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

Yanfen Liu,*† Xinfeng Chen,*† Dao Wang,‡ Hong Li,* Jianmin Huang,* Zhen Zhang,* Yingjin Qiao,§ Hongling Zhang,∥ Ying Zeng∥ Chao Tang¶,†† Shuangning Yang,* Xiaoqun Wan,¶ Youhai H. Chen,# and Yi Zhang*†††††

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 3.19×10^9/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 hydroscarca caused by CAR-T-cell therapy. All side effects disappeared within days following hemofiltration. Hemofiltration helped quickly clear cytokines, speeded up patient recovery, 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)

(J Immunother 2018;41:406-410)

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. Immuno-therapies, 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 malignancies. Meanwhile, chimeric antigen receptor T (CAR-T) cell therapies targeting CD19 successfully induced high response rates of B-ALL. 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. 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. 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.

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, hypoten- sion, coagulopathy and capillary leak, and these phenomena have been reported to occur in 54%-91% of patients, including severe CRS in 8.3%-43%. 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. 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.
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-l) 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 γ-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/m² days −7 to −5 and CTX 500 mg/m² days −7 to −6. The patient received a total of 3×10⁸ T cells, of which 30.9% were transduced by specific vector, for a total of 9.27×10⁷ transduced cells (3.19×10⁶ 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 CD8α hinge 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.
The patient was given methylprednisolone at a dose of 2 mg/kg/d and immunoglobulin 10 g/d from day 1. 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, convolution lasting 3–5 minutes per 1–2 hour times. Cerebrospinal fluid was not measured because of the twitch condition, but the brain computed tomography did not show obvious abnormality (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 (85–100/50–60 mmHg).

### 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; convolution; 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.
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 hydropsarca. 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. Hemofiltration was reported to decrease the temperature and the mortality of patients with hyperthermic septic shock. 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. 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 at high risk of severe CRS. Cardiac monitoring by telemetry might need from the time of CAR-T-cell infusion until 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.

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.

ACKNOWLEDGMENTS

The authors thank the staff members of Binde Biotech for their technical support.

Conflicts of Interest/Financial Disclosures

Supported by grants from the National Natural Science Foundation of China (no. 81711986, no. 81771781, no. 81702810), research grant from the Ministry of Public Health (no. 201501004) Shenzhen special funds for industry of the future (2015971); Nanshan pilot team project (LHTD20160001).

All authors have declared that there are no financial conflicts of interest with regard to this work.

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. Tisagenleucel 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. 2015;18:47–62.
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.