Dual specific CD19/CD22-targeted chimeric antigen receptor T-cell therapy for refractory diffuse large B-cell lymphoma: A case report

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Abstract. Clinical trials of chimeric antigen receptors (CARs) targeting CD19 have produced impressive results in hematological malignancies, including diffuse large B-cell lymphoma (DLBCL). However, a notable number of patients with DLBCL fail to achieve remission after CD19 CAR T-cell therapy and may therefore require a dual targeted CAR T-cell therapy. A 31-year-old man with refractory DLBCL was assessed in the present case report. The patient was treated with sequential infusion of single CD19 CAR T cells followed by dual CD19/CD22-targeted CAR T cells. The outcome was that the patient achieved partial remission after the first single CD19 CAR T-cell infusion and complete remission after the dual CD19/CD22-targeted CAR T-cell infusion. Grade 1 cytokine release syndrome (CRS) was observed after the single CD19 CAR T-cell infusion, while grade 3 CRS and hemophagocytic syndrome were observed after the dual targeted CAR T-cell infusion, but these adverse effects alleviated after the treatments. To the best of our knowledge, the present case report is the first to describe the successful application of dual CD19/CD22-targeted CAR T-cell therapy for the treatment of refractory DLBCL. The report suggests that dual CD19/CD22-targeted CAR T-cell therapy may represent a promising option for the treatment of refractory DLBCL; however, caution should be taken due to potential CRS development.

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Key words: diffuse large B-cell lymphoma, chimeric antigen receptor T cell, cytokine release syndrome, therapy, CD19, CD22

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma worldwide with the morbidity frequently in elderly people (1). DLBCL is an aggressive malignancy of large transformed B-lymphocytes that often originates from the lymph nodes, and that exhibits a notable molecular heterogeneity in gene profiles and clinical outcomes (2). DLBCL is potentially curable. Patients with DLBCL at an early stage usually undergo a short course of chemotherapy consisting of four drugs (cyclophosphamide, doxorubicin, vincristine and prednisone) known as CHOP, or chemo-immunotherapy, which is a combination of chemotherapy and the monoclonal antibody rituximab (Rituxan®) (3). For patients with late-stage DLBCL with a higher risk of recurrence after treatment, high-dose chemotherapy followed by a stem cell transplant is provided as an option (2). Allogeneic transplantation from a sibling or matched unrelated donor may be considered for patients with refractory disease, early relapse or relapse after autologous stem cell transplantation (4). All these therapeutic strategies have greatly improved the survival time of patients with DLBCL (5). Although DLBCL can now be successfully treated in ~50% of patients, certain individuals, especially those with relapsed or refractory DLBCL, fail to respond to these conventional treatments or to achieve long-term outcomes (2).

A number of novel therapies or procedures are being tested in various clinical trials for DLBCL, including immunomodulators, tyrosine kinase inhibitors, BCL2 inhibitors and immune checkpoint inhibitors (6). Chimeric antigen receptor (CAR) T-cell therapy is one of the most promising immunotherapies for patients with DLBCL (7). As of December 10, 2019, clinicaltrials.gov has registered a total of 896 CAR T-cell-associated clinical trials worldwide, including 43 for DLBCL. There are currently 15 clinical trials being performed in China for DLBC, including one using CD19- and CD22-targeted sequential treatment (8), nine against CD19 (9), two against CD22, two against CD20 and one against CD19/22 (10). The principle of CAR T-cell therapy is to genetically modify autologous T cells with a recombinant receptor construct composed of an antibody-derived extracellular single-chain variable fragment

(scFv) linked to intracellular T-cell signaling domains of the T-cell receptor. The T cell-antigen interaction is independent from molecules of the major histocompatibility complex, and is therefore not regulated by the immune escape promoted by tumor cells (11). Choosing the right tumor antigen as a target is the key to designing safe and effective CAR T-cell therapies. B-cell malignancies commonly express the surface antigens CD19 and CD22, which are not expressed on other non-B cells (such as hematopoietic stem cells) (12). At present, CD19 CAR T-cell therapy is widely used in clinical trials of malignant B-cell tumors, including B-cell acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, mantle cell lymphoma, multiple myeloma and B-cell non-Hodgkin's lymphoma, particularly for aggressive B-cell lymphomas (13). Single- and multi-center clinical trials using anti-CD19 CAR T-cell therapy have demonstrated the effectiveness of this cell therapy, it has great efficacy and long-term remissions in patients with poor-risk DLBCL, when no other effective treatment options are available (14). With no other effective treatment options available, the single and multi-center clinical trials have demonstrated that the anti-CD19 CAR T-cell therapy can provide long-term remission in patients with poor-risk DLBCL (15,16). As a synergistic targeting strategy, compared with targeting a single antigen, dual specific CD19- and CD22-targeted CAR T-cell therapy may represent a potential approach to improve the outcomes in patients with DLBCL with heterogeneous expression of CD19 and CD22 on leukemic blasts (16).

Cytokine release syndrome (CRS) is a systemic inflammatory response that can be triggered after infusion of antibody-based therapies, such as CAR T-cell therapy. According to the ZUMA-1 (Yescarta®) trial data published in January 2019, 83% of the 101 patients with assessable efficacy achieved an objective response and 58% achieved a complete response (14). Among the 108 patients whose safety could be assessed, 48% developed grade ≥3 serious adverse events and 11% of patients exhibited grade ≥3 CRS (17). CRS represents one of the most frequent serious adverse effects and is one of the challenges of using bispecific antibody (such as CD19/CD22) CAR T-cell therapies (18-20). To the best of our knowledge, the present case report describes the first clinical case of a patient with refractory DLBCL who underwent both single CD19- and dual CD19/CD22-targeted CAR T-cell therapies after multi-line chemotherapy regimens, and who achieved complete remission (CR) with minor CRS-associated adverse events.

