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Clinical Implications of Molecular markers in Acute Myeloid Leukemia- A Single Centre Prospective Study

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ABSTRACT

Background: Acute myeloid leukemia (AML) represents a heterogeneous group of hematologic malignancies where molecular markers play an increasingly crucial role in risk stratification and treatment decisions. This study evaluated the clinical implications of molecular markers in AML patients treated with standard induction chemotherapy.

Methods: This prospective observational study enrolled 44 adult AML patients treated with standard 3+7 induction regimen over 18 months. Comprehensive molecular profiling including FLT3, NPM1, CEBPA, and other mutations was performed at diagnosis. Post-induction bone marrow assessment and minimal residual disease (MRD) evaluation were conducted. Clinical outcomes including complete remission rates, duration of hospitalization, and survival were analyzed in relation to molecular markers.

Results: Among 44 patients (median age 30 years, 56.8% males), molecular mutations were detected in 56.8% of cases. NPM1 mutations (20.4%) and FLT3 mutations (15.9%) were most frequent. Complete remission was achieved in 72.1% of patients, with median time to remission of 27 days. Patients with favorable-risk markers (27.3%) showed superior remission rates compared to high-risk patients (20.5%). Infectious complications occurred in 63.6% of patients, with 40.9% requiring ICU admission. The presence of FLT3-ITD was associated with lower remission rates and increased treatment-related toxicity.

Conclusion: Molecular markers significantly influence treatment outcomes in AML. Integration of comprehensive molecular profiling at diagnosis enables risk-adapted treatment strategies and improves prognostication. Early MRD assessment post-induction provides valuable information for subsequent treatment decisions.

KEYWORDS: *Study Population and Sample Size, Baseline Assessment and Molecular Profiling*.

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1. INTRODUCTION

Acute myeloid leukemia (AML) represents a biologically and clinically heterogeneous group of aggressive hematologic malignancies characterized by clonal expansion of myeloid blasts in the bone marrow and peripheral blood[1]. The disease accounts for approximately 80% of acute leukemias in adults, with an age-adjusted incidence of 4.3 per 100,000 population annually. Despite significant advances in our understanding of AML pathogenesis and the development of novel therapeutic approaches, the overall five-year survival rate remains suboptimal at approximately 30% for adults under 60

years and less than 10% for those over 60 years[2].

The landscape of AML diagnosis and management has undergone a paradigm shift over the past two decades, driven primarily by the identification of recurrent genetic abnormalities that not only define disease biology but also serve as powerful prognostic and predictive biomarkers. The evolution from morphology-based classification to molecular-driven risk stratification has fundamentally transformed how clinicians approach this disease. The 2016 World Health Organization (WHO) classification and its subsequent 2022 revision reflect this transformation, incorporating specific molecular markers as defining features of distinct AML entities[3].

Cytogenetic analysis has traditionally served as the cornerstone of risk stratification in AML, dividing patients into favorable, intermediate, and adverse risk categories based on chromosomal abnormalities. However, approximately 45-50% of AML patients present with normal karyotype (NK-AML), previously considered intermediate risk, creating a significant prognostic uncertainty. The advent of next-generation sequencing technologies has revealed that these cytogenetically normal cases harbor a complex landscape of molecular mutations, with studies demonstrating that somatic mutations can be identified in over 97% of AML cases when comprehensive genomic profiling is performed[4].

Among the molecular markers that have gained clinical prominence, mutations in FMS-like tyrosine kinase 3 (FLT3), nucleophosmin 1 (NPM1), CCAAT/enhancer-binding protein alpha (CEBPA), and runt-related transcription factor 1 (RUNX1) have emerged as critical determinants of prognosis and treatment selection. FLT3 mutations, particularly internal tandem duplications (FLT3-ITD), occur in approximately 30% of AML cases and are associated with increased relapse risk and inferior overall survival. The prognostic impact of FLT3-ITD is further modified by the allelic ratio, with higher ratios conferring worse outcomes[5]. Conversely, NPM1 mutations, found in approximately 30-35% of AML cases and 50-60% of NK-AML, generally confer a favorable prognosis, particularly in the absence of FLT3-ITD. The interplay between NPM1 and FLT3 mutations creates distinct prognostic subgroups that have been incorporated into contemporary risk stratification systems[6].

