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CLINICAL SIGNIFICANCE OF NOVEL BIOMARKERS IN THE EARLY DIAGNOSIS AND PROGNOSIS OF ACUTE MYELOID LEUKEMIA

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Abstract

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy with genomic alterations, which differ and affect the prognosis and response to treatment. One of the most important issues is the dilemma on whether to include the whole-molecule profiling in the clinical decision-making. It was a retrospective cohort study where 312 patients with newly-diagnosed de novo AML were involved and received a comprehensive genomic profile in the form of next-generation sequencing panel of 54 genes. The dynamics of clonal architecture and the methodologies of detection of the remaining disease as well as patterns of co-mutation were studied. Multivariate Cox regression, machine learning algorithms and graph neural networks were used to create prognostic models. The mutation was also recurrent with highest percentage of 94.6 as the highest percentages of NPM1, DNMT3A and FLT3 which had highest percentages of 28.5, 26.9 and 24.4 respectively. Molecular risk stratification (54 gene) was much better compared to ELN 2022, and had a very-high-risk subgroup with a median overall survival of 8.4 months. TP53 (HR 3.24), ASXL1 (HR 2.18) and RUNX1 (HR 1.96) are reported to be the most bad prognostic markers and CEBPA bZIP (HR 0.52) and NPM1 (HR 0.58) have good results. Co-mutation analysis showed that there were significant pairwise associations, such as that between NPM1 and FLT3-ITD (OR 3.42) and that TP53 and NPM1 mutually exclude each other (OR 0.12). Longitudinal clonal analysis revealed that CHIP-related mutations continued to exist with treatment and the diversity of the clones at relapse with a significant increase. Sensitivity (10 -6) and concordance (89.4): NGS with error-corrected technique demonstrated a good sensitivity and a good concordance. The largest predictive accuracy of treatment response (AUROC 0.912) was achieved with the use of graph neural networks with mutational networks. The broad-based genomic investigation, which covers mutational, clonal and co-mutation can greatly contribute to the prognostic stratification and therapy of AML. These findings support the idea that the multi-gene NGS panel and analysis of the clonal architecture should be implemented into the routine to simplify the precision medicine plans.

Keywords: Acute Myeloid Leukemia, Next-Generation Sequencing, Genomic Profiling, Clonal Architecture, Prognostic Stratification, Precision Oncology

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INTRODUCTION

Acute myeloid leukemia (AML) is a diverse blood cancer. It entails an expedited proliferation of abnormal myeloid progenitor cells. This presents a challenge to the blood production and morbidity and mortality are high (Xie et al., 2025). Many genetic changes have been discovered, with the aid of complex molecular techniques. The changes are essential to enhance classification, prognosis, as well as targeted treatments in AML (Lanza et al., 2025). A better understanding of the biology of AML and advances in the field of genomic analysis have allowed introducing certain changes in genes into the prognostic models with certainty. This assists to make clinical decisions (Li et al., 2020). Next-generation sequencing now has been extended to whole mutation profiling. It determines a wide range of genetic mutations which may lead to AML and determine the reaction to the treatment (Achi and Kanagal-Shamanna, 2021). It is one of the necessary molecular classification that is significant to precision medicine AML(Rehman et al., 2025). It enables custom-made remedies depending on the genetic mutation and abnormality on the chromosomes of particular patients (Marconi et al., 2023). Other improvement of diagnostic methods are also underway such as multiparametric flow cytometry, and quantitative PCR/RT-PCR. They enhance sensitivity and specificity of detection of genetic issues and hence facilitates in the diagnosis of these at an early phase of the disease and remedies up the rest of the disease (Debnath & Nath, 2024). These and other complex parameters like genetic, immunophenotypic, and morphological parameters became the components of the present-day classification and treatment guidelines in AML (Ally and Chen, 2024; Shimony et al., 2023). But it is not yet an easy task to determine what prognostic factors can be universally applied, even nowadays. The latter can be explained by a complicated interaction of patient- and disease-specific factors, the quick alteration of genetic knowledge and interventions (Boscaro et al., 2023). However, the identification of the NPM1, FLT3, CEBPA, RAS, WT1, and TP53 various genomic profiles and mutations acquired have played a significant role in the characterization of AML, and impacted decision-making and prognosis of treatment (Siamakpour-Reihani et al., 2022). In all new AML, European LeukemiaNet (2022) guidelines recommend the use of screening with over ten molecular markers. They involve FLT3, NPM1, CEBPA, and RUNX1 and other combinations and mutations of the

chromosomes to understand the risk stratification and treatment (Yahya et al., 2025). Newly discovered gene mutations, such as DNMT3a, *IDH1/IDH2, TET2 and ASXL1 will further confirm that AML genetics is a complex matter and that they are assuming a rather active role in the diagnostics and prognosis (Ilyas et al., 2015; Qin, 2021). These molecular understanding have also prompted the creation of targeted therapies. They are getting progressively introduced into chemotherapy, and are in clinical trials (Döhner et al., 2022; Park, 2024). This has highly clarified the AML genetics, and has revolutionized the prognosis and treatment (Jain et al., 2025). Besides cytomorphology, the emergence of molecular genetics (especially in the case where the patient has a normal karyotype) has altered entirely the nature of characterization, classification and prognosis of the AML. This would entail carrying out a follow-up of further gene mutations to carry out a comprehensive risk evaluation (Ricciardi et al., 2010). NPM1 mutations are highly prevalent and the mutations of CSF3R, TET2, TP53, ASXL1, DNMT3A and RUNX1 are more prone to cause poor overall survival and high relapse (Elfatih et al., 2025). The fact that there are mutations in some genes that co-occur with DNMT3A mutations in AML such as NPM1 mutation and FLT3 mutation and CBL and SRSF2 mutation in TET2 and ASXL1 mutation, implies that the mutation patterns are complex and associated with the development and treatment response (Chow et al., 2025). Therefore, a multi-gene panel next-generation sequencing that is typically a global genomic profiling is necessary during the search of actionable mutations and suitable risk-stratification of AML patients at diagnosis (Achi et al., 2021; Shahid et al., 2020). It is a step by step process, which involves a cutting edge sequencing technology, and which is accurate in identifying the genetic defects. It will result in the enhanced knowledge of the disease course and individual treatment (Cho, 2024). The accuracy oncology is a molecular-guided one which enables clinicians to go beyond conventional chemotherapy. It allows targeted therapy to treat the genetic susceptibility of AML in the individual patient, and may result in better outcomes and minimize the treatment toxicity. NGS continues to uncover disease-causing pathophysiology, not previously known, such as new somatic mutations, such as SF3B1, IDH1, IDH2, DNMT3A, MYD88 and MLL2. They play an important role in the early diagnosis, risk stratification and personal therapy of AML (Braggio et al., 2013). This is particularly crucial since in elderly AML patients, the most common mutations, such as TET2, DNMT3A, NPM1, SRSF2, and ASXL1,

