

Sustained Therapeutic Efficacy of Humanized Anti-CD19 Chimeric Antigen Receptor T Cells in Relapsed/Refractory Acute Lymphoblastic Leukemia **A** **E**



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ABSTRACT

Purpose: Immunogenicity derived from the murine scFv, a major molecular component of chimeric antigen receptors (CARs), may limit the persistence of CAR T cells, resulting in tumor relapse of patients in complete remission (CR). In this study, we developed a humanized anti-CD19 scFv CAR-T (hCAR-T) to treat patients with relapsed/refractory acute lymphoblastic leukemia (r/r ALL).

Patients and Methods: In this one-arm, open-labeled study, we infused the T cells modified with hCAR to patients with r/r ALL. Patients were evaluated with long-term follow-up for response and safety of the treatment. The study was registered at Clinicaltrials.gov (NCT02349698).

Results: Ten patients with r/r ALL were recruited for this study. All were response evaluable and all achieved CR; eight

patients remained CR, and six were in CR for over 18 months without further treatment. A long-term persistence of hCAR T cells was observed in most of the patients. Among these patients, four of them with high tumor burden and rapidly progressive disease (median, 58%) experienced grade 3–4 cytokine release syndrome (CRS) and neurotoxicity. These severe CRSs were successfully controlled by tocilizumab, glucocorticoid, and plasma exchange.

Conclusions: T cells expressing the humanized anti-CD19 scFv CARs exhibited sustained therapeutic efficacy in the treatment of r/r ALL. Low relapse rate was associated with the long-term persistence of CAR T cells.

Introduction

Chimeric antigen receptors (CARs) are genetically modified receptors that couple a single-chain Fv (scFv) domain to intracellular cell signaling domains of the T-cell receptor. T cells engineered to express these receptors have shown significant therapeutic efficacy in treating relapsed/refractory acute lymphoblastic leukemia (r/r ALL) with complete remission (CR) rates reaching 80%–90% at first month, 65%–75% at 6 months, and

40%–50% at 12 months (1–5). However, relapse still remains the major concern of CD19 CAR T-cell therapy.

One of the causes of relapse is CD19-negative B-ALL progression (6); Maude and colleagues (1, 2) and Lee and colleagues (4) report that this was the case in approximately 10%–30% of relapsed patients. Another common cause is the loss of CAR T cells, leading to CD19-positive ALL regression. Multiple mechanisms may be responsible for the inability of CAR T cells to persist *in vivo*. One such mechanism is the development of humoral and/or cellular immune responses against some specific epitopes of the scFv in the CAR structure, which is derived from a murine antibody (7–12). Besides this, as reported by others, patients with a high and rapidly progressive tumor burden are at high risk for severe adverse events and early relapse after accepting CAR T-cell therapy (5, 13).

In this study, we developed an optimal humanized anti-CD19 CAR structure derived from FMC63 to reduce the immunogenicity of CARs. Ten patients with r/r ALL were treated with humanized anti-CD19 CAR-T (hCAR T) cells to evaluate its efficacy and safety. Our data showed that long-term sustained therapeutic efficacy could be achieved. Cytokine release syndrome (CRS) and neurotoxicity in patients with a high tumor burden and rapidly progressive disease could be controlled by tocilizumab, glucocorticoid, and plasma exchange (PE).

Patients and Methods

Study design

This clinical trial was an investigator-initiated trial for the treatment of CD19⁺ r/r ALL and was approved by the institutional review board (IRB) of the Southwest Hospital of Third Military Medical University (Chongqing, China), and all patients signed informed consents. The study was registered at Clinicaltrials.gov (NCT02349698) and

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Translational Relevance

CAR-T against CD19 has shown significant antitumor activity in relapsed/refractory acute lymphoblastic leukemia (r/r ALL). However, relapse still remains the major concern of anti-CD19 CAR T-cell therapy. One mechanism for relapse is the development of humoral and/or cellular immune responses against some specific epitopes of scFv in the CAR structure, which are derived from a murine antibody. In this investigator-initiated trial, we developed a humanized anti-CD19 scFv CAR T (hCAR T) cells and infused these cells to patients with r/r ALL. Sustained B-cell aplasia and long-term persistence of hCAR T cells were observed in these patients. Moreover, four patients with high tumor burden and rapidly progressive disease experienced grade 3–4 of cytokine release syndrome (CRS). These severe CRSs were successfully controlled by tocilizumab, glucocorticoid, and plasma exchange. Our data provide a potential method to reduce the relapse rate for patients accepting CAR T-cell therapy.

performed according to the principles of the Declaration of Helsinki. All patients or their guardians provided written informed consent before they were recruited into this study.

All patients participating in this study volunteered and had been diagnosed with B-ALL through clinical manifestations and the bone marrow (BM) examination. All the enrolled patients had relapsed or refractory disease. In this study, CR was defined as less than 5% marrow blasts and absence of circulating blasts, and no extramedullary sites of disease, regardless of cell count discovery. Ten patients with ALL were enrolled, including three females and seven males and the median age was 16 years. Further patient information is presented in **Table 1**.

Eight patients received an FC (fludarabine 25 mg/m², cyclophosphamide 900 mg/m²) preconditioning treatment, except for patients P3 and P4, who were offered and accepted Hyper-CVAD-A [cyclophosphamide 300 mg/(m²/12 hours), vincristine 2 mg/day, epirubicin 30 mg/day, dexamethasone 20 mg/day] preconditioning treatment because of an extremely high tumor burden. Day at infusion was defined as day 0. The hCAR T cells were administered by intravenous infusion in two split-dose manners, during which either 10%, 30% and 60%, or 40% and 60%, of the total cells were infused on continuous days. The patients were followed by disease assessment via BM and peripheral blood (PB) every month for the first 2 months, every 2 months from months 3 to 6 and every 3 months afterward. Adverse events, including neurotoxicity, were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, and are listed in Supplementary Table S1. CRS was graded according to MSKCC CRS grading system. Severe CRS was defined as \geq grade 3.

