Research article

Next-generation sequencing revealed factors associated with cumulative incidence of relapse and leukemia-free survival in patients with newly diagnosed acute myeloid leukemia

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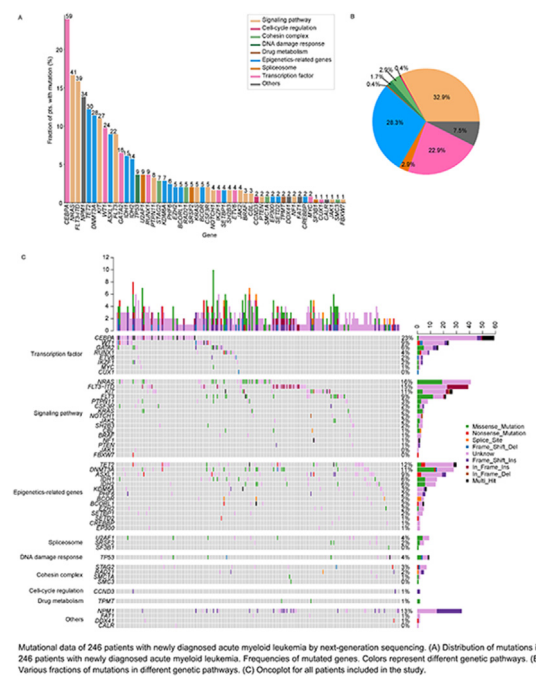[c] Chinese PLA Medical School, Beijing 100853, China
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HIGHLIGHTS

- Factors associated with the cumulative incidence of relapse and leukemia-free survival in real-world patients with acute myeloid leukemia (AML), who received the traditional "3 + 7" regimen were analyzed.

- Serine/arginine-rich splicing factor 2 (SRSF2) mutations were predictive of an increased risk of relapse, inferior leukemia-free survival (LFS) rates, and non-relapse mortality in patients with newly diagnosed AML.

GRAPHICAL ABSTRACT

Mutational data of 246 patients with newly diagnosed acute myeloid leukemia by next-generation sequencing. (A) Distribution of mutations in 246 patients with newly diagnosed acute myeloid leukemia. Frequencies of mutated genes. Colors represent different genetic pathways. (B) Various fractions of mutations in different genetic pathways. (C) Oncoplot for all patients included in the study.

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ABSTRACT

Background: Several prognostic biomarkers have been validated for acute myeloid leukemia (AML), a heterogeneous hematopoietic malignancy. However, the factors associated with the cumulative incidence of relapse (CIR) and leukemia-free survival (LFS) in real-world patients with AML have not been well defined.

Methods: This study examined clinical and mutational data of 246 patients with newly diagnosed AML who received the traditional "3 + 7" regimen in PLA General Hospital from January 2008 to August 2020. Factors associated with CIR and LFS in patients newly diagnosed with AML were analyzed using next-generation sequencing.

Results: Additional sex combs-like 1 (*ASXL1*) and Serine/arginine-rich splicing factor 2 (*SRSF2*) mutations were found to be associated with an increased risk of CIR and a reduced LFS in univariate analysis, while only *SRSF2* mutations were associated with these factors in the multivariate analysis. Hyperleukocytosis maintained an independent effect on LFS in the multivariate analysis. Hematopoietic stem cell transplantation conferred a significant prognostic benefit on both CIR and LFS in our cohort. Furthermore, we validated the risk classification of patients with AML receiving traditional induction regimens across a broad age range. Based on next-generation sequencing results, we concluded that *SRSF2* mutations were predictive of an increased risk of relapse, inferior LFS rates, and non-relapse mortality in patients with newly diagnosed AML.

Conclusion: These findings indicate that patients with *SRSF2* mutations might not benefit from the conventional "3 + 7" regimen. Our results may help in developing molecular stratification strategies and could guide treatment decisions for patients with newly diagnosed AML.

Introduction

Acute myeloid leukemia (AML) is categorized as a heterogeneous hematopoietic malignancy based on its molecular characteristics and clinical outcomes, with approximately 80% of newly diagnosed patients are predicted to have induction mortality rates of around 30% with intensive chemotherapy.[1] Treatment decisions for this disease are currently guided by several well-established prognostic markers, which include both genetic mutations and cytogenetic events.[2] Chromosomal aberrations have been detected in about 55%–60% of AML cases. Further, next-generation sequencing (NGS) studies have revealed several markers, providing a thorough understanding of the genomic landscape of AML and leading to the development of different characterization methods for the disease.[3] When used in combination with recurrent cytogenetic events, genetic mutations detected via NGS have remarkable prognostic value and can guide clinical decisions.[3,4] The recently revised European Leukemia Net (ELN$_{2017}$) stratifying patients into favorable, intermediate, and adverse risk groups based on cytogenetic events and mutations in *FLT3-ITD*, *NPM1*, *CEBPA*, *RUNX1*, additional sex combs-like 1 (*ASXL1*), and *TP53*, has been widely accepted for the diagnosis and treatment of adult patients with AML.[5–11]

The standard intensive chemotherapy combining cytarabine and an anthracycline, popularly called the "3 + 7" regimen, has been used in patients with newly diagnosed AML for more than 40 years.[12] The complete remission (CR) rate for this therapy ranges from 60% to 85% in adults <60 years of age, while the cure rate ranges from 35% to 40%. For patients aged >60 years, the CR and cure rates range from 40% to 60% and 5%–15%, respectively.[13] For patients with newly diagnosed AML, NGS-based stratification with clear treatment recommendations for precise prognostication and treatment has been applied in routine clinical practice. However, there is a paucity of real-world NGS-based data focusing on the prognostic factors associated with the cumulative incidence of relapse (CIR) and leukemia-free survival (LFS) in patients who received the traditional "3 + 7" regimen.

