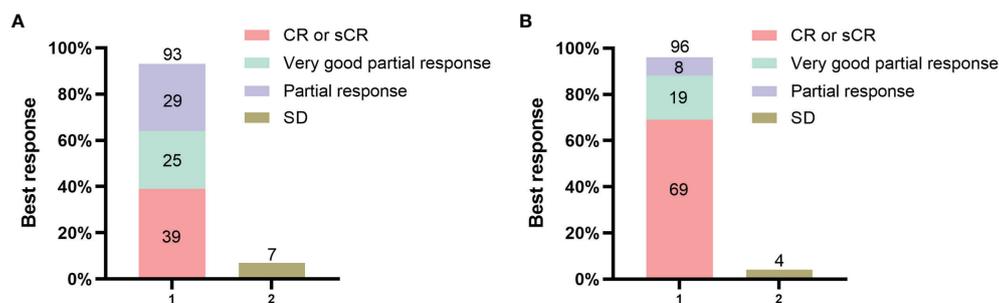

FIGURE 1
Response to the combination of anti-BCMA and anti-CD19 chimeric antigen receptor (CAR) T cells. Panel (A) shows the rates of overall response and no response in the PHT group. Panel (B) shows the rates of overall response and no response in the non-PHT group. All responses were confirmed and assessed on the basis of the International Myeloma Working Group Uniform (IMWG) Response Criteria for Multiple Myeloma.
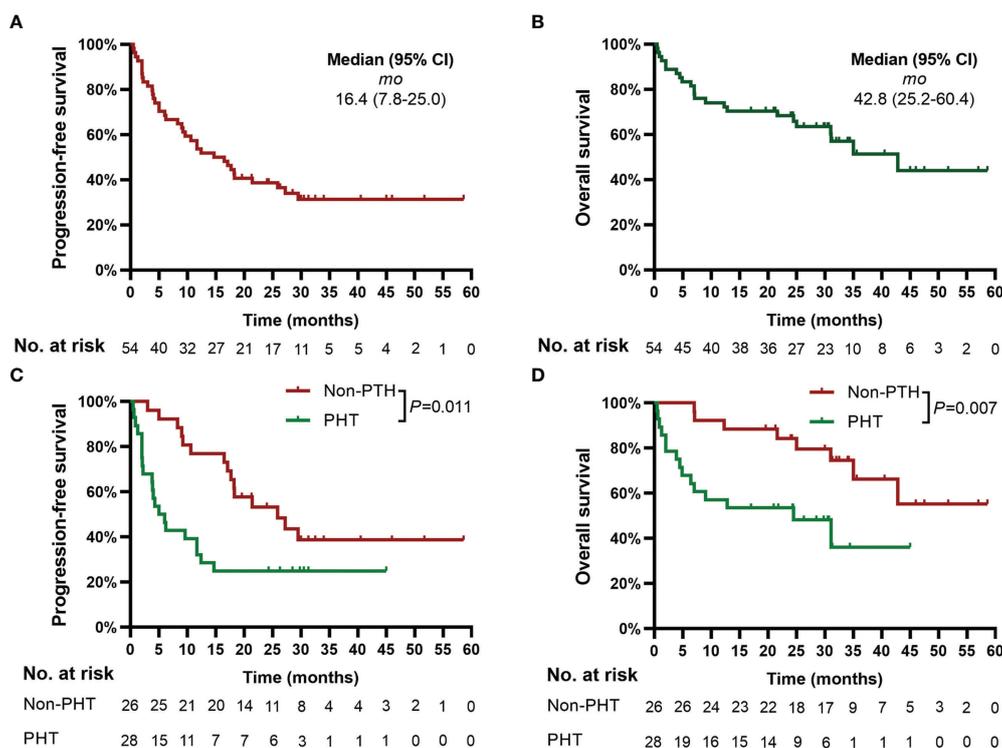
FIGURE 2
Progression-free survival (PFS) and overall survival (OS). Panels **(A, B)** show the Kaplan–Meier curves for PFS and OS in the 54 patients, respectively. Panels **(C, D)** show the Kaplan–Meier curves of PFS and OS, respectively, according to PHT. Two-sided P values were calculated based on the log-rank test. PHT, prolonged hematological toxicity.

8 (31%) died, all of whom died from disease progression or associated complications (Table 2).

