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Prognostic impact of co-mutations in adults with *IDH1/2*-mutated acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) is characterized by the accumulation of cytogenetic and molecular abnormalities. Isocitrate dehydrogenase 1 and 2 (*IDH1/2*) mutations occur in 11% to 20% of adults with AML. The outcome of *IDH1/2*-mutated AML is heterogeneous and affected by co-mutational patterns. We retrospectively analyzed 118 patients with *IDH1/2*-mutated AML who were retrieved from 1597 patients newly diagnosed with AML and treated with intensive chemotherapy. Univariate analysis revealed the *NPM1* mutation was a favorable factor (p = 0.019) for overall survival (OS), whereas the *DNMT3A* mutation was consistently associated with a poor outcome (3-year OS, 52.0%; 3-year relapse-free survival [RFS], 44.8%; and 3-year cumulative incidence of relapse [CIR], 42.6%). Interestingly, the *DNMT3A* mutation still identified patients with a poorer prognosis, even when measurable residual disease (MRD) was negative after 2 courses of chemotherapy. In a multivariate regression model, age, *DNMT3A* mutation and MRD positivity were retained as independent adverse markers for OS, RFS, and CIR. In the absence of the *DNMT3A* or *FLT3*-ITD mutations, the *NPM1* mutation identified patients with a very favorable OS (3-year OS, 96.3% and 86.3%, respectively). Finally, hematopoietic stem cell transplantation in first complete remission significantly improved RFS (p = 0.015) and there was a trend toward improvement in OS (p = 0.282) for patients with the *DNMT3A* mutation but it did not benefit 2 subgroups with the *IDH1/2*+/*NPM1*+/*DNMT3A*– and *IDH1/2*+/*NPM1*+/*FLT3*-ITD– genotypes. In summary, this study provides a reference for risk stratification and treatment implications for patients with *IDH1/2*-mutated AML as well as for comparison with results of IDH inhibitor- or venetoclax-based combination therapy.

Key Words: Acute myeloid leukemia; Co-mutation; DNMT3A mutation; IDH1/2 mutation; Measurable residual disease

1. INTRODUCTION

Acute myeloid leukemia (AML) is a heterogenous hematological malignancy and is characterized by the accumulation of cytogenetic and molecular abnormalities.[1,2] Mutations in the isocitrate dehydrogenase 1 and 2 genes (*IDH1/2*) account for 11% to 20% of adults with AML.[2,3] *IDH1/2* mutation exerts an enzymatic activity that reduces α-ketoglutarate (α-KG) to the oncometabolite 2-hydroxyglutarate. Accumulation of the oncometabolite competitively inhibits α-KG-dependent enzymatic reactions, thus leading to global alterations in DNA and histone methylation.[4–6] This could finally block hematopoietic differentiation and promote transformation to leukemia cells.[6] There has been increased interest in this subtype of AML with the advent of IDH inhibitors, such as ivosidenib and enasidenib, in the upfront and refractory/relapsed (R/R) settings.[7–10] Despite the well-established pathophysiological role of the *IDH1/2* mutation, there remain uncertainties regarding the prognostic significance of the *IDH1/2* mutation in AML.[11–14] In particular, diverse combinations of gene mutations further complicate this situation, generating substantial heterogeneity of outcomes in patients with *IDH1/2*-mutated AML.[1,15]

IDH1/2 mutation frequently co-occurs with *NPM1*, *DNMT3A*, and *FLT3*-ITD mutations.[2,3,12] *NPM1* mutation was demonstrated as a favorable prognostic factor for patients with *IDH1/2*-mutated AML.[13,15] The profound study conducted by Papaemmanuil et al[1] on genomic prognosis in AML revealed the poorer outcome conferred by co-mutation of *IDH2*-R140 and *DNMT3A*. However, inconsistent results regarding the prognostic impact of the *DNMT3A* mutation were reported, including a negative impact or no impact.[16,17] This might stem from the different types of *IDH* mutations and co-mutation contexts.[13,15,18] Complex genetic interplay and whether 3-way gene interactions further stratify the clinical outcome of patients with

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Written informed consent was attained from all patients.

J.W.: Advisor for AbbVie. The other authors declare that they have no conflict of interest.

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The detailed clinical and mutation data of the current study are provided upon rational request.

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IDH1/2-mutated AML has not been fully elucidated.[15,18] Triple mutation of *IDH1/2*, *DNMT3A*, and *NPM1* was reported to predict poorer survival of AML.[18] *FLT3*-ITD, only when accompanied by the *NPM1* mutation, was associated with an unfavorable outcome in patients with *IDH1/2*-mutated AML.[3] However, these studies did not examine how combinations of mutations should inform treatment decisions, particularly allogeneic hematopoietic stem cell transplantation (allo-HSCT) at the first complete remission (CR1).[15,18]

Increasing evidence has demonstrated that the presence of measurable residual disease (MRD) during remission is a powerful indicator for relapse and poorer survival in AML.[19–22] This also raises the question as to whether patients designated as high risk by baseline markers have a relatively favorable outcome if they achieve CR that has no MRD, and whether the absence of MRD can be used to spare patients with an adverse genetic risk from HSCT.[23,24] The *IDH2*-R140 mutation is a clonal hematopoiesis-relevant mutation, which has no prognostic value as a marker of MRD.[25,26] Unlike specific mutational markers, assessment of MRD based on multiparameter flow cytometry (MFC) is much more applicable and widely used.[22] However, little data regarding the prognostic role of MFC-MRD and the interaction with baseline genetic markers are available in patients with *IDH1/2*-mutated AML.

Here, we aimed to address the heterogeneity of outcomes of 118 patients with *IDH1/2*-mutated AML, among 1597 patients newly diagnosed with AML, by analyzing the prognostic significance of pre-treatment molecular factors and post-treatment MRD status, together with an assessment of the effect of HSCT in molecular subgroups.

