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Research article

Next-generation sequencing reveals relapse and leukemia-free survival risks in newly diagnosed acute myeloid leukemia treated with CAG regimen combined with decitabine☆

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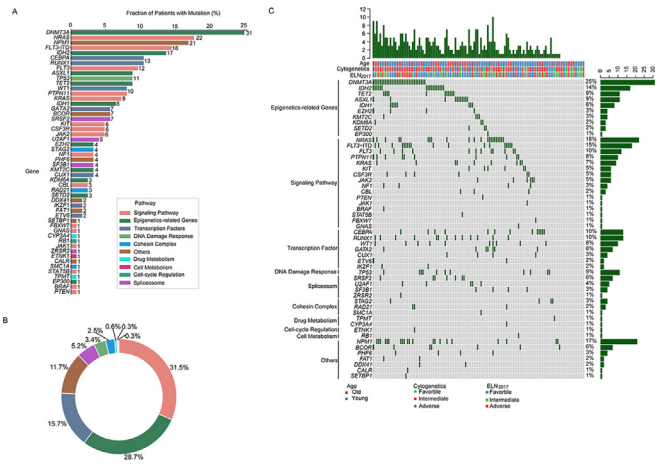
HIGHLIGHTS

- Factors associated with relapse and leukemia-free survival (LFS) risks in decitabine, cytarabine, aclarubicin, and granulocyte colony-stimulating factor (DCAG) regimen-treated patients with acute myeloid leukemia (AML) were analyzed.
- *FLT3-ITD*, *KIT*, *PTPN11*, *GATA2*, and *IDH1* were associated with a higher risk of cumulative incidence of relapse (CIR) or shorter LFS.
- *PTPN11* mutation was solely significant in multivariable analysis in patients newly diagnosed with AML.

GRAPHICAL ABSTRACT



Genetic distribution of mutations in 124 patients newly diagnosed with AML. (A) Gene mutation frequencies depicted with varied colors denoting diverse gene pathways. (B) Diverse-colored segments illustrate the proportional distribution of mutations within distinct gene pathways. (C) An oncoplot illustrating the genetic profile of the 124 patients.AML: Acute myeloid leukemia; ELN2017: European Leukemia Net.

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ABSTRACT

Background: Acute myeloid leukemia (AML) is a heterogeneous hematopoietic malignancy whose prognosis is associated with several biomarkers. Decitabine, a deoxyribonucleic acid (DNA) methyltransferase (DNMT) inhibitor, combined with cytarabine, aclarubicin hydrochloride, and granulocyte colony-stimulating factor (DCAG), has been used in patients newly diagnosed with AML. This regimen has been especially used in older and fragile patients who are immunocompromised or have co-morbidities, as well as those with specific gene mutations. However, the integration of molecular risk stratification and treatment guidance for the DCAG regimen has not been well defined. Therefore, this study aimed to investigate the genetic mutations associated with AML and establish appropriate treatment strategies for patients newly diagnosed with AML.

Methods: This study analyzed the clinical data and genetic mutations based on next-generation sequencing (NGS) in 124 newly diagnosed patients with AML who received the DCAG regimen at the People's Liberation Army (PLA) General Hospital from January 2008 to August 2020. Factors associated with the cumulative incidence of relapse (CIR) and leukemia-free survival (LFS) in patients newly diagnosed with AML were analyzed.

Results: The most adverse prognosis of DCAG-treated patients was observed in those with *FLT3-ITD, KIT, PTPN11, GATA2,* or *IDH1* mutations during univariable analysis, whereas *PTPN11* mutation was solely significant in multivariable analysis, with an increased likelihood of CIR ($P = 0.001$) and reduced LFS duration ($P = 0.077$). Hyperleukocytosis was maintained as an independent risk factor for increased CIR risk ($P = 0.044$) and decreased LFS duration ($P = 0.042$) in multivariable analysis. In this study, we validated the risk classification of patients with AML receiving an epigenetic modifier-based induction regimen across a broad age range.

Conclusion: NGS demonstrated a dismal overall outcome in patients with the rare *PTPN11* mutations, indicating the need for new therapies that target this high-risk subtype of AML. These results offer a potential molecular stratification and treatment guidance for patients with AML.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematopoietic malignancy characterized by substantial heterogeneity in molecular anomalies and clinical progressions.[1] Chromosomal aberrations, detected in approximately 55–60% of patients with AML, are well-established prognostic markers that guide treatment decisions.[2] Moreover, next-generation sequencing (NGS) has revealed better characterization and understanding of the genomic landscape of AML.[3] Combined with recurrent cytogenetic events, gene mutations have great prognostic significance and potential to guide therapeutic decisions.[3,4] The European Leukemia Net (ELN_{2017}) guidelines have been broadly accepted for the diagnosis and management of AML in adult patients aged 18–60 years. These guidelines categorize patients into favorable, intermediate, and adverse risk cohorts based on cytogenetic profiles and the mutational status of *FLT3-ITD*, *NPM1*, *CEBPA*, *RUNX1*, *ASXL1*, and *TP53*.[5–11] However, clinical prognostic factors of patients in all age groups were not included in the ELN_{2017} guidelines. Similarly, the induction regimen recommendations for different gene mutations are not provided. Thus, further research is needed to extend the study to younger and older patients and to determine a suitable induction regimen to improve the precision of risk stratification guidelines.

