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ORIGINAL RESEARCH

The Prognostic Value of Integrating Copy Number Alteration Profiles in *NPM1*-Mutated Acute Myeloid Leukemia: An Exploratory Study

Chunxia Zhang*, Li Gao*, Ming Gong, Chundi Liu, Dongmei Zhang, Zhenling Li

Department of Hematology, China-Japan Friendship Hospital, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhenling Li, Department of Hematology, China-Japan Friendship Hospital, No. 2 Yinghua East Road, Beijing, People's Republic of China, Tel +86 10 84205522, Fax +86 10 84205021, Email zryyxy2013@163.com

Background: Characteristic genetic events underpin acute myeloid leukemia (AML) heterogeneity and enable precise risk stratification. However, prognostic assessment remains ambiguous in many patients due to inadequate integration of specific genetic information.

Materials and Methods: Eighty *NPM1*-mutated AML patients were enrolled. Copy number alterations (CNAs) were detected via shallow whole-genome sequencing (sWGS), and concurrent mutations via targeted deep sequencing of myeloid malignancy-associated genes. Clinical and laboratory parameters were integrated with genomic data for statistical analysis, with the aim of assessing the potential clinical significance of CNA profiles in prognostic stratification.

Results: *NPM1* mutation subtypes A, B, and D were the most prevalent, with all patients harboring at least two concurrent mutations (4–5 mutations being the most frequent), and these mutations commonly co-occurred with those in *FLT3, DNMT3A, TET2, IDH2*, and *NRAS*. Forty-one samples (51%) exhibited CNAs across diverse genomic regions, with dup(18)(p11.23) identified as the most recurrent locus. No significant differences in FAB classification, hematologic parameters, demographic characteristics (gender, age), co-mutation profiles, complete remission (CR) rates, or survival outcomes were observed between the CNA-positive and CNA-negative groups. Univariate survival analysis revealed patients with ≥2 CNAs, or *FLT3*-internal tandem duplication (*FLT3*-ITD) had significantly shorter overall survival (OS). Notably, integrative analysis of CNAs with mutational profiles showed that patients harboring both *FLT3*-ITD and ≥2 CNAs had the poorest OS, followed by those with *FLT3*-ITD and <2 CNAs, and multivariate Cox regression analysis suggests a potential association between ≥3 CNAs and adverse outcomes; however, given the limited sample size of cases with high CNA burden, this result should be interpreted with caution.

Conclusion: This exploratory study suggests that combining CNAs and gene mutation profiles may potentially improve the existing prognostic evaluation system for *NPM1*-mutated AML patients. Confirmation of these results requires additional validation in larger prospective cohorts.

Keywords: *NPM1* mutation, acute myeloid leukemia, copy number alteration, prognosis

Introduction

Acute myeloid leukemia (AML), a malignant clonal hematopoietic disorder arising from hematopoietic progenitor cells, is primarily characterized by infection, hemorrhage, anemia, and extramedullary organ infiltration.[1,2] In recent years, notable advancements in cytogenetics and molecular biology have significantly deepened our understanding of AML pathogenesis, disease stratification, and prognostic determinants, thereby establishing a critical framework for risk stratification, minimal residual disease (MRD) monitoring, and precision therapeutic strategies.[3,4] Research focus has expanded from chromosomal translocations to copy number alterations (CNAs) and shifted from mutations in pan-cancer -associated genes—such as *TP53, NRAS*, and *BRAF*—to those specifically implicated in hematopoietic malignancies, including *NPM1* and *JAK2*.[5,6] Over the past half-century, the evolution of genetic and molecular research methodologies

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—from traditional chromosome banding and fluorescence in situ hybridization (FISH) to high-throughput sequencing technologies—has yielded an extensive landscape of genetic and molecular insights. This progress has significantly advanced AML subtyping, refined prognostic assessment models, and enabled the implementation of personalized treatment paradigms.[7–9] Despite these strides, a considerable subset of AML patients remains "mis-stratified", which delays the initiation of optimal therapeutic interventions and impairs clinical outcomes.

NPM1, widely expressed in various cell types and primarily located in the nucleus, shuttles between the nucleus and cytoplasm to perform critical physiological functions. These include preventing nucleolar aggregation, regulating ribosomal protein assembly, facilitating protein transport between the nucleus and cytoplasm, and initiating centrosome folding.[10,11] Since its first discovery by Falini et al in 2005 in some AML patients, numerous studies have demonstrated that *NPM1* aberrations were detectable in approximately one-third of adult AML patients and about half of cases with a normal karyotype (NK).[12–14] *NPM1* mutations have been identified across almost all FAB subtypes of AML. Notably, current research suggests that the isolated presence of *NPM1* mutations is associated with a better prognosis and a higher remission rate.[13] However, when *NPM1* mutations coexist with other genetic variants or chromosomal abnormalities, these cases require separate consideration. In the context of ELN (European Leukemia Net) risk stratification, *NPM1* mutations can span across low-, intermediate-, and high-risk groups, depending on the accompanying cytogenetic and genetic events.[15,16] Extensive prior investigations have consistently demonstrated that *NPM1* mutations occurring in isolation are strongly associated with favorable clinical outcomes, while the co-occurrence of *NPM1* mutations with *FLT3*-ITD significantly attenuates this prognostic benefit, patients harboring chromosomal aberrations linked to adverse prognosis should be stratified into the high-risk category, and when *NPM1* mutations coexist with other clinically actionable gene mutations, prognostic implications remain context-dependent and necessitate individualized assessment.[17–20] Despite significant advancements in research and clinical applications of *NPM1* mutations in AML over the past decade, many challenges remain that hinder accurate clinical diagnosis and treatment. *NPM1* mutations exhibit substantial clinical heterogeneity, and the prognosis of patients harboring these mutations is strongly linked to concurrent cytogenetic and genetic events.

For over half a century, clinically impactful CNAs have been established as pivotal molecular drivers underlying a spectrum of diseases, spanning cardiovascular disorders to malignancies.[21] In AML, specific CNAs are well-recognized as key determinants of disease heterogeneity and prognostic stratification. For instance, deletions of chromosome 7q (del (7q)) or 17p (del(17p)), and monosomies of chromosomes 5 or 7, are established adverse prognostic factors, while gains of chromosome 8 (trisomy 8) are typically associated with intermediate risk according to international guidelines such as those from the European LeukemiaNet (ELN).[22,23] Beyond these individual recurrent lesions, the overall genomic complexity, often quantified as the total number or burden of CNAs, has emerged as an important prognostic parameter. A growing body of studies has demonstrated that a higher burden of CNAs is independently associated with worse overall survival (OS) in AML patients.[24] However, most of these findings are derived from mixed AML cohorts, and the prognostic role of CNAs in the *NPM1*-mutated subgroup remains understudied and with inconsistent results. Furthermore, cytogenetic abnormalities are often under-detected in routine clinical testing, which have stifled progress in improving clinical diagnostic precision and therapeutic stratification,[25,26] leading to "false isolated cases" and biased clinical decision-making. In contrast, the implementation of shallow whole-genome sequencing (sWGS) for CNA profiling confers complementary utility to traditional methodologies, alleviating the underdetection of critical cytogenetic aberrations and furnishing actionable insights for disease subtyping and prognostic refinement in AML.[27,28] Hence, whether the integration of gene mutation profiles and CNAs can augment prognostic utility for risk stratification and clinical outcomes in *NPM1*-mutated AML represents a current research priority.

Against this scientific backdrop, the present study sought to systematically characterize CNA landscapes in bone marrow specimens from AML patients with *NPM1* mutations via sWGS. Subsequent integrative statistical analyses integrated hematological parameters, myeloid malignancy-associated gene mutations, and other relevant covariates to delineate CNA signatures with potential clinical relevance. The overarching objective was to explore two core questions: (1) Do CNAs (or CNA burden) independently predict prognosis in *NPM1*-mutated AML? (2) Does integrating CNA data with mutation profiles improve risk stratification compared to mutations alone? The goal was to refine existing prognostic frameworks for this genetically distinct subtype.