2. METHODS

2.1. Patients

We retrospectively reviewed 1597 adults (≥18 years) with non-acute promyelocytic AML diagnosed between January 2016 and March 2023, according to the 2016 WHO classification in the Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences. The exclusion criteria were as follows: patients without an available karyotype or targeted sequencing data (n = 53), patients who did not receive chemotherapy or received low-intensity chemotherapy (n = 214), and patients who died within 30 days after the initiation of induction therapy or were followed up for less than 30 days (n = 247). All patients were screened for *IDH1/2* mutations and 118 patients with *IDH1/2*-mutated AML were included in the study. Most patients (72.0%) were treated in clinical trials: NCT02432872 (n = 11) and NCT03021330 (n = 74).[27] Treatment details of the remaining patients are summarized in Table S1, http://links.lww.com/BS/A116. Allo-HSCT was recommended based on the criteria of the National Comprehensive Cancer Network.[28] Written informed consent was obtained from all patients in accordance with the principles of the Declaration of Helsinki and approved by the Hospital Ethics Committee (NKRDP2021005-EC-2).

2.2. MRD testing based on multiparametric flow cytometry

Bone marrow samples were obtained after the second course of chemotherapy and processed according to the standard procedure at the central MFC laboratory in our institution.[27] The leukemia-associated immunophenotype-based and different from normal-based method was used to detect MRD. All monoclonal antibodies were purchased from Beckman Coulter (Miami, Florida) or BD Biosciences (San Jose, California). Processed samples with a minimum of 5×10^5 nucleated cells were assessed using a BD FACS Canto flow cytometer, and the results were analyzed using KALUZA software (Beckman Coulter). The sensitivity was 10^{-4} and any detectable MRD was defined as MRD-positive.[27]

2.3. Targeted DNA sequencing and data processing

All targeted DNA sequencing data were provided by the Clinical Testing Center of our institution. The panel gene list and data processing have been described previously.[29] Briefly, sequencing data were processed using hg19 as a reference genome to obtain VCF files for downstream analysis at the Clinical Testing Center. ANNOVAR software was used to annotate the variants. The variants were then filtered using previously detailed criteria to avoid false positives. Finally, the mutational landscape was visualized using the maftools R package.

2.4. Statistical analysis

The Kruskal–Wallis test was used to compare continuous variables and the chi-square or Fisher exact test was used to compare categorical variables. Patient survival was analyzed using the Kaplan–Meier method and compared using the log-rank test. Overall survival (OS) was calculated from the time of diagnosis to the time of death or last follow-up. Relapse-free survival (RFS) was calculated from the time of the first remission to the time of relapse, death, or last follow-up. The cumulative incidence of relapse (CIR) was measured using the Fine-Gray model, where death was considered a competing risk. Initial prognostic variable selection was conducted using a univariate Cox regression model for OS and RFS, and Fine-Gray regression model for CIR, including demographic, clinical, cytogenetic risk, recurrent gene mutations (present in ≥10 patients), and MRD status. Baseline covariates with $p < 0.1$ and MRD status were subjected to multivariate Cox or Fine-Gray analysis using the backward stepwise procedure based on the Akaike information criterion. Allo-HSCT at CR1 was analyzed as a time-dependent variable and the effect was visualized with a Simon–Makuch plot.[30,31] The p values of the multiple comparisons were adjusted to calculate q values using the Benjamini and Hochberg method.[32] A $p < 0.05$ was considered to signify statistical significance. R software (version 4.3.1) was used for the statistical analysis.

3. RESULTS

3.1. Clinical characteristics and mutational landscape of the patients

A total of 118 patients with *IDH1/2*-mutated AML were analyzed in this study, including 56 with *IDH1*-mutated AML, 61 with the *IDH2*-mutation, and 1 with dual mutations of *IDH1* and *IDH2* (**Fig. 1A**). The median age of the cohort was 46.5 (range, 18–64) years. The majority of patients (89.8%) had intermediate cytogenetic risk.[33] According to the European Leukemia Network 2022 risk stratification,[34] 28.0%, 44.0%, and 28.0% of patients were categorized into the favorable, intermediate, and adverse risk groups, respectively. Ninety (76.3%) patients achieved CR or CR with incomplete hematologic recovery (CRi) within 2 courses of induction therapy, of whom 72 (83.7%, 72/86) were MRD-negative after the second course of chemotherapy. An additional 8 patients achieved CR/CRi after salvage chemotherapy, and another 5 achieved CR/CRi after salvage HSCT (Table 1). The median OS for the 118 patients was not reached, while the median RFS for 103 patients who finally achieved CR/CRi was 46.3 (95% confidence interval [CI], 38.8–not applicable) months with a median follow-up of 41.9 (95% CI, 33.2–47.2) months. Notably, the baseline characteristics and clinical outcomes were similar between patients with the *IDH1* and *IDH2* mutations (Table S2 and Fig. S1, http://links.lww.com/BS/A116).