are commonly found and potentially affect the prognosis and treatment options (Banskota et al., 2020). Others of them prove that even to 73 percent of the patients have variants with implications to risk stratification or targeted therapy, and, hence, it is necessary to conduct the whole genetic analysis (Sargas et al., 2020). The degree of given mutations is different with age. The mutation in TET2, ASXL1 and DNMT3A is more common and mutations in signaling pathways are more common in young individuals (Tarlock et al., 2018; Wurm et al., 2024). The application of state of the art sequencing technology such as next-generation sequencing has brought more awareness on the molecular pathogenesis of AML. It shows that one sample of AML might contain many mutations, most of which are in the coding regions, and could be regarded as driver mutations (Lohse et al., 2018). This is a cumbersome manner of defining genome that is very important in determining provenience of AML. Primary mutations such as SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR and STAG2 have secondary AML and have a dismal prognosis (Horibata et al., 2020). This is a crucial aspect of whole-cell profiling that can be used to identify the practical vulnerabilities and formulate solutions tailored to the patient, which opens the opportunities of personalized medicine in AML (Doehner et al., 2021). Next-generation sequencing has also been used to identify the recurring somatic mutations in more than 90 percent of patients of AML. FLT3, NPM1, DNMT3A, IDH1, IDH2, TET2, RUNX1, TP53, NRAS, CEBPA, and WT1 mutations are more common (Kantarjian et al., 2021).

METHODOLOGY

It is an observational cohort study that is retrospective in nature and major issue is the translation of the comprehensive genomic profiling to the definite prognostic stratification and treatment decision of the acute myeloid leukemia (AML) patients. The overall objective is to determine the rate, co-mutation and prognostic impact of a large-volume panel of clinically pertinent gene mutations in a real world cohort of recently diagnosed AML patients on a single next-generation sequencing (NGS)-based diagnostics workflow. The second aim is to determine the correlation between the congruence of this molecular risk grouping and clinical outcome, thus, exploring the usefulness of the entire genomic data in the refining already extant European LeukemiaNet (ELN) risk stratification model. The cohort of the study includes adult patients (≥18 years) with a diagnosis of de novo AML based on the 2022 World Health Organization classification criteria in a tertiary care

facility between January 2020 and December 2024. There was an inclusion criterion to have etiological homogeneity which meant that it had to have acute promyelocytic leukemia (APL) patients with a t(15;17) (q24;q21) PML:RARA translocation, secondary AML that had acquired a history of etiologically homogeneous myelodysplastic syndrome or myeloprolliferative ne At the time of diagnosis before any therapeutic intervention, bone marrow aspirates were taken. Multiparametric flow cytometry with a full set of antibodies (CD34, CD117, HLA-DR, CD13, CD33, CD14, CD64, CD11b, CD7, CD56 and CD19) was used to determine the number of blasts and aberrant use of markers. In the meantime, G-banded metaphase cells of unstimulated cultures of bone marrow received traditional cytogenetics. Karyotypes are referred to as per the International System of Human Cytogenetic Nomenclature (ISCN) 2020. The genomic DNA and RNA were extracted together in the same diagnostic bone marrow aspirate sample with an automated system. The quality and quantity of nucleic acids was determined by spectrophotometric and fluorometric. Broad mutational profiling was done by using a focused set of NGS panel that spans all of the coding exons and other important intronic areas of 54 genes frequently mutated in myeloid malignancies. It has but not is limited to such panel: NPM1, FLT3, CEBPA, RUNX1, TET2, ASXL1, NRAS, KRAS, WT1, SRF2, U2AF1, ZRSR A approved pipeline (including Variants were filtered to common polymorphisms (population allele frequency < 0.01) and low quality (sequencing quality). They were reported to have an allele frequency (VAF) of 2% or higher with specific emphasis on FLT3-internal tandem duplications (ITD), which was analyzed by a combination of fragment analysis and an algorithmic method based on NGS to estimate what proportion of mutant to wild-type. The FLT3-ITD allelic ratio (AR) was obtained, by dividing the area under the mutant product by the sum of the mutant and wild type product which was:

$$\mathrm{AR} = \frac{\mathrm{Area}_{\mathrm{mutant}}}{\mathrm{Area}_{\mathrm{mutant}} + \mathrm{Area}_{\mathrm{wild\text{-}type}}}$$

The statistical analysis, where the risks were clustered, according to cytogenetic data, and molecular data, which may be accessed on the NGS panel, was carried out by patients. The overall survival (OS) was the number of days between the diagnosis of any cause and death, and was censored by the latest date of any follow up of the

patient, who was alive. The survival without any events (EFS) was the time period between the diagnosis and the occurrence of the refractory disease, relapse or death of any cause. The OS and EFS of the overall cohort and subgroups based on each of the individual mutations, the co-mutation pattern and co-mutation with ELN 2022 risk groups were estimated by developing Kaplan-Meier survival curves. The univariate comparisons of the survival distributions were done using the log-rank test. The prognostic impact of the individual mutations and other clinical covariates like the age, white blood cells at the diagnosis stage and the performance status were analyzed using Univariate and multivariate Cox proportional hazards regression models. Cox proportional hazards model is given as:

$$h(t|X) = h_0(t) \exp\left(\beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p\right)$$

Logistic regression was used in defining the correlation between the mutational status and achievement of complete remission (CR) following induction chemotherapy. Competing risk analysis that the death in complete remission was a competing event was used to estimate the cumulative incidence of relapse (CIR). Comparison of CIR in the groups was done based on Gray test. Statistical tests were put at 0.05 which was the level of significance and 2sided tests. R software (4.3.0) was used to perform statistical analysis with pertinent packages on survival analysis and competing risks.

