

Biological and clinical influences of *NPM1* in acute myeloid leukemia patients with *DNMT3A* mutations

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Purpose: *DNMT3A* and *NPM1* mutations are known to impact the prognosis of acute myeloid leukemia (AML). *DNMT3A* mutations are negative prognostic factors, while *NPM1* mutations are low-risk factors and inclined to concurrently appear with *DNMT3A* mutations. In this study, we aimed to find out how *NPM1* mutations affect patients' outcomes in the background of *DNMT3A* mutations.

Patients and methods: We screened The Cancer Genome Atlas (TCGA) database and found 51 AML patients with *DNMT3A* mutations. Of them, 28 patients had a combination of *NPM1* mutations.

Results: In all, *NPM1* had the highest mutation frequency (n=28, 54.9%). *DNMT3A*^{mut}/*NPM1*^{mut} patients had higher bone marrow (BM) blasts ($P=0.015$), higher *FLT3-ITD/TKD* rate ($P=0.004$), and lower *IDH2* mutation rate ($P=0.014$) than the *DNMT3A*^{mut}/*NPM1*^{wild} patients, while their prognoses were the same as the *DNMT3A*^{mut}/*NPM1*^{wild} patients ($P>0.1$). All 51 patients benefited from hematopoietic stem cell transplantation (HSCT) treatment ($P=0.005$ and 0.001 for event-free survival [EFS] and overall survival [OS], respectively). In the 23 patients with *DNMT3A*^{mut}/*NPM1*^{wild}, those who received HSCT had prolonged EFS and OS ($P=0.043$ and 0.008, respectively), while HSCT treatment did not produce a positive impact on EFS and OS in the remaining 28 patients with *DNMT3A*^{mut}/*NPM1*^{mut} ($P=0.056$ and 0.053, respectively).

Conclusion: Our study found that *NPM1* mutations influenced BM blasts' percentage, *FLT3-ITD/TKD* rate, and *IDH2* mutation rate in AML patients with *DNMT3A* mutations but made little difference to the overall prognosis. While HSCT treatments benefited all *DNMT3A*^{mut} patients, it was better for *DNMT3A*^{mut}/*NPM1*^{wild} patients to extend their EFS and OS.

Keywords: AML, DNA methyltransferases 3A, nucleophosmin 1, next-generation sequencing, prognosis

Introduction

Acute myeloid leukemia (AML) is a kind of heterogeneous disease characterized by the clonal expansion of hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs) in the bone marrow (BM), blood, and other tissues without differentiation.¹

As sequencing methods have technologically advanced in the past four decades, next-generation sequencing (NGS) is now available for a detailed understanding of the molecular pathogenesis of AML.

DNMTs are genes that encode DNMTs for the methylation of CpG islands. As methylations reduce the expression of downstream genes, spontaneous defects in

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DNMTs lead to the instability of genome structure and thus increase the possibility of cancer.² Since Ley et al³ confirmed that *DNMT3A* mutations were highly recurrent in patients with de novo AML and associated with a poor outcome, many prognostic studies about *DNMT3A*-mutated AML have been carried out.^{4–10} Occurring in ~20% of AML patients and having a high proportion of *R882* mutation,^{11,12} *DNMT3A* mutations are often accompanied by *FLT3-ITD*, *NPM1*, *IDH1*, and *IDH2* mutations.¹³ *NPM1* mutations were more common in patients with *DNMT3A* mutations than in *DNMT3A* wild-type patients.¹⁴ High-risk *NPM1/FLT3* mutation (*NPM1*^{wild}/*FLT3-ITD*^{negative}, *NPM1*^{wild}/*FLT3-ITD*^{positive}, or *NPM1*^{mut}/*FLT3-ITD*^{positive}) was thought to be related with poor prognosis.⁵ Hematopoietic stem cell transplantation (HSCT) has been shown to benefit the survival of all AML patients with *DNMT3A* mutations.^{8,9} Gale et al¹⁵ argued that the presence of *DNMT3A* mutations should be considered as a poor-risk prognostic factor, irrespective of the *NPM1* genotype as it may disturb the outcome. There have been few studies concerning *DNMT3A* mutations alone in AML patients together with studying the biological and clinical features with companion genes. Therefore, understanding the biological and clinical characteristics of these patients with *DNMT3A* mutations is important for estimating their event-free survival (EFS) and overall survival (OS) and likely advantageous in deciding the most suited clinical treatment.