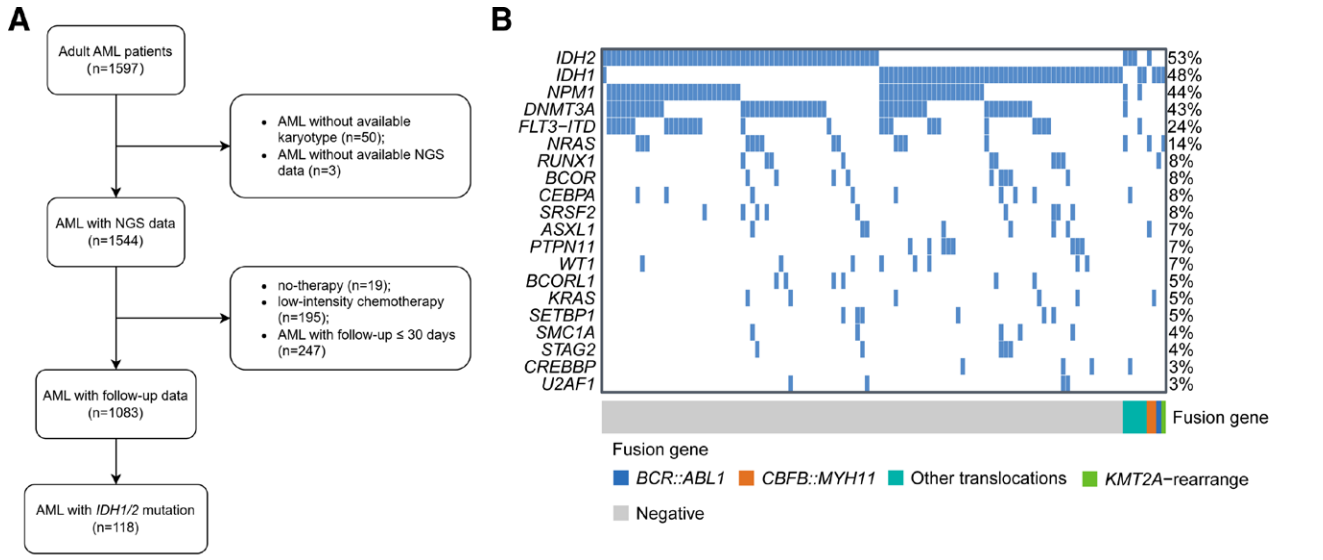

Figure 1. Flow diagram of the study population (A) and mutational landscape of the entire cohort with *IDH1/2*-mutated AML (B). Each column represents a patient with mutations being shown in blue box. AML = acute myeloid leukemia, NGS = next-generation sequencing.

The majority of *IDH1* mutations occurred at residue R132 (89.3%, 50/56), whereas 70.5% (43/61) and 29.5% (18/61) of *IDH2* mutations occurred at residues R140 and R172, respectively. The frequency of each *IDH* mutation type was consistent with that reported in previous studies.[3,12,35] One patient had a dual mutation, *IDH1*-R132 and *IDH2*-R140. A total of 296 co-mutations were detected in the 118 patients with *IDH1/2*-mutated AML. The five most frequent mutations were *NPM1* (44.1%), *DNMT3A* (43.2%), *FLT3*-ITD (23.7%), *NRAS* (13.6%), and *RUNX1* (8.5%) (**Fig. 1B**). The incidence of these 5 common mutations did not differ between patients with *IDH1*- and *IDH2*-mutations (Table S2, http://links.lww.com/BS/A116).

3.2. Prognostic factors for outcome

Univariate Cox regression analysis was performed to identify the prognostic factors for the patients with *IDH1/2*-mutated AML. Cytogenetic risk poorly stratified patients with *IDH1/2*-mutated AML with intermediate and adverse risks. Age and the *DNMT3A* mutation were associated with poorer OS, poorer RFS, and higher CIR (**Fig. 2A**). The OS, RFS, and CIR at 3 years were 52.0% (95% CI, 38.9–69.5), 44.8% (95% CI, 31.0–64.8), and 42.6% (95% CI, 28.9–59.5), respectively, for patients with the *DNMT3A* mutation, compared with 77.9% (95% CI, 68.2–88.9, p = 0.007), 73.0% (95% CI, 62.0–85.9, p = 0.003), and 23.2% (95% CI, 14.1–36.7, p = 0.008), respectively, for patients with wild-type *DNMT3A* (**Fig. 2B–D**). *RUNX1* mutation (p < 0.001) was an adverse factor for OS. *NPM1* mutation was the only favorable factor for OS (p = 0.019) in patients with *IDH1/2*-mutated AML (**Fig. 2A** and Fig. S2, http://links.lww.com/BS/A116). Apart from the pre-treatment factors, post-second course MRD positivity was associated with trends towards poorer OS (p = 0.054), poorer RFS (p = 0.097), and higher CIR (p = 0.103) (**Fig. 2A** and Fig. S3, http://links.lww.com/BS/A116).

Multivariate models were then fitted to determine the independent prognostic factors, where age, sex, clinical variables (eg, white blood cell count), cytogenetic risk, mutational subgroups, and MRD status were considered. Compared with the wild-type *DNMT3A* group, mutated *DNMT3A* independently predicted poorer OS (hazard ratio [HR] = 3.42, 95% CI, 1.41–8.30, p = 0.005), poorer RFS (HR = 3.61, 95% CI, 1.77–7.39, p < 0.001), and higher CIR (HR = 3.68, 95% CI, 1.64–8.26, p = 0.002). Also, MRD positivity was retained as an independent adverse factor for OS (HR = 3.72, 95% CI, 1.37–10.10, p = 0.019), RFS (HR = 3.14, 95% CI, 1.35–7.32, p = 0.015), and CIR (HR = 3.65, 95% CI, 1.50–8.90, p = 0.004) (**Fig. 2E**).

3.3. Prognostic impact of combinations of baseline gene mutations and post-treatment MRD status

To assess the interactions between pre-treatment gene mutations and post-treatment MRD, the outcomes were analyzed according to combinations of these two aspects. Within the *NPM1* mutated subgroup, patients with a positive MRD had a significantly poorer OS (p = 0.008) and RFS (p = 0.032) and a 3-year CIR of 58.3% (95% CI, 29.2–89.1) (**Fig. 3A–C**). Notably, the *DNMT3A* mutation still identified patients with a poorer OS (q = 0.016), poorer RFS (q = 0.002), and a higher CIR (q = 0.004) in the MRD-negative subgroup. As expected, the dual presence of the *DNMT3A* mutation at diagnosis and MRD after the second course predicted a very poor OS and RFS and high CIR (vs *DNMT3A*–/MRD–, q = 0.002 for OS, q < 0.001 for RFS, q < 0.001 for CIR; vs *DNMT3A*+/MRD–, q = 0.214 for OS, q = 0.164 for RFS, q = 0.019 for CIR; vs *DNMT3A*–/MRD+, q = 0.250 for OS, q = 0.164 for RFS, q = 0.072 for CIR) (**Fig. 3D–F**). Patients with an *FLT3*-ITD mutation and subsequent positive MRD had very poor outcomes compared to those with isolated *FLT3*-ITD or MRD positivity and dual negativity (OS, p = 0.042; RFS, p = 0.014; CIR, p = 0.006) (**Fig. 3G–I**).