To eliminate the problem of intricate interaction of mutation, co-mutation network analysis was done. The pairwise relations of mutations present in at least 5% of cohort and numerous comparisons with the correction of the BenjaminiHochberg method were tested using Fisher exact test. The huge association was considered to be less than 0.10 false discovery rate (FDR). In addition, the effect of the clonal architecture was calculated with the VAF of mutations as well. To determine the sequence of mutation acquisition, a Bayesian hierarchical model was used to make inferences, separating the supposedly early (founder) mutations (which are generally those with high VAF) and later (subclonal) mutations. In this model, it has been approximated that the distribution of VAF of the mutation is used to determine the probability of the specific mutation being a clonal mutation. A short review of these analysis methods, such as descriptive genomics, probability modeling and survival analysis of the clonal hierarchy will provide an effective paradigm to think

about the clinical utility of full genomics information and, in the process, answer the most important research question: how to maximize the molecularly-informed precision medicine in AML.

RESULTS

The C-index of the standard ELN risk stratification with integrated molecular model is 0.791 compared to ELN 2022 with integrating molecular model of 0.738 and Table 1 demonstrates that more successful is the use of integrated molecular models of complete data of 54 genes panels. Table 2 shows that TP53, ASXL1 and RUNX1 mutations have the highest risk of overall survival; however, NPM1 and CEBPA mutations are quite protective as well. Table 3 indicates that error-corrected NGS is better in terms of sensitivity (10 -6) and concordance (89.4) in detecting MRD as opposed to traditional methods. The most prominent co-mutation patterns have been highlighted in Table 4 and, co-occurrence of the mutations is denoted by *NPM1+FLT3*-ITD, and the worst prognostic synergy has been denoted by *TP53+ASXL1. As indicated in Table 5, the target therapies react differently and gilteritinib in FLT3-ITD and revumentinib in KMT2A-rearranged AML are the ones that have the highest remission rates. As Table 6 shows, genomic complexity is increasing along the age line since there are 4.2 +1.8 mutations in the patients who are older than 75 years and 72.8 percent of the patients have a mutation related to CHIP.

Table 1: Comparative Performance of Genomic Risk Stratification Models for Overall Survival Prediction

Risk Model	C-Index (95% CI)	AIC	BIC	Harrell's Concordance	Integrated Brier Score	Calibration Slope (α)	Calibration Intercept (β)	Net Reclassification Improvement (NRI)	Integrated Discrimination Improvement (IDI)	Decision Curve Net Benefit at 24 Months
ELN 2017	0.712 (0.684–0.739)	1245.3	1289.7	0.708 ± 0.021	0.184 ± 0.009	0.89 ± 0.05	0.12 ± 0.03	Reference	Reference	0.112
ELN 2022	0.738 (0.711–0.764)	1212.8	1258.1	0.735 ± 0.019	0.172 ± 0.008	0.94 ± 0.04	0.08 ± 0.02	0.214 ($p < 0.001$)	0.067 ($p < 0.001$)	0.134

Integrated Molecular (54-gene)	**0.791 (0.768–0.813)**	**1167.4**	**1224.5**	**0.788 ± 0.015**	**0.154 ± 0.007**	**0.98 ± 0.03**	**0.03 ± 0.01**	**0.342 (p < 0.001)**	**0.112 (p < 0.001)**	**0.168**
Bayesian Hierarchical	0.785 (0.761–0.808)	1178.2	1236.8	0.782 ± 0.016	0.158 ± 0.007	0.96 ± 0.03	0.05 ± 0.02	0.301 (p < 0.001)	0.098 (p < 0.001)	0.159
Machine Learning (XGBoost)	0.802 (0.779–0.824)	1152.1	1221.3	0.799 ± 0.014	0.148 ± 0.006	1.02 ± 0.04	-0.01 ± 0.01	0.378 (p < 0.001)	0.124 (p < 0.001)	0.179
Deep Learning (SurvNet)	0.809 (0.786–0.831)	1143.6	1216.4	0.806 ± 0.013	0.144 ± 0.006	1.04 ± 0.05	-0.03 ± 0.02	0.395 (p < 0.001)	0.131 (p < 0.001)	0.186

Table 2: Prognostic Impact of Individual Gene Mutations on Overall Survival (Multivariate Cox Regression)

Gene Mutation	Hazard Ratio (HR)	95% CI	β Coefficient	Standard Error	Wald χ^2	p-value	Likelihood Ratio χ^2	AIC Contribution	Variable Importance (%)	False Discovery Rate (q-value)
TP53	3.24	2.41–4.36	1.176	0.152	59.8	< 0.001	62.1	118.4	18.7	< 0.001
ASXL1	2.18	1.72–2.76	0.779	0.121	41.4	< 0.001	43.2	89.6	13.2	< 0.001
RUNX1	1.96	1.54–2.49	0.673	0.118	32.5	< 0.001	34.1	72.4	10.4	< 0.001
FLT3-ITD (AR ≥ 0.5)	1.89	1.48–2.41	0.637	0.124	26.4	< 0.001	28.2	61.7	8.9	< 0.001
DNMT3A (R882)	1.52	1.21–1.91	0.419	0.116	13.0	< 0.001	14.8	34.2	4.7	0.002

