Validation and Refinement of the European LeukemiaNet 2022 Genetic Risk Stratification of AML

Gojiri Mawalankar, MD[1,2]; Aarti Achrekar, MSc[1]; Seema Biswas, MD[1]; Vishram Terse, PhD[1]; Swapnali Joshi, MSc[1]; Priyanka Ugale, MSc[1]; Prasanna Bhanshe, MSc[1]; Shruti Chaudhary, PhD[1]; Saurabh Kusurkar, MD[1]; Shadma Shahin, MD[1]; Vaibhav Gawde, MSc[1]; Dhanlaxmi Shetty, PhD[3]; Nishant Jindal, MD, DM[2,4]; Sumeet Mirgh, MD, DM[2,4]; Alok Shetty, MD, DM[2,4]; Anant Gokarn, MD, DM[2,4]; Sachin Punatar, MD, DM[2,4]; Lingaraj Nayak, MD, DM[2,4]; Hasmukh Jain, MD, DM[2,4]; Bhausaheb Bagal, MD, DM[2,4]; Manju Sengar, MD, DM[2,4]; Navin Khattry, MD, DM[2,4]; Sweta Rajpal, MD, DM[1,2]; Gaurav Chatterjee, MD, MSc[1,2]; Prashant Tembhare, MD[1,2]; Papagudi G Subramanian, MD[1,2]; and Nikhil Patkar, MD, DNB[1,2]

DOI https://doi.org/10.1200/GO-25-00443

ABSTRACT

PURPOSE The 2022 European LeukemiaNet (ELN22) genetic risk stratification for AML makes critical changes, including removal of *FLT3*-internal tandem duplications (ITD) allelic ratio and inclusion of mutations in myelodysplasia-related (MR) genes and in-frame bZIP *CEBPA* mutations. We evaluated the applicability of ELN22 in a uniformly treated younger AML cohort and explored refinements to improve risk prediction.

METHODS We retrospectively analyzed 473 adult patients with AML treated with intensive therapy. A combination of cytogenetics and next-generation sequencing was used to stratify patients into the ELN17 and ELN22 risk. In addition, we also evaluated leukemic stem cell (LSC) burden at diagnosis and postinduction measurable residual disease by multiparametric flow cytometry.

RESULTS A total of 77 cases (16.3%) were reclassified from ELN17, primarily because of changes associated with *FLT3*-ITD, *CEBPA*, and MR gene mutations. As per ELN22, 56.7% of patients were classified as favorable, 28.3% intermediate, and 15.0% adverse risk. ELN22 adverse-risk patients had significantly inferior overall survival and relapse-free survival compared with intermediate- and favorable-risk patients. High LSC burden at diagnosis, presence of *WT1* mutations, and *DNMT3A-FLT3*-ITD comutated *NPM1* dichotomized the ELN22 intermediate-risk group.

CONCLUSION We conclude that ELN22 is prognostically valid in intensively treated younger patients with AML. Incorporation of *WT1* mutation status, triple-mutated *NPM1*, and LSC burden may improve risk stratification, particularly within the heterogeneous intermediate-risk category.

ACCOMPANYING CONTENT

Data Supplement

Accepted November 24, 2025
Published February 20, 2026

JCO Global Oncol 12:e2500443

INTRODUCTION

AML is characterized by a neoplastic transformation of hematopoietic stem cells or early committed myeloid progenitors resulting accumulation of blast cells or blast equivalents with a myeloid immunophenotype. AML is biologically heterogeneous, characterized by chromosomal alterations, translocations, and gene mutations which often co-occur. Heterogeneous treatment outcomes reflect this diverse biology. Risk stratification of this disease is challenging and must consider developments in biology as well as contemporary therapy.

The European LeukemiaNet (ELN) remains the cornerstone of AML risk stratification and is periodically updated to reflect recent advances in AML biology and therapy. The 2010 ELN recommendations incorporated cytogenetics and mutations in *FLT3*, *NPM1*, and *CEBPA* and stratified AML into four classes.[1] Studies indicated that the ELN 2010 classification—specifically the intermediate I and intermediate II risk groups—demonstrated limited reproducibility across age cohorts.[2,3] Consequently, the 2017 ELN revision (ELN17) integrated these two subgroups into a unified intermediate-risk category.[4] This version also introduced mutations in *ASXL1*, *RUNX1*, and *TP53* as adverse-risk features and substratified AML harboring *FLT3*-internal tandem duplications (ITD) based on allelic ratio. These recommendations found widespread application in clinical practice.

The latest iteration of the ELN classification, published in 2022 (ELN22), continues along this theme and risk-stratifies

CONTEXT

Key Objective

To demonstrate the applicability of the 2022 ELN genetic risk stratification of AML in a single-center adult AML cohort and explore possible refinements to improve risk stratification of these patients.

Knowledge Generated

In our cohort, 16.3% of patients classified as per ELN17 stratification got reclassified as per the ELN22 revisions. ELN22 risk stratification was highly predictive of outcomes; however, inclusion of high leukemic stem cell burden at diagnosis, presence of *WT1* mutations, and *DNMT3A-FLT3*-internal tandem duplications comutated *NPM1* class refined this stratification schema, particularly the intermediate-risk group.

Relevance

These findings can help in better prediction of outcomes, further aiding clinical decision making.

AML into three groups.[5] Major changes in ELN22 are as follows. *NPM*-mutated AML with *FLT3*-ITD is now an intermediate-risk AML irrespective of allelic ratio; furthermore, *NPM1* with adverse-risk cytogenetics is now placed in the adverse-risk group. Only in-frame insertions in the bZIP domain of the *CEBPA* gene are now classified as favorable risk. Another major change is the inclusion of secondary type or AML myelodysplasia-related (MR) mutations in the adverse-risk category. It also introduced newer entities of AML driven by t(8;16)(p11;p13)/*KAT6A::CREBBP* and t(3q26.2;v)/*MECOM(EVI1)* rearrangements and excluded hyperdiploid karyotype from adverse genetic risk.

Some groups have demonstrated the applicability of ELN22 to their respective cohorts.[6-9] However, this classification remains imperfect, and there remains scope for refinement. Recently, Ruhnke et al[6] have suggested a variant allele fraction-based cutoff for MR-defining mutations. Wangulu et al[9] have recommended that the ELN22 adverse-risk category be further dichotomized based on *TP53* mutation status. Furthermore, the prognostic impact of MR-defining mutations in ELN22 favorable-risk AML is unclear, with conflicting reports.[6,10-12]

Leukemic stem cells (LSCs) are rare, dormant, chemoresistant cells that lack immunogenicity. A few studies indicate that the quantitation of these cells at diagnosis or early into chemotherapy is a powerful predictor of outcome.[13-16] ELN22 suggests evaluation of LSCs during monitoring but makes no comment on the baseline burden in a newly diagnosed case of AML.

