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CORRESPONDENCE

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CD19 CAR-T in relapsed t(8;21) AML: a single-center prospective phase II clinical trial

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Abstract

Approximately 78.3% of patients with t(8;21) acute myeloid leukemia (AML) express CD19, making it a potential target for chimeric antigen receptor (CAR)-T cell therapy focused on CD19. This prospective phase II trial (NCT03896854) evaluated the safety and efficacy of CD19 CAR-T cell treatment in 10 relapsed CD19-positive t(8;21) AML patients. This study enrolled eight patients with hematologic and two with molecular relapsed AML. The median bone marrow blast percentage was 12.4% (0.1–50.2%), and the blasts exhibited a median CD19 positivity of 55.7% (22.6–97.1%). Genetic profiling revealed *TP53* alterations ($n=1$), *KIT* ($n=3$) and *FLT3*-ITD ($n=1$) mutations. After lymphodepletion with fludarabine and cyclophosphamide (FC), $5–20\times10^6$ cells per kilogram of CAR-T cells were administered. All patients experienced grade 3 or higher hematologic toxicities following tumor-reduction chemotherapy and the FC regimen, which were managed for a median of two weeks after CAR-T treatment. Non-hematological toxicities were mild and reversible. Eight patients presented with mild (grade 1–2) cytokine release syndrome (CRS), and one experienced grade 3 CRS. The immune effector cell-associated neurotoxicity syndrome was not observed. All patients achieved complete remission (CR) after CAR-T, with 60% achieving a molecularly MRD-negative CR. *RUNX1::RUNX1T1* fusion transcript levels demonstrated a median 2.5-log reduction (range: 0.7–4.5 log; $P=0.002$). At a median follow-up of 64.6 months (range: 11.2–88.8 months), the median overall survival and leukemia-free survival were 11.6 and 3.8 months, respectively. The 12-month cumulative incidence of relapse was 53.3%. These findings indicated that CD19 CAR-T was a safe and effective option for relapsed CD19-positive t(8;21) AML.

Keywords Chimeric antigen receptor T cells, CAR-T CD19, t(8;21) translocation, Acute myeloid leukemia, Relapsed

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To the editor

Despite the initial response to standard chemotherapy in most t(8;21) acute myeloid leukemia (AML) patients, approximately 35% experience relapse or persistent measurable residual disease (MRD) positivity, conferring a dismal 5-year overall survival rate of ≤ 20% in this setting [1–3]. This poor prognosis stems from the limited therapeutic options available for this molecularly defined subgroup.

Notably, CD19 expression is observed in 78.3% of t(8;21) AML cases [4], representing a potential therapeutic target for CD19-directed chimeric antigen receptor (CAR)-T cell treatment. We first demonstrated the efficacy of CD19 CAR-T cells in two relapsed t(8;21) AML patients, and this finding was subsequently revalidated by Israeli investigators [5, 6]. Based on these early proof-of-concept results, we conducted this prospective trial to evaluate CD19 CAR-T treatment in CD19 + relapsed t(8;21) AML.

This single-center, Phase II trial (NCT03896854, ClinicalTrials.gov) enrolled 10 relapsed t(8;21) AML patients between October 18, 2017, and December 11, 2023, at the First Affiliated Hospital of Soochow University, China. The study protocol received institutional review board approval in accordance with the Declaration of Helsinki guidelines. Baseline characteristics, including prior therapies, cytogenetic/molecular profiles, pretreatment, blast percentages, CAR-T cell source, CAR-T cell dose, response rates and survival, are tabulated (Table 1). The methodological components, including subject selection criteria, study protocol, CAR construct design, CAR-T cell production procedures, statistical approaches, and endpoint evaluations, were thoroughly documented in the supplementary materials. The study cohort comprised ten patients (eight males and two females). The median age was 31 years (range: 13–52 years). The median follow-up duration was 64.6 months (range: 11.2–88.8 months).