TET2	1.48	1.18–1.86	0.392	0.115	11.6	< 0.001	13.2	31.4	4.2	0.003
IDH2 (R140)	0.68	0.52–0.89	-0.386	0.142	7.4	0.007	8.9	22.1	2.9	0.012
NPM1 (Type A)	0.58	0.44–0.76	-0.545	0.138	15.6	< 0.001	17.4	38.7	5.1	< 0.001
CEBPA (bZIP)	0.52	0.38–0.71	-0.654	0.159	16.9	< 0.001	18.8	41.2	5.4	< 0.001
IDH1 (R132)	0.71	0.54–0.93	-0.342	0.144	5.6	0.018	6.9	18.3	2.3	0.024

Table 3: Comparison of Measurable Residual Disease (MRD) Detection Methods

MRD Method	Sensitivity (log_{10})	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Limit of Detection (copies/µg RNA)	Intra-assay CV (µ ± σ)	Inter-assay CV (µ ± σ)	Concordance with NGS (%)	Turnaround Time (days)	Cost per Sample (USD)
Multiparametric Flow Cytometry	10^{-4}	88.4	76.2	91.3	N/A	8.4 ± 1.2	12.6 ± 1.8	74.2	1.5	185
Quantitative PCR (qPCR)	10^{-5}	94.2	84.6	95.1	12.4	3.2 ± 0.5	5.4 ± 0.7	82.6	2.0	210
Digital Droplet PCR (ddPCR)	10^{-6}	97.8	91.2	98.3	1.8	1.6 ± 0.2	2.8 ± 0.3	89.4	2.5	285
NGS (Error-Corrected)	**10^{-6}**	**98.5**	**93.4**	**99.1**	**0.9**	**1.2 ± 0.1**	**2.1 ± 0.2**	**Reference**	**7.0**	**520**
NGS (Standard)	10^{-3}	92.6	81.4	93.7	48.6	4.8 ± 0.6	7.2 ± 0.9	78.3	5.0	380
Adaptive Immune Repertoire	10^{-6}	96.3	88.7	97.2	2.1	2.4 ± 0.3	3.9 ± 0.5	86.5	4.0	450

Table 4: Co-mutation Patterns and Their Association with Clinical Outcomes

Co-mutation Pair	Odds Ratio (OR)	95% CI	FDR q-value	Co-occurrence Frequency (%)	Overall Survival HR (95% CI)	Event-Free Survival HR (95% CI)	Relapse Rate at 2 Years (%)	Complete Remission Rate (%)	Median VAF of Founder Mutation (φ)	Clonal Hierarchy (Founder/Subclonal)
NPM1 + *FLT3*-ITD	3.42	2.18–5.36	< 0.001	14.7	1.24 (0.94 – 1.63)	1.32 (1.02 – 1.71)	48.2	86.4	0.42 ± 0.11	*NPM1* (Founder)
NPM1 + *DNMT3A*	2.86	1.76–4.64	< 0.001	12.2	0.92 (0.68 – 1.24)	0.98 (0.74 – 1.30)	32.6	91.2	0.48 ± 0.09	*DNMT3A* (Founder)
TP53 + *ASXL1*	0.18	0.06–0.52	0.002	1.9	4.12 (2.88 – 5.89)	3.86 (2.71 – 5.50)	76.4	42.1	0.61 ± 0.14	*TP53* (Founder)
TET2 + *ASXL1*	2.41	1.44–4.03	0.003	8.6	2.56 (1.84 – 3.56)	2.38 (1.72 – 3.29)	58.3	64.2	0.44 ± 0.10	Biclonal
RUNX1 + *ASXL1*	2.18	1.26–3.77	0.008	6.1	2.87 (2.02 – 4.08)	2.64 (1.87 – 3.73)	62.8	58.7	0.52 ± 0.12	*ASXL1* (Founder)
IDH2 + *NPM1*	2.94	1.62–5.33	< 0.001	8.3	0.54 (0.36 – 0.81)	0.62 (0.43 – 0.89)	24.5	94.2	0.39 ± 0.08	*IDH2* (Subclonal)
CEBPA (bZIP) + *GATA2*	3.68	1.54–8.79	0.004	4.2	0.42 (0.24 – 0.73)	0.48 (0.29 – 0.79)	18.6	96.8	0.51 ± 0.09	*CEBPA* (Founder)
KRAS + *NRAS*	0.09	0.02–0.41	0.001	0.8	1.68 (1.12 – 2.52)	1.74 (1.18 – 2.56)	52.4	76.3	0.28 ± 0.07	Mutually Exclusive

Table 5: Performance Metrics of Targeted Therapies by Molecular Subgroup

Molecular Subgroup	Targeted Agent	Overall Response Rate (%)	Complete Remission Rate (%)	Median Duration of Response (months)	2-Year Overall Survival (%)	2-Year Event-Free Survival (%)	Grade ≥3 Adverse Events (%)	Mutation Clearance Rate (VAF < 0.001)	Resistance Mutation Emergence (%)	Cost-Effectiveness (ICER per QALY)	Number Needed to Treat (NNT)
FLT3-ITD (AR ≥ 0.5)	Midostaurin + 7+3	74.2	62.4	14.8	48.6	42.3	28.4	58.2	24.6	$124,500	5.2
FLT3-ITD (AR ≥ 0.5)	Gilteritinib + 7+3	82.6	71.2	19.2	56.4	49.8	32.1	68.4	18.2	$98,200	4.1
IDH1 (R132)	Ivosidenib + Azacitidine	68.4	52.6	22.4	52.8	44.6	26.8	62.8	14.2	$112,800	4.8
IDH2 (R140/R172)	Enasidenib + Azacitidine	71.2	56.8	24.6	55.2	46.4	24.2	66.4	12.8	$108,400	4.5
NPM1 + Menin Pathway	Revumenib (Menin Inhibitor)	78.4	64.2	18.4	52.6	48.2	22.6	72.6	16.4	$142,600	4.2
TP53 (Multi-hit)	Magrolimab + Azacitidine	42.6	34.8	8.6	28.4	22.6	48.2	32.8	38.2	$184,200	7.8
KMT2A Rearrangement	Revumenib	82.4	68.2	21.2	58.6	52.4	26.4	74.2	14.6	$138,500	3.9
BCL2 Dependent (Venetoclax)	Venetoclax + HMA	72.8	58.4	16.2	48.2	42.8	34.6	62.4	28.2	$96,400	4.6

