Durable deep molecular response via CD19 chimeric antigen receptor T-cell therapy combined with a third-generation tyrosine kinase inhibitor in acute leukemia of ambiguous lineage with the Philadelphia chromosome: a potential strategy for transplant-ineligible patients

Acute leukemias of ambiguous lineage (ALAL) represent a rare and aggressive subgroup of acute leukemias characterized by blasts expressing markers of multiple lineages. The presence of the Philadelphia chromosome (Ph), resulting in the *BCR-ABL1* fusion gene, further stratifies these patients into a high-risk category. Current management typically involves acute lymphoblastic leukemia (ALL)-type induction chemotherapy combined with tyrosine kinase inhibitors (TKI), followed by allogeneic hematopoietic stem cell transplantation (HSCT) in first remission to mitigate the substantial risk of relapse.[1] However, a significant proportion of patients are ineligible for HSCT due to advanced age, comorbidity, or severe complications during chemotherapy. For these patients, effective consolidation strategies are lacking. Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of B-cell malignancies, yet its application in ALAL, particularly in combination with potent third-generation TKI as a frontline consolidation strategy, remains unexplored. We describe a case of Ph^+ ALAL with central nervous system (CNS) involvement where CD19 CAR T therapy combined with olverembatinib induced durable molecular remission, offering a blueprint for treating transplant-ineligible populations.

The study was approved by the Ethics Committee of the Blood Diseases Hospital, Chinese Academy of Medical Sciences (IIT2024019-EC-1). Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

A 60-year-old female presented with profound leukocytosis (white blood cell count [WBC] 261.72×10^9/L) and 91% circulating blasts. Bone marrow aspiration revealed 92.5% blasts expressing B-cell (CD19, CD79a, CD10, CD22) and myeloid (CD13, CD33, MPO) markers. Cytochemical staining showed a mixed lineage immunophenotype: strong NEDD8-activating enzyme (NAE) activity (56%) with partial sodium fluoride sensitivity (68% suppression), minimal CE expression (1%), weak myeloperoxidase reactivity (7%), and moderate glycogen deposition (PAS 28%). Cytogenetic analysis revealed an abnormal karyotype: 46, XX, t (9,22) (q34.1; q11.2) [4]/45, idem, der (16;18) (p10; q10) [4]/46~48, idem, -16, -der (22)t(9;22), +1~3mar[cp12]. Molecular testing identified a *BCR-ABL1* fusion transcript (p190 isoform) with a quantitative burden of 87.101%. According to the 5th edition of the World Health Organization Classification, diagnosis of Ph-positive (Ph^+) ALAL was rendered.

