

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

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 co-administration 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 (characterized 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 co-administered 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 (*Online Supplementary Figure S1*). All fulfilled the diagnostic criteria² 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 (*Online Supplementary Table S1*). 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 (*Chinese Clinical Trial Registry: ChiCTR2000032211*).

CAR T cells were manufactured from autologous peripheral blood T cells transduced with CD19- or CD22-directed lentiviral vectors containing a 4-1BB co-stimulatory domain and CD3 ζ signaling domain, as detailed in our prior report.⁹ The anti-CD19 and anti-CD22 scFv 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 immunoglobulin (Ig)G and complement. Pt.1 and Pt.2 initially received methylprednisolone (10 mg/kg/day $\times$ 3 days) and IVIG (1 g/kg/day $\times$ 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 2 weeks, followed by a 2-month taper to discontinuation, rituximab at 375 mg/m²/week for 3 weeks, and bortezomib for 4 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 af-

ter 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 3 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 (*Online Supplementary Figure S2*). No new autoimmune manifestations were recorded. These findings indicate that the co-administration 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

Table 1. Clinical and laboratory characteristics of patients with autoimmune hemolytic anemia.

Parameter	Pt.1	Pt.2	Pt.3
Clinical characteristics			
Diagnosis	B-ALL	B-ALL	B-ALL
Sex	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, months			
ALL relapse after HSCT	8.7	6.4	None
AIHA after HSCT	10.6	6.6	5.1
CAR T after HSCT	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

*Cyclophosphamide is used for lymphodepleting chemotherapy. 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; CAR T: chimeric antigen receptor T-cell; Lym: lymphocyte count; Pt.: patient; PLT: platelet count; CR: complete response; TTV: Torque teno virus.

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 com-

partment, 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

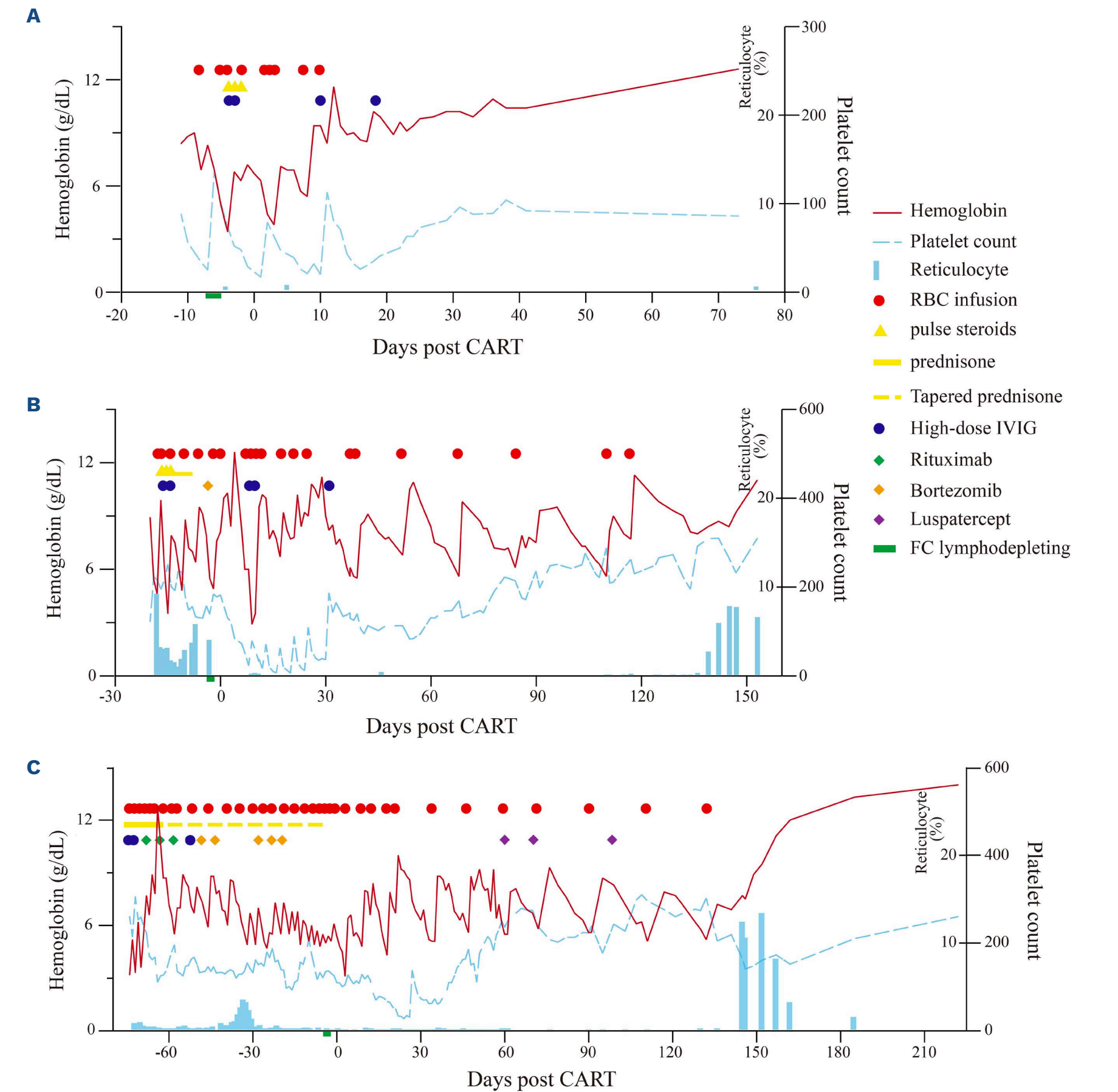


Figure 1. Treatment timeline. The hemoglobin, reticulocyte, and platelet counts of 3 patients, as well as the various treatments they received over time, are shown (A-C, respectively). Chimeric antigen receptor T-cell (CAR T) infusion on day 0. IVIG: intravenous immunoglobulin.

Table 2. Management and outcomes of patients with post-hematopoietic stem cell transplantation autoimmune hemolytic anemia treated with CD19/CD22-targeted chimeric antigen receptor T-cell therapy.

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			
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. AIHA: autoimmune hemolytic anemia; BM: bone marrow; CR: complete response; CAR: chimeric antigen receptor; CRS: cytokine release syndrome; HSCT: hematopoietic stem cell transplantation; ICANS: immune effector cell-associated neurotoxicity syndrome; DAT: direct antiglobulin test; Pt.: patient.

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 3 months despite early DAT conversion and B-cell aplasia suggests co-existence 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 2 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, co-administration 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.

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Disclosures

No conflicts of interest to disclose.

Contributions

BL and JC designed the study and did the interpretation. BL and YT provided financial and administrative support. CL, XH, MS, XQ, JZ, KA, JQ, and YT were responsible for clinical diagnosis and

treatment. LY and Xinyu Wan performed clinical data analysis and wrote the manuscript. LY, TW, WS, JY, RJ, and CF assisted in the clinical management; Xinyu Wan and Xiang Wang helped with laboratory examinations analysis. All authors read and approved the final manuscript.

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Data-sharing statement

For original data, please contact the corresponding author.

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.