The generation of hCAR T cells

Lentivirus was produced and purified from the supernatant of HEK293T cell cultures with transfection of a pCDH plasmid vector consisting of the humanized anti-CD19 scFv (only containing 25% of murine sequence), CD8 hinge, CD8 transmembrane domain, CD137 costimulatory signal, and CD3 zeta intracellular domains. PB mononuclear cells (PBMC) were isolated and activated by anti-CD3/CD28 paramagnetic beads followed by lentivector transduction and expansion *in vitro* with IL7 and IL21 for approximately 10–14 days to generate hCAR T cells. Phenotypes of hCAR T cells were analyzed

Table 1. Patient characteristics.

Patient	Gender	Age (Years)	Gene detection	Prior intensive therapies	HCT before	Marrow blasts before lymphodepletion	Marrow blasts after lymphodepletion	Dose level ($\times 10^6$ /kg)	CRS grade	Depletion therapy	Initial response
1	Male	16	WT1/ABL	5	YES	13%	1%	3.74	2	FC	CR, MRD-
2	Male	16	—	9	NO	1.2%	—	2.46	2	FC	CR, MRD-
3	Female	8	GLP TEL/AML1; MLL/MLLT1	11	NO	90%	—	41.67	3	Hyper-CVAD-A	CR, MRD-
4	Female	12	WT1	6	NO	90%	95%	12.3	4	Hyper-CVAD-A	CR, MRD-
5	Male	17	—	6	YES	6%	—	2.48	2	FC	CR, MRD-
6	Male	18	GLP TEL/AML1 WT1	7	NO	26.5%	69.5%	6.35	3	FC	CR, MRD-
7	Female	5	—	12	NO	3%	10.1%	1.68	2	FC	CR, MRD-
8	Male	40	E2A-PBX1; WT1	5	NO	2%	—	0.23	2	FC	CR, MRD-
9	Male	40	BCR/ABL; GLP16/9q21; WT1	13	NO	0.7%	—	0.5	2	FC	CR, MRD-
10	Male	16	E2A-PBX1	5	NO	3%	63%	0.86	3	FC	CR, MRD-

Note: The BM blasts in this table are calculated on the basis of marrow morphologic tests or FCM. The MRD in initial response was determined by both flow cytometry and RT-PCR.

before administration. A representative image is presented in Supplementary Fig. S1. The materials and methods of flow cytometry (FCM) are provided in Supplementary Information.

Quantitative PCR for detection of DNA copies

Blood samples were obtained following the trial procedure. Genomic DNA was extracted from the blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, 51106) according to the provided protocol. We applied TaqMan probes for quantitative real-time PCR (qPCR) in an ABI QuantStudio 5 (Applied Biosystems). TaqMan primers used were 50-CTTCCGGTATGGCTTCATTTTCTC-30 (forward) and 50 CGCCACGTTGCCTGACA-30 (reverse). The probe was 50-FAM-ACGGGCCACAACCTCCT-30. The TaqMan method was performed in accordance with the published protocol (14). CDKN1A was used as the control and produced a correction factor for DNA copy number. Healthy donor DNA was used as a negative control and to define the pre-infusion acceptance ranges of the qPCR method. The lower limit of quantification (LLOQ) was determined as 1 copy/ng genomic DNA.

Cytokine detection

Plasma samples were stored at -80°C and were analyzed in batches using a customized kit containing a 15-plex human cytokine magnetic bead panel (Milliplex, SPR372) with the MAGPIX instrument (Luminex). The plasma levels of cytokines IL2, IL6, IL10, FLT3LG, Fractalkine, GM-CSF, IFN γ , TNF α , IL1a, IP-10, MCP-1, MIP-1a, and MIP-1b were measured in the experiment. Samples were undiluted, and each was assayed in duplicate. Data analysis was conducted following the related protocol and algorithm using MILLIPLEX Analyst 5.1 software.

Human anti-mouse antibody detection

Cryopreserved serum samples of patients treated with hCAR T cells or murine-derived CAR T cells [these patients had been registered in a previous study (15) and all achieved CR] were measured for human anti-mouse antibodies (HAMA) using an ELISA assay [LEGEND MAX Human Anti-Mouse Ig (HAMA) ELISA Kit; Biolegend, 438308] specific for human IgG, according to the kit instructions.

Statistical analysis

All statistics were analyzed in GraphPad Prism 7. Descriptive statistics (median/range, percentage) were reported for variables. Comparison of cytokines in the two groups was performed using the Mann–Whitney test. Statistical significance was defined as $P < 0.05$. For analysis of leukemia-free survival (LFS), an event was defined as relapse. Death was the event for analysis of overall survival (OS). LFS and OS were determined by the Kaplan–Meier method.

Results

Characteristics of patients and hCAR T cells

From June 2017, 10 patients, ages from 5 to 40 years old, with r/r ALL were enrolled in this study. All the patients had received 5 to 13 intensive therapies including chemotherapy regimens; 2 of them had undergone HSCT before hCAR T cell infusion. The proportion of marrow blasts ranged from 0.7% to 90%, median 4.5%, and minimal residual disease (MRD) was detected positively in 10 patients by FCM and/or ALL fusion gene detection (Table 1). Among these patients, three (patients 2, 7, and 9) had central nervous system leukemia (CNSL) and accepted intrathecal chemotherapeutic drugs injection regularly to relieve symptoms such as dizziness and headache.