Materials and Methods

Patients Characteristics

This study was designed as a retrospective analysis and was approved by the Clinical Ethics Committee of China-Japan Friendship Hospital (Approval Nos.: 2023-KY-200 and 2022-KY-229-1). A total of 80 patients with newly diagnosed *NPM1*-mutated AML were consecutively recruited. All participants were treatment-naive and received clinical care at our institution between 2011 and 2023. For the follow-up period, the cutoff date was June 1, 2025, with a median follow-up duration of 23 months and a maximum follow-up period of 108 months. In this study involving 80 patients, 11 received supportive care, exemplified by hydroxyurea administration. Twenty-nine underwent conventional chemotherapy regimens, namely the Idarubicin and Cytarabine (IA) regimen, the Daunorubicin and Cytarabine (DA) regimen, the Mitoxantrone and Cytarabine (MA) regimen, and the low-intensity CAG regimen (comprising Aclarubicin, low-dose Cytarabine, and Granulocyte Colony-Stimulating Factor). Twenty-one were treated with demethylating agents alone, while 9 received a combination of demethylating agents and conventional chemotherapy. Ten patients were on BCL-2 inhibitor-based combination therapy, paired with either conventional chemotherapy or demethylating treatment. Additionally, 6 of the 80 patients incorporated FLT3 inhibitor treatment into the previously mentioned regimens. Moreover, 6 patients within the cohort underwent allogeneic hematopoietic stem cell transplantation. Detailed baseline clinical characteristics of the study cohort are summarized in Table 1.

Table 1 Comparison of Clinical and Laboratory Characteristics in *NPM1*-Mutated Acute Myeloid Leukemia (AML) Patients Based on Copy Number Alteration (CNA) Status

Characteristic	Overall (n=80)	CNA-Positive (n=41)	CNA-Negative (n=39)	P-values	Q-Values*
Median age (range, years)	64 (27–88)	62 (27–88)	66 (41–84)	0.217	0.582
Age≥60, n	48	23	25	0.465	0.678
Age≥70, n	32	14	18	0.273	0.615
Sex (male/female), n	37/43	21/20	16/23	0.361	0.648
White cell count ($\times 10^9$/L)	52.32 (1.23–417.23)	58.64 (1.39–206.45)	45.69 (1.23–417.23)	0.379	0.654
Hemoglobin (g/L)	75 (35–118)	78 (46–118)	72 (35–108)	0.109	0.458
Platelets ($\times 10^9$/L)	75 (4–784)	84 (10–784)	67 (4–300)	0.461	0.678
Bone marrow blasts (%)	64.5 (30–97)	63.4 (30–97)	65.7 (30.5–96.6)	0.662	0.778
NPM1 mutation type, n					
Type A	60	30	30	0.698	0.788
Type B	6	2	4	0.426	0.672
Type D	4	3	1	0.616	0.772
Others	10	6	4	0.800	0.824
FAB subtype, n					
M2	18	10	8	0.678	0.788
M4	8	3	5	0.655	0.782
M5	45	24	21	0.673	0.788
Others	9	4	5	0.937	0.838

(*Continued*)

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Table 1 (Continued).

Characteristic	Overall (n=80)	CNA-Positive (n=41)	CNA-Negative (n=39)	P-values	Q-Values*
Previous hematological disorder, n	16	7	9	0.502	0.692
Induction chemotherapy, n					
Supportive care	11	6	5	0.814	0.830
Conventional chemotherapy (DA, IA, MA, or CAG)	29	17	12	0.320	0.632
Demethylation monotherapy	21	7	14	0.056	0.392
Demethylating agents combined with conventional chemotherapy	9	6	3	0.483	0.682
BCL2 inhibitor combination therapy (+demethylation, or +chemotherapy)	10	5	5	1.000	1.000
FLT3 inhibitor	6	3	3	1.000	1.000
Allo-HSCT, n	6	4	2	0.432	0.674
CR, n	37	21	16	0.361	0.648
1-year relapse, n	15	8	7	0.729	0.810
2-year overall survival, n	9	5	4	0.784	0.822

Notes: * *Q*-values were derived from *P*-values via Benjamini-Hochberg (BH) False Discovery Rate (FDR) Correction.
Abbreviations: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete remission; AML, acute myeloid leukemia; CAG, the low-intensity CAG regimen (comprising Aclarubicin, low-dose Cytarabine, and Granulocyte Colony-Stimulating Factor); DA, the Daunorubicin and Cytarabine regimen; FAB, French-American-British cooperative group; IA, the Idarubicin and Cytarabine regimen; MA, the Mitoxantrone and Cytarabine (MA) regimen.

DNA Extraction, Targeted Next-Generation Sequencing, and Shallow Whole-Genome Sequencing

Genomic DNA was extracted from bone marrow specimens using the Tiangen DP318-02 Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China). Targeted next-generation sequencing (NGS) was performed to profile mutations in myeloid malignancy-associated genes, including the 36 genes recommended by the European LeukemiaNet (ELN).[29] The detailed list of genes analyzed in this study is provided in Supplementary Table 1. Sequencing was conducted on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) with a minimum coverage depth of 1000×. Single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) were identified using the Genome Analysis Toolkit (GATK). Variants were classified into pathogenic/likely pathogenic, variant of uncertain significance (VUS), or benign/likely benign categories in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP); only pathogenic or likely pathogenic mutations were included in subsequent analyses.[30] A variant allele frequency (VAF) cutoff of 2% was applied for pathogenic mutations. For sWGS, approximately 500 ng of high-quality genomic DNA was used for library construction with the KAPA Hyper Prep Kit (KAPA Biosystems, Wilmington, MA, USA).[27] Significantly recurrent amplifications and deletion regions in myeloid malignancy patients were analyzed using GISTIC 2.0 (Version 2.0.23).[31]

Chromosome Karyotype Analysis and Fluorescence in situ Hybridization Detection

Bone marrow cells were harvested following 24–48 hours of unstimulated culture. Conventional karyotype analysis was performed on G-banded metaphase cells prepared from unstimulated bone marrow aspirate cultures using standardized

protocols. A minimum of 20 metaphases were analyzed per sample, and cytogenetic results were reported in accordance with the International System for Human Cytogenetic Nomenclature (ISCN) guidelines.[32] Additionally, fluorescence in situ hybridization (FISH) assays were performed in 45 patients. The FISH probe panels utilized included: XL 5q31/5q33/5p15, XL del(7)(q22q31), XCE 8 Orange, XL ATM/TP53, XL ETV6 (12p13.2), XL RB1/DLEU/LAMP, XL 20q12/20qter, XL RUNX1, and XCE X/Y (Metasystems, Altlussheim, Germany).

Statistical Analysis

Fisher's exact test or one-way analysis of variance (ANOVA) was used to compare categorical and continuous variables, including hematologic parameters, mutated genes, treatment regimens, complete response (CR) rates, and survival rates, across different subgroups. For multiple comparisons involving these variables, the Benjamini-Hochberg (BH) False Discovery Rate (FDR) Correction was applied to adjust *P*-values and minimize the risk of false positive results. OS was defined as the time from the date of diagnosis to the occurrence of death (event) or last follow-up (censored). Kaplan–Meier methods were employed to estimate OS distributions, and the Breslow test (also referred to as the Breslow-Wilcoxon test) was used to compare survival curves between groups. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to quantify survival differences between groups. Multivariate analyses were performed using binary logistic regression and Cox proportional hazards models, with a stepwise backward elimination procedure applied to exclude redundant variables. All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Prevalent *NPM1* Subtypes and Co-Mutation Patterns in 80 Patients with Acute Myeloid Leukemia

NGS was applied to profile the 36 genes in bone marrow specimens from 80 patients with AML. Among these 80 cases, *NPM1* mutation subtypes A, B, and D were the most prevalent, identified in 60 (75%), 6 (7%), and 4 (5%) patients, respectively (Figure 1A). The majority of patients harbored 4 or 5 concurrent gene mutations, accounting for 35% (28 cases) and 25% (20 cases) of the cohort, respectively (Figure 1B). With respect to co-mutation patterns, the most frequently mutated genes alongside *NPM1* included *FLT3* (52.5%), *DNMT3A* (46.2%), *TET2* (28.8%), *IDH2* (26.2%), and *NRAS* (23.8%) (Figures 1C and D). Notably, mutations in *ANKRD26, CSF3R, PHF6, PPM1D*, or *ZRSR2* were not detected in any of the 80 patients enrolled in this study.