Patients and methods

Patients

We selected and enrolled 51 patients with diagnosed *DNMT3A*-mutated AML from The Cancer Genome Atlas (TCGA) database (<https://cancergenome.nih.gov/>). We collected data including age, gender, AML French-American-British classification subtypes, karyotype, cytogenetics' risk, white blood cell (WBC) count, BM blasts percentage, peripheral blood (PB) blasts percentage, relapse, mutated recurrent genes, and combined mutation genes, which were highly related to AML such as *FLT3*, *IDH1/2*, *TET2*, *NRAS*, *CEBPA*, *PTPN11*, *KRAS*, *U2AF1*, *SMC1A*, *SMC3*, and *RAD21*, with their clinical figures to include EFS and OS. Gene mutations were detected by NGS. Written informed consent was obtained from all patients. This study was approved by the Human Research Ethics Committee of Washington University.

Statistical analyses

The end points were EFS and OS. EFS is the time from the date of diagnosis to removal from the study due to the absence

of complete remission, relapse, or death. OS is the time from the date of diagnosis to death due to any cause.

We compared different biological and clinical characteristics by using different statistical methods. The Student's *t*-test was applied to two group comparisons, and Chi-square test was used to compare the rate between them. Survival analysis was performed by Kaplan–Meier method. A two-sided *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed by the SPSS Version 20.0 software.

Results

Biological and clinical characteristics

In all of the 51 patients, *NPM1* was the most frequently combined mutation gene (n=28, 54.9%), followed by *FLT3* (n=21, 41.2%), *IDH1* (n=11, 21.6%), and *TET2* (n=6, 11.8%). The mutational spectrum of all genes with >5% mutation frequency is shown in Figure 1. The biological and clinical characteristics are summarized in Table 1.

Patients were divided into two groups depending on their *NPM1* mutation states. There were 23 patients with *DNMT3A*^{mut}/*NPM1*^{wild} and 28 patients with *DNMT3A*^{mut}/*NPM1*^{mut}. The median age of the cohort was 58 years (24 men and 27 women; age range: 21–81 years), and 47.1% of the patients were ≥60 years. WBC counts ranged from 1.2 to 298.4×10⁹/L with a median of 45.0×10⁹/L, and 19 cases were not <50×10⁹/L. The median BM blast percentage and PB blast percentage were 76 and 36%, respectively, and ranged from 32 to 100% and 0 to 97%; there were 30 (58.8%) cases with BM blasts ≥70% and six (30.0%) cases with PB blasts ≥70%. In 28 of the 51 patients (58.8%) who had relapses, days from collection to first relapse ranged from 53 to 1230 days with a median of 324.7 days. There were two (8.7%) cases with *FLT3-ITD* mutation and two (8.7%) cases with *FLT-TKD* mutation. HSCT treatments were received by 23 (45.1%) cases.

Upon comparing the two groups with *DNMT3A* mutations, we found that patients with *DNMT3A*^{mut}/*NPM1*^{mut} had higher BM blasts (*P*=0.015), higher *FLT3-ITD/TKD* (*P*=0.004), and lower *IDH2* mutation (*P*=0.014). Other biological or clinical characteristics showed no significant differences between the two groups.

Comparison of EFS and OS between different biological and clinical characteristics

To determine what kind of biological or clinical characteristics were easily influenced by *NPM1* mutation, we chose

