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RESEARCH ARTICLE

Allogeneic hematopoietic stem cell transplantation could overcome the poor prognosis of *DNMT3A*^{mut}*NPM1*^{mut}*FLT3*-ITD^{mut} in acute myeloid leukemia: real-world multicenter analysis in China

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Abstract **The cooccurrence of *NPM1*, *FLT3*-ITD, and *DNMT3A* mutations (i.e., triple mutation) is related to dismal prognosis in patients with acute myeloid leukemia (AML) receiving chemotherapy alone. In this multicenter retrospective cohort study, we aimed to identify whether allogeneic hematopoietic stem cell transplantation (allo-HSCT) could overcome the poor prognosis of *DNMT3A*^{mut}*NPM1*^{mut}*FLT3*-ITD^{mut} AML across four transplant centers in China. Fifty-three patients with triple-mutated AML receiving allo-HSCT in complete remission were enrolled. The 1.5-year probabilities of relapse, leukemia-free survival, and overall survival after allo-HSCT were 11.9%, 80.3%, and 81.8%, respectively. Multivariate analysis revealed that more than one course of induction chemotherapy and allo-HSCT beyond CR1 were associated with poor survival. To our knowledge, this work is the largest study to explore the up-to-date undefined role of allo-HSCT in patients with triple-mutated AML. Our real-world data suggest that allo-HSCT could overcome the poor prognosis of *DNMT3A*^{mut}*NPM1*^{mut}*FLT3*-ITD^{mut} in AML.**

Keywords allogeneic hematopoietic stem cell transplantation; acute myeloid leukemia; *DNMT3A* mutation; *NPM1* mutation; *FLT3*-ITD mutation; triple mutation

Introduction

NPM1, *FLT3*-ITD, and *DNMT3A* mutations are the most common genomic lesions in *de novo* acute myeloid leukemia (AML) and play key roles in the pathogenesis and evolution of the disease, particularly in AML with normal cytogenetics [1–3]. According to limited real-world data, the cooccurrence of these three mutations (i.e., triple-mutated AML) occurs in approximately 5.9%–7% of patients with *de novo* AML [4]. Triple-mutated AML is associated with typical clinical features, such as significantly high white blood cell count and prevalence in young women [4].

Although triple-mutated AML generally has a poor prognosis, it is still categorized as intermediate-risk AML according to the European LeukemiaNet (ELN) 2022 risk classification [5]. The *DNMT3A* mutation is not

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considered a poor prognostic factor in the ELN 2022 risk classification for AML; however, some studies have reported that overlapping *DNMT3A* mutations with *NPM1* or *FLT3*-ITD mutations are associated with a poor prognosis [6,7].

The prognostic effects of individual mutations are often significantly altered by other mutations. Such gene–gene interactions are especially pronounced in *NPM1*-mutated AML [6,8,9]. Triple-mutated AML shows a unique differentiation response to *FLT3* inhibitors [10] and increased sensitivity to ibrutinib [11]. Transcriptomic and immunophenotypic data indicate that triple-mutated blasts are associated with a high frequency of leukemia stem cells and the synergistic upregulation of a specific leukemia stem cell regulator [12]. Specific DNA methylation signatures have been characterized in triple-mutated AML [13]. The genetic interactions among these three mutations have been documented in a mouse model [14] and in humans [6,15,16], further suggesting that triple-mutated AML might represent a distinct entity with poor outcomes.

Large cohort studies identified a poor prognosis for triple-mutated AML [4,6,17–21]. According to a report of 507 consecutive patients with AML from five Brazilian centers, 35 patients were diagnosed with triple-mutated AML; the cumulative incidence of relapse was as high as 85%, and the overall survival (OS) rate was only 4% [4]. *FLT3* inhibitors can improve the clinical outcomes of patients with AML having *FLT3* mutations, but whether they exhibit the same effect on patients with triple-mutated AML remains controversial. Some authors reported that patients with triple-mutated AML may benefit from sorafenib maintenance [19]; however, adding midostaurin to first-line therapy did not improve the clinical outcomes of triple-mutated AML [22].