This study aimed to determine the mutational profiles of a large cohort of patients with newly diagnosed AML treated with the traditional "3 + 7" regimen. We analyzed the pre-treatment characteristics, clinical outcomes, and mutational data of 246 patients with newly diagnosed AML to determine a more precise stratification method and evaluate its impact on the prognosis of these patients in routine clinical practice.

Methods

Patients and treatment

Pre-treatment bone marrow or peripheral blood samples were collected from 246 patients with newly diagnosed AML at the PLA General Hospital from January 2008 to August 2020. The inclusion criteria were as follows: patients diagnosed with AML according to the French–American–British (FAB) and World Health Organization (WHO) criteria. Patients diagnosed with acute promyelocytic leukemia were excluded. Risk stratification was based on the ELN$_{2017}$ risk stratification. All patients received the conventional "3 + 7" regimen—daunorubicin ($45 \, \text{mg} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) or idarubicin ($10 \, \text{mg} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$)—for 3 days and cytarabine ($100 \, \text{mg} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) for 7 days. Patients eligible for hematopoietic stem cell transplantation (HSCT) received allogeneic or autologous-HSCT (allo- or auto-HSCT, respectively).

Next-generation sequencing (NGS)

Genetic mutations were detected via targeted capture deep sequencing using NGS at Acornmed Biotechnology Co. Ltd. (Tianjin, China) [Supplementary Table 1]. Multiplex libraries were sequenced on a NovaSeq instrument (Illumina, USA). Raw variant results were then filtered using the following criteria: average effective sequencing depth on target per sample ≥1000 ×, mapping quality ≥30, base quality ≥30, and variant allele frequency ≥1% for single nucleotide variations (SNVs) or insertions and deletions (InDels). Burrows–Wheeler alignment (BWA, version 0.7.12) was used to align the trimmed reads. The MarkDuplicates tool from Picard (version 2.1.0) was used to mark polymerase chain reaction (PCR) duplicates. IndelRealigner and BaseRecalibrator from the Genome Analysis Toolkit (GATK, version 3.8) were used for realignment and recalibration of the BWA data, respectively. Mutect2 (version 3.8) was used to call variants, including SNVs and InDels. All variants were annotated using the ANNOVAR software (version 0722) with COSMIC, 1000G projects, PolyPhen, and SIFT.

Definition of clinical endpoints

Complete remission was defined as the presence of less than 5% bone marrow (BM) blasts, normal maturation of all cell lineages, absolute neutrophil count $>1.0 \times 10^9$/L, platelet count $>1.0 \times 10^{11}$/L, no blasts in the peripheral blood, and no extramedullary leukemia. Partial remission

was defined as a decrease of at least 50% in the percentage of BM blasts to 5%–25% and the normalization of blood counts, as noted above. Relapse was defined as the reappearance of >5% blasts in bone marrow blasts, the reappearance of blasts in the peripheral blood, or extramedullary leukemia in patients with previous CR. The CIR was calculated from the date of CR to the date of relapse, with non-relapse mortality (NRM) considered a competing event. NRM was calculated from the date of CR to the date of death; in CR, relapse was considered a competing event. LFS was defined as the time from the first CR to relapse, censoring at death in CR, or the last follow-up. Patients were followed up until May 31, 2022.

Statistical analysis

Statistical analyses were performed using R software (http://cran.R-project.org, version 3.6.1, The R Foundation for Statistical Computing, Vienna, Austria). The chi-squared test or Fisher's exact test was used for categorical variables. Survival analysis was performed using the Kaplan–Meier method, and differences were assessed using the log-rank test. Competing risk analysis was performed to calculate the CIR and NRM using Gray's test. Odds ratios (ORs) or hazard ratios (HRs) and 95% confidence intervals (CI) were also calculated for the endpoints. Variables with $P < 0.2$ in the univariate analysis were subsequently examined via multivariate analysis using a Cox proportional hazards model to identify the statistically significant parameters for LFS. In contrast, the Fine–Gray model was used for the CIR and NRM. All statistical tests in this study were two-sided, and statistical significance was set at $P < 0.05$.

