CASE REPORT
HEMATOLOGY

Plasma Exchange to Treat Cytokine Release Syndrome and Immune Effector Cell-Associated Neurotoxicity Syndrome after Anti-CD19 Chimeric Antigen Receptor-T Cell Infusion: A Case Report

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Abstract: Adoptive cell immunotherapy with chimeric antigen receptor-T (CAR-T) cells has shown remarkable clinical outcomes. However, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are the two most significant toxicities during this therapy and can be life-threatening. We described a 12-year-old juvenile who had been diagnosed with relapsed and refractory B-cell acute lymphocytic leukemia (r/r B-ALL). The patient was recruited into our phase I clinical trial concerning ssCAR-T-19 (anti-CD19 CAR-T cells with shRNA targeting IL-6), and 5*10⁶/kg of engineered ssCAR-T-19 cells were administered. After infusion, the patient underwent a typical CRS reaction, with fever and increased cytokine levels. He was treated with antipyretic drugs, methylprednisolone, and tocilizumab, but the effect was limited. He developed coagulation abnormalities, multiple organ dysfunction, lung infection and ICANS. Apart from the necessary supportive and symptomatic treatment, plasma exchange was performed three times in four days while methylprednisolone pulse was performed for two consecutive days. After that, the body temperature, heart rate, and especially the cytokine levels declined. But digestive tract hemorrhage occurred to him and he was transferred to intensive care unit. To make things worse, he developed acute respiratory failure and received intubation and mechanical ventilation. In addition, symptomatic treatment such as suppression of stomach acid and anti-infection was given. The bleeding was controlled, and his respiratory function improved, and the CRS and ICANS-related symptoms were relieved. He received extubation and was transferred back to the general ward. Additionally, a bone marrow smear showed no lymphoblast cells, and minimal residual disease in bone marrow was negative on day +22 and day +30. The patient was eventually discharged in a normal condition. In conclusion, CRS and ICANS as two most common toxicities after CAR-T therapy, which often cause patient death. Several methods such as anti-IL-6 therapy and/or corticosteroids have been adopted in the management guidelines of CRS and ICANS except plasma exchange. This case shows the validity of plasma exchange in a patient with severe CRS and ICANS after receiving ssCAR-T.

Keywords: Plasma exchange; Chimeric antigen receptor T cell therapy; Cytokine release syndrome; Immune effector cell-associated neurotoxicity syndrome.

Introduction

Autologous T cells expressing a chimeric antigen receptor (CAR) have emerged as a powerful living drug to treat malignant diseases. Clinical trials employing anti-CD19 CAR-T cells have shown promising results in patients with relapsed or refractory B-cell acute lymphoblastic leukemia (r/r B-ALL), chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin lymphoma (B-NHL) [1-8]. Due to their outstanding clinical effectiveness, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved four anti-CD19 CAR-T cell products to treat patients ≤ 25 years old with r/r B-ALL (Kymriah), adult patients with r/r diffuse large B-cell lymphoma (r/r DLBCL) (Kymriah, Yescarta and Breyanzi) [9-11], adult patients with r/r mantle-cell lymphoma (KTE-X19) [12].

However, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), the two...
most common adverse events associated with CAR-T therapy, could be life-threatening. The incidence of CRS is between 58% and 95% \cite{3,13,14} while the incidence of ICANS is reported to be between 12% and 64% \cite{3,13,15}.

Various cytokines observed in the serum such as IL (interleukin) -6, IL-10 and IFN (interferon)-γ contribute to the different clinical symptoms associated with CRS \cite{16}. A study demonstrated that the monocytes in the circulation are the main source of IL-6 during CRS \cite{17}. IL-6 caused many of the key symptoms of CRS, like vascular leakage and disseminated intravascular coagulation (DIC) \cite{18,19}. Furthermore, the release of large amounts of cytokines such as IL-6, IFN-γ, and TNF-α caused endothelial activation, vascular damage, and disruption of blood-brain barrier (BBB), resulting in a failure of the BBB to shield the cerebrospinal fluid (CSF) from cytokines and CAR-T cells \cite{20}. The disruption of the BBB may lead to ICANS.

Therefore, corresponding treatment measures including anti-IL-6 therapy and corticosteroids have been recommended (NCCN guidelines Version 3.2021). However, plasma exchange which can remove inflammatory cytokines has not been proposed as a standard measure for CRS and ICANS.

Moreover, it has been proven that the efficiency of anti-CD19 CAR-T with IL-6 knockdown (ssCAR-T-19) to reduce IL-6 release by monocytes in vitro and decrease IL-6 levels in circulation in mice \cite{21}, which may reduce the incidence of severe CRS and ICANS.