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most important curative treatment for adult patients with AML. For AML with intermediate- or adverse-risk cytogenetics, the clinical outcomes of patients receiving allo-HSCT are better than those of patients receiving chemotherapy alone [23–25]. However, whether patients with triple-mutated AML could benefit from allo-HSCT remains unknown.

In this multicenter real-world study, we aimed to identify the clinical outcomes of patients with triple-mutated AML who underwent allo-HSCT.

Materials and methods

Patients

This multicenter retrospective study was designed by the Peking University Institute of Hematology, the First Affiliated Hospital of Soochow University, Wuhan Tongji Hospital, and Shanghai Ruijin Hospital. Consecutive patients diagnosed with AML from January 2017 to June 2022 were screened, and the eligibility criteria were as follows: (1) aged ⩾ 16 years; (2) intermediate-risk *de novo* AML based on ELN 2022 classification [5]; (3) achieved complete remission (CR) before HSCT; and (4) comutation of *DNMT3A*, *NPM1*, and *FLT3*-ITD (*DNMT3A*^{mut}*NPM1*^{mut}*FLT3*-ITD^{mut}). The exclusion criteria were as follows: (1) age < 16 years; (2) patients who did not meet the criteria for intermediate-risk AML as defined by the ELN 2022 classification; (3) therapy-related AML or a previous history of myelodysplasia syndrome; and (4) incomplete medical information. Patients with triple-mutated AML receiving consolidation chemotherapy alone during the same period were also enrolled in the control cohort (n = 22), and only six of them achieved CR. Given the small sample size of patients receiving chemotherapy alone, only the allo-HSCT and chemotherapy-based groups were compared using descriptive statistics. The final follow-up was conducted on July 31, 2023. This study was approved by the institutional review board of each participating hospital and conducted in accordance with the *Declaration of Helsinki*. All patients provided written informed consent for the use of their clinical data as part of an ongoing quality improvement program.

Genotyping

DNMT3A mutations were detected as previously reported [26,27]. Next-generation sequencing was performed to detect concurrent mutations. The predominant variants (A, B, and D) within the *NPM1* gene were precisely identified using real-time fluorescence quantitative polymerase chain reaction (PCR) [28] (Supplementary Methods). *FLT3*-ITD mutations were detected using PCR [29] (Supplementary Methods). The allelic ratio for *FLT3*-ITD was determined by calculating the ratio of the area under the curve for the mutant alleles to that of the wild-type alleles (*FLT3*-ITD^{mut}/*FLT3*^{wt}).

Transplant regimen

The major preconditioning regimens included cytarabine, busulfan, cyclophosphamide, semustine, and anti-thymocyte globulin [30,31] (Supplementary Methods). The protocol for graft-versus-host disease (GVHD) and infection prophylaxis has been reported previously [32–35] (Supplementary Methods).

MRD monitoring protocols

MRD status was monitored before allo-HSCT; at 1, 2, 3, 4.5, 6, 9, and 12 months after allo-HSCT; and at 6-month intervals thereafter [3,36,37]. *NPM1* was identified by real-time quantitative PCR according to the ELN MRD

working party. A positive qPCR result was defined as a cycling threshold of < 40 in at least two of three replicates [38]. Multiparameter flow cytometry (MFC) for leukemia-associated aberrant immunophenotypes and/or those different from normal with 0.1% was used as a threshold to distinguish MRD positivity [39].

Data collection

The investigators at each hospital utilized the institutional electronic medical records and clinical databases. The collected data included information on patient demographics; diagnosis; mutated status of *DNMT3A*, *NPM1*, and *FLT3*-ITD; chemotherapy before allo-HSCT; transplant regimens; MRD status before and after allo-HSCT; maintenance and preemptive therapies; and clinical outcomes. All data were independently reviewed by two physicians with extensive experience in allo-HSCT.