Results

Patient- and disease-related variables

In total, 246 newly diagnosed patients were enrolled in this study. The patient characteristics are described in Table 1. In this cohort, the median age was 38 years (range, 10–73 years), and 11 (4.5%) patients were older than 60 years. Of all the enrolled patients, 154 (62.6%) were male, 235 (95.5%) had *de novo* AML, 11 (4.5%) had secondary AML, and 19 (7.7%) had extramedullary disease. The median white blood cell (WBC) count, hemoglobin (Hb), platelet (PLT) count, and bone marrow blasts at the time of diagnosis were 17.15 (range, 0.39–405.1 $\times 10^9$/L), 86 (range, 35–156 g/L), 40 (range, 4–924 $\times 10^9$/L), and 62 (range, 20–96%), respectively. Patients were further stratified using cytogenetic risk stratification; 57 (23.2%) of these patients showed favorable cytogenetics, 142 (57.3%) showed intermediate-risk cytogenetics, and 47 (19.1%) showed adverse cytogenetics. Patients were stratified according to the ELN$_{2017}$ risk stratification guidelines to investigate the role of mutational signatures in AML prognosis. A total of 107 (43.5%) patients had favorable risk, 58 (23.6%) had intermediate risk, and 81 (32.9%) had adverse risk. In the "3 + 7" cohort, the rates of CR, partial remission (PR), and persistent disease after initial induction chemotherapy were 62.6%, 11.8%, and 25.6%, respectively, based on bone marrow biopsy results collected on day 14. A total of 187 (76.0%) patients received HSCT, of which 57 (30.5%) received transplants from matched sibling donors (MSDs), and 125 (66.8%) received transplants from alternative donors (including 114 haploidentical donors and 11 suitably matched unrelated donors). A total of 170 (90.9%) patients received HSCT in CR status. At the last follow-up, 71 patients (28.9%) relapsed. With a median follow-up time of 37.98 months (range, 2.87–145.8 months), 142 (57.7%) patients survived, and 104 (42.3%) died.

Mutation topography

A total of 52 mutated genes were detected in the 246 patients included in this study. Among these, 13 genes were mutated in >10 patients [Figure 1A, Supplementary Table 2]. The top five common mutations were in *CEBPA* (24.0%, *n*= 59), *NRAS* (16.7%, *n*= 41), *FLT3-ITD* (15.9%, *n*= 39), *NPM1* (13.8%, *n*= 34), and *TET2* (12.2%, *n*= 30)

for the entire cohort [Table 2, Supplementary Table 2]. The median number of mutated genes per patient was 2 (range, 0–8). The mutated genes were further categorized by their functions, and 32.9% were found to be involved in signaling pathways, including JAK-STAT, NOTCH, RAS signaling pathway, etc.; 28.3% in epigenetics-related

Table 1

Characteristics of patients with newly diagnosed acute myeloid leukemia.

Characteristics (*n*= 246)	Value,*n (%)*
Median age, years (range)	38 (10–73)
<60 years	235 (95.5)
≥60 years	11 (4.5)
Gender	
Male	154 (62.6)
Female	92 (37.4)
AML type	
De novo	235 (95.5)
Secondary	11 (4.5)
WBC, $\times 10^9$/L	
median (range)	17.2 (0.4–405.1)
≥100 $\times 10^9$/L	24 (9.8)
Hb, g/L	
median (range)	86 (35–156)
PLT, $\times 10^9$/L	
median (range)	40 (4–924)
Bone marrow blasts, %	
median (range)	62 (20–96)
Extramedullary disease	19 (7.7)
Skin	5 (2.0)
CNS	4 (1.6)
Lymph node	5 (2.0)
Spleen	2 (0.8)
Others	3 (1.3)
Cytogenetic risk stratification	
Favorable	57 (23.2)
t(8; 21) (q22; q22.1)	46 (18.7)
inv(16) (p13.1q22)	11 (4.5)
Intermediate	142 (57.7)
t(9; 11) (p21.3; q23.3)	1 (0.4)
Entities not classified as favorable or adverse	141 (57.3)
Adverse	47 (19.1)
t(6; 9) (p23; q34.1)	2 (0.8)
t(v; 11q23.3)	3 (1.3)
t(9; 22) (q34.1; q11.2)	1 (0.4)
inv(3) (q21.3q26.2) or t(3; 3) (q21.3; q26.2)	1 (0.4)
−5 or del(5q)	1 (0.4)
−7 or add(7q)/del(7q)	2 (0.8)
−17 or abn(17p)	0 (0.0)
Complex karyotype	25 (10.2)
monosomal karyotype	12 (4.8)
ELN-2017 risk stratification guidelines	
Favorable	107 (43.5)
Intermediate	58 (23.6)
Poor/Adverse	81 (32.9)
Response to first induction	
CR	154 (62.6)
PR	29 (11.8)
Persistent disease	63 (25.6)
CR achievement	225 (91.5)
Relapse	71 (28.9)
Outcome of all patients	
Alive	142 (57.7)
Dead	104 (42.3)
NRM	49 (19.9)
Relapse mortality	55 (22.4)
HSCT	
Yes	187 (76.0)
No	59 (24.0)
Donor of HSCT	
MSD	57 (30.5)
Alternative donor	125 (66.8)
Auto	5 (2.2)
Status of disease prior to HSCT	
CR	170 (90.9)
R/R	17 (9.1)

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Table 1 (*continued*)

Characteristics (n= 246)	Value,n (%)
Outcome of HSCT	
Alive	127 (67.9)
Dead	60 (32.1)
TRM	21 (11.2)
Relapse mortality	31 (16.6)
Others	8 (4.3)
Follow-up, months, median (range)	38.0 (2.9–145.8)

AML: Acute myeloid leukemia; CR: Complete remission; CNS: Central nervous system; ELN: European Leukemia Net; Hb: Hemoglobin; HSCT: Hematopoietic stem cell transplantation; MSD: Matched sibling donor; NRM: Non-relapse mortality; PLT: Platelet; PR: Partial remission; TRM: Treatment-related mortality; WBC: White blood cell.

pathways; and 22.9% in transcription factor pathways [Figure 1B and C]. Furthermore, in the gene association analysis, the top five significant gene associations were *BCORL1-BCOR*, *DNMT3A-NPM1*, *JAK2-ASXL1*, *FLT3-ITD-NPM1*, and *IDH2-DNMT3A* (P < 0.001) [Figure 2, Supplementary Table 3].

