RESEARCH ARTICLE

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The impact of *DNMT3A* variant allele frequency and two different comutations on patients with de novo cytogenetically normal acute myeloid leukemia

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Abstract

To refine the biological and prognostic significance of *DNMT3A* mutations in acute myeloid leukemia (AML), we assessed the impact of *DNMT3A* variant allele frequency (VAF) and its comutations in this study. Using targeted next-generation sequencing, we analyzed 171 adult patients with de novo cytogenetically normal AML for *DNMT3A* mutations and associated comutations. $DNMT3A^{mut}$ was detected in 35 patients. $DNMT3A^{mut}$ patients were divided into $DNMT3A^{High}$ and $DNMT3A^{Low}$ using a cut-off VAF value of 42%. We observed that $DNMT3A^{High}$ patients at diagnosis had increasing white blood cell (WBC) counts ($p < 0.001$) and a higher lactate dehydrogenase (LDH) level ($p = 0.027$), and were associated with lower complete remission (CR) rate ($p = 0.015$) and shorter overall survival (OS) ($p = 0.032$) than $DNMT3A^{Low}$ patients. We classified two different comutated genotypes, including $DNMT3A^{mut}NPM1^{mut}FLT3\text{-}ITD^{mut}$ and $DNMT3A^{mut}IDH1/IDH2^{mut}$. Patients with $DNMT3A^{mut}NPM1^{mut}FLT3\text{-}ITD^{mut}$ showed worse OS ($p = 0.026$) and relapse-free survival (RFS) ($p = 0.003$) than those with $DNMT3A^{mut}IDH1/IDH2^{mut}$, and showed a shorter OS ($p = 0.027$) than those with $DNMT3A^{wt}NPM1^{mut}FLT3\text{-}ITD^{mut}$. We also observed that patients with $DNMT3A^{mut}IDH1/IDH2^{mut}$ had higher platelet counts ($p = 0.009$) and a lower BM blast percentage ($p = 0.040$) than those with $DNMT3A^{wt}IDH1/IDH2^{mut}$. In multivariate analyses, $DNMT3A^{High}$ was independently associated with a lower CR rate (OR = 5.883; $p = 0.004$) and shorter OS (HR = 3.768; $p < 0.001$). $DNMT3A^{mut}NPM1^{mut}FLT3\text{-}ITD^{mut}$ independently affected worse OS (HR = 6.030; $p < 0.001$) and RFS (HR = 8.939; $p < 0.001$). Our findings might be potentially useful for predicting clinical outcomes.

KEYWORDS
acute myeloid leukemia, *DNMT3A* mutations, variant allele frequency

1 | INTRODUCTION

DNMT3A belongs to a family of *DNMTs*, including *DNMT1*, *DNMT3A*, and *DNMT3B*, which encodes a DNA methyltransferase that is thought to regulate de novo DNA methylation modification, rather than maintenance methylation.[1,2] By regulating DNA methylation, *DNMT3A* may regulate the growth of hematopoietic stem cells (HSCs) into a predominantly granulocytic lineage during normal hematopoiesis.[3–5] Studies in mice have demonstrated that deletion of *DNMT3A* could cause HSC persistent self-renewal and inefficient differentiation.[6–8]

Somatic *DNMT3A* mutations are observed in various types of adult myeloid and lymphoid neoplasms. They are more frequently occurred (23%–37%) in adult patients with cytogenetically normal acute myeloid leukemia (CN-AML),[9–14] but very rarely found in pediatric or adolescent blood cancers.[15–17] *DNMT3A* mutations are usually heterozygous in AML.[9,18–20] In leukemogenesis, the mutant protein can dimerize with wild-type DNMT3A, but homotetramers with more potent activity cannot be formed. The resulting low level of DNMT3A homotetramer results in significantly reduced methyltransferase activity and genome-wide hypomethylation in patients.[21–24] In contrast to wild-type DNMT3A, more recent research suggests that it could interact with EZH2, which is the catalytic component of Polycomb repressive complex 1, leading to the down-regulation of genes associated with hematopoietic differentiation.[25]

Although DNMT3A mutations are more common in clonal hematopoiesis and appear to be relatively early events in leukemogenesis, the clinical effect of *DNMT3A* mutations on CN-AML remains inconclusive. Previous studies vary regarding the impact of *DNMT3A* mutations. Some studies found a significantly worse overall or event-free survival (EFS).[9,20,26–33] Still, others found no significant association with overall and EFS.[11,18,34–36] These conflicting results may be due to the genetic heterogeneity of *DNMT3A*-mutated CN-AML. Hence, it is crucial to further refine the genetic subclassification for a better understanding of the clinical effect of *DNMT3A* mutations in adult CN-AML.

We analyzed a cohort of 171 adult patients with de novo CN-AML for *DNMT3A* mutations and associated comutations using targeted next-generation sequencing (NGS). We further investigated the clinical impact of *DNMT3A* variant allele frequency (VAF) and two different comutated genotypes on these patients, which might help to further refine biological and prognostic implications of *DNMT3A* mutations in de novo CN-AML.

