Received: 28 June 2022 | Revised: 29 July 2022 | Accepted: 24 August 2022

DOI: 10.1002/cncr.34495

ORIGINAL ARTICLE

Molecular profiling of patients with cytogenetically normal acute myeloid leukemia and hyperleukocytosis

Friederike Pastore MD[1] | Alessandro Pastore MD[1] | Maja Rothenberg-Thurley PhD[1] | Klaus H. Metzeler MD[2] | Bianka Ksienzyk[1] | Stephanie Schneider PhD[1,3] | Stefan K. Bohlander MD[4] | Jan Braess MD[5] | Maria C. Sauerland PhD[6] | Dennis Görlich PhD[6] | Wolfgang E. Berdel MD[7] | Bernhard Wörmann MD[8] | Michael S. von Bergwelt-Baildon MD, PhD[1] | Wolfgang Hiddemann MD, PhD[1] | Karsten Spiekermann MD[1]

[1]Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, Ludwig Maximilian University Munich, Munich, Germany

[2]Department of Hematology and Cell Therapy, University Leipzig, Leipzig, Germany

[3]Institute of Human Genetics, Ludwig Maximilian University Munich, Munich, Germany

[4]Leukemia and Blood Cancer Research Unit, Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand

[5]Department of Oncology and Hematology, Hospital Barmherzige Brüder, Regensburg, Germany

[6]Institute of Biostatistics and Clinical Research, University of Münster, Münster, Germany

[7]Department of Medicine A, Hematology and Oncology, University of Münster, Münster, Germany

[8]Department of Medicine, Hematology, Oncology, and Tumor Immunology, Charite–Universitätsmedizin Berlin, Berlin, Germany

Correspondence
Karsten Spiekermann, Laboratory for Leukemia Diagnostics, Department of Internal Medicine III, Ludwig-Maximilians-University Munich, Munich, Germany.
Email: Karsten.Spiekermann@med.uni-muenchen.de

Funding information
Open Access funding enabled and organized by Projekt DEAL.

Abstract

Background: Acute myeloid leukemia (AML) with initial hyperleukocytosis is associated with high early mortality and a poor prognosis. The aims of this study were to delineate the underlying molecular landscape in the largest cytogenetic risk group, cytogenetically normal acute myeloid leukemia (CN-AML), and to assess the prognostic relevance of recurrent mutations in the context of hyperleukocytosis and clinical risk factors.

Methods: The authors performed a targeted sequencing of 49 recurrently mutated genes in 56 patients with newly diagnosed CN-AML and initial hyperleukocytosis of ≥100 G/L treated in the AMLCG99 study. The median number of mutated genes per patient was 5. The most common mutations occurred in *FLT3* (73%), *NPM1* (75%), and *TET2* (45%).

Results: The predominant pathways affected by mutations were signaling (84% of patients), epigenetic modifiers (75% of patients), and nuclear transport (*NPM1*; 75%) of patients. AML with hyperleukocytosis was enriched for molecular subtypes that negatively affected the prognosis, including a high percentage of patients presenting

with co-occurring mutations in signaling and epigenetic modifiers such as *FLT3* internal tandem duplications and *TET2* mutations.

Conclusions: Despite these unique molecular features, clinical risk factors, including high white blood count, hemoglobin level, and lactate dehydrogenase level at baseline, remained the predictors for overall survival and relapse-free survival in hyperleukocytotic CN-AML.

KEYWORDS
acute myeloid leukemia (AML), hyperleukocytosis, molecular profiling, normal karyotype

INTRODUCTION

Acute myeloid leukemia (AML) is the second most common form of leukemia in adult patients. AML originates in the bone marrow, where specific somatic genetic changes in hematopoietic stem cells cause clonal proliferation and a differentiation block. This results in bone marrow failure as well as complications derived from the excessive blast production, such as leukostasis, tumor lysis syndrome, and disseminated intravascular coagulation.[1,2]

Overall, outcomes in AML are poor, especially for patients older than 60 years, who have a cure rate of only 5%–15% versus 35%–40% in younger patients.[1] Primary refractory AML (10%–40% of newly diagnosed AML cases) and relapses after an initial response are the major causes of the short overall survival (OS) and remain challenges in the treatment of these patients.

Besides the cytogenetic and molecular heterogeneity that affects outcomes in AML,[3–6] clinical risk factors such as an older age and an Eastern Cooperative Oncology Group (ECOG) performance status of ≥2 have been shown to affect the prognosis.[7] One laboratory risk factor associated with adverse outcomes in AML, a high early death rate, and a high relapse rate is an initial hyperleukocytosis (HL) with a white blood count (WBC) ≥100 G/L, which occurs in 8%–18% of patients with AML.[2,7–10] HL can cause leukostasis, a medical emergency with microcirculation disturbances and reduced tissue perfusion.

Previous analyses have revealed an association between HL in AML and the French–American–British M4/M5 subtype,[11] the presence of a *FLT3* internal tandem duplication (*FLT3*-ITD),[12,13] or *KMT2A* rearrangements.[12,14]

However, a comprehensive molecular assessment in a homogeneously treated study cohort to delineate the mutational landscape of cytogenetically normal acute myeloid leukemia (CN-AML) with HL and to identify specific driver mutations of this particular AML type has not been performed yet.

MATERIALS AND METHODS

Patients

Analyses were based on a total of 86 patients with CN-AML and HL (WBC ≥ 100 G/L) at diagnosis who had been enrolled in the AMLCG99 study (NCT00266136).[15] The study protocol was approved by the Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM). Details regarding patients, treatments, and patient selection are provided in the supplementary appendix and Figure S1. In a subset of 56 of these 86 patients (65%), bone marrow that could be used for a targeted sequencing approach was available. For 30 of the 86 patients, no further specimens were available.

Targeted sequencing

For the 56 patients with additional bone marrow available, DNA was extracted, and targeted sequencing was performed. A total of 49 genes known to be recurrently mutated in AML[16] were included in our panel (Table S1). The entire coding region (for 16 of these genes) and recurrently mutated regions (for 33 genes) were sequenced with a custom, amplicon-based, targeted enrichment kit (design 27327-1399408228, 1–500 kb, HaloPlex, Agilent, Böblingen, Germany) and an Illumina MiSeq instrument (Illumina, San Diego, California).

For patient characteristics and further details on the definitions of the clinical endpoints and statistical, molecular, and computational analyses, see the supplementary appendix.

