Association Analysis of Persistent Cytopenia With Efficacy and Side Effects of Anti-CD19 CAR T Therapy in Relapsed/refractory Diffuse Large B-Cell Lymphoma Patients.

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Research Article

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Abstract

Background: Anti-CD19 chimeric antigen receptor (CAR) T cell therapy has been an effective salvage therapy for relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL). Hematological toxicity is one of the adverse events (AEs) of this therapy. But the underlying pathophysiologic mechanism of persistent cytopenia is unclear. The association of persistent cytopenia with the efficacy and side effects of anti-CD19-CAR T cell therapy should be concerned about.

Patients and methods: In this work, 38 R/R DLBCL patients were enrolled in clinical trial of anti-CD19-CAR T-cell therapy. Before CAR T-cell therapy, all the patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide. The degree and duration of cytopenia, clinical response, proportions of CAR T-cells and levels of interleukin-6 (IL-6), AEs and follow up were observed following the anti-CD19-CAR T-cell therapy.

Results: The 3-4 grade of persistent cytopenia occurred in 14 R/R DLBCL patients (more than 8 weeks after CAR-T cell infusion) and recovered at 8-18 weeks after CAR-T cell infusion. All these 14 patients achieved objective response rate (ORR) in their anti-CD19-CAR T cell therapy. In the 26 patients obtained ORR, the incidence of 3-4 grade of persistent cytopenia was higher in patients with high tumor load than that of patients without high tumor load. The mean peak of IL-6 and anti-CD19-CAR T cells, the grade of CRS in patients with 3-4 grade of persistent cytopenia were higher than that of patients without such persistent cytopenia. Most patients whose anti-CD19-CAR T cells could be observed at 21 and 28 days after infusion were associated with 3-4 grade of persistent cytopenia. The PFS and OS were higher in patients with 3-4 grade of persistent cytopenia.

Conclusion: All the R/R DLBCL patients who occurred 3-4 grade of persistent cytopenia after anti-CD19-CAR T cell therapy achieved ORR in this therapy. The 3-4 grade of persistent cytopenia was associated with high tumor load, higher peaks of IL-6 and anti-CD19-CAR T cells, higher grades of CRS, the higher PFS and OS. Our study is helpful in predicting efficacy and severe hematologic side effects of anti-CD19-CAR T-cell therapy in R/R DLBCL patients.

Trial registration: The study was registered at http://www.chictr.org.cn/index.aspx as *ChiCTR-ONN-16009862* and http://www.chictr.org.cn/index.aspx as *ChiCTR1800019622*.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a common type of B-cell non-Hodgkin lymphoma (B-NHL). Although combination chemotherapy based on R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone) achieved a higher objective response rate (ORR) in DLBCL, several patients developed to relapsed/refractory (R/R) DLBCL after standard combined chemotherapy [1–3]. Anti-CD19 chimeric antigen receptor (CAR) T cell therapy has been an effective salvage therapy for R/R DLBCL [4–7]. But its utility is hampered by the adverse events (AEs). These AEs include cytokine release syndrome (CRS), immune effector cell associated neurotoxic syndrome (ICANS), hematological toxicity, B-cell

aplasia, and so on [8–14]. Hematologic toxicity is a factor associated with secondary infection after anti-CD19-CAR T-cell therapy. In long-term activity and safety outcomes of the ZUMA-1 study, there was less than 20% of patients had grade 3 or worse cytopenias three months after this therapy [4]. But the underlying pathophysiologic mechanism of prolonged cytopenias is unclear. Fortunately, none of these patients had serious clinical sequelae due to this persistent cytopenia [15, 16]. Could this persistent cytopenia be explained by lymphodepleting chemotherapy with fludarabine and cyclophosphamide alone? Some studies before showed that the risk factors associated with persistent cytopenia include CRS/ICANS grade, baseline cytopenia, prior allogeneic stem cell transplantation, higher peak C-reactive protein or ferritin levels [17, 18]. The association of persistent cytopenia with the efficacy and side effects of anti-CD19-CAR T cell therapy should be of clinical concern.

Patients And Methods

Medical history of the patients enrolled in this study

Thirty-eight R/R DLBCL patients were admitted in our hospital and enrolled in clinical trials of anti-CD19-CAR T-cell therapy *(ChiCTR-ONN-16009862 and ChiCTR1800018059)* from January 2017 to May 2021. All these patients were diagnosed as R/R DLBCL. None of the patients received hematopoietic stem cell transplant (HSCT) before and after our study. Follow-up was carried out from the CAR-T cell infusion day to the date of cutoff or the date of death. The cutoff date was November 30, 2021 in this study.

Anti-CD19-CAR T cell therapy

All 38 R/R DLBCL patients provided their autologous peripheral blood mononuclear cells (PBMCs) for their anti-CD19-CAR T-cell therapy. The lenti-CD19-2rd-CAR was provided by Shanghai Genbase Biotechnology Co., Ltd. Shanghai, CHINA. After 12-15 days cell culture in vitro, the transduction efficiency of the anti-CD19-CAR was analyzed by flow cytometry (FCM). Before anti-CD19-CAR T-cell therapy, all the patients received lymphodepleting chemotherapy with fludarabine (30 mg/m^2) and cyclophosphamide (400 mg/m^2) from day -4 to day -2. Anti-CD19-CAR T cells were infused on day 0 ($2x10^6$ cells/kg) in all R/R DLBCL patients.

Clinical response criteria

From the date of anti-CD19-CAR T cells infusion, follow-up was done until the patients died. Two months after the anti-CD19-CAR T cell therapy, the efficacy was evaluated in all the 38 R/R DLBCL patients. Disease status was defined as complete response (CR), partial remission (PR), stable disease (SD) and progression of disease (PD) according to Lugano Revised Criteria for Response Assessment [19]. In our study, we observed the progression free survival (PFS) and overall survival (OS) after the anti-CD19-CAR T-cell therapy.