Here, we report a case involving a patient with r/r B-ALL who developed severe CRS (sCRS) and ICANS after infusing ssCAR-T-19 cells. In addition to tocilizumab and methylprednisolone pulse, plasma exchange was performed for 3 times. The patient’s cytokine levels decreased dramatically, and his CRS and ICANS-related symptoms were relieved in combination with other supportive treatment. Moreover, minimal residual disease (MRD) in bone marrow (BM) were negative. Ultimately, the patient was discharged in a normal condition.

Case presentation

B-ALL treatment history
A 12-year-old juvenile, who presented with swollen and painful left cheek, was initially admitted to Anhui Provincial Hospital in July 2019. He was diagnosed as B-ALL with IKZF1 mutation and was treated with chemotherapy (vincristine + daunorubicin + l-asparaginase + dexamethasone *1 course, cyclophosphamide + cytarabine + 6-mercaptopurine *2 courses, methotrexate *1 course). After sustained remission, he received allogeneic hematopoietic stem cell transplantation (allo-HSCT) on 19th November, 2019. Unfortunately, the tumor recurred four months later with 93% of blast in BM. Subsequently, the patient underwent another three courses of chemotherapy (cyclophosphamide + idarubicin + vincristine + prednisone), but the effects were poor, and an ultrasound revealed bilateral swollen testicle. He developed r/r B-ALL and was admitted to the Department of Hematology of the First Affiliated Hospital of Soochow University on July 8th, 2020.

The results of his baseline laboratory tests were as follows: (i) The white blood cell (WBC), hemoglobin (Hb), and platelet (PLT) levels were 0.98*10^9/L, 64 g/L, and 27*10^9/L, respectively; (ii) A BM smear showed that lymphoblast cells accounted for 1% of non-erythroid cells (NEC) (Fig. 1A); (iii) Immunophenotyping via flow cytometry indicated that malignant cells accounted for 74.36% of all examined cells (Fig. 1B), exhibiting the expression pattern CD34’ HLA-DR’CD10’CD19’CD79a’CD25’CD22’; (iv) Conventional cytogenetic analysis demonstrated 45, XY, 6q-, -7, der(9), t(7; 9)(p22; p21) [3] / 46, XY [7] karyotype; (v) Genotyping via second-generation sequencing revealed NOTCH2 mutations; (vi) A liver function test showed elevated alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT). Based on all of these findings, the patient was diagnosed with r/r B-ALL with a high tumor burden and was recruited into our CAR-T clinical trial (ClinicalTrials.gov number, NCT03275493) for r/r B-ALL.

ssCAR-T-19 cell therapy and related toxicity management
During the clinical trial, the T cells from the patient’s own were used to construct ssCAR-T-19 cells after approval by the ethics committee of our hospital. Generally, cells were enriched by anti-CD3 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). CD3+ T cells were stimulated with anti-CD3/CD28 monoclonal antibodies (Miltenyi Biotec) for 24 h and then transduced with recombinant lentiviral vectors consisting of the CD19-specific CAR, consisting of an anti-CD19 single-chain variable fragment (scFv), 4-1BB costimulatory moiety, CD3ζ activation domain, and IL-6 shRNA element, all manufactured by
UniCar Therapy Ltd (Shanghai, P.R. China). ssCAR-T-19 cells were cultured in AIM-V media (Gibco, Grand Island, NY, USA) supplemented with 10% autologous serum, 100 IU/ml IL-2 (Peprotech, Rocky Hill, USA), 5 ng/ml IL-7 (Peprotech), and 5 ng/ml IL-15 (Peprotech) for 12 days.

Before ssCAR-T-19 cell infusion, the patient underwent lymphodepleting chemotherapy for 3 days with fludarabine (30 mg/m²/d) and cyclophosphamide (300 mg/m²/d). Five days later, the patient received ssCAR-T-19 cell infusion on day 1 (30%) and day 2 (70%) at a total dose of 5×10⁶ cells/kg (Fig. 2A).

