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CASE REPORT

Novel Loop-Structure-Based CD19/CD22 Dual-Target CAR-T Therapy for High-Risk Diffuse Large B-Cell Lymphoma Presenting with Hemophagocytic Lymphohistiocytosis: A Case Report

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Objective: To investigate the efficacy and safety of novel loop-structure-based CD19/CD22 dual-target chimeric antigen receptor T-cell (CD19/CD22 BS LoopCAR-T) therapy in high-risk diffuse large B-cell lymphoma (DLBCL) presenting with hemophagocytic lymphohistiocytosis (HLH).

Methods: We analyzed the clinical data of a high-risk DLBCL patient presenting with HLH treated with CD19/CD22 BS LoopCAR-T at the Affiliated Nanshan Hospital of Shenzhen University in December 2023.

Results: The patient, a 59-year-old female, was diagnosed with myelodysplastic syndromes with multilineage dysplasia in October 2022. Following six cycles of azacitidine treatment, her bone marrow and hemogram returned to normal, and the disease was stable In August 2023, she presented with recurrent fever for over a month and was diagnosed with high-risk DLBCL stage IVB presenting with HLH. After receiving the HLH-1994 protocol followed by one cycle each of R-CHOP and R-DA-EPOCH regimens, the patient underwent infusion of CD19/CD22 BS LoopCAR-T cells at a dose of 1.73×10^8 cells. She experienced a rapid response, developing grade 1 cytokine release syndrome (CRS) and no immune effector cell-associated HLH-like syndrome (IEC-HS), and achieved disease stabilization following aggressive treatment. Bone marrow and peripheral blood flow cytometry at one and three months post-CAR-T therapy showed complete remission (CR). PET-CT at three months post-CAR-T therapy also indicated CR. The patient was followed up until April 2025, and the disease-free survival time after CAR-T treatment exceeded 16 months.

Conclusion: The novel CD19/CD22 BS LoopCAR-T therapy is safe and effective in treating high-risk DLBCL patients presenting with HLH.

Keywords: hemophagocytic lymphohistiocytosis, diffuse large B-cell lymphoma, CD19/CD22 dual target, chimeric antigen receptor T cells

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disease mediated by immune dysfunction. Diffuse large B-cell lymphoma (DLBCL) represents one of the most common types of non-Hodgkin lymphoma. However, its occurrence in conjunction with HLH is relatively rare, often presenting with complex clinical challenges. Despite standard first-line immunochemotherapy with R-CHOP, 30–40% of DLBCL patients experience relapse or refractory disease,[1] especially those in the intermediate-high-risk group, where salvage therapy outcomes are often suboptimal. Although CD19 CAR-T therapy has shown promising efficacy in relapsed/refractory DLBCL, about half of patients still relapse within one year after

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treatment. Considering that DLBCL exhibits a high expression of CD22, numerous clinical studies have indicated that CD19/CD22 dual-targeting CAR-T cell therapy may improve clinical outcomes in these patients.[2–6]

While CAR-T cell therapy is promising in treating B-cell malignancies, its toxicity profile should be acknowledged and managed. In addition to cytokine release syndrome (CRS) and immune-effector-cell-associated neurotoxicity syndrome (ICANS), other hematologic toxicities are clinically significant.[7] Immune effector cell-associated hemophagocytic lymphohistiocytosis like syndrome (IEC-HS), also known as CAR T-cell-associated HLH, is defined by the expert panel of the American Society for Transplantation and Cell Therapy as a pathological and biochemical hyperinflammatory syndrome that occurs independently of CRS and ICANS.[8] This condition is characterized by features of macrophage activation/HLH, and is associated with progression or new onset of cytopenias, hyperferritinemia, coagulopathy with hypofibrinogenemia, and/or transaminitis. They proposed that the distinction between CRS and IEC-HS is largely a difference in timing, IEC-HS often presents with a delayed onset, manifesting as CRS is resolved or resolving.[9] It is more prevalent after CD22 CAR-T therapy than after CD19 CAR-T therapy, for unknown mechanisms.[10,11] Given the similarities between IEC-HS, CRS, and primary HLH, its clinical recognition is essential.

Herein, we report a case of novel loop-structure-based dual-target CAR-T therapy (CD19/CD22 BS LoopCAR-T) against CD19 and CD22 in a high-risk DLBCL patient presenting with HLH. After receiving CD19/CD22 CAR-T therapy, the patient experienced grade I CRS and finally achieved sustained complete remission (CR). In conclusion, this study serves as a reference for CAR-T therapy in r/r DLBCL presenting with HLH and enhances the understanding of the distinctions between HLH and IEC-HS.

Case Presentation

General Information

The study was certified by the Ethics Committee with ethics number *XJS-2021034*. Informed consent was obtained for this case report and the patient has signed a written informed consent form. Written informed consent for publication of their details was obtained from the patient. The patient, a 59-year-old female, presented in October 2022 due to pancytopenia. Further bone marrow examination revealed approximately 2.7% myeloid blasts with a normal karyotype. The diagnosis was myelodysplastic syndromes with multilineage dysplasia (Intermediate-1 risk by IPSS, Intermediate risk by WPSS, High risk by IPSS-R). She completed six cycles of treatment with azacitidine 100 mg (75 mg/m^2) for 7 days. Follow-up bone marrow examination showed no abnormal myeloid blasts, and blood counts returned to normal. In August 2023, she began experiencing intermittent fever lasting over a month, with temperatures reaching up to 40°C, accompanied by fatigue and night sweats. On September 26th, peripheral blood flow cytometry revealed 13.6% abnormal mature B-cells expressing CD5, CD19, CD22, CD20, CD79a, CD79b, and Lambda. Upon admission, her blood work showed WBC 6.5×10^9/L (normal range: 3.5–9.5×10^9/L), Hb 66 g/L ↓ (normal range: 110–150 g/L), PLT 59×10^9/L ↓ (normal range: 125–350×10^9/L), CRP 73.16 mg/L ↑ (normal range: <5 mg/L); coagulation: FIB 1.11 g/L ↓ (normal range: 2–4 g/L), INR 1.15 (normal range: 0.85–1.15); ferritin: 4471.80 ng/mL ↑ (normal range:10–300 ng/mL); triglycerides 7.90 mmol/L ↑ (normal range: <1.7 mmol/L); liver enzymes and synthetic function: total bilirubin 9.1 μmol/L (normal range: 3–22 μmol/L), ALT 24 U/L (normal range: 0–35 U/L), AST 52 U/L ↑ (normal range: 14–36 U/L), albumin 29.8 g/L ↓ (normal range: 35–50 g/L); LDH 3705 U/L ↑ (normal range:120–246 U/L); β2-M 4.47 mg/L ↑ (normal range: 1–3 mg/L); soluble interleukin-2 receptor (sCD25) 22695 U/mL ↑ (normal range: 223–710 U/mL); NK cell activity: 14.13% ↓ (normal range≥15.11%); The qPCR tests for CMV and EBV returned negative results, metagenomic next-generation sequencing for pathogen detection in peripheral blood was negative, and autoimmune antibody screening was negative. CT scans revealed a left frontotemporal-parietal subdural hematoma and bilateral pulmonary multifocal infections. Fever persisted despite antimicrobial therapy.