The conventional intensive chemotherapy regimen, which merges cytarabine with an anthracycline and is recognized as the "3 + 7" protocol, has been used to treat AML for over 4 decades.[12] Complete remission and cure rates range from 60 to 85% and 35–40% respectively for adults under 60 years old and from 40 to 60% and 5–15% respectively for those older than 60 years.[13] Decitabine, a hypomethylating agent, is a deoxyribonucleic acid (DNA) methyltransferase (DNMT) inhibitor that can pharmacologically reverse DNA methylation and increase the chemosensitivity of leukemic cells.[14] Decitabine has become an appropriate treatment for all subtypes of AML over the past decade.[15] With well-tolerated toxicity and combined with cytarabine, aclarubicin, and granulocyte colony-stimulating factor (G-CSF), the decitabine, cytarabine, aclarubicin, and granulocyte colony-stimulating factor (DCAG) regimen was designed to improve the response rate by exploiting the synergy among these agents. In addition, a 70–80% overall rate of response and a clinical complete remission (CR) rate of up to 64.7% have been observed in older patients.[16–19] However, there is an absence of NGS-derived categorizations suitable for routine clinical applications. Furthermore, there are unequivocal therapeutic guidelines for individuals recently diagnosed with AML. This deficiency has hindered substantial advancements in prognosis and treatment strategies.

This study aimed to identify genetic mutations and establish appropriate treatment strategies for several individuals recently diagnosed with AML. Our analysis included pretreatment attributes, clinical results, and mutational insights of 124 patients with AML in the early stages of diagnosis. This study further aimed to refine our ability to categorize patients more accurately and to gauge the prognostic significance of employing the DCAG induction regimen, a therapeutic approach intended for everyday clinical application.

Methods

Patients and treatment

Pretreatment bone marrow or peripheral blood specimens were collected from 124 newly diagnosed patients with AML at the PLA General Hospital from January 2008 to August 2020. The inclusion criterion was a recent AML diagnosis, whereas patients diagnosed with acute promyelocytic leukemia were excluded. Patients were diagnosed according to the French–American–British and World Health Organization guidelines. Risk stratification was based on the revised Medical Research Council (MRC) prognostic classification and ELN_{2017} risk stratification.[4] All patients received DCAG regimens as follows for the first two chemotherapy cycles: decitabine (20 mg $\cdot$ m^{-2} $\cdot$ day^{-1}) for 5 days, cytarabine (100 or 200 mg/m^2) every 12 h for 5 days, aclarubicin (10 mg $\cdot$ m^{-2} $\cdot$ day^{-1}) for 5 days, and Granulocyte Colony Stimulating Factor (G-CSF) 300 μg/day from Day 0 to neutrophil recovery. Patients who were suitable for hematopoietic stem cell transplantation (HSCT) underwent allogeneic HSCT (allo-HSCT). For patients who were not considered suitable for allo-HSCT and those who were found eligible for transplantation but lacked a donor, high-dose cytarabine was used for consolidation, and the DNMT inhibitor decitabine was used as maintenance therapy.

Next-generation sequencing

Mutations in genomic DNA isolated from the bone marrow diagnostic samples were detected using targeted capture deep sequencing with NGS at Acornmed Biotechnology Co., Ltd. (Tianjin, China) [Supplementary Table 1]. The NovaSeq platform (Illumina, San Diego, CA, USA) was used

to sequence the multiplex libraries. Initial variant outputs were filtered based on the following criteria: a minimum average effective sequencing depth of 1000 × per sample in the target region, a base quality threshold of 30, a mapping quality threshold of 30, and a variant allele frequency exceeding 1% for single nucleotide variations (SNVs), insertions, or deletions (InDels). The Burrows–Wheeler alignment (BWA) tool (version 0.7.12) was used to align the processed reads. The MarkDuplicate tool (version 2.1.0) from Picard was used to account for the polymerase chain reaction (PCR) duplicates. Recalibration of base qualities and realignment of BWA-aligned data were conducted using BaseRecalibrator and IndelRealigner, respectively, from the Genome Analysis Toolkit (GATK; version 3.8; Broad Institute, Cambridge, MA, USA). Variants encompassing both SNVs and InDels were identified using Mutect2 software (version 3.8). After variant calling, all identified variants were annotated using ANNOVAR software, incorporating data from COSMIC (the Catalogue Of Somatic Mutations In Cancer), the 1000 Genomes (1000G) project, and predictive scores from PolyPhen and SIFT (sorts intolerant from tolerant) (version 0722).

Definition of clinical endpoints

Complete remission (CR) was operationally defined as the achievement of a <5% bone marrow (BM) blast reconstitution with concurrent normal maturation observed across all cellular lineages. This was concomitant with an absolute neutrophil count $>1.0 \times 10^9$/L, a platelet count $>100 \times 10^9$/L, and the absence of blasts in the peripheral blood or extramedullary leukemia manifestations. Relapse was defined as the re-emergence of >5% blasts in the BM, detection of blasts in the peripheral blood, or the manifestation of extramedullary leukemia in patients who had previously demonstrated CR. To assess the probability of relapse, the cumulative incidence of relapse (CIR) was calculated from the time of CR until relapse. Leukemia-free survival (LFS) was temporally defined as the interval between the initial attainment of CR and the onset of relapse, with data censored for patients who died while in CR or were subjected to the latest follow-up. The most recent patient follow-up was conducted on October 31, 2022.

