haematologica
Journal of the Ferrata Storti Foundation

CD19/CD22-targeted CAR T cells in autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation

by Liu Yang, Xinyu Wan, Chengjuan Luo, Xiaohang Huang, Meng Su, Xia Qin, Jing Zhang, Kang An, Juan Qian, Tianyi Wang, Wenhua Shi, Jing Yang, Rujun Jia, Chengming Fei, Xiang Wang, Yanjing Tang, Jing Chen and Benshang Li

Received: November 4, 2025.
Accepted: March 9, 2026.

Citation: Liu Yang, Xinyu Wan, Chengjuan Luo, Xiaohang Huang, Meng Su, Xia Qin, Jing Zhang, Kang An, Juan Qian, Tianyi Wang, Wenhua Shi, Jing Yang, Rujun Jia, Chengming Fei, Xiang Wang, Yanjing Tang, Jing Chen and Benshang Li. CD19/CD22-targeted CAR T cells in autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation
Haematologica. 2026 Mar 19. doi: 10.3324/haematol.2025.287983 [Epub ahead of print]

CD19/CD22-targeted CAR T cells in autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation

Liu Yang[1]*, Xinyu Wan[1]*,Chengjuan Luo[1], Xiaohang Huang[1], Meng Su[1], Xia Qin[1], Jing Zhang[1], Kang An[1], Juan Qian[1], Tianyi Wang[1], Wenhua Shi[1], Jing Yang[1], Rujun Jia[1], Chengming Fei[1], Xiang Wang[1], Yanjing Tang[1#], Jing Chen[1#], and Benshang Li[1#].

* contributed equally to this work as first authors.

# contributed equally to this work as senior authors.

1

Department of Hematology/Oncology, National Health Committee Key Laboratory of Pediatric Hematology & Oncology, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Running heads: CAR-T therapy in AIHA

Correspondence to: Benshang Li,

Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, 200127, China.

Tel.: 86-138-160-09925, Email: libenshang@scmc.com.cn

Data Sharing Statement:

For original data, please contact libenshang@scmc.com.cn

Disclosures: None.

Clinical trail information: Chinese Clinical Trial Registry: ChiCTR2000032211

Author Contributions:

B. L. and J.C. designed the study and did the interpretation; B.L. and Y.T. provided financial and administrative support. C.L., X.H., M.S., X.Q., J.Z., K.A., J.Q., and Y.T.

were responsible for clinical diagnosis and treatment; L.Y. and Xinyu Wan performed clinical data analysis and wrote the manuscript; L.Y., T.W., W.S., J.Y., R.J., and C.F. assisted in the clinical management; Xinyu Wan and Xiang Wang helped with laboratory examinations analysis. All authors read and approved the final manuscript. No other potential conflict of interest relevant to this article was reported.

Funding:

Supported by the National Natural Science Foundation of China (grant No. 81670174, B.L.; grant No. 81670136, J.T. And J.C.)

Autoimmune hemolytic anemia (AIHA) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents a rare but severe immune complication, frequently refractory to standard immunosuppressive therapy and associated with high mortality[1,2]. Given the pivotal function of autoreactive B cells in the development of post-transplant autoimmunity, B-cell-directed chimeric antigen receptor (CAR) T-cell therapy offers a potential therapeutic modality to eliminate pathogenic B-cell clones and attain a long-term immune reset[3,4]. Herein, we report three pediatric cases of refractory post-HSCT AIHA that were successfully managed with the coadministration of CD19- and CD22-targeted CAR T cells, resulting in complete and sustained remissions with minimal toxicity. To the best of our knowledge, this represents the first documented evidence demonstrating the therapeutic potential of dual-targeted CAR T-cell therapy in post-transplant AIHA.

AIHA affects approximately 2-6% of pediatric HSCT recipients, typically manifesting within the first year post-transplantation[5]. The pathogenesis of this condition involves the production of anti-erythrocyte antibodies by donor-derived autoreactive B cells, often accompanied by complement fixation. Standard corticosteroid therapy yields responses in fewer than 50% of patients; while second-line agents like rituximab improve initial response rates, durable remissions occur in only about one-third of cases[6]. This underscores the limited efficacy and significant toxicity of current standard immunosuppressive regimens. Mortality is typically associated with refractory hemolysis, infection, or disease relapse. As the immune system following transplantation remains in a state of immaturity (characterised by delayed recovery of T-regulatory cells and impaired self-tolerance), new strategies are required to eliminate autoreactive B cells while minimizing additional immunosuppression[7]. CAR T cells targeting CD19 or CD22 have demonstrated high efficacy in B-cell

malignancies and, more recently, in autoimmune diseases like systemic lupus erythematosus, via profound B-cell depletion and subsequent immune reconstitution[4,8]. These outcomes provide the rationale for investigating CAR T-cell therapy in the context of post-HSCT AIHA.