Case report

A 31-year-old man with no prior medical history presented with persistent epigastric pain for 1 week was admitted to the Fourth Hospital of Hebei Medical University on April 3rd, 2015. Imaging scans demonstrated a large mass-like conglomerate in the abdomen, with the maximum clast length measuring up to 13 cm (Fig. 1A). After that, immunohistochemistry examination of the biopsy specimen was performed as described below. Formalin-fixed (at 4˚C for 24 h) and paraffin-embedded tissues were cut into 5 μm thick sections. After drying at 65˚C for 2 h, tissues were deparaffinized and hydrated in graded alcohol and PBS. The sections were blocked at room temperature with 0.3% hydrogen peroxide to inhibit endogenous peroxidase activity for 5 min. EDTA pre-incubated with 5% normal bovine serum (Wuhan Boster Biological Technology Ltd.) was applied for antigen retrieval at room temperature for 20 min. Sections were subsequently incubated with antibodies against CD20 (cat. no. IS60430-2; 1:200; Dako; Agilent Technologies, Inc.), CD19 (cat. no. 551520; 1:100; Ventana Medical Systems, Inc.), CD22 (cat. no. 563941; 1:100; Ventana Medical Systems, Inc.), CD10 (cat. no. 561002; 1:1; Ventana Medical Systems, Inc.), BCL2 (cat. no. IS61430-2; 1:10; Agilent Technologies, Inc) and BCL6 (cat. no. 1306055; 1:75; Santa Cruz Biotechnology, Inc.), overnight at 4˚C. The sections were subsequently incubated with secondary antibody (cat. no. KIT-5220; 1:200; Maxim Biotech, Inc.) for 20 min at room temperature. The reaction products were treated with diaminobenzidine and counterstained with hematoxylin at room temperature for 5-10 min. Tissue sections were observed under a light microscope (magnification, x20). The results of immunohistochemistry stains revealed the infiltration of large atypical pleomorphic lymphoid cells, which expressed CD20, CD19, CD22 and BCL2, but not BCL6 and CD10. Furthermore, >70 and >50% of cells were positive for Ki-67 and c-Myc staining, respectively. Chest computed tomography (CT) scan revealed a shadow in the right upper lobe of the lung (Fig. 1B), while pathology tests of CT-guided percutaneous lung biopsy revealed epithelioid granulomas. The purified protein derivative skin test was negative, while a more accurate T cell-based test of tuberculosis infection was positive, indicating a prior mycobacteria infection (21). According to Ann Arbor staging system (22), the patient who diagnosed with DLBCL was classified as stage I after the biopsy procedure and immunohistochemical analysis. This type of DLBCL was also characterized as a non-germinal center B-cell-like (non-GCB) subtype (23). Due to the age of the patient, the international prognostic index was evaluated as 2 (2), and due to the persistent residual mass in the abdomen, the patient was considered to be at high-intermediate risk. The flow diagram of the treatments used is presented in Fig. 2.

The patient only achieved partial remission after two cycles of standard therapy, including 750 mg/m^2 of cyclophosphamide, 1.4 mg/m^2 of vincristine (max dose of 2 mg), 50 mg/m^2 of doxorubicin, 100 mg of prednisone, and 375 mg/m^2 of rituximab (R-CHOP). After that, an intensified immunochemotherapy regimen therapy was applied, as shown by cyclophosphamide 1200 mg/m^2, vincristine 2 mg/m^2 (max dose of 2 mg), doxorubicin 75 mg/m^2, prednisone 60 mg, and rituximab 375 mg/m^2 (R-ACVBP). The patient finally achieved CR after two courses of the R-ACVBP regimen, followed by another two courses of chemotherapy for consolidation. At 2 months post-CR, imaging scans revealed that the abdominal mass was ~7.6x5.1 cm in size (Fig. 1C), which was considered as a recurrence. The patient received sequential salvage chemotherapies, including two cycles of rituximab, ifosfamide, carboplatin and etoposide, two cycles of rituximab and lenalidomide, one cycle of gemcitabine, dexamethasone and cisplatin, and one cycle of etoposide, methyl prednisolone, cisplatin and cytarabine. Despite many attempts at treatment, the patient with refractory DLBCL exhibited no significant response to the salvage therapies.