2 | METHODS

2.1 | Patients group

We conducted a retrospective review of NGS analyses performed on clinical bone marrow samples from adult patients with De novo CN-AML who presented to The Secondary Hospital of Shanxi Medical University Hematology Center in China between February 2017 and January 2021 in this study. We identified 171 newly diagnosed adult patients with de novo CN-AML (90 males and 81 females; median age, 53 years; age range, 19–86 years). The clinical samples were unpaired design. All patients provided informed written consent. This study complied with the Helsinki declaration and was approved by the ethical board of the Second Hospital of Shanxi Medical University. The patient′s data including age, sex, hematological parameters, blasts in bone marrow aspirates, and prior history of cytotoxic chemotherapy or radiotherapy were obtained from the medical records at diagnosis. De novo AML defined the patient as having no antecedent myeloid malignancy, cytotoxic therapy or radiotherapy before the diagnosis. A total of 168 patients were subjected to anticancer therapies. Among these patients, 125 were treated with high-intensity induction chemotherapy regimens and 43 received low-intensity induction chemotherapy regimens. The dose and course of treatment were performed according to the Chinese Guidelines for the Diagnosis and Treatment of adult acute myeloid leukemia (non-acute promyelocytic leukemia) (2017 edition). Twenty-one patients without concomitant *DNMT3A* mutations but in the intermediate or adverse-risk group underwent allogous-hematopoietic stem cell transplantation (allo-HSCT) after complete remission (CR). The CR and recurrence were determined according to the ELN2017 recommendations. Overall survival (OS) was calculated as the period from the date of diagnosis to death or to the date of last observation. Relapse-free survival (RFS) was calculated as the period from the first CR to relapse, death or the last observation.

2.2 | Molecular analysis

A targeted NGS study was used to analyze the DNA of fresh bone marrow samples at initial diagnosis. Blood genomic DNA was isolated by Mini Blood DNA kit (Qiagen or OMEGA) and quantified with the NanoDrop spectrophotometer. We sequenced the mutation hotspots or entire coding regions of 34 genes associated with myeloid leukemia, which contains *FLT3, JAK2, KIT, MPL, CALR, CSF3R, PDGFRA, CEBPA, NRAS, KRAS, NPM1, TP53, RUNX1, GATA2, WT1, TET2, DNMT3A, IDH1, IDH2, ASXL1,*

BOCR, BOCRL1, CBL, ETV6, EZH2, MLL, NOTHCH2, PHF6, SF3B1, SRSF2, SH2B3, SETBP1, U2AF1, ZRSR2. The regions analyzed included mutational hotspots or the coding sequence of 34 genes. In Brief, 50 ng genomic DNA was used for each reaction. DNA samples from all patients were sequenced and analyzed on a high-throughput sequencing platform, the MiSeq next-generation sequencing instrument (Illumina). VAF was observed with a specific DNA sequence variation matching divided by the percentage of the overall coverage of the site. VAF greater than 5% was considered to be the presence of a mutation. VAF cut-off value was to use the optimal cutoff method with the Cutoff Finder web application (http://molpath.charite.de/cutoff). Data of patients with DNMT3A mutations were uploaded from a tab-separated table. The VAF of DNMT3A mutations and OS survival variable were selected from the table. The optimal cut-off point was determined by choosing "ROC curve (Manhattan distance)" method.

2.3 | Statistical analysis

The clinical features of the patients were described using descriptive statistics. Differences between groups were analyzed by the chi-squared or Fisher exact test for categorical variables and non-parametric Mann–Whitney U test for continuous variables. Survival analysis using the Kaplan–Meier method and the log-rank test, including the OS and RFS. Regarding the multivariate analysis of prognostic factors, a Cox-proportional hazards regression model was used for survival endpoints and a logistic regression model was used for CR. Bilateral $p < 0.05$ prompt difference was statistically significant. All statistical procedures were performed using SPSS software package version 25.0 and Graphpad Prism™ 8.30.

3 | RESULTS

3.1 | DNMT3A mutations characterization in adult patients with de novo CN-AML

DNMT3A mutations were identified in 35 of 171 (20%) patients with de novo CN-AML. Thirty-two patients carried single mutations in the DNMT3A gene, and three patients harbored two DNMT3A variants (double mutations). R882 missense mutations were more common variants (Figure 1A). Other non-R882 variants were detected in one patient respectively, including W893S,C911Y, E863K,Q842R,T835M, R792C,F755S,A741G,R736C,A571P,R326L,K299Q,P627fs, G511fs,R598X,W581X, A571-575del (Figure 1A).