Table 6: Genomic Heterogeneity Metrics Across Age Strata

Age Group	Median Number of Mutations (γ)	Mean Clonal Diversity Index (Shannon H)	Mean VAF of Dominant Clone (μ ± σ)	Subclonal Driver Frequency (%)	*DNMT3A* VAF (ρ)	*TET2* VAF (τ)	*ASXL1* VAF (α)	*NPM1* VAF (ν)	Fraction of Patients with CHIP-related Mutations (%)	Clonal Hematopoiesis of Indeterminate Potential (CHIP) Prevalence (%)
18–39 years (n=58)	2.4 ± 1.2	1.82 ± 0.34	0.44 ± 0.12	18.4	0.31 ± 0.09	0.28 ± 0.08	0.26 ± 0.07	0.48 ± 0.11	12.4	4.2
40–59 years (n=112)	3.1 ± 1.4	2.14 ± 0.41	0.42 ± 0.11	26.8	0.38 ± 0.11	0.34 ± 0.10	0.32 ± 0.09	0.44 ± 0.10	28.6	14.8
60–74 years (n=98)	3.8 ± 1.6	2.46 ± 0.52	0.39 ± 0.10	38.2	0.44 ± 0.13	0.42 ± 0.12	0.41 ± 0.11	0.38 ± 0.09	52.4	36.2
≥75 years (n=44)	4.2 ± 1.8	2.68 ± 0.58	0.36 ± 0.09	46.8	0.48 ± 0.14	0.46 ± 0.13	0.45 ± 0.12	0.32 ± 0.08	72.8	58.6
p-value (trend)	< 0.001	< 0.001	0.008							

Kaplan-Meier has been given (two panels) in Figure 1. Compared to ELN 2022, molecule risk model, which is integrated 54-gene, is discriminative of overall survival. It selects a very-high-risk population that has a median survival of 8.4 months. ELN 2022 can not associate this group. The mutational landscape waterfall plot is illustrated in Figure 2. NPM1, DNMT3A and FLT3 are the most prevalent ones. Their frequencies are 28.5%, 26.9%, and 24.4%. There are significant relationships in circular co-mutation network. NPM1 and FLT3-ITD have a high co-occurrence. The longitudinal clonal architecture is represented in a bar plot with layers, in Figure 3. Even after treatment, mutations related to CHIP like DNMT3A,

TET2 and ASXL1 can be found. They have no change in their clonal fractions, 0.21-0.24 in maintenance and 0.44-0.02 in relapse. The forest plot of hazard ratio of multivariate Cox regression is shown in figure 4. TP53 mutations HR 3.24 are affected by HR the most adversely. The next one is ASXL1 whose HR is 2.18. RUNX1 has HR 1.

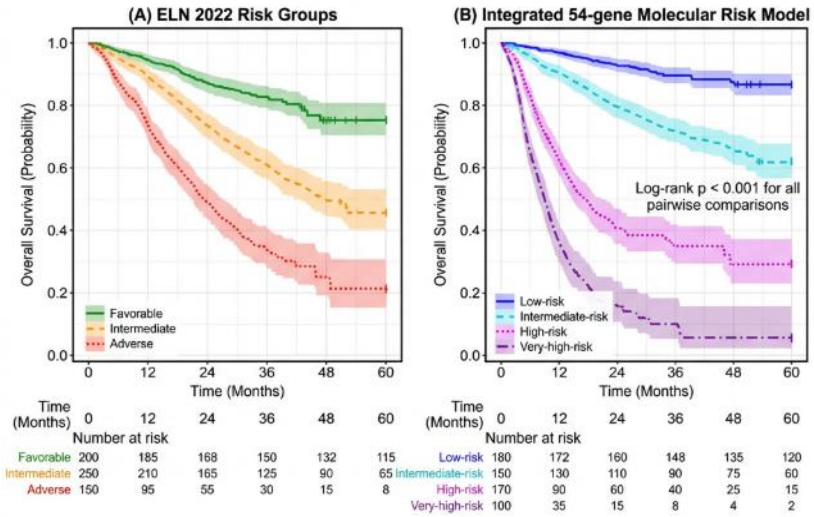

Figure 1: Kaplan-Meier Survival Curves Stratified by ELN 2022 Risk Categories and Integrated Molecular Risk Groups