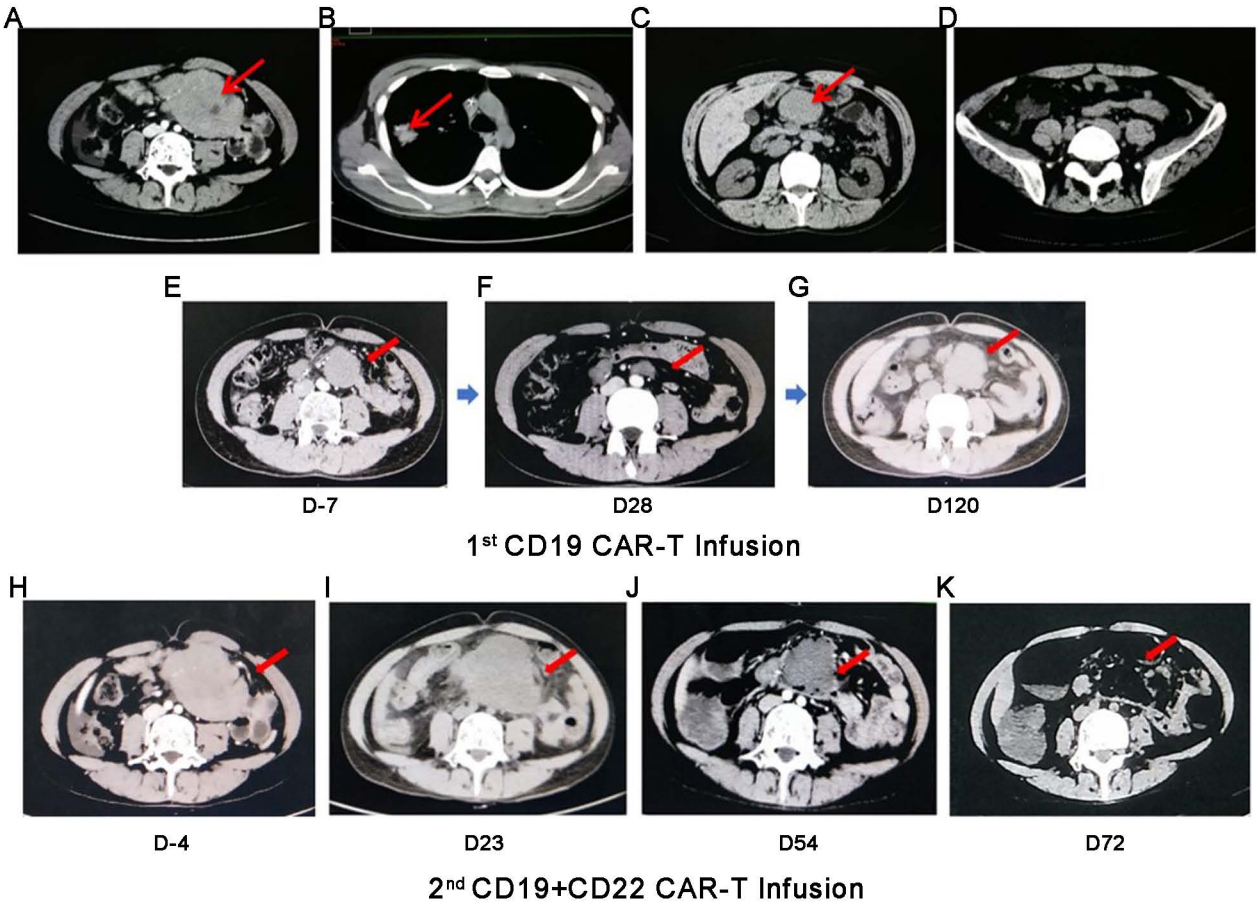

Figure 1. Axial images of CT chest and abdomen scan after two rounds of CAR-T infusion. (A) Imaging studies showed a large mass-like conglomerate with a maximum length of 13 cm in the patient's abdomen. (B) Chest CT scan showed the shadow of the right upper lobe of the lung. (C) The imaging showed that the abdominal mass was ~7.6x5.1 cm. (D) The patient's tumor burden decreased. (E) Full-body CT scans revealed enlarged mesenteric lymph nodes located in the abdomen compared with the CT scans before CAR T-cell infusion. (F) On day 28 post-infusion, imaging showed that enlarged lymph nodes in the abdominal cavity were smaller. (G) On day 120 post-infusion, imaging showed a conglomerate mass with a maximum length of 4.89 cm in the patient's enterocoelia. (H) Imaging before the second CART treatment showed that the abdominal mass was significantly larger than before. (I) Full body CT scans on day 23 revealed that the abdominal mass had increased in size (maximum diameter, 9 cm). (J) There was a marked reduction in the size of the abdominal mass after 54 days. (K) A full-body CT scan on day 72 demonstrated complete remission. CT, computed tomography.

Instead of stem cell transplantation, the patient received radiotherapy at a dose of 45 Gy in 25 fractions in order to treat the retroperitoneal soft-tissue masses. Although the tumor burden decreased (Fig. 1D), the course of radiotherapy was interrupted due to the development of severe bone marrow suppression and gastrointestinal intolerance. As one of the most common acute side effects of radiation therapy, a reduction of T cells was observed in the peripheral blood of the patient, and the biopsy of the abdominal mass resulted positive for CD19 expression in the non-GCB subtype DLBCL. Additionally, full-body CT scans revealed enlarged mesenteric lymph nodes located in the abdomen, as compared with prior CT scans (Fig. 1E).

The CAR construct used in the present study was composed of a CD19-scFv (FMC63), the costimulatory domains of 4-1BB and the endodomain of CD3-θ (24,25). After careful physical examination, the patient was recruited for a CD19 CAR T-cell therapy clinical trial (NCT03121625). Peripheral blood mononuclear cells (100 ml) were collected to prepare CD19-directed CAR T cells. A lymphodepleting pretreatment (25 mg/m^2 fludarabine on days-4 to -2, and 900 mg/m^2 cyclophosphamide on days-2 to -1) was administered prior to a 2x10^6 cells/kg CAR T-cell infusion on day 0. Within 12 h after infusion, the patient developed grade 1 CRS with fever (26). On day 28 post-infusion, the patient exhibited partial remission. An imaging test revealed that the size of the enlarged lymph nodes in the abdomen was decreased (Fig. 1F).

At 4 months post-infusion, the patient experienced disease progression, assessed via imaging examination revealing enlarged lymph nodes (maximum diameter, 4.89 cm; Fig. 1G). Immunohistochemistry results from the biopsy demonstrated that the infiltrates around the abdominal mass were CD19 and CD22 double-positive cells. Therefore, a dual CD19/CD22-targeted CAR T-cell therapy with the same dose of the FC regimen (fludarabine, 25 mg/m^2; cyclophosphamide, 250 mg/m^2) was administered to the patient 150 days after the first CD19 infusion. Imaging before the second CART treatment showed that the abdominal mass was significantly larger than before (Fig. 1H). The patient received 2x10^6/kg of both CAR T cells on day 0. The patient developed grade 3 CRS with shivering, hypotension and hyperpyrexia, and therefore received anti-infection