3.4. Prognostic impact of different co-mutational patterns of NPM1, DNMT3A, and FLT3-ITD

Correlations between the *IDH1/2* mutation and *NPM1*, *DNMT3A*, and *FLT3*-ITD mutations have long been recognized,[3,12] and were also identified in the current study (Fig. S4, http://links.lww.com/BS/A116). Therefore, the impact of different combinations of these gene mutations on the outcome of patients with *IDH1/2*-mutated AML was analyzed. Surprisingly, patients with the *IDH1/2*+/*NPM1*+/*DNMT3A*– genotype had a favorable outcome (3-year OS: 96.3%, 95% CI, 89.4–100.0; 3-year RFS: 84.3%, 95% CI, 71.2–99.9; and 3-year CIR: 15.7%, 95% CI, 6.1–36.9) compared to patients with a single mutation of *IDH1/2*, dual mutation of *IDH1/2* and *DNMT3A*, and triple mutation of *IDH1/2*, *NPM1*, and *DNMT3A* (OS: p = 0.004;

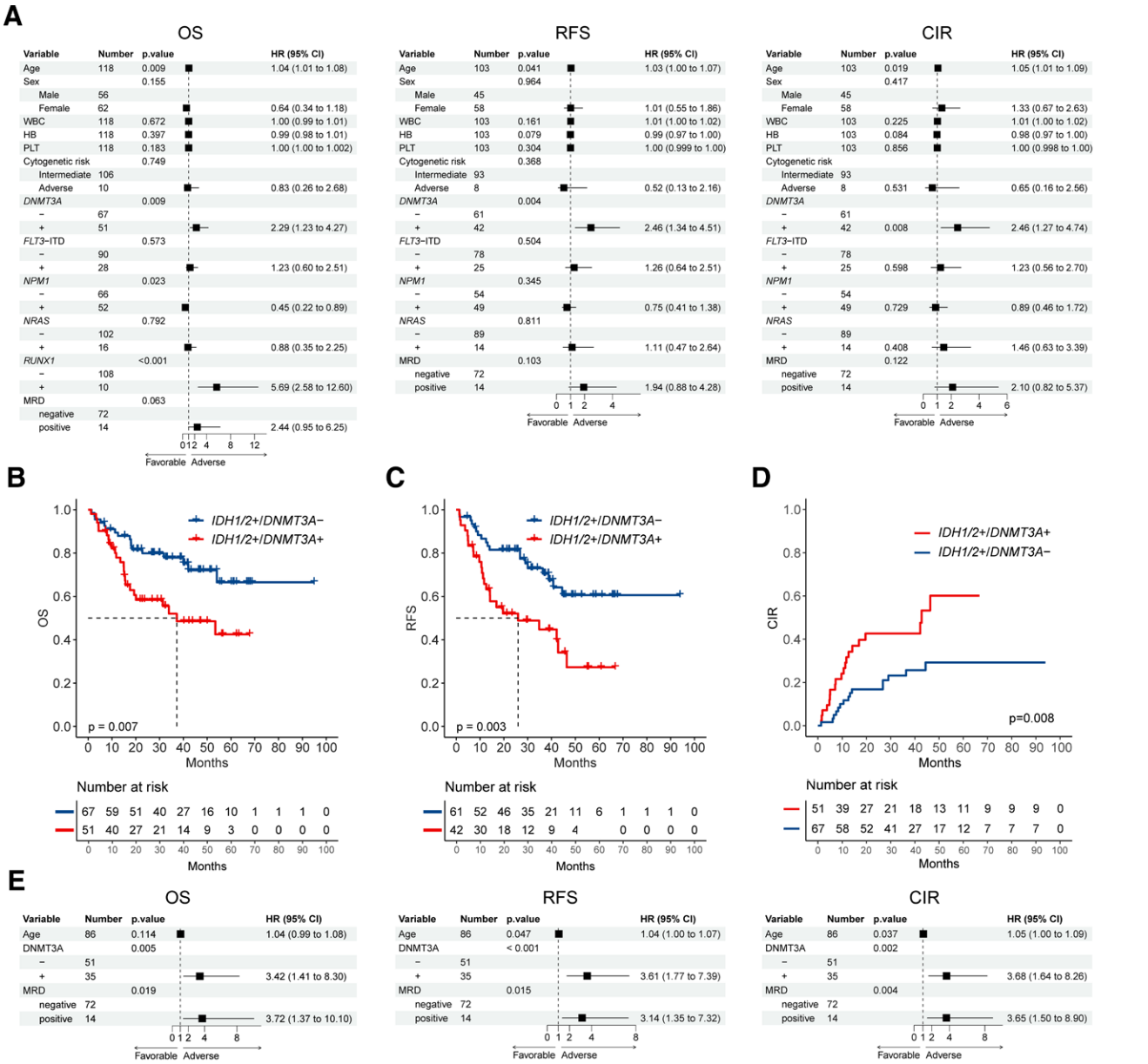

Figure 2. Factors associated with prognosis of AML patients with *IDH1/2* mutations. (A) Forest plots showing results of univariate regression analysis on OS, RFS, and CIR. (B–D) Kaplan–Meier plots showing impact of *DNMT3A* mutation on OS (B), RFS (C), and CIR (D). (E) Forest plots showing results of multivariate regression analysis on OS, RFS, and CIR. *Cytogenetic risk stratification based on refined Medical Research Council system. + = mutated, − = wide-type, AML = acute myeloid leukemia, CI = confidence interval, CIR = cumulative incidence of relapse, HB = hemoglobin level, HR = hazard ratio, MRD = measurable residual disease post 2 courses of chemotherapy, OS = overall survival, PLT = platelet count, RFS = relapse-free survival, WBC = white blood cell count.