Definition

Maintenance therapy was defined as patients who were persistently MRD-negative and received therapy for relapse prophylaxis after allo-HSCT. MRD positivity was defined by MFC-MRD or *NPM1*-qPCR positivity. Preemptive therapy was defined as patients who were MRD-positive and received therapy after allo-HSCT to prevent hematologic relapse, including donor lymphocyte infusion [40], interferon-α [41,42], *FLT3* inhibitors, or hypomethylating agents. Relapse was defined as the recurrence of > 5% bone marrow blasts, reappearance of blasts in the peripheral blood, development of extramedullary disease, or recurrence of pretransplantation chromosomal abnormalities. Nonrelapse mortality (NRM) was defined as death without disease progression or relapse. Leukemia-free survival (LFS) was defined as survival with continuous CR. OS events were defined as death from any cause.

Statistical analysis

Data were censored at the time of death or last available follow-up. The primary outcome was the relapse rate. The secondary outcomes included MRD positivity, NRM, EFS, LFS, and OS. Frequencies and percentages were used to describe patient characteristics. The Kaplan–Meier estimator was applied to calculate the probabilities of survival, and the cumulative incidence function was employed to calculate the incidence of relapse and NRM using competing risk analysis. Univariate and multivariate Cox regression analyses were performed to determine the impact of potential prognostic factors on clinical outcomes (Table S1). Two-sided P values were used. Independent variables with $P > 0.1$ were sequentially excluded from the model, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed with R software 4.2.0 and SPSS 26 (SPSS Inc., IBM, Armonk, NY, USA).

Results

Patients' characteristics

Fifty-three patients with intermediate-risk triple-mutated AML receiving allo-HSCT were enrolled, and their characteristics are shown in Table 1. The distribution of other comutant molecular abnormalities is presented in Table S2. Nine patients received maintenance therapy (seven with FLT3 inhibitors and two with hypomethylating agents) after allo-HSCT. The median follow-up duration was 865 days (range, 775–955) days.

Engraftment and GVHD

Among the patients, 52 (98.1%) achieved neutrophil engraftment, and the median time from transplantation to neutrophil engraftment was 12.5 days (range, 10–25 days). In addition, 52 patients (98.1%) achieved platelet engraftment, and the median time from transplantation to platelet engraftment was 14 days (range, 8–106 days).

The 100-day cumulative incidences of grades I–IV and III–IV acute GVHD after allo-HSCT were 30.2% (95% CI: 17.7%–42.7%) and 9.4% (95% CI: 1.5%–17.4%), respectively. The 3-year cumulative incidences of total and moderate or severe chronic GVHD after allo-HSCT were 26.4% (95% CI: 13.9%–38.9%) and 6.1% (95% CI: 0%–12.9%), respectively.

MRD occurrence and relapse

All 53 patients achieved CR before allo-HSCT, among which, 38 (71.7%) and 15 (28.3%) were MRD-negative and MRD-positive, respectively. Seven patients (13.2%) showed MRD positivity after allo-HSCT (MFC positivity alone, one; *NPM1* positivity alone, six), and two of these seven patients showed persistent MRD positivity and relapse after allo-HSCT (Table S2). Five patients achieved MRD negativity: one achieved MRD negativity after preemptive donor lymphocyte infusion, one achieved MRD negativity after sorafenib treatment, and the other three achieved MRD negativity without preemptive interventions.

Eight patients experienced relapse within a median time of 165 days (range, 32–398 days) after allo-HSCT. The 1.5-year cumulative incidence of relapse after allo-HSCT was 11.9% (95% CI: 2.8%–21.0%) and was comparable between the patients who were MRD positive and MRD negative before allo-HSCT (Fig. 1A; Table S3). The cumulative incidence of relapse after allo-HSCT was comparable between the patients with R882 and