Adverse events (AEs) of anti-CD19-CAR T-cell therapy

The AEs were observed more than 120 days following the anti-CD19-CAR T-cell infusion. Cytokine release syndrome (CRS) grade was determined according to the National Cancer Institute Common Terminology Criteria for AE v4.03 [20]. And the neurotoxicity syndrome was determined according to the Immune effector cell associated neurotoxic syndrome (ICANS) [21].

The expansion of anti-CD19-CAR T cells and levels of cytokines

The proportions anti-CD19-CAR T cells in peripheral blood were examined by FCM on 0, 7, 14, 21, 28 and 60 days after CAR-T cell infusion. Levels of interleukin-6 (IL-6) was measured on day 0, 7, 14, 21 and 28 after CAR T-cell infusion by enzyme linked immunosorbent assay (ELISA).

Criteria of CAR T-cell therapy mediated hematotoxicity and persistent cytopenia

The criteria of CAR T-cell therapy mediated hematotoxicity was graded according to the joint ASCO/IDSA consensus guidelines for cancer-related infection risk [22,23]. In our study, the persistent cytopenia was defined as 3-4 grade of cytopenia and hematotoxicity lasting more than 56 days after CAR-T cell infusion (Because in most patients, AEs in addition to hematotoxicity disappeared at 21-28 days after CAR-T cell infusion).

Statistical analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software was used for all the statistical analyses. T test was used for the measurement data conforming to normal distribution, and rank sum test was used for the measurement data not conforming to normal distribution. Chi-square test was used for counting data. All values were expressed as mean ± SD. $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Baseline characteristics of the 38 R/R DLBCL patients enrolled in this clinical trial were listed in Table 1. The age, the molecular subtypes, the stages according to the modified Ann Arbor staging system, the international prognostic index (IPI) scores, adverse prognostic factors of double hit, triple hit or double expression, triple expression and the lines of therapies were listed. None of the patients had any level of cytopenia when they were enrolled. In addition to the mean max tumor diameter, there was no difference in characteristics between the 14 R/R DLBCL patients with 3-4 grade of persistent cytopenia and the 24 R/R DLBCL patients without such persistent cytopenia after their anti-CD19-CAR T-cell therapy (Table 2).

Transduction efficiency, amplification and infusion of the anti-CD19-CAR T cells

The mean CD19-CAR transduction efficiency in the final products of the 38 R/R DLBCL patients was 49.85±13.35%. All the 38 patients received a dose of $2.19\pm0.42\times10^6$ cells/kg anti-CD19-CAR T cells infusion on day 0 after lymphodepleting chemotherapy.

Hematological toxicity of anti-CD19-CAR T-cell therapy

The hematological toxicity was diagnosed with 0-4 grade after anti-CD19-CAR T-cell therapy. It occurred from 4 to 10 days post anti-CD19-CAR T-cell infusion. 13 patients were defined as 3-4 grade of neutropenia, 9 patients were defined as 3-4 grade of anemia and 11 patients were defined as 3-4 grade of thrombocytopenia in this study (Figure 1a). The 3-4 grade of persistent cytopenia occurred in 14 patients in all these 38 R/R DLBCL patients (the hematotoxicity lasting more than 8 weeks after CAR-T cell infusion). The different types and persistent time of cytopenia in all these 14 patients who were defined as 3-4 grade of cytopenia are shown in Figure 1b. The trend of blood cell changes in patients with 3-4 grade of neutropenia, anemia and thrombocytopenia is shown in Figure 1c d e. We could see that the trend curve of grade 3-4 neutropenia has two valleys in seven patients with persistent neutropenia (Pt 2, 4, 14, 16, 21, 32, 34). Mean value of the first valley of neutropenia was $0.66\pm0.21\times10^9$/L at 7 days after CAR-T infusion, while the second valley of neutropenia was $0.21\pm0.11\times10^9$/L at 28-56 days after CAR-T infusion (Figure 1c). Such change trend was also observed in three patients (Pt 17, 21, 34) with persistent thrombocytopenia (Figure 1d). But this change trend was not observed in patients with persistent anemia.

There is no evidence of severe marrow dysplasia or relapse by bone marrow smear 8 weeks after CAR-T cell infusion in all these14 patients with 3-4 grade of persistent cytopenia. All these 14 patients with 3-4 grade of persistent cytopenia received supportive therapy to recover their persistent cytopenia. It was recovered at 8-18 weeks after CAR-T cell infusion.

After anti-CD19-CAR T-cell infusion, 3 patients who were diagnosed with 3 and 4 grade of neutropenia and 1 patients who were diagnosed with 2 grade of neutropenia were diagnosed as bacterial infections after anti-CD19-CAR T-cell therapy. All of these infections occurred in the period of their 3-4 grade of neutropenia. None of them had been diagnosed with invasive fungal disease. Only one patient (Pt 10) died of bacterial septicemia when the disease progressed again, but her neutropenia had been recovered at that time.

The clinical response of CAR-T cells

Two months after the anti-CD19-CAR T cell infusion, the efficacy of all the 38 R/R DLBCL patients was evaluated. 18 patients (18/38, 47.37%) obtained complete response (CR), while 8 patients (8/38, 21.05%) obtained partial remission (PR) in their anti-CD19-CAR T cell therapy. The ORR was 68.42% (26/38) in our study. 6 patients (6/38, 15.79%) obtained stable disease (SD), while other 6 patients (6/38, 15.79%) obtained progression of disease (PD) in .

All the 14 patients with 3-4 grade of persistent cytopenia obtained CR and PR in our study, while no patient who obtained SD and PD had 3-4 grade of persistent cytopenia after their anti-CD19-CAR T-cell therapy (Figure 2a).