Copy Number Alteration Profiles and Their Associations with Clinical and Genomic Characteristics in *NPM1*-Mutated Acute Myeloid Leukemia

Among the 80 *NPM1*-mutated AML samples, 41 patients (51%) were found to harbor CNAs, whereas 39 patients (49%) had no detectable CNAs (Figure 2A). Specifically, 22 patients (27%) carried one CNA, 11 patients (14%) had two CNAs, 3 patients (4%) presented with three CNAs, and 5 patients (6%) exhibited ≥4CNAs. GISTIC analysis identified dup(18)(p11.23) as the most recurrent amplification event, detected in 3 cases, with no high-frequency deletion regions observed (Figure 2B).

The 80 patients were stratified into two cohorts: the CNA-positive group (n=41) and the CNA-negative group (n=39). A comparative analysis of *NPM1* mutation subtypes between these two groups revealed no statistically significant differences (Table 1). Similarly, no significant disparities were observed in terms of the FAB classification, sex, age, white blood cell count, platelet count, hemoglobin level, complete remission (CR) rate, 1-year relapse rate, or 2-year overall survival rate. In this study, an examination of the mutational landscape across both cohorts demonstrated that mutations in *FLT3, DNMT3A, TET2, IDH2*, and *NRAS* were frequently detected in both the CNA-positive and CNA-negative groups (Figures 2C and D). We analyzed genetic alterations in 80 AML patients with *NPM1* mutations, with stratification based on CNA status (Table 2). Unfortunately, there were no significant differences in their prevalence between the two groups.

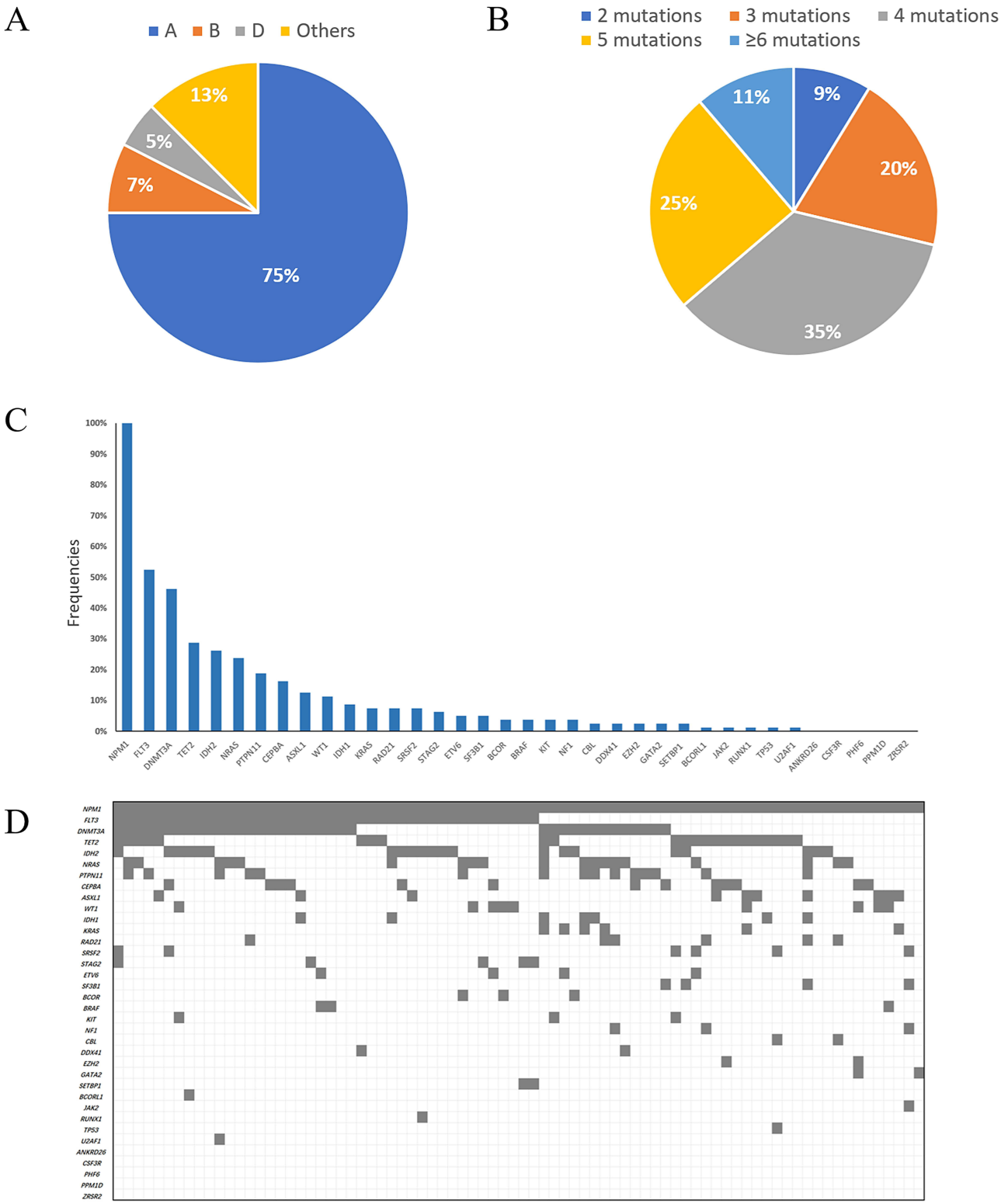

Figure 1 Genetic mutation profiles in acute myeloid leukemia (AML). (**A**) Distribution of *NPM1* mutation subtypes (A, B, D, and Others) in the AML cohort. Subtype A is the most prevalent, comprising 75% of cases. Subtypes B, D, and Others account for 7%, 5%, and 13%, respectively. (**B**) Stratification of AML cases by the total number of somatic mutations detected. Cases with 2 mutations represent 9%, 3 mutations 20%, 4 mutations 35%, 5 mutations 25%, and those with ≥6 mutations 11%. (**C**) Mutation prevalence of genes recurrently altered in myeloid malignancies within the AML cohort, with key genes exhibiting distinct mutation frequencies. (**D**) Heatmap visualization of mutation profiles across 80 AML patients, focusing on a panel of genes associated with myeloid disorders. Rows denote genes, columns denote individual patients, and gray squares indicate the presence of mutations. This heatmap facilitates the evaluation of mutation co-occurrence patterns and the overall genomic profile of the cohort.