Table 1 Characteristics of patients with triple-mutated AML receiving allo-HSCT

Characteristics	*n* = 53
Age, *n* (%)	
16–54 years	43 (81.1)
⩾ 55 years	10 (18.9)
Median age at allo-HSCT, years (range)	47 (22–61)
Gender, *n* (%)	
Male	22 (41.5)
Female	31 (58.5)
Courses of induction chemotherapy before first CR, median (range)	1 (1–4)
Disease status before allo-HSCT, *n* (%)	
CR1	50 (94.3)
> CR1	3 (5.7)
HCT-CI scores before allo-HSCT, *n* (%)	
0 (low risk)	38 (71.7)
1–2 (intermediate risk)	11 (20.8)
⩾ 3 (high risk)	4 (7.5)
Donor type, *n* (%)	
Matched sibling donor	14 (26.4)
Haploidentical related donor	37 (69.8)
Unrelated donor	2 (3.8)
Donor/recipient gender matching, *n* (%)	
Female donor/male recipient combination	7 (13.2)
Others	46 (86.8)
Blood group disparity, *n* (%)	
Matched	28 (52.8)
Minor mismatched	8 (15.1)
Major mismatched or minor and major mismatched	17 (32.1)
Conditioning regimen, *n* (%)	
Chemotherapy-based regimen	50 (94.3)
TBI-based regimen	3 (5.7)
Graft type, *n* (%)	
PB	17 (32.1)
BM + PB	36 (67.9)
MNC counts in graft, median (range, × 10^8/kg)	10.9 (3.4–28.7)
$CD34^+$ cell counts in graft, median (range, × 10^6/kg)	4.8 (1.3–14.9)

Allo-HSCT, allogeneic hematopoietic stem cell transplantation; BM, bone marrow; CR, complete remission; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; MNC, mononuclear cell; PB, peripheral blood; TBI, total body irradiation.

non-R882 mutations (Fig. 2A; Table S4). In addition, the patients who received consolidation chemotherapy alone had a higher cumulative incidence of relapse than those who received allo-HSCT (Fig. 3A; Table S5).

In the multivariate analysis, no risk factors were associated with relapse after allo-HSCT (Table 2).

NRM, LFS, and OS

Four patients died of NRM (infection, *n* = 3; acute GVHD, *n* = 1). The 1.5-year cumulative incidence of NRM after allo-HSCT was 7.8% (95% CI, 0.4%–15.2%). The 1.5-year probabilities of LFS and OS after allo-HSCT were 80.3% (95% CI: 70.0%–92.1%) and 81.8% (95% CI, 71.7%–93.3%), respectively. The 1.5-year probabilities of NRM, LFS, and OS were all comparable between the patients who were MRD positive and MRD negative before allo-HSCT (Fig. 1B–1D; Table S3) and between the patients with R882 and non-R882 mutations (Fig. 2B–2D; Table S4). In addition, the patients who