In all the 18 R/R DLBCL patients who achieved CR, 15 patients survived in CR state until the cutoff date, the other three patients died of disease relapse (Pt 7,25 with persistent cytopenia, Pt 4 without persistent

cytopenia). Only three patients who achieved PR (Pt 22,26,35 with persistent cytopenia) and two patients who achieved SD (Pt 8,13 without persistent cytopenia) survived until the cutoff date. All patients who achieved PD died of disease progression again (Figure 2b).

In all the 26 R/R DLBCL patients who obtained ORR in their anti-CD19-CAR T cell therapy, 14 patients had high tumor load and 12 patients had no high tumor load. In these 26 patients, 3-4 grade of persistent cytopenia occurred in 11(11/14, 78.57%) patients with high tumor load, while in three (3/12, 25.00%) patients without high tumor load. Incidence of 3-4 grade persistent cytopenia was higher in patients with high tumor load than that of in patients without high tumor load in these 26 patients obtained ORR (P=0.006) (Figure 2c).

AEs of anti-CD19-CAR T-cell therapy

The AEs were observed more than 120 days following the anti-CD19-CAR T-cell infusion. The patients developed fever, headache, fatigue, nausea, anorexia, dyspnea, cough, tachycardia and other symptoms. In addition to hematologic toxicity, there was no difference in characteristics between the 14 R/R DLBCL patients with 3-4 grade of persistent cytopenia and the 24 R/R DLBCL patients without such persistent cytopenia after anti-CD19-CAR T-cell therapy (Table 3). In most patients, AEs in addition to hematological toxicity disappeared after 14-21 days of CAR-T cell infusion.

The mean peak of IL-6 in R/R DLBCL patients with 3-4 grade of persistent cytopenia (54.44±28.96 pg/mL) was higher than that of in patients without such persistent cytopenia (17.06±8.22 pg/mL) (P 0.0001) (Figure 3a).

In addition to five patients developed 3 grade of CRS, all the other patients developed 0-2 grade of CRS in their anti-CD19-CAR T-cell therapy. Two patients developed 2 grade of ICANS, seven patients developed 1 grade of ICANS, then the other patients developed 0 grade of ICANS in this anti-CD19-CAR T-cell therapy. The grades of CRS were higher in patients with 3-4 grade of persistent cytopenia than that of patients without such persistent cytopenia (P_{CRS} 0.0001). But we did not observe this result in patients with different grades of ICANS (P_{ICANS}=0.0740) (Figure 3bc). None of the patients died of any level of CRS or ICANS during their anti-CD19-CAR T-cell therapy. Only patients who developed 3 grade of CRS and 2 grade of ICANS received glucocorticoid or tocilizumab in anti-CD19-CAR T-cell therapy.

Anti-CD19-CAR T cell amplification in this study

Proportions of anti-CD19-CAR T cells in peripheral blood was observed on 0, 7, 14, 21, 28 and 60 days post CAR-T cell infusion. The median amplification peak of anti-CD19-CAR T cells in $CD3^+$ T cells was 14.1 (IQR 3.3-21.6)% on 4 or 7 days after CAR-T cell infusion. The median amplification peak of anti-CD19-CAR T cells was higher in patients with 3-4 grade of persistent cytopenia (24.1 (IQR 16.3-33.5)%) than that of patients without such persistent cytopenia (8.2 (IQR 2.5-12.2)%) (P=0.0006) (Figure 3d). Proportion of anti-CD19-CAR T cells could still be observed (more than 1% by FCM) at 21 days post CAR-T cell infusion in 14 patients, while it could still be observed at 28 days post CAR-T cell infusion in 6

patients. In our study, the result of patients whose CAR-T cells could be observed at 21 and 28 days after CAR-T cell infusion were associated with 3-4 grade of persistent cytopenia (Figure 3ef). All these 20 R/R DLBCL patients whose CAR-T cells could be observed at 21 and 28 days post CAR-T cell infusion were the patients who obtained CR or PR during this therapy.

Follow up

By the cutoff date, the median PFS and OS in all 38 patients was 14.32 (IQR 3-54) months and 15.11 (IQR 2-54) months. In patients with 3-4 grade of persistent time of hematological toxicity, the 1-year PFS and OS rates were 83.91% and 91.78% respectively. But in patients without such toxicity, the 1-year PFS and OS rates were 33.50% and 32.60% respectively. The PFS and OS were higher in patients with 3-4 grade of persistent cytopenia than that of patients without such persistent cytopenia (P_{PFS}=0.0019 and P_{OS}=0.0006) (Figure 4ab).

Discussion

Hematological toxicity is not an uncommon condition after anti-CD19-CAR T-cell therapy. Most (90.4%) persistent cytopenia occurred between day 31 to 180 after CAR-T cell infusion in a study of toxicities in anti-CD19-CAR T-cell therapy [24]. In ZUMA-1 study [6, 12, 25], grade 3 or higher cytopenia is frequently in first 30 days after anti-CD19-CAR T-cell infusion and is associated with lymphodepleting chemotherapy with fludarabine and cyclophosphamide probably. It has been reported that the cytopenia could generally recover within several weeks even after six cycles of fludarabine and cyclophosphamide in chronic lymphocytic leukemia patients [26]. But in ZUMA-1 study, the cytopenia might persist for three months or later without evidence of marrow dysplasia or relapse [4]. Whether there is hematologic toxicity unrelated to the lymphodepleting chemotherapy?

The definition of prolongation and severity of cytopenia varies in diffenent studies [13, 27–29]. A high incidence of persistent CTCAE grade ≥3 neutropenia (30-38%), thrombocytopenia (21-29%), and anemia (5-17%) after day 21 has been reported. In our study, 14 in 38 R/R DLBCL patients developed 3-4 grade of persistent cytopenia after their anti-CD19-CAR T-cell therapy. To distinguish the persistent cytopenia from the hematological toxicity caused by lymphodepleting chemotherapy with fludarabine and cyclophosphamide, the persistent cytopenia in our study was defined as hematotoxicity lasting more than 56 days after CAR-T cell infusion. Meanwhile most AEs in addition to hematotoxicity had disappeared at 21-28 days after CAR-T cell infusion. The 3-4 grade of persistent cytopenia duration and recovery, and the clinical characteristics related to 3-4 grade of persistent cytopenia after anti-CD19-CAR T-cell therapy were analyzed in these 14 R/R DLBCL patients.