Diagnosis and Treatment of HLH

The diagnosis of HLH conformed to the HLH-2004 criteria, meeting 7 out of 8 criteria: ① Persistent fever for 1 month; ② Splenomegaly (The CT scan suggested a spleen size of 8 costal units. Palpation of the spleen indicated that it was palpable

approximately 2 cm below the left subcostal margin).; ③ Cytopenia affecting two or more blood cell lineages; ④ Hypertriglyceridemia; ⑤ Decreased natural killer (NK) cell activity; ⑥ Ferritin levels ≥ 500ug/L; ⑦ Soluble interleukin-2 receptor (sCD25) levels ≥ 2400 IU/mL. Chemotherapy according to the HLH-1994 protocol was initiated on October 9, 2023, with the following specific doses: etoposide 100 mg (75 mg/m^2) biweekly and dexamethasone 10 mg/m^2/d for weeks 1–2, 5 mg/m^2/d for weeks 3–4, followed by gradual tapering of treatment.

Diagnosis and Treatment of DLBCL

On September 30, 2023, bone marrow flow cytometry revealed approximately 54.6% mature B lymphocytes, with the immunophenotype CD19+, CD20+, CD5+ (partial), CD10-, CD23-, partial FMC7+, CD79b+, partial CD200+, and restricted expression of Lambda light chain immunoglobulin on the cell membrane. (Figure 1A). Bone marrow biopsy showed diffuse proliferation of cells positive for CD20, CD79a, PAX-5, MUM-1, CD22, partially positive for CD43, approximately 70% positive for BCL-2, approximately 30% positive for C-myc, weakly positive for approximately 20% of cells for BCL-6, and negative for CD19, CD10, CD15, CD30, TdT, Lysozyme, CD117, CD34, and P53. Sporadic positivity for CD3 was observed, with approximately 80% of tumor cells positive for Ki67 in hotspot areas. No hemophagocytic cells were identified in the hemophagocytic smear. Reticular fiber staining indicated Grade 3, consistent with Diffuse Large B-cell Lymphoma of Non-GCB type (Figure 1B). On October 8, 2023, whole-body PET-CT revealed multiple enlarged lymph nodes throughout the body, the largest measuring approximately 12×11mm with a SUVmax of 8.8. Splenomegaly with diffuse metabolic increase (SUVmax 8.5) and uneven metabolic increase in the whole-body bone marrow (SUVmax 6.3) were observed. Additionally, involvement of the subcutaneous fat layer of the right temporal region, posterior wall of the nasopharynx, bilateral palatine tonsils, and nasal cavity, suggestive of lymphoma infiltration, was noted. (Figure 1C). Based on the above findings, the patient was diagnosed with DLBCL (Non-GCB type, stage IVB, IPI 4, high risk). On October 13, 2023, the patient underwent R-CHOP regimen chemotherapy, with the following specific doses: rituximab 500 mg (375 mg/m^2) on day 0, cyclophosphamide 700 mg (500 mg/m^2) on day 1, liposomal doxorubicin 20 mg (15 mg/m^2) on day 1, vincristine 1.4mg (1.0 mg/m^2) on day 1, and dexamethasone 10 mg/m^2/d for 5 days (dexamethasone dosage according to the above-mentioned HLH-1994 protocol). The second cycle of chemotherapy was administered on November 5, 2023, following the R-DA-EPOCH regimen with specific doses: rituximab 500 mg (375 mg/m^2) on day 0, etoposide 70 mg (50 mg/m^2) on days 1–3, vincristine 0.5 mg (0.36 mg/m^2) on days 1–3, liposomal doxorubicin 10 mg (7.2 mg/m^2) on days 1–3, cyclophosphamide 700 mg (500 mg/m^2) on day 4, and dexamethasone 12 mg (9 mg/m^2) on days 1–5. Bone marrow minimal residual disease (MRD) assessment after two cycles of chemotherapy showed negativity. PET-CT revealed suppressed activity of lymphoma lesions throughout the body (Deauville score 3–4). (Figure 1D). Additionally, the patient's HLH condition had been effectively managed.

Preparation of CD19/CD22 BS LoopCAR-T Cells

A retroviral vector was employed to encode the gene sequence for the CD19/CD22 BS LoopCAR-T cells. As depicted in Figure 2A, the novel CD19/CD22 BS LoopCAR-T comprises a CD19 single-chain variable fragment (scFv), a CD22 nanobody, a CD8 hinge region, a CD8 transmembrane region, a 4–1BB costimulatory domain and a CD3ζ signaling region. The FMC63 scFv and Nb25 nanobody were connected by β-stranded linkers.[12]

For retrovirus packaging, a two-step method involving the sequential use of Phoenix-ECO cells and PG13 cells was employed to produce stable PG13 RV producer cell lines.[13] Retrovirus were harvested and used for the transduction of activated human T cells. Autologous peripheral blood mononuclear cells (PBMCs) were isolated from the patient's whole blood. The freshly extracted PBMCs were activated with OKT3 48h prior to transduction, and the plates were coated with RetroNectin the day before. Following T-cell activation, the retrovirus and activated T cells were added to the coated plates, and T cell transduction was performed through two centrifugations. The transduction efficiency of CAR-T cells was analyzed by flow cytometry (Figure 2B). IL-2 (final concentration 500U/mL) was added throughout in vitro culture. Once the cells recovered and the expanded sufficiently, they were infused back into the patient.