Here, in a single-center study, we aimed to validate the ELN22 genetic risk stratification in a cohort of de novo adult AML. We also suggest possible refinements to this stratification scheme on the basis of our observations.

METHODS

Patients

We retrospectively analyzed patients with adult AML (>18 years), treated with standard 3 + 7 induction therapy, recruited between 2013 and 2023 over three research studies at the Tata Memorial Centre. Patients who were treated with less intensive therapy or had a limited follow-up duration (<90 days) were excluded from the analysis. Clinical history, examination findings, and complete blood counts were noted from the electronic medical records. Blood and bone marrow morphology and immunophenotypic analysis findings were also noted. Patients were followed up till January 2025. Table 1 shows a brief overview of this cohort with relevant clinical details.

Diagnosis of AML

Patients were diagnosed as AML as per WHO 2017 and subsequently WHO 2022 criteria, as previously described.[17-19]

Cytogenetic Analysis

Conventional karyotyping and fluorescence in situ hybridization were performed as per standard recommendations as previously described.[17-20]

Next-Generation Sequencing

Sequencing was performed using a 50-gene myeloid panel of single-molecule molecular inversion probes (from 2013 to 2020) and analyzed as previously described.[18,21] For samples received between 2021 and 2023, target enrichment was performed using a 137-gene panel (Data Supplement, Table S1) using biotinylated capture baits (Supplementary Methods). For *FLT3*, *NPM1*, and *CEBPA*, fragment length analysis was performed as previously described.[18]

TABLE 1. Summary of Clinical, Laboratory, and MRD Characteristics of Patients Accrued in This Study

Parameter	Observation
Demographics	
Age, years, median (range)	35 (18-65)
Sex ratio	
Male	1.64
Female	1
Clinical characteristics	
Total no. of patients accrued	N = 473
Treatment details (3 + 7 followed by), No. (%)	
HiDAC	393 (83.1)
(HiDAC + FLT3 inhibitors)	19/393 (4.8)
SCT	09 (1.9)
Azacytidine	25 (5.3)
Palliative	22 (4.6)
No treatment details available	24 (5.1)
Remission characteristics, No. (%)	
CR	114 (24.1)
CR with incomplete hematologic recovery	359 (75.9)
Laboratory characteristics, No. (%)	
Blood counts at presentation	
More than 50,000/mm^3	95 (20.1)
Less than 50,000/mm^3	387 (79.9)
Classification according to cytogenetic risk, No. (%)	
Favorable risk	150 (31.7)
Intermediate risk	278 (58.8)
Poor risk	45 (9.5)
Classification according to ELN17 genetic risk, No. (%)	
Favorable risk	293 (61.9)
Intermediate risk	115 (24.3)
Poor risk	65 (13.8)
Classification according to ELN22 genetic risk, No. (%)	
Favorable risk	268 (56.7)
Intermediate risk	134 (28.3)
Poor risk	71 (15.0)
Postinduction flow MRD	n = 471
MRD positive, No. (%)	199 (42.2)
MRD negative, No. (%)	272 (57.8)

Abbreviations: CR, complete remission; MRD, measurable residual disease.

Diagnostic Quantification of LSC Burden

LSC burden was evaluated at diagnosis using a 10-color 2-tube (113 cases, Data Supplement, Table S2) or 16-color single tube immunophenotypic assay (55 cases, Data Supplement, Table S3). The patterns of maturation of normal CD34+CD38− populations were studied in 10 post-therapy (induction or consolidation) samples of acute lymphoblastic leukemia, between the two assays for reproducibility. The analytical equivalence of these two assays and receiver operating characteristic analysis for threshold determination are shown in the Data Supplement (Figs S1 and S2).

Multiparametric Flow Cytometry–Based Measurable Residual Disease Assessment

Measurable residual disease (MRD) was evaluated at post-induction (PI) time point using 10-color 2-tube[18] or 16-color single-tube assay (Data Supplement, Table S4).

ELN Genetic Risk

All the cases were risk stratified as per the ELN17 and ELN22 recommendations.[4,5]

Statistical Analysis

We studied associations between ELN22 risk groups and other patient characteristics (WBC counts, age, sex, multiparametric flow cytometry [MFC] MRD status, etc) using the Chi-square test for categorical and Kruskal-Wallis test for continuous variables. Overall survival (OS) and relapse-free survival (RFS) were calculated as previously described.[17-19,21] Patients who were not in morphologic remission PI or who relapsed and had no follow-up during the last 6 months of the study period were assumed to be deceased. Survival analyses for OS and RFS were performed using the Kaplan-Meier method, with comparisons between groups made using the log-rank test. Multivariate analysis of prognostic factors for OS and RFS was conducted using Cox proportional hazards regression models.

RESULTS

This study was approved by the Institutional Ethics Committee (IEC III project # 900163 and # 900616). Table 1 provides an overview of the clinical, laboratory, and genetic findings of our cohort.

Patient Details

The final cohort comprised 473 patients with a median follow-up of 37.1 months. The median OS was 33.4 months (95% CI, 26.3 to 38.7), and the median RFS was 27.6 months (95% CI, 21.3 to 35.4). Only a small number of patients could undergo allogeneic bone marrow transplantation, and their analysis is pooled with the rest of the patients.

WHO HAEM5 and ELN22 Classification

The frequency of various AML subtypes based on WHO 2022[22] in our cohort is shown in the Data Supplement (Table S7). Based on the ELN22 stratification, most patients (n = 268, 56.7%) had a favorable genetic risk, whereas 134 (28.3%) had an intermediate risk and 71 (15%) had an adverse risk (Data Supplement, Fig S3). Patients in ELN22 adverse-risk group had significantly lower total leukocyte count compared with the other two groups,

whereas intermediate-risk patients presented with higher median blast counts at diagnosis (Data Supplement, Fig S4).