Univariate analysis revealed factors associated with CR, CIR, LFS, and NRM

To investigate the prognostic effects of mutation topography and clinical variables, we analyzed the impact of several prognostic variables on CR, CIR, LFS, and NRM in patients treated with the "3 + 7" regimen using univariate analysis. Compared with the rest of the patients, those

Figure 1. Distribution of mutations in 246 patients with newly diagnosed acute myeloid leukemia. (A) Frequencies of mutated genes. Colors represent different genetic pathways. (B) The colored sector displays the various fractions of mutations in different genetic pathways. (C) Oncoplot for all patients included in the study. Each column represents one patient, and each row corresponds to a mutation in the defined genes. Colors represent mutated genes for missense variants (green), nonsense variants (bright red), frameshift indel variants (blue), in-frame indel variants (brown), frameshift ins (purple), and in-frame ins (dark red). The top bar indicates the number of mutations, and the right bar indicates the frequency of different mutated genes.

Table 2

Myeloid mutations in patients with newly diagnosed acute myeloid leukemia.

Myeloid mutations	Value, *n* (%)
CEBPA	
Wild type	187 (76.0)
Mutated (monoallelic)	7 (2.8)
Mutated (biallelic)	52 (21.2)
NRAS	
Wild type	205 (83.3)
Mutated	41 (16.7)
DNMT3A	
Wild type	218 (88.6)
Mutated	28 (11.4)
FLT3-ITD	
Wild type	207 (84.1)
Mutated	39 (15.9)
NPM1	
Wild type	212 (86.2)
Mutated	34 (13.8)
TET2	
Wild type	216 (87.8)
Mutated	30 (12.2)
Other *FLT3*	
Wild type	224 (91.1)
Mutated	22 (8.9)
WT1	
Wild type	222 (90.2)
Mutated	24 (9.8)
ASXL1	
Wild type	224 (91.1)
Mutated	22 (8.9)
KIT	
Wild type	219 (89.0)
Mutated	27 (11.0)
IDH2	
Wild type	232 (94.3)
Mutated	14 (5.7)
GATA2	
Wild type	230 (93.5)
Mutated	16 (6.5)
IDH1	
Wild type	231 (93.9)
Mutated	15 (6.1)
RUNX1	
Wild type	237 (96.3)
Mutated	9 (3.4)
TP53	
Wild type	237 (96.3)
Mutated	9 (3.4)
PTPN11	
Wild type	238 (96.7)
Mutated	8 (3.3)
SRSF2	
Wild type	241 (98.0)
Mutated	5 (2.0)

over 60 years of age had a higher risk of CIR (HR, 4.406; 95% CI, 1.598–12.144; $P = 0.032$), shorter LFS (HR, 4.256; 95% CI, 0.715–25.325; $P = 0.001$), and high reduction in CR rate (OR, 10; 95% CI, 2.781–35.962; $P < 0.001$). Similarly, patients with extramedullary disease had shorter LFS (HR, 2.137; 95% CI, 0.824–5.542; $P = 0.029$), and those who underwent HSCT had lower risk of CIR (HR, 0.382; 95% CI, 0.228–0.64; $P < 0.001$) and shorter LFS (HR, 0.421; 95% CI, 0.226–0.784; $P < 0.001$), with no difference in NRM ($P = 0.766$). Regarding the status of disease prior to HSCT, patients who were resistant or relapsed (R/R) before HSCT had a significantly higher risk of CIR (HR, 2.651; 95% CI, 1.293–5.434; $P = 0.015$) and shorter LFS (HR, 2.156; 95% CI, 0.907–5.123; $P = 0.016$) than those in CR. The other clinical factors analyzed, including sex, sAML *vs. de novo* AML, WBC count, Hb, PLT count, and donor of HSCT, were not significant predictors of CIR, LFS, and NRM. Although there was no significant impact on CIR, LFS, and NRM according to the cytogenetic risk stratification and ELN$_{2017}$ risk stratification guidelines, patients with adverse risk in both