RESULTS

Patient selection and comparison of selected and nonselected patients

Out of a total of 86 patients with CN-AML and HL, 56 had material available for targeted sequencing. Among these selected patients, the median age was 54 years, 41% were female, 52% had an ECOG performance status of 0–1, and most had de novo AML (96%). *NPM1* mutations, *FLT3*-ITDs, *FKT3* tyrosine kinase domain (*FLT3*-TKD) mutations, *KMT2A* partial tandem duplications, and biallelic *CEBPA* (bi*CEBPA*) mutations, which were routinely assessed in all patients in the clinical diagnostic laboratory, were present in 75%, 59%, 16%, 4%, and 0% of the patients, respectively (Table 1 and Table S2). Fifty-seven percent of the patients received double induction therapy (Table 2 and Table S3). In patients younger than 60 years, double induction was the standard therapy according to the protocol,

TABLE 1 Baseline characteristics of younger and older patients with hyperleukocytosis

	All 56 selected patients				34 patients < 60 years old				22 patients ≥ 60 years old				
	No.	%	Median	Range	No.	%	Median	Range	No.	%	Median	Range	*p*
Age, years			54	20–78			45	20–58			68	60–78	
WBC, $\times 10^9$/L			147	104–798			166	104–798			142	111–786	NS
Platelet count, $\times 10^9$/L			54	10–238			51	22–232			71	10–238	NS
Hemoglobin level, $\times 10^9$/L			94	50–142			96	50–142			92	62–131	NS
Bone marrow blasts, %			90	30–100			90	40–100			85	30–100	NS
LDH, U/L			1008	186–7343			1082	186–5560			794	278–7434	NS
Females	23	41.1			18	52.9			15	68.2			NS
ECOG													NS
0 or 1	29	51.8			19	55.9			10	45.5			
2–4	27	48.2			15	44.1			12	54.5			
FAB													NS
M0	0	0			0	0			0	0			
M1	17	30.4			8	23.5			9	40.9			
M2	9	16.1			5	14.7			4	18.2			
M4	18	32.1			13	38.2			5	22.7			
M5	12	21.4			8	23.5			4	18.2			
M4/M5	30	81.1			21	61.8			9	40.9			NS
Type of disease													NS
De novo	54	96.4			33	97.1			21	95.5			
sAML/tAML	2	3.6			1	2.9			1	4.5			
NPM1-mutated	42	75.0			26	76.5			16	72.7			NS
FLT3-ITD	33	58.9			20	58.8			13	59.1			NS
FLT3-TKD	9	16.1			8	23.5			1	4.5			NS
KMT2A-PTD	2	3.8			2	5.9			0	0			NS
bi*CEBPA*	0	0			0	0			0	0			NS

Abbreviations: bi*CEBPA*, biallelic *CEBPA*; ECOG, Eastern Cooperative Oncology Group; FAB, French–American–British classification; ITD, internal tandem duplication; LDH, lactate dehydrogenase; NS, not significant; PTD, partial tandem duplication; sAML, secondary acute myeloid leukemia evolving from a previous hematological disorder; tAML, therapy-related acute myeloid leukemia; TKD, tyrosine kinase domain; WBC, white blood count.

regardless of the response. In patients 60 years old or older, a second induction therapy was administered only in the case of persistent disease in the bone marrow.

The median follow-up time was 61.9 months (95% CI, 48.1–75.7 months). Seven percent, 16%, and 21% of the patients died within 7, 30, and 60 days, respectively, after the start of therapy (Table 2 and Table S3). Thirteen patients underwent allogeneic hematopoietic stem cell transplantation (HSCT) as consolidation therapy during their first complete remission (CR; 23% of the 56 patients), whereas three patients underwent allogeneic HSCT because of primary progressive AML. The median OS was 10.7 months (95% CI, 5.5–15.9 months), and the median relapse-free survival (RFS) for 34 patients who achieved CR was 7.8 months (95% CI, 0.8–15.2 months) (Table 2 and Table S3). Twenty-eight patients (82%) in CR relapsed or died.

There were no significant differences between the 56 selected patients and the 30 patients not selected with respect to the following: age; gender; ECOG performance status; origin of AML (de novo AML vs. secondary AML/therapy-related AML), mutations in *NPM1*, *FLT3*, and *KMT2A*; and clinical outcomes (Tables S2 and S3). The selected patients did not show any bi*CEBPA* mutations (0% vs. 19% for nonselected patients; $p = .001$). European LeukemiaNet (ELN) risk groups were distributed similarly among the 56 patients with HL and material available for next-generation sequencing and the 30 patients with HL who were excluded from the study because no material was available (Table S4A). When comparing all CN-AML patients with HL to patients without HL treated in the AMLCG99 study, we observed a significant enrichment of the favorable ELN subgroup *NPM1*mut/*FLT3*-ITD^{low} and the intermediate-risk groups

TABLE 2 Therapy and outcomes in younger and older patients with hyperleukocytosis

	All 56 selected patients				34 patients < 60 years old				22 patients ≥ 60 years old				
	No.	%	Median	95% CI	No.	%	Median	95% CI	No.	%	Median	95% CI	*p*
No. of induction cycles													.002
1	24	42.9			9	26.5			15	68.2			
2	32	57.1			25	73.5			7	31.8			
CR	34	60.7			20	58.8			14	63.6			NS
Early death until Day 7	4	7.1			3	8.8			1	4.5			NS
Early death until Day 30	9	16.1			6	17.6			3	13.6			NS
Early death until Day 60	12	21.4			8	23.5			3	13.6			NS
Total allogeneic SCT	16	28.6			14	41.2			2	9.1			.009
In first CR	13	23.2			12	35.3			1	4.5			.002
Primary refractory	3	5.4			2	5.9			1	4.5			NA
Median OS, months			10.7	5.5–15.9			11.8	NA			7.2	1.8–12.7	NS
Median RFS, months			7.8	0.8–15.2			11.5	0.7–22.3			5.7	1.1–10.2	NS

Abbreviations: CR, complete remission; NA, not applicable; NS, not significant; OS, overall survival; RFS, relapse-free survival; SCT, stem cell transplantation.

*NPM1*mut/*FLT3*-ITD^{high} and *NPM1*wt/*FLT3*-ITD^{low} in patients with HL (Table S4B). The frequency of the double-negative *NPM1*wt/*FLT3*wt group was significantly lower in patients with HL (Table S4B). No patient with HL CN-AML belonged to the *NPM1*wt/*FLT3*-ITD^{high} adverse-risk group according to ELN 2017, whereas eight patients with non-HL CN-AML did; however, this was not significantly different (Table S4B).