All patients experienced grade 3 or higher hematologic toxicities following tumor-reduction chemotherapy and lymphodepletion regimen with fludarabine and cyclophosphamide (FC), including anemia in 8 patients (80%), thrombocytopenia in 8 patients (80%), and neutropenia in 9 patients (90%). The median time of red blood cell transfusions (hemoglobin < 70g/L) was 11 days (range: 8–26 days), and for platelet transfusion independence (platelet counts ≥ 20×10^9/L) was 14 days (range: 0–30 days). The median time to neutrophil recovery (ANC ≥ 0.5×10^9/L) was 12 days (range: 1–24 days). Ultimately, the median time for recovery from all hematologic toxicities was 14 days (range: 1–30 days).

The non-hematological toxicities were transient and manageable, including pulmonary infection (grade 2;

Table 1 Patient baseline characteristics and treatment outcomes

Characteristics	*N* = 10 (%)
Median age (range) years	31 (13–52)
Female sex	2 (20.0%)
ECOG performance status ≤ 2	10 (100.0%)
Median percentage of CD19 positive blasts (%)	55.7% (22.6–97.1%)
Relapsed status	
Relapsed after chemotherapy	8 (80.0%)
Relapsed after transplantation	2 (20.0%)
Hematological relapse	8 (80.0%)
Molecular relapse	2 (20.0%)
Molecular Abnormalities	
RUNX1::RUNX1T1	10 (100.0%)
TP53 alteration	1 (10.0%)
KIT mutation	3 (30.0%)
ASXL1 mutation	4 (40.0%)
FLT3-ITD mutation	1 (10.0%)
NRAS mutation	1 (10.0%)
Tumor reduction chemotherapy before CD19 CAR-T infusion	
HMA + HAAG	5 (50.0%)
HMA + ECAG	1 (10.0%)
HMA + High dose Ara-C	1 (10.0%)
HMA + Venetoclax + Selinexor	1 (10.0%)
None	2 (20.0%)
Conditioning regimen	
FC	10 (100.0%)
Source of CD19 CAR-T	
Autologous	9 (90.0%)
Donor	1 (10.0%)
Median CD19 CAR-T dose (range)	10 (5–20) × 10^6/kg
Clinical responses	
CR	10 (100.0%)
CR_{MRD-}	6 (60.0%)
Maintenance therapy	2 (20.0%)
Decitabine alone	1 (10.0%)
Decitabine after allo-HSCT	1 (10.0%)
Survival Status	
Alive	3 (30.0%)
Death	7 (70.0%)
- Relapse-related	6 (85.7%)
- GVHD-related	1 (14.3%)
Relapse after CAR-T	
- CD19-negative	5/7 (71.4%)

Abbreviations: *ECOG* Eastern Cooperative Oncology Group, *HMA* Hypomethylating agent, *HAAG* Homoharringtonine (H), Cytarabine (A), Aclarubicin (A), Granulocyte colony-stimulating factor (G), *ECAG* Etoposide (E), Cytarabine (C), Aclarubicin (A), Granulocyte colony-stimulating factor (G), *Ara-C* Cytarabine; FC: Fludarabine (F), Cyclophosphamide (C), *CR* Complete remission, CR_{MRD-} Measurable residual disease (MRD)-negative complete remission, *allo-HSCT* Allogeneic hematopoietic stem cell transplantation

$n=1$), transient hepatotoxicity (grade 1; $n=1$) and hypotension (grade 3; $n=1$). Cytokine release syndrome (CRS) occurred in 9 patients (90%), with grades 1 in 4, 2 in 4, and 3 in 1. The grade 3 event was managed with steroid intervention (methylprednisolone 1 mg/kg/day intravenous for 3 days) and supportive care. No immune effector cell-associated neurotoxicity syndrome (ICANS) (ASTCT criteria) [7] was observed.

Ten out of 10 patients achieved complete remission (CR) (100% CR rate) post-CAR-T treatment, with 60% attaining molecular MRD-negative CR (CR_{MRD-}) (Fig. 1a, b, Table S2). *RUNX1::RUNX1T1* fusion transcript levels showed a median 3.27-log reduction (range: 0.7–4.5 log; $P=0.002$) post-CAR-T (Fig. 1c).