stratifications had worse CR rates (OR, 5.5; 95% CI, 1.434–21.098; $P = 0.007$; and OR, 4.084; 95% CI, 1.822–9.153; $P < 0.001$, respectively) compared to those with favorable risk. Univariate analyses of genetic mutations demonstrated that patients carrying mutations in the *ASXL1* gene had a significantly higher risk of CIR (HR, 2.271; 95% CI, 1.16–4.444; $P = 0.045$) and shorter LFS (HR, 2.109; 95% CI, 0.958–4.639; $P = 0.01$) than did those without the *ASXL1* gene. Similarly, patients with Serine/arginine-rich splicing factor 2 (*SRSF2*) mutations had a significantly higher risk of CIR (HR, 5.499; 95% CI, 1.991–15.185; $P < 0.001$) and shorter LFS (HR, 4.709; 95% CI, 0.576–38.507; $P = 0.001$) than did those without the *SRSF2* gene. Interestingly, we did not observe any difference in the NRM between patients with mutations in *ASXL1* or *SRSF2*. These results indicate that patients over 60 years of age who have extramedullary disease and mutations in the *ASXL1* or *SRSF2* genes are at an increased risk of relapse and have decreased LFS rates [Figure 3, Table 3, and Supplementary Table 4].

Multivariate analysis of survival

Multivariate analysis for CIR, LFS, and NRM was performed using prognostic factors (those with $P < 0.200$ in the univariate analysis). HSCT was found to be associated with a decreased risk of CIR (HR, 0.4354; 95% CI, 0.2465–0.769; $P = 0.0042$) and an increased LFS (HR, 0.4855; 95% CI, 0.2873–0.8204; $P = 0.0069$). Mutations in the *SRSF2* gene were still associated with an increased risk of CIR (HR, 4.306; 95% CI, 1.331–13.930; $P = 0.015$), a reduced LFS (HR, 3.411; 95% CI, 1.018–11.43; $P = 0.0467$), and an increased risk of NRM (HR: 6.279; 95% CI, 1.141–34.56; $P = 0.035$). Among the clinical variables, a high WBC count was a poor prognostic factor of LFS in the multivariate model, with an HR of 1.004 (95% CI, 1–1.008; $P = 0.0324$) [Table 4].

Impact of SRSF2 mutations on chemotherapy and transplant outcomes

Among the study cohort of 246 patients with AML, 187 (76.0%) underwent HSCT. We compared prognostic factors based on transplant status and *SRSF2* mutation status in the survival analysis. Patients with *SRSF2* mutations who underwent chemotherapy only ($n = 4$) had a higher risk of CIR ($P = 0.0013$) and inferior LFS rates ($P = 0.002$), with a median LFS of 3.3 months. In contrast, evaluable patients with wild-type *SRSF2* who underwent chemotherapy only ($n = 55$) had a median LFS of 11.2 months [Supplementary Fig. 1].

Discussion

With the increasing use of NGS-based mutational signatures in patients with newly diagnosed AML, several genetic mutations providing important prognostic information have been detected.[14,15] Risk stratification via genetic abnormalities presented in the updated ELN$_{2017}$ classification has been widely adopted and is currently incorporated into the United States National Comprehensive Cancer Center Network (NCCN) clinical practice guidelines for AML.[16] Although several breakthroughs have been made in understanding its molecular pathogenesis, diagnosis, monitoring, and treatment, AML remains a complicated and difficult-to-treat hematopoietic malignancy.[17,18] Pre-treatment conditions of patients and genetic mutations are being increasingly recognized as important factors in AML treatment and prognosis.[19,20] In recent years, the current induction regimen, the conventional "3 + 7" regimen, has been widely used in patients with highly heterogeneous AML. However, the implementation of mutational signatures in this induction regimen is still lacking. In this study, we described the mutational landscape of a well-characterized population-based cohort of 246 patients with newly diagnosed AML treated with the "3 + 7" induction regimen.

