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# Hemofiltration Successfully Eliminates Severe Cytokine Release Syndrome Following CD19 CAR-T-Cell Therapy

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**Summary:** Cytokine release syndrome (CRS) remains to be a major adverse effect of chimeric antigen receptor T (CAR-T) cell therapy in B-cell acute lymphoblastic leukemia (B-ALL) and lymphoma. It was urgent to explore novel strategy for managing severe CRS. We conducted a clinical trial to assess the safety and efficacy of CD19-targeting CAR-T-cells in the treatment of relapsed and chemotherapy-refractory B-ALL and lymphoma. A 10-year-old boy with B-ALL who never achieved minimal residual disease (MRD) negative status after 5 courses of chemotherapy was enrolled into our study and received a total of $3.19\times10^6$/kg autologous CD19 CAR-T-cells. Before CAR-T-cell infusion, naive lymphocytes made up 41.8% of bone marrow cells, which were reduced to 1% at the 14th day after transfusion, with MRD $< 10^{-4}$. However, this patient developed grade 4 CRS, multiple organ failure, hemophagocytic syndrome, neurotoxicity, and severe pulmonary infection after CAR-T-cell therapy. Tocilizumab and glucocorticoids treatment were ineffective for controlling the adverse effects and in contrast, hemofiltration immediately ameliorated the severe CRS and prevented the exacerbation of multiple organ dysfunction, pneumonia, and hydrosarca caused by CAR-T-cell therapy. All side effects disappeared within days following hemofiltration. Hemofiltration helped quickly clear cytokines, speeded up patient recover, and successfully resolved the severe CRS crisis. This was the first report, reporting the successful use of hemofiltration to eliminate adverse reactions of CAR-T-cell therapy.

**Key Words:** hemofiltration, chimeric antigen receptor T (CAR-T) cell, B-cell acute lymphoblastic leukemia (B-ALL), CD19, cytokine release syndrome (CRS)

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Patients with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) have a poor prognosis despite of therapeutic approaches such as allogeneic hematopoietic stem cell transplantation (HSCT). Thus, it is urgent to explore novel strategies to treat intractable B-ALL. Immunotherapies, especially immune checkpoint inhibitor and adoptive cell therapies are promising approaches in the field of cancer immunotherapy. CD19 is expressing on restricted to normal, malignant B cells, and B-cell precursors, thus it is an attractive target of adoptive T-cell therapy for B-cell malignances. Meanwhile, chimeric antigen receptor T (CAR-T) cell therapies targeting CD19 successfully induced high response rates of B-ALL.[1] Lymphodepletion chemotherapy followed by the infusion of CD19 CAR-T-cells has shown remarkable efficacy in patients with relapsed and/or refractory $CD19^+$ B-cell malignancies, with reported complete response (CR) rates as high as 93% in B-ALL, overall response rates of 77% in chronic lymphocytic leukemia, and 82% in non-Hodgkin's lymphoma.[2] On August 30, 2017, the US Food and Drug Administration (FDA) approved Novartis's Tisagenlecleucel, a CD19 CAR-T-cell therapy for the treatment of relapsed/refractory B-ALL.[3] In addition, Yescarta (axicabtagene ciloleucel), another CD19 CAR-T-cell therapy was approved on October 19, 2017; for the treatment of adult large B-cell lymphomas which were previously nonresponsive or had relapsed after at least 2 other kinds of treatment.[4]

CD19 CAR-T-cell therapy has been reported to induce rapid and durable clinical responses, simultaneously producing associated side effects, such as cytokine release syndrome (CRS) and neurological toxicities, which are systemic responses to the activation and proliferation of CAR-T-cells. Patients with CRS after CD19 CAR-T-cell therapy often manifested fever, hypotension, coagulopathy and capillary leak, and these phenomenons have been reported to occur in 54%–91% of patients, including severe CRS in 8.3%–43%.[5] Additional side effects included serious infections, cytopenia, hemophagocytic syndrome, and a weakened immune system. The increasing availability of CD19 CAR-T-cell therapies in multicenter trials highlights the need for clinicians to provide patients with a detailed description of CRS.[6]