RFS: p = 0.014; and CIR: p = 0.035) (**Fig. 4A–C**). Likewise, patients with the *IDH1/2*+/*NPM1*+/*FLT3*-ITD− genotype had superior OS (3-year OS: 86.3%, 95% CI, 74.6–99.8) compared to patients with the *IDH1/2*+/*NPM1*−/*FLT3*-ITD− (q = 0.040) and *IDH1/2*+/*NPM1*−/*FLT3*-ITD+ genotypes (q = 0.040), whereas the RFS (p = 0.522) and CIR (p = 0.747) did not differ significantly among the 4 subgroups. Additionally, triple mutations in *IDH1/2*, *NPM1*, and *FLT3*-ITD also correlated with a trend toward a more superior OS than for those with dual mutations in *IDH1/2* and *FLT3*-ITD (q = 0.295) (**Fig. 4D–F**). Patients with triple-mutated *IDH1/2*, *DNMT3A*, and *FLT3*-ITD had extremely poor OS (median: 13.5 months, 95% CI, 7.9–not applicable, p = 0.002) and RFS (median: 7.9 months, 95% CI, 3.7–not applicable, p < 0.001) compared to those with other combination of mutations (**Fig. 4G–I**).

3.5. Effect of HSCT at CR1

Overall, 48 patients underwent HSCT at CR1 (Table 1). When analyzed as a time-dependent variable, allo-HSCT at CR1 improved RFS (HR = 0.27, 95% CI, 0.13–0.59, p < 0.001), and trended toward an improvement in OS (HR = 0.59, 95% CI, 0.26–1.37, p = 0.237) for patients with the *IDH1/2* mutation (Fig. S5, http://links.lww.com/BS/A116). Given the consistently poor outcomes of patients with AML and concurrent *IDH1/2* and *DNMT3A* mutations, whether HSCT

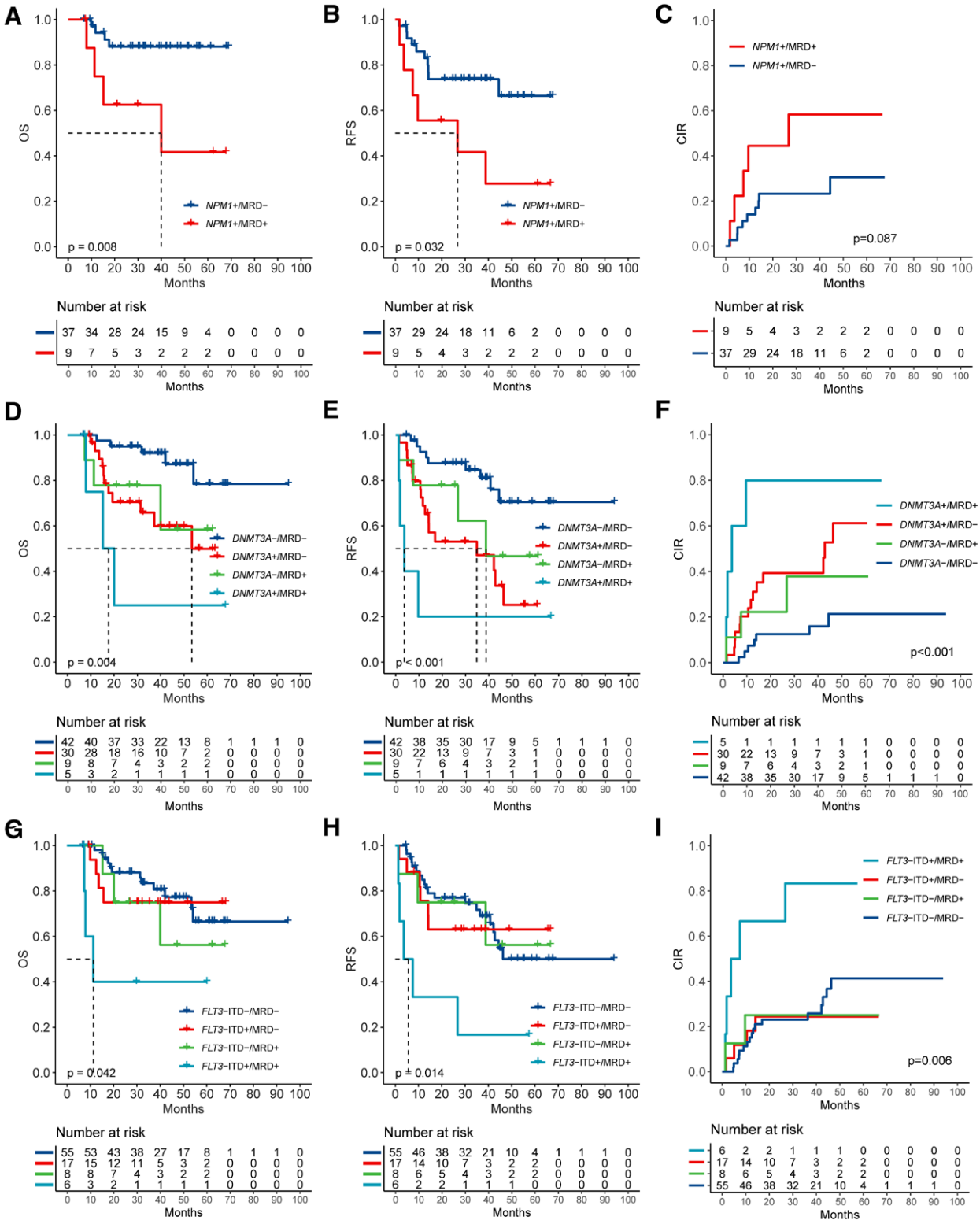

Figure 3. Prognostic impact of combinations of baseline gene mutations and post-treatment MRD status. (A–C) OS (A), RFS (B), and CIR (C) of *NPM1*-mutated patients grouped by MRD status. (D–F) OS (D), RFS (E), and CIR (F) of patients grouped by *DNMT3A* mutational and MRD status. (G–I) OS (G), RFS (H), and CIR (I) of patients grouped by *FLT3*-ITD mutational and MRD status. CIR = cumulative incidence of relapse, *DNMT3A*− = wide-type *DNMT3A*; *DNMT3A*+ = mutated *DNMT3A*, *FLT3*-ITD− = negative for *FLT3*-ITD mutation, *FLT3*-ITD+ = positive for *FLT3*-ITD mutation, MRD = measurable residual disease, MRD− = negative for MRD, MRD+ = positive for MRD, *NPM1*+ = mutated *NPM1,* OS = overall survival, RFS = relapse-free survival.