All 35 patients with $DNMT3A^{mut}$ harbored at least one or more companion comutations, with an average total of three mutations per patient (range:1–6), which was higher than $DNMT3A^{wt}$ patients with (mean:2; range: 1–6) ($p < 0.001$). A detailed mutations profiling is provided in Figure 1B,C. Notably, compared to those with $DNMT3A^{wt}$, patients with $DNMT3A^{mut}$ more frequently harbored NPM1 (69% vs. 18%; $p < 0.001$), IDH1/IDH2 (34% vs. 9%; $p < 0.001$), FLT3-ITD (26% vs. 12%; $p = 0.037$), NRAS (29% vs. 8%; $p = 0.029$) mutations. In a similar fashion, patients with $DNMT3A^{mut}$ less frequently had CEBPA mutations (6% vs. 32%; $p = 0.002$). In this cohort, the median VAF value of DNMT3A mutations in 35 patients was 45% (6%–63%). Additionally, we observed DNMT3A mutations were presented as an ancestral mutation with higher or similar VAFs compared with other co-mutations in 35 patients (data not shown). Based on data from comutations and their VAFs, we further identified three comutated patterns with high frequency in 35 patients, including $DNMT3A^{mut}IDH1/IDH2^{mut}$ ($N = 12$, 34%), $DNMT3A^{mut}NPM1^{mut}FLT3-ITD^{mut}$ ($N = 9$, 26%), and $DNMT3A^{mut}NRAS^{mut}$ ($N = 8$, 23%). Among these three comutations, $DNMT3A^{mut}IDH1/IDH2^{mut}$ and $DNMT3A^{mut}NPM1^{mut}FLT3-ITD^{mut}$ existed independently of each other in $DNMT3A^{mut}$ patients of this cohort, whereas $DNMT3A^{mut}NRAS^{mut}$ and $DNMT3A^{mut}IDH1/IDH2^{mut}$ had overlaps in two patients, but was mutually exclusive with $DNMT3A^{mut}NPM1^{mut}FLT3-ITD^{mut}$. Furthermore, we also observed that the ratios of FLT3-ITD were lower than 0.5 in all of nine patients with $DNMT3A^{mut}NPM1^{mut}$ $FLT3-ITD^{mut}$. In addition, there were no significant differences in the VAFs of DNMT3A mutations between patients with or without NPM1(44.5% vs. 45%; $p = 0.320$), FLT3-ITD (43% vs. 46.5%; $p = 0.503$), or IDH1/IDH2 (46.5% vs. 44%; $p = 0.469$).

3.2 | Clinical impact of DNMT3A VAF

We used the optimal cut-off method to set the VAF of 42% as the cut-off value, and further divided $DNMT3A^{mut}$ patients into two subgroups: $DNMT3A^{High}$ patients ($DNMT3A$ VAF > 42%) and $DNMT3A^{Low}$ patients ($DNMT3A$ VAF ≤42%). To classify double DNMT3A mutant cases into the $DNMT3A^{High}$ or $DNMT3A^{Low}$ group, the higher VAF of the two mutations was used.

Biological features and patient outcomes were compared between these two subgroups (Table 1). To exclude the clinical effects of DNMT3A mutation types and comutations, here we evaluated these mutational features between the both subgroups. Our data showed that the number of patients with R882 or non-R882, comutated genes in the $DNMT3A^{High}$ subgroup were similarly
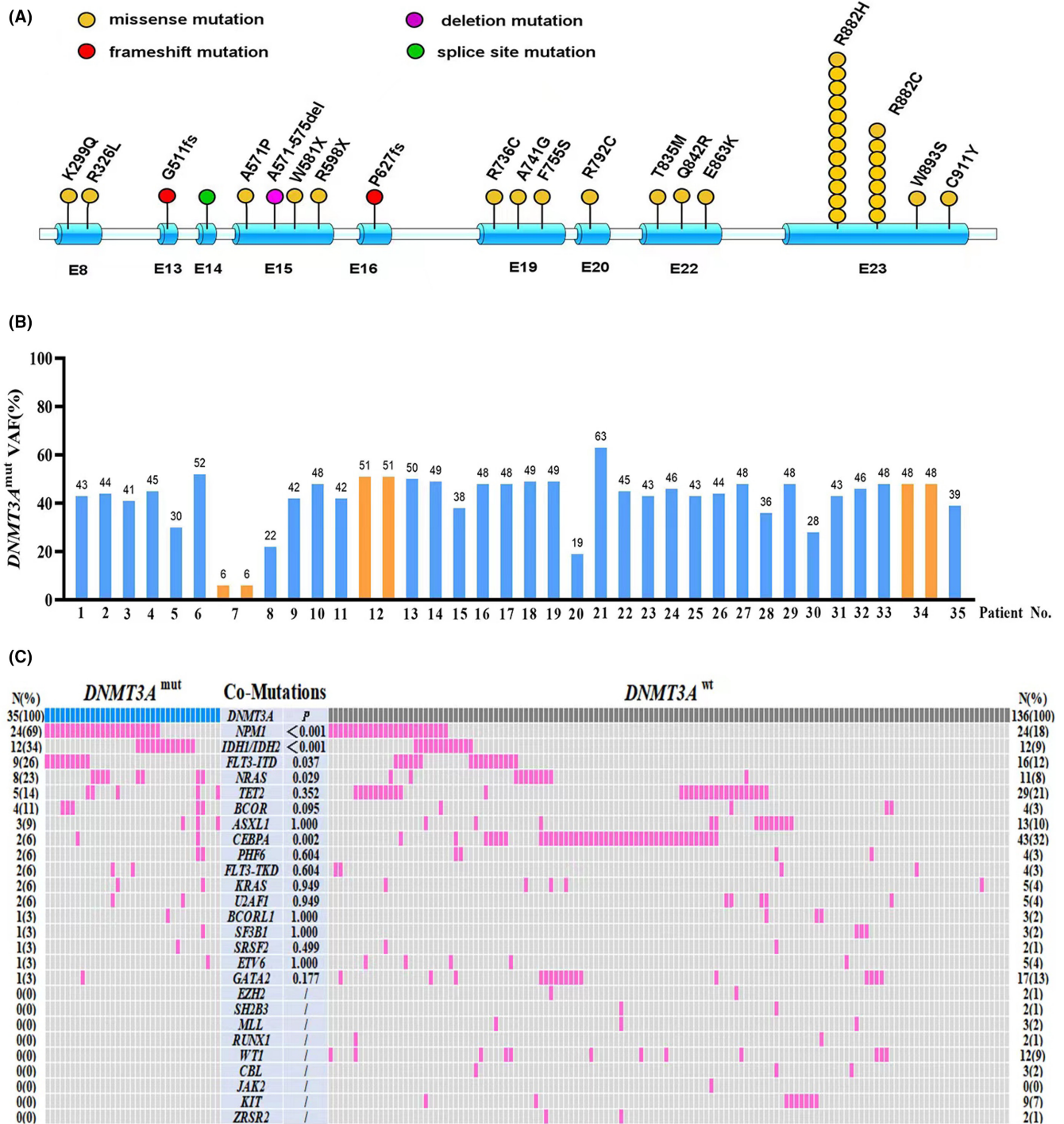
FIGURE 1 *DNMT3A* mutations and associated co-mutations in 171 adult patients with de novo CN-AML. (A) Structural diagram showing the location of mutations in *DNMT3A*. Each patient with *DNMT3A* mutation is designated with a circle. The color of the circle indicates the different types of mutations. (B) *DNMT3A* VAFs of all patient in this cohort. Blue bar indicates that the patient has single *DNMT3A* mutation. Orange bar indicates that the patient has two *DNMT3A* mutations. (C) Comparison of associated comutations and their frequency between *DNMT3A*^mut and *DNMT3A*^wt patients.