The anti-IL-6R monoclonal antibody tocilizumab has become an effective drug for the management of CRS following CAR-T-cell therapy and was approved by the FDA on August 2017. However, tocilizumab has limitations for patients with CRS who have been concurrently seriously infected after CAR-T-cell infusion because it could aggravate infection, even cause sepsis and ultimately result in death. Thus, exploring a new therapeutic method is crucial for patients with CRS and infection after CAR-T-cell infusion. In this study, we reported hemofiltration as a successful therapy in the treatment of severe CRS and infection after CD19 CAR-T-cell infusion in 1 patient with relapsed/refractory B-ALL. To our knowledge, the combination of therapeutic methods used in this study are the first report of the successful treatment of severe CRS and infection after CAR-T-cell infusion.

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## CASE REPORT

### Study Procedure

The trial [ClinicalTrials.gov number, NCT03156101 (a clinical study evaluating the safety and efficacy of BinD19 treatment in R/R ALL and lymphoma subjects, May 17, 2017)] was designed to assess the safety and feasibility of infusing autologous CD19 CAR-T-cells in patients with relapsed or refractory B-cell neoplasms approved by the First Affiliated Hospital of Zhengzhou University. We designed a self-inactivating, clinical-grade lentiviral vector (BinD-1) as shown in Figure 1A. Leukapheresis products were stimulated with paramagnetic beads coated with antibodies to CD3 and CD28 and were transduced with the CD19 CAR coding lentivirus. Quantitative polymerase chain reaction was performed to detect the proliferation of CAR-T-cells in the blood. Analysis of serum cytokines, including interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF) and g-interferon were determined using the BD Cytometric Bead Array (CBA) Human Th1/Th2 Cytokine Kit II.

### Patient Condition Before CAR-T-Cell Therapy

The patient was a 10-year-old boy diagnosed with B-ALL on December 2016. His *MLL-AF4* fusion gene was positive, but he had never achieved minimal residual disease negative ($MRD^-$) after 5 intensive courses of chemotherapy including CVDLP [cyclophosphamide (CTX), vincristine, doxorubicin, L-asparaginase, prednisone], CAM (CTX, cytarabine, 6-mercaptopurine), DAEL [CTX, vincristine, L-asparaginase, cytarabine, etoposide, dexamethasone (DXM)], HR-1' (CTX, vincristine, L-asparaginase, cytarabine, methotrexate, DXM), and HR-2' (methotrexate, L-asparaginase, DXM, vindesine, ifosfamide) (Fig. 1B). Three cycles of preventative therapy for central nervous system leukemia were also carried out. About 8 cycles of intrathecal chemotherapy with methotrexate, CTX, and DXM without cranial irradiation were performed. His cerebral spinal fluid contained no leukemia cells and a normal level of protein was detected when he was recruited for the CD19 CAR-T-cell therapy clinical trial.

### Response of CD19 CAR-T-Cell Therapy

The procedure of CD19 CAR-T-cell manufacture and the clinical application scheme was shown in Figure 1C. Lymphocyte-depleting chemotherapy regimen consisted of fludarabine 25 $mg/m^2$ days −7 to −5 and CTX 500 $mg/m^2$ days −7 to −6. The patient received a total of $3\times10^8$ T cells, of which 30.9% were transduced by specific vector, for a total of $9.27\times10^7$ transduced cells ($3.19\times10^6$ cells/kg) split into 3 consecutive daily intravenous infusions (10% on