Spectrum of driver mutations and associated pathways in 56 selected patients

A total of 269 variants (166 of which were unique variants) were identified in 37 of the 49 assessed genes. Predominantly, missense mutations (60.9%), frameshift insertions (17.1%), and in-frame insertions (13.8%) were detected (Figure S2A). The majority of the mutations were single-nucleotide variants (64.7%), which were followed by insertions and deletions in 30.9% and 4.4% of the cases, respectively (Figure S2B). Nucleotide changes occurred most frequently as C to T or T to C transitions (Figure S2C). The median number of variants per sample was 5 (range, 2–8) (Figures S2D and S3A). The median number of missense mutations, frameshift insertions, and in-frame insertions per sample was 3, 1, and 1, respectively (Figure S2E).

Among the 37 genes that showed mutations, 32 were mutated in >3% of the patients, and 13 genes were mutated in >10% of the patients (Figure 1A). Twelve genes in our sequencing panel (*BRAF*, *CFS3R*, *CSFR1*, *ETV6*, *GATA1*, *GATA2*, *JAK1*, *JAK2*, *KRAS*, *MPL*, *TERC*, and *U2AF1*) did not show mutations in any of the patients. The median number of mutated genes per patient was 5 (range, 1–8) (Figure S3B). All patients showed at least one driver mutation (Figure S3B). The top 11 recurrently mutated genes included *NPM1* (75%), *FLT3* (73%), *TET2* (45%), *BCORL1* (27%), *SF3A1* (23%), *BCOR* (18%), *KIT* (16%), *IDH2* (12%), *IDH1* (11%), *SMC3* (11%), and *ASXL1* (11%) (Figure 1A,B). Notably, 57% of all 56 patients who underwent targeted sequencing (32 of 56) had mutations in both *NPM1* and *FLT3*, and no bi*CEBPA* mutation was detected. Thirty-three of the 56 patients were positive for *FLT3*-ITD. In 32 of the 33 *FLT3*-ITD–positive patients for whom the *FLT3*-ITD allelic ratio (AR) was available, the median *FLT3*-ITD AR was 0.41 (range, 0.02–0.98) (Table S5A). In 24 patients with co-occurring *NPM1* mutations and *FLT3*-ITD, the median *FLT3*-ITD AR was 0.46 (range, 0.04–0.98) (Table S5A).

There was no significant difference in WBCs between patients with a *FLT3*-ITD AR <0.50 and those with a *FLT3*-ITD AR ≥0.50 among all patients who underwent targeted sequencing ($p = .075$; Table S5B). When analyses were restricted to *FLT3*-ITD–positive patients, the median WBC among *FLT3*-ITD–positive patients was slightly higher for patients with a *FLT3*-ITD AR of <0.50 versus ≥0.50 ($p = .040$; Table S5B). In patients with co-occurring *NPM1* mutations and *FLT3*-ITD, there was no difference in WBCs between patients with a *FLT3*-ITD AR <0.50 and patients with a *FLT3*-ITD AR ≥0.50 ($p = .114$; Table S5B).

Forty-seven percent of the patients (26 of 55) were classified as having favorable-risk AML according to the current ELN risk classification, 36% (20 of 55) showed an intermediate-risk profile, and 16% (9 of 55) were classified as having an adverse risk[6](Table S5A).

The three most frequent mutations—*NPM1*, *FLT3*, and *TET2*—were not significantly associated with any of the other sequenced mutations in pairwise comparisons (Table S6A–C).

Eighty-four percent of the patients had mutations in genes associated with activated cellular signaling (Figure 2A). Other pathways affected by these gene mutations included nuclear transport (*NPM1*;

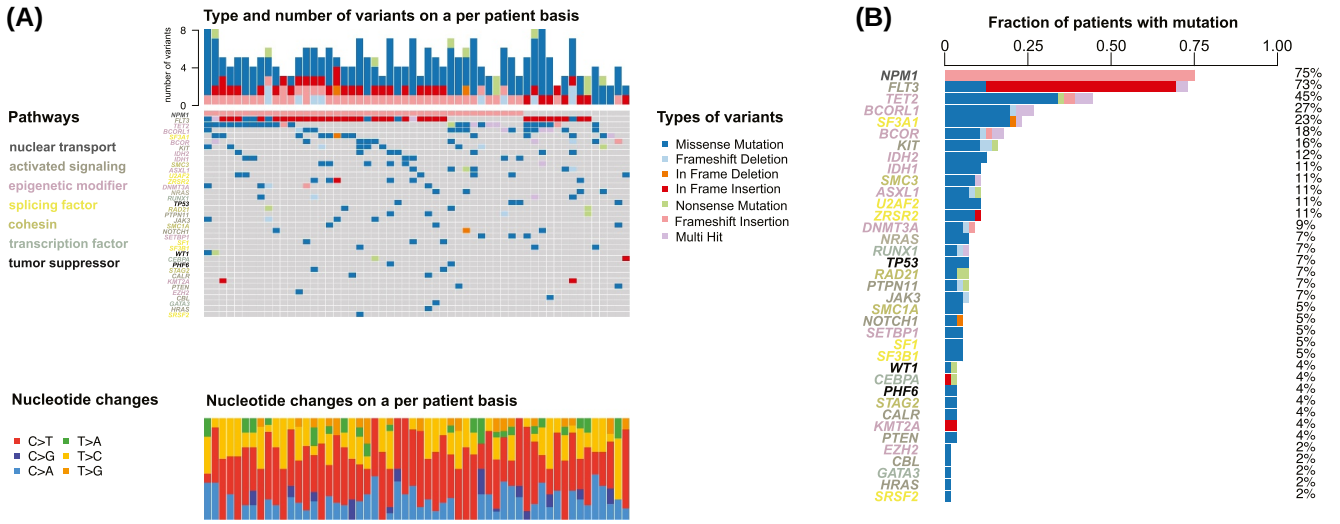

FIGURE 1 Overview of driver gene mutations identified by targeted sequencing in 56 patients with cytogenetically normal acute myeloid leukemia and initial hyperleukocytosis. (A) An oncoplot depicts driver gene mutations and their associated pathways, numbers and types of identified variants, and single nucleotide changes per patient. (B) A histogram depicts the frequency of driver gene mutations detected in >1% of the patients. The bars are colored according to the functional category assigned to each driver gene

75%), mutations in genes encoding epigenetic modifiers (75%), splicing factors (48%), members of the cohesion complex (25%), tumor suppressors (14%), and transcription factors (13%) (Figure 2A). Mutations within nuclear transport and/or activated signaling and/or epigenetic modifier pathways were present in the majority of the patients (96% [54 of 56]) (Figure 2B). The median number of pathways affected in a patient was 3 (range, 1–6) (Table S7). Predominantly, mutations occurred in combinations of three or four different pathways (30.4% and 43% of patients, respectively) (Table S7).