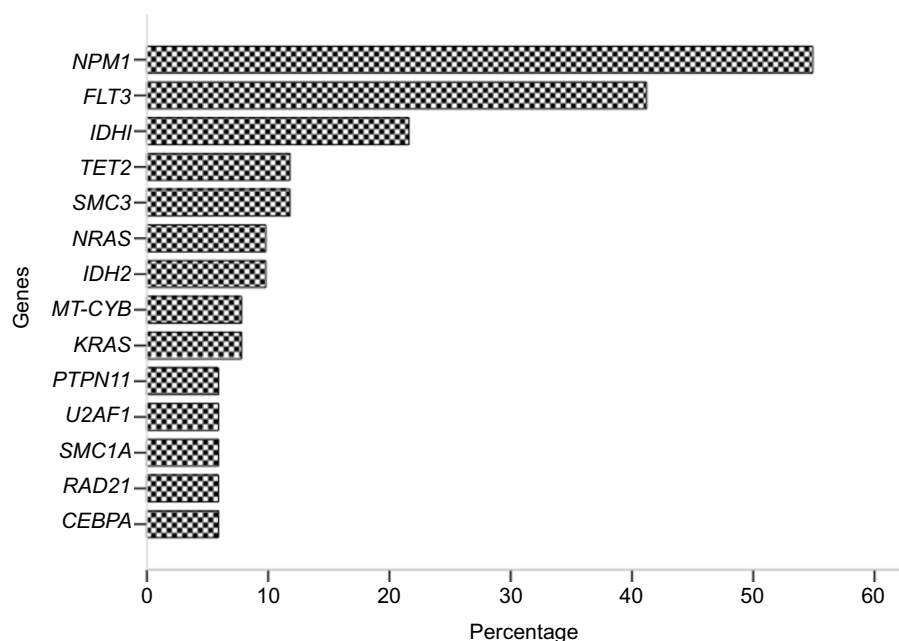


Figure 1 Mutational spectrum of all genes with >5% mutation frequency.

Note: NPM1 was the most frequently combined mutation gene (n=28, 54.9%), followed by FLT3 (n=21, 41.2%), IDH1 (n=11, 21.6%), TET2 (n=6, 11.8%), SMC3 (n=6, 11.8%), IDH2 (n=5, 9.8%), NRAS (n=5, 9.8%), KRAS (n=4, 7.8%), MT-CYB (n=4, 7.8%), CEBPA (n=3, 5.9%), PTPN11 (n=3, 5.9%), U2AF1 (n=3, 5.9%), SMC1A (n=3, 5.9%), and RAD21 (n=3, 5.9%).

the following parameters for EFS and OS analyses: age (<60 vs ≥60 years), cytogenetic risk (intermediate vs poor), WBC count (<50×10⁹ vs ≥50×10⁹/L), BM blasts percentage (<70 vs ≥70%), PB blasts percentage (<70 vs ≥70%), mutated recurrent genes (<5 vs ≥5), genes with no <10 (19.6%) cases harboring *FLT3-ITD/TKD* and *IDH1* changes (mutation vs wild type), and HSCT (yes vs no). The results are shown in Table 2.

The *NPM1* mutation had no influence on any of the abovementioned parameters in *DNMT3A*-mutated AML patients. EFS and OS between *DNMT3A*^{mut}/*NPM1*^{wild} and *DNMT3A*^{mut}/*NPM1*^{mut} groups showed no difference ($P=0.504$ and 0.586 , respectively, Figure 2A and B). Furthermore, there was also no difference in EFS and OS between groups of patients with only chemical therapy ($P=0.938$ for EFS and $P=0.942$ for OS, Figure 2C and D) or HSCT treatment ($P=0.940$ for EFS and $P=0.790$ for OS, Figure 2E and F).

HSCT was an effective way for the treatment of *DNMT3A*-mutated AML patients ($P=0.005$ for EFS and $P=0.001$ for OS) in all of the 51 patients (Figure 3A and B). We also compared the influence of different treatments in each group. The group of people with *DNMT3A*^{mut}/*NPM1*^{wild} derived the best of HSCT treatment and had longer EFS and OS than patients who received only chemical therapy ($P=0.043$ for EFS and $P=0.008$ for OS, Figure 3C and D).

Patients in the other group with *DNMT3A*^{mut}/*NPM1*^{mut} did not show similar results ($P=0.056$ for EFS and $P=0.053$ for OS, Figure 3E and F).

For further detailed analysis, we considered another subgroup of patients with *DNMT3A*^{mut}/*FLT3*^{mut}/*NPM1*^{wild} from the existing cohort of 51 patients. We compared the EFS and OS between *DNMT3A*^{mut}/*FLT3*^{wild}/*NPM1*^{wild} patients (n=19) and *DNMT3A*^{mut}/*FLT3*^{mut}/*NPM1*^{wild} patients (n=4) and found that there were significant differences between these two groups ($P_2=0.004$ for EFS in Figure 4A and $P_2=0.001$ for OS in Figure 4B), but there was no difference between the outcomes for the *DNMT3A*^{mut}/*FLT3*^{wild}/*NPM1*^{wild} and *DNMT3A*^{mut}/*FLT3*^{mut}/*NPM1*^{mut} groups (n=17, $P_1>0.1$, Figure 4A and B).

Discussion

Our study showed that *DNMT3A* mutations in AML had higher BM blasts, higher *FLT3-ITD/TKD* mutation rate, and higher *IDH2* mutation rate when combined with *NPM1* mutation, but little difference in prognoses compared with *NPM1* wild type. HSCT treatment benefited all patients who carried the *DNMT3A* mutation and was a better choice for the *DNMT3A*^{mut}/*NPM1*^{wild} group.