Consistent with the results of previous studies focusing on patients with newly diagnosed AML, the five most frequently mutated genes were *CEBPA*, *NRAS*, *FLT3-ITD*, *NPM1*, and *TET2*.[21,22] Mutations in the *BCOR*
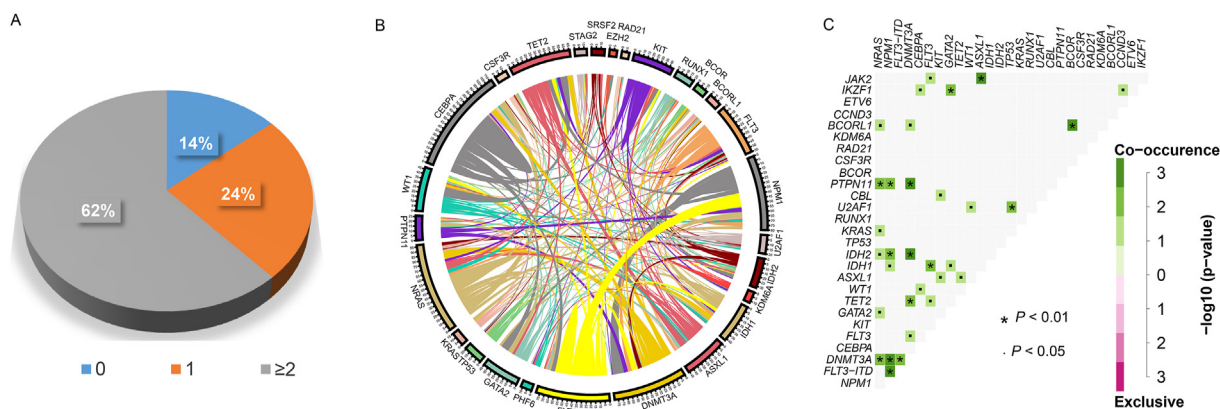
Figure 2. Association among acute myeloid leukemia cases. (A) The colored sector shows the distribution of patients with the different number of mutations. (B) Pairwise associations among genetic mutations. The co-occurrence of each association is color coded, and the significance level is indicated by an asterisk or dot in each field. (C) The circos plot corresponds to the pairwise co-occurrence of mutations and the relative frequency. The length of the arc indicates the frequency of mutations in the first gene, and the width of the ribbon represents the percentage of patients with mutations in the second gene.
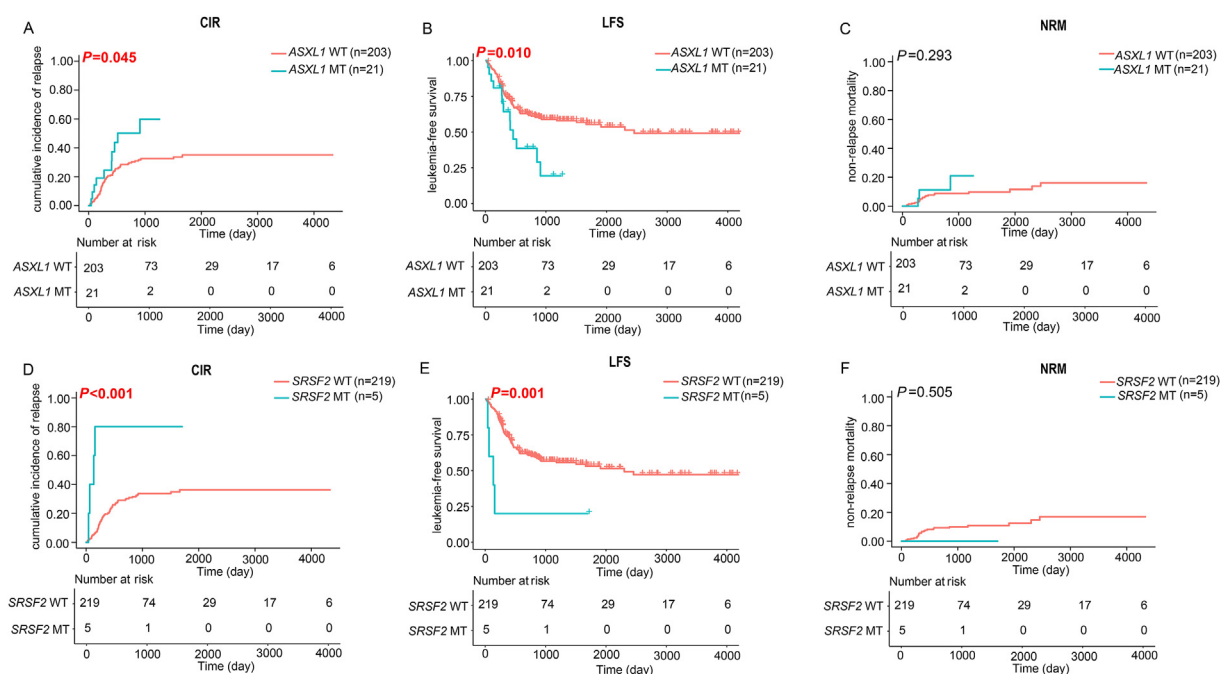
Figure 3. Influence of *ASXL1* and *SRSF2* mutations on clinical outcomes of patients with acute myeloid leukemia. (A) CIR in patients with *ASXL1* mutations. (B) LFS in patients with *ASXL1* mutations. (C) NRM in patients with *ASXL1* mutations. (D) CIR in patients with *SRSF2* mutations. (E) LFS of patients with *SRSF2* mutations. (F) NRM in patients with *SRSF2* mutations. CIR: Cumulative incidence of relapse; LFS: Leukemia-free survival; NRM: Non-relapse mortality.

and *BCORL1* genes were the most common ones detected in this study. Other frequent mutations were found in *DNMT3A-NPM1* and *FLT3-ITD-NPM1*, which were the same as those detected in the study by Folta et al.[1] The genes detected in these co-occurrence analyses could potentially be used as surrogate biomarkers for patients with several molecular signatures.

For developing new effective drugs and combination regimens, it is vital to consider NGS-based molecular features within the current treatment paradigm.[23] Standard first-line AML therapy is rapidly evolving; hence many prognostic factors derived from the era of the "3 + 7" regimen may have already become irrelevant.[24] In the present study, we found that mutations in both the *ASXL1* and *SRSF2* genes predicted a higher risk of CIR and a shorter LFS in the univariate analysis, with the effect of *SRSF2* mutations becoming more prominent in the multivariate analysis. In the survival analysis, we found that patients with *SRSF2* mutations had inferior CIR and LFS rates in the chemotherapy-only cohort, while HSCT abrogated the adverse impact of *SRSF2* mutations

on the prognosis of patients. Mutations in *SRSF2* can be found in many myeloid diseases.[25] As *SRSF2* is a splicing factor, mutations in this gene can cause abnormal splicing associated with exon skipping events.[26] *SRSF2* mutations are usually associated with worse outcomes following traditional chemotherapy, consistent with our results.[27]

Here, we described the clinical and molecular characteristics and the prognosis of patients with newly diagnosed AML. However, this study has some potential limitations, which could be mainly attributed to its retrospective nature. First, the study was carried out at a single center, which limits its generalizability. Second, the small effective sample size is the main challenge in assessing the impact of less frequent mutations on the prognosis of patients with AML. For instance, we could not prove the significance of mutations in the CR rate. Our ability to further develop co-concurrence and mutually exclusive analyses areare limited. The future research directions are carried out at more centers throughout the country and including more patients with newly diagnosed AML, etc.