at CR1 could ameliorate survival in this high-risk group was assessed. Among patients who underwent HSCT at CR1, the *DNMT3A* mutation lost prognostic significance (OS, $p = 0.594$; RFS, $p = 0.583$; CIR, $p = 0.942$). However, the *DNMT3A* mutation still conferred poorer RFS ($p = 0.036$) and a trend toward poorer OS ($p = 0.091$), and higher CIR ($p = 0.074$) in patients who did not receive HSCT at CR1 (Fig. S6, http://links.lww.com/BS/A116). Overall, 13 patients harboring concomitant

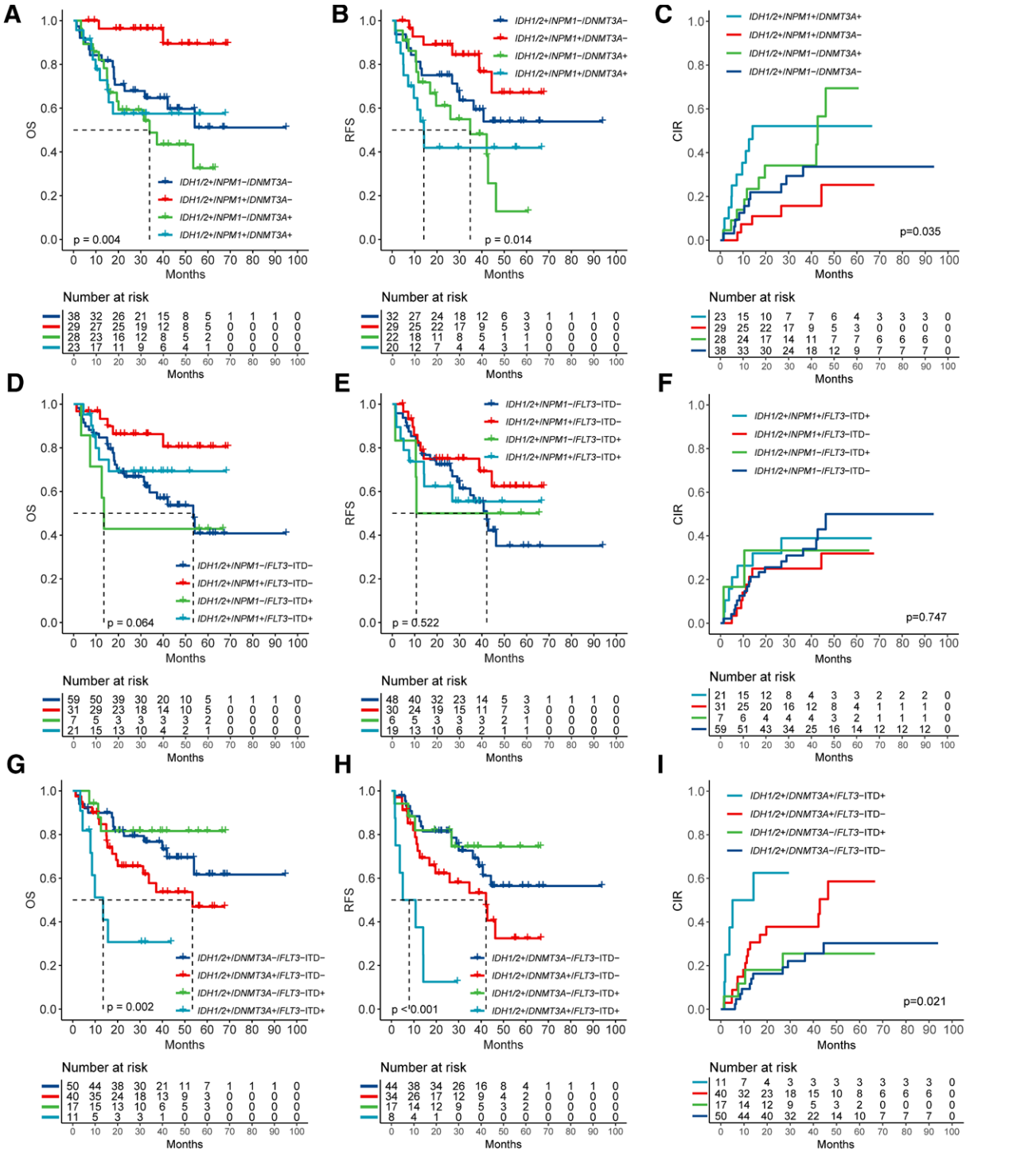

Figure 4. Prognostic impact of different co-mutational patterns of *NPM1*, *DNMT3A,* and *FLT3*-ITD. (A–C) OS (A), RFS (B), and CIR (C) of patients grouped by *NPM1* and *DNMT3A* mutational status. (D–F) OS (D), RFS (E), and CIR (F) of patients grouped by *NPM1* and *FLT3*-ITD mutational status. (G–I) OS (G), RFS (H), and CIR (I) of patients grouped by *DNMT3A* and *FLT3*-ITD mutational status. + = mutated, − = wild-type, CIR = cumulative incidence of relapse, OS = overall survival, RFS = relapse-free survival.

IDH1/2 and *DNMT3A* mutations underwent HSCT at CR1. The baseline characteristics, CR/CRi, and MRD-negative rates were similar between the HSCT and non-HSCT groups (Table S3, http://links.lww.com/BS/A116). HSCT at CR1 was associated with improved RFS (HR = 0.23, 95% CI, 0.07–0.81, $p = 0.015$) and a trend toward prolonged OS (HR = 0.49, 95% CI, 0.14–1.76, $p = 0.282$) (**Fig. 5**). Moreover, in a multivariate Cox regression model accounting for age, clinical and molecular variables, HSCT at CR1 independently predicted improved RFS (HR = 0.14, 95% CI, 0.04–0.55, $p = 0.005$) but not OS (Table S4, http://links.lww.com/BS/A116).