The hCAR T cell dose levels ranged from 2.3×10^5 cells/kg to 4.17×10^7 cells/kg, median 2.47×10^6 cells/kg (Table 1). The manufacturing procedure is shown in Fig. 1A. After cultivation, the phenotype of hCAR T cell products was detected, including naive T cells (Tn), central memory T cells (Tcm), effector memory T cells (Tem), and effector T cells (Teff; Fig. 1B; Supplementary Fig. S1). Inhibitory markers such as PD-1, Tim-3, and Lag-3 on the T cells were also checked (Fig. 1C). The median ratio of CD4 $^{+}$ and CD8 $^{+}$ T cells was 0.70 (range, 0.17–4.6) and 0.68 (range, 0.17–2.33) in CAR $^{+}$ and CAR $^{-}$ T cells, respectively (Fig. 1D).

Treatment with hCAR T cells achieved long-term CR

On day 28 ± 7 after hCAR T cell infusion, the clinical responses were evaluated in the 10 patients. All were response-evaluable and achieved morphologic CR initially, and the CR rate was 100% (Fig. 2A).

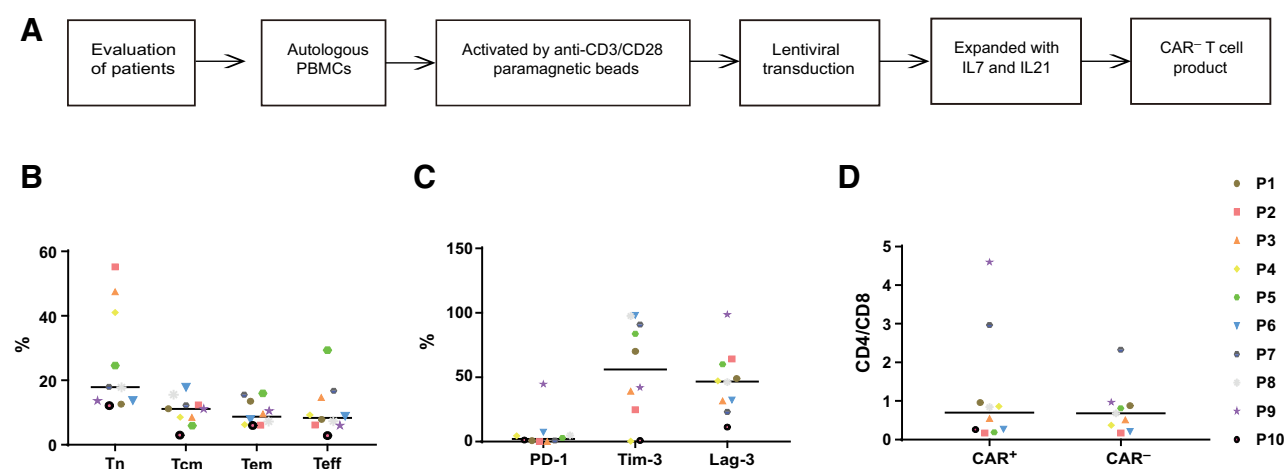


Figure 1.

Manufacturing procedure and characteristics of hCAR T cells before infusion. **A**, Manufacturing procedure for hCAR T cells. **B**, Percentage of Tn, Tcm, Tem, and Teff cells in hCAR-T products. **C**, Percentage of PD-1, Tim-3, and Lag-3 in hCAR-T products. **D**, Ratio of CD4 $^{+}$ and CD8 $^{+}$ cells in CAR $^{+}$ and CAR $^{-}$ T cells. PD-1, programmed cell death protein 1; Tim-3, T-cell immunoglobulin and mucin-domain containing-3; Lag-3, lymphocyte-activation gene 3.