The median time to peak levels of CAR-T cells in blood was 8 days after infusion (range: 4–24 days) (Fig. 1d). Longitudinal cytokine profiles (e.g., IL-6) are shown in Fig. 1e and Figure S3. The median peak of IL-6 levels occurred on day 5 (range: 1–10). Figure S1

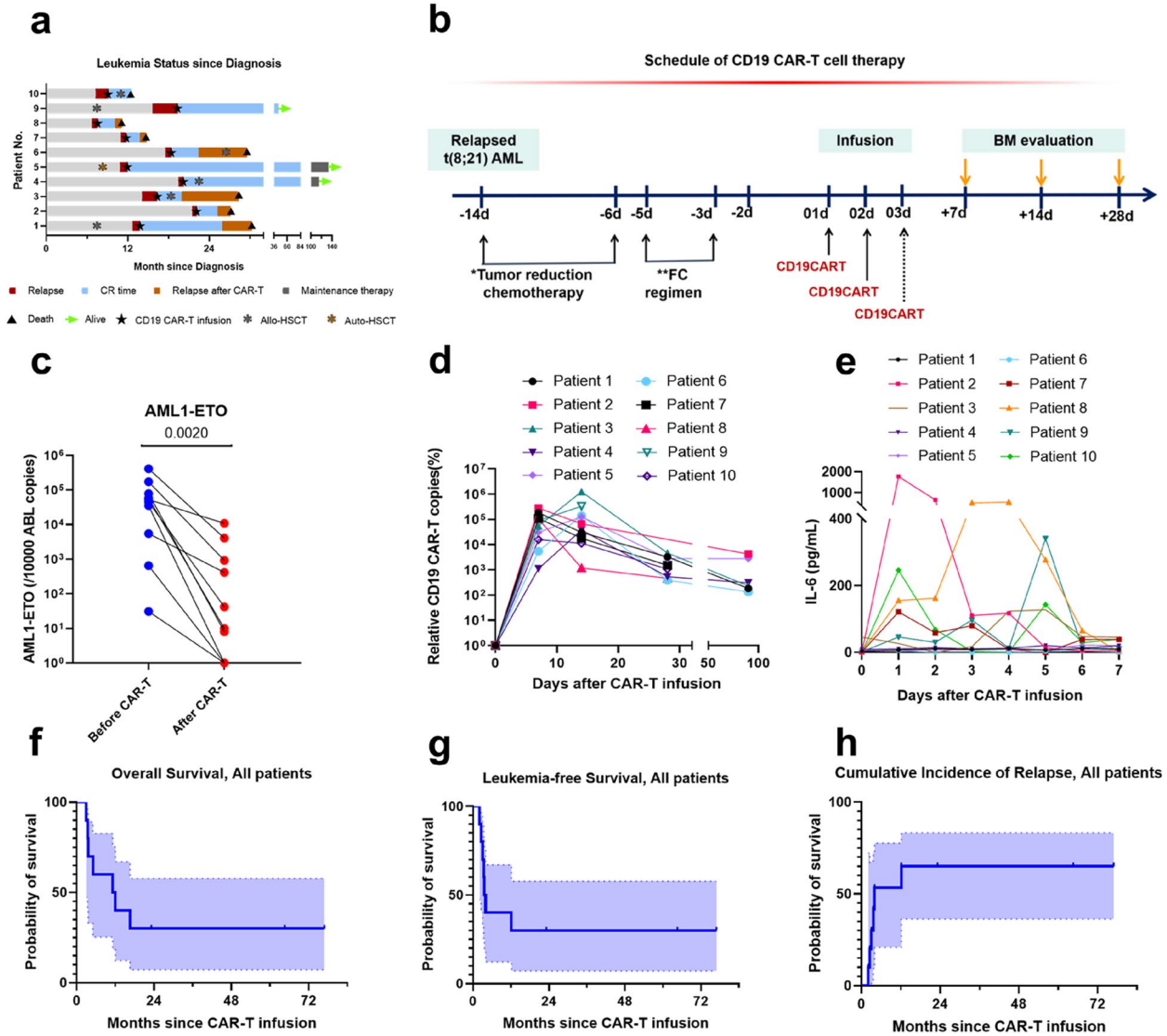

Fig. 1 Treatment Response and Survival of Patients. **a** Complete remission (CR) status of patients post-CAR-T infusion. Patients 1, 3, 4, 5 and 8 achieved molecular remission. Patients 2, 3, 6, 7 and 8 relapsed within four months, and Patient 1 experienced relapse at 12.2 months after the CD19 CAR-T treatment. **b** Schedule of the CD19 CAR-T treatment regimen. **c** Variation in *RUNX1::RUNX1T1* fusion gene expression in the bone marrow (BM) measured by quantitative real-time PCR (RT-PCR). **d** CAR-T cell copy numbers were detected using vector copy numbers (VCN) analysis. **e** The IL-6 cytokine results during CAR-T cell treatment. **f** & **h** 12-month overall survival (OS) and leukemia-free survival (LFS) rates were 45.0% and 46.7%, respectively. **h** The cumulative incidence of all patients is displayed

demonstrated CD19 dynamics and MRD clearance in a representative case (patient 4).

After CAR-T treatment, three patients (patients 3, 4, and 10) underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT), 2 received decitabine-based regimens, and 5 declined further consolidation treatment. Among the three allo-HSCT patients, the patient 10 died of grade IV acute intestinal graft-versus-host disease (GVHD) at 1.5 months post-transplantation, the patient 3 with *TP53* alterations succumbed to relapse at 2 months post-transplantation, and the patient 4 receiving decitabine maintenance remained alive in remission. Six patients who underwent allo-HSCT (patients 1, 2, 3, 6, 7, and 8) experienced relapse at a median time of 3.4 months (ranging: 2.1–12.2 months) post-CAR-T therapy. This included five cases of CD19-negative relapse and one case of CD19-positive relapse (patient 8). Several salvage treatments were administered to patients who relapsed after CAR-T, including venetoclax with hypomethylating agent (patients 1, 3), decitabine plus HAAG (Homoharringtonine, Cytarabine, Aclarubicin, combined with Granulocyte colony-stimulating factor) (patient 3), salvage transplantation (patient 6), decitabine combination with CD38 CAR-T (patient 3), and palliative care (patients 2, 7, 8). Finally, seven patients died, and three patients (patients 4, 5, 9) survived after CAR-T treatment. (Figure S2).