Genomic Landscape

An oncoplot of somatic mutations observed in our cohort, stratified by the ELN22 genetic risk, can be appreciated in Figure 1A. Most patients (n = 462 [97.7%]) had an identifiable somatic mutation, whereas 11 (2.3%) patients had no detectable mutations. *NPM1* mutation was the commonest followed by *FLT3*, *NRAS*, and *KIT* mutations. Two patients harboring *NPM1* mutation with *BCR::ABL1* fusion were classified as ELN22 adverse risk.

Figure 1B illustrates the interaction of somatic mutations within this AML cohort. Co-occurrence and mutual exclusivity patterns were noted. *NPM1* mutations frequently co-occurred with *FLT3*, *DNMT3A*, *IDH1*, *IDH2*, *TET2*, and *PTPN11* mutations, consistent with our previous observation.[21]

Such unique patterns were also noted among mutations in MR genes, which showed a tendency to co-occur. For example, *BCOR* mutation often co-occurred with mutations in *RUNX1*, *U2AF1*, *IDH2*, and *EZH2* genes. Similar observations were made for other MR genes. *CEBPA* mutations were frequently observed with *CSF3R* and *GATA2* mutations. *KIT* and *ASXL2* mutations also co-occurred, as well as *WT1* and *FLT3*-ITD (Fig 1B). Analogous to these observations, patterns of mutual exclusivity were noted for signaling pathway genes like *FLT3*, *CSF3R*, *KIT*, as well as *WT1*, suggesting the presence of distinct molecular subtypes. The interactions here highlight established mutational relationships in AML and indicate the presence of co-operation and antagonism between leukemic clones.

Reclassification of ELN17 Cases to ELN22

We then reclassified patients of AML from the ELN17 to the ELN22 classification, resulting in 77 patients (16.3%) being reclassified (Fig 2).

Among the 268 patients classified as favorable risk by ELN22, 11 patients were reclassified from intermediate-risk in ELN17 to favorable in ELN22 as they harbored monoallelic, in-frame *CEBPA* mutations in the bZIP domain. Two additional patients previously categorized as adverse risk were reclassified as favorable risk in ELN22 based on the same *CEBPA* mutational profile, despite adverse risk cytogenetics.

The ELN22 intermediate-risk group (n = 134) included 38 cases previously classified as favorable, primarily *NPM1*-mutated AML with low allelic ratio *FLT3*-ITD because ELN22 no longer considers allelic ratio in risk stratification. Additionally, nine cases classified as adverse risk in ELN17, because of high allelic ratio *FLT3*-ITD without other defining abnormalities, shifted to intermediate-risk under ELN22.

Of the 71 patients in the ELN22 adverse-risk group, 17 were reclassified because of the incorporation of MR gene mutations.

Clinical Impact of the ELN22 Risk Stratification

The clinical relevance of cytogenetic risk, ELN17, and ELN22-based genetic risk stratification schema can be seen in Table 2. Patients with adverse-risk cytogenetics had significantly inferior OS and RFS compared with the favorable-risk group ($P < .001$; Data Supplement, Figs S5A and S5B).

We confirm that the ELN22 risk stratification is applicable to our cohort and is highly predictive of outcomes (Table 2). Patients in the adverse-risk group had inferior OS and RFS as compared with the intermediate- and favorable-risk groups (Figs 3A and 3B, Table 2).

However, the consolidation therapies varied among patients in our cohort. Outcome data in patients stratified by type of consolidation therapy are shown in the Data Supplement (Table S5).

Baseline LSC Analysis

LSC quantitation at diagnosis could be performed on 168 samples. Of these, 98 samples (58.3%) had LSC burden of >0.1% (LSC-high). LSC-high patients had an inferior OS (hazard ratio [HR], 1.9 [95% CI, 1.3 to 3.1]; $P = .003$; Data Supplement, Fig S6A) and RFS (HR, 1.9 [95% CI, 1.1 to 3.1]; $P = .01$; Data Supplement, Fig S6B). This impact was most notable in the ELN22 intermediate-risk patients (Data Supplement, Figs S6C-S6H).

MFC MRD

A total of 471 samples could be processed for MFC MRD at the end of induction. Of these, 199 (42.2%) samples had detectable MRD. Patients who were MRD positive had significantly inferior OS (HR, 1.8 [95% CI, 1.4 to 2.4]; $P < .001$) and RFS (HR, 1.4 [95% CI, 1.1 to 1.9]; $P = .005$; Data Supplement, Fig S7, Table 2) as compared with MRD-negative patients. PI MFC MRD positivity rates differed significantly across ELN22 risk groups ($P < .0001$; Data Supplement, Table S6). MFC MRD was detected in 34.3% (n = 92) of favorable-risk, 40.7% (n = 55) of intermediate-risk, and 74.2% (n = 52) of adverse-risk patients.

MFC MRD status could not be determined in two cases in view of technical difficulties while sample processing. Both cases were in morphological remission after induction therapy. One of the patients received 2# HiDAC PI, relapsed after 9.4 months of induction, and deceased 4 months after the event. The other patient was treated with palliative intent in view of persistent cytopenias PI. The patient deceased at 11.34 months after diagnosis.