The clinical implementation of molecular markers extends beyond initial risk assessment. The European LeukemiaNet (ELN) 2022 recommendations have refined risk stratification by incorporating not only the presence of specific mutations but also their co-occurrence patterns and variant allele frequencies. Furthermore, the identification of targetable mutations has ushered in an era of precision medicine in AML, with the approval of FLT3 inhibitors such as midostaurin and gilteritinib, and IDH1/2 inhibitors like ivosidenib and enasidenib, fundamentally changing the treatment landscape[7].

The assessment of measurable residual disease (MRD) represents another critical advancement in AML management where molecular markers play an essential role. MRD detection using multiparameter flow cytometry or molecular techniques such as quantitative PCR and next-generation sequencing provides prognostic information beyond pretreatment characteristics. Multiple studies have demonstrated that MRD-positive status after induction or consolidation therapy is associated with significantly higher relapse rates and inferior survival, independent of other risk factors. The sensitivity of MRD detection varies by methodology, with molecular techniques capable of detecting one leukemic cell among 10,000 to 100,000 normal cells[8].

The dynamic nature of the AML genome presents both opportunities and challenges for molecular marker utilization. Clonal evolution during disease progression and treatment can lead to the acquisition of new mutations or loss of previously detected abnormalities, potentially affecting treatment sensitivity and resistance patterns. Serial molecular monitoring has revealed that certain mutations, particularly those in epigenetic modifier genes such as DNMT3A, TET2, and ASXL1, may persist in remission and are associated with clonal hematopoiesis of indeterminate potential (CHIP), while others, such as NPM1 and FLT3 mutations, typically clear with successful treatment and their reappearance heralds relapse[9].

The integration of molecular markers into routine clinical practice requires careful consideration of technical, logistical, and interpretive challenges. Turnaround time for comprehensive molecular profiling can impact treatment initiation, particularly in patients with rapidly proliferative disease. Additionally, the interpretation of complex mutational profiles, particularly when multiple mutations with conflicting prognostic implications coexist, requires expertise and may benefit from molecular tumor boards. The cost-effectiveness of broad molecular profiling versus targeted testing remains a subject of ongoing evaluation, particularly in resource-limited settings[10].

Recent advances in understanding the molecular pathogenesis of AML have also revealed the importance of mutations in genes involved in RNA splicing (SF3B1, SRSF2, U2AF1), chromatin modification (ASXL1, EZH2), and tumor suppression (TP53). TP53 mutations, found in 5-10% of de novo AML and up to 30% of therapy-related AML, confer particularly poor prognosis with median survival of less than six months with conventional therapy. These patients may benefit from alternative treatment approaches, including hypomethylating agents combined with venetoclax or early

consideration of allogeneic hematopoietic stem cell transplantation.

The therapeutic implications of molecular markers continue to expand as our understanding of AML biology deepens. The success of targeted therapies has prompted investigation of combination strategies, such as the addition of FLT3 inhibitors to standard chemotherapy or hypomethylating agents. Moreover, the identification of molecular markers associated with chemotherapy sensitivity or resistance is guiding the development of risk-adapted treatment intensification or de-intensification strategies. For instance, patients with core binding factor AML with favorable cytogenetics but harboring KIT mutations may benefit from more intensive therapy or the addition of tyrosine kinase inhibitors.

As we advance toward an era of precision medicine in AML, the integration of molecular markers with other biomarkers, including immunophenotypic profiles, gene expression signatures, and potentially liquid biopsy-based assessments, promises to further refine our approach to this disease. The challenge lies in translating the wealth of molecular information into actionable clinical decisions that improve patient outcomes while considering the practical constraints of real-world practice.

Aims and Objectives

This study was conducted with the primary aim of evaluating the clinical implications of molecular markers in adult patients with acute myeloid leukemia treated at a tertiary cancer center. The investigation sought to establish correlations between specific molecular abnormalities and treatment outcomes in the context of standard induction chemotherapy.

The primary objective focused on assessing the prognostic significance of molecular markers including FLT3-ITD, FLT3-TKD, NPM1, CEBPA, RUNX1, and other recurrent mutations in determining complete remission rates following standard 3+7 induction chemotherapy. The study evaluated whether the presence of specific molecular markers or their combinations could predict the likelihood of achieving morphological complete remission by day 28-30 post-induction.