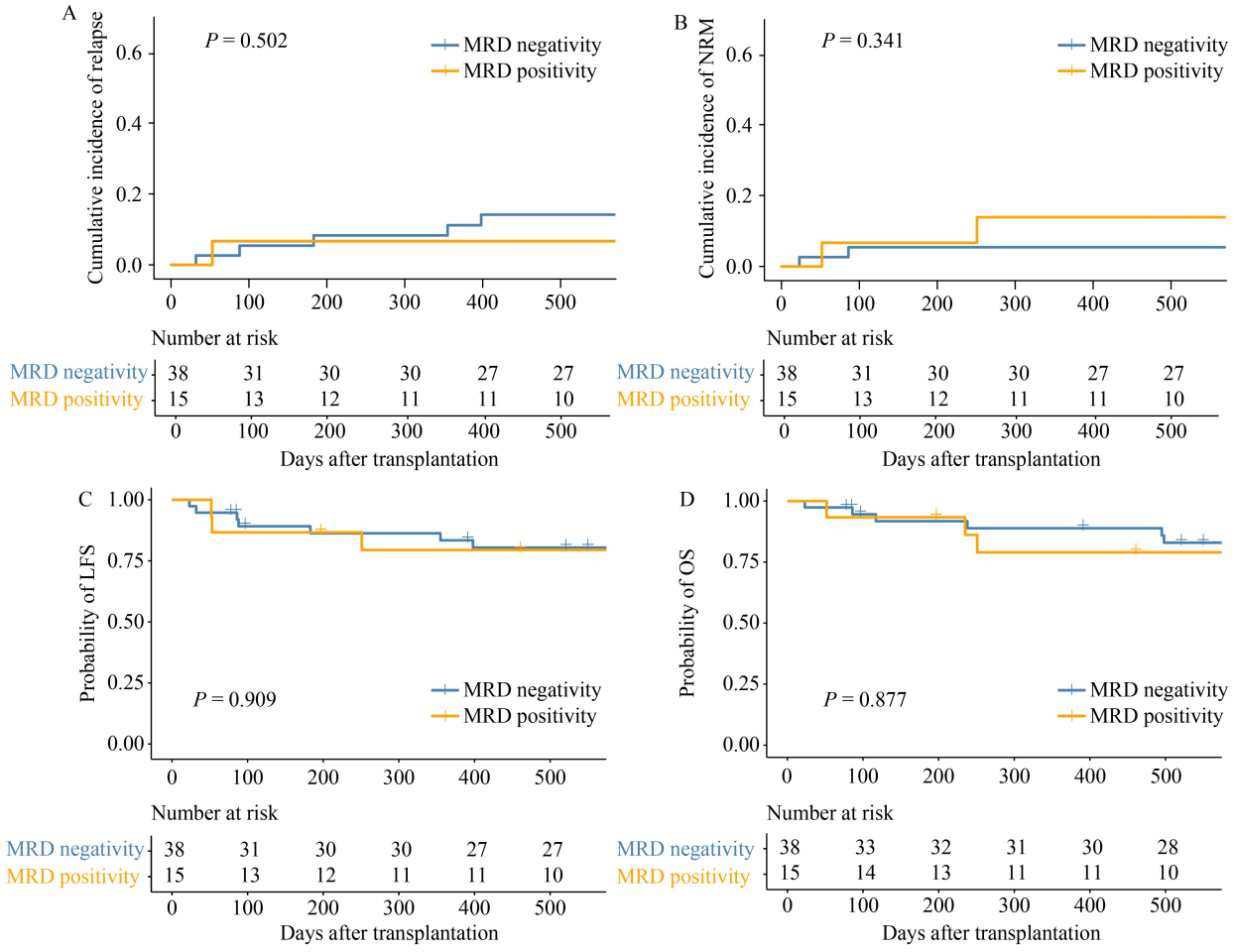

Fig. 1 1.5-year clinical outcomes in patients with triple-mutated AML who were positive and negative for MRD before allo-HSCT. (A) relapse, (B) non-relapse mortality, (C) leukemia-free survival, and (D) overall survival.

received consolidation chemotherapy alone had worse LFS and OS than those receiving allo-HSCT (Fig. 3C and 3D; Table S5).

Multivariate analysis revealed that many courses of induction chemotherapy and allo-HSCT beyond CR1 were associated with poor LFS and OS. No risk factors were associated with NRM (Table 2).

Discussion

In this study, we observed that the 1.5-year probabilities of relapse, NRM, LFS, and OS after allo-HSCT were 11.9%, 7.8%, 80.3%, and 81.8%, respectively. To our knowledge, this work is the largest study to explore the undefined role of allo-HSCT in patients with triple-mutated AML.

The LFS of triple-mutated AML is poor, and the incidence of relapse could be as high as 70%–85% in patients without allo-HSCT [4, 6]. In the present study, the relapse risk was reduced to 11.9% after allo-HSCT. The EBMT Acute Leukemia Working Party conducted a large retrospective research on a subgroup of 324 normal-cytogenetic allo-HSCT recipients with *FLT3*-ITD and *NPM1* comutation; the results showed that 244 were triple-positive (75%) and LFS and OS were not significantly different between the patients with and without *DNMT3A* mutation (2-year LFS: 62% vs. 63%, HR: 0.98, $P = 0.95$; 2-year OS: 70% vs. 73%, HR: 1.17, $P = 0.58$) [43]. All these data suggested that allo-HSCT could substantially reduce the relapse risk of triple-mutated AML to the level of intermediate-risk AML.