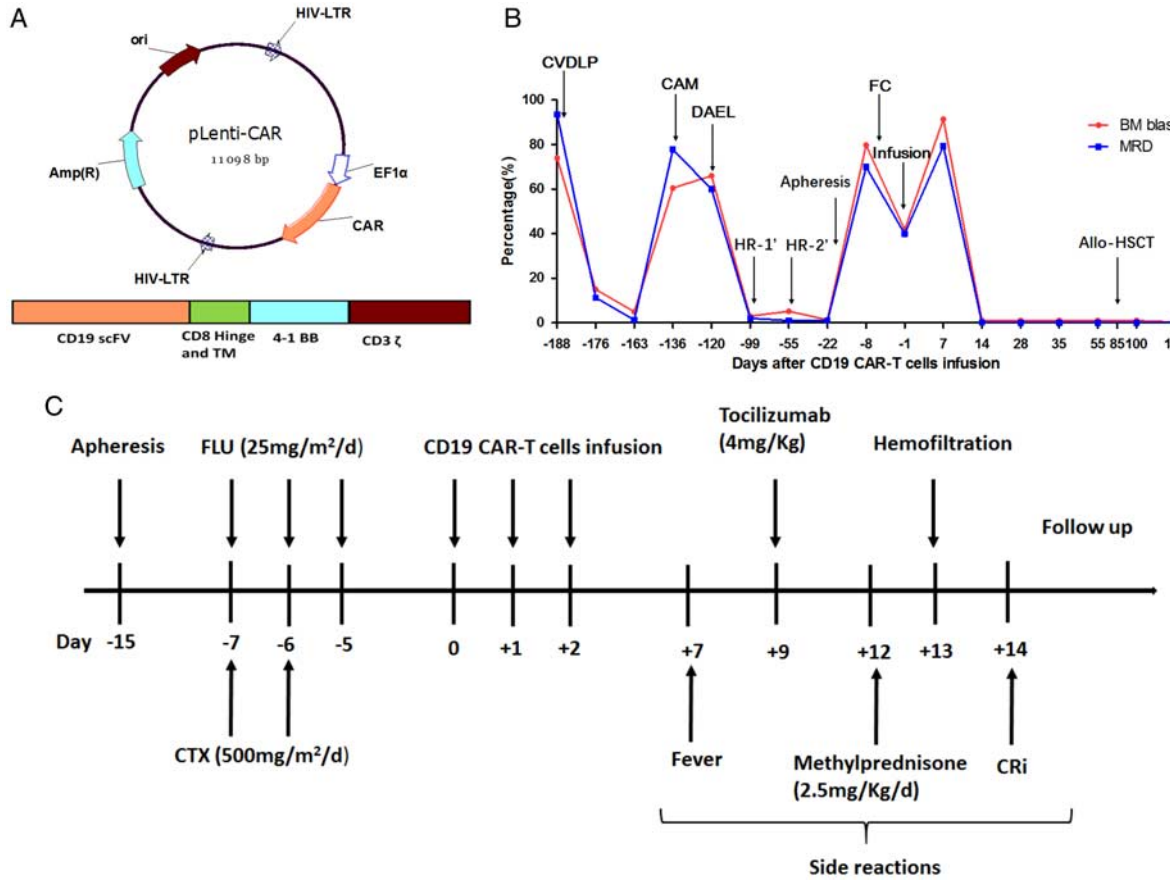


**FIGURE 1.** Efficacy of chemotherapy and CD19 CAR-T-cell therapy in the patient (boy, 10 years old). A, Lentiviral vector used to infect T cells from the patient. A pseudotyped, clinical-grade lentiviral vector directing expression of anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8αhinge and transmembrane domain, and human 4-1 BB and CD3ζ signaling domains were produced. B, The percentage of BM blast and MRD were detected after chemotherapy and CD19 CAR-T-cell therapy. C, Procedure of CD19 CAR-T-cell manufacture and the clinical application scheme. BM indicates bone marrow; CAM, cyclophosphamide, cytarabine, 6-mercaptopurine; CAR-T, chimeric antigen receptor T cell; CRi, complete response with incomplete count recovery; CTX, cyclophosphamide; CVDLP, CTX, vincristine, doxorubicin, l-asparaginase, prednisone; DAEL, cyclophosphamide, vincristine, l-asparaginase, cytarabine, etoposide, dexamethasone; HIV-LTR, longterminal repeats of human immunodeficiency virus; HR-1', cyclophosphamide, vincristine, l-asparaginase, cytarabine, methotrexate, dexamethasone; HR-2', methotrexate, l-asparaginase, dexamethasone, vindesine, ifosfamide; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; scFV, single-chain fragment variable.