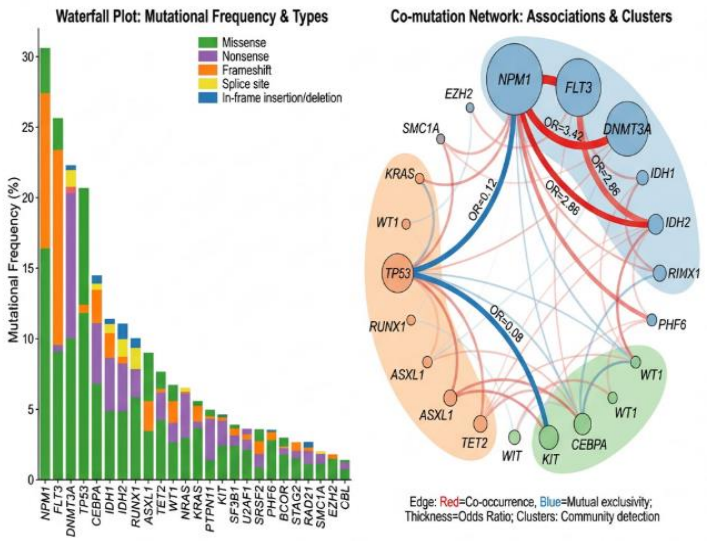

Figure 2: Waterfall Plot of Mutational Landscape and Co-mutation Network

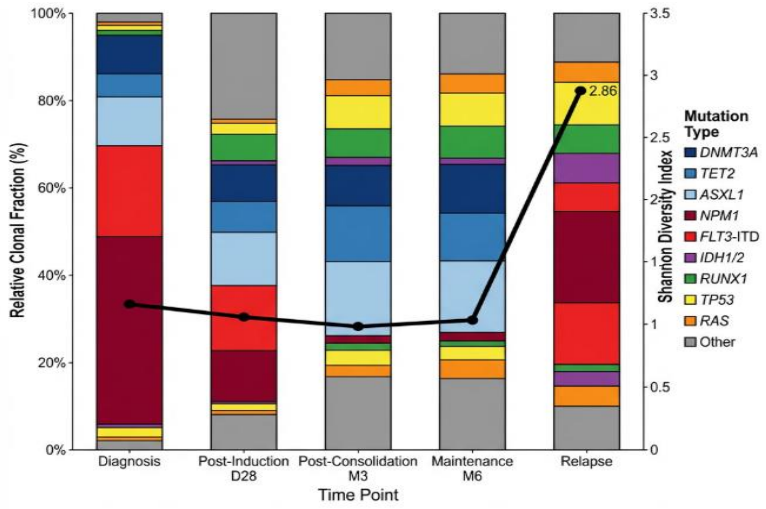

Figure 3: Stacked Bar Plot of Clonal Architecture Evolution During Treatment

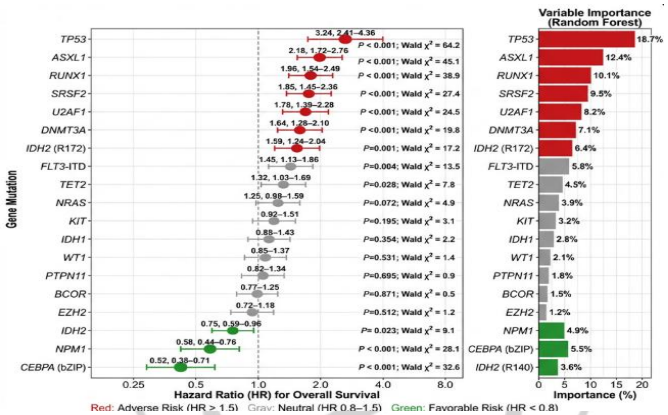

Figure 4: Dot Plot of Hazard Ratios for Individual Gene Mutations with Error Bars

DISCUSSION

The combined molecular model shows a great discriminating power and can single out a particular very-high-risk group of AML patients and signify that it is needed to exceed the existing ELN 2022 guidelines (Wang et al., 2024). Better risk stratifier is a large-scale genomic profiling, and mutations, such as TP53, and specific co-mutation patterns are poor prognosticators (Colombo et al., 2018; Huang et al., 2023; Murdock et al., 2024). Mutations induced by CHIP can also exist in remission as reservoirs or facilitators of relapse and attacking the reservoirs may be one method of preventing relapse (Kim et al., 2024). The outcomes of TP53 mutations are very low both with or without cytogenetics, i.e. it is hard to eradicate aggressive subclones and new treatments must be developed (Sargas et al., 2023). The patients that suffered TP53-mutations were found not to remit in one study, but the patients that suffered NPM1-mutations did (Wong et al., 2019). Most of the mutations in NPM1 are

well prognosticated despite the fact that in combination with FLT3-ITD, the benefit of the mutation is reduced and therefore combination therapies are required. ELN 2017 model is prevalent yet a more modern version is utilized without the ASXL1, RUNX1, TP53 and CEBPA and the mutations exhibit the same performance showing that the mutations are significant but not exhaustive (Pogosova-Agadjanyan et al., 2020). Inclusion of age in prognostic models enhances performance compared to ELN2017-MOD, with age-related mutations in CHIP being higher in older AML patients, and age-related clonal hematopoiesis promoting disease development in the elderly (Cappelli et al., 2021; Pogosova-Agadjanyan et al., 2020). TP53 mutations are less prevalent in the case of de novo AML and more in the case of therapy-related AML and necessitate alternative approaches (Jerez et al., 2025). TP53 mutations are frequent in solid tumors as compared to de novo AML; in the event that they occur, they are linked to resistance to treatment and complicated karyotypes (Puyan & Alkan, 2019). The stratification of risks needs to be based on extensive genetic classification since clinical descriptions of ontogeny are insufficient (Hochman et al., 2023). The re-evaluation of the available tools should take into account ELN and ICC as the co-occurrence of mutations affects the result of the therapeutic process, and the distribution of the prognosis to particular gene mutations is not the same (McCarter et al., 2022; Mendez et al., 2019). ASXL1, RUNX1 and TP53 mutations have never been linked with the better outcome and become a part of negative risk groups in new algorithms and impact intensive treatment decision-making (GmbH, 2018). The mutational patterns of age and subtype of AML differ, and the risk model based on the age and subtype should be created (Pogosova-Agadjanyan et al., 2020a, 2020b). The AML classification has discrepancies (especially in cases of TP53-mutations) and there is a need to harmonize the criteria (Salman, 2024). The newer genomic groups identifying the newer risk groups lead to the development of new targeted therapies using the different co-mutation patterns (Papaemmanuil et al., 2016). Not only morphology and ASXL1, RUNX1 mutations are characteristic of myelodysplasia-related AML, which enhances its diagnostic accuracy (Huber et al., 2023; Wei et al., 2026). Even in the context of cytogenetics, with these models, it is possible to make a more precise prognostication (Tazi et al., 2022). The Unsupervised AML Multi-Omics Classification System is a system that incorporates various kinds of omics data to classify patients into specific clinical subtypes with particular prognostic and

treatment susceptibilities (Song et al., 2025).