In Gale et al's¹⁵ study, 80% of 272 patients with *DNMT3A* mutation concomitantly had the *NPM1* mutation. We analyzed the biological and clinical features of 51 patients

Table 1 Biological and clinical characteristics

Characteristic	<i>DNMT3A</i> ^{mut} / <i>NPM1</i> ^{wild} , median (range) or n/%	<i>DNMT3A</i> ^{mut} / <i>NPM1</i> ^{mut} , median (range) or n/%	<i>t/χ</i> ²	<i>P</i>
Age (years)	63 (42–81)	57 (21–81)	1.700*	0.095
<60	10/43.5	17/60.7		
≥60	13/56.5	11/39.3		
Gender			0.440 [§]	0.580
Male	12/52.2	12/42.9		
Female	11/47.8	16/57.1		
AML FAB subtypes				
M0	3/13.0	0	3.880 [§]	0.085
M1	7/30.4	6/21.4	0.539 [§]	0.529
M2	5/21.7	6/21.4	0.001 [§]	1.000
M4	5/21.7	7/25.0	0.075 [§]	1.000
M5	2/8.7	9/32.1	4.104 [§]	0.084
M7	1/4.3	0	1.242 [§]	0.451
Karyotype				
Normal	10/43.5	23/85.2	9.628 [§]	0.003
Complex	3/13.0	1/3.7	1.472 [§]	0.322
Trisomy 8	4/17.4	1/3.7	2.585 [§]	0.167
-7/7q	2/8.7	0	2.446 [§]	0.207
Others	4/17.4	2/7.4	1.172 [§]	0.395
Risk (cytogenetics)			3.224 [§]	0.121
Good	0	0		
Intermediate	17/73.9	25/92.6		
Poor	6/26.1	2/7.4		
WBC count (×10 ⁹ /L)	14.9 (1.2–202.7)	69.8 (2.6–298.4)	1.948*	0.057
<50	19/82.6	13/46.4		
≥50	4/17.4	15/53.6		
BM blasts	62 (32–99)	81.5 (41–100)	2.531*	0.015
<70	14/60.9	7/25.0		
≥70	9/39.1	21/75.0		
PB blasts	32 (0–97)	49 (0–91)	0.692*	0.492
<70	17/73.9	18/66.7		
≥70	6/26.1	9/33.3		
Relapse	11/47.8	17/60.7	0.847 [§]	0.407
Mutated recurrent genes	6 (2–10)	5 (2–11)	1.569*	0.123
<5	5/21.7	10/35.7		
≥5	18/78.3	18/64.3		
<i>FLT3</i>			9.785 [§]	0.004
<i>FLT3-ITD</i>	2/8.7	10/35.7		
<i>FLT3-TKD</i>	2/8.7	7/25.0		
Wild type	19/82.6	11/39.3		
<i>IDH2</i>			6.749 [§]	0.014
Mutation	5/21.7	0/0		
Wild type	18/78.3	28/100		
<i>IDH1</i>			0.506 [§]	0.514
Mutation	6/26.1	5/17.9		
Wild type	17/73.9	23/82.1		
<i>TET2</i>			4.015 [§]	0.079
Mutation	5/21.7	1/3.6		
Wild type	18/78.3	27/96.4		
<i>NRAS</i>			0.058 [§]	1.000
Mutation	2/8.7	3/10.7		
Wild type	21/91.3	25/89.3		

(Continued)

Table 1 (Continued)

Characteristic	<i>DNMT3A</i> ^{mut} / <i>NPM1</i> ^{wild} , median (range) or n/%	<i>DNMT3A</i> ^{mut} / <i>NPM1</i> ^{mut} , median (range) or n/%	<i>t/χ</i> ²	<i>P</i>
<i>CEBPA</i>			0.599 [§]	0.583
Single mutation	1/4.3	1/3.6		
Double mutation	1/4.3	0/0		
Wild type	21/91.3	27/96.4		
<i>PTPN11</i>			2.618 [§]	0.242
Mutation	0/0	3/10.7		
Wild type	23/100	25/89.3		
<i>KRAS</i>			0.042 [§]	1.000
Mutation	2/8.7	2/7.1		
Wild type	21/91.3	26/92.9		
<i>U2AF1</i>			0.599 [§]	0.583
Mutation	2/8.7	1/3.6		
Wild type	21/91.3	27/96.4		
<i>SMC1A</i>			0.178 [§]	1.000
Mutation	1/4.3	2/7.1		
Wild type	22/95.7	26/92.9		
<i>SMC3</i>			0.066 [§]	1.000
Mutation	3/13.0	3/10.7		
Wild type	20/87.0	25/89.3		
<i>RAD21</i>			0.178 [§]	1.000
Mutation	1/4.3	2/7.1		
Wild type	22/95.7	26/92.9		
HSCT			2.208 [§]	0.166
Yes	13/56.5	10/35.7		
Allo-HSCT	11/47.8	8/28.6		
Auto-HSCT	2/8.7	2/7.1		
No	10/43.5	18/64.3		

Notes: *Student's t-test. [§]Chi-square test.