The maintenance of sorafenib therapy after allo-HSCT could further decrease the incidence of relapse and improve the LFS [19,44] of patients with AML and *FLT3* mutation; however, only seven patients received sorafenib maintenance after allo-HSCT. The 1.5-year probability of relapse was only 11.9%, and the OS rate was as high as 81.8%. This finding suggested that most of the patients in our triple-mutated AML cohort achieved persistent LFS without maintenance. One explanation is that most of the patients (94.3%) were MRD negative before allo-HSCT, and the proportion of women was also high (nearly 60%).

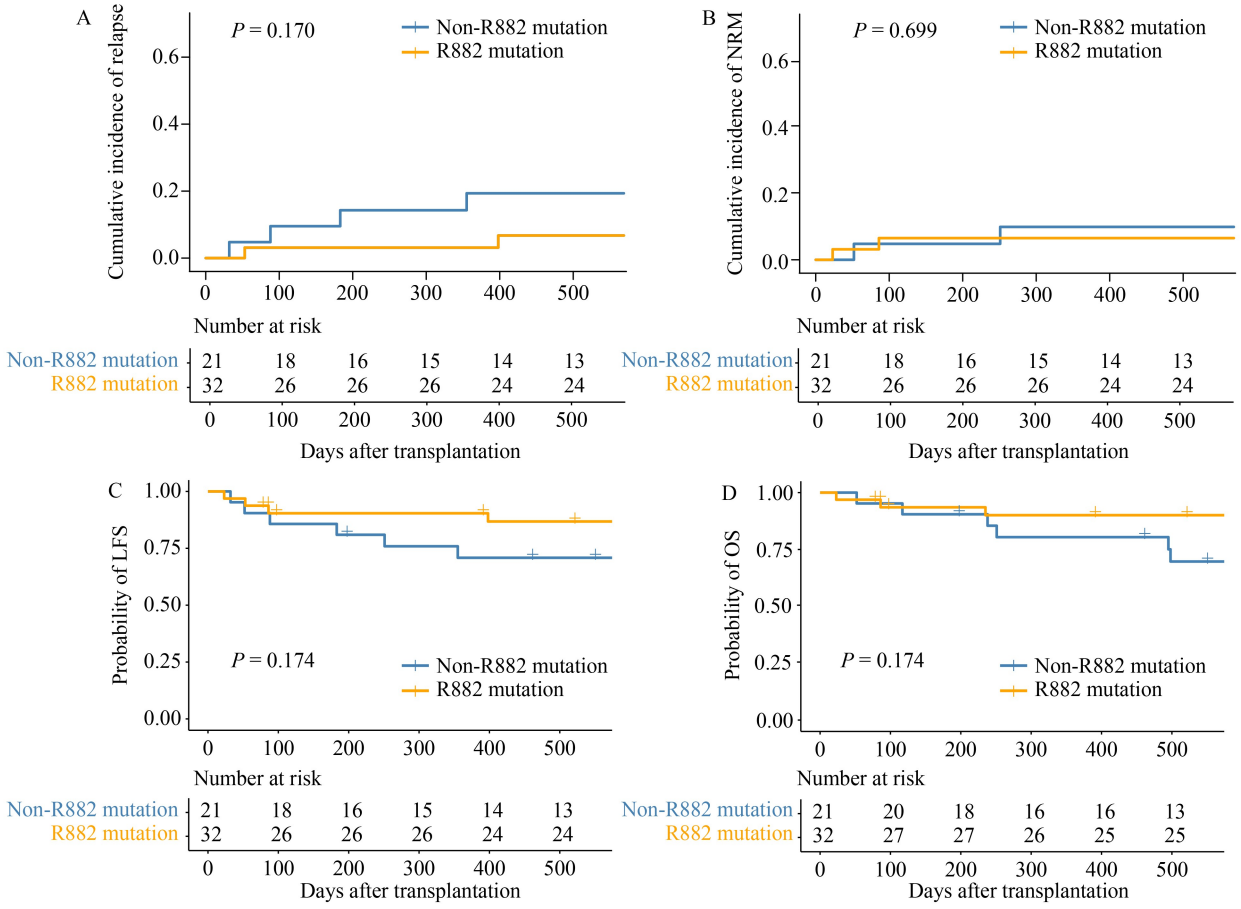

Fig. 2 1.5-year clinical outcomes after allo-HSCT in patients with triple-mutated AML and R882 or non-R882 mutations. (A) relapse, (B) non-relapse mortality, (C) leukemia-free survival, and (D) overall survival.