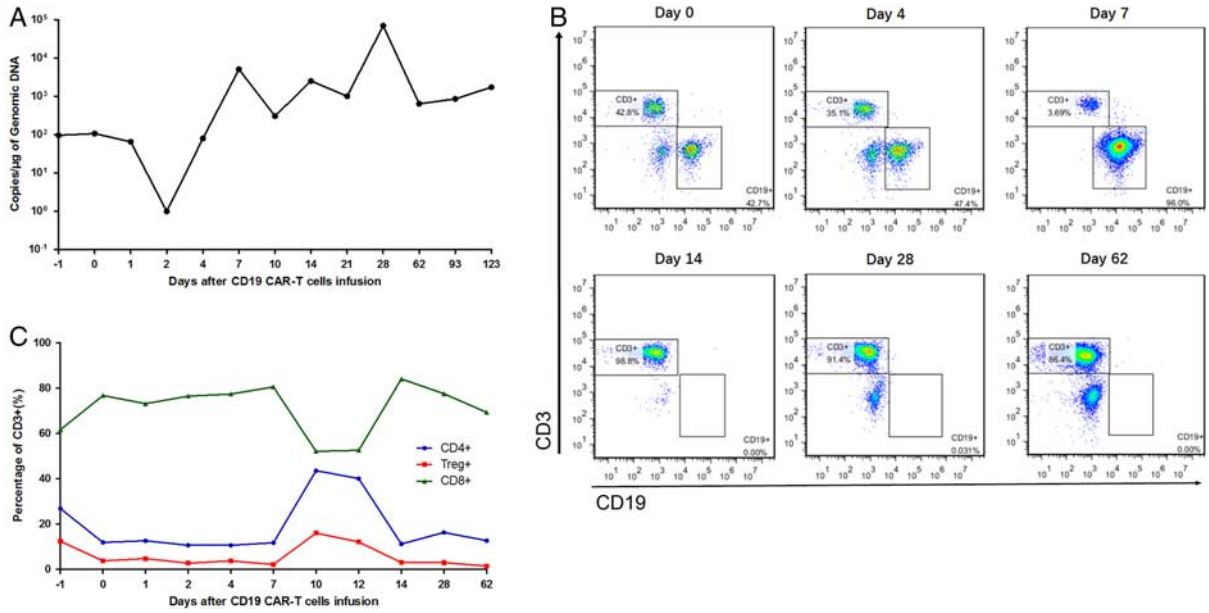


**FIGURE 2.** Expansion and persistence of CD19 CAR-T-cells in vivo. A, The presence of CD19 CAR-T-cells in peripheral blood was assessed by means of a quantitative real-time polymerase chain reaction assay. Genomic DNA was isolated from samples of whole blood collected at serial timepoints before and after CD19 CAR-T-cell infusion. The *y*-axis shows a log 10 scale. B, The percentage of $CD19^+$ B cell and $CD3^+$ T cells in $CD45^+$ PBMCs by Flow-cytometric analysis before and after CD19 CAR-T-cell infusion. C, Changes in the composition of T cells in peripheral blood before and after CD19 CAR-T-cell infusion. CAR-T indicates chimeric antigen receptor T cell; PBMCs, peripheral blood mononuclear cells; Treg, regulatory T cell.