We next assessed associations between different pathways. Pairwise comparisons revealed that co-occurring mutations most commonly led to alterations in the following three pathways: activated signaling, nuclear transport, and epigenetic modifiers. In detail, 64%, 63%, and 59% of the patients had mutations in both activated signaling and nuclear transport, activated signaling and epigenetic modifiers, or nuclear transport and epigenetic modifiers, respectively (Table S8). Mutations in epigenetic modifiers and splicing factors tended to be mutually exclusive (odds ratio, 0.17; 95% CI, 0.03–0.70), but this did not reach significance when we adjusted for multiple testing. In addition, mutations affecting epigenetic modifiers and cohesin, mutations affecting epigenetic modifiers and tumor suppressors, and mutations of tumor suppressors and activated signaling tended to be mutually exclusive (Table S8).

Forty-eight percent of the patients (27 of 56) showed co-occurring mutations in all three pathways of nuclear transport, signaling, and epigenetic modifiers (Figure 2B). In 10 of these 27 patients, no other pathway was affected. Fourteen of these 27 patients showed one additional pathway affected, with splicing factors most commonly involved (7 of 14). In three of these triple-pathway-mutated patients, two or three additional pathways were altered. In 23 patients (41%) who showed dual combinations of activated signaling, nuclear transport, and epigenetic modifiers, the pathways that were most commonly altered included splicing factors (12 of 23) and cohesin complex (8 of 23).

We next assessed the spectrum of mutations in younger (61%) and older patients (39%).

In patients younger than 60 years, the top 10 recurrently mutated genes were *FLT3* (77%), *NPM1* (74%), *TET2* (43%), *SF3A1* (23%), *IDH2* (17%), *SMC3* (17%), *BCORL1* (17%), *IDH1* (14%), *KIT* (14%), and *ASXL1* (14%) (Figure 3A,B).

Among the younger patients, 62% (21 of 34) showed mutations in both *NPM1* and *FLT3*, and no bi*CEBPA* mutation was detected. Forty-four percent (15 of 34) were classified as having favorable-risk AML according to the current ELN risk classification, 35% (12 of 34) showed an intermediate-risk profile, and 21% (7 of 34) belonged to the adverse-risk group[6] (Table S9A).

In patients 60 years old or older (n = 22), similarly, the most frequently mutated genes included *NPM1* (76%), *FLT3* (67%), and *TET2* (48%), which were followed by mutations in *BCORL1* (43%), *BCOR* (24%), *SF3A1* (24%), *KIT* (19%), *DNMT3A* (14%), *RAD* (14%), and *PTPN11* (14%) (Figure 3C,D). Fifty percent of the patients (11 of 22) had both *NPM1* and *FLT3* mutations. Fifty-two percent (11 of 21), 38% (8 of 21), and 10% (2 of 21) were classified as having AML with favorable, intermediate, and adverse ELN risk, respectively[6] (Table S9B). There were no significant differences in single-mutation frequencies or frequencies of affected pathways when we compared younger and older patients (data not shown).

Association of mutations with clinical features and outcomes

We further assessed associations between detected gene mutations and baseline clinical characteristics. *PTEN* mutations tended to occur