Presentation, management, and outcome of CRS of the patient are shown in Fig. 2B. Fever ≥38°C was the first objective sign of CRS which occurred on d+4 accompanied by tachycardia (Fig. 2B, 3A) and antipyretic drugs were administered to control the fever. However, the body temperature peaked up to 40.3°C and the patient developed hypoxia requiring low-flow nasal cannula on d+6. Because the body temperature remained high, 20 mg of methylprednisolone was administered on d+7. But the effect was limited. The patient complained of abdominal pain, dysphagia, paroxysmal dry cough and eyelid edema, and developed hypotension on d+8. Apart from the daily routine test after ss-

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**Fig. 2. Medication protocol relative to the timing of ssCAR-T therapy and presentation, management, and outcome of CRS.**

A. Lymphodepleting chemotherapy include fludarabine and cyclophosphamide was performed 7 days prior to ssCAR-T-19 infusion. ssCAR-T-19 cells were infused at a split-dose of 30% on day 1, 70% on day 2 (total 5×10⁶/kg). F, fludarabine. C, cyclophosphamide.

B. Different colors on the swimmer plot indicate the CRS grades on each day through 18 days after ssCAR-T-19 infusion. Interventions with methylprednisolone, tocilizumab, plasma exchange, and the duration of neurotoxicity are indicated. Doses of medications: methylprednisolone 20 mg on d+7, 100mg on d+8, 120mg on d+9, 240mg on d+10 and d+11 intravenously (green trapezoid indicated the dose escalation); tocilizumab 320mg on d+8, d+9 and d+10 intravenously; plasma exchange 2000ml on d+8, 1500ml on d+10 and 2000ml on d+11. NT, neurotoxicity.
CAR-T-19 cell infusion, electrocardiogram, chest X-ray, abdominal ultrasound, myocardial enzyme and amylase were ordered immediately. He suffered from coagulation abnormalities, cardiac dysfunction, liver dysfunction manifested by prolongation of the activated partial thromboplastin time (APTT) and prothrombin time (PT), falling fibrinogen concentration (Fig. 3B,C,D), and elevated N-terminal pro-brain natriuretic peptide (NT-pro BNP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Table 1). Besides, ultrasound showed the ascites while X-ray indicated bilateral lobular infection. The patient was then

![Graphs showing clinical and laboratory parameters](image)

**Fig. 3.** Clinical and laboratory parameters before and after ssCAR-T-19 cells.
A. The trends of the patient’s temperature in centigrade (°C) degrees and heart rate.
B-D. Coagulation parameters including APTT, PT and fibrinogen.
E. Cytokines including IL-6 and IL-10, IFN-γ, and ferritin.
F. C-reactive protein (CRP).
administered with platelets, cryoprecipitate, cedilanid, diuretic, and hepatic protectant. Antibiotics were escalated while continuous intravenous infusion of norepinephrine was administered since d+8 to increase blood pressure. Significantly, the cytokine levels and C-reactive protein (CRP) were observed to elevate extremely when compared those of d1 (e.g. IL-6 1075-fold increase, IFN-γ 1964-fold increase, IL-10 138-fold increase, ferritin 80-fold increase, CRP 24-fold increase) and peak between d+8 and d+9 (Fig. 3E,F). Correspondingly, the CRS progressed from grade 1 to grade 3. Therefore, 100 mg methylprednisolone was administered and 320 mg tocilizumab was initiated since d+8 (in total four times) to control CRS. To our disappointment, improvement in the patient’s condition was not achieved. Therefore, plasma exchange using 2000 ml fresh frozen plasma as the replacement solution were performed. On d+9, the second body peak temperature occurred. The dosage of methylprednisolone was increased to 140 mg. However, cytokine levels continued to increase on d+9 and the neurotoxicity including depressed level of consciousness and impaired cognitive skills presented on d+10. Therapy was strengthened with 240 mg of methylprednisolone pulse for two consecutive days and 100 mg of mannitol was initiated. Aggressively, the second and third plasma exchange (1500 ml & 2000 ml) were performed on two consecutive days and cytokine levels decreased dramatically accompanied by declined body temperature. Therefore, the administration of methylprednisolone was terminated. However, on d+12, the patient developed digestive tract hemorrhage manifested by melena. In addition, his arterial oxygen saturation (SaO₂) fluctuated around 90% with the support of high flow oxygen via facemask and his blood pressure (BP) fluctuated round 80-96/36-58 mmHg with the support of norepinephrine. His NT-proBNP and AST continued to rise. He was transferred to intensive care unit (ICU) due to the hourly worsening condition. He was fasted and received MAP, platelets, plasma, VII factor, cryoprecipitate, fibrinogen, somatostatin and proton pump inhibitors (PPI) to control the hemorrhage, recombinant human natriuretic peptide, dobutamine to treat heart failure. Later at night on d+13, the patient developed acute respiratory failure and received pressure-controlled mechanical ventilation after intubation. Gradually after that, the bleeding was controlled, his respiratory function improved and the CRS and ICANS-related symptoms.