Between April 2021 and September 2023, three children with refractory or relapsed B-lineage acute lymphoblastic leukemia (B-ALL) complicated by AIHA after allo-HSCT were treated at Shanghai Children's Medical Center with a single infusion of coadministered CD19- and CD22-targeted CAR T cells. The last follow-up was conducted on June 30, 2024. Before infusion, two patients (Pt.1 and Pt.2) had concurrent leukemia relapse, while the remaining case (Pt.3) failed both first- and second-line AIHA treatments (**Figure S1**). All fulfilled the diagnostic criteria[2] for warm AIHA, including a positive direct antiglobulin test (DAT) and laboratory evidence of hemolysis. The median interval from transplantation to AIHA onset was 6.6 months (range, 5.1-10.6). Notably, all three patients exhibited complete donor chimerism, full blood-type conversion, and no signs of graft-versus-host disease (GVHD) at AIHA diagnosis. Viral screenings detected human parvovirus B19 in Pt. 1 and Torque teno virus (TTV) in Pt. 3 (**Supplemental Table 1**). Pt.1 and Pt.2 developed AIHA in the context of post-transplant leukemia relapse, whereas Pt.3 exhibited isolated, refractory AIHA after failure of both steroid and rituximab-based regimens. Informed consent for compassionate CAR T-cell use was obtained from the patients' guardians, and the study was approved by the institutional ethics committee in accordance with the Declaration of Helsinki (ChiCTR2000032211).

CAR T cells were manufactured from autologous peripheral blood T cells transduced with CD19- or CD22-directed lentiviral vectors containing a 4-1BB costimulatory domain and CD3ζ signaling domain, as detailed in our prior report[9]. The anti-CD19

and anti-CD22 scFvs were derived from the FMC63 and m5/44 clones, respectively. The two products were expanded separately for 5-7 days and subsequently infused together at a 1:1 ratio, following lymphodepleting chemotherapy with fludarabine (40 mg/m2, days -4 to -2) and cyclophosphamide (500 mg/m2, days -4 to -3). The time interval from the diagnosis of AIHA to CAR-T cell infusion for the three patients was 5 days, 17 days, and 72 days, respectively. Efficacy assessment followed international consensus definitions for AIHA response[10]. Quantification of CAR T-cell persistence in peripheral blood and cytokine profiling were screened after CAR T-cell infusion. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were graded according to the 2019 American Society for Transplantation and Cellular Therapy consensus guidelines[11].

Clinical characteristics are summarized in **Table 1**. At AIHA diagnosis, all patients presented with severe anemia (hemoglobin levels were 3.4 g/dL, 4.6 g/dL, and 3.3 g/dL, respectively) and DAT positivity for IgG and complement. Pt.1 and Pt.2 initially received methylprednisolone (10mg/kg/day ×3 days) and IVIG (1g/kg/day ×2 days). Pt.1 exhibited transient improvement, whereas Pt.2 remained refractory, despite an additional dose of bortezomib. Both proceeded to CAR T-cell infusion to control relapsed leukemia and concurrent AIHA. Pt.3 developed refractory AIHA 5.1 months post-transplantation and underwent both first- and second-line therapies (Methylprednisolone at a dosage of 2 mg/kg/day for two weeks, followed by a 2-month taper to discontinuation, Rituximab at 375 mg/m2/week for three weeks, and Bortezomib for four doses), remaining unresponsive after 72 days of treatment. Based on the favorable therapeutic response to AIHA observed in Pt.1 and Pt.2, Pt. 3 also received infusion of CD19/CD22-targeted CAR T cells. For detailed clinical interventions after CAR T-cell infusion, refer to **Figure 1.**