3.3 Adverse events and interventions

3.3.1 CRS and neurotoxicity

In the PHT group, 23 patients (82%) developed mild CRS, and 5 patients (18%) developed severe CRS. The median time to CRS onset was 6 days (range 0-13), with a median duration of 5 days (range 1-12). Twelve patients (43%) received tocilizumab, and 17 patients (61%) received glucocorticoids. Moreover, 2 patients (7%) developed grade 3 and grade 4 neurotoxicity. In the non-PHT group, 24 patients (92%) sustained mild CRS, and 2 patients (8%) sustained severe CRS. The median time to CRS onset was 7 days (range 0-13), with a median duration of 4 days (range 1-8). Four patients (15%) received tocilizumab, and 6 patients (23%) received glucocorticoids. None of the patients developed neurotoxicity (Table 2).

3.3.2 Incidence and temporal characteristics of HT

Before lymphodepleting chemotherapy, in the PHT group, the median ANC was 1.82 (range, 0.37-6.87) $\times 10^9$/L, the median

hemoglobin serum concentration was 91 (range, 47-149) g/L, and the median platelet count was 81 (range, 13-230) $\times 10^9$/L. After lymphodepletion chemotherapy, the incidence of severe neutropenia significantly increased from 11% to 46% (P<0.05), while no significant changes were detected with respect to severe anemia (11% $vs.$ 21%) or severe thrombocytopenia (18% $vs.$ 21%). After CAR-T-cell infusion, the total incidence of HT increased remarkably (82% for severe neutropenia, 46% for severe anemia, and 61% for severe thrombocytopenia, P<0.05) (Figure 3). Moreover, 27 (96%) patients received granulocyte colony-stimulating factor (G-CSF), 17 (61%) received packed red blood cell (PRBC) transfusions, and 16 (57%) received platelet transfusions (Table 2). In the non-PHT group, the median ANC was 2.42 (range, 0.97-3.98) $\times 10^9$/L, the median hemoglobin serum concentration was 108 (range, 59-158) g/L, and the median platelet count was 153 (range, 79-329) $\times 10^9$/L. After lymphodepletion chemotherapy, no significant changes were detected with respect to severe neutropenia (0% $vs.$ 15%), severe anemia (4% $vs.$ 4%) or severe thrombocytopenia (0% $vs.$ 0%). After CAR-T-cell infusion, the total incidence of HT did not increase remarkably (19% for SN, 4% for severe anemia, and 0% for severe thrombocytopenia). Among these patients, 18 (69%) received G-CSF, 2 (8%) received PRBC transfusions and 2 (8%) received platelet transfusions. Of

TABLE 2 PHT with other adverse events and interventions after CAR-T-cell infusion.

Characteristic	PHT (n = 28)	Non-PHT (n = 26)	P value
Adverse events			
CRS, no. (%)			0.480
Grade 1-2	23 (82)	24 (92)	
Grade 3-5	5 (18)	2 (8)	
Median time to CRS onset, median (range)	6 (0-13)	7 (0-13)	0.875
Median duration, median (range)	5 (1-20)	4 (1-8)	0.463
Neurotoxicity, no. (%)	2 (7)	0	0.491
Infection, n (%)	18 (64)	8 (31)	0.014
Unspecified pathogen	6 (21)	1 (4)	
Viral	4 (14)	3 (12)	
Bacterial	9 (32)	4 (15)	
Fungal	2 (7)	1 (4)	
Hemorrhage, n (%)	2 (7)	0	0.491
Interventions			
G-CSF, n (%)	27 (96)	18 (69)	0.021
PRBC transfusions, n (%)	17 (61)	2 (8)	< 0.001
Platelet transfusions, n (%)	16 (57)	2 (8)	< 0.001

PHT, prolonged hematological toxicity; CRS, cytokine release syndrome; G-CSF, granulocyte colony-stimulating factor; PRBC, packed red blood cell. Data are described as n (%). P values were tested by the chi-square test and Fisher's exact test for categorical variables.

note, compared with PHT patients, patients without PHT showed a lower requirement for blood transfusion or G-CSF support (P<0.005) (Table 2). In the PHT group and non-PHT group, the median time from infusion to recovery from neutropenia, anemia and thrombocytopenia was 42 *vs.* 15 days (range 10-159 *vs.* 6-71), 53 *vs.* 34 days (range 9-213 *vs.* 10-90) and 53 *vs.* 21 days (range 7-210 *vs.* 6-45), respectively.