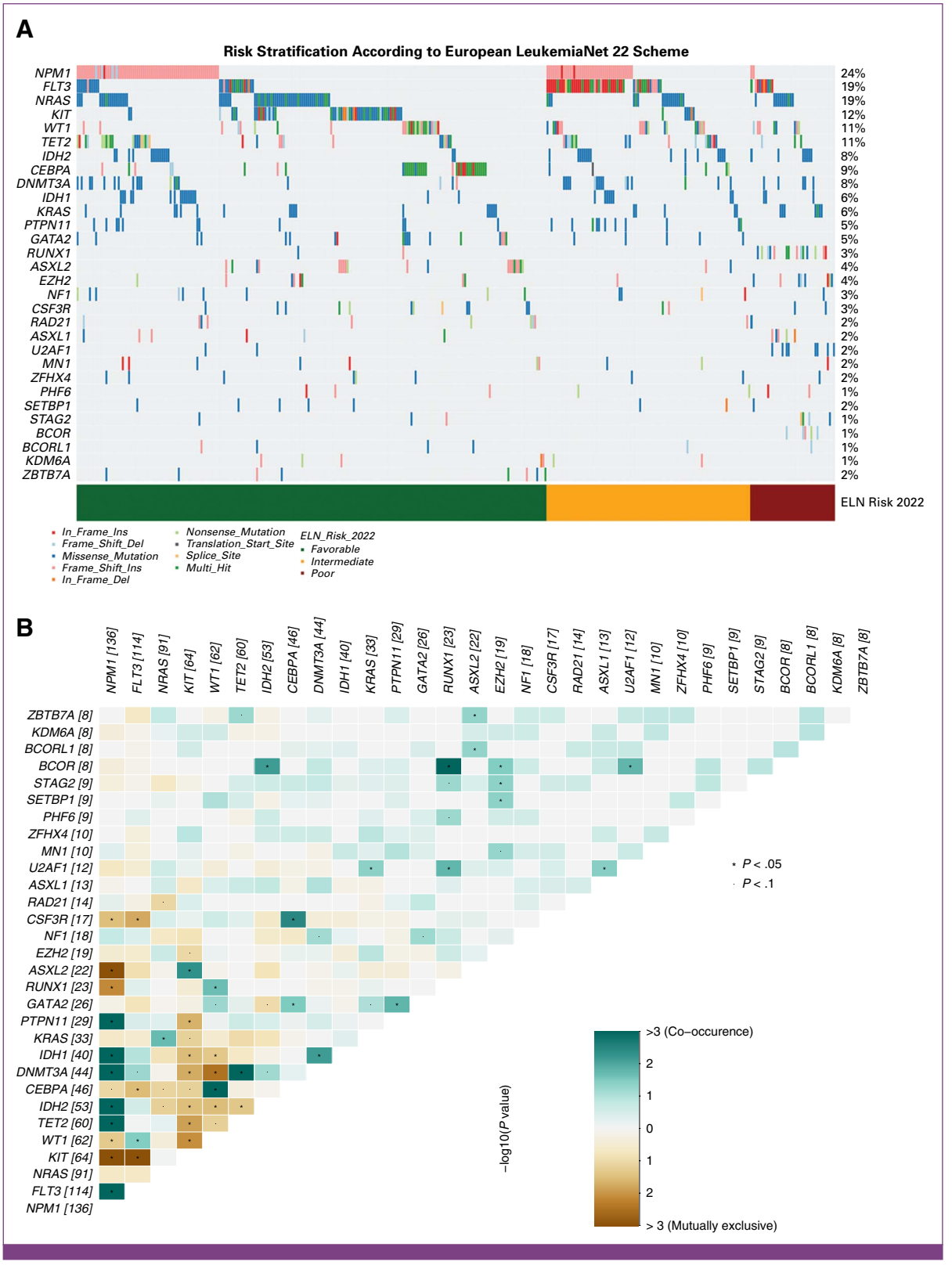

FIG 1. (A) Oncoplot showing the distribution of 30 most frequent mutations in various ELN22 risk stratification groups; (B) somatic interaction plot showing interaction of mutations at baseline. Co-occurrence is indicated in green color and mutual exclusivity is indicated in brown. ELN22, 2022 European LeukemiaNet.

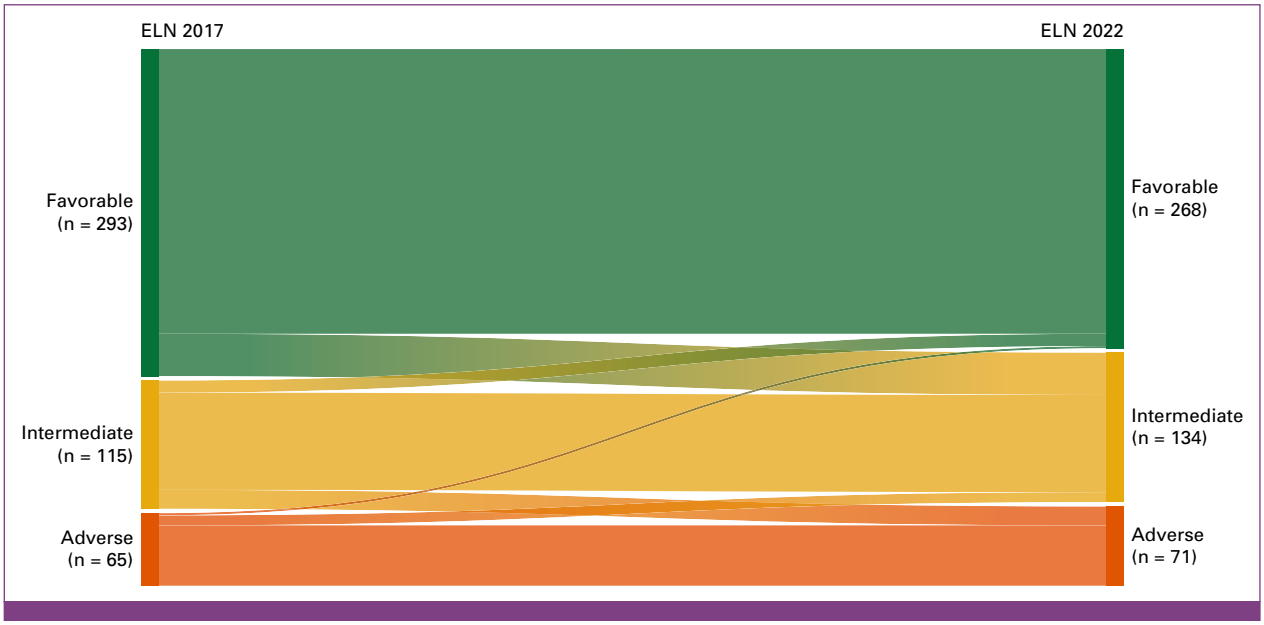

FIG 2. Sankey diagram showing the restratification of patients from ELN17 to ELN22 risk categories. ELN17, 2017 European LeukemiaNet; ELN22, 2022 European LeukemiaNet.

TABLE 2. Univariate Cox Analysis

	OS		RFS	
Parameter	HR (95% CI)	*P*	HR (95% CI)	*P*
Cytogenetic risk				
Favorable	1	<.0001	1	<.0001
Intermediate	1.9 (1.4 to 2.4)		1.5 (1.1 to 1.9)	
Adverse	3.2 (1.9 to 5.3)		3.4 (1.8 to 6.4)	
ELN17 risk				
Favorable	1	<.0001	1	<.0001
Intermediate	1.8 (1.3 to 2.4)		1.5 (1.1 to 2.1)	
Adverse	2.9 (1.9 to 4.5)		2.9 (1.7 to 5.1)	
ELN22 risk				
Favorable	1	<.0001	1	<.0001
Intermediate	1.8 (1.4 to 2.5)		1.5 (1.1 to 2.0)	
Adverse	3.6 (2.4 to 5.6)		3.9 (2.2 to 7.1)	
MFC MRD				
Negative	1	<.0001	1	.005
Positive	1.8 (1.4 to 2.4)		1.4 (1.1 to 1.9)	
WT1 mutation				
Absent	1	<.0001	1	<.0001
Present	2.1 (1.4 to 3.2)		2.2 (1.4 to 3.6)	
Triple mutated AML with *NPM1* mutation				
Absent	1	.0001	1	<.0001
Present	3.0 (1.3 to 7.4)		3.5 (1.3 to 9.2)	

Abbreviations: ELN22, 2022 European LeukemiaNet; MFC, multiparametric flow cytometry; MRD, measurable residual disease; OS, overall survival; RFS, relapse-free survival.