Statistics

Statistical analyses were executed using R (version 3.6.1, http://cran.R-project.org). Categorical variables underwent scrutiny through either the chi-square test or Fisher's exact test. Survival analysis entailed the utilization of the Kaplan–Meier method, with distinctions assessed through the log-rank test. A competing-risk analysis was employed using Gray's test to CIR. For categorical variables, odds ratios (ORs) or hazard ratios (HRs) were reported as pertinent to the endpoints under consideration. Parameters exhibiting a significance level of $P < 0.2$ through univariate analysis underwent subsequent scrutiny via multivariate analysis. Specifically, Cox proportional hazards modeling was applied to discern statistically significant parameters for LFS, whereas the Fine–-Gray model was utilized for CIR determination. All statistical assessments adhered to a two-sided approach. $P < 0.05$ was considered statistically significant.

Results

Patient- and disease-related variables

In total, 124 newly diagnosed patients were enrolled in this study. The patient characteristics are shown in Table 1. The median age was 55 years (range, 39–78 years), and 67 patients (54%) were male. *De novo* AML constituted 82.3% of the cohort, and three (2.4%) patients harbored extramedullary disease. The median white blood cell (WBC), hemoglobin (Hb), platelet (Plt), and BM blast counts at diagnosis were 5.68 (0.25–208.40 × 10^9/L), 80 (26–161 g/L), 54 (3–621 × 10^9/L) and 51.6 (20–96), respectively. Here, 32 (25.8%) patients had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≥2. Eighteen (14.5%) patients harbored favorable cytogenetics, 74 (59.9%) had intermediate-risk cytogenetics, and 32 (25.6%) had adverse cytogenetics. According to ELN_{2017}, 39 (31.5%), 33 (26.6%), and 52 (41.9%) patients had favorable, intermediate, and adverse risks, respectively. The initial induction of DCAG resulted in CR, partial remission (PR), and persistent disease rates of 45.2%, 16.1%, and 38.7%, respectively, on the Day 14 BM biopsy. Furthermore, 40 (32.3%) patients underwent HSCT. Among these patients, nine (22.5%) received transplants from matched sibling donors (MSDs), whereas 30 (75%) underwent transplants from alternative donors (ADs) (including 27 haploidentical donors and three suitably matched unrelated donors). Additionally, 33 patients (85%) who underwent HSCT achieved CR. After the most recent follow-up, 34 patients (27.4%) experienced relapse. The median duration of follow-up was extended to 41.12 months (3.33–108.60), revealing that survival was achieved by 46 patients (37.1%), whereas 78 patients (62.9%) succumbed to the disease [Table 1].

Mutation topography

A comprehensive analysis of a 52-gene panel was carried out in 124 patients newly diagnosed with AML. A subset of 13 genes demonstrated mutations in >10 patients [Figure 1A; Supplementary Table 2]. The most frequent mutations were *DNMT3A* (25%, $n = 31$), *NRAS* (17.7%, $n = 22$), *NPM1* (16.9%, $n = 21$), *FLT3-ITD* (14.5%, $n = 18$), and *IDH2* (13.7%, $n = 17$) [Supplementary Tables 2 and 3]. The median number of mutated genes per patient was 2 (range, 0–8). Subsequent functional categorization of the genetic landscape revealed that 31.5% of the mutated genes participated in activated signaling pathways, 28.7% were associated with epigenetic-related pathways, and 15.7% were implicated in transcription factor pathways [Figure 1B and C]. Thus, a gene association analysis was conducted to uncover the potential synergistic relationships between these genetic aberrations. Significant co-existence patterns were observed for *GATA2-CEBPA*, *SRSF2-RUNX1*, *NPM1-DNMT3A*, *SRSF2-ASXL1*, and *FLT3-ITD-NPM1* ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.001$, and $P = 0.001$, respectively) [Figure 2; Supplementary Table 4].

Univariable analysis revealed factors associated with cumulative incidence of relapse and leukemia-free survival

Univariate analysis revealed that patients with a higher WBC count had a higher risk of CIR (HR, 1.013; $P = 0.001$) and shorter LFS (HR, 1.010; $P = 0.006$). Specifically, patients with hyperleukocytic AML (initial WBC count $\geq 100 \times 10^9$/L) had a significantly higher risk of CIR (HR, 13.517; $P < 0.001$) and shorter LFS (HR, 6.942; $P < 0.001$). Similarly, patients with extramedullary disease had a higher risk of CIR (HR, 3.441; $P = 0.044$). Furthermore, patients with an R/R status had a significantly higher risk of CIR (HR, 5.777; $P = 0.001$) and shorter LFS (HR, 4.380; $P < 0.001$) than those with CR. HSCT was not a predictor of CIR and LFS in this cohort, nor was the HSCT donor. Additional clinical variables assessed, including sex and secondary AML (sAML) *vs. de novo* cases, Hb, Plt, and BM blast counts, did not exhibit noteworthy predictive significance for the cumulative incidence of CIR and LFS.

Univariate analyses focusing on gene mutations revealed that patients with *FLT3-ITD* mutations experienced a statistically significant reduction in LFS (HR, 2.326; $P = 0.025$). Similarly, patients with *KIT* mutations exhibited a significant increase in CIR (HR, 4.534; $P = 0.015$), concomitant with a significant decrease in LFS (HR, 3.104; $P = 0.011$). Similar results were observed in patients with *PTPN11* mutations, who had a higher risk of CIR (HR, 4.868; $P = 0.021$) and shorter LFS (HR, 3.379; $P = 0.012$). Patients with mutations in *GATA2* and *IDH1* had a significantly higher risk of CIR (HR, 3.927; $P = 0.034$ and HR, 2.332; $P = 0.039$, respectively). These results indicate that patients who had higher WBC counts and mutations in *FLT3-ITD*, *KIT*, *PTPN11*, *GATA2*, or *IDH1* had a significantly higher risk of relapse or shorter LFS [Figure 3; Table 2].