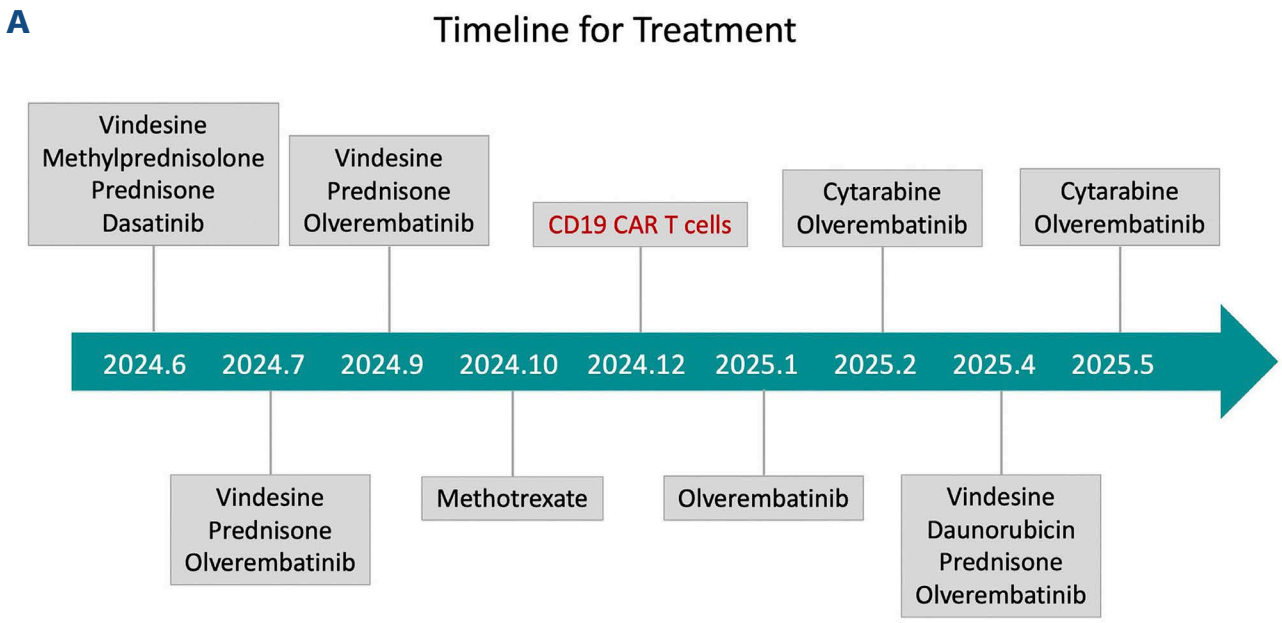
Before induction, the patient suffered a large cerebral infarction resulting in left-sided hemiparesis and dysphagia. Although conservative management stabilized her neurological condition, this severe comorbidity rendered her ineligible for consolidative HSCT. Following reduced-intensity induction chemotherapy with the second-generation TKI (dasatinib) (Figure 1A), the patient achieved morphologic remission and was negative for residual disease by flow cytometry. The emergence of massive pleural effusion led to the TKI switch of the third-generation TKI (olverembatinib). Cerebrospinal fluid (CSF) analysis confirmed CNS leukemia involvement (Figure 1D). However, the disease proved refractory at the molecular level: *BCR-ABL1* transcripts remained persistent, and high-sensitivity next-generation sequencing (NGS) detected residual immunoglobulin (Ig) H gene rearrangements (Figure 1B, C).

Since the patient was ineligible for consolidative HSCT due to severe comorbidity, she received murine-derived CD19 CAR T cells as an innovative consolidation regimen to eliminate residual blast cells in both the bone marrow and CSF. The patient underwent lymphodepletion with fludarabine (30 mg/m^2/d) and cyclophosphamide (0.3 g/m^2/day) for 3 consecutive days before 2×10^6 cell/kg autologous CD19 CAR T cells were administered on day 0.

The CAR T cells expanded robustly *in vivo*, peaking at day 10 (Figure 2A-C) without significant toxicities such as cytokine release syndrome or immune effector cell-associated neurotoxicity syndrome. Despite the pre-infusion undetectability of *BCR-ABL1* transcripts and *IgH* rearrangements, CAR T-cell infusion provided critical consolidation and was followed by durable complete molecular remission (CMR) throughout follow-up. The treatment also effectively eradicated leukemic cells in the CNS by following multiple intrathecal injections. To suppress potential myeloid clone resurgence and sustain remission, maintenance chemotherapy with olverembatinib was initiated 1-month post infusion, supplemented by two courses of cytarabine (1 g/m^2 twice per day for 3 days). As of the latest follow-up (1 year post infusion), the patient maintains a stringent

molecular remission.

While most ALAL cases are still classified by immunophenotype according to the 2022 World Health Organization and International Consensus Classification, *BCR*::*ABL1* fusion defines a distinct genetically defined ALAL entity. In parallel, the identification of founding lesions in primitive hematopoietic progenitors supports the early progenitor origin of ALAL.[2,3] Recent research has increasingly focused on delineating biologically distinct ALAL subtypes through genetic and epigenetic heterogeneity to inform precision therapy;[4,5] however, standardized classification frameworks and well-established therapeutic strategies remain lacking. In addition, lineage switch resulting from insufficient control of multilineage disease during treatment, as well as