In contrast, no benefit of transplantation at CR1 was observed in the favorable group with the *IDH1/2+/NPM1+/DNMT3A−* genotype (OS: $p = 0.999$; RFS: $p = 0.442$) (Fig. S7, http://links.lww.com/BS/A116). Likewise, OS ($p = 0.589$) was similar between the HSCT and non-HSCT groups among patients with

Table 1

Characteristics of AML patients with *IDH1/2* mutations.

	Overall
N	118
Age (median [range])	46.5 [18.0–64.0]
Sex (%)	
Male	56/118 (47.5)
Female	62/118 (52.5)
WBC (median [range], $\times 10^9$/L)	5.98 [0.580, 121]
HB (median [range], g/L)	84.5 [49.0, 156]
PLT (median [range], $\times 10^9$/L)	70.0 [14.0, 1430]
Cytogenetic risk*	
Favorable	2 (1.7%)
Intermediate	106 (89.8%)
Adverse	10 (8.5%)
ELN2022 risk (%)	
Favorable	33/118 (28.0)
Intermediate	52/118 (44.0)
Adverse	33/118 (28.0)
DNMT3A+ (%)	51/118 (43.2)
NPM1+ (%)	52/118 (44.1)
FLT3-ITD+ (%)	28/118 (23.7)
CR/CRi (%)	90/118 (76.3)
Negative MRD (%)	72/86 (83.7)
HSCT at CR1 (%)	48/98 (49.0)

+ = mutation, AML = acute myeloid leukemia, CR = complete remission, CR1 = first complete remission, CRi = CR with incomplete hematologic recovery, ELN2022 risk = 2022 European LeukemiaNet risk stratification, HB = hemoglobin level, HSCT = hematopoietic stem cell transplantation, MRD = measurable residual disease after 2 courses of chemotherapy, PLT = platelet count, WBC = white blood cell count.

*Cytogenetic risk stratification based on refined Medical Research Council system.

the *IDH1/2*+/*NPM1*+/*FLT3*-ITD– genotype despite a trend toward an improved RFS (HR = 0.18, 95% CI, 0.02–1.46, $p = 0.082$) (Fig. S8, http://links.lww.com/BS/A116). Two patients in the *IDH1/2*+/*DNMT3A*+/*FLT3*-ITD+ subgroup underwent HSCT at CR1 but died within 1 year after transplantation.

4. DISCUSSION

In this study, the clinical outcomes of patients with *IDH1/2*-mutated AML were comprehensively analyzed and the *DNMT3A* mutation and MRD positivity were identified as adverse prognostic factors. HSCT at CR1 mitigated the negative effects of the *DNMT3A* mutation. Also, we identified 2 molecular subgroups with the genotypes *IDH1/2*+/*NPM1*+/*DNMT3A*– and *IDH1/2*+/*NPM1*+/*FLT3*-ITD– that exhibited superior OS. These patients could benefit from a chemotherapy-based consolidation regimen.

Notably, a strong association between the *DNMT3A* mutation and inferior outcomes in patients with *IDH1/2*-mutated AML was confirmed. Moreover, the adverse effects of the *DNMT3A* mutation were not abrogated by the *NPM1* mutation (**Fig. 4A–C**).[18] Previous studies revealed that HSCT at CR1 could benefit the outcome of patients with *IDH1/2*-mutated AML; however, few studies have evaluated its role in the *DNMT3A*-mutated subgroup.[15] Zhang et al[17] reported that patients with dual mutations in *DNMT3A* and *IDH1/2* did not benefit from HSCT. However, their study was limited by a small sample size. We demonstrated that HSCT at CR1 significantly improved RFS in patients with co-mutated *IDH1/2* and *DNMT3A* while there was a trend toward a prolonged OS. The absence of a statistically significant improvement in OS could be explained by the fact that a considerable proportion of patients with relapsed disease responded well to salvage HSCT or novel therapies (salvage HSCT, n = 5; clinical trials, n = 2). Single-cell DNA analysis revealed that co-occurring *DNMT3A-IDH* mutations were associated with clonal dominance, which might underpin clonal sweep at disease recurrence and confer a poor prognosis.[36,37] The concomitant loss of *Dnmt3a* and mutation in *Idh2* in a murine model resulted in accelerated onset of disease and a more severe phenotype of myeloid malignancies through epigenomic and metabolomic dysregulation.[38] Considering the promising results of IDH inhibitors in the upfront and R/R settings, together with *IDH1/2* and *DNMT3A* mutations as potential immunotherapeutic targets,[7–10] prospective trials are