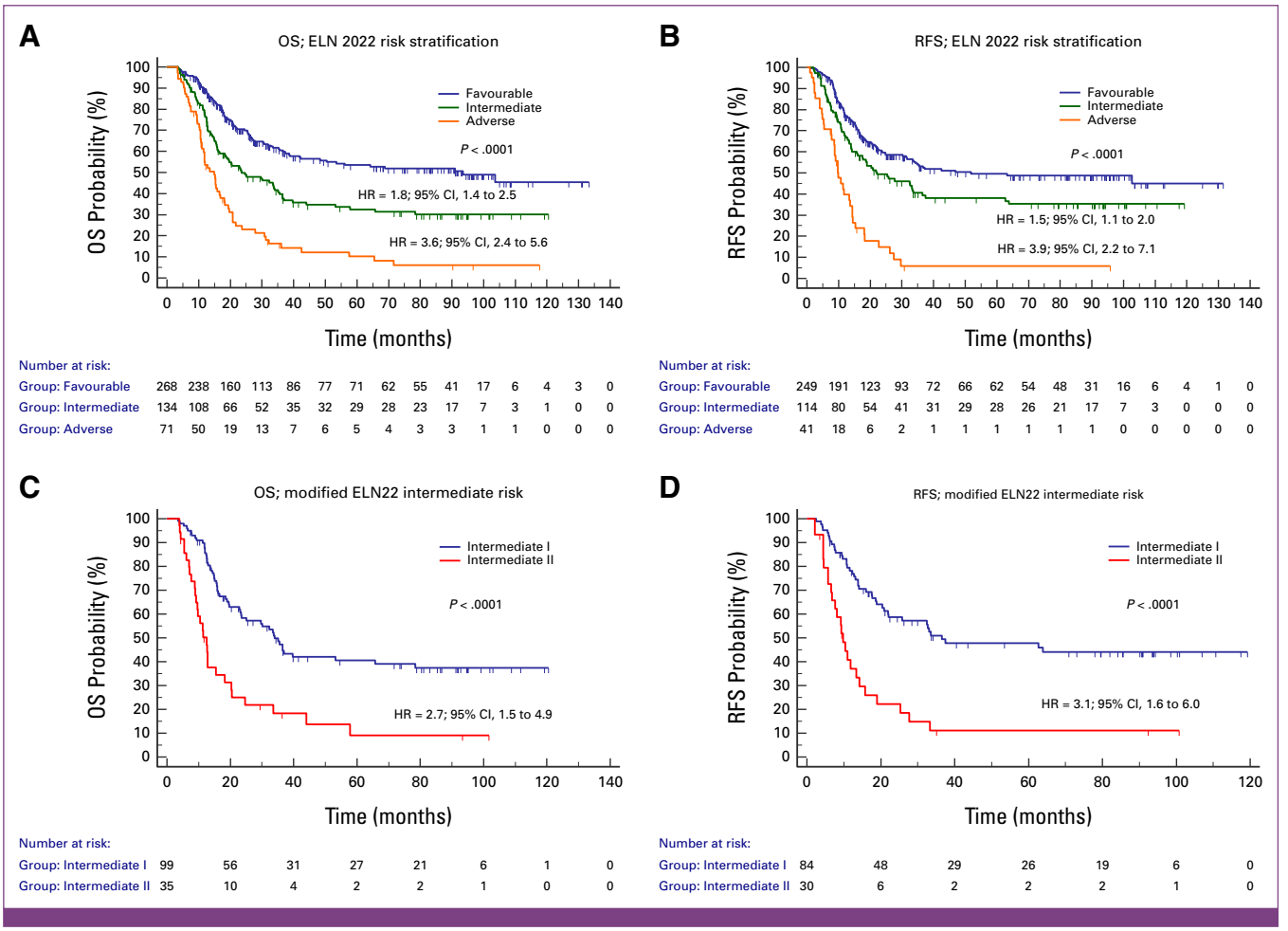

FIG 3. Kaplan-Meier curves showing the impact of the different ELN22 groups on (A) OS and (B) RFS; presence of *WT1* mutation and/or triple mutated *NPM1* cases formed a separate subgroup in ELN22 intermediate risk (intermediate II) with inferior (C) OS and (D) RFS compared with the rest of the cases in the intermediate risk group. ELN22, 2022 European LeukemiaNet; HR, hazard ratio; OS, overall survival; RFS, relapse-free survival.

Clinical Relevance of Other Mutations in the Context of ELN22

Based on a previous observation[15] highlighting molecular heterogeneity in *NPM1*-mutated AML, we analyzed patients who were comutated for *NPM1*, *DNMT3A*, and *FLT3*-ITD as one group (triple-mutated *NPM1*). This subset accounted for 11.4% (n = 15/132) of all *NPM1*-mutated AML, all ELN22 intermediate risk, and had a significantly inferior OS (HR, 3.0 [95% CI, 1.3 to 7.4]; $P = .0001$) and RFS (HR, 3.5 [95% CI, 1.3 to 9.2]; $P < .0001$) as compared with other *NPM1*-mutated AML (Data Supplement, Fig S8).

The presence of *WT1* mutations (n = 62, 13.1%) was predictive of inferior OS and RFS (Data Supplement, Figs S9A and S9B). When further divided by ELN22 risk, favorable (n = 27), intermediate (n = 22), and adverse risk (n = 13), the presence of *WT1* mutation in intermediate risk was associated with an inferior OS (Data Supplement, Figs S9C-S9H).