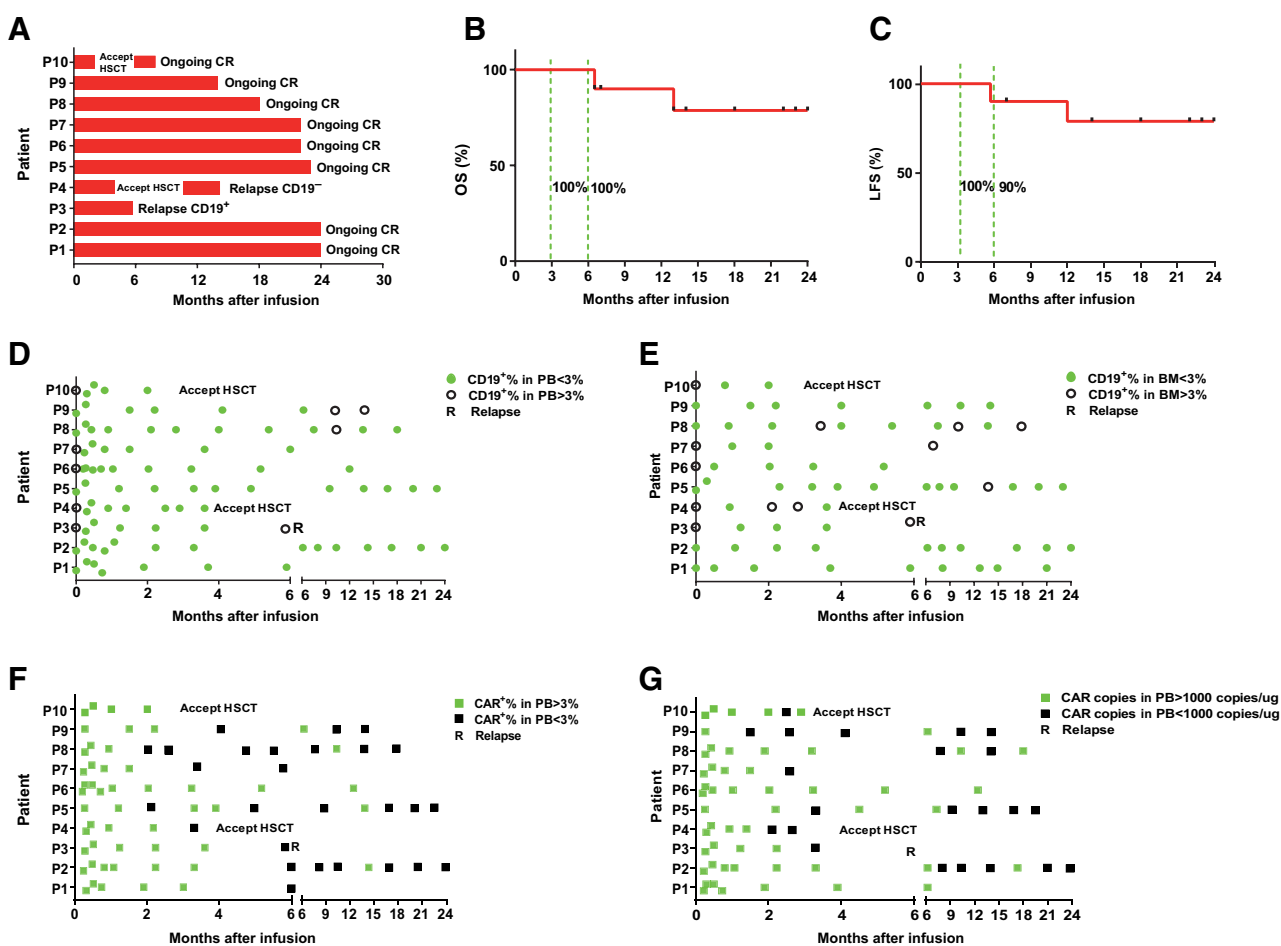


Figure 2.

Clinical responses of 10 patients with r/r ALL. **A**, Clinical outcomes of the 10 patients. OS rate (**B**) and LFS rate (**C**) of the 10 patients. The OS and LFS rates at 3 and 6 months were calculated. **D** and **E**, Percentage of CD19⁺ cells of nucleated cells in the PB and BM, respectively. Negative results were defined as less than 3% of CD19⁺ B cells in PB and BM. **F**, Percentage of hCAR⁺ T cells in the peripheral lymphocytes was detected by FCM. **G**, DNA copies of hCAR-T were detected by qPCR in the PB. A positive result was defined as more than 3% of CART cell in peripheral lymphocytes and more than 1,000 hCAR-T DNA copies per microgram genomic DNA.

Detection of MRD by multiparametric FCM and PCR was negative in all 10 patients. Three patients with CNSL had no detectable cerebral diseases. During the follow-up period, 1 patient (P3) experienced CD19 positive relapse at 6 months after infusion; and 2 patients (P4 and P10) proceeded to HSCT at 4 and 3 months, respectively, after infusion. Patient 4 had a CD19⁻ relapse at 12 months, and P10 remained CR. Both the OS and LFS rate were 100% at 3 months, and the OS and LFS rate were 100% and 90% at 6 months, respectively (Fig. 2B and C). Long-term remissions (>12 months) were observed in seven patients (P1, P2, P5, P6, P7, P8, and P9). Among those patients, six have remained sustained LFS for over 18 months, and two patients have maintained disease-free survival for 2 years. Another patient (P10) has been in remission for 8 months. In the PB and BM of these patients, B-cell aplasia was observed (Fig. 2D and E).

Long-term persistence of hCAR T cells was observed in these patients

Dynamic changes of CD19⁺ cells in the peripheral lymphocytes and BM were shown in the Fig. 3. DNA copies of hCAR T cells were

also detected after infusion. In most patients, the DNA copy numbers had a rapid increase in first 2 weeks after infusion but declined quickly during the next few weeks (Fig. 2F and G). However, in some patients (P2, P4, P5, P8, and P9), the DNA copies of hCAR T cells experienced several rounds of fluctuation during the follow-up time. Meanwhile, we observed the re-appearance of CD19⁺ cells in PB or BM (Fig. 3B, D, E, H and I). For example, the DNA copy number dramatically increased to 319,881 copies/μg after 520 days of infusion along with a sharp decrease of CD19⁺ cells in BM of patient 2 (Fig. 3B). However, DNA copies in patient 6 declined relatively slower than other patients and remained a high level for more than 12 months, whereas consistent CD19⁺ cells aplasia was observed in PB or BM (Fig. 3F).

Furthermore, for each patient who had a recovery of CD19⁺ cells during the follow-up period, we conducted multiparametric FCM tests to verify whether these cells were normal or malignant B cells. For patients P8 and P9 who had a recovery of CD19⁺ cells at the last follow-up, we did multiparametric FCM tests to analyze these immature cells and found that they were early pre-B cells and pre-B cells without any sign of malignance (Supplementary Fig. S2). The E2A-PBX1 gene