Table 1
Characteristics of newly diagnosed patients with acute myeloid leukemia.

Characteristics	Value
Age (years), median (range)	55.0 (39.0–78.0)
<60 years, *n* (%)	83.0 (66.9)
≥60 years, *n* (%)	41.0 (33.1)
Sex, *n* (%)	
Male	67.0 (54.0)
Female	57.0 (46.0)
AML type, *n* (%)	
De novo	102 (82.3)
Secondary	22 (17.7)
WBC ($\times 10^9$/L)	
Median (range)	5.7 (0.3–208.4)
$\geq 100 \times 10^9$/L, *n* (%)	5 (4.0)
Hb (g/L)	
Median (range)	80.0 (26.0–161.0)
PLT, 10^9/L	
Median (range)	54.0 (3.0–621.0)
Bone marrow blasts, %	
Median (range)	51.6 (20.0–96.0)
Extramedullary disease, *n* (%)	3.0 (2.4)
Skin	1.0 (0.8)
CNS	1.0 (0.8)
Lymph node	1.0 (0.8)
ECOG PS, *n* (%)	
0	52.0 (41.9)
1	40.0 (32.3)
2	24.0 (19.3)
3	8.0 (6.5)
Cytogenetic risk stratification, *n* (%)	
Favorable	18.0 (14.5)
t(8;21) (q22;q22.1)	15.0 (12.1)
inv(16) (p13.1q22)	3.0 (2.4)
Intermediate	74.0 (59.9)
Entities not classified as favorable or adverse	74.0 (59.9)
Adverse	32.0 (25.6)
t(6;9) (p23;q34.1)	2.0 (1.6)
t(v;11q23.3)	1.0 (0.8)
inv(3) (q21.3q26.2) or t(3;3) (q21.3;q26.2)	3.0 (2.4)
Complex karyotype	15.0 (12.0)
Monosomal karyotype	11.0 (8.8)
ELN_{2017} risk stratification guidelines, *n* (%)	
Favorable	39.0 (31.5)
Intermediate	33.0 (26.6)
Adverse	52.0 (41.9)
Response to first induction, *n* (%)	
CR	56.0 (45.2)
PR	20.0 (16.1)
Persistent disease	48.0 (38.7)
CR achievement	88.0 (71.0)
Relapse	34.0 (27.4)
Outcome of all patients, *n* (%)	
Alive	46.0 (37.1)
Dead	78.0 (62.9)
Relapse mortality	29.0 (23.4)
HSCT, *n* (%)	
Yes	40.0 (32.3)
No	84.0 (67.7)
Donor of HSCT, *n* (%)	
MSD	9.0 (22.5)
Alternative donor	30.0 (75.0)
Auto	1.0 (2.5)
Status of disease prior to HSCT, *n* (%)	
CR	34.0 (85.0)
R/R	6.0 (15.0)
Outcome of HSCT, *n* (%)	
Alive	25.0 (62.5)
Dead	15.0 (37.5)
TRM	4.0 (10.0)
Relapse mortality	9.0 (22.5)
Others	2.0 (5.0)
Follow-up (months), median (range)	41.1 (3.3–108.6)

AML: Acute myeloid leukemia; CNS: Central nervous system; CR: Complete remission; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ELN_{2017}: European Leukemia Net; Hb: Hemoglobin; HSCT: Hematopoietic stem cell transplantation; MSD: Matched sibling donor; PLT: Platelets; PR: Partial remission; R/R: Relapsed/refractory; TRM: Transplant-related mortality; WBC: White blood cell.