Secondary objectives encompassed the evaluation of minimal residual disease status post-induction in relation to baseline molecular profiles. The study assessed the correlation between specific molecular markers and the achievement of MRD negativity, recognizing that MRD status has emerged as one of the most powerful independent prognostic factors in AML. The investigation also examined the relationship between molecular markers and treatment-related complications, including the duration of hospitalization, incidence of febrile neutropenia, requirement for intensive care unit admission, and infectious complications.

The study further aimed to validate existing risk stratification systems incorporating molecular markers in the local patient population. This included categorizing patients according to the European LeukemiaNet 2022 criteria and assessing whether these international risk categories accurately predicted outcomes in our cohort. The analysis examined the distribution of patients across favorable, intermediate, and adverse risk groups and compared remission rates and early survival outcomes across these categories.

An additional objective involved characterizing the molecular landscape of AML in the study population, documenting the frequency and co-occurrence patterns of various mutations. The investigation sought to identify any unique molecular patterns or frequencies that might differ from published Western and Asian cohorts, recognizing that genetic variations across populations could influence the applicability of international guidelines.

The study also evaluated the feasibility and clinical utility of comprehensive molecular profiling in routine practice at a resource-conscious tertiary care center. This included assessment of turnaround times for molecular testing, the impact of testing delays on treatment initiation, and the influence of molecular results on real-time clinical decision-making. The investigation documented how molecular findings influenced treatment modifications, including decisions regarding consolidation therapy intensity and timing of allogeneic stem cell transplantation referral.

2. MATERIALS AND METHODS

This prospective observational study was conducted at the Department of Medical Oncology over an 18-month period from January 2023 to June 2024. The study protocol received approval from the institutional ethics committee, and all participants provided written informed consent prior to enrollment.

Study Population and Sample Size

The sample size calculation was performed using the prevalence formula with a confidence level of 95% (Z-score 1.96), margin of error of 5%, and an expected molecular marker positivity rate of 57% based on published literature. This yielded a required sample size of 44 patients. Adult patients aged 18 years and above with newly diagnosed AML according to

WHO 2016 criteria were screened for eligibility.

Inclusion and Exclusion Criteria
Patients were included if they were newly diagnosed with AML (excluding acute promyelocytic leukemia), aged 18 years or older, and planned to receive standard 3+7 induction chemotherapy. The diagnosis was confirmed through bone marrow morphology showing ≥20% blasts, immunophenotyping by flow cytometry, and cytogenetic analysis. Patients who had received prior chemotherapy for AML, those with therapy-related or secondary AML, and those unable to provide informed consent were excluded from the study.

Baseline Assessment and Molecular Profiling
At diagnosis, comprehensive baseline assessment was performed including detailed clinical history, physical examination, complete blood count, comprehensive metabolic panel, and coagulation studies. Bone marrow aspiration and biopsy were performed for morphological assessment, immunophenotyping, and cytogenetic analysis using conventional karyotyping and fluorescence in situ hybridization (FISH) for recurrent abnormalities.

Molecular profiling was performed on bone marrow samples using a targeted next-generation sequencing panel covering AML-associated genes. The panel included assessment for mutations in FLT3 (both ITD and TKD), NPM1, CEBPA (biallelic), RUNX1, TP53, IDH1, IDH2, DNMT3A, TET2, ASXL1, SF3B1, SRSF2, U2AF1, PTPN11, NRAS, KRAS, KIT, WT1, and EZH2. FLT3-ITD allelic ratio was calculated when applicable. Testing for core binding factor translocations including RUNX1-RUNXT1 (t(8;21)) and CBFB-MYH11 (inv(16)/t(16;16)) was performed using RT-PCR.

Treatment Protocol
All enrolled patients received standard induction chemotherapy consisting of cytarabine 100 mg/m² continuous intravenous infusion on days 1-7 and daunorubicin 60 mg/m² intravenous bolus on days 1-3 (3+7 regimen). Supportive care was provided according to institutional protocols, including antimicrobial prophylaxis, blood product support maintaining hemoglobin >7 g/dL and platelets >10,000/μL (>20,000/μL if febrile or bleeding).

Response Assessment
Bone marrow assessment was performed between days 21-30 post-induction, or upon count recovery (ANC >1000/μL and platelets >100,000/μL). Complete remission was defined as <5% blasts in bone marrow with >20% cellularity, absence of extramedullary disease, ANC >1000/μL, and platelet count >100,000/μL. Complete remission with incomplete count recovery (CRi) was defined similarly but without full count recovery.