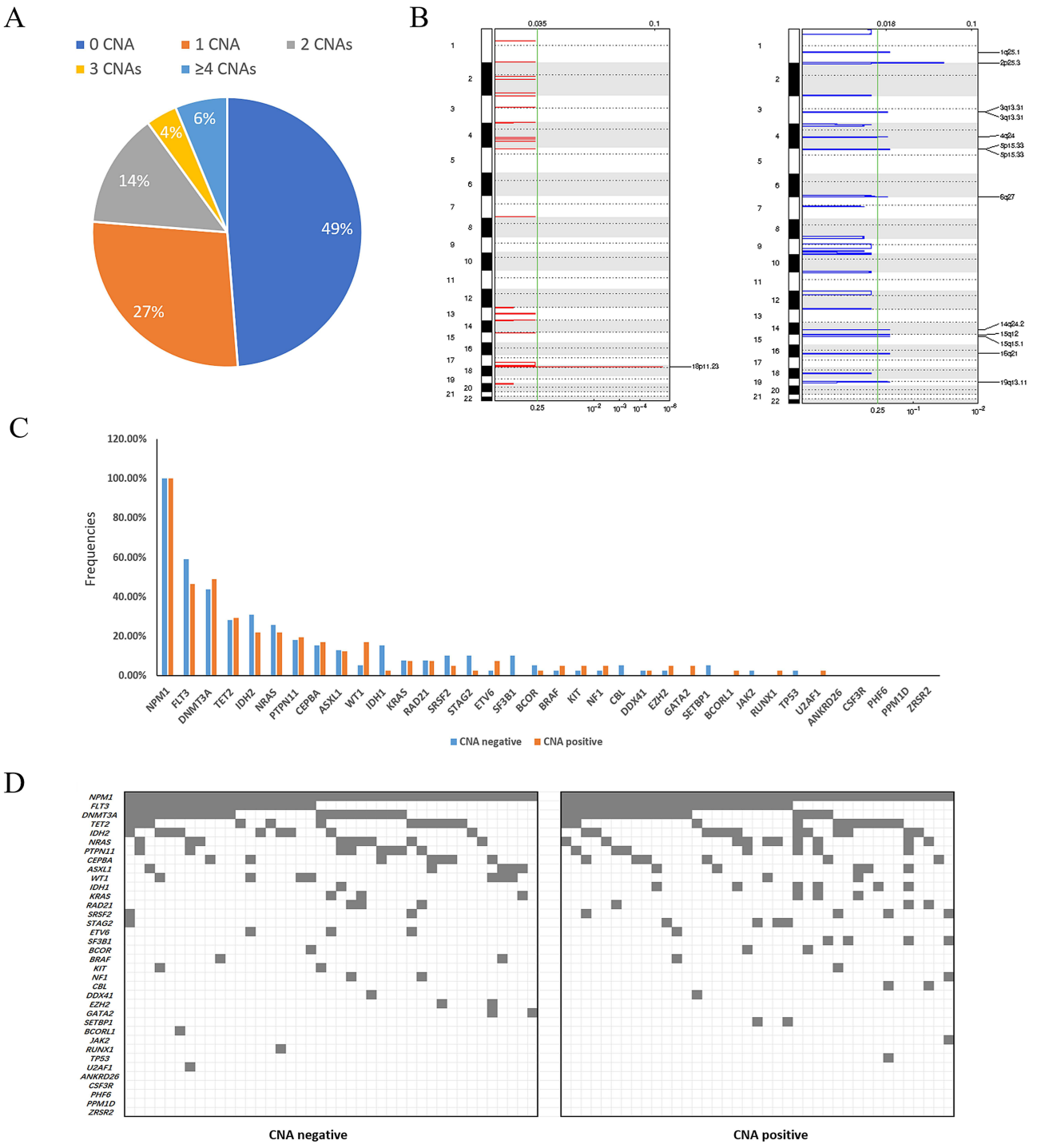

Figure 2 Genomic alterations and mutation profiles stratified by copy number alteration (CNA) status in acute myeloid leukemia (AML). (**A**) Distribution of CNA counts across the AML cohort. Among the cases, 49% harbor 0 CNA (no detectable CNA), 27% have 1 CNA, 14% present with 2 CNAs, 4% show 3 CNAs, and 6% exhibit ≥4 CNAs. (**B**) Recurrent duplication (red) and deletion (blue) regions identified by GISTIC analysis in CNA-positive cases. The left panel displays duplication regions, and the right panel shows deletion regions. Vertical green lines denote the significance threshold (q-value=0.25). (**C**) Comparison of mutation frequencies of genes recurrently altered in myeloid malignancies between the CNA-negative and CNA-positive subgroups. (**D**) Visualization of mutation profiles across CNA-negative (left) and CNA-positive (right) subgroups. Rows represent genes, columns represent individual cases, and gray squares indicate the presence of mutations.

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Table 2 Genetic Alterations in *NPM1*-Mutated Acute Myeloid Leukemia (AML) Patients: Comparison by Copy Number Alteration (CNA) Status

Variant	Whole Cohort (n=80)	CNA-Positive (n=41)	CNA-Negative (n=39)	P-values	Q-Values*
FLT3	42	19	23	0.258	0.596
DNMT3A	37	20	17	0.642	0.780
TET2	23	12	11	0.916	0.838
IDH2	21	9	12	0.370	0.652
NRAS	19	9	10	0.698	0.788
PTPN11	15	8	7	0.858	0.834
CEBPA	13	7	6	0.838	0.834
ASXL1	10	5	5	1.000	1.000
WT1	9	7	2	0.156	0.488
IDH1	7	1	6	0.054	0.392
KRAS	6	3	3	1.000	1.000
RAD21	6	3	3	1.000	1.000
SRSF2	6	2	4	0.426	0.672
STAG2	5	1	4	0.195	0.488
ETV6	4	3	1	0.616	0.772
SF3B1	4	0	4	0.052	0.392

Notes: *Q-values were derived from *P*-values via Benjamini-Hochberg (BH) False Discovery Rate (FDR) Correction.

G-Banding and FISH Detection Analysis in 80 Patients

Conventional karyotype analysis using G-banding was performed for all 80 patients. Thirteen patients were identified with abnormal karyotypes, whereas the remaining 67 cases exhibited normal karyotypes. Among the 80 patients, 45 underwent FISH testing, with 7 samples demonstrating abnormalities in the targeted regions. By integrating G-banding and FISH results, 14 patients were found to harbor chromosomal abnormalities across diverse genomic regions, while 66 patients showed no cytogenetic aberrations. Furthermore, we compared FISH findings with CNAs detected by sWGS in five FISH-positive patients, who carried one or more of the following abnormalities: del(20q13.2), +8, +X, or -Y. The concordance between FISH and sWGS results was consistent with expectations.

Survival Analysis

The 80 patients were stratified into high- and low-variant allele frequency (VAF) groups based on the VAF levels of *NPM1* mutations, followed by univariate survival analysis. No significant difference in survival outcomes was observed between these two groups (P=0.121, Figure 3A). We further investigated the association between *NPM1* mutation subtypes and OS; however, no significant correlation was identified between these subtypes and OS outcomes (Supplementary Figures 1A and 1B). Additionally, patients were stratified into distinct pairwise subgroups according to their mutation burden: <3 mutations versus ≥3 mutations, and <4 mutations versus ≥4 mutations. None of these comparative analyses yielded statistically significant differences in OS (Supplementary Figures 1C and 1D). Notably, the comparison between the <5 mutations group and the ≥5 mutations group, which is presented in Figure 3B of this study (P=0.238), also failed to demonstrate a statistically significant disparity in OS. Further subgroup survival analyses, based on co-mutational profiles, revealed that patients harboring *FLT3*-ITD mutations exhibited shorter OS (P=0.031,