3.3.3 Infection

In the PHT group, 18 of the 28 patients (64%) experienced infections, mainly including 10 (36%) with lung infections, 2 (7%) with upper respiratory tract infections, 2 (7%) with bacteremia, 1 (4%) with a urinary tract infection and 3 (11%) with skin soft-tissue infections after CAR-T-cell infusion. Moreover, 9 patients (32%) had bacterial infections, 2 (7%) had fungal infections, 2 (7%) had herpes zoster virus infections, 1 (4%) had a hepatitis B virus infection, 1 (4%) had hepatitis B virus activation, and 6 (21%) had unspecified pathogen infections. In the non-PHT group, 8 patients (31%) were infected with 4 (15%) lung infections, 1 (4%) bacteremia infection and 2 (8%) urinary tract infections. Among these patients, 4 patients (15%) had bacterial infections, 1 (4%) had a fungal infection, 1 (4%) had a herpes zoster virus infection, 1
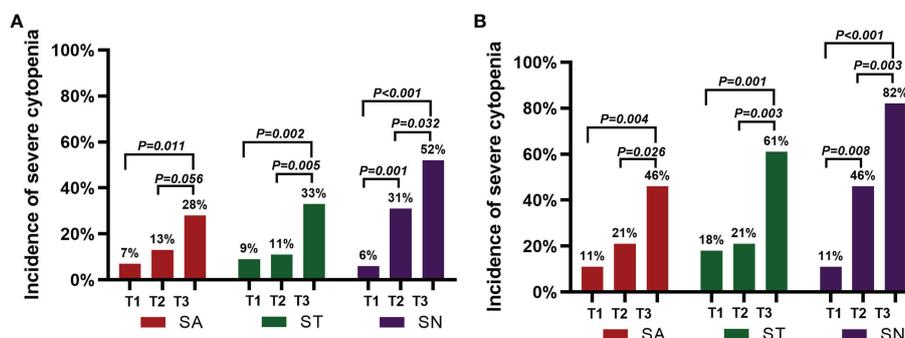

FIGURE 3
Incidence of severe cytopenia before and after CAR-T-cell infusion. **(A)** shows the percentage of severe cytopenia changes after lymphodepletion chemotherapy and CAR-T-cell infusion in the 54 patients. **(B)** shows the percentage of severe cytopenia changes after lymphodepletion chemotherapy and CAR-T-cell infusion in the 28 patients with PHT. A two-sided P value was determined *via* the Pearson chi-square test.

(4%) had a hepatitis B virus infection, 1 (4%) had a cytomegalovirus infection and 1 (4%) had an unspecified pathogen infection (Table 2).

3.4 PHT with PFS and OS

Patients with PHT had significantly poorer PFS (median of 5.0 months [95% CI, 2.1 to 7.9] *vs.* 45.9 months [95% CI, 13.4 to 38.4], *P*=0.011) and OS (median of 24.5 months [95% CI, 4.1 to 44.9] *vs.* not reached, *P*=0.007) than patients without PHT (Figures 2C, D).

Further analysis demonstrated that the patients with severe neutropenia, severe anemia and severe thrombocytopenia had an inferior PFS (severe neutropenia: median of 5.0 months [95% CI, 1.7 to 8.3] *vs.* 25.9 months [95% CI, 13.0 to 38.8], *P*=0.010; severe anemia: median of 2.2 months [95% CI, 0.0 to 5.5] *vs.* 18.2 months [95% CI, 11.9 to 24.5], *P*=0.018; severe thrombocytopenia: median of 3.8 months [95% CI, 1.4 to 6.2] *vs.* 25.9 months [95% CI, 14.3 to 37.5], *P*<0.001) and OS (severe neutropenia: median of 24.5 months [95% CI, 16.2 to 32.0] *vs.* not reached, *P*=0.018; severe anemia: median of 4.9 months [95% CI, 0.0 to 9.8] *vs.* 42.8 months [95% CI, 27.0 to 58.6], *P*=0.009; severe thrombocytopenia: median of 6.4 months [95% CI, 3.0 to 9.8] *vs.* not reached, *P*<0.001) than patients without severe neutropenia, severe anemia and severe thrombocytopenia at 28 days after CAR-T-cell infusion (Figure S2).

Furthermore, Cox regression analyses showed that PHT (HR: 2.762; 95% CI: 1.355-5.631; *P*=0.005), baseline tumor burden (HR: 3.635; 95% CI: 1.498-8.821; *P*=0.004) and having high-risk cytogenetic (HR: 2.945; 95% CI: 1.387-6.255; *P*=0.005) were independent risk factors for PFS. Moreover, PHT (HR: 3.347; 95% CI: 1.318-8.503; *P*=0.011) and the severity of CRS (HR: 3.084; 95% CI: 1.004-9.474; *P*=0.049) were also independent risk factors for OS (Tables S1, 2).