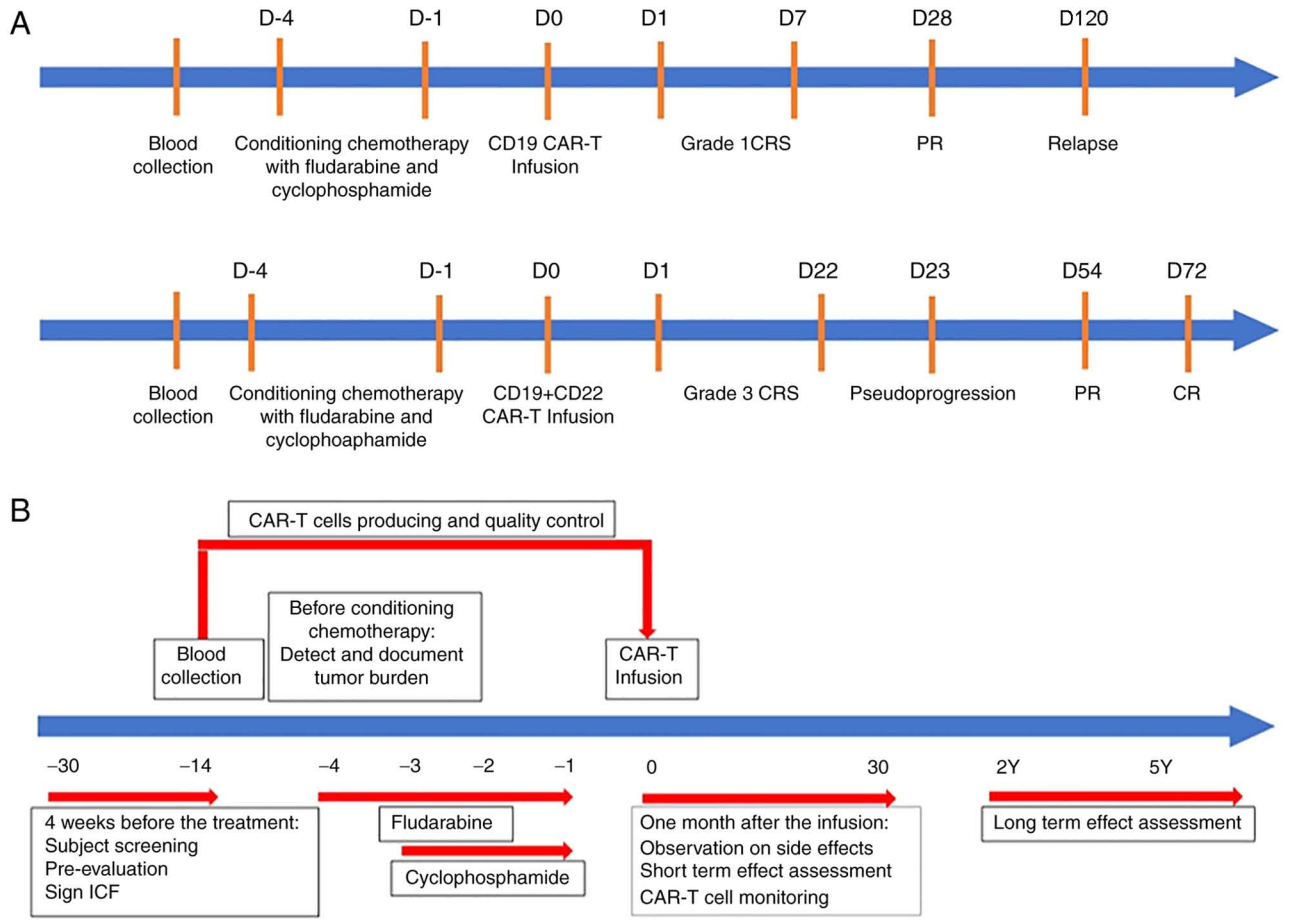

Figure 2. Schematic representation of the patient's progress and clinical protocol design. (A) Record of patient's progress after single CD19 CAR-T infusion or CD19 + CD22 CAR-T infusion treatment. Blood was collected from the patient, and conditioning chemotherapy with fludarabine and cyclophoamide was conducted, following by giving either CD19 CAR-T infusion or CD19+CD22 CAR-T infusion treatment; the grade of CRS, PR or pseudoprogression and CR or relapse were recorded. (B) Clinical protocol design with time frame ranged from -30 days to 5 years. 4 weeks before the treatment, patient had screening, pre-evaluation and signed ICF, followed by detection and documentation the size of tumor burden before conditioning chemotherapy. One month after the infusion, any side effects and short-term effects were observed and assessed. At the conclusion of the CAR-T cell monitoring, the patient was asked for long-term follow up (2-5 years). CAR, chimeric antigen receptor; PR, partial remission; CR, complete remission; CRS, cytokine release; ICF, informed consent form; D, day; M, month; Y, years.

and rehydration treatments. Full body CT scans on day 23 revealed that the abdominal mass had increased in size (maximum diameter, 9 cm), suggesting a poorer prognosis (Fig. 1I). Accordingly, the patient had persistent fever for >1 week, with pancytopenia, a decreased fibrinogen level (<1.5 g/l; normal range, 2-4 g/l) and an elevated serum ferritin level (23,410.00 ng/ml; normal range, 15-200 ng/ml for adult male). The proliferation of peripheral blood CAR T cells was analyzed via quantitative (q)PCR. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, and reversed transcribed into cDNA using the reverse transcription kit (cat. no. 18091050; Thermo Fisher Scientific, Inc.). cDNA was amplified using EmeraldAmp® PCR Master Mix (Thermo Fisher Scientific, Inc.). The algorithm $2^{-\Delta\Delta Cq}$ method (27) was used to normalize the relative expression of genes to GAPDH. The following primer sequences were used for qPCR: CAR forward, 5'-CATCCTCCCTGT CTGCCTCT-3;' and reverse, 5'-GCCTCCGCCATCTTA TCTTT-3'; GAPDH forward, 5'-TGCATTCGCCCTCTT AA-3' and reverse, 5'-CATCACGCCACAGTTTCC-3'; and CAR FQ-PCR forward, 5'-GGATTCGCCAGCCTCCAC-3' and reverse, 5'-AAACTTGGCTCTTGGAGTTGT-3'. CAR FQ-PCR-Probe: 5'-(FAM)-TCCCAGCCACTCCAGACC CTT-(MGB)-3'. Additionally, qPCR was carried out with an ABI 7500 machine (Applied Biosystems, Carlsbad, CA). The following thermocycling conditions were applied: 1 cycle at 50˚C for 2 min, 1 cycle at 95˚C for 10 min and 40 cycles at 95˚C for 15 s, while 60˚C for 60 s (27). The data demonstrated that both CD19 and CD19/CD22 CAR T cells began to expand after 4 days, peaked on days 7 and 18, and then gradually decreased (Fig. 3A-C). Notably, dual-CAR T cells were mixed after individual transduction, and their expansion was ~5 times higher than single CD19 CAR T cells; additionally, both $CD19^+$ and $CD22^+$ CAR T cells remained sustained at higher levels for 2 further months. After infusion of both CAR T cells, the patient exhibited an intermittent high fever during the first few days but, subsequently, the body temperature gradually returned to normal and remained stable (Fig. 3D and E). The levels of lymphocytes, ferritin and C-reactive protein gradually decreased after day 7 for CD19 single CAR T-cell infusion (Fig. 4A), and for dual CAR T-cell infusion, the level of platelets drastically decreased after day 18, while the levels of ferritin and C reactive protein