Xuan *et al*. [19] reported that women who were MRD negative before or after allo-HSCT did not benefit from sorafenib maintenance therapy. In the MORPHO study, researchers also observed that the benefits of gilteritinib maintenance were restricted to patients who were MRD positive before allo-HSCT [45]. Zhang *et al*. [46] reported that the clinical outcomes after HID HSCT were comparable between patients with AML with or without FLT3 mutation, suggesting that HID HSCT could partially overcome the poor prognosis of FLT3 mutation. In the present work, more than half of the patients underwent HID HSCT. The patients who received regular MRD monitoring after allo-HSCT and those who were MRD positive all received preemptive therapy, which could also help prevent relapse in the absence of maintenance therapies. Given that the pathogenic mechanism may be different between patients with triple-mutated AML and those with *FLT3* mutation alone, the benefit of *FLT3* inhibitor maintenance therapy should be verified in patients with triple-mutated AML, particularly in the era of novel *FLT3* inhibitors (including quizartinib). However, we could not further identify its efficacy in patients with triple-mutated AML because of the small number of patients receiving sorafenib maintenance therapy.

Owing to the paucity of patients with triple-mutated AML, our multicenter study was limited by the sample size. Thus, we did not restrict the therapy protocols to induction and consolidation regimens, which might have had an impact on posttransplant outcomes. Nonetheless, 94.3% (n = 50) of the patients achieved CR, with 71.7% (n = 38) being MRD negative before allo-HSCT. The disease status before allo-HSCT was uniform, which may have partially offset the impact of different induction and consolidation protocols before allo-HSCT.

In conclusion, this large-scale real-world study indicated that patients with triple-mutated AML in CR could benefit from allo-HSCT. The results confirmed the efficacy and safety of allo-HSCT in a disease-specific population of patients with triple-mutated AML. Therefore, sequential allo-HSCT might provide an ideal workflow for this AML subset. Further prospective multicenter randomized controlled trials could help confirm our results. In addition, novel pre- and post-transplant treatments, such as new *FLT3* inhibitors, menin inhibitors, and immunomodulation, may further improve