What's interesting in our study is that the trend curve of grade 3-4 neutropenia has two valleys in seven patients with persistent neutropenia at 7 days and 28-56 days after CAR-T infusion respectively. This 3-4 grade of persistent cytopenia was recovered at 8-18 weeks after CAR-T cell infusion with supportive therapy. The first valley of grade 3-4 neutropenia might be related to the lymphodepleting chemotherapy

with fludarabine and cyclophosphamide. But what is the cause of the second valley of grade 3-4 neutropenia? Most of the infections after anti-CD19-CAR T-cell therapy occurred after their neutropenia had occurred in this study. Meanwhile, there was no evidence of severe marrow dysplasia or relapse in bone marrow at the second valley of grade 3-4 neutropenia in these patients. So the second valley of grade 3-4 neutropenia was not caused by the lymphodepleting chemotherapy, the infections and the marrow dysplasia or relapse, while it is most likely caused by the anti-CD19-CAR T-cell therapy. But the underlying mechanism is still unclear.

Other factors such as cytotoxic therapies, autologous or allogeneic HSCT have been hypothesized to cause the development of cytopenia [10, 28, 30]. In our study, other baseline characteristics of the R/R DLBCL patients did not differ between patients with and without 3-4 grade of persistent cytopenia, except that tumor load was larger in R/R DLBCL patients with persistent cytopenia before the anti-CD19-CAR T-cell therapy. Our results provide further evidence that anti-CD19-CAR T-cell therapy itself might lead to 3-4 grade of persistent cytopenia. A study reported that the increasing grade of CRS and ICANS, baseline cytopenias, CAR construct, higher peak C-reactive protein or ferritin levels were associated with persistent cytopenia for more than one month [18]. Another previous study demonstrated the same result that the grade ≥4 CRS was associated with delayed hematopoietic recovery [30]. Anti-CD19-CAR T-cell induced cytolysis of CD19 positive lymphoma cells facilitates antigenic spreading, which might be associated with severe CRS [31–33]. Yet another study proved that the severe neutropenia until 60 days after anti-CD19-CAR T-cell therapy was not associated with the incidence and severity of CRS, ICANS and the peak cytokine levels absolutely, while it was associated with hematopoietic reserve and baseline inflammation [23].

In our study, all R/R DLBCL patients who occured 3-4 grade of persistent cytopenia obtained ORR in their anti-CD19-CAR T-cell therapy. Furthermore, in all 26 patients who obtained ORR, the incidence of 3-4 grade of persistent cytopenia was higher in patients with high tumor load than that of patients without high tumor load. The other factors associated with 3-4 grade of persistent cytopenia include the peak level of IL-6, the grade of CRS, and the peak proportion of anti-CD19-CAR T cells in anti-CD19-CAR T-cell therapy. In addition, the persistent existence of CAR-T cells in peripheral blood at 21 and 28 days after infusion was associated with 3-4 grade of persistent cytopenia. Whereas, persistent cytopenia was not associated with grade of ICANS.

In a long-term follow-up study in patients after anti-CD19-CAR T-cell therapy, 16% of patients with persistent CR had sustained significant persistent cytopenia after infusion [30]. Similar results were obtained in our study. The PFS and OS were longer in patients with 3-4 grade of persistent cytopenia than that of patients without such persistent cytopenia.

Conclusion

In our study, 3-4 grade of persistent cytopenia occurred in 14 R/R DLBCL patients after anti-CD19-CAR T-cell therapy. All these 14 patients achieved ORR in this therapy. Our research proved that the 3-4 grade of

persistent cytopenia was associated with high tumor load, higher peaks of IL-6 and CAR T cells, higher grades of CRS, and longer PFS and OS. None of these patients developed severe infection or death as a direct result of infection in our study. Our study is helpful in predicting efficacy and severe hematologic side effects of anti-CD19-CAR T-cell therapy in R/R DLBCL patients.

Abbreviations

DLBCL: Diffuse large B-cell lymphoma, R/R: Relapsed/refractory, Chimeric antigen receptor T cell: CAR T-cell, AEs: Adverse events, CRS: Cytokine release syndrome, ICANS: Immune effector cell associated neurotoxic syndrome, ORR: Objective response rate, PFS: Progression free survival, OS: Overall survival, IL-6: Interleukin-6.

Declarations

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Authors' contributions

XML, MJL and QD contributed to the conception and design of the study and to the draft of the manuscript. XML and MJL were responsible for writing the manuscript. QD, XML, RC, CCL and JW were responsible to patients' enrollment and treatment, clinical data and management of patients. NM carried out statistical analysis. MJL and QL collected data and the follow-up of patients. All authors agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are

available within the article.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of the Tianjin First Center Hospital (Tianjin, China). (Approved No. of ethic committee: 2015002X and 2018N105KY).

Informed consent was obtained from the participants. The patients agreed to participate in our Clinical trials. They agreed to the use of their data for our study. All the data andmaterial have been performed in accordance with the Declaration of Helsinki and conformed to relevant aspects of the ARRIVE guidelines.

This Clinical trials is registered at http://www.chictr.org.cn/index.aspx as ***ChiCTR-ONN-16009862*** and http://www.chictr.org.cn/index.aspx as ***ChiCTR1800019622***.

Consent for publication

Not applicable.

Conflict of Interest

The authors have no conflict of interest to report.