Table 1. The results of NT-proBNP, ALT and AST in the patient

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NT-proBNP, N-terminal pro-brain natriuretic peptide; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Fig. 4. BM smear and flow cytometric analysis of MRD after ssCAR-T-19 cell infusion.
A-B. The result of BM smear performed on d+22, and d+30 after infusion.
C-E. MRD in the patient by flow cytometry analysis on d+1, d+22, and d+30 respectively.
were relieved. The laboratory parameters also improved, and CRS grade declined to grade 3. Therefore, he was transferred from ICU to general ward on d+18.

Response
Bone marrow morphology analysis suggested complete remission (CR) on d+22 and d+30 (Fig. 4A,B). Bone marrow aspiration analysis by flow cytometry showed the MRD was $5 \times 10^{-7}$ on d+1, $<1.2 \times 10^{-3}$ on d+22 and $<6.8 \times 10^{-5}$ on d+30 (Fig. 4C,D,E).

Expansion and subsets of ssCAR-T-19 cells
The level of ssCAR-T-19 cells in the patient’s peripheral blood was monitored daily after infusion. ssCAR-T-19 cells started to expand rapidly on d+5 and the peak expansion occurred on d+13. High level of ssCAR-T-19 cells (copies $\geq 1 \times 10^5$/L) maintained for 5 days (Fig. 5A). The subsets of ssCAR-T-19 cells were analyzed by flow cytometry on d+17 after infusion (Fig. 5B). The majority of ssCAR-T-19 cells were CD8$^+$ and effect memory T cells (Tem), accounted for 90.31% and 99.80%, respectively.

Discussion
Anti-CD19 CAR-T cells are widely used in the treatment of r/r B-ALL due to their powerful ability to kill tumor cells. A variety of studies have shown that the complete remission was 54.5%-92.3% [22-24]. However, various fatal toxic effects were frequently observed after CAR-T cell therapy. The CRS and ICANS were two common toxicities associated with CAR-T cells therapy. CRS was defined as activation of T cells and release of large amounts of inflammatory factors resulting in a systemic inflammatory response, resulting in organ damage due to CAR-T therapy. Patients with CRS often presented with fever, headache and other life-threatening conditions and was grad-
ed according to the patient’s symptoms after CAR-T infusion [25]. The driving cytokine underlying CRS is thought to be IL-6, which is derived from monocytes, initiating a pro-inflammatory signaling cascade that underlies the myriad of symptoms of CRS [27,19]. CAR-T with IL-6 knockdown could significantly reduce IL-6 release from monocytes in vitro and lower IL-6 levels were found in serum of mice without decreasing anti-tumor efficiency [21].

However, the patient in this case developed sCRS despite the use of IL-6 knocking down CD19 CAR-T. Previous research have demonstrated that higher disease burden, cyclophosphamide/fludarabine lymphodepletion, higher CAR T-cell dose and higher peak expansion of CAR-T cells were associated with sCRS [26]. After analysis, we found that the patient had high tumor burden and it may contribute to sCRS. The BM smear of the patient showed that bone marrow blasts accounted for 1%. Yet, the bone marrow aspiration analysis by flow cytometry showed the malignant cells accounted for 74.36%. Blood dilution may be a cause of lower bone marrow tumor burden. Moreover, bilateral swollen testicle suggested the presence of extramedullary lesions, considering that the patient had a high tumor burden.

ICANS was defined as damage to the central nervous system caused by cytokines and CAR-T cells that diffuse into the central nervous system after CAR-T, resulting in a range of clinical symptoms. ICANS was often observed in association with and after CRS. Patients with ICANS mainly presented with altered attention, impaired consciousness, seizures, motor weakness and cerebral edema [25]. High CD19$^+$ cells in bone marrow, CRS, high CAR-T cell dose, and pre-existing neurologic comorbidities were proved to be risk factors for the development of ICANS [20]. The patient in this case also had severe ICANS after ssCAR-T-19 infusion, mainly presenting with unconsciousness and cognitive depression. As mentioned before, the high percentage of malignant cells in the flow analysis as well as extramedullary lesions were indicative of a high tumor burden in the patient. In addition, he developed sCRS after ssCAR-T-19 cell infusion, mainly manifest-
ed by a dramatic rise in cytokines and impaired consciousness.
High tumor burden and severe CRS may be related to his severe ICANS.