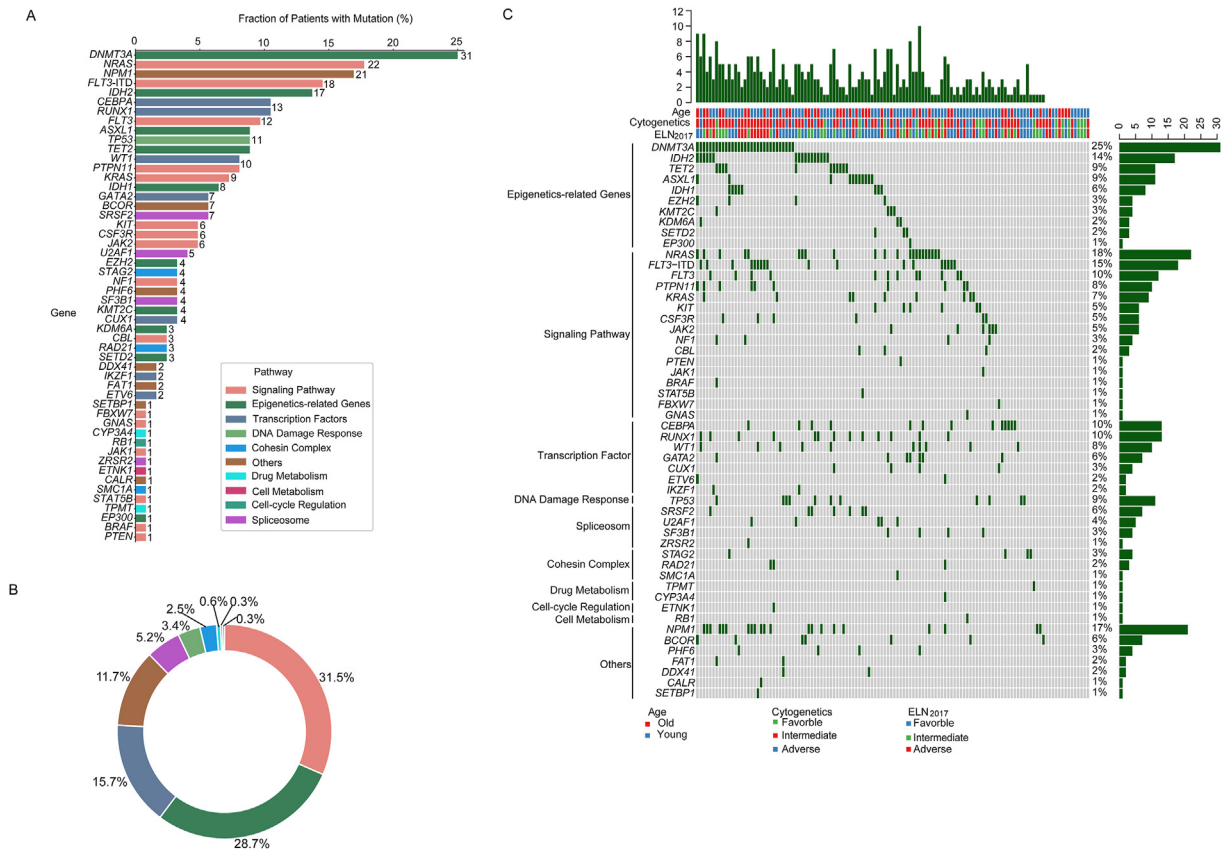

Figure 1. Genetic distribution of mutations in 124 patients newly diagnosed with AML. (A) Gene mutation frequencies depicted with varied colors denoting diverse gene pathways. (B) Diverse-colored segments illustrate the proportional distribution of mutations within distinct gene pathways. (C) An oncoplot illustrating the genetic profile of the 124 patients. Each patient is represented by a column, whereas rows correspond to mutations in predefined genes. The upper bar denotes mutation frequency (mutations per megabase of DNA), and the lateral bar illustrates the frequency of distinct mutated genes. The oncoplot provides patient stratification based on age, cytogenetics, and ELN_{2017}. AML: Acute myeloid leukemia; ELN: European Leukemia Net.

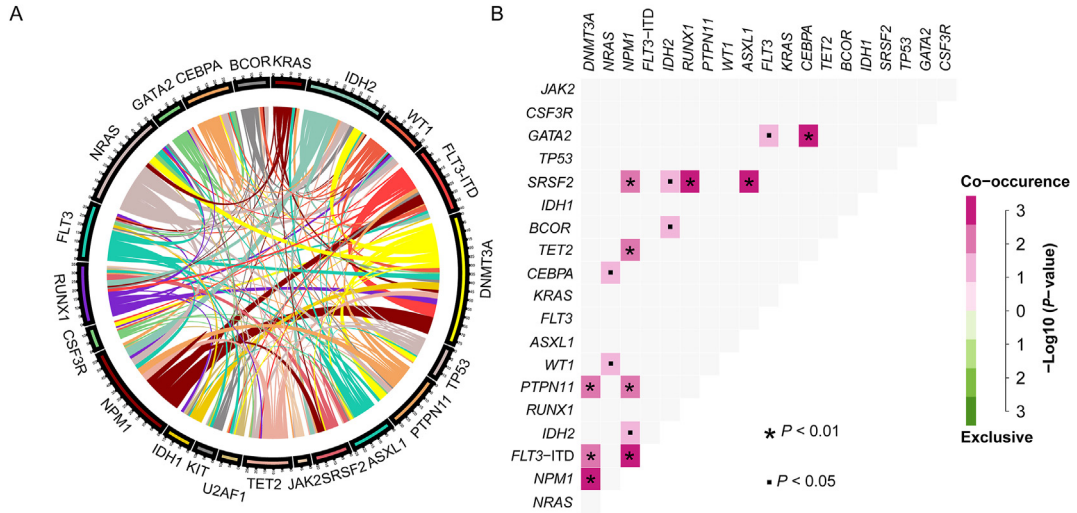

Figure 2. Correlation among mutations in cases of AML. (A) Presentation of pairwise associations between gene mutations, with color-coded indications of co-occurrence. (B) The Circos plot visually represents the pairwise co-occurrence of mutations and their relative frequencies. Statistical significance is denoted by asterisks or dots in each respective field. AML: Acute myeloid leukemia.

As the CIR and LFS after allogeneic stem cell transplantation were affected by variables beyond leukemia biology, univariate analyses were performed separately for non-transplanted patients, and survival analyses were censored for transplantation. In univariate analyses of non-transplant patients, patients with higher WBC counts, as well as *GATA2* and *TP53* mutations had an elevated propensity for CIR and correspondingly reduced LFS [Supplementary Table 5]. Similar results were observed in survival analyses censored for transplantation, patients who were ≥60 years old, and hyperleukocytosis [Supplementary Table 6].