We can also confirm that *FLT3*-ITD allelic ratio did not have any prognostic bearing on outcome (Data Supplement, Figs S10A and S10B). In the ELN22 favorable-risk group, 27 of 268 (10.1%) patients harbored an MR-defining mutation. These included AML with *RUNX1::RUNX1T1* fusion (n = 15, 55.6%), AML with *NPM1* mutation (n = 9, 33.3%), AML with *CEBPA* mutation (n = 2, 7.4%), and AML with *CBFB::MYH11* fusion (n = 1, 3.7%). The presence of MR-mutations did not influence outcomes in the ELN22 favorable-risk group (Data Supplement, Figs S10E and S10F).

On multivariate analysis, ELN22 risk, MFC MRD status, and *WT1* mutation status predicted for an inferior OS and RFS (Table 3).

DISCUSSION

In this study, we demonstrate the application of ELN22 recommendations for the genetic risk stratification of AML in a single-center study and identify heterogeneity mainly in the existing ELN22 intermediate risk.

TABLE 3. Multivariate Cox Analysis

	OS		RFS	
Parameter	HR (95% CI)	*P*	HR (95% CI)	*P*
Cytogenetic risk				
Intermediate	1.2 (0.8 to 1.7)	.4	1.1 (0.7 to 1.6)	.6
Adverse	1.2 (0.6 to 2.1)	.6	1.5 (0.7 to 2.7)	.2
ELN17 risk				
Intermediate	0.8 (0.5 to 1.2)	.3	0.9 (0.5 to 1.4)	.5
Adverse	0.7 (0.4 to 1.4)	.3	0.7 (0.3 to 1.5)	.3
ELN22 risk				
Intermediate	1.9 (1.2 to 3.0)	.003*	1.5 (1.0 to 2.5)	.05
Adverse	3.6 (1.9 to 6.9)	.0001*	3.8 (1.8 to 8.2)	.0004*
MFC MRD				
Positive	1.6 (1.2 to 2.1)	.001*	1.3 (1.0 to 1.8)	.05
WT1 mutation				
Present	1.9 (1.3 to 2.6)	.0004*	1.9 (1.3 to 2.7)	.001*

Abbreviations: ELN22, 2022 European LeukemiaNet; MFC, multiparametric flow cytometry; MRD, measurable residual disease; OS, overall survival; RFS, relapse-free survival.
*$P < .05$.

The median age of our cohort is younger (35 years) as compared with published studies.[6-9] This observation, however, is not limited to our center but has been reiterated by other adult AML studies from India.[23] This may represent a referral bias rather than a genuine biologic phenomenon. Our previous work on adult AML is consistent with this finding (median age range, 29-41 years).[17-19,21,24] The median OS and RFS of this adult AML cohort are 33.4 and 27.6 months, respectively. In comparison, the 3-year median event-free survival (EFS) and OS of a pediatric AML (pAML) cohort from our center was 39.8 and 42.0 months, respectively. The incidence of genetic abnormalities in our cohort as compared with pediatric cohort can be seen in the Data Supplement (Table S7). We observed a higher incidence of fusions in pAML cohort, with *RUNX1::RUNX1T1* fusion (33%) being the commonest AML defining abnormality in pAML. *NPM1* mutation was the most common genetic abnormality in our cohort (27.9%), which was seen only in 6.3% of pediatric patients.[25] Consistent with the observation by Ruhnke et al,[6] adverse genetic risk was associated with significantly lower WBC counts in our cohort. By contrast, the intermediate-risk group showed significantly higher bone marrow blast percentages, whereas Ruhnke et al[6] reported lower blasts in adverse-risk patients.

A total of 77 cases (16.3%) in our cohort were reclassified from ELN17 to ELN22. Although Lachowiez et al[8] reported a similar percentage of restratification cases in their cohort (15%), other studies reported a higher percentage of restratification.[6,7]

One of the major changes included in ELN22 was the elimination of *FLT3*-ITD allelic ratio. This change was implemented in part due to the widespread use of targeted therapies, which have been shown to improve outcomes across all ELN risk groups. The revision has since been supported and validated by multiple studies, including ours (Data Supplement, Figs S10A and S10B).[6,26-29]

Lindsley et al[30] demonstrated that MR-associated mutations have a distinct ontogeny associated with poor prognosis.[30-32] Seventeen patients in our cohort were reclassified as adverse risk because of the presence of MR-defining mutations and showed outcomes similar to those of other adverse-risk cases (Data Supplement, Figs S10C and S10D). However, the presence of these mutations had no significant impact on outcomes when they co-occurred with the favorable-risk group (Data Supplement, Figs S10E and S10F). There is a discordance in the literature regarding the impact of these mutations in favorable-risk AML groups, especially AML with *NPM1* mutation.[10,11] However, Eckardt et al[10] studied this phenomenon on one of the largest cohorts of *NPM1*-mutated AMLs till date and found no significant impact of secondary type mutations on this favorable-risk AML subgroup. Lachowiez et al[8] reported similar findings. Loghavi et al[33] proposed that higher variant allelic fractions of MR mutations were responsible for their negative impact on outcomes in *NPM1*-mutated AMLs.

We found that high LSC burden at diagnosis could predict inferior OS and RFS, especially in the ELN22 intermediate-risk group. Although Zeijlemaker et al[14] found that baseline LSC status affected OS, Terwijn et al[15] demonstrated its impact on RFS. In addition, Reuvekamp et al[34] demonstrated that LSC positivity at diagnosis in elderly patients treated with hypomethylating agents was also predictive of shorter EFS and higher cumulative incidence of relapse. The cutoff used to determine high LSC burden in these studies was 0.03%, as defined by Terjwin et al.[15] Kamel et al[16] evaluated the effect of various immunophenotypic marker expression in predicting outcomes. They concluded that only CD123+ LSCs predicted for an inferior OS and disease-free survival. In our cohort, the 0.1% threshold demonstrated optimal discriminative performance for OS. Taken together, baseline LSC burden may be used as an early marker for prognostication in patients with AML as well as for the development of targeted therapy against specific markers.

We demonstrate that triple-mutated *NPM1* has a distinctly inferior outcome in line with several studies that have reported the negative impact.[3,35,36] Poor prognosis of *WT1* mutation has also been reported in the literature by various groups, particularly in the intermediate-risk group.[37,38] We demonstrate that the presence of either *WT1* mutation or triple-mutated *NPM1* dichotomized the ELN22 intermediate-risk category into two subgroups with significantly different outcomes (Figs 3C and 3D).