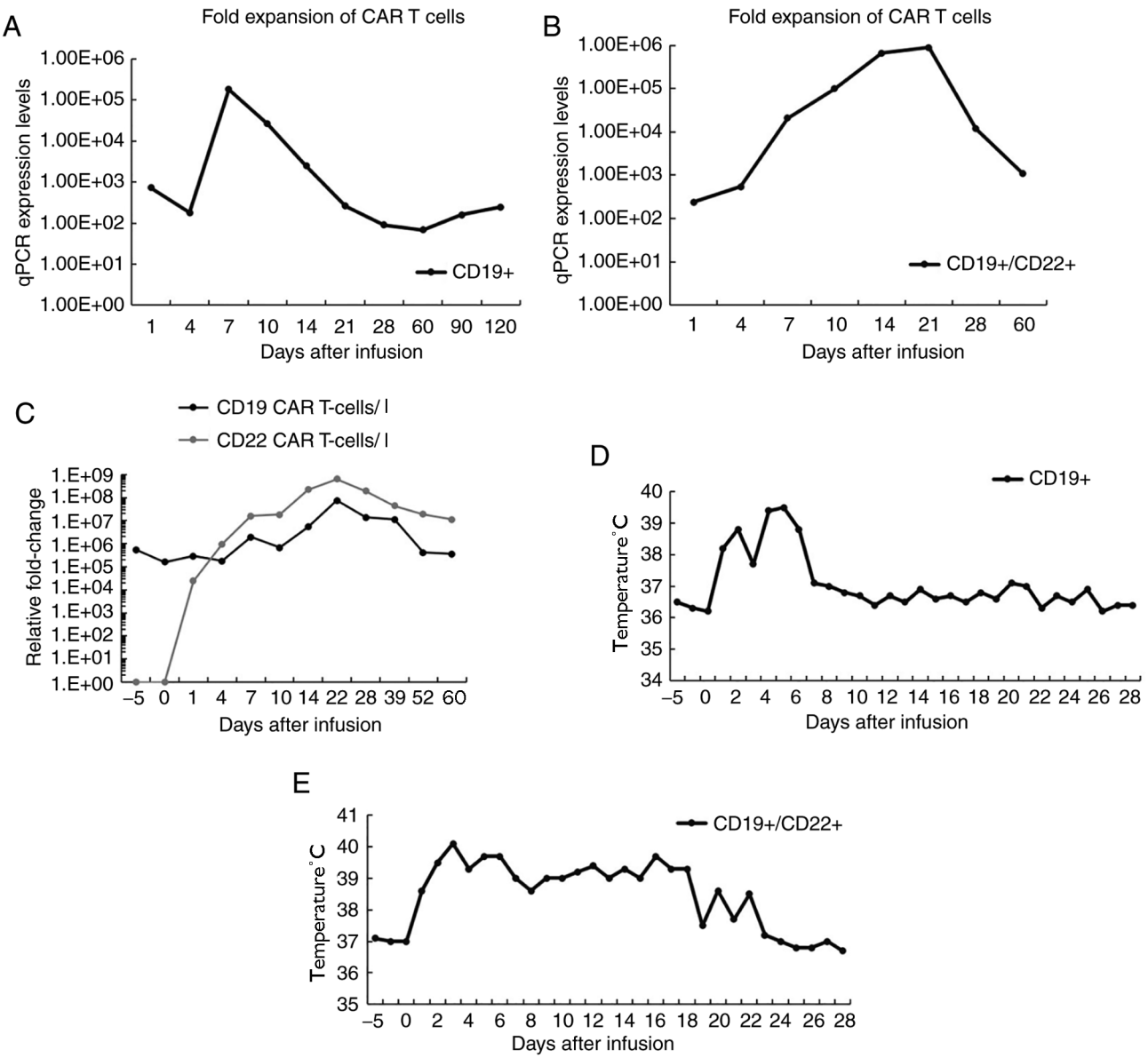

Figure 3. Monitoring of circulating T-cell subsets and body temperature after CAR T-cell infusion. T-cell expansion measured via qPCR after (A) CD19$^+$ and (B) CD19$^+$/CD22$^+$ CAR T-cell infusion, and (C) via flow cytometry. Body temperature change after (D) CD19$^+$ and (E) CD19$^+$/CD22$^+$ CAR T-cell infusion. qPCR, quantitative PCR; CAR, chimeric antigen receptor.

decreased after day 20 (Fig. 5A). Although no immediate infusion-associated toxic effect was observed, a febrile syndrome with elevated cytokine levels was subsequently observed after CAR T-cell infusions (Figs. 4B and 5B). Overall, the present results indicated that the patient developed grade 1 CRS after single CD19 CAR T-cell infusion, and grade 3 CRS after dual CAR T-cell infusion.

Additionally, hemophagocytosis was observed in the patient's bone marrow, supporting a diagnosis of hemophagocytic syndrome. The patient was treated with dexamethasone (20 mg/day), which was later replaced by methylprednisolone (80 mg/day) on day 33. The body temperature of the patient was well controlled from day 48. Additionally, the patient developed gastrointestinal bleeding and was therefore administered proton pump inhibitors, intravenous fluids and electrolytes, while the oral intake of liquids or solids was prohibited. There was a marked reduction in the size of the abdominal mass after day 54 (Fig. 1J). The hemophagocytosis symptom was not observed after day 57, while at the same time several blood tests, including ferritin levels and coagulation function, returned to normal. A full-body CT scan on day 72 demonstrated CR (Fig. 1K) that continued until day 100. Flow cytometric analysis (28) revealed that most of the lymphocytes before infusion were CD3$^+$ and CD3$^+$/CD4$^+$ T cells, and these cells were replaced by CAR T cells rapidly after infusion (Fig. 6). The proportion of CAR T cells increased and then decreased within 1-month post-infusion for single CAR T-cell infusion but remained high in dual CAR T-cell infusion for at least 2 months. Overall, the present data suggest a synergistic efficacy of CD19- and CD22-targeted CAR T cells in the present patient. After monitoring (up to D60) and infecting with H1N1 (at D85), the patient was transferred to the Hebei Provincial Chest Hospital because of gastrointestinal hemorrhage.