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; FAB, French-American-British classification; HSCT, hematopoietic stem cell transplantation; PB, peripheral blood; WBC, white blood cell.

with *DNMT3A* mutations and found *NPM1* to be the most common accompanying mutation (n=28, 54.9%). This may be due to a relatively high percentage of patients older than 60 years in our study (47.1 vs 3%). Many genes participate in AML development.¹⁶ Mutations in *NPM1*, *IDH2*, and biallelic *CEBPA* are favorable risk factors;¹³ while *FLT3-ITD* positive, *IDH1*, *TET2*,¹³ *KRAS*,¹⁷ *U2AF1*, and *PTPN11* are always associated with poor outcomes.^{18,19} Although showing different mutation rates in AML patients, genes such as *NRAS*,²⁰ *SMC1A*, *SMC3*, and *RAD21* showed no influence in the prognosis.²¹

Holmberg et al²² did a basic clinical experiment on the relationship between *DNMT3A* and *NPM1* and found that deficiency in *DNMT3A* enhanced the rearrangement of heterochromatin, which was triggered by the loss of *NPM1*. In our study, 35.7% of *DNMT3A*^{mut}/*NPM1*^{mut} patients harbored the *FLT3-ITD* mutation and this rate was similar to that reported by Gale et al¹⁵ (37%). Thol et al⁵

Table 2 Kaplan–Meier analysis for EFS and OS between different biological and clinical character groups

Characteristic	EFS (months)				OS (months)			
	DNMT3A ^{mut} / NPM1 ^{wild}	DNMT3A ^{mut} / NPM1 ^{mut}	χ^2	P	DNMT3A ^{mut} / NPM1 ^{wild}	DNMT3A ^{mut} / NPM1 ^{mut}	χ^2	P
Age (years)								
<60	14.90	8.70	0.019	0.891	21.50	12.00	0.018	0.892
≥60	17.20	2.50	1.863	0.172	8.40	6.30	2.319	0.128
Risk (cytogenetics)								
Intermediate	9.30	18.00	0.279	0.598	16.40	10.20	0.330	0.565
Poor	4.00	0.60	2.527	0.112	4.00	0.60	1.232	0.267
WBC count (×10 ⁹ /L)								
<50	9.30	8.00	0.517	0.472	20.10	10.20	0.677	0.410
≥50	4.00	7.50	1.632	0.201	6.60	7.90	0.739	0.390
BM blasts (%)								
<70	9.60	7.80	1.153	0.283	20.20	10.20	1.188	0.276
≥70	5.20	7.50	0.424	0.515	8.10	7.80	0.500	0.479
PB blasts (%)								
<70	9.30	8.00	0.091	0.763	17.40	10.20	0.063	0.802
≥70	4.00	5.70	0.072	0.789	6.60	7.50	0.020	0.888
Mutated recurrent genes								
<5	8.50	5.30	0.363	0.547	20.20	7.50	0.443	0.506
≥5	8.60	8.20	0.059	0.807	15.80	10.20	0.055	0.815
FLT3-ITD/TKD								
Present	4.00	6.90	1.443	0.230	6.60	7.90	1.817	0.178
Absent	9.60	8.00	0.269	0.604	20.10	10.20	0.551	0.458
IDH1								
Mutation	4.90	13.80	1.134	0.287	5.20	24.80	1.781	0.182
Wild type	9.30	6.90	1.620	0.203	16.40	7.50	1.910	0.167
HSCT								
Yes	14.90	10.20	0.006	0.938	21.50	16.30	0.005	0.942
No	5.90	5.30	0.006	0.940	7.70	6.30	0.071	0.790

Abbreviations: BM, bone marrow; EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; OS, overall survival; PB, peripheral blood; WBC, white blood cell.

also found that patients with *DNMT3A* mutations were more likely to have mutations in *NPM1* with a trend toward a higher *FLT3* mutation rate. In our study, the *IDH2* mutation was seen in 21.7% patients in the *DNMT3A*^{mut}/*NPM1*^{wild} group, while those in the *DNMT3A*^{mut}/*NPM1*^{mut} group did not harbor the *IDH2* mutation. Another study showed that the percentage of *IDH2* mutations were 11.5 and 33.3% for *DNMT3A*^{mut}/*NPM1*^{wild} and *DNMT3A*^{mut}/*NPM1*^{mut}, respectively.¹⁵ We were not very consistent with their *IDH2* mutation rates, and this may be related to the small number of cases.