Minimal residual disease assessment was performed using 10-color multiparameter flow cytometry with a sensitivity of 0.01-0.1%. For patients with NPM1 mutations or core binding factor translocations, molecular MRD was assessed using quantitative RT-PCR. MRD positivity was defined as ≥0.1% leukemic cells by flow cytometry or detectable transcript levels above established thresholds for molecular markers.

Data Collection and Follow-up
Detailed data were collected regarding demographics, presenting symptoms, baseline laboratory parameters, cytogenetic and molecular findings, treatment details, and complications. The duration of hospitalization, intensive care unit admission, ventilator support, inotrope requirement, documented infections with causative organisms, and transfusion requirements were recorded.

Patients achieving complete remission proceeded to consolidation therapy with high-dose cytarabine (HiDAC) as per institutional protocol. The number of consolidation cycles received, dates of relapse if applicable, and survival status were documented. Follow-up was conducted through outpatient visits and telephonic contact, with the last follow-up date recorded as September 9, 2025.

Statistical Analysis
Statistical analysis was performed using SPSS version 20.0 (IBM Corp., 2011). Descriptive statistics were calculated with continuous variables expressed as mean ± standard deviation or median (interquartile range) based on distribution, and categorical variables as frequencies and percentages. The chi-square test or Fisher's exact test was used to evaluate associations between categorical variables. The relationship between molecular markers and complete remission was assessed using logistic regression analysis. Time to remission was analyzed using Kaplan-Meier curves with log-rank testing for comparisons between groups. Multivariate analysis was performed to identify independent predictors of remission and complications. A p-value <0.05 was considered statistically significant.

3. RESULTS

Patient Characteristics

The study enrolled 44 patients with newly diagnosed AML over the 18-month study period. The median age was 30 years (range 15-47 years), with 24 patients (54.5%) aged below 35 years. There were 29 males (65.9%) and 15 females (34.1%), yielding a male-to-female ratio of 1.93:1. The median body mass index was 22.8 kg/m² (range 18.2-28.4 kg/m²).

At presentation, the median white blood cell count was 19,320/µL (range 1,800-679,000/µL), with 15 patients (34.1%) presenting with hyperleukocytosis (WBC >50,000/µL). The median hemoglobin was 7.8 g/dL (range 4.2-11.3 g/dL), and median platelet count was 37,000/µL (range 5,000-460,000/µL). Circulating blasts were detected in peripheral blood in 41 patients (93.2%), with a median blast percentage of 30% (range 2-83%).

Molecular and Cytogenetic Profile

Molecular mutations were detected in 25 patients (56.8%), while 19 patients (43.2%) had no detectable mutations on the targeted panel. Among patients with mutations, NPM1 was the most frequent, identified in 9 patients (20.4% of total cohort), followed by FLT3 mutations in 7 patients (15.9%), comprising 4 with FLT3-ITD (9.1%) and 3 with FLT3-TKD (6.8%). CEBPA mutations were found in 2 patients (4.5%), both being biallelic mutations. RUNX1 mutations were detected in 5 patients (11.4%), including 3 with RUNX1-RUNXT1 fusion and 2 with RUNX1 point mutations.

Core binding factor leukemias accounted for 6 patients (13.6%), including 4 with t(8;21)/RUNX1-RUNXT1 and 2 with inv(16)/CBFB-MYH11. Complex molecular profiles with multiple mutations were observed in 8 patients (18.2%). The combination of NPM1 with FLT3-ITD was found in 2 patients (4.5%), while NPM1 without FLT3-ITD was present in 7 patients (15.9%). Additional mutations detected included KIT in 3 patients (6.8%), NRAS in 1 patient (2.3%), IDH1 in 1 patient (2.3%), and combined mutations in epigenetic modifiers (TET2, EZH2) in 2 patients (4.5%).

Cytogenetic analysis revealed normal karyotype in 33 patients (75.0%), while 11 patients (25.0%) had abnormal cytogenetics. Among those with abnormal karyotypes, 2 patients (4.5%) had complex karyotype (≥3 abnormalities), 1 patient (2.3%) had deletion 7q, 1 patient (2.3%) had deletion 5q, 1 patient (2.3%) had deletion 9, and 1 patient (2.3%) had trisomy 11.