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Tables

Table 1. Patients baseline characteristics

	Sex	Age	Subtype	Stage	IPI	Prelines of therapy	Poor prognosis
P1	M	56	Non-GCB	III	3	3	C-MYC BCL6 (DE),TP53
P2	M	25	GCB	IV	2	3	No
P3	M	33	Non-GCB	III	2	3	C-MYC BCL6 (DH)
P4	M	45	GCB	IV	3	4	C-MYC BCL6 (DE)
P5	M	72	Non-GCB	II	2	2	No
P6	M	49	GCB	III	2	4	C-MYC BCL6 (DE),TP53
P7	F	79	GCB	III	2	3	C-MYC BCL2 (DE)
P8	M	55	GCB	IV	3	3	C-MYC BCL2 BCL6 (TH)
P9	M	64	Non-GCB	IV	3	4	No
P10	F	29	GCB	III	3	4	C-MYC BCL2 (DE)
P11	F	33	Non-GCB	III	2	3	No
P12	M	40	Non-GCB	IV	3	2	C-MYC BCL6 (DT)
P13	M	46	Non-GCB	III	3	4	C-MYC BCL6 (DE)
P14	M	22	GCB	IV	3	5	No
P15	M	35	Non-GCB	II	3	2	C-MYC BCL6 (DH) ,TP53
P16	M	41	Non-GCB	III	2	2	C-MYC BCL2 (DE)
P17	M	46	GCB	III	2	4	No
P18	M	56	Non-GCB	IV	3	3	C-MYC BCL2 (DH) ,TP53
P19	M	38	GCB	IV	2	3	No
P20	F	72	Non-GCB	III	3	3	TP53
P21	M	50	Non-GCB	IV	4	3	C-MYC BCL6 (DE)
P22	M	41	Non-GCB	III	3	5	TP53
P23	F	55	GCB	IV	2	4	No
P24	F	35	GCB	II	2	3	No
P25	M	62	Non-GCB	III	3	4	No
P26	F	52	Non-GCB	III	3	5	C-MYC BCL2 (DH)
P27	F	70	Non-GCB	IV	2	3	No
P28	M	58	GCB	IV	2	4	C-MYC BCL6 (DE)

P29	F	71	GCB	III	3	3	C-MYC BCL2 BCL6 (TH)
P30	M	76	Non-GCB	IV	3	3	C-MYC BCL2 (DE)
P31	M	51	Non-GCB	III	3	4	C-MYC BCL6 (DE),TP53
P32	F	66	GCB	III	3	3	No
P33	M	52	Non-GCB	II	2	4	C-MYC BCL2 BCL6 (TH),TP53
P34	M	45	GCB	III	2	3	C-MYC BCL6 (DT)
P35	F	72	GCB	IV	2	5	C-MYC BCL2 (DE)
P36	M	61	GCB	IV	3	3	TP53
P37	M	57	Non-GCB	III	2	5	C-MYC BCL2 (DH) ,TP53
P38	M	79	Non-GCB	III	4	3	No

NOTE: GCB: Germinal center B-cell-like lymphoma**, Non-GCB:**Non-Germinal center B-cell-like lymphoma**,DH:**Double Hit, **TH:**Triple Hit, **DE:**Double expression**, TE:** Triple expression, **TP53**:TP53 is mutation or deletion

Table 2. Comparison of baseline characteristics between the two groups

	With persistent cytopenia (n=14)	Without persistent cytopenia (n=24)	*P* value
Sex			
Male:Femal	9:5	16:8	1.000
Age (years)	50.6±17.8	53.4±13.8	0.595
Subtype			
Non-GCB: GCB	6:8	15:9	0.317
Stage			
I-II□III-IV	0:14	4:20	0.275
IPI(Score)			
1-2:3-4	5:9	12:12	0.505
Poor prognosis			
With□Without	8:6	17:7	0.486
Max tumor diameter(cm)	12.89±4.91	5.04±1.88	**0.001**
Prelines of therapy	2.78±0.69	2.50±0.51	0.337

NOTE:Poor prognosis including:DH: Double Hit, **TH:**Triple Hit, **DE:**Double expression, **TP53:**TP53 is mutation or deletion

Table 3. AEs in the process of anti-CD19 CAR-T cell therapy

Events	With persistent cytopenia (n=14)	Without persistent cytopenia (n=24)	*P* value
General condition			
Temperature ≥38 °C (fever)	13/14 (92.86%)	20/24 (83.33%)	0.663
Chills	5/14 (35.71%)	9/24 (37.50%)	1.000
Rash	3/14 (21.43%)	3/24 (12.50%)	0.65
Muscular weakness	5/14 (35.71%)	8/24 (33.33%)	1.000
Hypotension	2/14 (14.29%)	0/24 (0.00%)	0.129
Hypoxia	2/14 (14.29%)	0/24 (0.00%)	0.129
Fatigue	11/14 (78.57%)	15/24 (62.50%)	0.472
Organ toxicities	4/14 (28.57%)		
Gastrointestinal	3/14(21.43%)		
Nausea	7/14 (50.00%)	3/24 (33.33%)	0.387
Vomiting	4/14 (28.57%)	1/24 (4.17%)	0.132
Decreased appetite	6/14 (42.86%)	4/24 (16.67%)	0.061
Diarrhea		1/24 (4.17%)	0.052
Abdominal pain		5/24 (20.83%)	0.266
Cardiac			
Tachycardia	3/14 (21.43%)	2/24 (8.33 %)	0.337
Arrhythmias	1/14 (7.14 %)	0/24 (0.00%)	0.368
Respiratory			
Dyspnoea	2/14 (14.29%)	0/24 (0.00%)	0.129
Cough	4/14 (28.57%)	5/24 (20.83%)	0.699
Pleural effusion	5/14 (35.71%)	6/24 (25.00%)	0.712
Renal			
Increased creatinine	3/14 (21.43%)	2/24 (8.33%)	0.337
Oliguria	5/14 (35.71%)	7/24 (29.17%)	0.728
Hepatic			
Increased serum ALT, AST	4/14 (28.57%)	2/24 (8.33 %)	0.167