Table 3

Univariate analysis of clinical variables and myeloid mutations in patients with newly diagnosed acute myeloid leukemia who received the "3 + 7" induction regimen.

Characteristics	CIR			LFS			NRM		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (continuous)	1.013	0.9931–1.034	0.200	1.018	1–1.036	0.049	0.974	0.9525–0.9958	0.020
≥60 vs. <60 years	4.406	1.598–12.144	0.032	4.256	0.715–25.325	0.001	6.021	0.801–45.244	0.482
Gender (male vs. female)	1.09	0.68–1.747	0.898	1.207	0.801–1.817	0.380	2.328	0.869–6.238	0.081
AML type (sAML vs. de novo)	1.026	0.373–2.822	0.713	0.775	0.318–1.89	0.614	0	0–Inf	0.181
WBC (× 10⁹/L) (continuous)	1.002	0.9985–1.006	0.2237	1.003	0.9995–1.006	0.096	0.998	0.9899–1.006	0.650
≥100 vs. <100	1.642	0.817–3.301	0.266	0.340	0.013–9.164	0.259	1.662	0.495–5.579	0.644
Hb (g/L) (continuous)	0.9959	0.9866–1.005	0.3939	0.997	0.989–1.005	0.492	1.004	0.9938–1.013	0.480
PLT (× 10⁹/L) (continuous)	0.9997	0.9971–1.002	0.8212	0.998	0.9859–1.002	0.366	1.001	0.9966–1.006	0.610
Bone marrow blasts (%) (continuous)	1.355	0.459–4.001	0.5822	1.244	0.4862–3.181	0.649	0.944	0.3021–2.949	0.920
Extramedullary disease (yes vs. no)	1.890	0.818–4.364	0.336	2.109	0.818–5.439	0.029	3.631	1.066–12.364	0.101
Cytogenetic risk stratification									
Adverse vs. Favorable	1.193	0.847–1.681	0.454	1.483	0.795–2.764	0.199	1.394	0.722–2.692	0.380
ELN₂₀₁₇ risk stratification guidelines									
Intermediate vs. Favorable	0.983	0.524–1.842	0.877	0.99	0.583–1.682	0.972	1.060	0.392–2.87	0.827
Adverse vs. Intermediate	1.673	0.877–3.193	0.111	1.544	0.905–2.634	0.120	1.133	0.379–3.38	0.837
Adverse vs. Favorable	1.300	1.005–1.68	0.059	1.562	0.974–2.507	0.050	1.081	0.671–1.744	0.959
Response to induction									
R/R vs. CR	1.419	0.681–2.958	0.748	1.556	0.777–3.113	0.135	2.978	1.111–7.98	0.062
HSCT (yes vs. no)	0.382	0.228–0.64	<0.001	0.421	0.226–0.784	<0.001	0.642	0.191–2.159	0.766
Donor of HSCT									
Alternative donors vs. MSD	0.955	0.539–1.692	0.638	1.175	0.723–1.91	0.523	2.350	0.78–7.08	0.096
Status of disease prior to HSCT									
R/R vs. CR	2.651	1.293–5.434	0.015	2.156	0.907–5.123	0.016	1.525	0.351–6.618	0.821
Myeloid mutations (yes vs. no)									
CEBPA	0.998	0.756–1.316	0.988	1.137	0.333–3.881	0.974	1.129	0.714–1.784	0.786
bi–CEBPA vs. WT	0.998	0.755–1.318	0.895	0.99	0.615–1.593	0.966	1.116	0.7–1.779	0.725
mono–CEBPA vs. WT	0.950	0.231–3.9	0.886	1.079	0.326–3.57	0.897	1.832	0.241–13.922	0.552
NRAS	0.851	0.448–1.617	0.723	0.831	0.488–1.413	0.520	0.67	0.199–2.252	0.588
DNMT3A	0.828	0.38–1.805	0.835	0.767	0.401–1.469	0.471	0.362	0.049–2.682	0.334
FLT3–ITD	0.796	0.396–1.601	0.658	0.754	0.428–1.326	0.375	0.544	0.128–2.316	0.471
NPM1	0.557	0.242–1.285	0.132	0.668	0.381–1.173	0.224	0.991	0.338–2.901	0.662
TET2	1.447	0.762–2.75	0.215	1.389	0.716–2.694	0.268	1.045	0.245–4.455	0.776
Other FLT3	0.648	0.236–1.775	0.410	0.79	0.375–1.666	0.574	1.040	0.242–4.463	0.755
WT1	1.669	0.829–3.362	0.174	1.467	0.705–3.052	0.228	1.218	0.286–5.186	0.930
ASXL1	2.271	1.16–4.444	0.045	2.109	0.958–4.639	0.010	3.004	0.879–10.268	0.293
KIT	0.979	0.469–2.045	0.838	1.148	0.607–2.171	0.652	1.772	0.592–5.301	0.265
IDH2	1.323	0.533–3.282	0.657	1.43	0.581–3.515	0.360	1.609	0.378–6.85	0.589
GATA2	0.341	0.084–1.391	0.127	0.42	0.193–0.913	0.126	0.568	0.076–4.236	0.785
IDH1	0.441	0.108–1.797	0.141	0.718	0.33–1.564	0.469	1.665	0.496–5.593	0.188
RUNX1	1.563	0.569–4.293	0.403	1.413	0.493–4.052	0.448	1.168	0.156–8.738	0.894
TP53	0	0–Inf	0.074	0.614	0.202–1.867	0.491	2.168	0.509–9.232	0.094
PTPN11	0.305	0.042–2.192	0.185	0.426	0.166–1.089	0.217	0.751	0.101–5.572	0.976
SRSF2	5.499	1.991–15.185	<0.001	4.709	0.576–38.507	0.001	0	0–Inf	0.505