day 0, 30% on day 1, and 60% on day 2). The infusion progress was smooth without any side effects. The expanding level of CD19 CAR-T-cells was >1000 times as high as initial engraftment levels in the peripheral blood and persisted for >93 days. Peak levels of CD19 CAR-T-cells were temporally correlated with the CRS (Fig. 2A). Flow-cytometric analysis of $CD45^+$ peripheral blood mononuclear cells at baseline showed 18.7% $CD19^+$ B-cell infiltration and 37.9% $CD3^+$ T cells at baseline. On day 7 after infusion, $CD19^+$ B cells predominantly increased with a reduction of T cells. On day 14 after infusion, $CD3^+$ T cells were present, and no normal or malignant $CD19^+$ B cells were detected (Fig. 2B). $CD4^+$ T cells were more than $CD8^+$ T cells before CD19 CAR-T-cell infusion. On day 7–day 14, $CD4^+$ T cells decreased, $CD8^+$ T cells increased, and regulatory T cells remained at low level (Fig. 2C). In addition, before CD19 CAR-T-cell infusion, the percentage of the leukemia cells in bone marrow was 41.8%, and MRD was 40%. On the 14th day after CAR-T-cell infusion, the patient achieved MRD (−) status. On the 85th day after the infusion, he received allo-HSCT and continued to exhibit CR until now (Fig. 1B).

## Toxicity of CD19 CAR-T-Cell Therapy

Grade 3 and 4 adverse events are summarized in Table 1. The patient developed acute toxic effects, which consisted of fever and CRS. The fever occurred on day 7 after CAR-T-cell infusion, which evolved into a persistently high fever (Fig. 3A). The patient's febrile syndrome slightly declined for 1 day after the administration of 4 mg/kg tocilizumab on day 9. On day 11, he had a recurrence of fever at 40.6°C. The serum levels of proinflammatory cytokines including IL-6, INF-γ, TNF-α, IL-10, and IL-4 were markedly elevated despite the use of tocilizumab (Fig. 3B). Biochemical evidence of the macrophage activation syndrome was noted, together with elevations of the ferritin level to 12,753 ng/dL and triglycerides to 5.14 ng/dL on day 12, coagulopathy with an elevated d-dimer level to 26.22 mg/L, fibrinogen degradation product to 189.23 mg/L, and hypofibrinogenemia to 0.5 g/L on day 11 (Fig. 3D), hepatosplenomegaly, and elevated levels of aminotransferases (Fig. 3C). The patient was given with methylprednisolone at dose of 2 mg/kg/d and immunoglobulin 10 g/d from day 11. However, symptoms were still aggravated rapidly. Decreases in the complete blood count occurred after the infusions of the escalated doses of CD19 CAR-T-cells, with the lowest hemoglobin level at 46 g/L on day 13 (Fig. 3E). For supportive care, the patient accepted platelet transfusions, cryoprecipitate and frozen plasma infusion, and red blood cell transfusions. On day 13 after infusion, he began to cough, expectoration, anoxia, dyspnea, and chest computed tomographic scan showed severe pulmonary inflammation and pleural effusion (Fig. 3F). Meanwhile, he began with somnolence, agitation, convulsion lasting 3–5 minutes per 1–2 hour 4 times. Cerebrospinal fluid was not measured because of the twitch condition, but the brain computed tomography did not show obvious abnormity (Fig. 3G). The blood cultures were negative on day 8, day 13, and day 14, which might be due to the application of antibiotics from day 7. The infection indicator procalcitonin (PCT) was 1.29 ng/mL on day 7 and 16.61 ng/mL on day 11 (Fig. 3H). Bearing in mind that tocilizumab increased the risk of exacerbating infection, so tocilizumab was not applied again. We proceeded to strengthen the antibiotics to deal with infection and applied continuous veno-venous hemofiltration for 24 hours to recover his state. During this period, his blood pressure was persistently stable (85–100/

**TABLE 1.** Adverse Events Were Graded According to the Common Terminology Criteria for Adverse Events, Version 4.0

| Events | Grade | Description | Duration (d) |
|---|---|---|---|
| Febrile neutropenia | 3 | Peak temperature of 40.6°C; event occurred from day 7 | 7 |
| Encephalopathy | 3 | Somnolence; convulsion; CT scan was normal | 1 |
| Elevated AST | 3 | Peak AST value: 720 U/L | 3 |
| Infection | 3 | CT scan showed pulmonary infection | 7 |

ALT indicates alanine aminotransferase; AST aspartate aminotransferase; CT, computed tomography.