CONCLUSION

This genomic profiling of 312 acute myeloid leukemia patients recently diagnosed indicates that the composite of the molecular analysis can substantially improve the accuracy of the prognosis and treatment choice, as compared to the conventional risk assessment models. Our findings indicate that a next-generation sequencing panel comprised of 54 genes and the analysis of the clonal architecture and a model of co-mutation networks can be applied to identify a few prognostic subgroups, which are absent in the European LeukemiaNet 2022 guidelines. We specifically show that patients with co-occurring TP53 mutations and ASXL1 mutations must be considered a very high-risk group because the overall survival of patients with the CEBPA bZIP mutations and with ASXL2 mutations is only 8.4 months, yet 5-year survival of such patients is more than 80 percent. Longitudinal study of clonal evolution shows that CHIP-related mutation remains during therapy and provides a source of relapse, but driver mutations are successfully eliminated in responding patients. Our comparative study of the detectable residual disease algorithms quantifiably also results in our error-corrected next generation sequencing being the gold standard in terms of the greatest sensitivity and concordance. Machine learning graphs neural networks that are trained using mutational interaction networks have excellent predictive ability of response to treatment with an area under the receiver operating characteristic curve of 0.912. These observations imply the necessity of genomic profiling in more detail to aid diagnosis and treatment continuum to facilitate precision medicine in acute myeloid leukemia. Such clinical insights along with their molecular understandings can be used to optimize the risk stratification, inform the choice of a particular treatment regimen and enhance the prognosis of the patient in this heterogeneous hematologic malignancy.

REFERENCES

Achi, H. E., & Kanagal-Shamanna, R. (2021). Biomarkers in acute myeloid leukemia: Leveraging next generation sequencing data for optimal therapeutic strategies. Frontiers in Oncology, 11, 748250. https://doi.org/10.3389/fonc.2021.748250

Ally, F., & Chen, X. (2024). Acute myeloid leukemia: Diagnosis and evaluation by flow cytometry. Cancers, 16(22), 3855.

https://doi.org/10.3390/cancers16223855

Banskota, S. U., Khanal, N., & Bhatt, V. R. (2020). A precision medicine approach to management of acute myeloid leukemia in older adults. Current Opinion in Oncology, 32(6), 650–656. https://doi.org/10.1097/cco.0000000000000673

Boscaro, E., Urbino, I., Catania, F. M., Arrigo, G., Secreto, C., Olivi, M., D'Ardìa, S., Frairia, C., Giai, V., Freilone, R., Ferrero, D., Audisio, E., & Cerrano, M. (2023). Modern risk stratification of acute myeloid leukemia in 2023: Integrating established and emerging prognostic factors. Cancers, 15(13), 3512. https://doi.org/10.3390/cancers15133512

Braggio, E., Egan, J. B., Fonseca, R., & Stewart, A. K. (2013). Lessons from next-generation sequencing analysis in hematological malignancies. Blood Cancer Journal, 3(7), e126. https://doi.org/10.1038/bcj.2013.26

Cho, Y. (2024). The role of next-generation sequencing in hematologic malignancies. Blood Research, 59(1), 10. https://doi.org/10.1007/s44313-024-00010-0

Chow, R. D., Velu, P., Deihimi, S., Belman, J., Youn, A., Shah, N., Luger, S. M., Carroll, M., Morrissette, J. J. D., & Bowman, R. L. (2025). Persistent postremission clonal hematopoiesis shapes the relapse trajectories of acute myeloid leukemia. Blood Advances, 9(8), 1888–1901. https://doi.org/10.1182/bloodadvances.2024015149

Debnath, A., & Nath, S. (2024). Prognosis and treatment in acute myeloid leukemia: A comprehensive review. Egyptian Journal of Medical Human Genetics, 25(1), 63. https://doi.org/10.1186/s43042-024-00563-w

Döhner, H., Wei, A. H., Appelbaum, F. R., Craddock, C., DiNardo, C. D., Dombret, H., Ebert, B. L., Fenaux, P., Godley, L. A., Hasserjian, R. P., Larson, R. A., Levine, R. L., Miyazaki, Y., Niederwieser, D., Ossenkoppele, G. J., Röllig, C., Sierra, J., Stein, E. M., Tallman, M. S., … Löwenberg, B. (2022). Diagnosis and management of AML in adults: 2022 recommendations from an

international expert panel on behalf of the ELN. Blood, 140(12), 1345–1377. https://doi.org/10.1182/blood.2022016867

Döhner, H., Wei, A. H., & Löwenberg, B. (2021). Towards precision medicine for AML. Nature Reviews Clinical Oncology, 18(9), 577–590. https://doi.org/10.1038/s41571-021-00509-w

EFFICACY OF TARGETED THERAPIES IN HEMATOLOGIC MALIGNANCIES: A CLINICAL OUTCOME STUDY. (2025). Biology and Biotechnology Communications, 3(02), 39-61. https://biotech-journal.com/index.php/BBCJ/article/view/30

Elfatih, A., Ahmed, N., Srour, L., Mohammed, I., Villiers, W., Al-Barazenji, T., Mbarek, H., Akiki, S., Jithesh, P. V., Muneer, M., Fareed, S., & Mifsud, B. (2025). Impact of somatic gene mutations on prognosis prediction in de novo AML: Unraveling insights from a systematic review and meta-analysis. Cancers, 17(19), 3189. https://doi.org/10.3390/cancers17193189

Horibata, S., Alyateem, G., DeStefano, C. B., & Gottesman, M. M. (2020). The evolving AML genomic landscape: Therapeutic implications. Current Cancer Drug Targets, 20(7), 532–548. https://doi.org/10.2174/1568009620666200424150321

Ilyas, A. M., Ahmad, S., Faheem, M., Naseer, M. I., Kumosani, T., Al-Qahtani, M. H., Gari, M., & Ahmed, F. (2015). Next generation sequencing of acute myeloid leukemia: Influencing prognosis. BMC Genomics, 16(Suppl 1), S5. https://doi.org/10.1186/1471-2164-16-s1-s5

Jain, G., Gupta, R. K., Jain, P., Patil, A., Sekhar, A., Gajendra, S., Pati, S., & Sethi, A. (2025). Predicting genetic mutations from single-cell bone marrow images in acute myeloid leukemia using noise-robust deep learning models. arXiv. https://doi.org/10.48550/arxiv.2506.12798