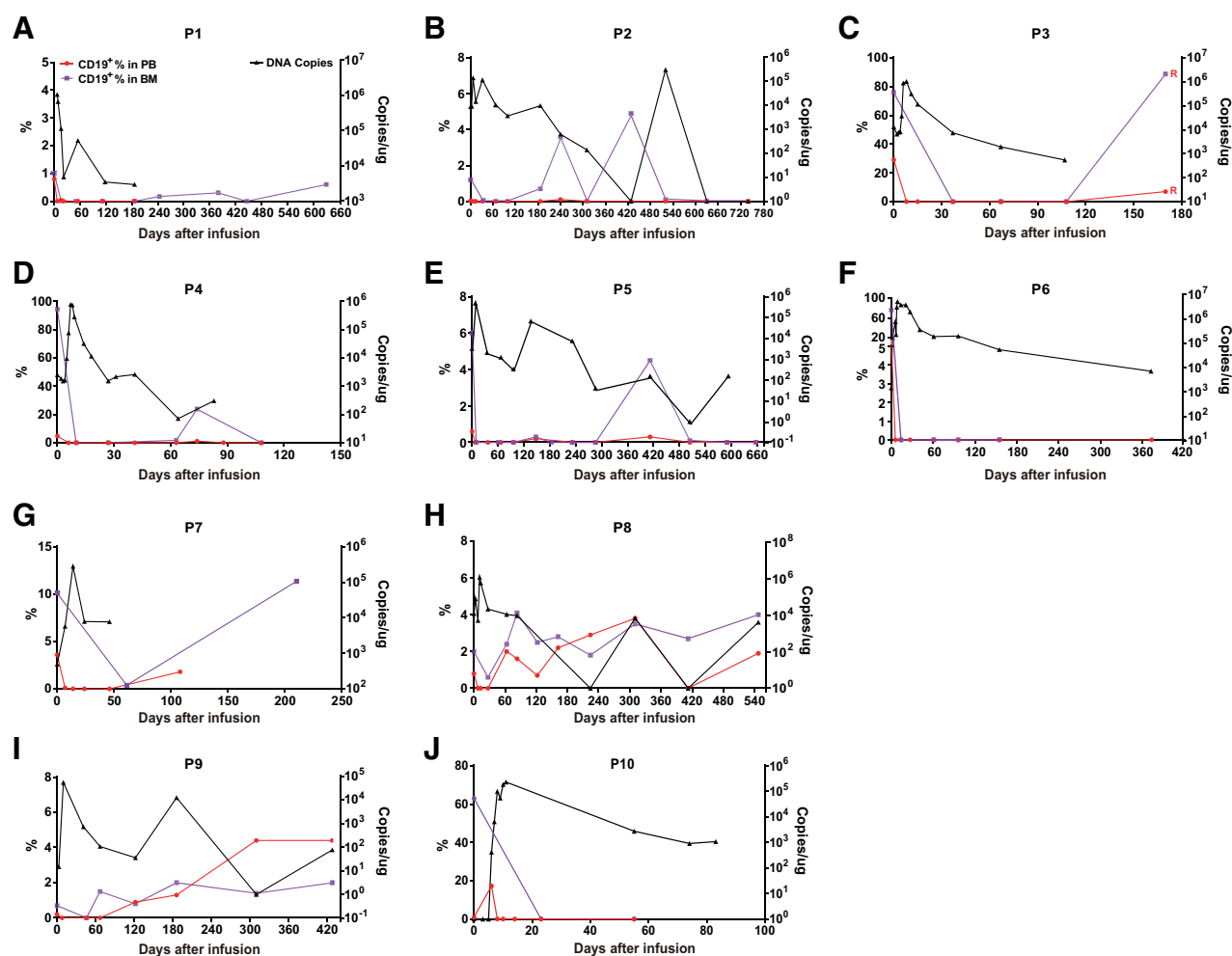


Figure 3.

Detection of DNA copies of hCAR-T and CD19⁺ cells in PB and BM during follow-up. **A–J**, Dynamics of DNA copies and CD19⁺ cells in the 10 patients. The percentage of CD19⁺ cells was calculated among lymphocytes in PB or BM nucleated cells. Except for P3, who had a relapse of CD19⁺ malignant B cells, the other patients' recurrent CD19⁺ B cells were verified as normal B cells by 12-parametric FCM. The DNA copy number of CAR T cells was detected using qPCR. R, relapse.

(positive for P8 when enrolled) and BCR-ABL gene (positive for P9 when enrolled) were also negative for patients P8 and P9 at the last follow-up (Supplementary Fig. S3).

Low level of HAMA was detected in serum of patients infused with hCAR T cells

Cryopreserved serum samples were analyzed for human IgGs specific for mouse proteins by ELISA. Results are shown in Supplementary Fig. S4A. For eight patients (except for patients P6 and P9), minimally positive IgG HAMA before infusion was detected and which had a significant increase after infusion. The peak level of HAMA was detected in the first 3 weeks postinfusion among most patients. For patients P1 and P5, the HAMA peaked at about 3 months after infusion. After that, the HAMA quickly decreased to the undetected level until the latest follow-up. For comparison, we also detected the level of HAMA in another five patients (labeled patients a–e) who had accepted mouse-derived CAR T cells (FMC63) and achieved CR, as described in our previous article (15). Similarly, a baseline level of HAMA was detected in four patients. However, the dynamic change of HAMA seemed to be irregular

but was always detectable during the follow-up (Supplementary Fig. S4B).

Rapidly ascending number of hCAR T cells in PB was a sign of recovery from systemic toxicity responses

Figure 4 shows the kinetics of DNA copies of hCAR-T and IL6 from 1 to 2 weeks after infusion. During the first 5 to 6 days after infusion, the DNA copies of hCAR T cells in PB remained at the low level, but some inflammatory cytokines, including IL6, dramatically increased and the systemic toxicities were apparent or severe in this period. Approximately 7 to 9 days after infusion, DNA copies in PB had a rapid rise and arrived at a maximum 1 or 2 days later. At the same time, the serum level of IL6 decreased dramatically and temperature also returned to the normal value. Systemic toxicities also turned to the endpoint and the damaged function of organs recovered gradually. This appeared to be due to the fact that the majority of CD19⁺ leukemic cells existed in the BM, and the primary and most intense antitumor responses had occurred in that stage. Once leukemic cells were eradicated completely, hCAR T cell responses would be attenuated, resulting in decline of cytokine production and inflammatory responses.