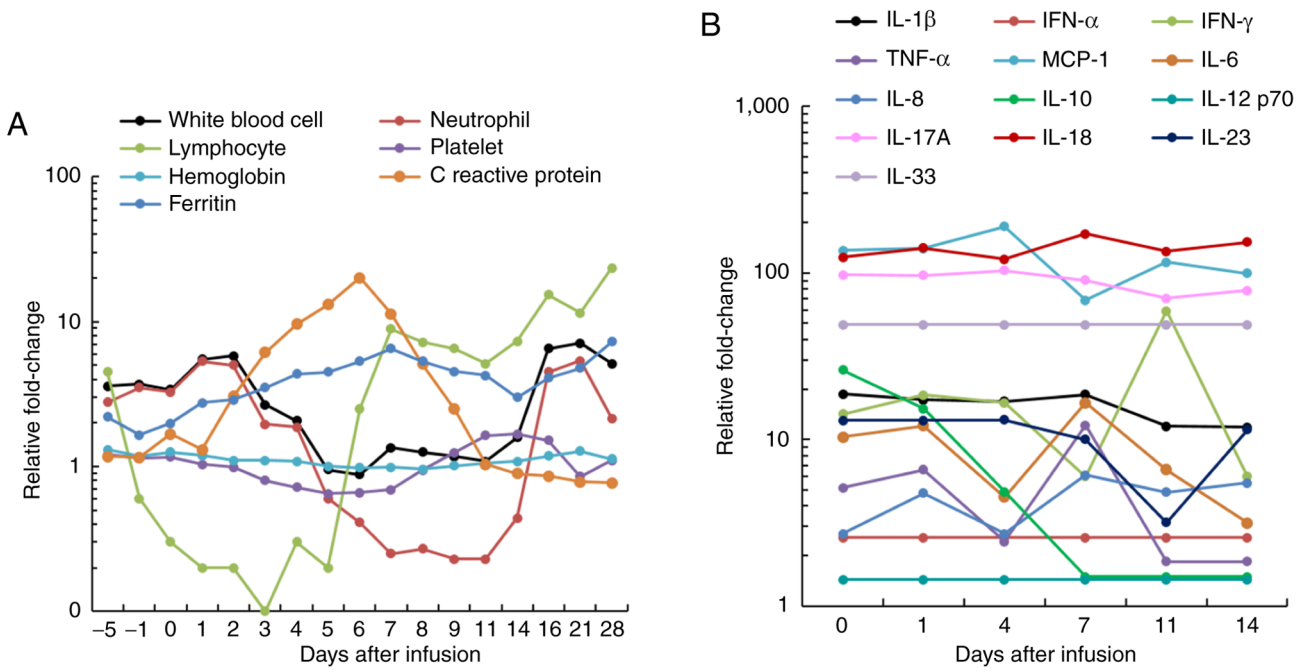

Figure 4. Routine blood test and serum cytokine monitoring after CD19$^+$ chimeric antigen receptor T-cell infusion. (A) Individual immune cell numerical expansion and serum factors fold-change values. (B) Concentrations of cytokines in serum were determined by a fluorescence-activated cell sorter. The fold-change is relative to the pre-infusion peripheral blood samples (baseline). IL, inte rleukin; IFN, interferon; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein-1.