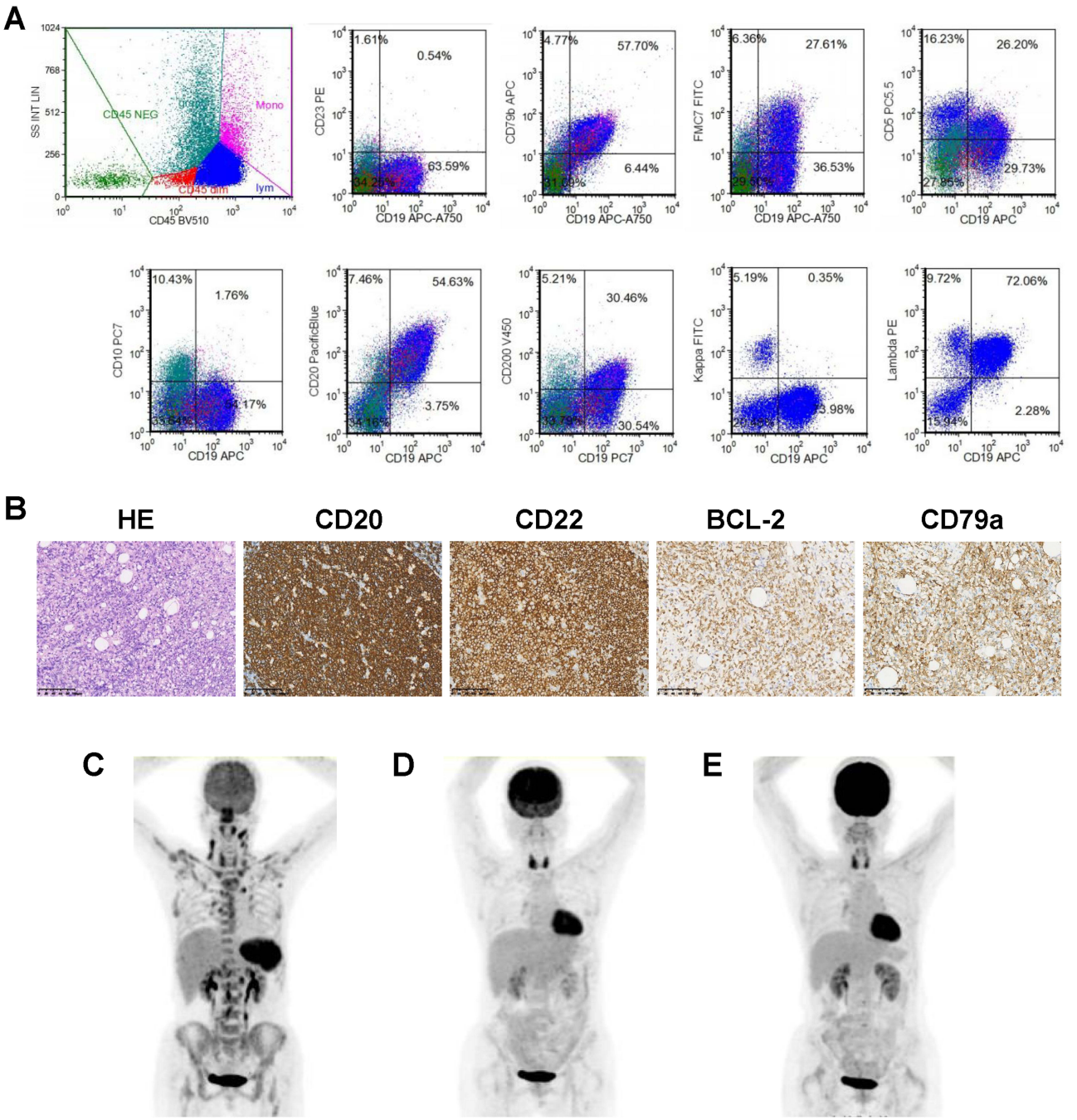

Figure 1 Hematopathology results of DLBCL patients presenting with HLH. (**A**) Bone marrow flow cytometry images from September 30, 2023, revealed approximately 54.6% mature B lymphocytes, with the immunophenotype CD19+, CD20+, CD5+ (partial), CD10-, CD23-, partial FMC7+, CD79b+, partial CD200+, and restricted expression of Lambda light chain immunoglobulin on the cell membrane. (**B**) Bone marrow pathology from October 8, 2023. The HE staining image shows patchy or focal proliferation of cells in the bone marrow, with medium-sized cells and pale nuclei. Some cells have a central nucleolus and sparse cytoplasm. The remaining images are immunohistochemical staining slides, show diffuse proliferation of cells positive for CD20, CD22, BCL-2 and CD79a. Scale = 100 μm. Shown are representative images taken at 20× resolution. (**C**) PET-CT image at the initial diagnosis of lymphoma on October 8, 2023. (**D**) PET-CT image after completion of 1 cycle of R-CHOP and 1 cycle of R-DA-EPOCH chemotherapy, prior to CAR-T treatment on December 2, 2023 (Deauville score: 3–4). (**E**) PET-CT image 3 months after CAR-T infusion on March 16, 2024 (Deauville score: 1).

In vitro Cytotoxicity and Cytokine Release of CD19/CD22 BS LoopCAR-T Cells

The CD19/CD22 BS LoopCAR-T cells were validated in vitro. CD19 CAR-T, CD22 CAR-T or CD19/CD22 BS LoopCAR-T cells were co-incubated with engineered CFSE-labeled ($CFSE^+$) K562 cell lines (K562-CD19, K562-CD22 or K562) at different E: T ratio for 24 hours. After incubation, the dead cells were excluded by 7-AAD staining.