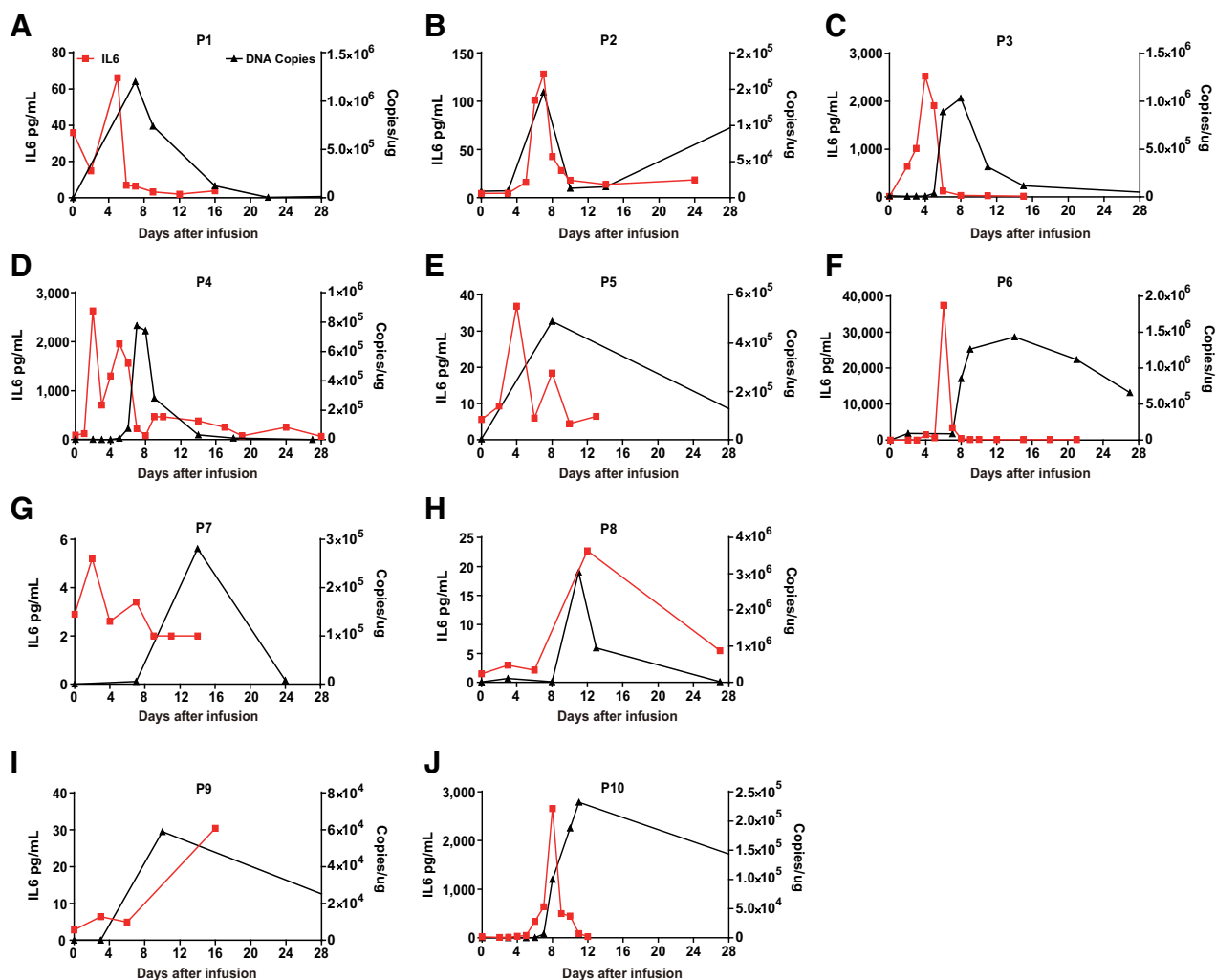


Figure 4.

The rapidly ascending number of hCAR T cells accompanied a decrease of IL6 in the PB. **A–J**, Kinetics of IL6 and DNA copies in PB of the 10 patients. After the sharp increase of CAR-T DNA copies in the PB, inflammatory cytokines such as IL6 rapidly decline and organ functions began to recover from CRS.

The incidence of CRS and neurotoxicity

CAR T cells can induce a clinical syndrome of fevers, hypotension, and hypoxia associated with marked elevations of serum cytokines in some patients. This syndrome has been termed CRS (16, 17). To analyze the severity of CRS clearly, patients in our study were divided into two groups according to the CRS grade. Four patients with grade 3–4 CRS were defined as the first group, whereas the other six patients with grade 1–2 CRS were defined as the second group. The temperature of each patient was recorded every 1 to 4 hours during the treatment (Supplementary Fig. S5A and S5B). Compared with patients who had grade 1–2 CRS, patients who had grade 3–4 CRS experienced fever earlier after hCAR T cell infusion and the fever reached a higher maximum temperature ($P < 0.05$) and was of longer duration (Supplementary Fig. S6A). We also measured the levels of IL6, CRP, and IFN γ regularly in all patients to help diagnose and treat CRS (Supplementary Fig. S5C–S5H). We found that IL6 in patients with grade 3–4 CRS increased much more rapidly ($P < 0.05$), and the peak levels of these cytokines were distinctly

higher than those of patients with grade 1–2 CRS ($P < 0.01$; Supplementary Fig. S6). The four patients with grade 3–4 CRS also developed grade 1–4 neurotoxicity. P3 and P4 developed multiple neurologic symptoms after CAR T cell infusion, including headache, nausea, vomiting, and epilepsy. Typically, neurotoxicity presented at the time point when the patients began to recover from the CRS. This was likely partly due to the long-term disruption to the blood–brain barrier from the inflammatory cytokines such as IL6 (18). The details of adverse events are listed in Supplementary Table S1.

Currently, temperature and IL6 are the two most used factors to diagnose CRS. In this study, we found that those patients who experienced grade ≥ 3 CRS also exhibited a higher concentration of IFN γ , IL10, fractalkine, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), and GM-CSF (Supplementary Fig. S6). This finding suggested that one or more of these cytokines could be used as clinically diagnostic biomarkers for grade ≥ 3 CRS, which was consistent with the results reported by Teachey and colleagues (19).