distributed as those in *DNMT3A*^Low subgroup. For biological features at diagnosis, patients with *DNMT3A*^High had significantly increased white blood cell (WBC) counts (median:50.915 vs. 2.33×10^9/L; $p < 0.001$) and a higher lactate dehydrogenase (LDH) level (median:582.85 vs. 333.5 U/

mL; $p = 0.027$) than those with *DNMT3A*^Low. Other biological features (including age, sex, Hb levels, platelet counts, BM blast percentages) were not significantly different between *DNMT3A*^High and *DNMT3A*^Low subgroups. In this cohort, 32 patients further received therapy, whereas

TABLE 1 Comparison of clinical impact between *DNMT3A*^{High} and *DNMT3A*^{Low}.

Characteristics	Total cohort (N = 35)	DNMT3A^{High} (VAF>42%) (N = 24)	DNMT3A^{Low} (VAF≤42%) (N = 11)	p value
Age				
≥60	16 (46)	13 (54)	3 (27)	0.138
<60	19 (54)	11 (46)	8 (73)	
Sex				
Male	16 (46)	11 (46)	5 (45)	0.983
Female	19 (54)	13 (54)	6 (55)	
Laboratory data median (range)				
Hb (g/L)	80.0 (46.0–139.0)	81.0 (46.00–138.00)	78.0 (46.0–139.0)	0.657
WBC (×10⁹/L)	24.6 (0.75–283.79)	50.915 (0.75–283.79)	2.33 (0.98–13.30)	**<0.001**
PLT (×10⁹/L)	76.0 (8.0–298.0)	76.5 (9.0–298.0)	70.0 (8.0–153.0)	0.831
BM blast (%)	49.0 (20.0–94.0)	57.5 (20.0–94.0)	40.0 (22.0–86.0)	0.095
LDH (U/mL)	427.0 (140.0–2210.0)	582.85 (140.0–2210.0)	333.5 (159.0–620.0)	**0.027**
Mutation type, *N* (%)				
R882	19 (54)	13 (54)	6 (55)	0.983
Non-R882	16 (46)	11 (46)	5 (45)	
Co-mutation				
NPM1	24 (69)	18 (75)	6 (55)	0.413
IDH	12 (34)	8 (33)	4 (36)	1.00
FLT3-ITD	9 (26)	5 (21)	4 (36)	0.576
NRAS	8 (23)	7 (29)	1 (9)	0.379
TET2	5 (14)	4 (17)	1 (9)	1.00
BCOR	4 (11)	1 (4)	3 (27)	0.082
CEBPA	2 (6)	1 (4)	1 (9)	0.536
PHF6	2 (6)	1 (4)	1 (9)	0.536
Treatment, *N* (%)				
High intensity	18 (51)	15 (63)	3 (27)	**0.044**
Low intensity	14 (40)	6 (25)	8 (73)	
Outcome				
CR, *N* (%)	18 (51)	9 (38)	9 (82)	**0.015**
Median OS (range), months	7 (1–28)	3 (1–28)	12 (2–28)	**0.032**
Median RFS (range), months	10 (1–25)	6 (1–16)	12 (5–25)	**0.642**

Note: The bold values indicate *p* value < 0.05.

three patients did not receive any therapy. *DNMT3A*^{High} subgroup had a greater number of patients receiving high-intensity induction than *DNMT3A*^{Low} subgroup, whereas most of patients with *DNMT3A*^{Low} receiving low-intensity induction due to associated hypoproliferative AML. In addition, none of patients received allo-HSCT in the entire *DNMT3A*^{mut} group. Among patients receiving induction therapy, patients with *DNMT3A*^{High} showed a significantly lower CR rate (38% vs. 82%; $p = 0.015$). In terms of survival, patients with *DNMT3A*^{High} had a shorter OS (median: 3 vs. 12 months; $p = 0.032$) than those with *DNMT3A*^{Low} (Figure 2 A1), but no statistical difference on RFS (median: 6 vs. 12 months; $p = 0.642$) was detected

between them (Figure 2 B1). In multivariable analyses, *DNMT3A*^{High} had independent effects on worse OS (hazard ratio [HR] = 3.768, 95% CI, 1.957–7.255; $p < 0.001$) (Figure 2 C1), and lower CR rate (odds ratio [OR] = 5.883, 95% CI, 1.733–19.970; $p = 0.004$) (Figure 2 C3).