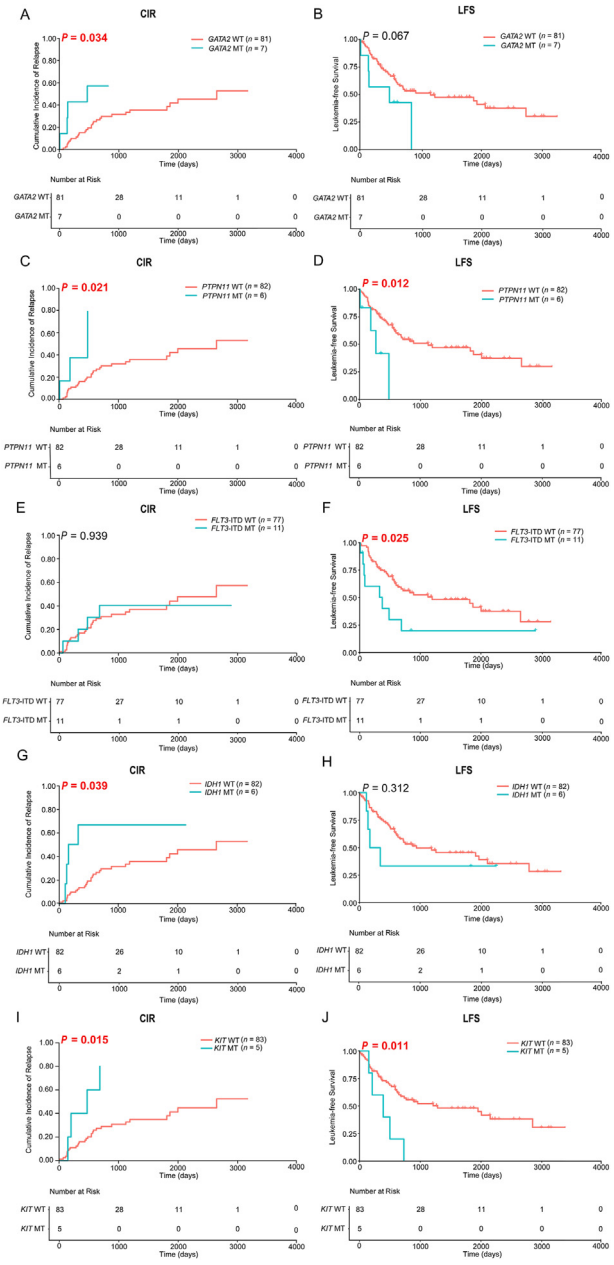

Figure 3. The effect of *GATA2*, *PTPN11*, *FLT3-ITD*, *IDH1,* and *KIT* mutations on outcomes in patients with AML. (A) CIR in *GATA2* mutation. (B) LFS in *GATA2* mutation. (C) CIR in *PTPN11* mutation. (D) LFS in *PTPN11* mutation. (E) CIR in *FLT3-ITD* mutation. (F) LFS in *FLT3-ITD* mutation. (G) CIR in *IDH1* mutation. (H) LFS in *IDH1* mutation. (I) CIR in *KIT* mutation. (J) LFS in *KIT* mutation. AML: Acute myeloid leukemia; CIR: Cumulative incidence of relapse; LFS: Leukemia-free survival; MT: Mutated type; WT: Wild type.

Multivariate analysis revealed factors associated with cumulative incidence of relapse and leukemia-free survival

Multivariate analysis was further developed using prognostic factors with $P < 0.200$ in the univariate analysis. A high WBC count was associated with an increased CIR risk (HR, 1.015; $P = 0.044$) and decreased LFS duration (HR, 1.009; $P = 0.042$). Patients with mutated *PTPN1*1 had an increased likelihood of CIR (HR, 14; $P = 0.001$) and reduced LFS duration (HR, 3.486; $P = 0.077$) [Table 3].

Discussion

The increasing use of mutational signatures based NGS in newly diagnosed AML enables the recurrent manifestation of mutated genes, offering substantive prognostic implications for patients with AML.[20,21] AML risk stratification through genetics in the ELN_{2017} updated classification has garnered widespread acceptance and integration into clinical practice guidelines for AML, such as those established by the U.S. National Comprehensive Cancer Center Network (NCCN).[22] AML remains a complex and challenging hematopoietic malignancy despite the advancements in studies on molecular pathogenesis, diagnostics, therapeutic interventions, and monitoring.[23,24] Pretreatment genetic mutation profiles in patients with AML have progressively assumed significance as a comprehensive perspective in the landscape of AML therapeutics.[25,26] Additionally, conventional "3 + 7" and the newly rising DCAG regimen have been widely used in patients with highly heterogeneous AML. When combined with cytarabine, aclarubicin hydrochloride, and granulocyte colony-stimulating factor, the DNMT inhibitor decitabine shows therapeutic potential in newly diagnosed AML. However, the integration of mutational signatures into recommendations for induction regimens requires further investigation. This study elucidates the mutational landscape within a meticulously characterized, population-based cohort comprising 124 consecutively diagnosed patients with AML who underwent induction therapy involving the DCAG regimen.