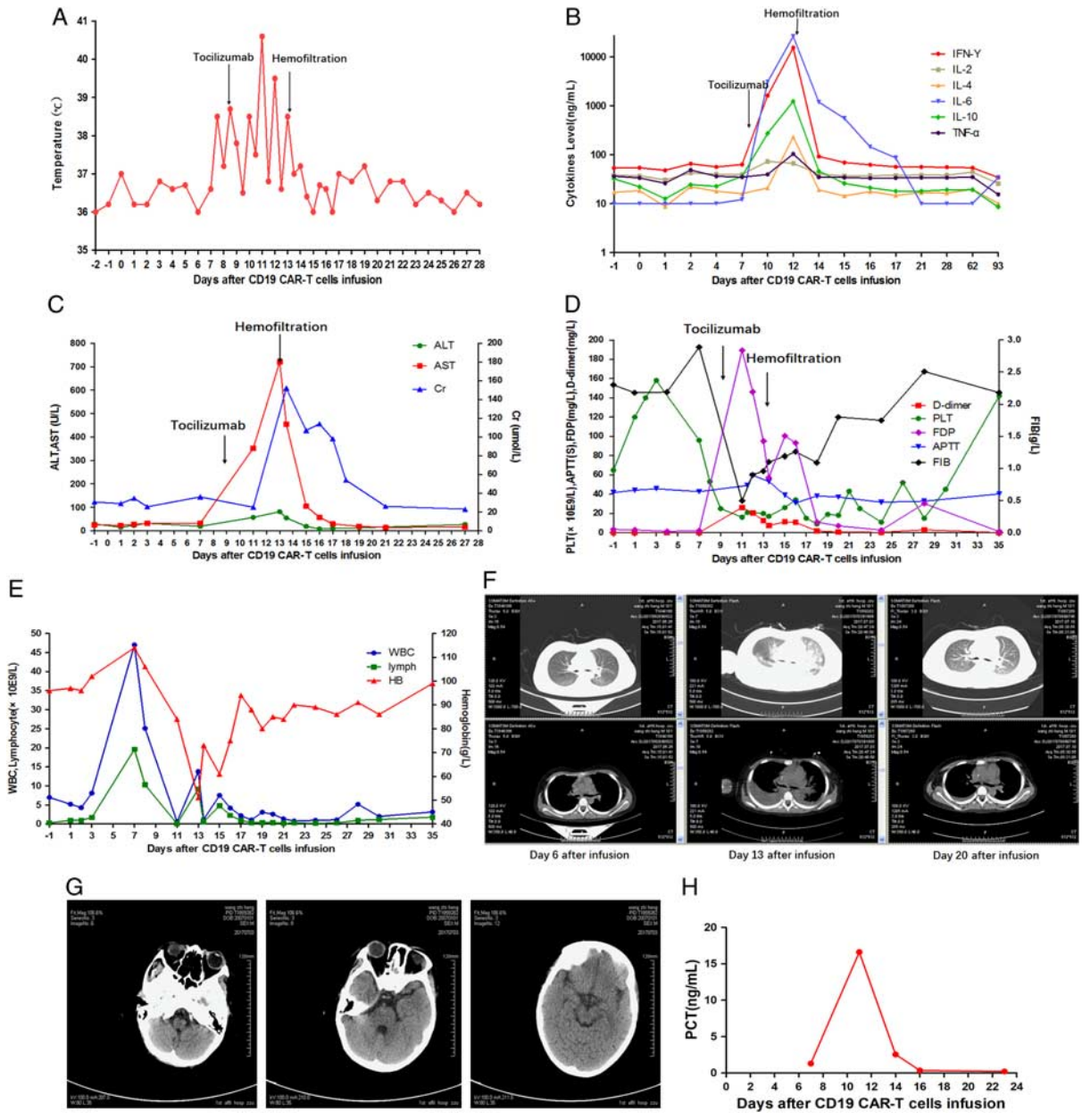


**FIGURE 3.** Changes in body temperature, liver and renal function, cytokines, coagulation, and the complete blood count during and after the CD19 CAR-T-cell infusion. A, Changes in the temperature and the relevance between temperature and tocilizumab and hemofiltration. B, Changes in the various cytokine levels before and after CD19 CAR-T-cell infusion. C, The left panel showed the alterations in ALT and AST, and the right panel shows the changes in Cr. D, The left panel showed the alterations in PLT, APTT, FDP, D-dimer and the right panel showed the changes in FIB. E, The left panel showed the alterations in WBC and lymphocyte counts, and the right panel showed the changes in the hemoglobin level. F, Changes in pulmonary inflammation after CD19 CAR-T-cell therapy. G, Image of brain computed tomographic scan on day 13 after CD19 CAR-T-cell infusion. H, Changes in the infection indicator procalcitonin after CD19 CAR-T-cell infusion. ALT indicates alanine transaminase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; CAR-T, chimeric antigen receptor T cell; Cr, creatinine; FDP, fibrinogen degradation product; FIB fibrinogen; PCT, procalcitonin; PLT, platelet; WBC, white blood cell.

55–65 mm Hg), 1000 mL plasma was infused to sustain colloid osmotic pressure. After the hemofiltration, cytokines significantly decreased; liver function improved significantly; twitch did not occur again, and body temperature returned to normal, PCT decreased significantly as 0.35 ng/mL on day 16. All the experimental markers gradually returned back to normal and his general condition was markedly improved. In addition, the clinical and laboratory abnormalities of the macrophage activation syndrome were resolved.

## DISCUSSION

Anti-CD19 CAR-T-cell therapy has been approved to treat CD19$^+$-B-ALL by FDA,[3] there are currently numerous ongoing clinical trials of CAR-T-cell targeting CD19$^+$ malignancies in the world. CAR-T-cell therapy could induce rapid and durable clinical responses, accompanying by unique acute toxicities, which would be severe or even fatal.[5]

Thus, it is urgent to explore novel strategies to effectively control the side reactions and to reduce the adverse effects while maintaining the efficacy of the treatment in B-ALL.