Following infusion, a rapid elimination of circulating CD19+ B cells was evidenced in all three patients (with respective times of 14 days, 7 days, and 6 days). Responses were observed between 9 and 40 days after infusion, and all achieved complete response (CR) with normalization of hemoglobin and disappearance of hemolytic markers after 18 days, 118 days, and 157 days, respectively. At the final follow-up (median 36.5 months, range 9.6-39.3), all patients remained alive and in sustained CR for AIHA. B-cell reconstitution occurred between 4.5 and 23.3 months post-infusion. None of them showed signs of GVHD after CAR T-cell infusion. Among the first two cases, both achieved minimal residual disease (MRD)-negative remissions, with overall survival of 39.3 and 36.5 months (**Table 2**). Pt.3 demonstrated gradual hematologic recovery with delayed reticulocyte rise approximately three months after DAT conversion, consistent with transient pure red cell aplasia (PRCA), which eventually resolved in parallel with sustained donor-type chimerism and stable hemoglobin levels. No recurrence of hemolysis was observed in any patient.

The severity of the CAR-T-associated toxicity was mild in all cases. CRS manifested at a grade of ≤1 and was effectively managed with supportive care or tocilizumab. No patient developed ICANS, prolonged cytopenias, or severe infection. The highest CAR T-cell expansion in peripheral blood occurred at a median of 7 days (range, 6-13) post-infusion, coinciding with maximal B-cell depletion (**Figure S2**). No new autoimmune manifestations were recorded. These findings indicate that the coadministration of CD19/CD22 CAR T cells can be performed safely in post-transplant patients with severe immune dysregulation.

Before the standard infusion, all patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide. While lymphodepleting chemotherapy may contribute to short-term stabilization, it is unlikely to induce the profound, sustained

B-cell clearance required for drug-free remission in refractory AIHA. The mechanism underlying post-HSCT AIHA involves impaired central and peripheral immune tolerance due to thymic damage, lymphodepleting therapy, and delayed T-cell reconstitution, resulting in unchecked expansion of autoreactive B cells[12,13]. Conventional immunosuppressive therapy fails to eliminate long-lived plasma cells or reset the B-cell compartment, leading to frequent relapse and dependency on chronic treatment. By contrast, CAR T cells can profoundly deplete B-lineage cells, including autoreactive clones and plasmablasts, thereby abrogating autoantibody production. The sustained remission and B-cell reconstitution observed in our patients suggests that CAR T-cell therapy can restore immune homeostasis following allo-HSCT.

The temporal dynamics of DAT negativity and hemoglobin recovery varied among patients, possibly reflecting differing degrees of marrow suppression, hemolysis, or concurrent disease burden. Notably, in Pt.3, the delayed reticulocyte recovery by approximately three months despite early DAT conversion and B-cell aplasia suggests coexistence of immune-mediated PRCA. Judging from complete donor-type chimerism, we speculate that both immune-mediated hemolytic anemia and PRCA may be caused by the same autoimmune mechanism[14]. This can be corroborated by the fact that the time interval for PRCA recovery coincides with the clearance of autoantibodies in the absence of other therapeutic interventions. Nevertheless, further investigation is necessary to elucidate the specific pathogenic mechanisms involved.

The absence of severe cytokine release or neurotoxicity in this cohort contrasts favorably with toxicity rates seen in malignancy-focused CAR T-cell trials, possibly due to lower disease burden and reduced antigenic stimulation. Furthermore, despite extended B-cell aplasia lasting up to two years in one case, no severe infections were recorded, indicating that residual plasma cells and protective antibodies may persist or

that immune reconstitution following CAR T therapy may suffice to prevent opportunistic infection. Nevertheless, continued monitoring and immunoglobulin replacement remain essential until full B-cell recovery.

Clinical trials for CAR T-cell therapy in rAIHA are progressively underway. Jun Shi et al.[15] were the first to report favorable therapeutic responses to CD19 CAR T cells in patients with rAIHA. Patients who experienced hemolytic recurrence after cell therapy achieved renewed remission with treatment using CM336, a novel bispecific antibody that targets CD3 and B-cell maturation antigen (BCMA). These results suggest that achieving rapid and sustained remission in refractory cases may require optimizing CAR T target combinations, such as incorporating BCMA, to target long-lived plasma cells and memory B cells.

In conclusion, coadministration of CD19/CD22 CAR T-cell therapy induced rapid, complete, and durable remissions of refractory AIHA following allo-HSCT in three pediatric patients, with manageable toxicity and concurrent leukemia control. These results provide preliminary evidence supporting the feasibility and potential efficacy of CAR T-cell therapy as a novel therapeutic strategy for post-transplant autoimmune cytopenias.