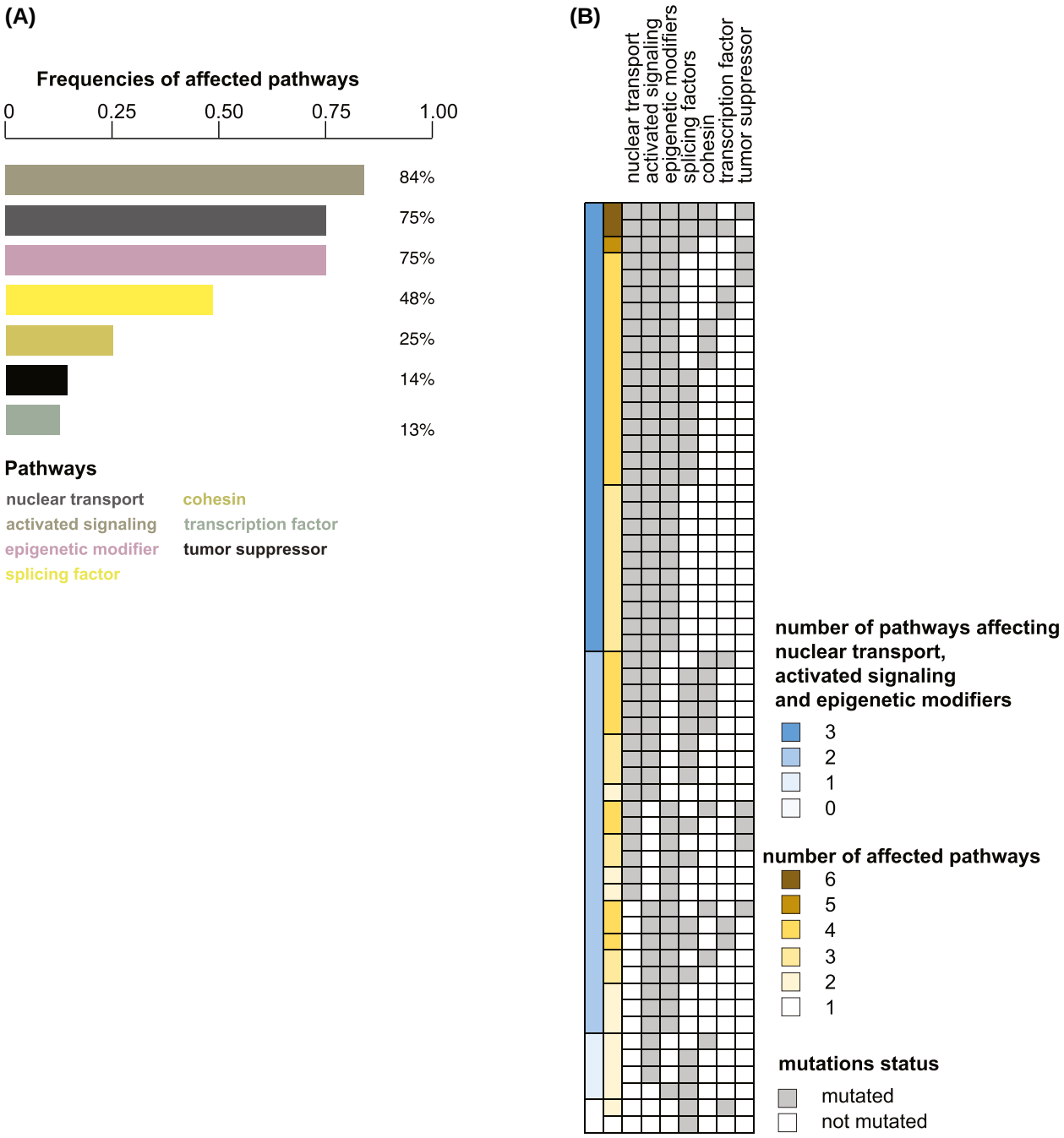

FIGURE 2 Overview of associated pathways that were affected by gene mutations in 56 patients with cytogenetically normal acute myeloid leukemia and initial hyperleukocytosis. (A) A histogram depicts the frequency of affected pathways. The bars are colored according to the functional category assigned to each pathway. (B) An oncoplot depicts the type and number of affected pathways per patient

more frequently in older patients versus younger patients, whereas *NRAS* mutations were associated with younger age. Mutations of *IDH1*, *U2AF2*, *CEBPA*, and *PHF6* were detected more frequently in men. *RUNX1*, *BCOR*, and *SMC1A* were associated with French–American–British types M4 and M5. *IDH1* mutations occurred less in patients with M4/M5. *SF3B1* mutations tended to be more common in patients with an ECOG performance status of 2–4, whereas *NOTCH1* mutations were less common in patients with a poor ECOG status. *CALR* and *SF3B1* mutations were associated with secondary or therapy-related AML. *SETBP1* and *KIT* mutations were associated with a lower median WBC, *SMC3* mutations were associated with a higher median hemoglobin count, and *SETBP1* mutations were associated with lower median bone marrow blasts in comparison with patients without mutations in these genes. However, none of these

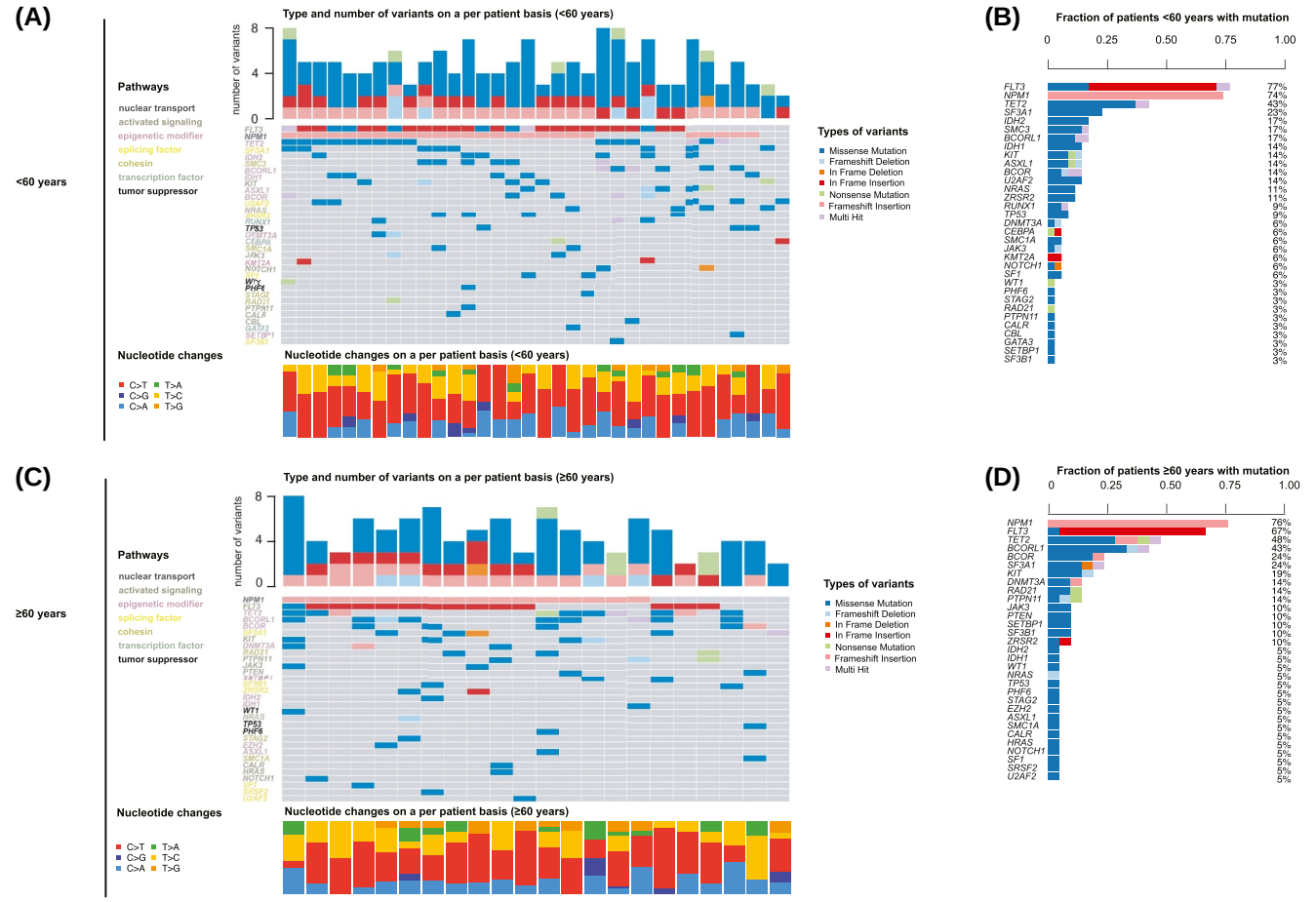

FIGURE 3 Overview of gene mutations identified by targeted sequencing in 34 younger patients and 22 older patients with cytogenetically normal acute myeloid leukemia and initial hyperleukocytosis. (A) An oncoplot depicts driver gene mutations and their associated pathways, numbers and types of identified variants, and single nucleotide changes for each patient younger than 60 years. (B) A histogram depicts the frequency of driver gene mutations detected in >1% of the patients younger than 60 years. (C) An oncoplot depicts driver gene mutations and their associated pathways, numbers and types of identified variants, and single nucleotide changes for each patient 60 years old or older. (D) A histogram depicts the frequency of driver gene mutations detected in >1% of the patients 60 years old or older. The bars are colored according to the functional category assigned to each driver gene

associations between individual gene mutations and clinical characteristics reached significance after adjustments for multiple testing (Table S10A,B).