In the present study, we evaluated patient-related clinical factors. In contrast to previous studies with few cytogenetic favorable-risk (14.5%) and ELN_{2017} favorable-risk (31.5%) patients, our patients had lower CR rates in response to first induction (45.2%), overall CR achievement (71.0%), and a higher mortality rate (62.9%) than the traditional "3 + 7" regimen. However, the relapse rate was almost equivalent (27.4%) to that of patients who received the "3 + 7" regimen, indicating its value in the achievement of striking response rates.[27]

Consistent with prior investigations focusing on patients newly diagnosed with AML, the five most frequently mutated genes were *DNMT3A*, *NRAS*, *NPM1*, *FLT3-ITD*, and *IDH2*. This finding aligns with the outcomes of the study by Metzeler et al., wherein the five foremost mutations were in *FLT3*, *NPM1*, *DNMT3A*, *NRAS*, and *RUNX1*.[28] We further found that *GATA2-CEBPA*, *SRSF2-RUNX1*, *NPM1-DNMT3A*, *SRSF2-ASXL1*, and *FLT3-ITD-NPM1* were significant co-existence mutations. This insightful exploration sheds light on the intricate genetic interactions within this AML cohort. These co-occurrence analyses have the potential to identify surrogate biomarkers in patients with multiple concurrent molecular signatures.

The combination of decitabine with cytarabine, aclarubicin, and G-CSF has emerged as a novel standard treatment for newly diagnosed AML. Therefore, a thorough understanding of the distinct gene mutation features that could serve as unique predictors of clinical outcomes in patients undergoing this regimen is important. Here, mutations in *FLT3-ITD*, *KIT*, *PTPN11*, *GATA2*, or *IDH1* were associated with a higher risk of CIR or shorter LFS, whereas *PTPN11* mutation was significant in multivariable analysis. *PTPN11* is located on chromosome 12q24 and plays a pivotal role in various crucial cellular communication processes within normal hematopoiesis. These processes encompass fundamental functions, such as proliferation, differentiation, and apoptosis.[29] *PTPN11* mutations may co-occur more commonly with *DNMT3A*, *NPM1*, and *FLT3-ITD* mutations.[30] Furthermore, *PTPN11* mutations exhibit a discernible link with diminished response to treatment interventions and a less favorable disease outcome in AML, with several reports fully defining the clinical characteristics and prognostic impact of *PTPN11*-mutated AML.[31–33] Studies on *PTPN11*-mutated juvenile myelomonocytic leukemia have shown that a combination of 5-Aza, trametinib, and chemotherapy might result in a better clinical response.[34] However, several widely adopted adverse gene mutations, such as *WT1*,

Table 2
Univariable analysis of clinical variables and myeloid mutations in newly diagnosed patients with AML who received the 'DCAG' induction regimen.

Characteristics	CIR			LFS		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (continuous)	1.010	0.987–1.033	0.415	1.008	0.989–1.028	0.393
≥60 *vs.* <60 years	1.956	0.956–4.002	0.057	1.650	0.829–3.285	0.104
Sex (male *vs.* female)	0.880	0.444–1.745	0.399	1.088	0.613–1.929	0.774
AML type (secondary *vs. de novo*)	1.125	0.433–2.924	0.603	0.969	0.416–2.260	0.943
WBC ($\times 10^9$/L), (continuous)	1.013	1.006–1.020	0.001	1.010	1.003–1.016	0.006
≥100 *vs.* <100	13.517	3.647–50.100	<0.001	6.942	0.377–127.845	<0.001
Hb (g/L), (continuous)	0.999	0.986–1.012	0.873	0.998	0.987–1.010	0.7518
PLT ($\times 10^9$/L), (continuous)	0.999	0.994–1.004	0.609	0.100	0.996–1.004	0.855
Bone marrow blasts (%), (continuous)	1.844	0.414–8.169	0.420	0.803	0.227–2.84	0.734
Extramedullary disease (yes *vs.* no)	3.441	0.809–14.626	0.044	2.456	0.282–21.372	0.197
Cytogenetic risk stratification						
Adverse *vs.* favorable	0.558	0.184–1.691	0.259	0.802	0.323–1.996	0.627
ELN_{2017} risk stratification guidelines						
Intermediate *vs.* favorable	1.187	0.466–3.027	0.991	1.421	0.636–3.178	0.365
Adverse *vs.* intermediate	1.131	0.456–2.806	0.692	1.044	0.502–2.169	0.909
Adverse *vs.* favorable	1.151	0.781–1.696	0.644	1.485	0.771–2.862	0.233
Response to induction						
R/R *vs.* CR	5.777	2.386–13.988	0.001	4.380	16.004–1.199	<0.001
HSCT (yes *vs.* no)	0.761	0.381–1.520	0.664	0.746	0.421–1.323	0.322
Donor of HSCT						
Alternative donors *vs.* MSD	0.623	0.207–1.873	0.194	0.929	0.329–2.628	0.887
Status of disease before HSCT						
R/R *vs.* CR	7.250	1.799–29.213	0.070	6.670	0.861–51.662	<0.001
Myeloid mutations (yes *vs.* no)						
CEBPA	0.734	0.393–1.370	0.605	0	0–0	0.365
bi-*CEBPA vs.* WT	0.762	0.413–1.406	0.489	0.572	0.251–1.305	0.267
mono-*CEBPA vs.* WT	0	0–Inf	0.466	0	0–0	0.374
NRAS	1.158	0.443–3.027	0.832	1.315	0.594–2.912	0.456
DNMT3A	0.814	0.333–1.993	0.809	0.834	0.407–1.71	0.636
FLT3-ITD	1.677	0.586–4.802	0.939	2.326	0.816–6.629	0.025
NPM1	0.936	0.360–2.436	0.873	0.757	0.348–1.644	0.521
TET2	1.311	0.392–4.386	0.977	1.556	0.561–4.315	0.302
Other *FLT3*	0.989	0.300–3.264	0.801	1.216	0.446–3.314	0.678
WT1	0.505	0.121–2.114	0.411	0.595	0.232–1.525	0.377
ASXL1	1.133	0.343–3.743	0.800	1.041	0.367–2.950	0.939
KIT	4.534	1.542–13.327	0.015	3.104	0.682–14.129	0.011
IDH2	0.782	0.275–2.226	0.947	0.585	0.255–1.344	0.299
GATA2	3.927	1.324–11.648	0.034	2.305	0.614–8.657	0.067
IDH1	2.332	0.815–6.676	0.039	1.683	0.466–6.075	0.312
RUNX1	0.833	0.198–3.509	0.567	1.054	0.370–3.005	0.919
TP53	2.039	0.609–6.824	0.143	1.407	0.361–5.490	0.563
PTPN11	4.868	1.406–16.856	0.021	3.379	0.575–19.843	0.012
SRSF2	–	–	–	0.270	0.037–1.969	0.197