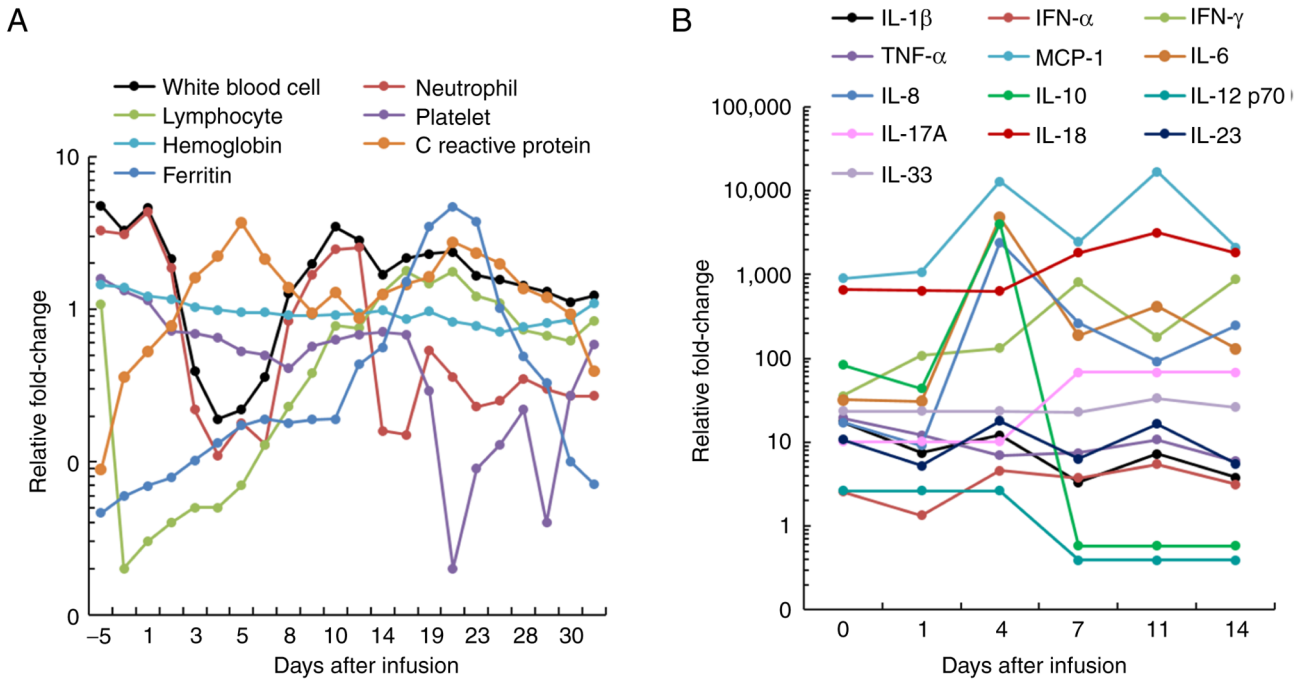

Figure 5. Routine blood test and serum cytokine monitoring after CD19$^+$/CD22$^+$ chimeric antigen receptor T-cell infusion. (A) Individual immune cell numerical expansion and serum factors fold-change values. (B) Concentrations of cytokines in serum were determined by a fluorescence-activated cell sorter. The fold-change is relative to the pre-infusion peripheral blood samples (baseline). IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; MCP-1, monocyte chemoattractant protein-1.

Discussion

Cancer immunotherapy is an emerging therapeutic strategy that has demonstrated significant efficiency compared with conventional treatments such as radiotherapy, chemotherapy and surgery (29). At present, >800 CAR T-cell studies are registered on clinicaltrials.gov, with one-third being CD19-targeted CAR T-cell trials. Tumor cells of B-cell malignancies typically express both CD19 and CD22 surface antigens (30), making dual targeted CAR T cells a more broadly active therapy (31).

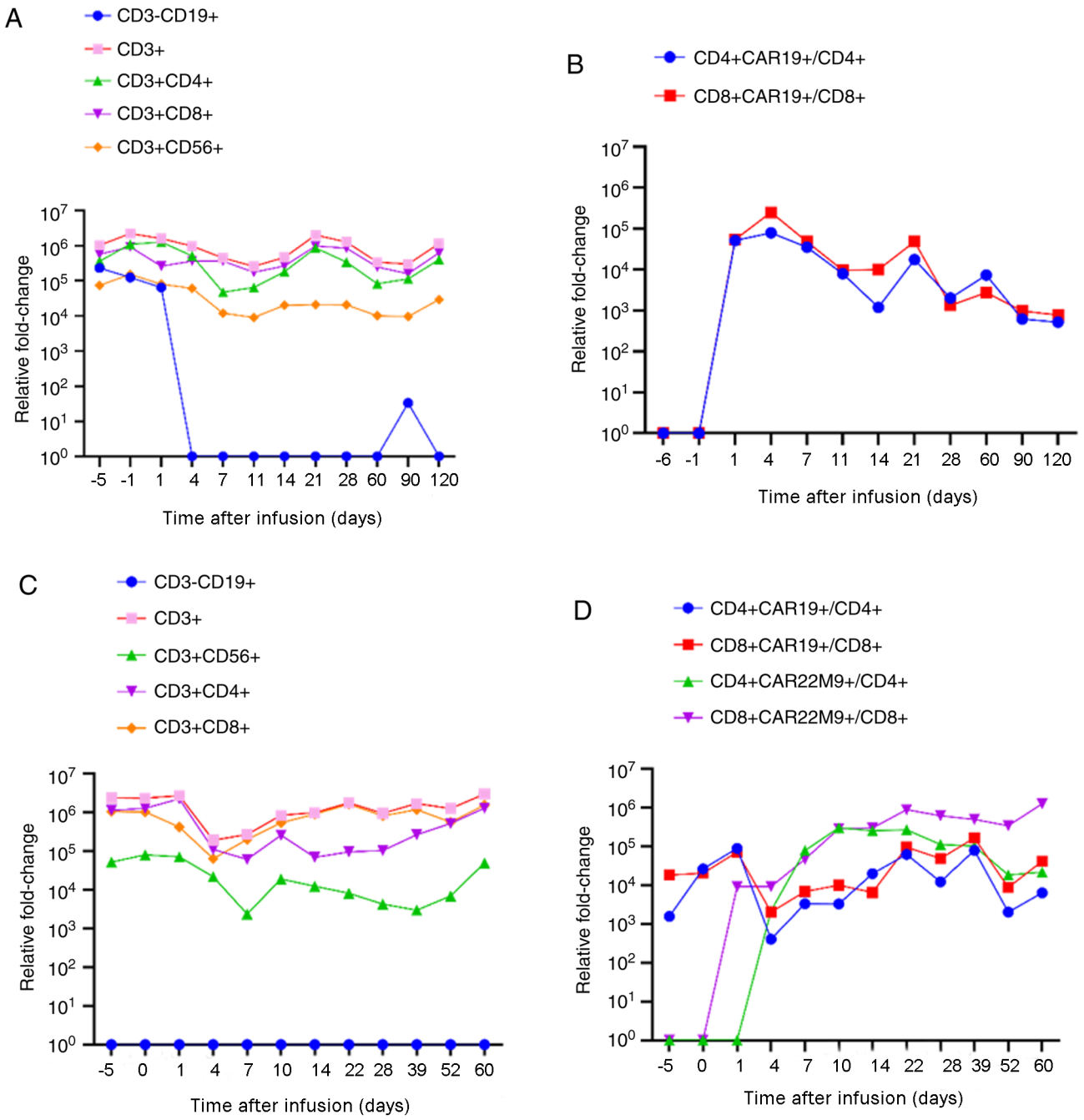

Figure 6. Monitoring of lymphocyte subsets after CAR T-cell infusion. Fold-change of lymphocyte subsets from peripheral blood was measured by flow cytometry after (A and B) CD19$^+$ and (C and D) CD19$^+$/CD22$^+$ CAR T-cell infusion. CAR, chimeric antigen receptor; M, month.

To the best of our knowledge, there are no reports regarding the efficacy of dual CD19/CD22-targeted CAR T-cell therapy in DLBCL. In addition, the toxicity and safety have not yet been investigated. The present case report describes the first clinical experience in a patient with DLBCL treated with bispecific CD19/CD22-targeted CAR T cells.