References

1. Brodsky RA. Warm Autoimmune Hemolytic Anemia. N Engl J Med. 2019;381(7):647-654.

2. Gonzalez-Vicent M, Sanz J, Fuster JL, et al. Autoimmune hemolytic anemia (AIHA) following allogeneic hematopoietic stem cell transplantation (HSCT): A retrospective analysis and a proposal of treatment on behalf of the Grupo Espanol De Trasplante de Medula Osea en Ninos (GETMON) and the Grupo Espanol de Trasplante Hematopoyetico (GETH). Transfus Med Rev. 2018;S0887-7963(17)30164-30165.

3. Mougiakakos D, Krönke, G, Völkl S, et al. CD19-Targeted CAR T Cells in Refractory Systemic Lupus Erythematosus. N Engl J Med. 2021;385(6):567-569.

4. Schett G, Mackensen A, Mougiakakos D. CAR T-cell therapy in autoimmune diseases. Lancet. 2023;402(10416):2034-2044.

5. Faraci M, Zecca M, Pillon M, et al. Autoimmune hematological diseases after allogeneic hematopoietic stem cell transplantation in children: an Italian multicenter experience. Biol Blood Marrow Transplant. 2014;20(2):272-278.

6. Reynaud Q, Durieu I, Dutertre M, et al. Efficacy and safety of rituximab in auto-immune hemolytic anemia: A meta-analysis of 21 studies. Autoimmun Rev. 2015;14(4):304-313.

7. Gabelli M, Ademokun C, Cooper N, et al. Pathogenesis, risk factors and therapeutic options for autoimmune haemolytic anaemia in the post-transplant setting. Br J Haematol. 2022;196(1):45-62.

8. Muller F, Taubmann J, Bucci L, et al. CD19 CAR T-Cell Therapy in Autoimmune Disease - A Case Series with Follow-up. N Engl J Med. 2024;390(8):687-700.

9. Wang T, Tang Y, Cai J, et al. Coadministration of CD19- and CD22-Directed

Chimeric Antigen Receptor T-Cell Therapy in Childhood B-Cell Acute Lymphoblastic Leukemia: A Single-Arm, Multicenter, Phase II Trial. J Clin Oncol. 2023;41(9):1670-1683.

10. Jager U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. Blood Rev. 2020;41:100648.

11. Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019;25(4):625-638.

12. Li Z, Rubinstein SM, Thota R, et al. Immune-Mediated Complications after Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2016;22(8):1368-1375.

13. Holbro A, Passweg JR. Management of hemolytic anemia following allogeneic stem cell transplantation. Hematology Am Soc Hematol Educ Program. 2015;2015:378-384.

14. Kako S, Kanda Y, Oshima K, et al. Late onset of autoimmune hemolytic anemia and pure red cell aplasia after allogeneic hematopoietic stem cell transplantation using in vivo alemtuzumab. Am J Hematol. 2008;83(3):247-249.

15. Zhang L, Gao Z, Pan H, et al. BCMA-Targeted T-Cell Engager for Autoimmune Hemolytic Anemia after CD19 CAR T-Cell Therapy. N Engl J Med. 2025;392(22):2282-2284.

Table 1. Clinical and laboratory characteristics of patients with AIHA.

Parameter	Pt. 1	Pt. 2	Pt. 3
Clinical characteristics			
Diagnosis	B-ALL	B-ALL	B-ALL
Gender	F	M	M
Donor type	MMRD	UCB	MMRD
Blood type (Recipient/Donor)	O+/O+	B+/AB+	A+/AB+
Timing of blood type conversion	Before AIHA	Before AIHA	Before AIHA
Age at ALL diagnosis (Months)	24.4	5.6	8.2
Age at HSCT (Months)	38.6	10.2	14.8
Time of event from HSCT			
ALL relapse after HSCT (Months)	8.7	6.4	None
AIHA after HSCT (Months)	10.6	6.6	5.1
CAR-T after HSCT(Months)	10.8	7.1	7.5
Laboratory Baseline at AIHA			
Hemoglobin (g/dL)	3.4	4.6	3.3
Lym $\times 10^9$/L	0.2	2.2	0.1
PLT$\times 10^9$/L	67	217	131
Ret%	0.4	9.2	0.8
Infection at AIHA	B19	None	TTV

Prior treatments			
Methylprednisolone	Yes	Yes	Yes
Gammaglobulin	Yes	Yes	Yes
Rituximab	No	No	Yes
*Cyclophosphamide	Yes	Yes	Yes
Others	No	Bortezomib	Bortezomib, Luspatercept

Abbreviations: AIHA, autoimmune hemolytic anemia; F, female; M, male; HLA, human leukocyte antigen; MMRD, mismatched related donor; UCB, umbilical cord blood transplant; ALL, acute lymphoblastic leukemia; HSCT, hematopoietic stem cell transplantation; HCT, red blood cell hematocrit; Lym, lymphocyte count; PLT, platelet count; CR, complete response; DAT, direct antigloblin test. TTV, torque teno virus.