3.3 | Clinical impact of two different DNMT3A^{mut} comutations

To address the clinical impact of *DNMT3A* mutations in more detail and because of the high prevalence and mutual exclusion of two comutations of *DNMT3A*^{mut}, we next
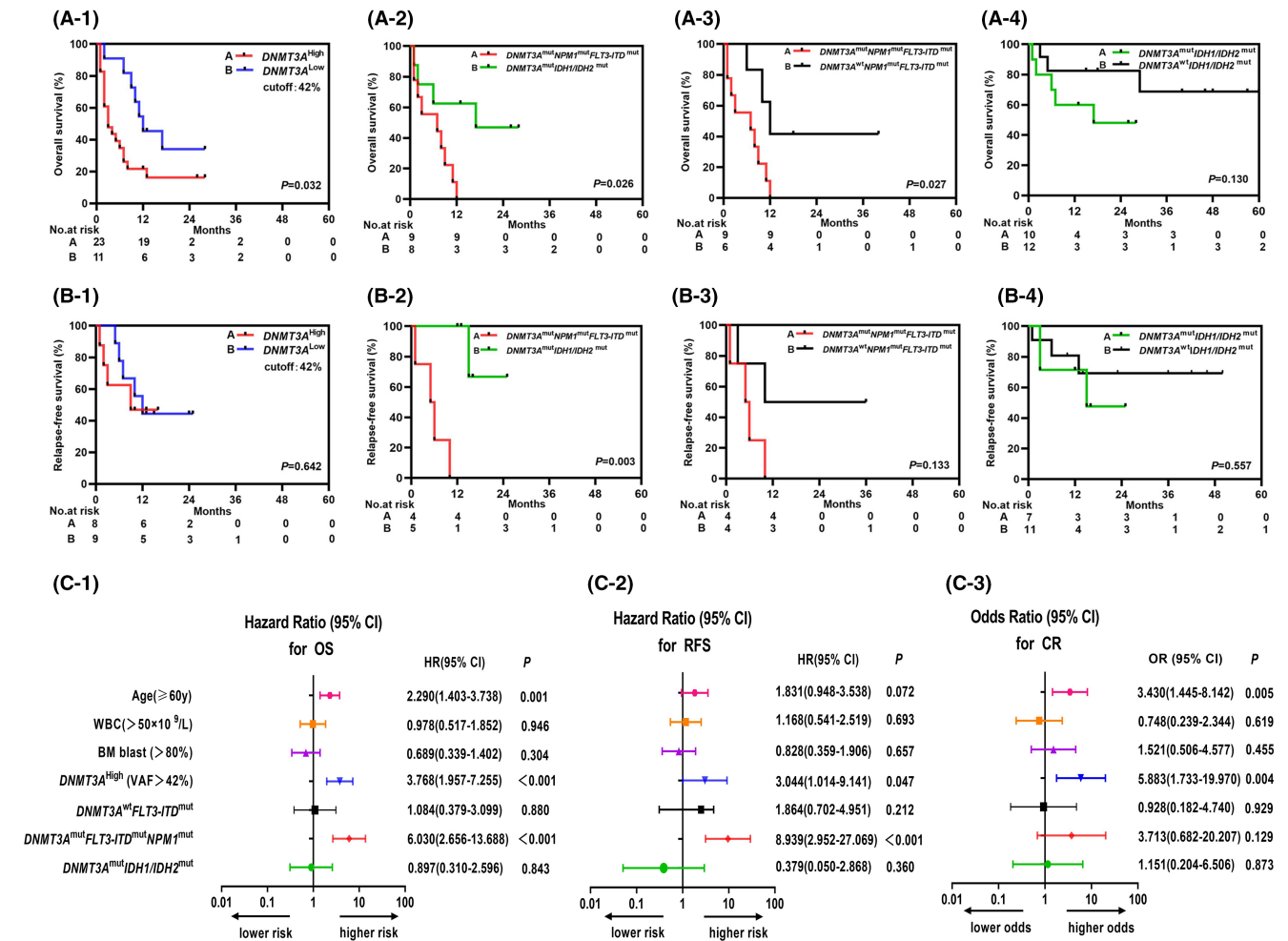
FIGURE 2 Prognosis effects of $DNMT3A^{mut}$ VAF and two comutations on adult patients with de novo CN-AML. Kaplan–Meier survival curves for OS and RFS in $DNMT3A^{High}$ versus $DNMT3A^{Low}$ (A-1,B-1); as well as in $DNMT3A^{mut}NPM1^{mut}FLT3$-$ITD^{mut}$ and $DNMT3A^{mut}IDH1/IDH2^{mut}$ (A-2,B-2), in $DNMT3A^{mut}NPM1^{mut}FLT3$-$ITD^{mut}$ and $DNMT3A^{wt}NPM1^{mut}FLT3$-$ITD^{mut}$ (A-3,B-3), in $DNMT3A^{mut}IDH1/IDH2^{mut}$ and $DNMT3A^{wt}IDH1/IDH2^{mut}$ (A-4,B-4). Cox-proportional hazard regression models were analyzed for OS(C-1) and RFS(C-2). Logistic regression models were analyzed for CR(C-3).

analyzed clinical features and outcomes of these two co-mutations, including $DNMT3A^{mut}NPM1^{mut}FLT3$-$ITD^{mut}$ and $DNMT3A^{mut}IDH1/IDH2^{mut}$.