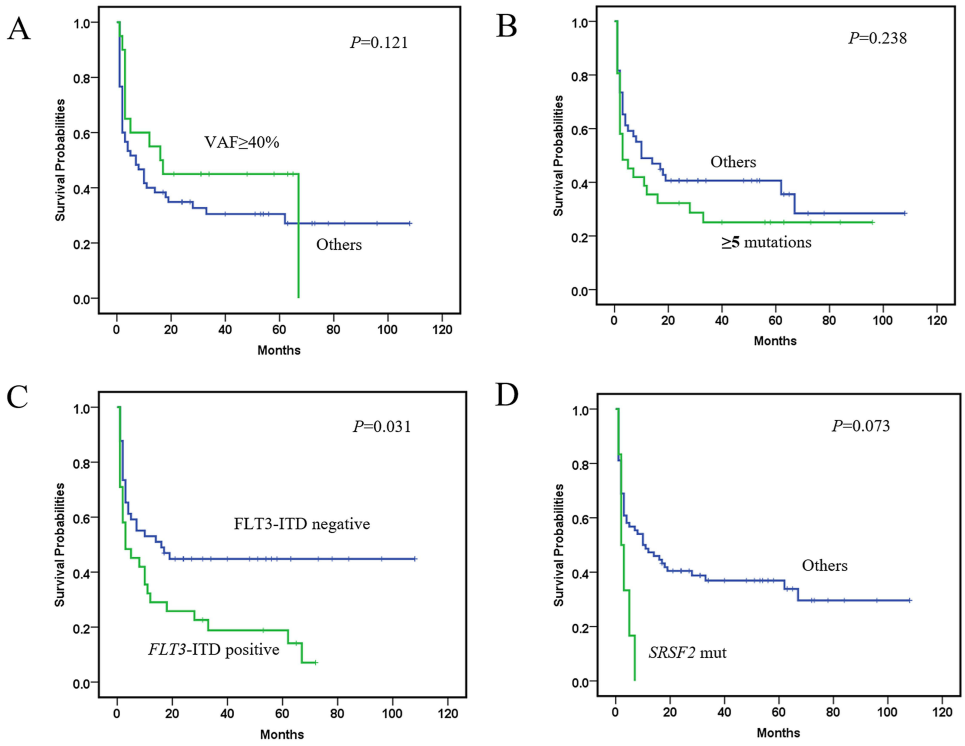

Figure 3 Overall survival (OS) analyses stratified by genomic features in *NPM1*-mutated acute myeloid leukemia (AML) cohorts. (**A**) Kaplan–Meier analysis illustrates overall survival probabilities stratified by *NPM1* variant allele frequency (VAF) levels. Patients were stratified into two groups: those with *NPM1* VAF ≥ 40% (green) and the "Others" group (blue). The Breslow test yielded a *P*-value of 0.121, indicating that different *NPM1* VAF levels do not correlate with significant OS differences. (**B**) The group with ≥5 mutations (green) was compared with the Others (blue). With a *P*-value of 0.238, there is no statistically significant OS difference between these two groups. (**C**) Kaplan–Meier curves compare OS between patients with *FLT3*-ITD-positive (green) and *FLT3*-ITD-negative (blue) statuses. The Breslow test resulted in a *P*-value of 0.031, demonstrating that *FLT3*-ITD positivity is associated with poorer OS. (**D**) This analysis assesses OS in patients with *SRSF2* mutations versus the "Others" group (blue). The Breslow *P*-value is 0.073, suggesting a non-significant trend toward shorter OS in patients with *SRSF2* muations.

Figure 3C). However, patients with concurrent *SRSF2* mutations did not exhibit a statistically significant reduction in OS (*P*=0.073, Figure 3D). Additionally, mutations in *DNMT3A, TET2, IDH2, RAS(NRAS/KRAS), PTPN11*, and *ASXL1* failed to show statistically significant differences in OS (Supplementary Figures 2A-2F). We also analyzed survival differences between the cytogenetically abnormal group and the cytogenetically normal group using traditional genetic testing platforms (G-banding and FISH). Unfortunately, no statistically significant difference in OS was observed between these two groups (Supplementary Figure 3A). Furthermore, we assessed age-related disparities in OS and found that patients aged 60 years or older had significantly shorter OS (Supplementary Figure 3B).

We further analyzed the impact of CNAs on OS. As part of this investigation, we first examined whether dup(18)(p11.23)—a recurrent amplification region—influenced OS. Initially, we performed a conventional comparison between the dup(18)(p11.23)-positive and dup(18)(p11.23)-negative groups. Regrettably, due to the small number of positive cases, the results showed no statistically significant difference in OS between these two groups. Subsequently, we conducted an additional exploratory analysis, comparing the dup(18)(p11.23)-positive group with the CNA-negative group. This design aimed to explore whether this specific amplification might still predict a different outcome, even when benchmarked against a relatively "clean" genomic background. The analysis presented in Figure 4A yielded a *P*-value of 0.271, with no statistically significant difference observed. However, the reliability of this result requires further validation in large-sample, multi-center studies.

To evaluate whether CNAs had a statistically significant impact on clinical survival, we divided the patients into CNA-positive and CNA-negative groups. The results indicated no significant difference in OS between these groups (*P*=0.898, Figure 4B). However, when we grouped patients based on the number of CNAs, those with ≥2 CNAs and ≥3 CNAs had shorter OS (*P*=0.026 and *P*=0.019, respectively) (Figures 4C and D). This study also combined gene mutation