We have seen that despite the updates in risk stratification by ELN over the years, the treatment outcomes in AML remain heterogeneous, especially in the ELN22 intermediate-risk

group. Our study demonstrates that triple-mutated *NPM1*, *WT1* mutation, and baseline LSC burden could possibly explain this heterogeneity, particularly in the intermediate-risk category and suggest a possible benefit of transplantation or targeted therapy in such patients.

The limitations of our study are the exclusion of older patients from the cohort because these patients are usually not candidates for intensive chemotherapy, and the lack of uniform LSC quantification. However, the relevance of LSC burden is evident even in a limited number of patients. Studies to validate these stratification criteria on older/unfit patients treated with less intensive chemotherapy also found that these patients could not be robustly grouped as per the ELN22 classes.[39,40] Döhner et al[41] have proposed a genetic risk classification for such patients. However, further validation and testing of this classification is needed to strengthen its clinical utility for patient management.

AFFILIATIONS

[1]Department of Hematopathology, ACTREC, Tata Memorial Centre, Navi Mumbai, India
[2]Homi Bhabha National Institute (HBNI), Mumbai, India
[3]Department of Cytogenetics, ACTREC, Tata Memorial Centre, Navi Mumbai, India
[4]Adult Hematolymphoid Disease Management Group, Tata Memorial Centre, Mumbai, India

CORRESPONDING AUTHOR

Nikhil Patkar, MD, DNB; e-mail: nvpatkar@gmail.com.

EQUAL CONTRIBUTION

G.M. and A.A. contributed equally to this work.

SUPPORT

Supported by Indian Council of Medical Research (ICMR-ICRC:5/13/9/NP/ICRC/2020/NCD-III) and the Wellcome Trust/DBT India Alliance Senior Fellowship (grant number IA/CPHS/21/1/505947) awarded to Dr Nikhil Patkar.

AUTHOR CONTRIBUTIONS

Conception and design: Nikhil Patkar
Financial support: Nikhil Patkar
Administrative support: Nikhil Patkar
Provision of study materials or patients: Dhanlaxmi Shetty, Nishant Jindal, Alok Shetty, Anant Gokarn, Hasmukh Jain, Bhausaheb Bagal, Manju Sengar, Prashant Tembhare, Nikhil Patkar
Collection and assembly of data: Gojiri Mawalankar, Aarti Achrekar, Seema Biswas, Swapnali Joshi, Priyanka Ugale, Prasanna Bhanshe, Shruti Chaudhary, Shadma Shahin, Sweta Rajpal, Gaurav Chatterjee, Papagudi G Subramanian, Nikhil Patkar
Data analysis and interpretation: Gojiri Mawalankar, Seema Biswas, Vishram Terse, Swapnali Joshi, Prasanna Bhanshe, Saurabh Kusurkar, Shadma Shahin, Vaibhav Gawde, Dhanlaxmi Shetty, Nishant Jindal, Sumeet Mirgh, Alok Shetty, Anant Gokarn, Sachin Punatar, Lingaraj Nayak, Hasmukh Jain, Bhausaheb Bagal, Manju Sengar, Navin Khattry, Sweta Rajpal, Gaurav Chatterjee, Prashant Tembhare, Papagudi G Subramanian, Nikhil Patkar
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/go/authors/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Sachin Punatar
Research Funding: Sun Pharma (Inst), Takeda (Inst)

Hasmukh Jain
Employment: ImmunoACT Private Limited (I)
Stock and Other Ownership Interests: Cipla Pharmaceuticals, Biocon Pharmaceuticals, Dr Reddys Laboratory
Consulting or Advisory Role: ImmunoACT Private Limited (I)
Research Funding: ImmunoACT, Intas, Zydus Pharmaceuticals

Gaurav Chatterjee
Research Funding: DBT Wellcome Trust India Alliance Intermediate Fellowship

Nikhil Patkar
Research Funding: Illumina, Thermo Fisher Scientific
Patents, Royalties, Other Intellectual Property: Dr Patkar is an inventor on these two pending patents (Applicant is ACTREC, Tata Memorial Center) 1. Method for Sequencing PreSelected Genomic Regions by Asymmetrically Looped Strand-Biased Oligonucleotides and Hemi-Nested PCR. 2. A method and a kit for generating nucleic acid for target capture (PCT Patent Filed: PCT/IN2025/051311) (Inst)

No other potential conflicts of interest were reported.

REFERENCES

1. Döhner H, Estey EH, Amadori S, et al: Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 115:453-474, 2010
2. Röllig C, Bornhäuser M, Thiede C, et al: Long-term prognosis of acute myeloid leukemia according to the new genetic risk classification of the European LeukemiaNet recommendations: Evaluation of the proposed reporting system. J Clin Oncol 29:2758-2765, 2011
3. Moualla Y, Moassass F, AL-Halbi B, et al: Prognostic relevance of DNMT3A, FLT3 and NPM1 mutations in Syrian acute myeloid leukemia patients. Asian Pac J Cancer Prev 23:1387-1395, 2022
4. Döhner H, Estey E, Grimwade D, et al: Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129:424-447, 2017
5. Döhner H, Wei AH, Appelbaum FR, et al: Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood 140:1345-1377, 2022
6. Ruhnke L, Bill M, Zukunft S, et al: Validation of the revised 2022 European LeukemiaNet risk stratification in adult patients with acute myeloid leukemia. Blood Adv 9:1392-1404, 2025
7. Song GY, Kim HJ, Kim T, et al: Validation of the 2022 European LeukemiaNet risk stratification for acute myeloid leukemia. Sci Rep 14:8517, 2024
8. Lachowiez CA, Long N, Saultz J, et al: Comparison and validation of the 2022 European LeukemiaNet guidelines in acute myeloid leukemia. Blood Adv 7:1899-1909, 2023