*Cyclophosphamide is used for lymphodepleting chemotherapy.

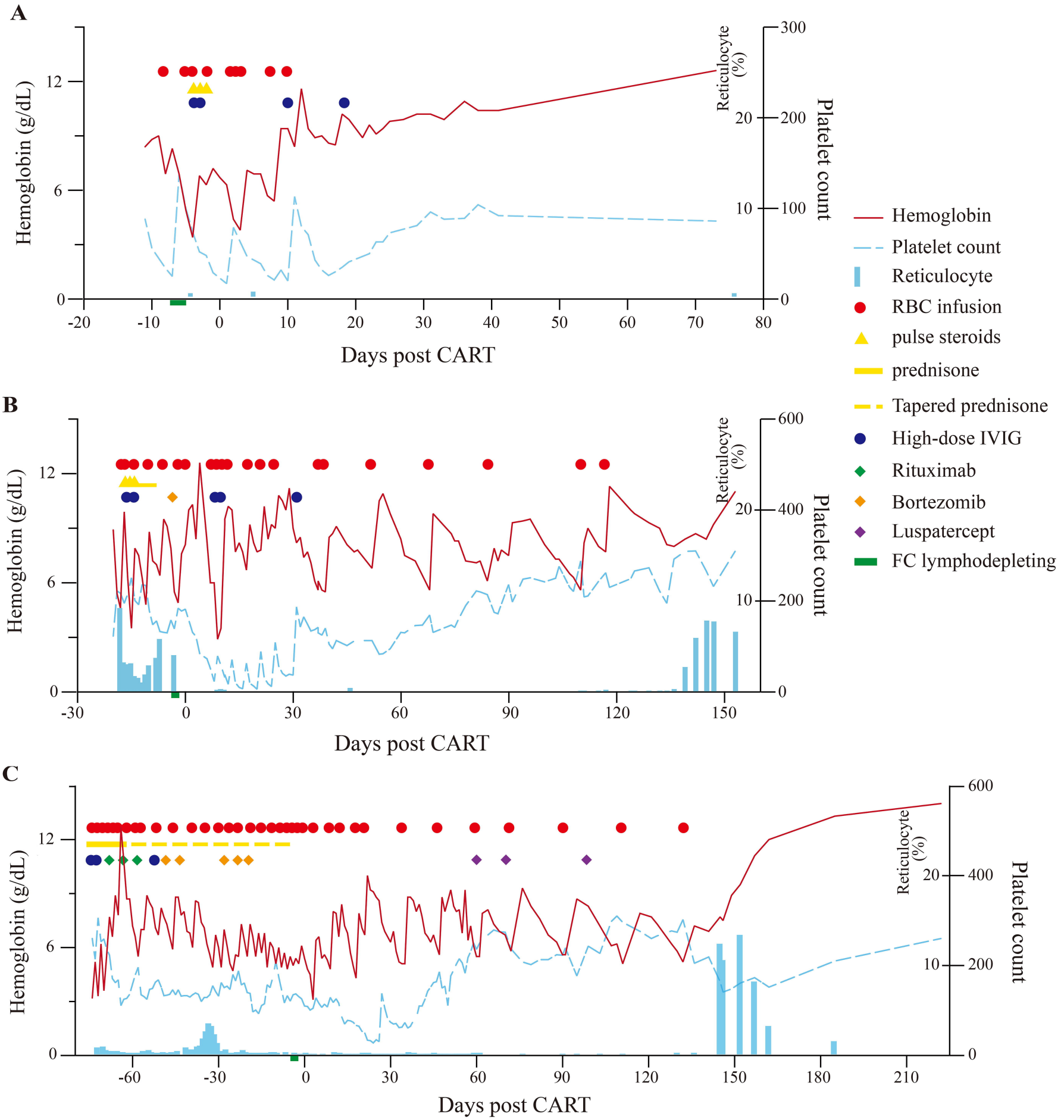
Table 2. Management and outcomes of patients with post-HSCT AIHA treated with CD19/CD22-Targeted CAR T-Cell.