3.5 Factors associated with the incidence of PHT

Next, we analyzed the patients' characteristics, prior therapies, serum cytokine levels and CAR-T-cell therapy-associated factors to identify the risk factors correlated with PHT.

Univariate analyses revealed that the severe HT after lymphodepletion chemotherapy (OR: 8.500; 95% CI: 2.299-31.431; *P*=0.001), grade of CRS (OR: 2.697; 95% CI: 1.152-6.312; *P*=0.022) and the levels of several serum biomarkers (including peak levels of IL-6 (OR: 1.008; 95% CI: 1.001-1.015; *P*=0.025), IL-8 (OR: 1.011; 95% CI: 1.002-1.020; *P*=0.019), IFNγ (OR: 1.036; 95% CI: 1.002-1.072; *P*=0.040), and MIP1α (OR: 1.066; 95% CI: 1.000-1.137; *P*=0.049) were significantly associated with the incidence of PHT. Multivariate analysis revealed that IFNγ (OR: 1.046; 95% CI: 1.002-1.093, *P*=0.042) and severe HT after lymphodepletion chemotherapy (OR: 0.054; 95% CI: 0.008-0.357; *P*=0.002) were independent risk factors for PHT (Table 3).

4. Discussion

In our previous study, 21 patients with R/R MM received an infusion of anti-BCMA and humanized anti-CD19 CAR-T cells. At a median follow-up of 179 days, 20 (95%) of the 21 patients had an overall response. In this retrospective study, we further expanded the sample size and demonstrated that 51 (95%) of 54 patients achieved a PR or better. Moreover, the occurrence of severe CRS and neurotoxicity was relatively low. These results indicated that the combined infusion of anti-BCMA and anti-CD19 CAR-T cells is feasible for patients with R/R MM.

During the management of patients with R/R MM who are receiving CAR-T-cell therapy, HT, in addition to CRS and neurotoxicity, is a major issue for clinicians and has a higher incidence. In the phase 2 study, for the 128 patients with R/R MM following idecabtagene vicleucel infusion (25), the incidences of HT were 89% for severe neutropenia, 60% for severe anemia and 52% for severe thrombocytopenia. Among all patients, 41% (52 patients) had persistent severe neutropenia, and 48% (62 patients) had persistent severe thrombocytopenia 1 month after infusion. In the phase 1 study, for the 33 patients with R/R MM following bb2121 infusion (6), the incidences of HT were 85% for severe neutropenia, 45% for severe anemia and 45% for severe thrombocytopenia, and PHT, with incidences of 3% for severe neutropenia and 35% for severe thrombocytopenia, was not resolved by Day +28 after cell therapy. In the phase 1 dose-climbing and expansion study following a bispecific CAR-T-cell therapy targeting BCMA and CD38 (BM38) in 23 patients with R/R MM (26), HT was the most common adverse event; severe neutropenia occurred in 83% of the patients, severe anemia occurred in 13% and severe thrombocytopenia occurred in 48%, with 40% of the patients with severe neutropenia and 55% with severe thrombocytopenia not having recovered within 1 month. In our studies, the incidences of HT were 52% for severe neutropenia, 28% for severe anemia and 33% for severe thrombocytopenia. Moreover, 46% of the patients with severe neutropenia, 30% with severe anemia and 31% with severe thrombocytopenia were not recovered by Day +28, which is consistent with the above reported study.

Previous studies have shown that patients with PHT have a poor prognosis after CAR-T-cell infusion. Sarah et al. (17) reported that the 1-year PFS and 1-year OS in patients with PHT were 24% and 36%, respectively, in 31 patients with R/R diffuse large B-cell lymphoma (DLBCL) who received tisagenlecleucel or axicabtagene ciloleucel. Moreover, patients without PHT had a longer 1-year OS of 81%. In a phase 1/2 study of 86 patients with R/R ALL who underwent CD19 CAR-

TABLE 3 Factors related to PHT.