Management of patients with severe CRS

For four patients with grade 3–4 CRS, the tumor loads in their BM were extremely high and rapidly progressive. As shown in Supplementary Fig. S7, all of these patients had received six to 11

rounds of chemotherapy previously. Although three patients had ever achieved CR after chemotherapy, the remission time was obviously short and they would become resistant to these regimens once the relapse occurred. Even worse, patient 4, whose tumor

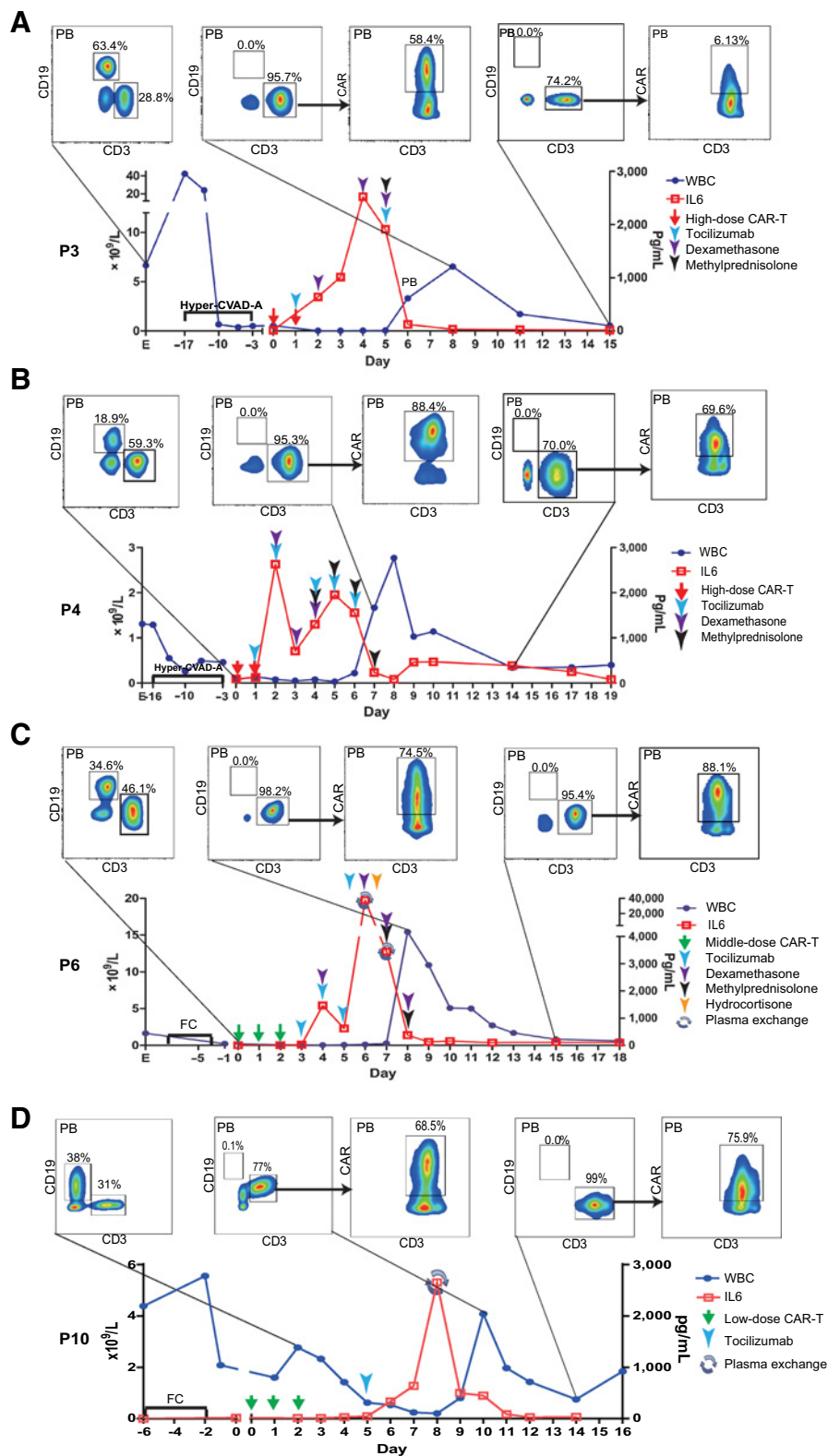


Figure 5.

Management details for CRS in four patients with grade 3–4 CRS. **A–D**, Kinetics of WBC and IL6 in PB of four patients of high tumor burden with grade ≥ 3 CRS are shown. Main interventions for toxicity management and analysis of cell phenotype in PB at key time points are labeled. For patients P3 and P4, Hyper-CVAD-A was chosen as the preconditioning regimen, whereas for patients P6 and P10, the regular FC regimen was given. Day at infusion was defined as day 0. WBC, white blood cell.

disease increased to 95% after lymphodepletion, had never achieved CR during the entire period of chemotherapy (Supplementary Fig. S7B). For patients P3 and P4, considering that the tumor load in their BM was extremely high ($\geq 90\%$) and the CRS would become uncontrollable, a more intensive preconditioning regimen Hyper-CVAD-A instead of FC was conducted in these two patients (Supplementary Fig. S7A and S7B).

For management of grade 3–4 CRS, early use of tocilizumab and glucocorticoid is recommended (20–22). After infusion of CAR T cells, the temperature responded rapidly to this therapy, and ibuprofen was used to further control the temperature. At the same time, tocilizumab and glucocorticoid were administered to these patients even though elevated cytokines were not observed. In addition to receiving tocilizumab and glucocorticoid, patients P3 and P4 also received mannitol to control cerebral edema of neurotoxicity (Fig. 5; Supplementary Fig. S8).