Increased serum bilirubin levels	2/14 (14.29%)	3/24 (12.50%)	1.000
Neurological			
Headache	6/14(42.86%)	9/24 (37.50%)	1.000
Dizziness	2/14 (14.29%)	0/24 (0.00%)	0.129
Confused	1/14 (7.14 %)	0/24 (0.00%)	0.368
Somnolence	2/14 (14.29%)	2/24 (8.33%)	0.616
Aphasia	1/14 (7.14 %)	0/24 (0.00%)	0.368
Disorientation	1/14 (7.14 %)	0/24 (0.00%)	0.368
Hematological			
Neutropenia (grade 3/4) （<1*10^9/L）	10/14 (71.43%)	3/24 (12.50%)	**0.000**
Anemia (grade 3/4) （<80g/L）	7/14 (50.00%)	2/24 (8.33 %)	**0.006**
Thrombocytopenia (grade 3/4) (<50*10^9/L)	9/14 (64.29%)	2/24 (8.33%)	**0.000**

NOTE: The AEs are according to the National Cancer Institute's Common Terminology

Criteria for Adverse Events (version 4.03).

Figures

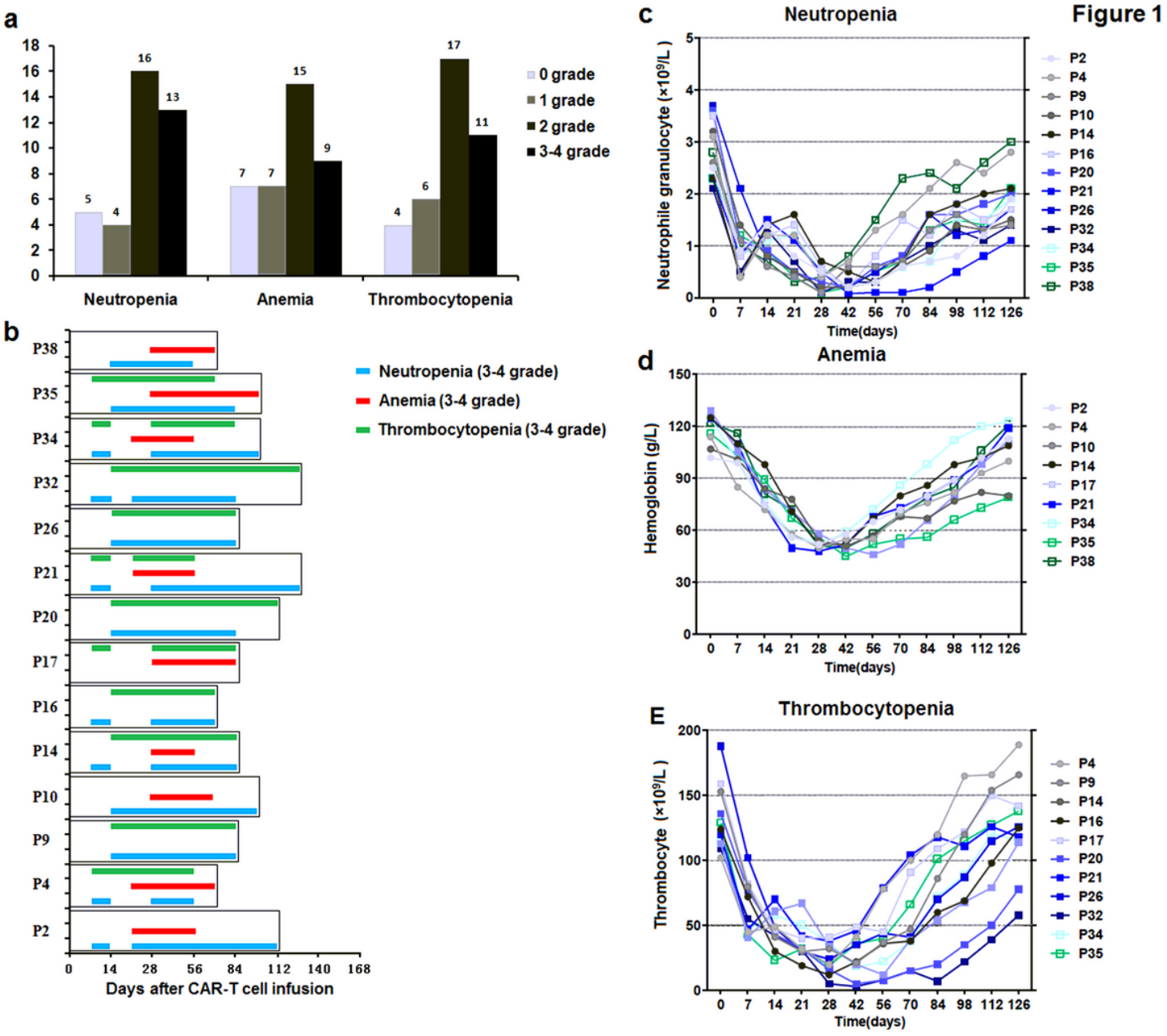

Figure 1

Hematological toxicity of anti-CD19-CAR T-cell therapy. a. The neutropenia, anemia and thrombocytopenia was diagnosed with 0-4 grade after anti-CD19-CAR T-cell therapy. **b.** The different types and persistent time of cytopenia in the 14 patients who were defined as 3-4 grade of persistent cytopenia**. c-e.** The trend of blood cell changes in patients with 3-4 grade of neutropenia, anemia and thrombocytopenia.