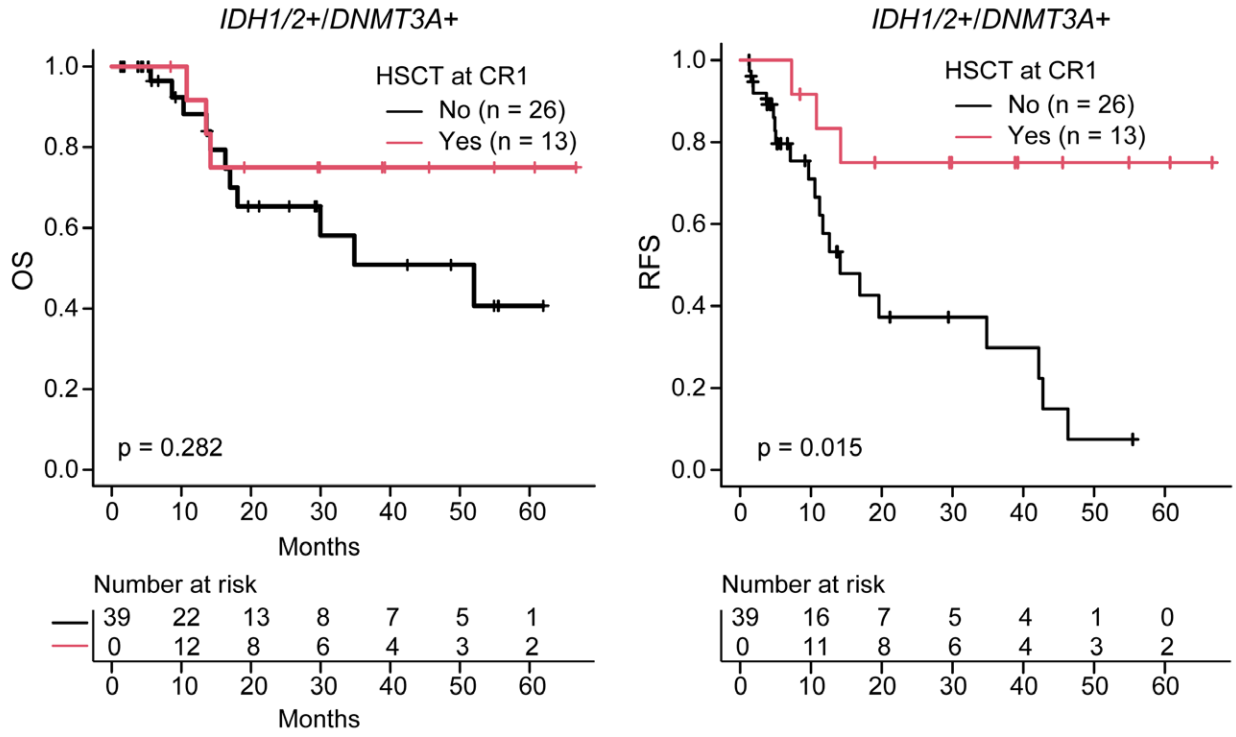

Figure 5. The effect of allogeneic HSCT at CR1 in patients with *DNMT3A* mutation. Patients who did not achieve CR or attained CR after HSCT were excluded from the analysis. CR1 = first complete remission, HSCT = hematopoietic stem cell transplantation, OS = overall survival, RFS = relapse-free survival.

required to examine the efficacy of combination regimens based on these novel therapies in this high-risk subgroup.

Post-treatment MRD serves as another useful prognostic indicator for AML.[21,22] Intriguingly, MFC-based MRD cannot entirely capture the negative impact of the *DNMT3A* mutation at presentation. *IDH1*-R132 and *IDH2*-R172 mutation status at HSCT have been suggested to predict a higher risk of relapse.[26] Given their frequent co-occurrence, the *NPM1* and *FLT3*-ITD mutations can also serve as MRD markers for patients with *IDH1/2*-mutated AML, allowing the identification of those at high risk.[24,25,39] The combined use of MFC-based and molecular MRD might increase sensitivity,[20] which, along with the interaction between baseline factors, warrants further investigation. Nevertheless, MFC-MRD positivity identified patients with poorer outcome in *NPM1*- and *FLT3*-ITD–mutated subgroup, which were supposed to confer favorable and neutral effects on survival, respectively.[15] Both of the subgroups had a 3-year CIR of over 40% and might require HSCT at CR1.[23] Taken together, our study highlights the importance of an integrated analysis of baseline genetic markers and post-treatment MFC-MRD status in terms of risk stratification of patients with *IDH1/2*-mutated AML, and highlights the necessity for a more sensitive method to detect MRD.

Multiple co-occurring mutations cooperate in leukemogenesis and constitute a mutation context that should be considered when analyzing the prognostic value of specific mutations. Although *FLT3*-ITD had no impact on relapse or survival, triple mutations in *IDH1/2*, *DNMT3A*, and *FLT3*-ITD were associated with very poor outcomes, possibly driven by the adverse effects of concomitant *DNMT3A* and *FLT3*-ITD mutations. The number of patients in this subgroup was too small to analyze the effect of HSCT; however, an allograft at CR1 was recommended for patients with concurrent *DNMT3A* and *FLT3*-ITD mutations.[40] In addition, the *NPM1* mutation was not retained as an independent factor for OS in the multivariate analysis in our study. However, in the absence of *DNMT3A* or *FLT3*-ITD mutations, the *NPM1* mutation identified 2 groups of patients with a very favorable OS, whereas allografts at CR1 yielded no benefit. This is important because it discriminates between patients who do not require transplantation for consolidation, thus reducing the number of allo-HSCT procedures and reducing the disease burden while maintaining a consistent prognosis.[41]

The limitations of this study are as follows. First, the sample sizes of some co-mutated genotypes and patients who underwent allo-HSCT were small; therefore, caution should be exercised when interpreting the results. Second, we included only patients who were treated with intensive chemotherapy. The prognostic value of specific mutations may change in patients treated with low-intensity chemotherapy, IDH inhibitors, or venetoclax-based combination therapy, thus warranting further re-evaluation.[42]

In conclusion, the prognostic factors identified in our study, including the *DNMT3A* mutation, MRD status, *IDH1/2*+*/NPM1*+*/DNMT3A*– and *IDH1/2*+*/NPM1*+*/FLT3*-ITD– genotypes, are treatment informative for patients with *IDH1/2*-mutated AML, providing reference for future study design.

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ETHICAL APPROVAL

The study was in accordance with the Declaration of Helsinki and approved by the Hospital Ethics Committee (NKRDP2021005-EC-2).

AUTHOR CONTRIBUTIONS

J.W. was responsible for designing the study. A.L. was responsible for analyzing and interpreting the data and writing the report. W.L. revised the manuscript. All authors contributed to the collection and assembly of clinical and mutational data. C.Z., Y.L., S.W., K.L., B.G., X.G., Y.L, G.Z., J.Z., R.G., S.Q., B.L., Y.W., H.W., Y.M. provided the patients and materials. All authors reviewed the article and approved the final version.

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