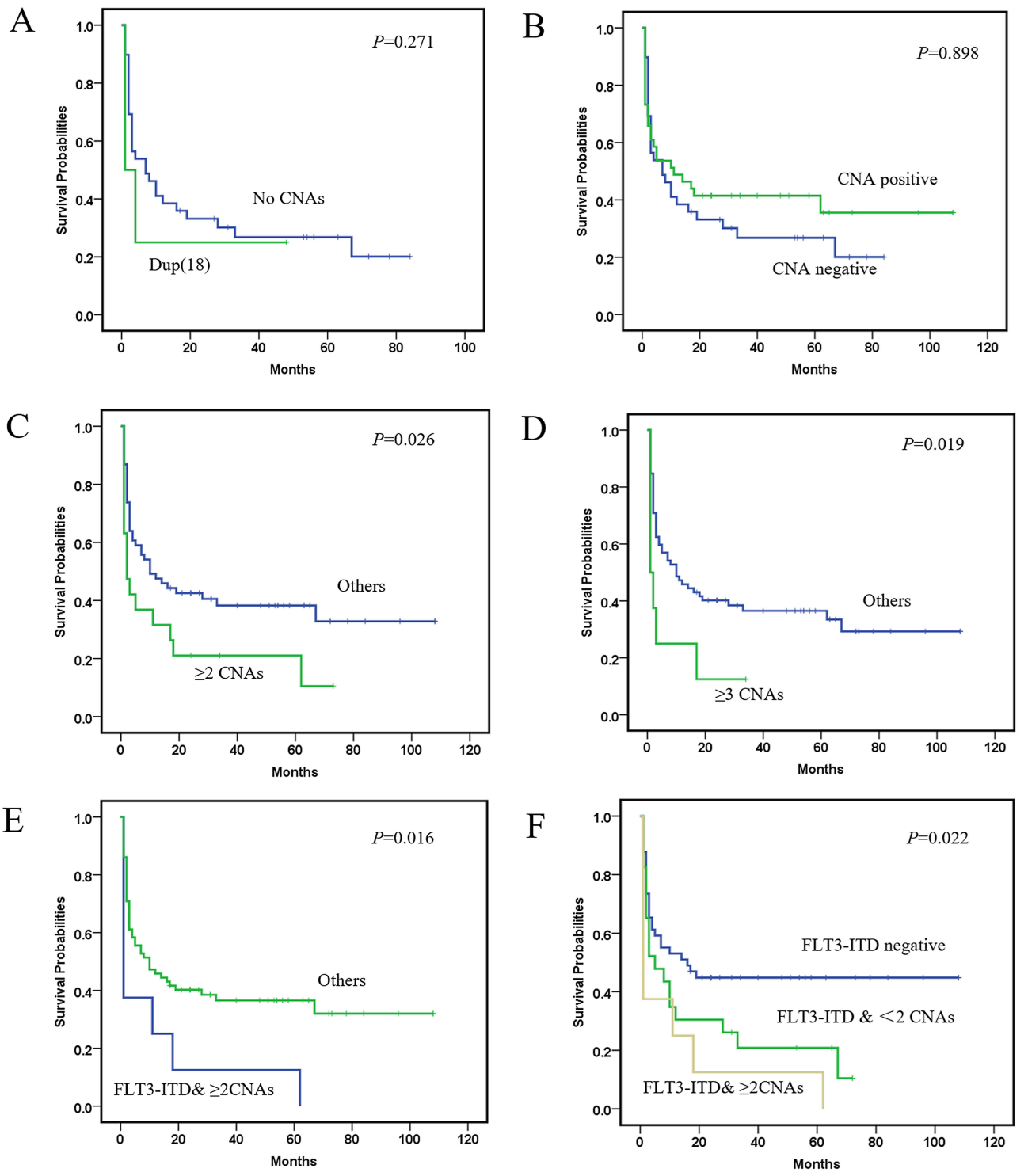

Figure 4 Overall survival (OS) analyses stratified by copy number alterations (CNAs) and *FLT3*-ITD status in *NPM1*-mutated acute myeloid leukemia (AML) cohorts. (**A**) Kaplan–Meier curves depict OS probabilities for patients with dup(18)(p11.23) (green) and those with no CNAs (blue). The Breslow test yielded a *P*-value of 0.271, indicating no significant OS difference between these two groups. (**B**) This panel illustrates OS probabilities for patients stratified into CNA-positive (green) and CNA-negative (blue) groups. With a Breslow *P*-value of 0.898, there is no statistically significant OS difference between the two groups. (**C**) Kaplan–Meier analysis compares OS between patients with ≥2 CNAs (green) and the Others (blue). A Breslow *P*-value of 0.026 shows that patients with ≥2 CNAs have significantly shorter survival. (**D**) OS probabilities are shown for patients with ≥3 CNAs (green) versus the Others (blue). The Breslow test resulted in a *P*-value of 0.019, reinforcing that a higher CNA burden (≥3 CNAs) correlates with shorter OS. (**E**) This panel evaluates OS in patients with both *FLT3*-ITD positivity and ≥2 CNAs (blue) versus the Others (green). The Breslow *P*-value is 0.016, indicating that the co-occurrence of *FLT3*-ITD and ≥2 CNAs is associated with poorer OS. (**F**) Kaplan–Meier curves compare three subgroups: patients with *FLT3*-ITD negative (best OS), those with *FLT3*-ITD positive & <2 CNAs, and patients with *FLT3*-ITD positive & ≥2 CNAs (worst OS). With a Breslow *P*-value of 0.022, patients without *FLT3*-ITD have better OS, while those with combined *FLT3*-ITD and ≥2 CNAs exhibit the poorest OS.

Table 3 Multivariate Cox Regression Analysis of Variables Predicting Overall Survival in 80 *NPM1*- Mutated AML Cases

Variables	P-values	Hazard Ratio (95% Confidence Interval)
Age (≥60 years old)	<0.001	4.167 (2.187–7.939)
FLT3-ITD	0.007	2.463 (1.403–4.321)
≥3 CNAs	0.013	2.872 (1.233–6.688)

Notes: Only variables with a *P*-value<0.5 on univariate analysis were incorporated into the multivariate Cox proportional hazards regression analysis.
Abbreviations: AML, acute myeloid leukemia; CNAs, copy number alterations.

data with CNA information, revealing that patients with both *FLT3*-ITD and ≥2 CNAs had the worst survival, whereas those without *FLT3*-ITD had the best OS (Figures 4Eand F).

In the present study, we enrolled 80 patients with *NPM1*-mutated AML, with a median follow-up duration of 23 months. Despite the relatively small sample size and short follow-up duration, we still performed an exploratory multivariate Cox regression analysis to identify potential prognostic factors for OS. Variables incorporated into the model included *FLT3*-ITD mutation, presence of ≥3 CNAs, age ≥60 years, administration of hypomethylating agent-containing regimens (including single-agent hypomethylating therapy or hypomethylating therapy combined with conventional chemotherapy), and receipt of BCL2 inhibitor-containing regimens (including BCL2 inhibitor combined with hypomethylating therapy or BCL2 inhibitor combined with conventional chemotherapy). As shown in Table 3, the analysis results demonstrated that: patients aged ≥60 years exhibited a significantly shorter OS (hazard ratio [HR]=4.167, 95% confidence interval [CI]: 2.187–7.939, *P*<0.001), those with *FLT3*-ITD mutations had a reduced OS (HR=2.463, 95% CI: 1.403–4.321, *P*=0.007), and patients with ≥3 CNAs also showed a significantly shorter OS (HR=2.872, 95% CI: 1.233–6.688, *P*=0.013). However, given the small sample size and limited follow-up duration, these results should be interpreted cautiously as exploratory, and future validation in larger, multi-center cohorts with prolonged follow-up is essential to solidify their clinical significance and inform precise risk stratification or individualized management strategies in this subset of AML.

Discussion

Over the past half-century, genetics and molecular biology have assumed an increasingly pivotal role in the diagnostic classification, risk stratification, and targeted therapeutic management of AML.[1,3,4] Prognostic evaluation models based on chromosomal aberrations, fusion genes, and somatic mutations have demonstrated substantial and irreplaceable clinical utility, as validated by extensive research.[9,33] For instance, the identification of *NPM1* mutations in 2005 represented a landmark advance, providing a critical impetus for investigations into AML diagnostic subtyping and prognostic assessment. In general, AML patients harboring isolated *NPM1* mutations—unaccompanied by adverse chromosomal abnormalities or *FLT3*-ITD—exhibit favorable prognoses. However, in the presence of concurrent genetic mutations or chromosomal aberrations, an integrated analysis of all genomic events is imperative.[11,16] In clinical practice, distinct genetic events (encompassing chromosomal abnormalities and gene mutations) correlate with divergent clinical outcomes, resulting in *NPM1*-mutated AML patients being stratified into favorable, intermediate, and adverse prognostic categories. The present study aimed to characterize CNA profiles using sWGS, evaluating whether CNA heterogeneity can facilitate refined prognostic stratification of *NPM1*-mutated AML patients from an alternative genomic perspective.

Among the 80 patients with *NPM1* mutations included in this study, type A mutations were the most prevalent, followed by type B and type D, consistent with previous studies.[34] Additionally, we observed that all patients carried two or more mutations, with four and five mutations being the most common. *NPM1* mutations were frequently associated with mutations in the *FLT3, DNMT3A, TET2, IDH2*, and *NRAS* genes. Both our findings and previous research support

the multi-step pathogenesis theory of AML, in which different groups of gene mutations, such as those involved in epigenetic regulation, RNA splicing, signaling molecules, and transcription factors, contribute to cancer development.[15]

Several studies have established that sWGS for CNA detection serves as a valuable adjunct to G-banding and FISH, providing more objective and comprehensive genomic profiles to support clinical dicision-making (enhance diagnostic precision, treatment response assessment, and prognostic stratification).[24,27,28] In the present study, we systematically characterized CNA profiles using sWGS and found that approximately half of the patients with *NPM1*-mutated AML harbored CNAs across diverse genomic loci; subsequently, we integrated CNA data with mutational profiles, FAB classification, bone marrow blast percentage, hematological parameters, CR rate, 1-year relapse rate, and 2-year OS rate for comprehensive analyses, though regrettably, no statistically significant associations were identified in these comparative analyses. The rationale for selecting the 2-year OS rate as our analytical endpoint lies in the specific characteristics of our cohort: this cohort had a median follow-up duration of 23 months and a median age of 64 years, with 54 out of 80 patients (67.5%) having succumbed to the disease by the data cutoff date of June 1, 2025. Notably, the 2-year OS rate exhibits better alignment with the completeness of actual follow-up data, thereby ensuring that our prognostic assessment maintains statistical robustness. Although the current follow-up provides reliable data for short-to-medium-term outcomes, longer follow-up periods in future prospective studies will be crucial for validating the long-term prognostic value of CNA burden (defined as the number of copy number alterations per patient) in *NPM1*-mutated AML. In the upcoming multi-center, large-scale cohort study, we will further explore clinically actionable CNA signatures, including potential high-frequency CNA loci. Concurrently, we plan to incorporate copy-neutral loss of heterozygosity (CN-LOH, a distinct genomic aberration that does not alter copy number but affects allele balance, complementary to conventional CNAs) into our detection and analysis objectives, and conduct multi-level analyses by integrating the VAF values of myeloid-related genes.