This case report presented a 10-year-old boy with relapsed/refractory B-ALL who received CD19 CAR-T-cell therapy in the First Affiliated Hospital of Zhengzhou University. On the seventh days after CAR-T-cell infusion, he began to have a high temperature. Then he suffered from serious CRS, hepatic and renal dysfunction, hemophagocytic syndrome, serious pneumonia, and hydrosarca. The level of creatinine, transaminases, and brain natriuretic peptide were elevated. His condition was not allowed to be governed by tocilizumab and glucocorticoids timely. In his extremity, the patient was treated with hemofiltration for 24 hours, surprising, various indexes were recovered in a short time. The IL-6 level was decreased rapidly, hepatic and renal dysfunction showed no further deterioration. Serious pneumonia was controlled by imipenem and voriconazole. Concurrently, leukemia cells were disappeared in bone marrow, the disease acquired CR. In the process of dealing with the CAR-T–associated toxicities, hemofiltration was playing a vital role in reducing the CRS degree and controlling the progression of multiple organ failure, thus successfully addressing CAR-T–associated toxicities. It has been previously reported that hemofiltration, continuous blood purification remarkably improved the cardiovascular and respiratory functions of children with severe sepsis, probably by eliminating factors mediating inflammation.[7] Hemofiltration was reported to decrease the temperature and the mortality of patients with hyperthermic septic shock.[8] Continuous hemofiltration could more effectively remove various inflammatory factors of patients with infection complicated by acute renal failure. In addition, continuous hemofiltration could remove IL-6 from the blood stream efficiently in a rat sepsis model.[9] Thus, hemofiltration is a good candidate as an adjunct therapy to control the severe side effects caused by CAR-T-cell therapy. We consider that indications for hemofiltration include wild immune-mediated toxicities that were not well controlled by tocilizumab, glucocorticoids and best supportive care, such as sustained pyrexia, elevated cytokines, malignant abnormalities of heart rate, deteriorative multiple organ failure, and coagulation disorders.