Comparison of EFS and OS between different biological and clinical characteristics groups showed no difference. Similar to Xu et al's²³ conclusion, we also confirmed that HSCT is a better option for patients with *DNMT3A* mutations. We found that chemical therapy and HSCT treatment had the same effect in the *DNMT3A*^{mut}/*NPM1*^{mut} group. We supposed that this may be due to high mutation rate of *FLT3* in the *NPM1*-mutated group. Previous studies suggested that *FLT3-ITD* always results in significantly worse clinical outcomes and weakens the curative effect of conventional

chemotherapy,²⁴ as well as shortening the EFS and OS in *DNMT3A*-mutated patients.²⁵ Only the *R140* mutation in *IDH2* seems to have prognostic meaning and is associated with a better outcome.¹³ In our study, we found that *IDH2* mutation only occurred in *DNMT3A*^{mut}/*NPM1*^{wild} patients and three of the five *IDH2* mutations happened at the *R140* locus. This could be another reason why the *DNMT3A*^{mut}/*NPM1*^{mut} group showed similar results to the *DNMT3A*^{mut}/*NPM1*^{wild} group.

There are some limitations to our study. First, the small sample size may have reduced the accuracy of our results. Second, our study has a retrospective design, the effectiveness of which is limited when compared to a prospective study.

Conclusion

All patients with the *DNMT3A* mutation benefited from HSCT, but those who also have *NPM1* mutations should be treated carefully given their high *FLT3-ITD/TKD* rate, and hence, HSCT may not be better than chemotherapy for this group of patients.