We first compared two subgroups of patients with these two different comutated-genotypes each other (Table 2). Similarly, we also compared $DNMT3A^{mut}$ VAF and type (*R882* and *non-R882*) between both subgroups, and no significant statistical differences were observed. Our data further showed that these two subgroups were similar for biological features at diagnosis, including age, sex, Hb levels, WBC counts, platelet counts, BM blast percentages, and LDH levels. Regarding treatment types, there were no differences for the number of patients who received different induction therapies between the two subgroups. We observed that patients with $DNMT3A^{mut}NPM1^{mut}FLT3$-$ITD^{mut}$ were associated with poorer OS (median:7 vs. 15 months; $p = 0.026$) (Figure 2 A2) and RFS (median: 5.5 vs. 15 months; $p = 0.003$) (Figure 2 B2) compared to those with $DNMT3A^{mut}IDH1/$

$IDH2^{mut}$, though there was no significant difference on CR rate between these two subgroups (Table 2).

To investigate the interaction impact of $DNMT3A$ mutations and comutations, we also compared biological and clinical outcomes of patients harboring $NPM1^{mut}FLT3$-ITD^{mut} (*FLT3-ITD* ratio <0.5) genotype when with or without $DNMT3A$ mutations, and patients harboring $IDH1/IDH2^{mut}$ when with or without $DNMT3A$ mutations (Table 3). When comparing $DNMT3A^{mut}NPM1^{mut}FLT3$-$ITD^{mut}$ and $DNMT3A^{wt}NPM1^{mut}FLT3$-$ITD^{mut}$ subgroups, most of the features were similar, including age, sex, Hb levels, WBC counts, platelet counts, BM blast percentages, and LDH levels, treatment, CR rate, RFS, only for OS, patients with $DNMT3A^{mut}NPM1^{mut}FLT3$-$ITD^{mut}$ revealed a shorter OS (median: 7 vs. 11 months; $p = 0.027$) than those with $DNMT3A^{wt}NPM1^{mut}FLT3$-$ITD^{mut}$ (Figure 2 A3). When comparing $DNMT3A^{mut}IDH1/IDH2^{mut}$ and $DNMT3A^{wt}IDH1/IDH2^{mut}$ subgroups, patients with

TABLE 2 Comparison of clinical impact between the two *DNMT3A*^{mut} comutaions.

Characteristics	*DNMT3A*^{mut} *NPM1*^{mut}*FLT3-ITD*^{mut} (*N* = 9)	*DNMT3A*^{mut} *IDH1/IDH2*^{mut} (*N* = 8)	*p* value
Age, *N* (%)			
≥60	2 (22)	4 (50)	0.335
<60	7 (78)	4 (50)	
Sex, *N* (%)			
Male	5 (56)	4 (50)	1.00
Female	4 (44)	4 (50)	
Laboratory data, Median (range)			
Hb (g/L)	78.5 (52.0–103.0)	77.0 (53.0–139.0)	1.00
WBC ($\times 10^9$/L)	21.7 (0.98–283.79)	7.815 (1.25–123.71)	0.386
PLT ($\times 10^9$/L)	52.0 (33.0–143.0)	85.0 (61.0–298.0)	0.124
BM blast (%)	49.0 (22.0–88.0)	42.9 (20.0–86.0)	0.630
LDH (U/mL)	339.0 (195.0–1981.0)	391.0 (157.0–866.0)	0.749
DNMT3A VAF			
High	5 (56)	4 (50)	1.00
Low	4 (44)	4 (50)	
Mutation type, *N* (%)			
R882	6 (67)	4 (50)	0.637
Non-R882	3 (33)	4 (50)	
Treatment, *N* (%)			
High intensity	4 (44)	5 (63)	0.637
Low intensity	5 (56)	3 (38)	
Outcome			
CR, *N* (%)	4 (44)	5 (63)	0.637
Median OS (range), mo	7 (1–12)	15 (1–28)	**0.026**
Median RFS (range), mo	5.5 (1–10)	15 (12–25)	**0.003**

DNMT3A^{mut}*IDH1/IDH2*^{mut} presented with higher platelet counts (median: 85.0 vs. 37.0×10^9/L; $p = 0.009$) and a lower BM blast percentages (median: 42.9 vs. 81.5%; $p = 0.040$) than those with *DNMT3A*^{wt}*IDH1/IDH2*^{mut}, but no significant differences were detected for other biological features and their impacts on clinical outcomes. In multivariable analyses, *DNMT3A*^{mut}*NPM1*^{mut}*FLT3-ITD*^{mut} independently affected worse OS with a HR for the risk of death of 6.030 (95% CI, 2.656–13.688; $p < 0.001$) (Figure 2 C1), and worse RFS with an HR of 8.939 (95% CI, 2.952–27.069; $p < 0.001$) (Figure 2 C2). In contrast, *DNMT3A*^{mut}*IDH1/IDH2*^{mut} was not an independent factor for impacting CR rates, OS, and RFS (Figure 2 C1–3).