In an effort to compare the molecular characteristics of early responders and nonresponders, we used the day sixteen (d16) early blast cell clearance, a biomarker shown to be a prognostic factor for predicting survival and responses to induction therapy.[17] No significant differences in the frequencies of gene mutations were detected in patients who showed an early d16 blast clearance of <10% versus ≥10% (Table S11A). Similarly, we did not observe a significant difference in activated pathways (Table S11B).

Next, we sought to assess the impact of driver mutations that were present in ≥3% of the patients on treatment outcomes in univariate analyses. The median OS and RFS for the 56 patients selected for mutational analysis were 11.8 months (95% CI, 5.9–17.6 months) and 10.7 months (95% CI, 4.9–16.6 months), respectively (Figure S4A,B). In all 86 patients with CN-AML and HL, the median OS and RFS were 10.8 months (95% CI, 5.9–17.7 months) and 10.7 months (95% CI, 7.1–14.3 months), respectively. Patients treated with chemotherapy displayed median OS and RFS times of 7.2 months (95% CI, 4.8–9.7 months) and 9.8 months (95% CI, 5.9–13.6 months), respectively. Patients who received chemotherapy and underwent allogeneic transplantation showed median OS and RFS times of 32.8 months (95% CI, 11.3–54.4 months) and 10.8 months (95% CI, 2.8–18.8 months), respectively (Figure S5A–F).

No significant association ($q < 0.05$) between individual gene mutations and OS, RFS, or the CR rate was observed when we corrected for multiple testing (Tables S12–S14). To account for the complexity of interactions, we performed multivariate analyses for OS and RFS, including mutations that were present in ≥20% of the patients together with clinical parameters (see the Materials and Methods section for details). These analyses revealed higher WBC ($p = .01$ and $p = .014$), hemoglobin level ($p = .036$ and $p = .005$), and lactate dehydrogenase (LDH) level ($p = .020$ and $p = .008$) at the

baseline as risk factors for significantly shorter OS and RFS, whereas no mutation reached the level of significance (Tables S15 and S16).

DISCUSSION

The aim of this study was to delineate the genetic landscape of driver mutations in CN-AML with HL. Selected patients with HL AML were enriched for the AML M4/M5 subtypes and did not show any bi*CEBPA* mutations in comparison with unselected patients. This bias was inevitable because our selection was based merely on the availability of bone marrow. Targeting sequencing was performed to assess 49 genes known to be recurrently mutated in AML. The median number of genes mutated per patient was 5, which is similar to published data for AML in general.[4]

Our gene panel included the *NPM1*, *FLT3*, and *CEBPA* genes as well as *TP53*, *RUNX1*, and *ASXL1*, which are required for the current ELN risk stratification of AML.[6] *NPM1* mutations are enriched in CN-AML, where they occur in 50%–60% of cases.[5,18–21] Mutations in the *FLT3* gene occur in approximately 30% of patients with AML; they include *FLT3*-ITDs in ~25% of patients and *FLT3*-TKDs in 7%–10% of patients.[22–24] Similarly to *NPM1* mutations, *FLT3*-ITDs have their highest frequency in CN-AML: 32%–38%.[22,23,25]

In our cohort of patients with HL AML, we observed higher frequencies of both *NPM1* mutations and *FLT3*-ITDs in comparison with the literature as well as compared to the data for all patients with CN-AML in the AMLCG99 study[7] (*NPM1*, 75% in our cohort vs. 52% in all AMLCG99 patients with CN-AML; *FLT3*-ITD, 59% in our cohort vs. 29% in all AMLCG99 patients with CN-AML). No biallelic mutation of *CEBPA* was detected.

NPM1 mutations have been shown to occur more frequently in patients with HL AML versus patients with non-HL AML (30% vs. 17%) in analyses performed by Tien et al.,[13] but their analyses were not restricted to CN-AML. We are the first to report that *NPM1* mutations are enriched in CN-AML with HL. The frequency of *NPM1* mutations in our HL CN-AML cohort was more than twice as high as that in the HL AML cohort described by Tien et al. (75% vs. 30%).

Although *FLT3*-ITDs are known to be associated with high baseline WBCs,[22,25] there was no positive association of a higher *FLT3*-ITD AR and WBCs in the context of HL CN-AML.

Signaling was the top pathway altered by mutations. Specifically, 84% of the patients showed mutations causing the activation of *FLT3* signaling (*FLT3* mutations, 73%), *KIT* signaling (*KIT* mutations, 16%), *RAS* signaling (*PTPN11* mutations, 11%; *NRAS* mutations, 7%; *HRAS* mutations, 2%), or *JAK/STAT* signaling (*JAK3* mutations, 7%; *CALR* mutations, 4%), besides others. In line with our results, Tien et al.,[13] who performed sequencing of 20 genes in 693 patients with AML, found higher frequencies of mutations affecting signaling: *FLT3*-ITD (33%), *NRAS* (20%), and *PTPN11* (7.4%) in 200 patients with HL versus 493 patients without HL. Notably, *KIT* mutations, which are typically associated with cytogenetically favorable CBF leukemias and are rare in CN-AML, were enriched in our cohort.[4] This was not observed by Tien et al., whose analyses included AML of all cytogenetic groups and used a lower WBC threshold than the one used by us (50 vs. 100 G/L).