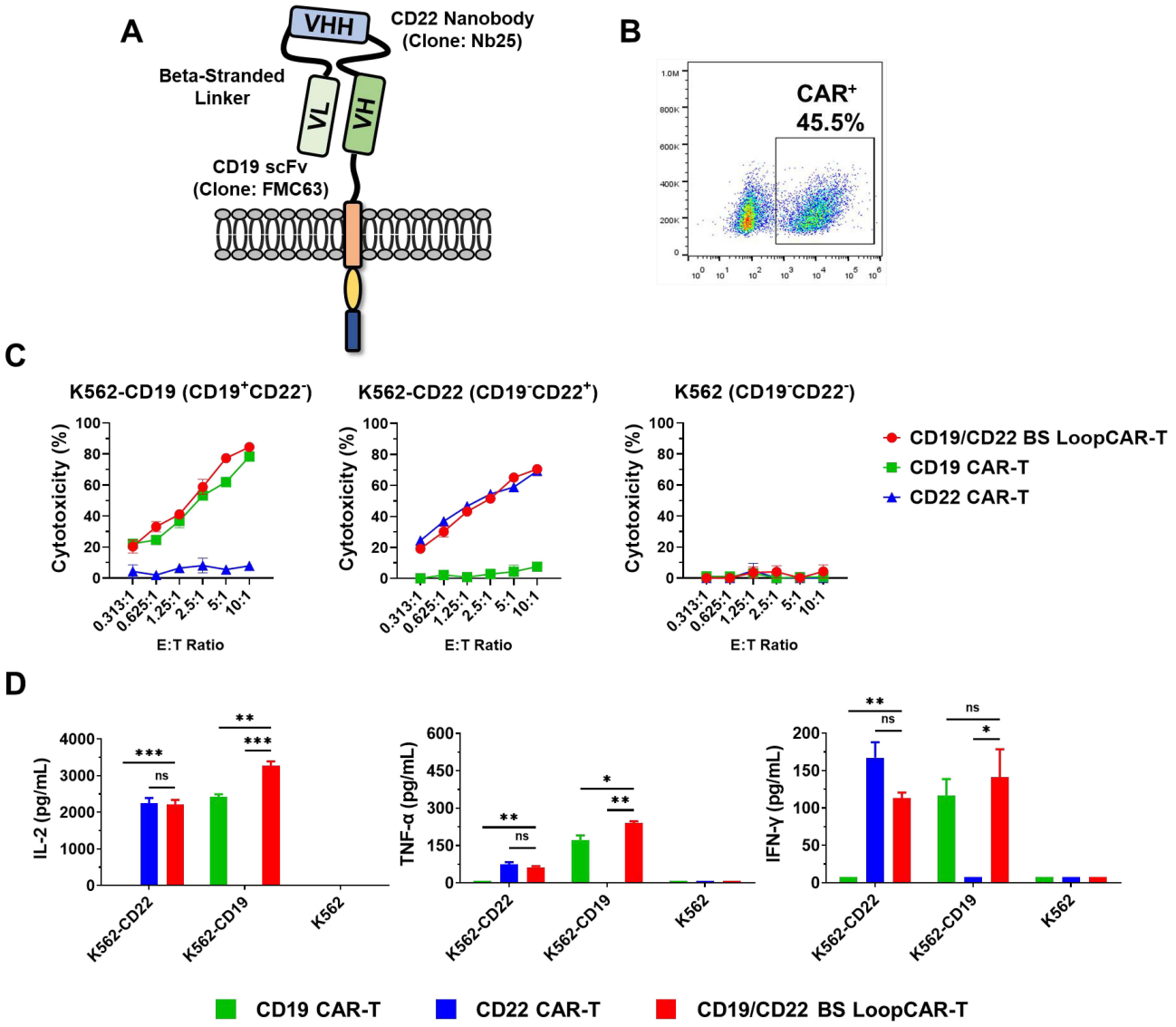

Figure 2 In vitro functional validation of CD19/CD22 BS LoopCAR-T cells. (**A**) Schematic diagram of CD19/CD22 BS LoopCAR-T cell structure. (**B**) Expression levels of CD19/CD22 BS LoopCAR-T cells. (**C**) Co-culture of CD19 CAR-T, CD22 CAR-T, or CD19/CD22 BS LoopCAR-T cells with K562-CD19, K562-CD22, or wild-type K562 cells at varying E:T ratios for 24 hours, followed by quantification of tumor cell killing percentage using flow cytometry. (**D**) Co-culture of CD19 CAR-T, CD22 CAR-T, or CD19/CD22 BS LoopCAR-T cells with K562-CD19, K562-CD22, or wild-type K562 cells at a 3:1 E:T ratio for 24 hours, followed by detection of IL2, TNF-α, and IFN-γ levels in the culture supernatant using ELISA.

Notes: Two-way ANOVA multiple comparisons in Dunnett correction were used to assess the significance. Error bars represent means ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001; ns indicates not significant (P ≥ 0.05).

CAR-T-cell-mediated cytotoxicity was evaluated using a flow cytometer by counting the residual live target cells (identified as 7-AAD$^-$CFSE$^+$). As shown in Figure 2C, CD19/CD22 BS LoopCAR-T exhibited comparable cytotoxicity to CD19 or CD22 single-target CAR-T cells in vitro, without nonspecific killing activity. Additionally, the different CAR-T cells were co-incubated with tumor cells (K562-CD19, K562-CD22 or K562) at an E:T ratio of 1:3 for 24 hours, and the culture supernatant was collected. ELISA cytokine kits (Thermo Fisher Scientific) were used to measure IL2, TNF-α and IFN-γ levels. Figure 2D demonstrates that CD19/CD22 BS LoopCAR-T cells induced significant cytokine release when interacting with either K562-CD19 or K562-CD22 cells, with release levels similar to those of CD19 or CD22 single-target CAR-T cells. These results indicate that CD19/CD22 BS LoopCAR-T cells not only offer the advantage of dual targeting but also maintain the single targeting capabilities of CD19 and CD22.