AML: Acute myeloid leukemia; CI: Confidence interval; CIR: Cumulative incidence of relapse; CR: Complete remission; ELN: European Leukemia Net; Hb: Hemoglobin; HR: Hazard ratio; HSCT: Hematopoietic stem cell transplantation; LFS: Leukemia-free survival; MSD: Matched sibling donor; NRM: Non-relapse mortality; PLT: Platelet; R/R: Resistant or relapsed; sAML: Secondary acute myeloid leukemia; WBC: White blood cell. Values in bold indicate significant difference determined at $P < 0.05$.

Table 4

Multivariate analysis of clinical variables and myeloid mutations in patients with newly diagnosed acute myeloid leukemia who received the "3 + 7" induction regimen.

Characteristics	CIR			LFS			NRM		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (continuous)	1.009	0.9877–1.031	0.400	1.016	0.9979–1.035	0.083	0.968	0.9433–0.9928	0.012
WBC (× 10⁹/L) (continuous)	1.003	0.9984–1.007	0.210	1.004	1–1.008	0.032	0.998	0.9909–1.005	0.580
PLT (× 10⁹/L) (continuous)	1.001	0.9984–1.003	0.600	0.999	0.9967–1.002	0.677	1.001	0.9951–1.006	0.800
Extramedullary disease (yes vs. no)	1.229	0.4841–3.119	0.660	2.317	1.127–4.764	0.223	0.512	0.1853–1.416	0.200
HSCT (yes vs. no)	0.435	0.2465–0.769	0.004	0.486	0.2873–0.8204	0.007	1.751	0.897–3.419	0.100
Myeloid mutations (yes vs. no)									
NPM1	0.696	0.2772–1.746	0.440	0.822	0.4079–1.655	0.582	0.582	0.1709–1.984	0.390
WT1	1.619	0.8071–3.247	0.170	1.208	0.6254–2.3330	0.574	1.910	0.724–5.038	0.190
ASXL1	1.393	0.6876–2.823	0.360	1.769	0.9158–3.4160	0.089	0.891	0.326–2.436	0.820
GATA2	0.420	0.1069–1.652	0.210	0.477	0.1468–1.5470	0.217	0.317	0.03417–2.931	0.310
IDH1	0.542	0.1193–2.465	0.430	1.018	0.3923–2.642	0.971	0.247	0.02211–2.758	0.260
SRSF2	4.306	1.331–13.930	0.015	3.411	1.018–11.43	0.047	6.279	1.141–34.56	0.035

CI: Confidence interval; CIR: Cumulative incidence of relapse; HR: Hazard ratio; HSCT: Hematopoietic stem cell transplantation; LFS: Leukemia-free survival; NRM: Non-relapse mortality; PLT: Platelet; WBC: White blood cell. Values in bold indicate significant difference determined at $P < 0.05$.

In conclusion, we identified different prognostic factors in AML patients treated with traditional induction regimens, further supporting existing evidence for the impact of mutational signatures based on NGS on the prognosis of patients with newly diagnosed AML. These findings may help in identifying suitable induction regimens for eligible patients.

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Author contributions

Sai Huang designed and performed the study and wrote the manuscript. Peng Chen and Lu Wang analyzed the data and revised the manuscript. Ling-min Xu, Ming-yu Jia, Jing Chen, Nan Wang, and Fei Li collected data. Lixia Liu, Jiayue Qin, Chengcheng Wang, and Shanbo Cao performed the NGS platform and statistical analysis. Li-ping Dou and Daihong Liu designed and supervised the research project. All authors reviewed the final manuscript.

Ethics statement

This study strictly followed the *Declaration of Helsinki,* and informed consent was obtained from all subjects. Ethical approval was provided by the regional ethical review board of PLA General Hospital (approval code 2019–338).

Data availability statement

The datasets used in the current study are available from the corresponding author on reasonable request.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or Acornmed Biotechnology Co., Ltd that can inappropriately influence our work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpt.2022.09.003.

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