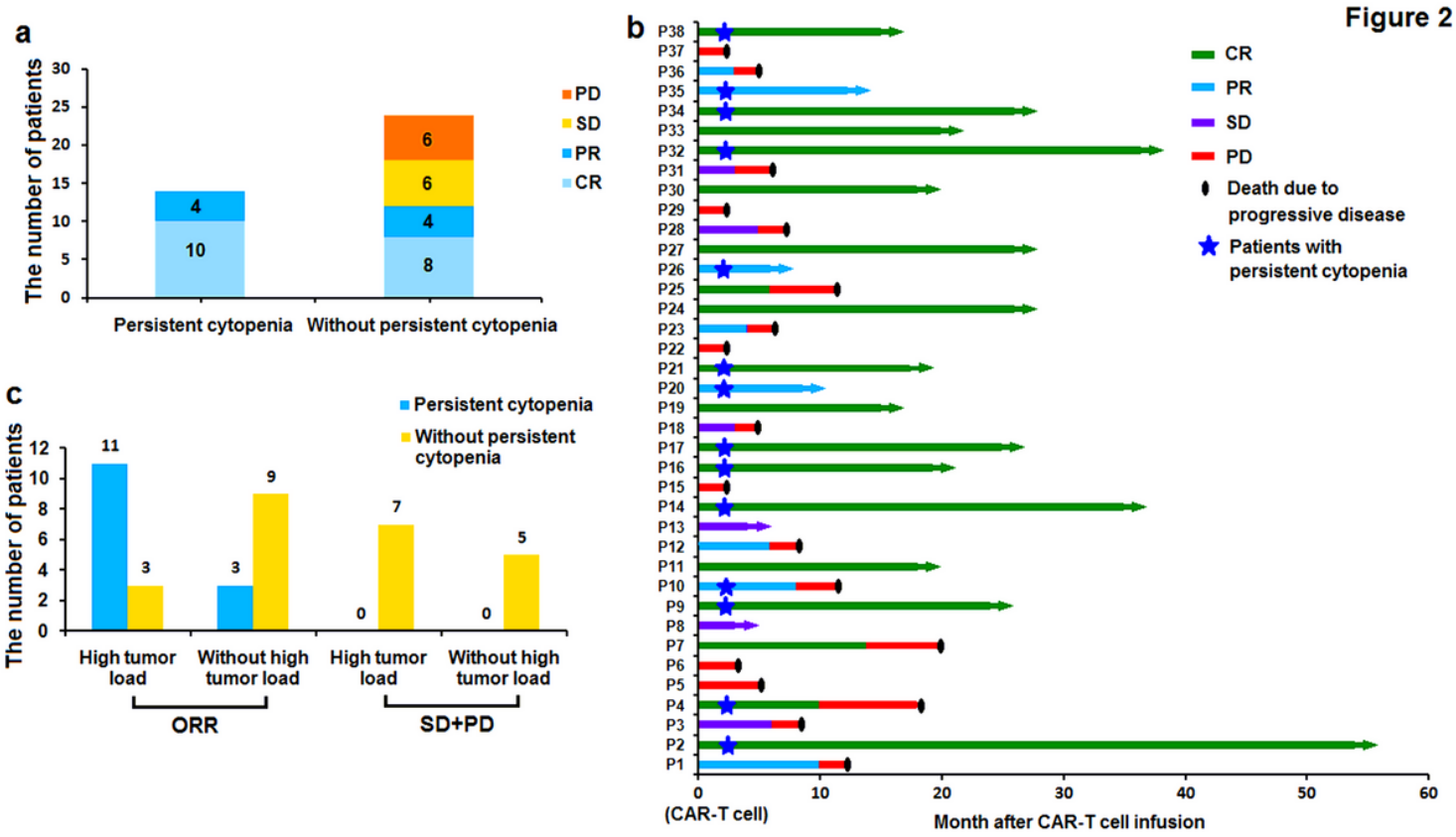

Figure 2

a. All the 14 patients with 3-4 grade of persistent cytopenia obtained CR and PR in our study, while no patient who obtained SD and PD had such cytopenia in CAR T-cell therapy**. b.** The clinical response to anti-CD19-CAR T-cell therapy, disease progression and survival time of all the 38 patients. **c.** The incidence of 3-4 grade of persistent cytopenia was higher in patients with high tumor load in 26 patients obtained ORR.