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CAR-T Therapy Process

PBMCs were collected on December 8, 2023. On December 9, 2023, the patient received chemotherapy utilizing the BR regimen as bridging therapy prior to CAR-T therapy: Rituximab 500 mg (375 mg/m^2) on day 0 and Bendamustine 100 mg (75 mg/m^2) on days 1–2. According to the ESMO guidelines, the patient exhibited a high-risk IPI score, involvement of multiple extranodal sites, and elevated LDH levels, indicating a high risk for central nervous system (CNS) recurrence and necessitating CNS prophylaxis. On December 11, 2023, a lumbar puncture with intrathecal injection of Methotrexate 10 mg, Cytarabine 50 mg, and Dexamethasone 5 mg was performed, revealing no tumor cells in the cerebrospinal fluid. On December 14, 2023, the patient received FC preconditioning: Fludarabine 30 mg (25 mg/m^2) and Cyclophosphamide 300 mg (250 mg/m^2) on days 1–3. On December 19, 2023, the patient was infused with 50mL CD19/CD22 BS LoopCAR-T cells (total number of CAR+ cells: 1.73×10^8cells) (Figure 3A and B). Peripheral-blood CAR levels were regularly monitored during this period (Figure 3C). Following the infusion, there was a notable decrease in white blood cells and platelets (Figure 4A). These were managed symptomatically with rhTPO for thrombocytopenia and intermittent granulocyte colony-stimulating factor for leukopenia, leading to gradual recovery of blood counts. As shown in Figure 4A, on day 30, the complete blood count, including leukocytes, neutrophils, and lymphocytes, has recovered and gradually returned to near-normal levels. On day 17 post-infusion, the patient developed a fever of 37.5°C, with normal blood pressure and oxygen saturation, diagnosed as grade I CRS. Her temperature normalized without specific treatment (Figure 4B and C). Several studies have shown that combining CAR-T therapy with BTK inhibitors can enhance the efficacy of CAR-T treatment for malignant tumors.[14,15] Therefore, starting one

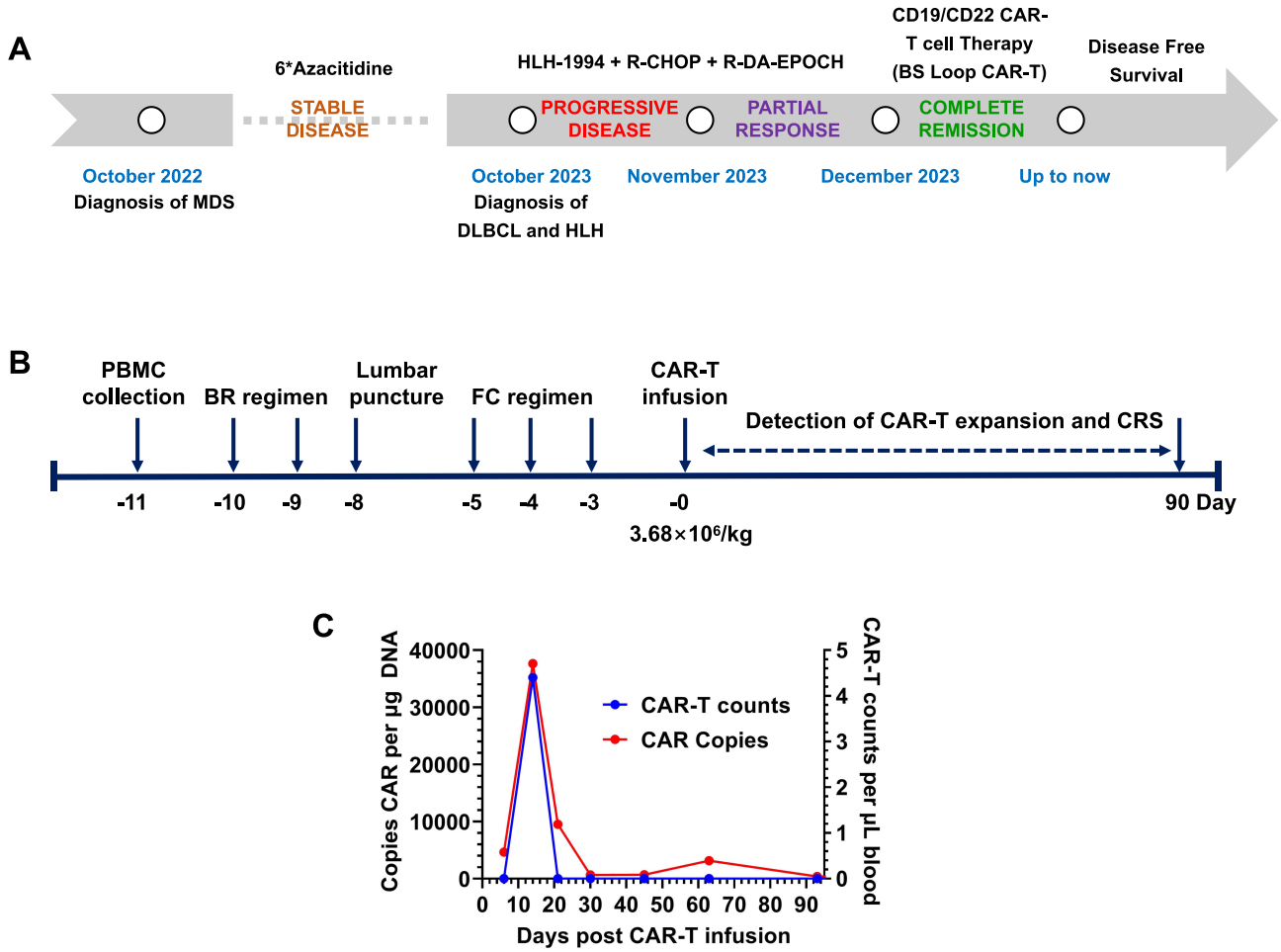

Figure 3 Patient course and CAR-T treatment process. (**A**) Timeline of patient's past treatment events. (**B**) Timeline of CAR-T treatment process. (**C**) Changes in peripheral blood CAR levels.