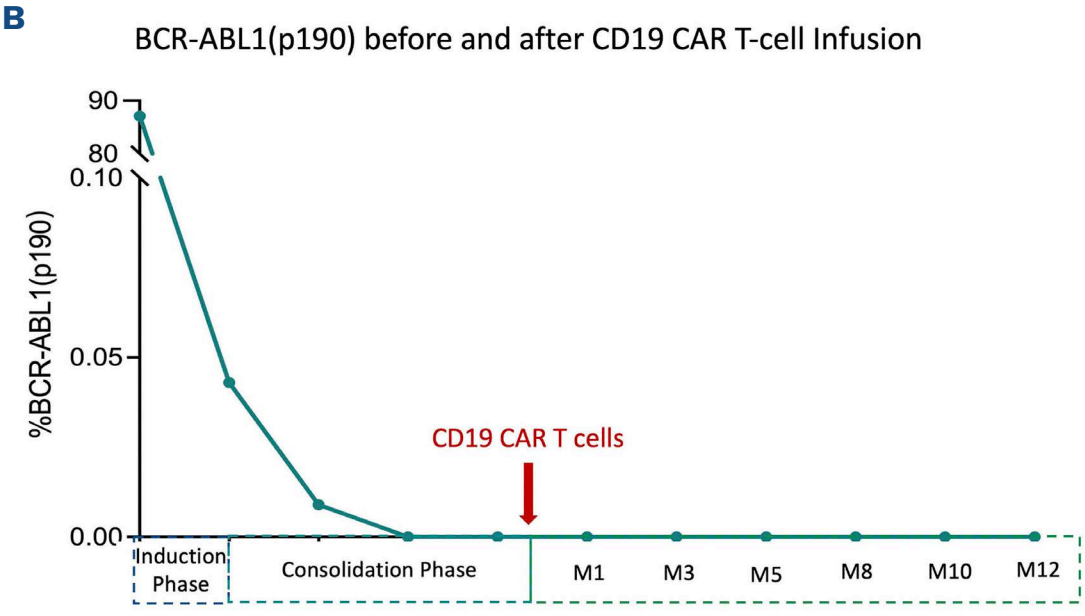
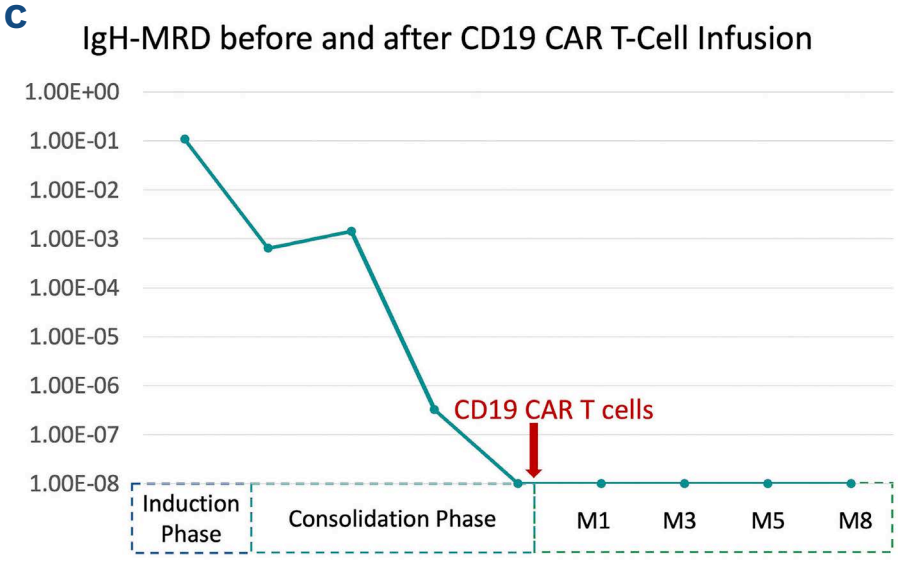
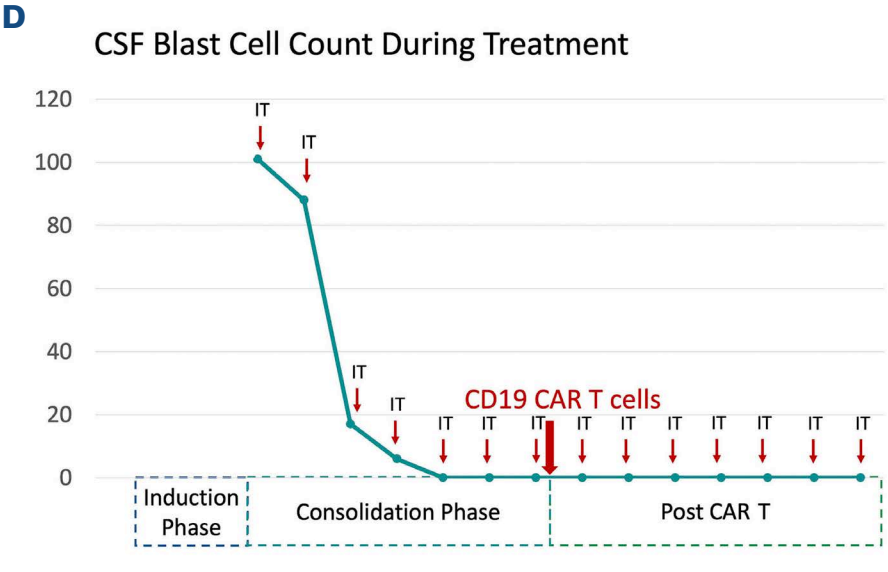