9. Wangulu C, Zhao D, Zhou Q, et al: Proposed refinement of 2022 European LeukemiaNet adverse-risk group of AML patients using a real-world cohort. Cancers 17:1405, 2025
10. Eckardt JN, Bill M, Rausch C, et al: Secondary-type mutations do not impact outcome in NPM1-mutated acute myeloid leukemia—Implications for the European LeukemiaNet risk classification. Leukemia 37:2282-2285, 2023
11. Chan O, Al Ali N, Tashkandi H, et al: Mutations highly specific for secondary AML are associated with poor outcomes in ELN favorable risk *NPM1* -mutated AML. Blood Adv 8:1075-1083, 2024
12. Rausch C, Rothenberg-Thurley M, Dufour A, et al: Validation and refinement of the 2022 European LeukemiaNet genetic risk stratification of acute myeloid leukemia. Leukemia 37:1234-1244, 2023
13. Li SQ, Xu LP, Wang Y, et al: An LSC-based MRD assay to complement the traditional MFC method for prediction of AML relapse: A prospective study. Blood 140:516-520, 2022
14. Zeijlemaker W, Grob T, Meijer R, et al: CD34+CD38− leukemic stem cell frequency to predict outcome in acute myeloid leukemia. Leukemia 33:1102-1112, 2019
15. Terwijn M, Zeijlemaker W, Kelder A, et al: Leukemic stem cell frequency: A strong biomarker for clinical outcome in acute myeloid leukemia. PLoS ONE 9:e107587, 2014
16. Kamel AM, Elsharkawy NM, Kandeel EZ, et al: Leukemia stem cell frequency at diagnosis correlates with measurable/minimal residual disease and impacts survival in adult acute myeloid leukemia. Front Oncol 12:867684, 2022
17. Patkar N, Kakirde C, Shaikh AF, et al: Clinical impact of panel-based error-corrected next generation sequencing versus flow cytometry to detect measurable residual disease (MRD) in acute myeloid leukemia (AML). Leukemia 35:1392-1404, 2021
18. Patkar N, Kakirde C, Bhanshe P, et al.: Utility of immunophenotypic measurable residual disease in adult acute myeloid leukemia—Real-world context. Front Oncol 9:450, 2019
19. Shaikh AF, Kakirde C, Dhamne C, et al: Machine learning derived genomics driven prognostication for acute myeloid leukemia with *RUNX1-RUNX1T1*. Leuk Lymphoma 61:3154-3160, 2020
20. Korf BR: Overview of clinical cytogenetics. Curr Protoc Hum Genet Chapter 8:Unit 8.1, 2001
21. Patkar N, Shaikh AF, Kakirde C, et al: A novel machine-learning-derived genetic score correlates with measurable residual disease and is highly predictive of outcome in acute myeloid leukemia with mutated NPM1. Blood Cancer J 9:79, 2019
22. WHO Classification of Tumours Editorial Board. Haematolymphoid Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer; 11, 2024
23. Philip C, George B, Ganapule A, et al: Acute myeloid leukaemia: Challenges and real world data from India. Br J Haematol 170:110-117, 2015
24. Patkar N, Kodgule R, Kakirde C, et al: Clinical impact of measurable residual disease monitoring by ultradeep next generation sequencing in NPM1 mutated acute myeloid leukemia. Oncotarget 9: 36613-36624, 2018
25. Srinivasan S, Aggarwal M, Patkar N, et al. Prevalence and prognostic impact of NPM1 mutation in childhood acute myeloid leukemia: Experience from a single tertiary cancer Centre in India. Clin Lymphoma Myeloma Leuk. 2025. doi:10.1016/j.clml.2025.10.015.
26. Döhner K, Thiede C, Jahn N, et al: Impact of NPM1/FLT3-ITD genotypes defined by the 2017 European LeukemiaNet in patients with acute myeloid leukemia. Blood 135:371-380, 2020
27. Mrózek K, Kohlschmidt J, Blachly JS, et al: Outcome prediction by the 2022 European LeukemiaNet genetic-risk classification for adults with acute myeloid leukemia: An Alliance study. Leukemia 37:788-798, 2023
28. Stone RM, Mandrekar SJ, Sanford BL, et al: Midostaurin plus chemotherapy for acute myeloid leukemia with a *FLT3* mutation. N Engl J Med 377:454-464, 2017
29. Sakaguchi M, Yamaguchi H, Najima Y, et al: Prognostic impact of low allelic ratio FLT3-ITD and NPM1 mutation in acute myeloid leukemia. Blood Adv 2:2744-2754, 2018
30. Lindsley RC, Mar BG, Mazzola E, et al: Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood 125:1367-1376, 2015
31. Gao Y, Jia M, Mao Y, et al: Distinct mutation landscapes between acute myeloid leukemia with myelodysplasia-related changes and de novo acute myeloid leukemia. Am J Clin Pathol 157:691-700, 2022
32. Tsai XCH, Sun KJ, Lo MY, et al: Poor prognostic implications of myelodysplasia-related mutations in both older and younger patients with de novo AML. Blood Cancer J 13:4, 2023
33. Loghavi S, Reville PK, Lachowiez CA, et al: The adverse effect of myelodysplasia-related mutations in *de novo* acute myeloid leukemia is associated with higher variant allelic frequency (VAF): A proposal for a numeric cutoff for variant allelic frequency. Blood 140:6306-6308, 2022 (suppl 1)
34. Reuvekamp T, Ngai LL, den Hartog D, et al: CD34+CD38- leukemia stem cells predict clinical outcomes in acute myeloid leukemia patients treated non-intensively with hypomethylating agents. Leukemia 39:972-975, 2025
35. Othman J, Potter N, Ivey A, et al: Molecular, clinical, and therapeutic determinants of outcome in *NPM1*-mutated AML. Blood 144:714-728, 2024
36. Yao Y, Zhou Y, Zhuo N, et al: Co-mutation landscape and its prognostic impact on newly diagnosed adult patients with NPM1-mutated de novo acute myeloid leukemia. Blood Cancer J 14:118, 2024
37. Atluri H, DiGennaro J, Patel KP, et al: Clinical and prognostic implications of *WT1* mutations in de novo and relapsed acute myeloid leukemia. Blood 142:959, 2023 (suppl 1)
38. Gannamani V, Baranwal A, Langer KJ, et al: Impact of Wilms Tumor (WT1) mutation on relapse and overall survival in acute myeloid leukemia patients following allogenic stem cell transplantation. Transplant Cell Ther 30:S112-S113, 2024
39. Jahn E, Saadati M, Fenaux P, et al: Clinical impact of the genomic landscape and leukemogenic trajectories in non-intensively treated elderly acute myeloid leukemia patients. Leukemia 37: 2187-2196, 2023
40. Döhner H, Pratz KW, DiNardo CD, et al: Genetic risk stratification and outcomes among treatment-naive patients with AML treated with venetoclax and azacitidine. Blood 144:2211-2222, 2024
41. Döhner H, DiNardo CD, Appelbaum FR, et al: Genetic risk classification for adults with AML receiving less-intensive therapies: The 2024 ELN recommendations. Blood 144:2169-2173, 2024