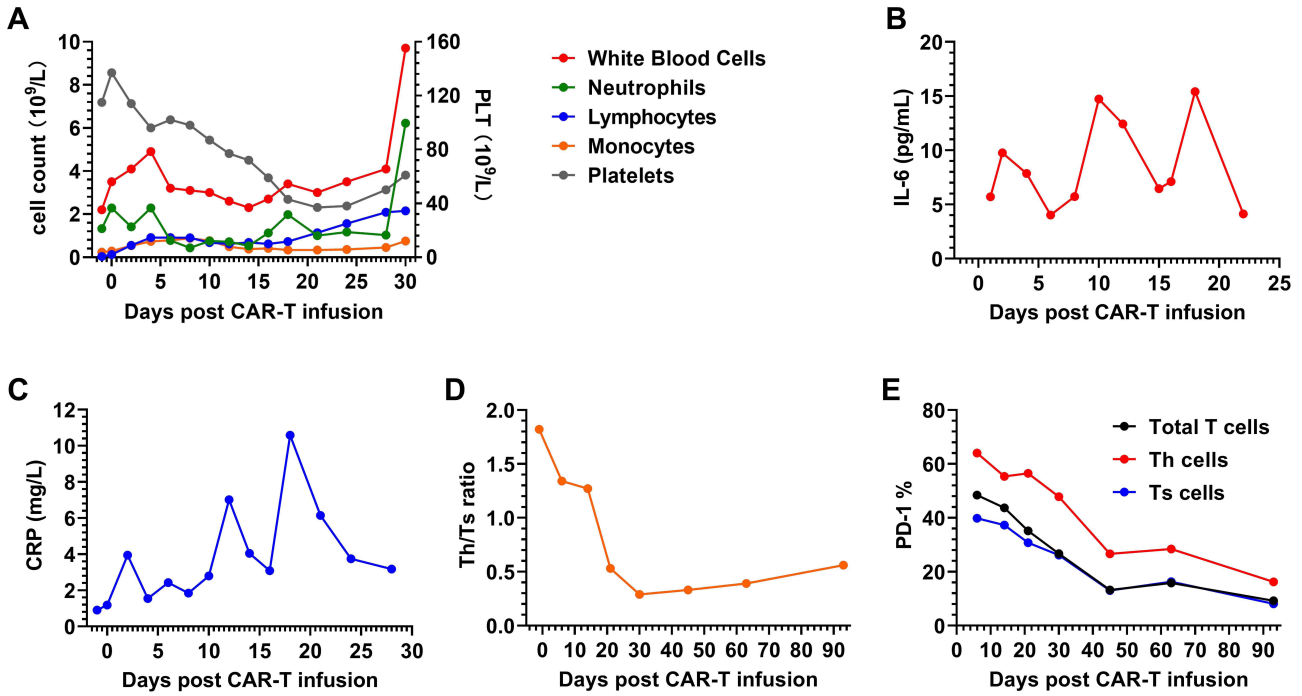

Figure 4 Changes in parameters before and after CAR-T therapy. (**A**) Changes in peripheral blood white blood cells, neutrophils, lymphocytes, monocytes and platelets (PLT) after CAR-T infusion. From days 5 to 25, the various parameters were in a declining phase. On day 30, re-evaluation revealed that white blood cells, neutrophils, and lymphocytes had recovered and were approaching normal levels. (**B**) Changes in IL-6 levels after CAR-T infusion. (**C**) Changes in CRP levels after CAR-T infusion. (**D**) Changes in the ratio of helper T lymphocyte (Th) to suppressor T lymphocyte (Ts) after CAR-T infusion. Helper T cells (Th) were identified as $CD45^+CD3^+CD4^+CD8^-$. Suppressor T cells (Ts) were identified as $CD45^+CD3^+CD8^+CD4^-$. (**E**) Changes in the level of PD-1 expression on T lymphocytes after CAR-T infusion.

month after the CAR-T infusion, the patient was maintained on oral therapy with the BTK inhibitor orelabrutinib 150 mg once daily to enhance the efficacy of CAR-T therapy. Additionally, research has shown that maintenance therapy with PD-1 inhibitors after CAR-T treatment can achieve better response rates and survival outcomes.[16] There are also reports indicating that the combination of BTK inhibitors and PD-1 inhibitors can synergistically enhance CAR-T efficacy.[17] Therefore, starting 4 months after infusion, the patient received maintenance therapy consisting of the PD-1 inhibitor toripalimab at a dose of 160mg (3mg/kg) every 3 weeks plus BTK inhibitor orelabrutinib at a dose of 150 mg daily. In the presence of the inhibitor, the Th/Ts ratio and PD-1% of peripheral blood T lymphocyte subsets were maintained at a low level, suggesting the long-lasting efficacy of CAR-T (Figure 4D and E).

Follow-Up and Outcomes

After whole-body PET-CT scan conducted three months after CAR-T therapy showed no increased metabolic activity, with a Deauville score of 1, indicating complete remission (Figure 1E). Bone marrow and peripheral blood flow cytometry at the first and third months post-infusion were both negative. There were no abnormalities in hemophagocytic markers, and the patient did not experience fever, splenomegaly, or abnormal blood counts. Follow-up until April 2025 revealed a disease-free survival of more than 16 months after CAR-T therapy.

Discussion

Lymphoma-associated hemophagocytic lymphohistiocytosis (LA-HLH) is a secondary HLH characterized by rapid progression and high mortality. Due to its nonspecific symptoms and significant overlap with lymphoma, LA-HLH is often misdiagnosed or underdiagnosed.[18] Early recognition, diagnosis, and timely intervention are crucial for improving LA-HLH prognosis. Most LA-HLH cases are attributed to T or NK cell lymphomas/leukemias, while secondary HLH from B cell lymphomas is relatively rare. Studies indicate that patients with B cell LA-HLH tend to be older, but clinical manifestations and laboratory findings are similar to those of T or NK cell LA-HLH.[19] The diagnostic criteria for HLH refer to the HLH-2004 standard, but its applicability to LA-HLH remains controversial.[20]