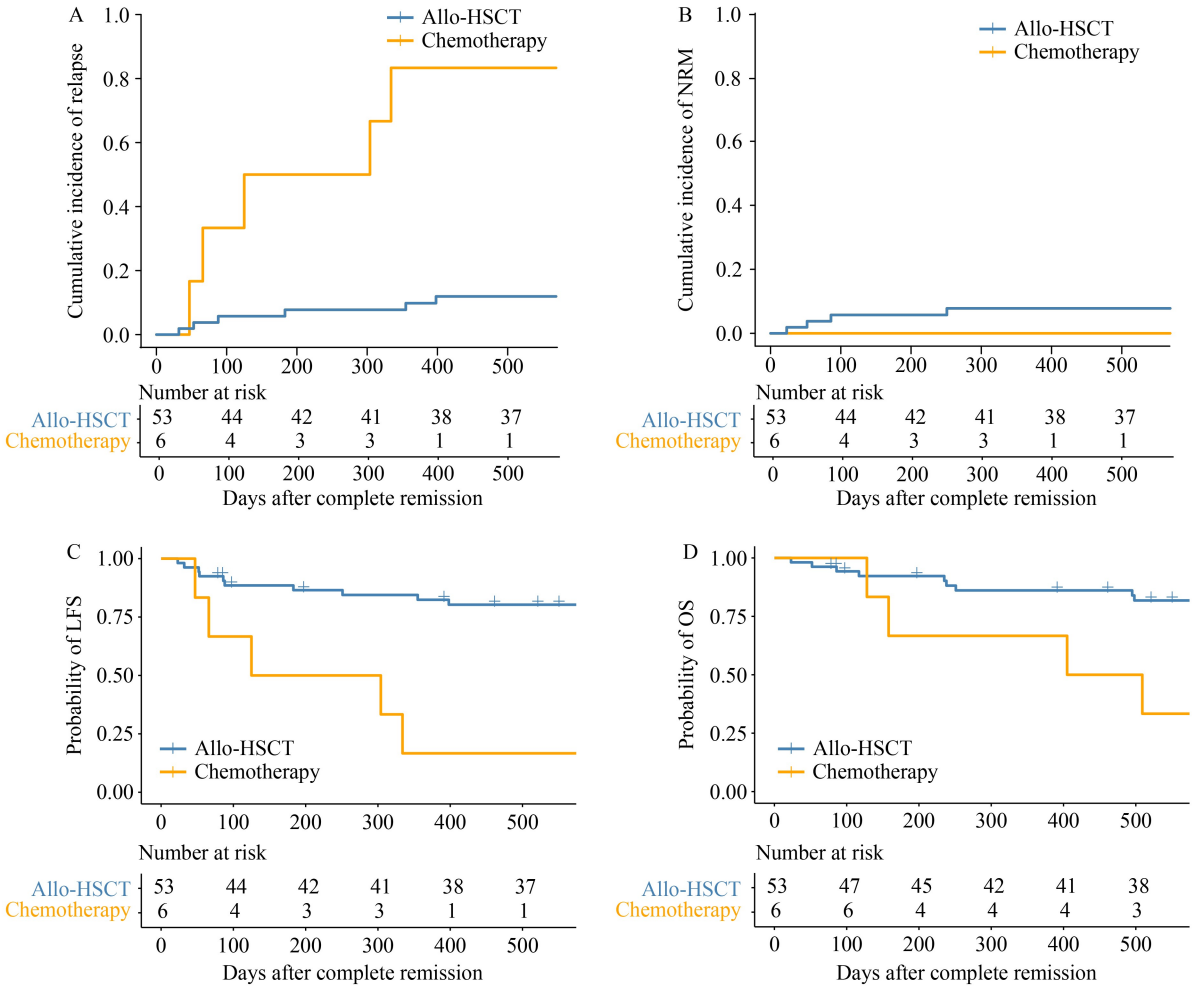

Fig. 3 1.5-year clinical outcomes after the first remission in patients with triple-mutated AML who received allo-HSCT or consolidation chemotherapy alone. (A) relapse, (B) nonrelapse mortality, (C) leukemia-free survival, and (D) overall survival.

Table 2 Multivariable analysis of clinical outcomes following allo-HSCT in patients with triple-muted AML

Outcomes	HR (95% CI)	*P*
Treatment failure as defined by leukemia-free survival		
Courses of induction chemotherapy before first CR		
1	1	0.010
> 1	5.77 (1.51–21.97)	
Disease status before allo-HSCT		
CR1	1	0.003
⩾ CR1	14.07 (2.51–78.89)	
Treatment failure as defined by overall survival		
Courses of induction chemotherapy before first CR		
1	1	0.012
> 1	5.55 (1.46–21.19)	
Disease status before allo-HSCT		
CR1	1	0.002
⩾ CR1	14.45 (2.58–80.95)	

CR, complete remission; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease.

the outcomes of patients with triple-mutated AML.

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Compliance with ethics guidelines

Conflicts of interset Wenxuan Huo, Yifan Shen, Jiayu Huang, Yang Yang, Shuang Fan, Xiaosu Zhao, Qi Wen, Luxiang Wang, Chuanhe Jiang, Yang Cao, Xiaodong Mo, Yang Xu, and Xiaoxia Hu declare no competing interests.

The study was approved by the Institutional Review Board of Peking University People's Hospital and the study was performed in accordance with the ethical standards as laid down in the 1964 *Declaration of Helsinki* and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants or their guardians included in the study.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-024-1091-5 and is accessible for authorized users.

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