In this study, we analyzed genetic alterations in 80 AML patients with *NPM1* mutations, stratified by CNA status. There were no significant differences in prevalence between the two groups. For example, mutations in *FLT3* (42 in the whole cohort, 19 in CNA-positive vs 23 in CNA-negative, P= 0.258, Q=0.596), *DNMT3A* (37 in total, 20 vs 17, P= 0.642, Q=0.780), and *TET2* (23 in total, 12 vs 11, P=0.916, Q=0.838) showed similar distribution patterns, suggesting these mutations may occur independently of CNA status in *NPM1*-mutated AML.

In the survival analysis, no association was observed between *NPM1* mutation subtypes, VAF, and OS. Additionally, although only 3 patients exhibited dup(18)(p11.23), we attempted to explore the potential correlation between this alteration and survival outcomes. Accordingly, patients were stratified into dup(18)(p11.23)-positive and -negative groups based on the presence of this amplification; however, no statistically significant difference in OS was detected. Furthermore, when comparing the dup(18)(p11.23) group with CNA-negative group, no positive findings were identified either. Given the extremely small number of positive cases, no reliable insights could be drawn. Thus, in future studies, statistical analyses with larger sample sizes may enable specific CNA loci to exhibit distinct clinical prognostic significance. Moreover, while CNA positivity alone failed to enable effective prognostic stratification, we still conducted a preliminary analysis of the impact of CNA burden on clinical outcomes. Encouragingly, in this study, patients with ≥2 CNAs or ≥3 CNAs had significantly shorter OS. It is important to emphasize that the observation of a potential association between ≥3 CNAs and adverse outcomes is an exploratory finding of this study, rather than a definitive conclusion. Given the small number of cases in this subgroup (ie, patients with ≥3 CNAs), this result should be viewed as a hypothesis-generating observation and requires further validation in cohorts with larger sample sizes and longer follow-up durations.

Over the past two decades, extensive research has explored the clinical prognostic implications of *NPM1* mutations in combination with other genetic aberrations, with numerous studies confirming that *FLT3*-ITD mutations exert an adverse impact on outcomes in patients with *NPM1*-mutated AML. However, subsets of *NPM1*-mutated patients lacking *FLT3*-ITD still exhibit poor prognosis, underscoring the need to investigate additional contributing factors, such as co-mutated genes and chromosomal abnormalities. The prevailing paradigm holds that adverse chromosomal aberrations negatively influence remission rates and survival in patients with *NPM1*-mutated AML.[35] In our study cohort, we attempted to analyze the association between mutation count and clinical outcomes; unfortunately, no correlation was observed

between total mutation number and survival. We anticipate that larger-scale studies will provide more meaningful clues to clarify this relationship in the future.

Despite consistent evidence that concurrent *NPM1* mutations and *FLT3*-ITD are associated with poor prognosis, significant heterogeneity persists within this subgroup. In the present study, we integrated CNA data with mutational profiles to perform survival analysis. Results demonstrated that *NPM1*-mutated patients harboring both *FLT3*-ITD and ≥2 CNAs had the worst outcomes, followed by those with *FLT3*-ITD but <2 CNAs. These findings suggest that integrating CNA burden with *FLT3*-ITD mutational status may enhance the accuracy of prognostic assessment for *NPM1*-mutated/*FLT3*-ITD-positive patients. Building on this encouraging observation, future studies will leverage large sample sizes to conduct detailed analysis and refinement of CNA results, while integrating *FLT3*-ITD mutation burden, specific copy number variations, and CN-LOH (e.g., 13q LOH). Our goal is to identify more reliable genetic markers, thereby supporting the clinical stratification and management of this AML subtype.

It is important to note that only 6 patients in the cohort harbored *SRSF2* mutations. When analyzing the association between *SRSF2* mutations and OS, the *P*-value did not drop below 0.05 but approached this significance threshold (P=0.073), suggesting that *SRSF2* mutations may have a potential adverse prognostic role that merits validation in larger cohorts. Although integrating multi-parametric genetic data is critical for refined risk stratification, the limitations of the current cohort have limited exploration of *SRSF2*-related associations. This gap will be addressed in our upcoming large-scale multi-center study, which will incorporate *SRSF2* mutations, CNA profiles, CN-LOH, and VAF values to identify multi-dimensional prognostic biomarkers.

This study has several limitations that warrant attention. First, the small sample size (n=80) limits statistical power, and this issue is notably more conspicuous in subgroups—such as patients with ≥3 CNAs (n=8). The insufficient sample size of this subgroup not only impairs the reliability of survival analysis results but also increases the risk of overfitting in the multivariate Cox regression model, potentially leading to overestimated effect sizes for variables like ≥3 CNAs. Therefore, although findings related to CNA burden are statistically significant in our model, they should still be considered exploratory and hypothesis-generating conclusions, and interpreted with caution. Second, due to the limited sample size, we only conducted an overall analysis of CNA burden and did not perform in-depth characterization of specific genomic regions linked to CNAs. Specifically, we anticipate that large-cohort studies will identify clinically meaningful variations in specific regions as well as CN-LOH. Additionally, this study uses a retrospective, single-center design, which may introduce selection bias and restrict the generalizability of the results. Although we controlled for known confounding factors via multivariate analysis, residual confounding from unmeasured variables cannot be excluded.

Given the aforementioned limitations, this study is best categorized as a descriptive, hypothesis-generating investigation. The observed association between high CNA burden (defined as ≥3 CNAs based on our survival analysis results) and poorer OS requires further validation through large-scale, multi-center prospective studies—with sample sizes determined by a priori power calculations. Such studies are critical for confirming the prognostic value of CNA quantification in *NPM1*-mutated AML and defining its role in risk stratification. Furthermore, mechanistic studies are needed to clarify the biological basis of the aggressive phenotype associated with high CNA burden (i.e., an increased number of CNAs) and complex karyotypic changes.

Beyond the limitations of small sample size and limited follow-up duration, treatment heterogeneity also warrants attention: although we documented the primary treatment regimens (e.g., conventional chemotherapy, hypomethylating agents) and controlled for these regimens as a covariate in multivariate analyses, residual heterogeneity persisted (e.g., individualized dosage adjustments, variable adherence to targeted agents such as FLT3 inhibitors). This residual treatment heterogeneity increases the risk of confounding the association between CNA burden and survival outcomes, as treatment-related differences in outcomes may be misinterpreted as genomic effects. However, the core objective of the current study is to explore the association between CNA burden and prognosis in *NPM1*-mutated AML, rather than to quantify treatment efficacy; the present exploratory finding—that "incorporating CNAs may potentially optimize prognostic stratification"—provides a critical hypothesis and direction for subsequent larger-scale, multi-center prospective studies, and its scientific value as a hypothesis-generating observation remains intact.

Conclusion

Genetic events including CNAs and somatic mutations play a crucial role in the molecular pathogenesis of AML.[3,36,37] These variations are the core basis for the current disease classification system and risk stratification framework. Over the past two decades, extensive research has been carried out on AML patients with *NPM1* mutations. However, many issues still await resolution. The findings of this study suggest that combining CNAs with gene mutations may offer additional reference information. This combination can facilitate the optimization of risk analysis and prognostic assessment for this subtype of AML. Furthermore, an association between high CNA burden and adverse clinical outcomes has been observed. This suggests that genomic instability (reflected by high CNA burden) may contribute to disease heterogeneity within the *NPM1*-mutated AML subgroup. Naturally, the aforementioned results are derived from a single-center cohort study with a limited sample size. Therefore, they should be regarded as preliminary exploratory conclusions. Before considering the translation of these findings into clinical decision-making, it is essential to conduct further validation in larger independent patient populations, particularly in the context of current treatment regimens.

Abbreviations

CNA, copy number alteration; CN-LOH, copy-neutral loss of heterozygosity; CR, complete remission; ELN, the European Leukemia Net; FAB, French-American-British; FISH, fluorescence in situ hybridization; *FLT3*-ITD, *FLT3* internal tandem duplication; HR, hazard ratio; MRD, minimal residual disease; NGS, next-generation sequencing; NK, normal karyotype; OS, overall survival; sWGS, shallow whole-genome sequencing; WBC, white blood cell.