The median OS and leukemia-free survival (LFS) were 11.6 months (95% CI, 18.4% to 75.3%) and 3.8 months (95% CI, 18.4% to 75.3%). The 12-month cumulative incidence of relapse (CIR) was 53.3% (95% CI, 20.9% to 77.7%) (Fig. 1f-h). By the last follow-up, three patients were alive (Table S2).

Our study was the first to report the effectiveness of CD19 CAR-T therapy in t(8;21) AML, expanding the research with a larger group, longer follow-up, and a focus on post-CAR-T consolidation. Although all patients achieved CR, the median LFS was only 3.8 months, highlighting the necessity for larger sample sizes and the need for multi-center, collaborative trials to refine treatment strategies. While sequential allo-HSCT or maintenance treatment may help consolidate CAR-T-induced remission, future trials should focus on larger cohorts to evaluate these strategies.

Abbreviations

CAR-T	Chimeric antigen receptor T cell
allo-HSCT	Allogeneic hematopoietic stem cell transplantation
CR	Complete remission
AML	Acute myeloid leukemia
FC	Fludarabine + cyclophosphamide
MRD	Measurable residual disease
OS	Overall survival
ANC	Absolute neutrophil count
CRS	Cytokine release syndrome
CR_{MRD-}	MRD-negative complete remission
IV	Intravenous
HAAG	Homoharringtonine (H), Cytarabine (A), Aclarubicin (A), Granulocyte colony-stimulating factor (G)
ECAG	Etoposide (E), Cytarabine (C), Aclarubicin (A), Granulocyte colony-stimulating factor (G)
GVHD	Graft-versus-host disease
LFS	Leukemia-free survival
CIR	Cumulative incidence of relapse

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13045-025-01708-z.

Supplementary Material 1.

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Authors' contributions
TXW and WDP were responsible for the concept and design of the study. YJ and CQY collected and analyzed the data and wrote the manuscript. CW and DHP treated the patients and assisted in the data collection. All the authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This clinical trial was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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