Along with tocilizumab or glucocorticoid, for patients P6 and P10, PE was administered to reduce the serum level of inflammatory cytokines. For patient P6, PE was conducted on days 6 and 7 (Fig. 5C). A total of 1,800 mL of plasma was exchanged, with the IL6 reducing from 37,510 to 375.9 pg/mL within 24 hours, and other symptoms like fever were quickly relieved. For P10, PE was administered on day 8, and 750 mL of plasma was exchanged (Fig. 5D). After PE, his serum IL6 level had an evident decrease from 2,656 to 499.5 pg/mL, accompanied with significant relief of other symptoms, indicating that PE might be an effective method in the management of CRS.

Discussion

CAR T-cell therapy has shown substantial clinical efficacy in treating B-lineage malignancies, particularly in *r/r* ALL, where a 70%–90% CR rate has been achieved in the first month after infusion. Despite the inspiring initial achievement, relapse remains a major challenge in some patients with the recovery of CD19⁺ leukemia cells due to the loss of the CAR T cells. There are many factors contributing to early loss of the CAR T cells, including the CAR structure itself, cultivation methods of CAR T cells, and the immunogenicity of CARs.

In this study, we focus on the immunogenicity of the CAR structure because most of the scFvs of CARs are derived from murine antibodies, which may develop an immune response specific for epitopes in murine scFvs and reduce the persistence of CAR T cells. To reduce the immunogenicity of CARs, we developed a humanized anti-CD19 scFv and found that it shows similar anti-leukemia efficacy in preclinical study, providing the basis for its clinical use.

To evaluate the efficacy and safety of anti-CD19 hCAR-T cells, we analyzed data from ten patients with a diagnosis of *r/r* ALL. Among them, all patients were response evaluable, and all achieved morphologic CR (100%) and MRD-negative CR (100%). With long-term follow-up, we observed that both the OS and LFS rate were 100% at 3 months, and the OS and LFS rate were 100% and 90% at 6 months, respectively. Previous study showed that treatment of *r/r* ALL with Tisagenlecleucel, FDA-approved CAR-T based on murine scFv, resulted in the overall remission rate at 81% within 3 months. The rates of OS and event-free survival were 90% and 73% at 6 months, respectively (2). In our study, the long-term persistence of hCAR T cells has been observed in six patients with more than 1,000 DNA copies/ μ g of CAR T cells detected in PB at or after 6 months.

Relapse remains a major challenge for CAR T-cell therapy. In this study, we found that hCAR-T treatment resulted in eight of 10 patients remaining CR until now and six in CR for over 18 months without further treatment. Only two relapses have been observed—in patients P3 and P4 during the follow-up. For P3, hCAR T cells were lost at 4 months and leukemia cells with CD19 positive were found at 5.7 months. For P4, early loss of hCAR T cells was also observed at 64 days after infusion with detection of less than 100 DNA copies/ μ g in PB. However, the DNA copies in P4 experienced a fivefold increase after the recovery of CD19⁺ cells at 83 days, and then CD19⁺ cells in the PB and in the BM descended to a level lower than 1%. Four months after infusion, patient P4 proceeded to accept HSCT and had a CD19⁺ relapse at 12 months after infusion. Considering the reasons for the early loss of hCAR T cells in these two patients, the most probable one is that these two patients were in a high disease burden and the ratio of CAR T cells to target CD19⁺ leukemia cells was lower than those with a low disease burden, potentially resulting in the early exhaustion of CAR T cells (5). Another reason may be that high-dose glucocorticoids have been used in these two patients because both of them had developed grade 3–4 CRS. Glucocorticoid is immunosuppressive and has been reported to negatively affect the survival of CAR-T cells (17, 23). These two patients were also treated with preconditioning regimen Hyper-CVAD-A instead of FC before CAR-T infusion, and it was reported that incorporation of fludarabine into the lymphodepletion regimen was associated with superior CAR T cell expansion and persistence and a lower risk of CD19-positive relapse (8, 24).

Management of toxicity is also an important part of the clinical application for CAR T cell therapy (25). As reported, the most common adverse events observed were CRS and neurotoxicity (5, 23). Recently, there have been some studies on the pathological and kinetic mechanism of CAR T-related toxicity (21, 26). Despite significant progress, there are still no good solutions for reducing the risk of CAR T-related toxicity, particularly for those with a bulk and rapid progressive tumor disease (5, 27). In this study, we found that early use of tocilizumab and glucocorticoid before cytokines elevations were effective for the treatment of severe CRS. Although the early use of tocilizumab and glucocorticoid could not reduce the secretion of cytokines such as IL6, this method could alleviate the inflammatory responses in the body and reverse the organ toxicities. Besides this effect, glucocorticoid could also reduce local inflammatory reactions in the brain, potentially helping to alleviate some neurotoxic symptoms.

Furthermore, in our study, we utilized PE to manage severe CRS. PE has been commonly used in the treatment of a variety of hematologic, neurologic, nephrologic, and rheumatologic disorders (28–30) but has only been reported to be applied for the management of CRS in a recent case report by Xiao and colleagues (31). The therapeutic effect of PE was likely achieved through the removal of pathogenic substances; thus, we speculated that PE could be used to control CRS by removing inflammatory factors. In the study, PE was first used to take control of life-threatening CRS in P6. The levels of IL6 and other inflammatory cytokines dramatically decreased, and other symptoms, including the fever and dyspnea, were quickly relieved. According to this successful practice, we conducted PE once again in P10 who was at high risk for grade ≥ 3 CRS because his tumor burden increased significantly from 3% to 63% after preconditioning therapy. After PE, his serum IL6 level had an evident decrease from 2,656 to 499.5 pg/mL. These results indicated PE was feasible and could be included in the management of grade ≥ 3 CRS.

Taken together, we demonstrated that T cells expressing the humanized anti-CD19 scFv CAR exhibited sustained therapeutic efficacy and low relapse in the treatment of r/r ALL. Low relapse rate was associated with the long-term persistence of CAR T cells in the treated patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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