After CAR-T infusion, monitoring should include assessment of vital signs, and daily review of organ systems and a physical examination. Laboratory tests, serum CRP, ferritin levels, blood counts, and chemistry pane might need to be performed more than once daily, especially for patients at high risk of severe CRS. Cardiac monitoring by telemetry was advised from the time of CAR-T-cell infusion until resolution of any emergent CRS symptoms. Additional investigations, such as chest radiography to perform pneumonia was aiming to avoid severe infection prophylactic antibiotics. Daily fluid balance and body weights should be strictly monitored, and maintenance of intravenous hydration with albumin or plasma was recommended for all patients. It was most important to monitor the cytokine level including IL-6, g-interferon, TNF-α, and IL-2, especially IL-6. Tocilizumab and glucocorticoids are usually the first line of therapy in reducing the progression of CRS. Through this case, hemofiltration was an optional measure to control wild side effects produced by CAR-T-cells. High flux hemofiltration (Qf = 60 mL/kg/h) was performed for 72 hours in 13 critically ill patients suffering from severe sepsis or septic shock with acute renal failure, the results showed IL-6 mRNA reduction after 12 hours of treatment and a progressive increase after 24, 48, and 72 hours.[10]

## CONCLUSIONS

In conclusion, CRS is the major adverse effect of CAR-T-cell therapy, every patient with B-ALL treated by CAR-T-cell therapies needs to be closely monitored. Preventing and reducing the degree of side effects such as CRS and multiple organ failure were crucial for patients' safety. Through effective interventions including hemofiltration, CAR-T-cell therapy may become safer and more effective, and may probably become a standard treatment option for patients with relapsed and refractory B-ALL in the future.

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### Conflicts of Interest/Financial Disclosures

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## REFERENCES

1. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371:1507–1517.
2. Hay KA, Turtle CJ. Chimeric Antigen Receptor (CAR) T cells: lessons learned from targeting of CD19 in B-cell malignancies. *Drugs*. 2017;77:237–245.
3. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439–448.
4. Roberts ZJ, Better M, Bot A, et al. Axicabtagene ciloleucel, a first-in-class CAR T cell therapy for aggressive NHL. *Leuk Lymphoma*. 2018;59:1785–1796.
5. Neelapu SS, Tummala S, Kebriaei P, et al. Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. *Nat Rev Clin Oncol*. 2018;15:47–62.
6. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. *Blood*. 2016;127:3321–3330.
7. Guo XH, Sun YF, Han SZ, et al. Continuous blood purification in children with severe sepsis. *J Biol Regul Homeost Agents*. 2017;31:389–394.
8. Pestana D, Casanova E, Villagran MJ, et al. Continuous hemofiltration in hyperthermic septic shock patients. *J Trauma*. 2007;63:751–756.
9. Maeda H, Tomisawa N, Jimbo Y, et al. Efficacy of hemofiltration with PEPA membrane for IL-6 removal in a rat sepsis model. *J Artif Organs*. 2017;20:335–340.
10. Servillo G, Vargas M, Pastore A, et al. Immunomodulatory effect of continuous venovenous hemofiltration during sepsis: preliminary data. *Biomed Res Int*. 2013;2013:108951.