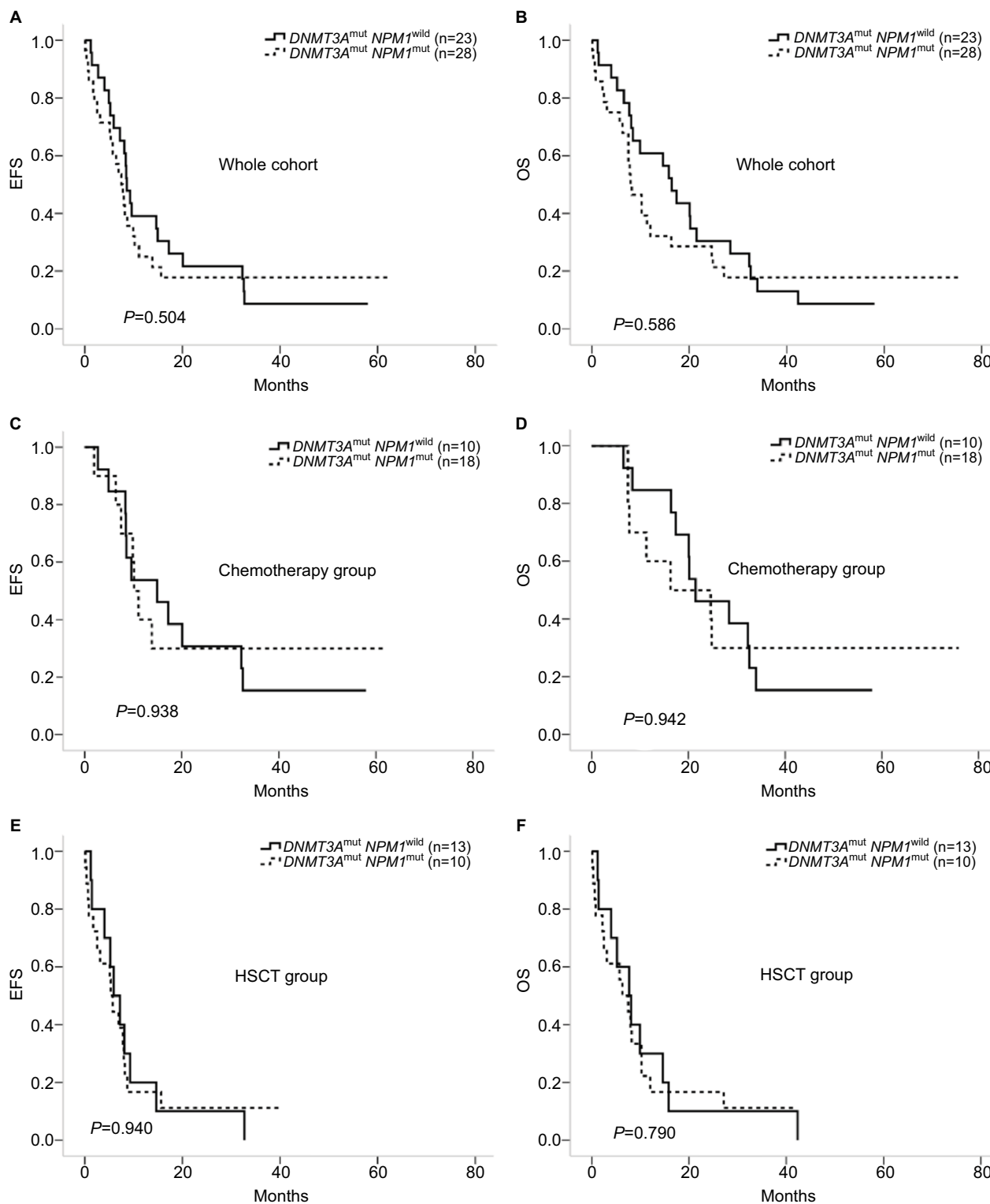


Figure 2 Comparison of EFS and OS between different biological and clinical character groups.

Notes: (A and B) EFS and OS of $DNMT3A^{mut}/NPM1^{wild}$ vs $DNMT3A^{mut}/NPM1^{mut}$ in all of the 51 patients. (C and D) EFS and OS of $DNMT3A^{mut}/NPM1^{wild}$ vs $DNMT3A^{mut}/NPM1^{mut}$ in 28 patients with chemotherapy. (E and F) EFS and OS of $DNMT3A^{mut}/NPM1^{wild}$ vs $DNMT3A^{mut}/NPM1^{mut}$ in 23 patients with HSCT.

Abbreviations: EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; OS, overall survival.