Figure 1. Treatment regimen and response monitoring during therapy. (A) Timeline for treatment including 1 course of induction chemotherapy and 3 courses of consolidation chemotherapy before chimeric antigen receptor (CAR) T-cell therapy. (B, C) Levels of molecular minimal residual disease (MRD) during treatment including *BCR-ABL1* transcripts and high-sensitivity next-generation sequencing detected residual immunoglobulin (Ig)H gene rearrangements. (D) Number of blast cells in the patient's cerebrospinal fluid (CSF) and frequency of intrathecal therapy during treatment.





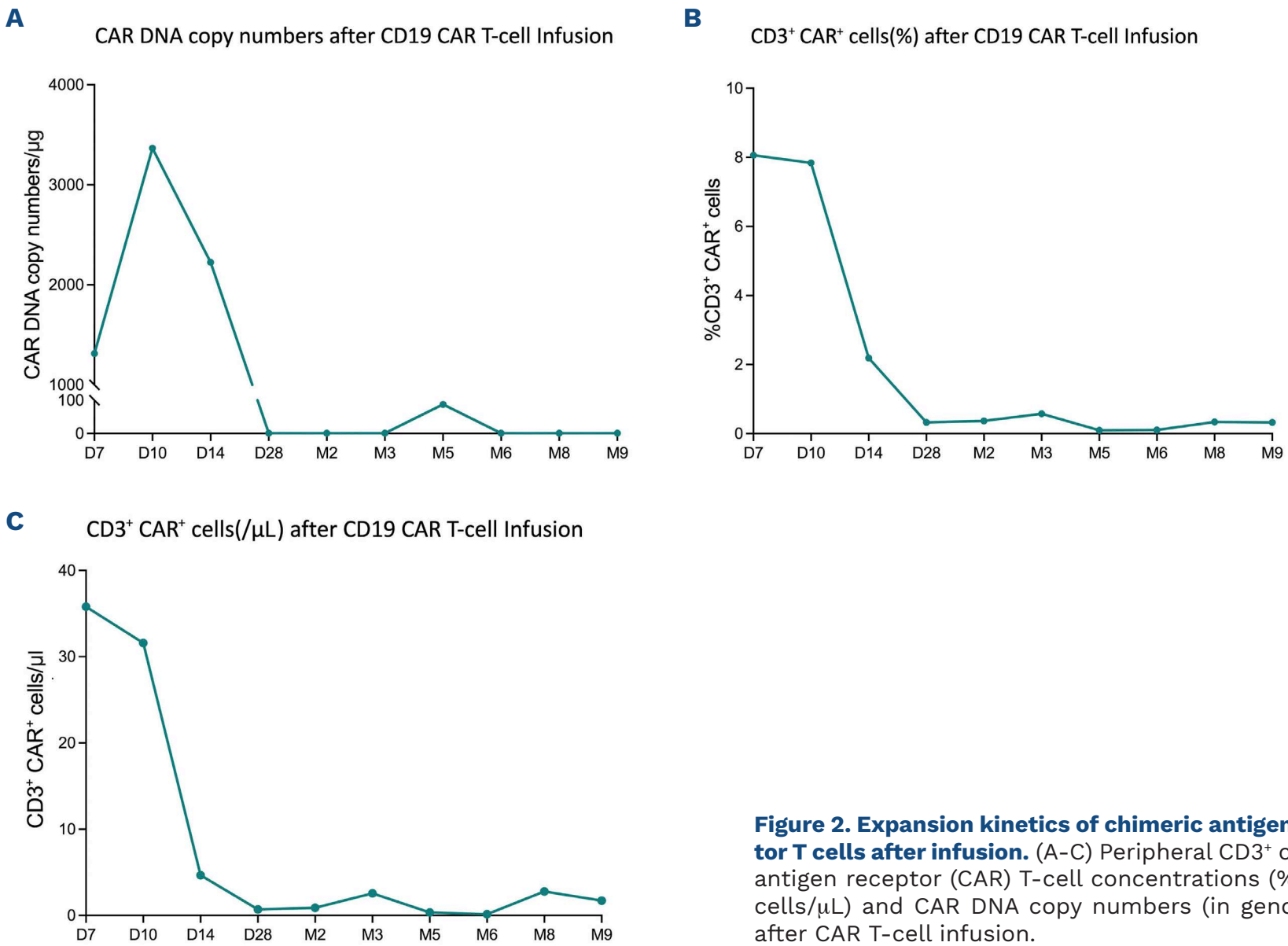


Figure 2. Expansion kinetics of chimeric antigen receptor T cells after infusion. (A-C) Peripheral CD3+ chimeric antigen receptor (CAR) T-cell concentrations (% and in cells/µL) and CAR DNA copy numbers (in genome/µg) after CAR T-cell infusion.

that occurring after CAR T therapy, has been increasingly recognized in ALAL, yet no standard management approach has been established.[6] Against this background, the present case suggests that our therapeutic strategy may offer a potential approach to addressing these challenges.

This case highlights the therapeutic dilemma posed by Ph+ ALAL patients who cannot undergo HSCT. While blinatumomab has shown promise in ALAL,[7,8] our report provides the first clinical evidence supporting CD19 CAR T therapy combined with a third-generation TKI as a definitive, transplant-free consolidation strategy. The rationale for this combination is synergistic: firstly, CD19 CAR T cells deeply deplete the B-lineage component of the leukemia, which often drives the bulk of the tumor burden in ALAL. Secondly, third-generation TKI (olverembatinib) potently inhibits the constitutively active BCR-ABL1 tyrosine kinase, targeting the underlying oncogenic driver across both lymphoid and myeloid