4 | DISCUSSION

In this study, we further assessed the genetic characteristics of *DNMT3A* mutations in adult patients with de novo CN-AML using targeted NGS with a panel of 34 genes associated with myeloid leukemia. In accordance with previous studies,[9,10,18,19,26–36] we detected *DNMT3A* mutations with a frequency of 20% in adult patients with primary CN-AML, and most of the mutations clustered at the *R882* site in exon 23. Moreover, all *DNMT3A*^{mut} patients of this cohort harbored one or more additional mutations, of which the majority of patients had a relatively higher or similar VAF compared with other comutated genes, strengthening previous data that reported *DNMT3A*^{mut} were presented as ancestral clone or preleukemia clone in AML.[12,37] Previous findings reported that *DNMT3A* mutations had a significant association with *NPM1* and *IDH1/IDH2* mutations, of which ~60% of *DNMT3A*^{mut} cases having *NPM1*, and more often (~30%) displaying a significant co-mutation pattern with *NPM1* and *FLT3-ITD* mutations, and had a mutually exclusive relationship with *CEBPA* mutations in AML patients.[18–20] Similarly, our data still showed that *DNMT3A* mutations had a significant association with *NPM1*, *FLT3-ITD*, and *IDH1/IDH2* mutations, and but an inverse correlation with *CEBPA*

TABLE 3 Comparison of clinical impact between two comutaions with or without $DNMT3A^{\text{mut}}$.

Characteristics	$DNMT3A^{\text{mut}}$ $NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$ (N = 9)	$DNMT3A^{\text{wt}}$ $NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$ (N = 6)	p value	$DNMT3A^{\text{mut}}$ $IDH1/IDH2^{\text{mut}}$ (N = 8)	$DNMT3A^{\text{wt}}$ $IDH1/IDH2^{\text{mut}}$ (N = 8)	p value
Age, N (%)						
≥60	2 (22)	1 (17)	1.00	4 (50)	1 (13)	0.282
<60	7 (78)	5 (83)		4 (50)	7 (87)	
Sex, N (%)						
Male	5 (56)	1 (17)	0.287	4 (50)	3 (38)	1.00
Female	4 (44)	5 (83)		4 (50)	5 (63)	
Laboratory data, Median (range)						
Hb (g/L)	78.0 (52.0–103.0)	75.0 (72.0–92.0)	0.841	77.0 (53.0–139.0)	73.5 (45.0–117.0)	0.494
WBC (×10⁹/L)	21.7 (0.98–283.79)	49.53 (2.20–133.17)	0.593	7.815 (1.25–123.71)	3.305 (2.05–95.26)	0.401
PLT (×10⁹/L)	52.0 (33.0–143.0)	40.0 (22.0–108.0)	0.096	85.0 (61.0–298.0)	37.0 (13.0–113.0)	**0.009**
BM blast (%)	49.0 (22.0–88.0)	83.0 (50.0–88.0)	0.181	42.9 (20.0–86.0)	81.5 (20.0–91.0)	**0.040**
LDH (U/mL)	339.0 (195.0–1981.0)	316.0 (258.3–1540.0)	0.808	391.0 (157.0–866.0)	364.5 (166.0–634.8)	0.817
Treatment, N (%)						
High intensity	4 (44)	5 (83)	0.287	5 (63)	5 (63)	1.00
Low intensity	5 (56)	1 (17)		3 (38)	3 (38)	
Outcome						
CR, N (%)	4 (44)	4 (67)	0.608	5 (63)	7 (88)	0.569
Median OS (range), months	7 (1–12)	11 (6–40)	**0.027**	15 (1–28)	16.5 (3–48)	0.130
Median RFS (range), months	5.5 (1–10)	10 (3–36)	0.133	15 (12–25)	14 (1–46)	0.557

Note: The bold values indicate p value < 0.05.

mutations in CN-AML patients. In addition, we found that *DNMT3A* mutations had a positive association with *NRAS* mutations in our patients. Furthermore, by an analysis of comutations, we identified two critical comutated gene-types with a high frequency, including $DNMT3A^{\text{mut}}IDH1/IDH2^{\text{mut}}$ and $DNMT3A^{\text{mut}}NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$. Although $DNMT3A^{\text{mut}}IDH1/IDH2^{\text{mut}}$ and $DNMT3A^{\text{mut}}NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$ comutations had been reported in other studies, their mutation features were rarely described. Here we found that $DNMT3A^{\text{mut}}IDH1/IDH2^{\text{mut}}$ and $DNMT3A^{\text{mut}}NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$ were mutually exclusive in $DNMT3A^{\text{mut}}$ patients, and that the mutation ratios of *FLT3-ITD* were lower than 0.5 in all of the patients with $DNMT3A^{\text{mut}}NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$. The above data strongly indicated that $DNMT3A^{\text{mut}}NPM1^{\text{mut}}FLT3\text{-}ITD^{\text{mut}}$ and $DNMT3A^{\text{mut}}IDH1/IDH2^{\text{mut}}$ might be two different genetic subgroups of $DNMT3A^{\text{mut}}$ patients with CN-AML.