Parameter	Pt. 1	Pt. 2	Pt. 3
Infusion information			
Treatment duration from CAR-T to AIHA (days)	5	17	72
CAR T-cell Concentration (cells/kg)	2.1×10^6	7.3×10^6	7.5×10^6
Cell source	Autogenous	Autogenous	Donor (Father)
Relapse site after HSCT	BM	Testicular	None
Responses to CAR T-cell			
AIHA Status	CR	CR	CR
Response after CAR-T (days)	9	40	22
Complete response after CAR-T (days)	18	118	157
Days to negative DAT test after CAR-T	51	117	52
Vital status	Alive	Alive	Alive
Safety assessment			
CRS	0	1	1
ICANS	0	0	0
B cell reconstitution (Months)	NA*	23.3	4.5

NA*: B cell reconstruction tests were not performed during follow-up.

Figure Legends

Figure 1. Treatment timeline. The hemoglobin, reticulocyte, and platelet counts of three patients, as well as the various treatments they received over time, are shown (A-C respectively). CAR T-cell infusion on day 0.


A
Hemoglobin (g/dL)
Reticulocyte (%)
Platelet count
Days post CART
B
Hemoglobin (g/dL)
Reticulocyte (%)
Platelet count
Days post CART
C
Hemoglobin (g/dL)
Reticulocyte (%)
Platelet count
Days post CART
Hemoglobin
Platelet count
Reticulocyte
RBC infusion
pulse steroids
prednisone
Tapered prednisone
High-dose IVIG
Rituximab
Bortezomib
Luspatercept
FC lymphodepleting

Supplemental Table 1 Additional clinical information.

Patient ID	Diagnosis	Gene type	Age at primary diagnosis (mos)	Age at HSCT (mos)	Gender of donor	Preparative regimen	GVHD prophylaxis regimen	Stem cell concentration (total CD34+ cells/kg)	Chimerism ratio at AIHA	DAT repeated at diagnosis, + or -
01	B-ALL	None	24.4	38.6	M	NA	NA	7.9×10^6	95.8%	Yes, +
02	B-ALL	*KMT2A-MLLT3*	5.6	10.2	F	BuCy/VP16	CSA/MMF	0.5×10^6	100.0%	Yes, +
03	B-ALL	*KMT2A-MLLT10*	8.2	14.8	M	Flu/Bu/Cy/Mel/ TBI(3Gy)	CSA/MMF	14.5×10^6*	99.6%	Yes, +

Patient ID	HCT(%)	Lym ($\times10^9$/L)	PLT ($\times10^9$/L)	Ret ($\times10^9$/L)	LDH (U/L)	Total bilirubin (umol/L)	Indirect bilirubin (umol/L)	Infection	Blood type at AIHA	GVHD at AIHA
01	15.5	0.2	67	3.8	808	40.9	22.3	parvovirus B19	O+	No
02	9.5	2.2	217	129.7	2220	60.0	48.7	None	AB+	No
03	13.7	0.1	131	8.8	631	34.4	17.2	TTV	AB+	No

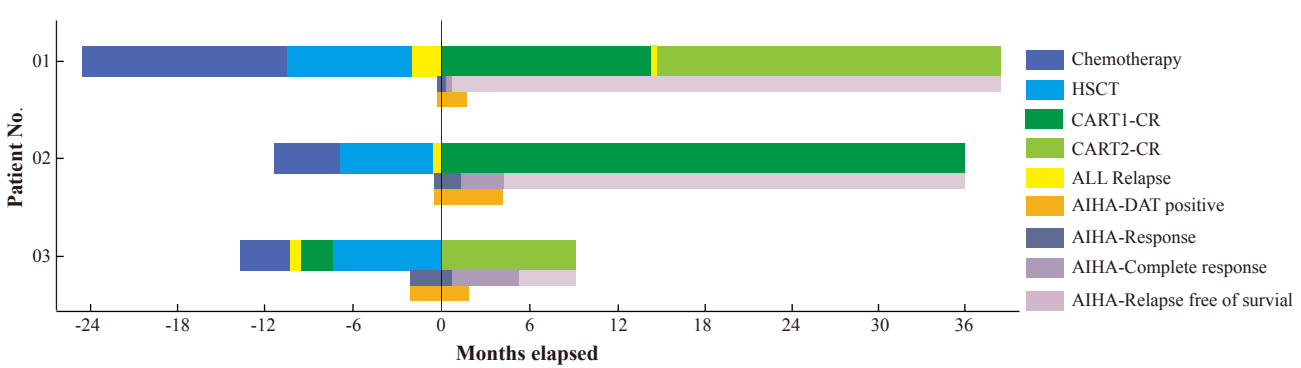
Abbreviations: HSCT, hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; mos, months; DAT, direct antiglobulin test; ALL, acute lymphoblastic leukemia; M, male; F, female; Bu, busulfan; Cy, cyclophosphamide; TBI, total body irradiation; Flu, Fludarabine; Mel, melphalan; Gy, Gray; CSA, cyclosporine; MMF, mycophenolate mofetil; TTV, Torque teno virus; NA, Not available.