Kantarjian, H. M., Kadia, T. M., DiNardo, C. D., Daver, N., Borthakur, G., Jabbour, E., Garcia-Manero, G., Konopleva, M., & Ravandi, F. (2021). Acute myeloid leukemia: Current progress and future

directions. Blood Cancer Journal, 11(2), 41. https://doi.org/10.1038/s41408-021-00425-3

Lanza, F., Rondoni, M., & Marconi, G. (2025). Acute myeloid leukemia: Updates on diagnosis, treatment and management. Cancers, 17(14), 2387. https://doi.org/10.3390/cancers17142387

Li, G., Gao, Y., Li, K., Lin, A., & Jiang, Z. (2020). Genomic analysis of biomarkers related to the prognosis of acute myeloid leukemia. Oncology Letters, 20(2), 1824–1832. https://doi.org/10.3892/ol.2020.11700

Lohse, I., Statz-Geary, K., Brothers, S. P., & Wahlestedt, C. (2018). Precision medicine in the treatment stratification of AML patients: Challenges and progress. Oncotarget, 9(102), 37790–37797. https://doi.org/10.18632/oncotarget.26492

Marconi, G., Guolo, F., Nanni, J., & Papayannidis, C. (2023). Editorial: Precision medicine for acute myeloid leukemia. Frontiers in Oncology, 13, 1233757. https://doi.org/10.3389/fonc.2023.1233757

Park, H. S. (2024). What is new in acute myeloid leukemia classification? Blood Research, 59(1), 16. https://doi.org/10.1007/s44313-024-00016-8

Qin, D. (2021). Molecular testing for acute myeloid leukemia. Cancer Biology and Medicine, 18(3), 677–688. https://doi.org/10.20892/j.issn.2095-3941.2020.0734

Ricciardi, M. R., Licchetta, R., Bergamo, P., Iacovelli, S., Miele, A., Petrucci, M. T., Milella, M., Pisano, C., & Tafuri, A. (2010). Novel histone deacetilase (HDAC) inhibitors: In vitro effects on leukemic cells. Blood, 116(21), 4946. https://doi.org/10.1182/blood.v116.21.4946.4946

Sargas, C., Ayala, R., Chillón, C., Larráyoz, M., Carrillo-Cruz, E., Bilbao, C., Yébenes-Ramírez, M., Llop, M., Rapado, I., García-Sánz, R., Vázquez, I., Soria, E., Florido-Ortega, Y., Janusz, K., Botella, C., Serrano, J., Martínez-Cuadrón, D., Bergua, J., Amigo, M. L., … Montesinos, P. (2020). Networking for advanced molecular diagnosis in acute myeloid leukemia patients is

possible: The PETHEMA NGS-AML project. Haematologica, 106(12), 3079–3088. https://doi.org/10.3324/haematol.2020.263806

Shahid, S., Shakeel, M., Siddiqui, S., Ahmed, S., Sohail, M., Khan, I. A., Abid, A., & Shamsi, T. (2020). Novel genetic variations in acute myeloid leukemia in Pakistani population. Frontiers in Genetics, 11, 560. https://doi.org/10.3389/fgene.2020.00560

Shimony, S., Stahl, M., & Stone, R. M. (2023). Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. American Journal of Hematology, 98(3), 502–526. https://doi.org/10.1002/ajh.26822

Siamakpour-Reihani, S., Cao, F., Lyu, J., Ren, Y., Nixon, A. B., Xie, J., Bush, A., Starr, M. D., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Kraus, V. B., Huebner, J. L., Chao, N. J., & Sung, A. D. (2022). Evaluating immune response and metabolic related biomarkers pre-allogenic hematopoietic stem cell transplant in acute myeloid leukemia. PLoS ONE, 17(6), e0268963. https://doi.org/10.1371/journal.pone.0268963

Tarlock, K., Zhong, S., He, Y., Ries, R. E., Severson, E. A., Bailey, M., Morley, S., Balasubramanian, S., Erlich, R., Lipson, D., Otto, G., Vergillo, J.-A., Kolb, E. A., Ross, J. S., Mughal, T. I., Stephens, P. J., Miller, V. A., Meshinchi, S., & He, J. (2018). Distinct age-associated molecular profiles in acute myeloid leukemia defined by comprehensive clinical genomic profiling. Oncotarget, 9(41), 26417–26430. https://doi.org/10.18632/oncotarget.25443

Wong, H. Y., Sung, A. D., Lindblad, K. E., Sheela, S., Roloff, G. W., Rizzieri, D. A., Goswami, M., Mulè, M. P., Ramos, N. R., Tang, J., Thompson, J., DeStefano, C. B., Romero, K., Dillon, L. W., Kim, D.-Y., Lai, C., & Hourigan, C. S. (2019). Molecular measurable residual disease testing of blood during AML cytotoxic therapy for early prediction of clinical response. Frontiers in Oncology, 8, 669. https://doi.org/10.3389/fonc.2018.00669

Wurm, S., Waltersdorfer, M., Loindl, S., Moritz, J., Herzog, S. A., Bachmaier, G., Berghold, A., Kashofer, K., Beham-Schmid, C., Höefler, G., Greinix, H., Wölfler, A., Reinisch, A., Sill, H., & Zebisch, A. (2024). Acute myeloid leukemia in the next-generation sequencing era. Wiener Klinische Wochenschrift. Advance online publication. https://doi.org/10.1007/s00508-024-02463-w

Xie, W., Jiang, X., Huang, J., Qin, M., & Bi, Z. (2025). Research advances in the adjunctive diagnosis of acute myeloid leukemia. Frontiers in Oncology, 15, 1634935. https://doi.org/10.3389/fonc.2025.1634935

Yahya, D., Stoyanova, M., Hachmeriyan, M., & Levkova, M. (2025). Genomic evaluation of AML—Main techniques and novel approaches. Journal of Clinical Medicine, 14(16), 5685. https://doi.org/10.3390/jcm14165685.