Factor	Univariate Analysis	Multivariate Analysis		
	P value *	Hazard Ratio	95% CI	P value
ISS Stage III	0.761			
High-risk **cytogenetic** #	0.675			
Previous ASCT	0.182			
Extramedullary disease	0.893			
High tumor burden¶	0.158	1.739	0.259-11.681	0.569#
Time since diagnosis	0.635			
≥5 yrs. versus <5 yrs.				
Previous therapy lines	0.219			
>3 versus ≤ 3				
Severe HT after lymphodepletion chemotherapy	0.001	0.054	0.008-0.457	0.002#
Severity of CRS	0.279			
Grade of CRS	0.022	2.099	0.608-7.253	0.241#
Cytokines, (pg/mL)				
IL-6	0.025	1.006	0.997-1.015	0.198#
IL-8	0.019	1.007	0.990-1.024	0.450#
IFN γ	0.040	1.046	1.002-1.093	0.042#
MIP1α	0.049	1.026	0.964-1.092	0.425#

PHT, prolonged hematological toxicity; CI, confidence interval; ISS, International Staging System, ASCT, autologous stem cell transplantation; HT, hematological toxicity; CRS, cytokine release syndrome. # The cytogenetic risk profile was reported by investigators on the basis of fluorescence in situ hybridization. A high-risk cytogenetic profile was defined by the presence of the following abnormalities: del(17p), t (4;14), or t (14;16). ¶ High tumor burden was defined as at least 50% clonal plasma cells or bone marrow plasma cells. * Two-sided P values were calculated on the basis of logistic regression. # A logistic regression model was used for multivariate analysis. The variables in which the P value was ≤ 0.1 by univariate analysis or the variables that may have affected the results were included.

T-cell therapy, the results demonstrated that persistent severe cytopenia was highly associated with a poor 1-year OS. However, in R/R MM patients, the correlation between PHT and prognosis is rarely reported. Notably, our results showed that patients with PHT had significantly poorer median PFS and OS than those without PHT. Moreover, patients with severe neutropenia, severe anemia, or severe thrombocytopenia at 28 days also had a shorter PFS and OS. Multivariate analysis revealed that PHT was an independent risk factor for poor PFS and OS. Therefore, additional measures are required to reduce PHT incidence after CAR-T-cell infusion to improve survival.

Prolonged cytopenias after CAR-T therapy have increasingly been reported at varying rates, and the pathogenesis of this complication is not yet well-understood but is likely contributed to by multiple factors. Sarah et al. (17). showed that the development of CRS, the administration of tocilizumab or steroids, and the levels of ferritin and CRP were positively associated with the occurrence of PHT in R/R DLBCL. Wang et al. (18). found that the baseline bone marrow tumor burden, CRS severity, and serum biomarker levels (including max lg CRP, IL-10, IFNγ, ferritin, and D-dimer levels) were associated with the incidence of PHT in R/R ALL. We also further analyzed the risk factors for PHT and found that severe HT after lymphodepletion chemotherapy, levels of serum cytokines, grade of CRS and infection were involved in the occurrence of PHT. However, the severity of CRS, time to CRS onset and

duration of CRS were not related to PHT, which is inconsistent with previous studies. This may be related to the lower incidence of severe CRS in our trial. Therefore, some possible measures to reduce severe HT after lymphodepletion chemotherapy should be considered, such as the optimization of the lymphodepletion chemotherapy and early application of promoting blood cell growth, including the usage of G-CSF, blood transfusions, antibiotics and so on. In this trial, patients with severe hematological toxicity received G-CSF, blood transfusions, and other treatments that improved the degree of cytopenia. However, whether the application of these supportive treatments can affect the long-term prognosis of patients will be an issue to be investigated after we expand the sample size.

Our study is limited because it was a retrospective single-center study based on a relatively large sample size and longer follow-up time. We demonstrated that the combined infusion of humanized anti-CD19 and anti-BCMA CAR-T-cells was feasible, safe and significantly effective in treating patients with R/R MM. Moreover, HT remains one of the most common AEs after CAR-T-cell infusion, and the occurrence of PHT is associated with a poor prognosis in patients with R/R MM. Accordingly, enhancing bridging therapy to reduce the baseline tumor burden, monitoring serum biomarker levels, optimizing lymphodepletion chemotherapy and providing appropriate supportive treatment may be essential to reduce the incidence of PHT and improve the outcome of CAR-T-cell therapy in R/R MM patients. The

preliminary results need to be confirmed in future prospective and multicenter clinical trials, and the mechanism of PHT after CAR-T-cell infusion requires further exploration.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Affiliated Hospital of Xuzhou University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HL, LZh, and ZS wrote the manuscript; LZ, YY, JW, TH, and SJ acquired the data; LZh, SW, ZS, HC, and MS interpreted the data and performed the statistical analyses. LL, LZe, QW, JQ, CC, KX, JZ, and JC helped revise the manuscript. All authors contributed to the article and approved the submitted version.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1019548/full#supplementary-material

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