Data Sharing Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of China-Japan Friendship Hospital (Approval No.: 2023-KY-200 and 2022-KY-229-1) and conducted in strict adherence to the principles outlined in the Declaration of Helsinki. As a retrospective study, and given that it involves neither the disclosure of patients' personal information nor the imposition of any direct or indirect harm to them, we were granted an exemption from obtaining informed consent.

Author Contributions

All authors participated in drafting the manuscript, critically revising it for important intellectual content, gave final approval of the version to be published, agreed on the journal to which the article was submitted, and are accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373:1136–1152. doi:10.1056/NEJMra1406184
2. Stubbins RJ, Francis A, Kuchenbauer F, Sanford D. Management of acute myeloid leukemia: a review for general practitioners in oncology. *Curr Oncol*. 2022;29:6245–6259. doi:10.3390/curroncol29090491
3. Shimony S, Stahl M, Stone RM. Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2023;98:502–526. doi:10.1002/ajh.26822

4. Padmakumar D, Chandraprabha VR, Gopinath P, et al. A concise review on the molecular genetics of acute myeloid leukemia. *Leuk Res*. 2021;111:106727. doi:10.1016/j.leukres.2021.106727
5. Kayser S, Levis MJ. The clinical impact of the molecular landscape of acute myeloid leukemia. *Haematologica*. 2023;108:308–320. doi:10.3324/haematol.2022.280801
6. Prada-Arismendy J, Arroyave JC, Röthlisberger S. Molecular biomarkers in acute myeloid leukemia. *Blood Rev*. 2017;31:63–76. doi:10.1016/j.blre.2016.08.005
7. Salman H. Comparative analysis of AML classification systems: evaluating the WHO, ICC, and ELN frameworks and their distinctions. *Cancers*. 2024;16:2915. doi:10.3390/cancers16162915
8. de Oliveira Lisboa M, Brofman PRS, Schmid-Braz AT, et al. Chromosomal instability in acute myeloid leukemia. *Cancers*. 2021;13:2655. doi:10.3390/cancers13112655
9. Ishikawa Y. Recent progress in AML with recurrent genetic abnormalities. *Int J Hematol*. 2024;120:525–527. doi:10.1007/s12185-024-03848-3
10. Falini B. NPM1-mutated acute myeloid leukemia: new pathogenetic and therapeutic insights and open questions. *Am Hematol*. 2023;98:1452–1464. doi:10.1002/ajh.26989
11. Falini B, Brunetti L, Sportoletti P, et al. NPM1-mutated acute myeloid leukemia: from bench to bedside. *Blood*. 2020;136:1707–1721. doi:10.1182/blood.2019004226
12. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352:254–266. doi:10.1056/NEJMoa042914
13. Sharma N, Liesveld JL. NPM1 mutations in AML-the landscape in 2023. *Cancers*. 2023;15:1177. doi:10.3390/cancers15041177
14. Wang R, Xu P, Chang LL, et al. Targeted therapy in NPM1-mutated AML: knowns and unknowns. *Front Oncol*. 2022;12:972606. doi:10.3389/fonc.2022.972606
15. Bullinger L, Döhner K, Döhner H. Genomics of acute myeloid leukemia diagnosis and pathways. *J Clin Oncol*. 2017;35:934–946. doi:10.1200/JCO.2016.71.2208
16. Patel SS, Kluk MJ, Weinberg OK. NPM1 Biology in Myeloid Neoplasia. *Curr Hematol Malig Rep*. 2020;15:350–359. doi:10.1007/s11899-020-00592-3
17. Kunchala P, Kuravi S, Jensen R, et al. When the good go bad: mutant NPM1 in acute myeloid leukemia. *Blood Rev*. 2018;32(3):167–183. doi:10.1016/j.blre.2017.11.001
18. Patel SS. NPM1-mutated acute myeloid leukemia: recent developments and open questions. *Pathobiology*. 2024;91:18–29. doi:10.1159/000530253
19. Heath EM, Chan SM, Minden MD, et al. Biological and clinical consequences of NPM1 mutations in AML. *Leukemia*. 2017;31:798–807. doi:10.1038/leu.2017.30
20. Nong T, Mehra S, Taylor J. Common driver mutations in AML: biological impact, clinical considerations, and treatment strategies. *Cells*. 2024;13:1392. doi:10.3390/cells13161392
21. Steele CD, Abbasi A, Islam SMA, et al. Signatures of copy number alterations in human cancer. *Nature*. 2022;606:984–991. doi:10.1038/s41586-022-04738-6
22. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129:424–447. doi:10.1182/blood-2016-08-733196
23. Tashakori M, Kadia T, Loghavi S, et al. TP53 copy number and protein expression inform mutation status across risk categories in acute myeloid leukemia. *Blood*. 2022;140:58–72. doi:10.1182/blood.2021013983
24. Xu X, Bryke C, Sukhanova M, et al. Assessing copy number abnormalities and copy-neutral loss-of-heterozygosity across the genome as best practice in diagnostic evaluation of acute myeloid leukemia: an evidence-based review from the cancer genomics consortium (CGC) myeloid neoplasms working group. *Cancer Genet*. 2018;228-229:218–235. doi:10.1016/j.cancergen.2018.06.004
25. Kanagal-Shamanna R, Hodge JC, Tucker T, et al. Assessing copy number aberrations and copy neutral loss of heterozygosity across the genome as best practice: an evidence based review of clinical utility from the cancer genomics consortium (CGC) working group for myelodysplastic syndrome, myelodysplastic/myeloproliferative and myeloproliferative neoplasms. *Cancer Genet*. 2018;228-229:197–217. doi:10.1016/j.cancergen.2018.07.005
26. Mikhail FM, Biegel JA, Cooley LD, et al. Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). *Genet Med*. 2019;21:1903–1916. doi:10.1038/s41436-019-0545-7
27. Lyu X, Li T, Zhu D, et al. Whole-genome sequencing as an alternative to analyze copy number abnormalities in acute myeloid leukemia and myelodysplastic syndrome. *Leuk Lymphoma*. 2022;63:2301–2310. doi:10.1080/10428194.2022.2080821
28. Van der Eecken K, Van der Linden M, Raman L, et al. Shallow whole-genome sequencing: a useful, easy to apply molecular technique for CNA detection on FFPE tumor tissue-a glioma-driven study. *Virchows Arch*. 2022;480:677–686. doi:10.1007/s00428-022-03268-w
29. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. 2022;140:1345–1377. doi:10.1182/blood.2022016867
30. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. doi:10.1038/gim.2015.30
31. Mermel CH, Schumacher SE, Hill B, et al. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol*. 2011;12:R41. doi:10.1186/gb-2011-12-4-r41
32. Simons A, Shaffer LG, Hastings RJ. Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 edition. *Cytogenet Genome Res*. 2013;141:1–6. doi:10.1159/000353118
33. Kurzer JH, Weinberg OK. Updates in molecular genetics of acute myeloid leukemia. *Semin Diagn Pathol*. 2023;40:140–151. doi:10.1053/j.semdp.2023.04.002
34. Mareschal S, Palau A, Lindberg J, et al. Challenging conventional karyotyping by next-generation karyotyping in 281 intensively treated patients with AML. *Blood Adv*. 2021;5:1003–1016. doi:10.1182/bloodadvances.2020002517
35. Angenendt L, Röllig C, Montesinos P, et al. Chromosomal abnormalities and prognosis in NPM1-mutated acute myeloid leukemia: a pooled analysis of individual patient data from nine international cohorts. *J Clin Oncol*. 2019;37:2632–2642. doi:10.1200/JCO.19.00416

36. Yang JJ, Park TS, Wan TS. Recurrent cytogenetic abnormalities in acute myeloid leukemia. *Methods Mol Biol*. 2017;1541:223–245. doi:10.1007/978-1-4939-6703-2_19
37. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374:2209–2221. doi:10.1056/NEJMoa1516192