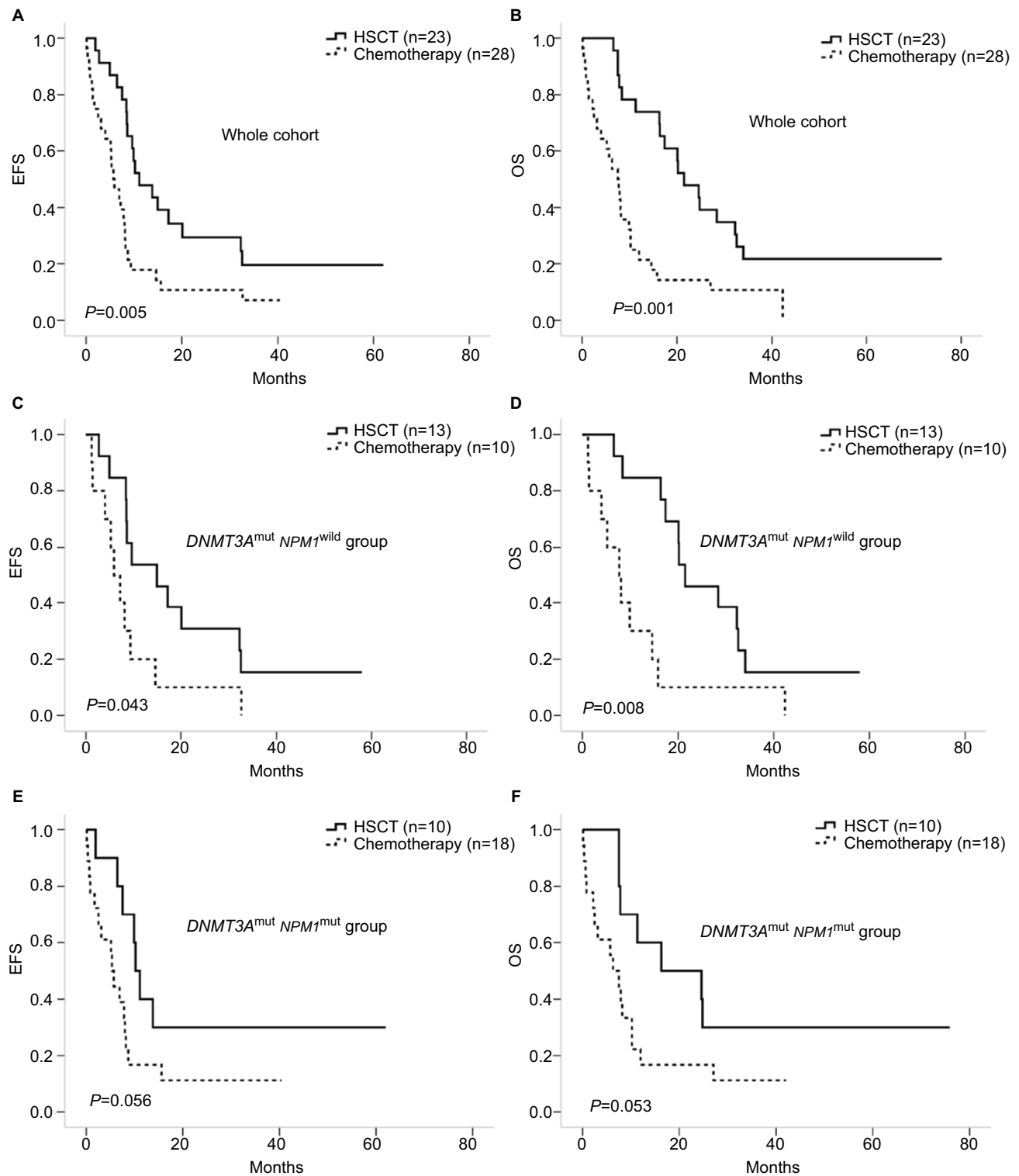


Figure 3 Comparison of chemotherapy and HSCT in different mutation groups.

Notes: (A and B) EFS and OS of all the 51 patients. (C and D) EFS and OS of the 23 patients with $DNMT3A^{mut}/NPM1^{wild}$. (E and F) EFS of the 28 patients with $DNMT3A^{mut}/NPM1^{mut}$.

Abbreviations: EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; OS, overall survival.

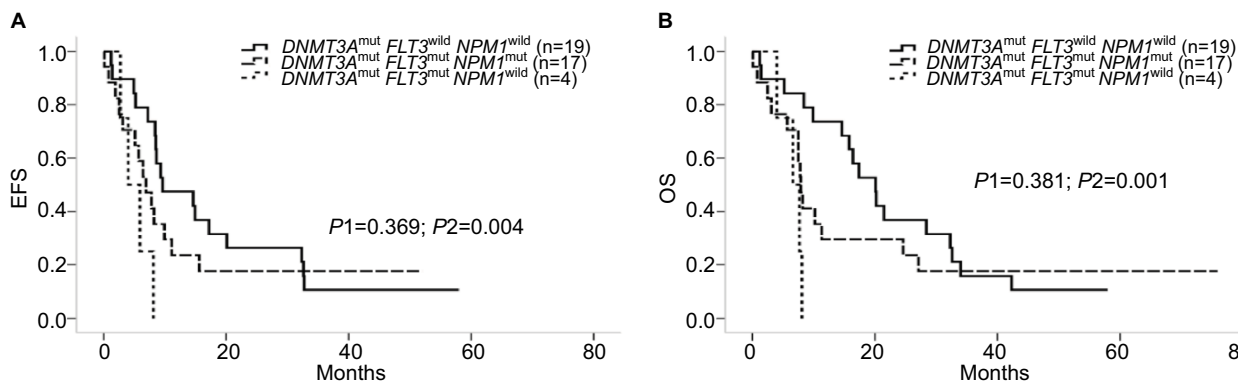


Figure 4 Influences of FLT3-ITD/TKD to the cohort.

Notes: (A and B) EFS and OS of $DNMT3A^{mut}/FLT3^{wild}/NPM1^{wild}$ vs $DNMT3A^{mut}/FLT3^{mut}/NPM1^{mut}$ vs $DNMT3A^{mut}/FLT3^{mut}/NPM1^{wild}$; P1 for comparison between $DNMT3A^{mut}/FLT3^{wild}/NPM1^{wild}$ and $DNMT3A^{mut}/FLT3^{mut}/NPM1^{mut}$; P2 for comparison between $DNMT3A^{mut}/FLT3^{wild}/NPM1^{wild}$ vs $DNMT3A^{mut}/FLT3^{mut}/NPM1^{wild}$.

Abbreviations: EFS, event-free survival; OS, overall survival.

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Disclosure

The authors report no conflicts of interest in this work.

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