lineages, including clones that might escape CD19-directed therapy, and thus may provide a means of suppressing lineage switch. Thirdly, CNS penetration: CD19 CAR T cells possess the ability to cross the blood-brain barrier, addressing the high risk of CNS relapse associated with Ph+ ALAL.

We demonstrate that the combination of CD19 CAR T-cell therapy and olverembatinib can achieve durable deep molecular response in Ph+ ALAL, overcoming the poor prognosis associated with persistent minimal residual disease and CNS involvement. This regimen represents a promising, potentially curative alternative for patients precluded from HSCT, warranting further investigation in prospective clinical trials.

Authors

Ao Zhang,* Benfa Gong,* Yuntao Liu, Guangji Zhang, Chunlin Zhou, Shouyun Li, Chengcai Guo, Huihui Yang, Yanhui Li, Lili Wang, Boming Zhang, Hui Wei, Jianxiang Wang and Shaowei Qiu

State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College and Tianjin Institutes of Health Science, Tianjin, China

**AZ and BG contributed equally as first authors.*

Correspondence:
S. QIU - qiushaowei@ihcams.ac.cn
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JW discloses advisory role with honoraria for AbbVie. All other authors have no conflicts of interest to disclose.

Contributions

AZ collected the data and wrote the manuscript. BG contributed to patient management. YL, GZ, CZ, SL, CG, HY, YL, LW, and BZ participated in patient care and data collection. HW, JW, and SQ supervised the study. SQ, as the corresponding author, was responsible for the overall design and supervision of the work and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Data-sharing statement

This research data is available upon request from the authors. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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