We first investigated the biological and clinical impact of $DNMT3A^{\text{mut}}$ VAF on CN-AML, which be stratified by the cut-off value of 42%. For biological features, we observed that $DNMT3A^{\text{High}}$ patients at diagnosis had a significantly higher WBC counts and a trend for higher BM blast percentage compared with $DNMT3A^{\text{Low}}$ ones,

further strengthing a previous report by Narayanan et al.[38] that that $DNMT3A^{\text{High}}$ (≥44%) patients presented with leukocytosis and higher blast counts, and they further demonstrated that *DNMT3A* VAF had a positive correlations with WBC counts in AML patients. Combining these two data sets suggested that higher WBC counts and BM blast percentage might be unique biological features for patients with CN-AML with $DNMT3A^{\text{High}}$. In addition, we also observed another striking feature with more elevated serum LDH levels in $DNMT3A^{\text{High}}$ cases in our cohort. As far as we know, this is the first description of such an association in $DNMT3A^{\text{mut}}$ AML patients. With regard to clinical outcomes, in univariable and multivariable analyses, we found that $DNMT3A^{\text{High}}$ conferred an unfavorable effect on CR rate and OS, but did not show its negative impact on RFS in these patients. Although the predictive results of *DNMT3A* VAF are rarely reported, our findings were similar to previous reports. An analysis of 104 patients with *DNMT3A*-mutated AML led by *Narayanan* et al.[38] showed that high *DNMT3A* VAF was associated with more inferior OS and EFS, but had no impact on CR rate in univariable analyses, but in multivariable analyses, the adverse effect of $DNMT3A^{\text{High}}$ only on OS but not on

EFS in the CN-AML subset. _Linch_ et al.[39] reported that high _DNMT3A R882_[mut] VAF (≥47%) also presented worse effects on CR rate and OS in univariable analyses, but were not significant in multivariable analyses. Yuan et al.[40] reported that higher _DNMT3A R882_[mut] VAF (≥39%) had a shorter OS than those with a lower _DNMT3A R882_[mut] VAF.

Based on the comutations features of _DNMT3A_[mut], we further investigated the clinical effects of two comutations, including _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] and _DNMT3A_[mut]_IDH1/IDH2_[mut], which were considered as the two genetic subgroups of _DNMT3A_[mut] patients with de novo CN-AML in this study. We observed that patients with _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] had significantly worse OS and RFS than those with _DNMT3A_[mut]_IDH1/ IDH2_[mut]. To better understand the effect of _DNMT3A_ mutations in CN-AML, we compared the clinical impact of _NPM1_[mut]_FLT3- ITD_[mut] and _IDH1/IDH2_[mut] when with or without the genetic context of _DNMT3A_ mutations. Recently, the 2022 ELN guideline has updated _FLT3-ITD_ as an intermediate risk marker irrespective of _NPM1_ mutational status.[41] Our data showed that _DNMT3A_ mutations had a significantly poorer effect on OS on patients with _NPM1_[mut]_FLT3-ITD_[mut] (_FLT3-ITD_-ratio <0.5) genotype, further suggesting that _DNMT3A_ mutations could reduce favorable prognosis effect of _NPM1_[mut]_FLT3-ITD_[mut] (_FLT3-ITD_-ratio <0.5) genotype. In contrast, we did not observe significant differences on clinical outcomes between _DNMT3A_[mut]_IDH1/IDH2_[mut] and _DNMT3A_[wt]_IDH1/IDH2_[mut] subgroups, indicating that _DNMT3A_ mutations could not change the clinical prognosis of _IDH1/IDH2_ mutations. In multivariable analyses, the _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] (_FLT3-ITD_-ratio <0.5) was still independently associated with worse OS and RFS in this cohort. The above data strongly indicated that _DNMT3A_ mutations could generate different prognostic effects when combined with different comutations. In addition, although _DNMT3A_[mut]_IDH1/ IDH2_[mut] had no significant prognosis impact, we found that patients with this mutated genotype presented biological features such as higher platelet counts and a lower BM blast percentage in comparison to those with _DNMT3A_[wt]_IDH1/IDH2_[mut]. To the best of our knowledge, the clinical impact of the _DNMT3A_[mut]_IDH1/IDH2_[mut] was barely reported, whereas the clinical implications of the _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] were described in only a few reports. A report led by Loghavi et al.[42] showed that _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] had a shorter effect on EFS and a trend for shorter OS among AML old patients in comparison to those within other mutation subgroups. Another recent study cohort conducted by Bezerra et al.[43] reported that the _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] had worse effects on OS and DFS, which was similar to our findings.

5 | CONCLUSIONS

In summary, this study more detailly refined the biological and clinical prognostic effects of _DNMT3A_[mut] in adult patients with de novo CN-AML. Our findings highlighted that _DNMT3A_[mut] VAF and its two comutations had their specific clinical consequences. We found that high _DNMT3A_ VAF was associated with higher WBC counts and BM blast percentage than low _DNMT3A_ VAF, and had an independent effect on lower CR rate and shorter OS. We also identified that _DNMT3A_[mut]_NPM1_[mut]_FLT3-ITD_[mut] exerted an independent worse impact on OS and RFS. In contrast, the patients with _DNMT3A_[mut]_IDH1/IDH2_[mut] had relatively favorable prognoses, but manifested as higher platelet counts and a lower BM blast percentage in CN-AML patients than those with _DNMT3A_[wt]_IDH1/ IDH2_[mut]. However, there were several limitations to the current study because of a small sample size and its retrospective nature. Our findings need to be validated in a more extensive and prospective cohort, which might be the potentially helpful in predicting clinical outcomes.

AUTHOR CONTRIBUTIONS

Xian Chen: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Chuchu Tian:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal). **Zhuanghui Hao:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Lingang Pan:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Minglin Hong:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal). **Wei Wei:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal). **Daniel Muteb Muyey:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal). **Hongwei Wang:** Conceptualization (equal); funding acquisition (equal); project administration (equal); writing – review and editing (equal). **Xiuhua Chen:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); writing – review and editing (equal).

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