AML: Acute myeloid leukemia; CI: Confidence interval; CIR: Cumulative incidence of relapse; CR: Complete remission; DCAG: Decitabine, cytarabine, aclarubicin, and granulocyte colony-stimulating factor; ELN_{2017}: European Leukemia Net; Hb: Hemoglobin; HR: Hazard ratio; HSCT: Hematopoietic stem cell transplantation; LFS: Leukemia-free survival; MSD: Matched-sibling donor; PLT: Platelets; R/R: Relapsed/refractory; WBC: White blood cell; WT: Wild type; -: No data.

Table 3
Multivariable analysis of clinical variables and myeloid mutations in newly diagnosed patients with AML who received the 'DCAG' induction regimen.

Characteristics	CIR			LFS		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (continuous)	1.012	0.9846–1.040	0.400	1.013	0.9902–1.036	0.265
WBC ($\times 10^9$/L), (continuous)	1.015	1.000–1.030	0.044	1.009	1.000–1.018	0.042
Extramedullary disease (yes *vs.* no)	5.289	0.885–31.610	0.068	0.997	0.136–7.298	0.998
HSCT (yes *vs.* no)	0.890	0.374–2.116	0.790	0.794	0.388–1.626	0.528
Myeloid mutations (yes *vs.* no)						
FLT3-ITD	0.470	0.152–1.4550	0.190	2.042	0.747–5.579	0.164
KIT	2.720	0.519–14.260	0.240	2.183	0.525–9.082	0.283
GATA2	2.807	0.755–10.430	0.120	2.348	0.783–7.041	0.128
IDH1	4.334	0.836–22.480	0.081	2.157	0.736–6.322	0.161
TP53	1.250	0.107–14.570	0.860	1.133	0.275–4.658	0.863
PTPN11	14.000	3.025–64.770	0.001	3.486	0.8743–13.9	0.077
SRSF2	–	–	–	0.231	0.030–1.777	0.159

AML: Acute myeloid leukemia; CI: Confidence interval; CIR: Cumulative incidence of relapse; DCAG: Decitabine, cytarabine, aclarubicin, and granulocyte colony-stimulating factor; HR: Hazard ratio; HSCT: Hematopoietic stem cell transplantation; LFS: Leukemia-free survival; WBC: White blood cell; -: No data.

ASXL1, and *TP53*, failed to show differences in CIR and LFS, indicating that the DCAG regimen might be able to abrogate their adverse prognosis.

This study describes the clinical and molecular characteristics, as well as clinical outcomes, of newly diagnosed AML. However, there were potential constraints in our study design, which were mainly related to

the retrospective nature of our analysis. These limitations included a small number of patients who received the DCAG induction regimen. In addition, this study was carried out at a single center, which limits the generalizability of our results. Another noteworthy challenge stems from our relatively small sample size, which hinders the ability to thoroughly evaluate the prognostic implications of infrequent mutations. Our ability to conduct comprehensive analyses of the concurrent presence and exclusive distribution of mutations was curtailed by these limitations.

In conclusion, the investigation has revealed distinct prognostic factors in AML treated with the DCAG induction regimen. This well-tolerated regimen for patients newly diagnosed with AML has shown similar results in terms of relapse of widely accepted adverse gene mutations such as *FLT3-ITD* and *TP53*. However, new approaches should be investigated to treat high-risk patients with *PTPN11* mutations and hyperleukocytosis. Our study supports existing evidence of the prognostic impact of mutational signatures based on NGS in newly diagnosed AML. Thus, eligible patients may benefit from a CAG combined with decitabine induction regimen. Finally, chemotherapy combined with DNMT inhibitors may represent a promising treatment for patients with newly diagnosed AML.

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Authors contribution

Sai Huang: conceptualization, methodology, writing - original draft preparation; Peng Chen: formal analysis, writing - reviewing and editing; Lu Wang, Lingmin Xu, Mingyu Jia, Jing Chen, Nan Wang, and Fei Li: data curation; and Liping Dou and Daihong Liu: conceptualization, and supervision. The final manuscript has been reviewed and its submission has been approved by all authors.

Ethics statement

Our study followed the *Declaration of Helsinki*, with informed consent obtained from all participants. Ethical approval was obtained from the regional ethical review board of the People's Liberation Army (PLA) General Hospital. The ethical approval code was 2019-338.

Data availability statement

The datasets produced or analyzed in the present study are available from the corresponding author upon reasonable request.

Conflict of interest

We declare that we have no financial and personal relationships with 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.2023.10.002.

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