The peak expansion of the ssCAR-T-19 cells occurred on the d+13. This is in line with the findings of previous researches, in which the median time to maximum expansion was 10 days in patients with a response [3]. The ssCAR-T-19 cells in the patient were mainly CD8+ T cells and Tem. Compared to the central memory T cells (Tcm), Tem cells have stronger IFN-γ secretion capacity and cytotoxicity, ensuring the ability of CAR-T cells to kill tumor cells [27]. Therefore, the potent secretion capacity and cytotoxicity of the high proportion of the CD8+ T cells and Tem may lead to sCRS and ICANS.

In this case, the serum biomarkers in the patient such as cytokines, CRP and ferritin increased sharply after ssCAR-T-19 infusion. IL-6, IL-10 and IFN-γ are core cytokines responsible for a range of clinical symptoms, with IL-6 playing a key role [16]. According to NCCN guidelines (Version 3.2021), sCRS and severe ICANS were recommended to be treated with anti-IL-6 therapy (tocilizumab), corticosteroids (dexamethasone or methyl-prednisolone) and other supportive treatments. Tocilizumab was approved by the FDA for the treatment of CRS since it ameliorated CRS without reducing T cells’ ability to kill tumor cells [29]. However, due to its inability to cross the BBB [30], the FDA did not approve tocilizumab for the treatment of ICANS. As for corticosteroids, their influences on CAR-T cells remain unclear by far. Early researchers believed that corticosteroids could inhibit the proliferation and function of CAR-T cells and were associated with early relapse after CAR-T therapy [30]. However, another study concluded that corticosteroids did not affect the efficiency and proliferation of CAR-T cells in patients with B-cell ALL [31].

The latest research demonstrated that early and prolonged corticosteroids application were associated with early relapse and shortened overall survival in patients with large B-cell lymphoma [32]. The patient in this case was treated not only with tocilizumab and corticosteroids, but also with plasma exchange. After receiving plasma exchange, the patient’s cytokines dropped rapidly and clinical symptoms such as fever were relieved. Compared with tocilizumab and corticosteroids, plasma exchange could have limited influences on T cells and were proved to be effective in reducing cytokine storm in this case. Previous studies demonstrated the feasibility of plasma exchange for the treatment of severe CRS without serious complications. Xia Xiao et al. reported that a r/r B-ALL patient’s cytokine levels decreased significantly while CAR-T cells continued to be sustained at high levels after receiving 9000 ml of fresh frozen plasma exchange [33]. In a study of anti-CD19 CAR-T therapy for r/r ALL, 2 patients who developed grade 3-4 CRS received 1800 and 750 ml of plasma exchange along with tocilizumab and corticosteroids, respectively. After plasma exchange, the patients’ CRS-related symptoms were relieved and serum IL-6 levels decreased obviously [34]. Therefore, plasma exchange should be considered for the management of sCRS and ICANS.

Various approaches have been adopted in clinical guidelines for the treatment of CRS and ICANS in recent years [35]. Nevertheless, plasma exchange was not included in the NCCN guidelines. This case showed the efficiency of plasma exchange in controlling cytokine storm and provided new ideas for the treatment of CRS and ICANS.

**Abbreviations**

allo-HSCT, allogeneic hematopoietic stem cell transplantation; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; B-ALL, B-cell acute lymphocytic leukemia; BBB, blood-brain barrier; BM, bone marrow; B-NHL, B-cell non-Hodgkin lymphoma; BP, blood pressure; CAR-T, chimeric antigen receptor-T cells; CLL, chronic lymphocytic leukemia; CR, complete remission; CRP, C-reactive protein; CRS, cytokine release syndrome; CSF, cerebrospinal fluid; DIC, disseminated intravascular coagulation; DLBCL, diffuse large B-cell lymphoma; EMA, European Medicines Agency; FDA, Food and Drug Administration; Hb, Hemoglobin; ICANS, immune effector cell-associated neurotoxicity syndrome; ICU, intensive care unit; MRD, minimal residual disease; NEC, non-erythroid cells; NT-pro BNP, N-terminal pro-brain natriuretic peptide; PLT, platelet; PPI, proton pump inhibitors; PT, prothrombin time; r/r, relapsed and refractory; SaO₂, arterial oxygen saturation; scFv, single-chain variable fragment; sCRS, severe CRS; ssCAR-T-19, anti-CD19 CAR-T with IL-6 knockdown; Tcm, central memory T cells; Teff, effect T cells; Tem, effect memory T; WBC, white blood cell.

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**Conflict of interest**

The authors declare no competing financial interests.

**Authors’ contributions**

S-L X designed the study; YQ and W-J G collected the data, made the figures, and wrote the manuscript; L-Q K provided essential materials; S-L X edited the manuscript; LY, JZ, A-N S and D-P W read the manuscript and gave comments; all authors reviewed the manuscript.

**References**