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Immune effector cell-associated hemophagocytic syndrome (IEC-HS) is a rare complication of CAR-T cell therapy, classified as secondary hemophagocytic lymphohistiocytosis (HLH).[9] This condition is also referred to as CAR-T-related HLH (carHLH). IEC-HS represents a life-threatening form of secondary HLH, characterized by markedly elevated serum ferritin levels, coagulopathy, cytopenia, hypertriglyceridemia, impaired lung function, and renal and/or hepatic dysfunction.[9] It is typically characterized by hemophagocytosis and other features resembling HLH following the resolution of acute CRS. With the increasing use of CAR-T therapy in the treatment of hematological malignancies, the distinctive phenomenon of IEC-HS has attracted increasing scholarly attention.[7] Although infrequent, reported incidences range from 1% to 3% and up to 35%, influenced by variations in underlying diseases, patient populations, CAR-T products, and other factors.[10] IEC-HS has been reported in patients treated with tisa-cel for B-ALL, axi-cel for LBCL, and ide-cel or cilta-cel for multiple myeloma.[21–23] Furthermore, rare fatal cases (≤1%) have been reported with axi-cel, ide-cel, and cilta-cel.[21,22,24] IEC-HS rarely manifests in the absence of CRS, suggesting that the two syndromes may share a common underlying mechanism; however, the specific triggers remain unclear.[10] In addition, it has been suggested that CD22 CAR-T therapy may have a higher incidence of IEC-HS than CD19 CAR-T therapy.[10,11] This event rate has not been reported for CD19/CD22 CAR-T therapy. In this case, only grade I CRS was observed, and no IEC-HS was noted. However, further investigation is needed to elucidate the mechanism underlying IEC-HS and to determine its potential association with CD22 targets.

While CD19 CAR-T therapy has proven to be an effective treatment for B-cell lymphoma,[25,26] antigen escape-mediated relapse remains a major challenge affecting its prognosis.[27] Several studies have shown that dual-target CD19/CD22 CAR-T cells can prevent antigen escape, and thereby improving clinical response rates.[2–6,28–30] Wei Guoqing et al reported the use of CD19/CD22 CAR-T cells in treating 16 patients with relapsed/refractory aggressive B-cell lymphoma,[5] achieving an objective response rate (ORR) of 87.5% and a CR rate of 62.5%. The 2-year overall survival and progression-free survival (PFS) rates were 77.3% and 40.2%, respectively, with manageable adverse reactions. Similarly, Zhang Ying et al treated 32 patients with relapsed/refractory B-cell non-Hodgkin lymphoma,[31] achieving an ORR of 79.3% and a CR rate of 34.5%. The 1-year PFS and OS rates were 40.0% and 63.3%, respectively. Spiegel et al reported a Phase I study involving 22 patients with relapsed/refractory B-cell malignancies treated with CD19/CD22 Loop CAR-T cell therapy,[3] achieving an ORR of 62% and a CR rate of 29%. A meta-analysis indicated that dual-target CD19/CD22 CAR-T cell therapy is more effective than single-target CAR-T cell therapy (CD19 or CD22 antigen) in treating relapsed/refractory B-cell malignancies.[32] Our preclinical studies suggested that the CD19/CD22 BS LoopCAR-T, which utilizes a highly hydrophilic flexible β-stranded linkers to connect the CD19 scFv and the CD22 nanobody in a loop structure, demonstrated significantly better performance compared to single-target and tandem dual-target CAR-T therapies. This includes superior results compare to the CD19/CD22 Loop CAR-T reported by Spiegel, showing the potential to further enhance dual-target recognition and anti-tumor activity.[12]

DLBCL presenting with HLH is rare and presents significant clinical challenges. In this case, the patient presented with HLH as an initial manifestation of elderly high-risk diffuse large B-cell lymphoma (DLBCL), involving bone marrow and developing into a leukemic state, which is very rare in clinical practice. The condition was critically severe with a poor prognosis, and the patient's tolerance to hematopoietic stem cell transplantation was low. The patient achieved rapid disease remission after CD19/CD22 BS LoopCAR-T treatment, with only transient grade I CRS and no IEC-HS. At 1- and 3-months post-treatment, bone marrow pathology, peripheral blood immunophenotyping, and whole-body PET/CT all showed CR. As of April 2025, the patient has been in complete remission for over 16 months following CAR-T therapy. Notably, this case may represent the first report of CD19/CD22 CAR-T therapy for high-risk DLBCL presenting with HLH achieving good results. We will continue to monitor the efficacy of this case long-term and plan to initiate larger clinical trials to benefit more patients.

Data Sharing Statement

The datasets generated during or analyzed during the current study are available from the corresponding author Liqiong Liu on reasonable request.