Seventy-five percent of the patients showed mutations in epigenetic modifiers, with the most common being mutations in *TET2* (45% of the patients). *TET2* mutations typically occur in 6%–36% of patients with CN-AML, are associated with a reduced response to chemotherapy and impaired OS,[26,27] and also have been shown to be more frequent in patients with AML and HL by Tien et al. (22%). Furthermore, we observed a high frequency of mutations in the transcriptional corepressors *BCOR* and *BCORL1*. Eighteen percent of our patients had *BCOR* mutations, which typically occur in CN-AML with a frequency of only approximately 4%. They are mutually exclusive with *NPM1* mutations and tend to be associated with a dismal prognosis.[28,29] *BCORL1* mutations were found in 27% of our patients versus approximately 6% of patients with AML in general.[30]

Mutations in epigenetic modifiers that alter hematopoietic differentiation have been shown to cooperate with dysregulated growth factor signaling and drive oncogenic transformation.[31–33] Interestingly, 62.5% of our cohort displayed alterations in both signaling and epigenetic modifiers. Notably, *FLT3* together with *TET2* mutations was the most common combination affecting signaling and epigenetic pathways. This combination, which has been shown to confer a poor prognosis in AML, was found in 30% of the patients (17 of 56) in our cohort versus a frequency of 3%–8% in patients with AML, including normal and abnormal cytogenetics.[4,34–36]

We did not observe significant differences in the frequencies of mutations between younger and older patients with AML, although a higher frequency of mutations in, for example, epigenetic modifiers in elderly patients has been reported.[16] This discrepancy might be due to the fact that our cohort was restricted to CN-AML with HL, so trends in frequencies did not reach statistical significance because of the smaller number of patients.

Despite molecular enrichment for mutations with a negative impact on prognosis, in a multivariate model for OS and RFS, only clinical risk factors such as WBC, hemoglobin level, and LDH level remained significant. This might be due to the fact that these risk factors have a strong impact on the development of leukostasis, which is associated with high early mortality.

A high WBC is a known risk factor for an adverse prognosis in AML.[37] Elevated hemoglobin levels have been linked to increases in blood viscosity causing microcirculatory disturbances and eventually life-threatening leukostasis.[38,39] In fact, Harris[40] reported the death of three patients with AML and a WBC >100 G/L who died after a blood transfusion; two had developed fatal cerebral leukostasis.[40] Furthermore, data from AML MRC trials 4 and 5 revealed that the mean hemoglobin levels of patients with a WBC >100 G/L who died within the first week were significantly higher than those of survivors.[40] Elevated serum lactate levels could indicate microcirculatory failure and leukostasis, which is associated with high early mortality in patients with AML and HL.[41]

In our cohort, patients who underwent allogeneic HSCT showed a median OS of 32.8 months, whereas patients who were treated with chemotherapy only showed a median OS of only 7.2 months.

This could suggest a role of allogeneic HSCT in ameliorating the prognosis in patients with HL AML. Yet, further data, ideally prospective, are needed to further elucidate the role of allogeneic HSCT in this patient population.

Altogether, CN-AML with initial HL is enriched for molecular subtypes that negatively affect the prognosis. This includes a high percentage of patients presenting with co-occurring mutations in signaling genes and epigenetic modifiers such as *FLT3*-ITD and *TET2* mutations. Despite these unique molecular features, clinical risk factors, including high WBC, hemoglobin levels, and LDH levels at the baseline, remain the main predictors for OS and RFS in HL CN-AML.

AUTHOR CONTRIBUTIONS

Friederike Pastore: Conceptualization, molecular and cytogenetic diagnostics and analyses, writing–original draft, and writing–review and editing. **Alessandro Pastore:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Maja Rothenberg-Thurley:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Klaus H. Metzeler:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Bianka Ksienzyk:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Stephanie Schneider:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Stefan K. Bohlander:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Jan Braess:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Maria C. Sauerland:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Dennis Görlich:** Molecular and cytogenetic diagnostics and analyses and writing–review and editing. **Wolfgang E. Berdel:** Supervision and writing–review and editing. **Bernhard Wörmann:** Supervision and writing–review and editing. **Michael S. von Bergwelt-Baildon:** Writing–review and editing. **Wolfgang Hiddemann:** Supervision and writing–review and editing. **Karsten Spiekermann:** Supervision, conceptualization, molecular and cytogenetic diagnostics and analyses, and writing–review and editing.

ACKNOWLEDGMENTS

We thank all the patients who participated in the AMLCG99 study.

Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

Klaus H. Metzeler has acted as a consultant for Novartis, AbbVie, Pfizer, Janssen Biotech, and Bristol-Myers Squibb. Michael S. von Bergwelt-Baildon has acted as a consultant for the Munich Center for Neurosciences. Jan Braess is a fiduciary officer for Krankenhaus Barmherzige Brüder. Karsten Spiekermann has acted as a consultant for the University Hospital Munich. Stefan K. Bohlander is supported by Leukaemia & Blood Cancer New Zealand and the Family of Marijana Kumerich. The other authors made no disclosures.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Friederike Pastore https://orcid.org/0000-0002-2220-2346

REFERENCES

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1136-1152. doi:10.1056/nejmra1406184
2. Bewersdorf JP, Zeidan AM. Hyperleukocytosis and leukostasis in acute myeloid leukemia: can a better understanding of the underlying molecular pathophysiology lead to novel treatments? *Cells*. 2020;9(10):2310. doi:10.3390/cells9102310
3. Byrd JC, Mrózek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100(13):4325-4336. doi:10.1182/blood-2002-03-0772
4. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-2074. doi:10.1056/nejmoa1301689
5. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23):2209-2221. doi:10.1056/nejmoa1516192
6. Dohner 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(4):424-447. doi:10.1182/blood-2016-08-733196
7. Pastore F, Dufour A, Benthaus T, et al. Combined molecular and clinical prognostic index for relapse and survival in cytogenetically normal acute myeloid leukemia. *J Clin Oncol*. 2014;32(15):1586-1594. doi:10.1200/jco.2013.52.3480
8. Ventura GJ, Hester JP, Smith TL, Keating MJ. Acute myeloblastic leukemia with hyperleukocytosis: risk factors for early mortality in induction. *Am J Hematol*. 1988;27(1):34-37. doi:10.1002/ajh.2830270109
9. Giles FJ, Shen Y, Kantarjian HM, et al. Leukapheresis reduces early mortality in patients with acute myeloid leukemia with high white cell counts but does not improve long-term survival. *Leuk Lymphoma*. 2001;42(1-2):67-73. doi:10.3109/10428190109097677
10. Burnett AK, Russell NH, Hills RK, et al. A randomized comparison of daunorubicin 90 mg/m^2 vs 60 mg/m^2 in AML induction: results from the UK NCRI AML17 trial in 1206 patients. *Blood*. 2015;125(25):3878-3885. doi:10.1182/blood-2015-01-623447
11. Inaba H, Fan Y, Pounds S, et al. Clinical and biologic features and treatment outcome of children with newly diagnosed acute myeloid leukemia and hyperleukocytosis. *Cancer*. 2008;113(3):522-529. doi:10.1002/cncr.23581
12. Pastore F, Pastore A, Wittmann G, Hiddemann W, Spiekermann K. The role of therapeutic leukapheresis in hyperleukocytotic AML. *PLoS One*. 2014;9(4):e95062. doi:10.1371/journal.pone.0095062
13. Tien FM, Hou HA, Tsai CH, et al. Hyperleukocytosis is associated with distinct genetic alterations and is an independent poor-risk factor in de novo acute myeloid leukemia patients. *Eur J Haematol*. 2018;101(1):86-94. doi:10.1111/ejh.13073
14. Sorensen PH, Hammond GD, Kersey JH, et al. Molecular rearrangements of the MLL gene are present in most cases of infant acute myeloid leukemia and are strongly correlated with monocytic or myelomonocytic phenotypes. *J Clin Invest*. 1994;93(1):429-437. doi:10.1172/jci116978
15. Krug U, Berdel WE, Gale RP, et al. Increasing intensity of therapies assigned at diagnosis does not improve survival of adults with acute myeloid leukemia. *Leukemia*. 2016;30(6):1230-1236. doi:10.1038/leu.2016.25

16. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128(5):686-698. doi:10.1182/blood-2016-01-693879
17. Kern W, Haferlach T, Schoch C, et al. Early blast clearance by remission induction therapy is a major independent prognostic factor for both achievement of complete remission and long-term outcome in acute myeloid leukemia: data from the German AML Cooperative Group (AMLCG) 1992 trial. *Blood*. 2003;101(1):64-70. doi:10.1182/blood-2002-02-0532
18. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352(3):254-266. doi:10.1056/NEJMoa041974
19. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. *Blood*. 2007;109(3):874-885. doi:10.1182/blood-2006-07-012252
20. Liso A, Bogliolo A, Freschi V, et al. In human genome, generation of a nuclear export signal through duplication appears unique to nucleophosmin (NPM1) mutations and is restricted to AML. *Leukemia*. 2008;22(6):1285-1289. doi:10.1038/sj.leu.2405045
21. Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. *Nature*. 2013;502(7471):333-339. doi:10.1038/nature12634
22. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United King. *Blood*. 2001;98(6):1752-1759. doi:10.1182/blood.v98.6.1752
23. Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood*. 2002;100(1):59-66. doi:10.1182/blood.v100.1.59
24. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia*. 2019;33(2):299-312. doi:10.1038/s41375-018-0357-9
25. Fröhling S, Schlenk RF, Breitruck J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood*. 2002;100(13):4372-4380. doi:10.1182/blood-2002-05-1440
26. Metzeler KH, Maharry K, Radmacher MD, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2011;29(10):1373-1381. doi:10.1200/JCO.2010.32.7742
27. Wang R, Gao X, Yu L. The prognostic impact of tet oncogene family member 2 mutations in patients with acute myeloid leukemia: a systematic-review and meta-analysis. *BMC Cancer*. 2019;19(1):389. doi:10.1186/s12885-019-5602-8
28. Grossmann V, Tiacci E, Holmes AB, et al. Whole-exome sequencing identifies somatic mutations of BCOR in acute myeloid leukemia with normal karyotype. *Blood*. 2011;118(23):6153-6163. doi:10.1182/blood-2011-07-365320
29. Tiacci E, Grossmann V, Martelli MP, Kohlmann A, Haferlach T, Falini B. The corepressors BCOR and BCORL1: two novel players in acute myeloid leukemia. *Haematologica*. 2012;97(1):3-5. doi:10.3324/haematol.2011.057901
30. Li M, Collins R, Jiao Y, et al. Somatic mutations in the transcriptional corepressor gene BCORL1 in adult acute myelogenous leukemia. *Blood*. 2011;118(22):5914-5917. doi:10.1182/blood-2011-05-356204
31. Lara-Astiaso D, Weiner A, Lorenzo-Vivas E, et al. Immunogenetics. Chromatin state dynamics during blood formation. *Science*. 2014;345(6199):943-949. doi:10.1126/science.1256271
32. Shih AH, Jiang Y, Meydan C, et al. Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. *Cancer Cell*. 2015;27(4):502-515. doi:10.1016/j.ccell.2015.03.009
33. Kelly MJ, So J, Rogers AJ, et al. Bcor loss perturbs myeloid differentiation and promotes leukaemogenesis. *Nat Commun*. 2019;10(1):1-14. doi:10.1038/s41467-019-09250-6
34. Weissmann S, Alpermann T, Grossmann V, et al. Landscape of TET2 mutations in acute myeloid leukemia. *Leukemia*. 2012;26(5):934-942. doi:10.1038/leu.2011.326
35. Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079-1089. doi:10.1056/NEJMoa1112304
36. Hou HA, Lin CC, Chou WC, et al. Integration of cytogenetic and molecular alterations in risk stratification of 318 patients with de novo non-M3 acute myeloid leukemia. *Leukemia*. 2014;28(1):50-58. doi:10.1038/leu.2013.236
37. Dutcher JP, Schiffer CAWP, Wiernik PH. Hyperleukocytosis in adult acute nonlymphocytic leukemia: impact on remission rate and duration, and survival. *J Clin Oncol*. 1987;5(9):1364-1372. doi:10.1200/jco.1987.5.9.1364
38. Stucki A, Rivier AS, Gikic M, Monai N, Schapira M, Spertini O. Endothelial cell activation by myeloblasts: molecular mechanisms of leukostasis and leukemic cell dissemination. *Blood*. 2001;97(7):2121-2129. doi:10.1182/blood.v97.7.2121
39. Ho CH. Relationship between blood cells and blood viscosity. *Am J Hematol*. 2004;75(4):264. doi:10.1002/ajh.20035
40. Harris AL. Leukostasis associated with blood transfusion in acute myeloid leukaemia. *Br Med J*. 1978;1(6121):1169-1171. doi:10.1136/bmj.1.6121.1169
41. Stemmler J, Wittmann GW, Hacker U, Heinemann V. Leukapheresis in chronic myelomonocytic leukemia with leukostasis syndrome: elevated serum lactate levels as an early sign of microcirculation failure. *Leuk Lymphoma*. 2002;43(7):1427-1430. doi:10.1080/1042819022386671

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pastore F, Pastore A, Rothenberg-Thurley M, et al. Molecular profiling of patients with cytogenetically normal acute myeloid leukemia and hyperleukocytosis. *Cancer*. 2022;128(24):4213-4222. doi:10.1002/cncr.34495