Single-agent MOR208 therapy has previously demonstrated a good clinical performance in patients with relapse/refractory (r/r) DLBCL and r/r follicular lymphoma, including in patients refractory to rituximab (32). A number of published reports have identified CD19 as a promising target for CAR T-cell therapy for most B-cell malignancies, including ALL (33-35). The peak of CAR T-cell expansion was positively correlated with post-treatment efficiency and survival time, in accordance with previous studies (36,37). Due to the short duration and small number of CD19 CAR T-cell expansions, the patient in the present case report only reached partial remission for 3 months.

Although most B-cell ALL cases can be targeted by CD19 CAR T-cell therapy, 5-10% of relapses occur in patients with absent or low cell-surface expression of CD19 (38,39). In the PLAT-02 clinical trial, 93% of patients with r/r ALL exhibited a good response after anti-CD19 CAR T-cell therapy; however, 50% of patients relapsed at the end of the trial (40). Instead of CD19 expression, CD22 expression was identified in patients with relapsed leukemia. The Stanford University School of

Medicine and the National Cancer Institute have launched a phase I clinical trial of anti-CD22 CAR T-cell therapy in patients with relapsed B-cell ALL and obtained significant progress. This includes CD-22 CAR that can mediate similar potent antineoplastic effects as CD19, while the dual CD19/CD22 targeted immunotherapeutic plays an important role to overcome the resistance to immunotherapy via antigen loss (41). In November 2019, Tongji Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China) published a single case report of HBV reactivation after sequential treatment with CD19 and CD22 in a patient with DLBCL; after 2.5 months of CAR T-cell treatment, the tumor condition remained stable and superficial lymph nodes could not be detected (42). In the present report, the proportion of CAR T cells remained high throughout the two CAR T-cell infusions, and gradually restored the body temperature of the patient to normal, thereafter remaining stable. Therefore, the synergistic efficacy of CD19 and CD22 CAR T-cell infusion was observed in the present patient with refractory DLBCL.

The dose of CAR T-cell infusion is dependent on body weight. Due to the different weight of patients, the total number of CAR T cells returned to each individual is not the same, making it difficult to analyze all patients by a specific number of CAR T cells and therefore having to rely on CAR T-cell expansion trends and patient symptoms as a marker of treatment efficacy. In the present report, the CAR T cells began to expand on days 4-7, peaked on days 7-10 and began to decline on day 14. CRS is one of the most notable adverse reactions in the clinical application of CAR T-cell technology (43,44). If patients experience severe CRS reactions, such as high fever, >20% blood pressure reduction, dyspnea and grade 4 organ damage, the test should be automatically suspended, and restorative treatment should be initiated immediately. In the present report, due to the large release of cytokines caused by T-cell expansion, the patient developed manageable CRS symptoms, such as fever, hypotension, myalgia and respiratory failure. Currently, although there are drugs that can control CRS, complications remain a barrier to standard treatment. It has been demonstrated that the degree of CRS severity is associated with disease burden at the time of infusion, as a higher tumor burden results in more serious CRS (45), suggesting that in the case of low tumor burden, such as early disease, the risk and severity of CRS in patients undergoing CAR T-cell therapy may be markedly reduced. Therefore, in the present study, radiotherapy was used prior to CAR T-cell therapy, successfully decreasing the tumor burden of the patient. It has been suggested that CRS is over-activated by immune effector cells, resulting in excessive release of inflammatory cytokines, such as interleukin (IL)-1, IL-2, IL-6, IL-10, IL-15, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) (46). Therefore, the associations between the number of CAR T-cell expansions and IL-6, TNF-α and IFN-γ levels were analyzed in the present report. The analysis revealed that when the patient was infused with a single CD19 CAR T-cell infusion for the first time, the qPCR amplification curve and the fold-change curve of IL-6 seemed to have the same trend, suggesting that there was no association between qPCR and the changes in the three factors (IL-6, IFN-γ and TNF-α). However, upon further analysis of the three cytokines and qPCR via one-way ANOVA, no statistical differences were observed (P>0.2), indicating that it may be due to insufficient sample size. IL-6 is a cytokine known to cause side effects, such as fever, hypotension, myalgia and respiratory failure (46). In addition to T cells, macrophages are a typical cell subset that produce IL-6 (47). In a mouse model, the severity of CRS was reduced when monocytes depleted, which provided the major source of IL-1 and IL-6 or can block IL-6 receptor with tociizumab, suggesting that IL-6 inhibitors or anti-IL-6 receptor antibodies may reverse the syndrome (48). Recently, two independent trials from two research teams at the San Rafael Institute of Science and the Memorial Sloan Kettering Cancer Center (MSKCC) demonstrated that CRS is triggered by the inflammatory molecule IL-1 (46,47). Anakinra is an IL-1 inhibitor that can be combined with CAR T-cell therapy and is effective in managing CRS and neurotoxicity (49). In addition, researchers from the MSKCC have designed CAR T cells that secrete an IL-1 inhibitor to prevent CRS.

The present case report demonstrates the efficacy and safety of dual CD19/CD22-targeted CAR T-cell therapy in the treatment of DLBCL. The present results provide evidence that dual CAR T-cell therapy may be a promising option for the treatment of relapsed or refractory DLBCL in patients who do not benefit from single CD19-targeted CAR T-cell therapy. However, CRS is the major adverse effect of dual CD19/CD22-targeted CAR T-cell therapy and caution should be taken for patients receiving this treatment. However, this was only one case report on a single patient; therefore, the optimal dose of CAR T cells and the follow-up treatment remain to be clarified in well-designed studies with larger sample sizes.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

CH designed the experiments and wrote the article. HZ performed the histopathological and serum marker analyses; JH, RL and LW performed the experiments. NK and MZ were responsible for data collection and analysis, and checked the references. LL and JL assisted with the study design and made the figures of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present report was approved by the Ethics Committee of Drug Clinical Trials of The Fourth Hospital of Hebei Medical University (Shijiazhuang, China; approval no. 2016040).

Patient consent for publication

Written informed consent was obtained from the patient for publication of the present report and any accompanying images.

Competing interests

The authors declare that they have no competing interests.

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