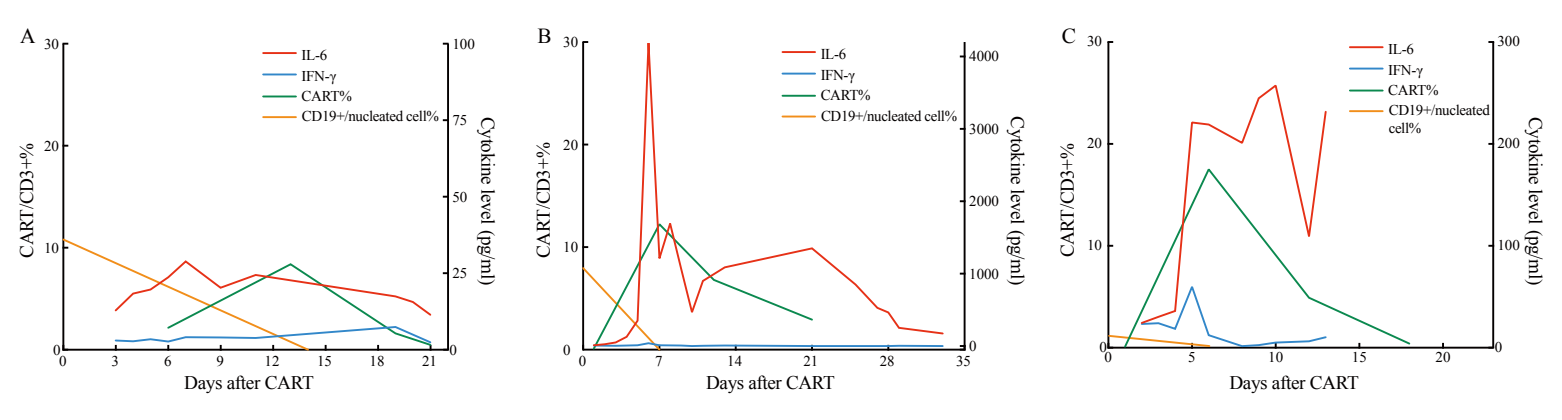
*T cell receptor alpha/beta depleted graft.

Supplementary Figure Legends

Figure S1. Treatment process and outcomes. The entire treatment process for three patients from the primary diagnosis to the cut-off time. The color and length of each bar respectively represent the status and duration of the response. CAR T-cell infusion on day 0. BMT: Bone marrow transplantation; CART-CR: Maintain complete remission after CAR T-cell infusion.

Figure S2. Changes in cytokine levels, expansion of CAR T cells and depletion of CD19+ B cells in 3 patients after CAR T-cell infusion (A-C represents Pt.1, Pt.2, and Pt.3, respectively).


Patient No.
01
02
03
-24
-18
-12
-6
0
6
12
18
24
30
36
Months elapsed
Chemotherapy
HSCT
CART1-CR
CART2-CR
ALL Relapse
AIHA-DAT positive
AIHA-Response
AIHA-Complete response
AIHA-Relapse free of survial


A
IL-6
IFN-γ
CART%
CD19+/nucleated cell%
CART/CD3+%
Cytokine level (pg/ml)
Days after CART
B
IL-6
IFN-γ
CART%
CD19+/nucleated cell%
CART/CD3+%
Cytokine level (pg/ml)
Days after CART
C
IL-6
IFN-γ
CART%
CD19+/nucleated cell%
CART/CD3+%
Cytokine level (pg/ml)
Days after CART