Acknowledgments

Chinese patent "Construction of A Novel Bispecific Chimeric Antigen Receptor and Uses Thereof," with the application number CN202210126836.0, is related to the present work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Wilson WH, Wright GW, Huang DW, et al. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell*. 2021;39(12):1643–1653.e3. doi:10.1016/j.ccell.2021.10.006
2. Dai H, Wu Z, Jia H, et al. Bispecific CAR-T cells targeting both CD19 and CD22 for therapy of adults with relapsed or refractory B cell acute lymphoblastic leukemia. *J Hematol Oncol*. 2020;13(1):30. doi:10.1186/s13045-020-00856-8
3. Spiegel JY, Patel S, Muffly L, et al. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. *Nat Med*. 2021;27(8):1419–1431. doi:10.1038/s41591-021-01436-0
4. Cordoba S, Onuoha S, Thomas S, et al. CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial. *Nat Med*. 2021;27(10):1797–1805. doi:10.1038/s41591-021-01497-1
5. Wei G, Zhang Y, Zhao H, et al. CD19/CD22 dual-targeted CAR T-cell therapy for relapsed/refractory aggressive B-cell lymphoma: a safety and efficacy study. *Cancer Immunol Res*. 2021;9(9):1061–1070. doi:10.1158/2326-6066.CIR-20-0675
6. Jia H, Wang Z, Wang Y, et al. Haploidentical CD19/CD22 bispecific CAR-T cells induced MRD-negative remission in a patient with relapsed and refractory adult B-ALL after haploidentical hematopoietic stem cell transplantation. *J Hematol Oncol*. 2019;12(1):57. doi:10.1186/s13045-019-0741-6
7. Rejeski K, Subklewe M, Locke FL. Recognizing, defining, and managing CAR-T hematologic toxicities. *Hematology Am Soc Hematol Educ Program*. 2023;2023(1):198–208. doi:10.1182/hematology.2023000472
8. Hines MR, Knight TE, McNerney KO, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant Cell Ther*. 2023;29(7). doi:10.1016/j.jtct.2023.03.006
9. Brudno JN, Kochenderfer JN. Current understanding and management of CAR T cell-associated toxicities. *Nat Rev Clin Oncol*. 2024;21(7):501–521. doi:10.1038/s41571-024-00903-0
10. Shah NN, Highfill SL, Shalabi H, et al. CD4/CD8 T-cell selection affects chimeric antigen receptor (CAR) T-cell potency and toxicity: updated results from a phase I anti-CD22 CAR T-cell trial. *J Clin Oncol*. 2020;38(17):1938–1950. doi:10.1200/JCO.19.03279
11. Lichtenstein DA, Schischlik F, Shao L, et al. Characterization of HLH-like manifestations as a CRS variant in patients receiving CD22 CAR T cells. *Blood*. 2021;138(24):2469–2484. doi:10.1182/blood.2021011898
12. Zhao L, Li S, Wei X, et al. A novel loop-structure-based bispecific CAR that targets CD19 and CD22 with enhanced therapeutic efficacy against B-cell malignancies. *Protein Cell*. 2024. doi:10.1093/procel/pwae034
13. Zhong N, Ma Q, Gong S, et al. Rapid response in relapsed follicular lymphoma to novel anti-CD19 CAR-T therapy with pseudo-progression and cytomegalovirus infection: a case report. *Int Immunopharmacol*. 2024;134:112174. doi:10.1016/j.intimp.2024.112174
14. Luo W, Li C, Wu J, et al. Bruton tyrosine kinase inhibitors preserve anti-CD19 chimeric antigen receptor T-cell functionality and reprogram tumor micro-environment in B-cell lymphoma. *Cytotherapy*. 2023;25(7):739–749. doi:10.1016/j.jcyt.2023.03.005
15. Zhou D, Zhu X, Xiao Y. CAR-T cell combination therapies in hematologic malignancies. *Exp Hematol Oncol*. 2024;13(1):69. doi:10.1186/s40164-024-00536-0
16. Xin X, Zhu X, Yang Y, et al. Efficacy of programmed cell death 1 inhibitor maintenance after chimeric antigen receptor T cells in patients with relapsed/refractory B-cell non-Hodgkin-lymphoma. *Cell Oncol*. 2024. doi:10.1007/s13402-024-00940-y
17. Zhang W, Huang C, Liu R, et al. Case report: CD19-directed CAR-T cell therapy combined with BTK inhibitor and PD-1 antibody against secondary central nervous system lymphoma. *Front Immunol*. 2022;13:983934. doi:10.3389/fimmu.2022.983934
18. Knauft J, Schenk T, Ernst T, et al. Lymphoma-associated hemophagocytic lymphohistiocytosis (LA-HLH): a scoping review unveils clinical and diagnostic patterns of a lymphoma subgroup with poor prognosis. *Leukemia*. 2024;38(2):235–249. doi:10.1038/s41375-024-02135-8
19. La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood*. 2019;133(23):2465–2477. doi:10.1182/blood.2018894618
20. Henter J-I, Horne A, Aricó M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124–131.
21. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531–2544. doi:10.1056/NEJMoa1707447
22. Hansen DK, Sidana S, Peres LC, et al. Idecabtagene vicleucel for relapsed/refractory multiple myeloma: real-world experience from the myeloma CAR T consortium. *J Clin Oncol*. 2023;41(11):2087–2097. doi:10.1200/JCO.22.01365
23. 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(5):439–448. doi:10.1056/NEJMoa1709866

24. Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet*. 2021;398(10297):314–324. doi:10.1016/S0140-6736(21)00933-8
25. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol*. 2023;20(6):359–371. doi:10.1038/s41571-023-00754-1
26. Guo Z, He M, Liu N, et al. In vitro functional validation of anti-CD19 chimeric antigen receptor T cells expressing lysine-specific demethylase 1 short hairpin RNA for the treatment of diffuse large B cell lymphoma. *Front Immunol*. 2024;15:1521778. doi:10.3389/fimmu.2024.1521778
27. Xu -P-P, Huo Y-J, Zhao W-L. All roads lead to targeted diffuse large B-cell lymphoma approaches. *Cancer Cell*. 2022;40(2):131–133. doi:10.1016/j.ccell.2022.01.013
28. Zhao L, Li S, Wei X, et al. A novel CD19/CD22/CD3 trispecific antibody enhances therapeutic efficacy and overcomes immune escape against B-ALL. *Blood*. 2022;140(16):1790–1802. doi:10.1182/blood.2022016243
29. Liu D, Qi X, Wei X, et al. A novel Her2/VEGFR2/CD3 trispecific antibody with an optimal structural design showed improved T-cell-redirecting antitumor efficacy. *Theranostics*. 2022;12(18):7788–7803. doi:10.7150/thno.75037
30. Cao YJ, Wang X, Wang Z, et al. Switchable CAR-T cells outperformed traditional antibody-redirected therapeutics targeting breast cancers. *ACS Synth Biol*. 2021;10(5):1176–1183. doi:10.1021/acssynbio.1c00007
31. Zhang Y, Li J, Lou X, et al. A prospective investigation of bispecific CD19/22 CAR T cell therapy in patients with relapsed or refractory B cell non-Hodgkin lymphoma. *Front Oncol*. 2021;11:664421. doi:10.3389/fonc.2021.664421
32. Nguyen TT, Thanh Nhu N, Chen C-L, Lin C-F. Effectiveness and safety of CD22 and CD19 dual-targeting chimeric antigen receptor T-cell therapy in patients with relapsed or refractory B-cell malignancies: a meta-analysis. *Cancer Med*. 2023;12(18):18767–18785. doi:10.1002/cam4.6497