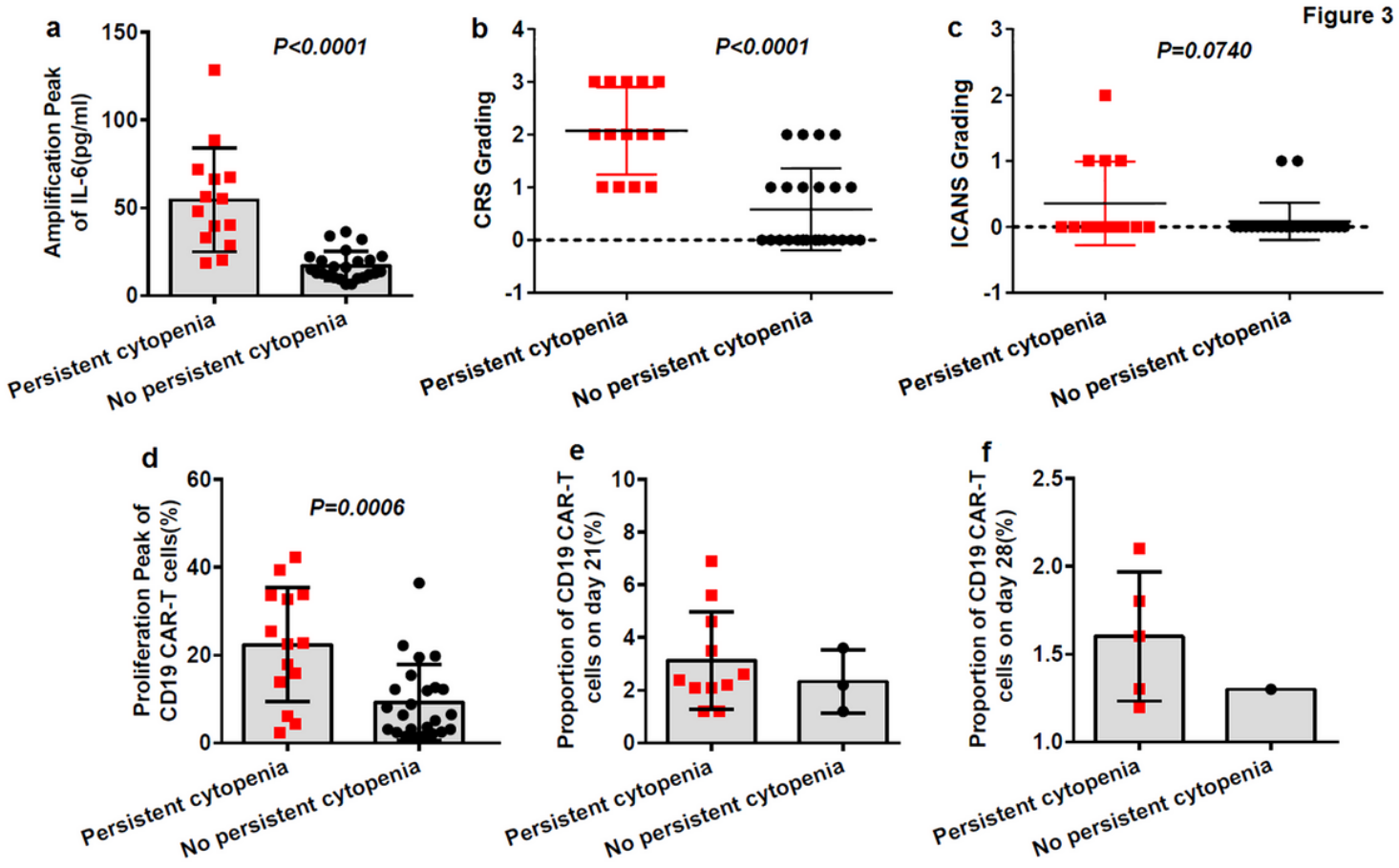

Figure 3

AEs of anti-CD19-CAR T-cell therapy. a. The mean of peak of IL-6 in patients with 3-4 grade of persistent cytopenia was more higher. **b.** The higher grades of CRS were higher in patients with 3-4 grade of persistent cytopenia than that of in patients without such persistent cytopenia. **c.** There was no difference of 3-4 grade of persistent cytopenia between different grades of ICANS. **d.** The median amplification peak of CAR T cells was higher in patients with 3-4 grade of persistent cytopenia than that of in patients without such persistent cytopenia. **e-f.** The majority of patients whose CAR-T cells could be observed at 21 and 28 days after CAR-T cell infusion were associated with 3-4 grade of persistent cytopenia.

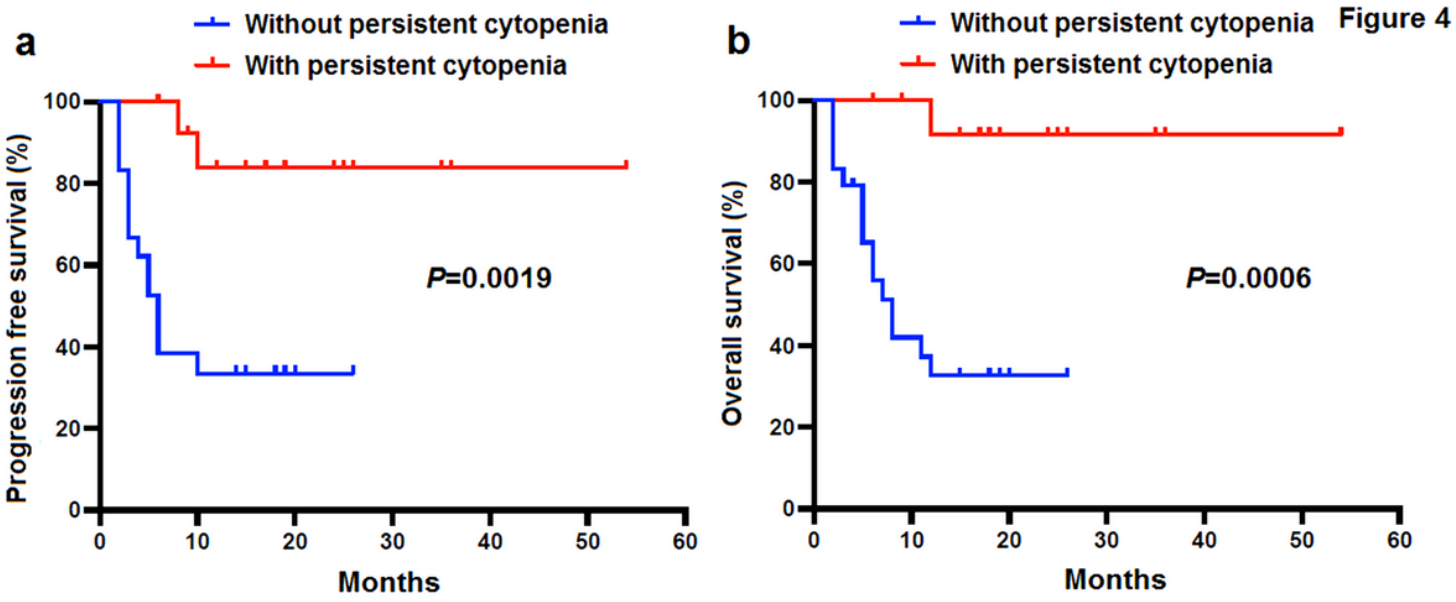

Figure 4

Figure 4

Follow up after anti-CD19-CAR T-cell therapy. a-b. The PFS and OS were more higher in patients with 3-4 grade of persistent cytopenia.