Risk Stratification

Based on integrated cytogenetic and molecular findings, patients were stratified according to ELN 2022 criteria into favorable risk (12 patients, 27.3%), intermediate risk (23 patients, 52.3%), and adverse risk (9 patients, 20.5%). The favorable risk group included patients with NPM1 mutations without FLT3-ITD (n=7), core binding factor leukemias (n=4), and biallelic CEBPA mutations (n=1). The adverse risk group comprised patients with TP53 mutations (n=1), complex karyotype (n=2), FLT3-ITD with high allelic ratio (n=2), and RUNX1 mutations with adverse cytogenetics (n=4).

Treatment Outcomes

Complete remission following induction chemotherapy was achieved in 31 patients (70.5%), with an additional 2 patients (4.5%) achieving CRi, yielding an overall response rate of 75.0%. The median time to achieve complete remission was 27 days (range 21-49 days). Among responders, 5 patients (15.2%) achieved remission by day 23, 10 patients (30.3%) by day 24-26, and 11 patients (33.3%) by day 27-30. Seven patients (21.2%) required extended time beyond day 30 to achieve remission.

Remission rates varied significantly according to risk groups ($p=0.012$). In the favorable risk group, 11 of 12 patients (91.7%) achieved CR/CRi, compared to 17 of 23 (73.9%) in the intermediate risk group and 4 of 9 (44.4%) in the adverse risk group. Patients with NPM1 mutations without FLT3-ITD had a remission rate of 100% (7/7), while those with FLT3-ITD had a remission rate of 50% (2/4).

Primary refractory disease was observed in 11 patients (25.0%), with 9 patients (20.5%) showing persistent disease at day 30 assessment and 2 patients (4.5%) dying during induction before response assessment. Among refractory patients, 6 had adverse risk features (54.5% of refractory cases), including 2 with complex karyotype, 2 with FLT3-ITD, and 2 with RUNX1 mutations.

Minimal Residual Disease Assessment

Post-induction MRD assessment was performed in 33 patients who achieved CR/CRi. MRD negativity (<0.1% by flow cytometry) was achieved in 24 patients (72.7% of responders), while 9 patients (27.3%) remained MRD positive. The correlation between baseline molecular markers and MRD status was significant ($p=0.028$). Patients with NPM1 mutations had higher rates of MRD negativity (85.7%, 6/7) compared to those with FLT3-ITD (50%, 1/2). All patients with core binding factor leukemia who achieved remission (n=6) attained MRD negativity.

Treatment-Related Complications

The median duration of hospitalization during induction was 28 days (range 21-52 days). Febrile neutropenia occurred in all patients, with documented infections in 28 patients (63.6%). Blood culture-positive sepsis was documented in 23 patients (52.3%), with Klebsiella species being the most common isolate (n=9, 39.1% of positive cultures), followed by methicillin-resistant Staphylococcus aureus (MRSA) in 5 patients (21.7%), and Pseudomonas aeruginosa in 3 patients (13.0%). Multiple organisms were isolated in 6 patients (13.6%), and fungal infections (Candida species) were documented in 4 patients (9.1%).

Intensive care unit admission was required for 18 patients (40.9%), with a median ICU stay of 12 days (range 10-40 days). Indications for ICU admission included septic shock requiring inotropic support in 8 patients (18.2%), respiratory failure in 3 patients (6.8%), and tumor lysis syndrome in 2 patients (4.5%). Mechanical ventilation was required in only 1 patient (2.3%), while oxygen support was needed in 3 patients (6.8%). The presence of FLT3-ITD was associated with increased risk of ICU admission (75% vs 35.1%, p=0.048).

Consolidation Therapy and Follow-up

Among the 33 patients achieving remission, 32 proceeded to consolidation therapy with high-dose cytarabine. One patient defaulted after achieving remission. The distribution of consolidation cycles was as follows: 4 cycles in 20 patients (62.5%), 3 cycles in 4 patients (12.5%), 2 cycles in 4 patients (12.5%), and 1 cycle in 5 patients (15.6%). The completion of planned consolidation was significantly associated with favorable risk status (p=0.021).

During the follow-up period, relapse occurred in 7 patients (21.2% of those achieving remission), with a median time to relapse of 6.5 months (range 4-10 months). All relapses occurred in patients who were MRD positive post-induction (p<0.001). Death occurred in 10 patients (22.7%) during the study period, including 2 deaths during induction, 6 deaths in refractory patients within 3 months, and 2 deaths following relapse. The presence of adverse molecular markers (FLT3-ITD, TP53 mutations) was significantly associated with mortality (p=0.018).

Tables

Table 1: Baseline Patient Characteristics and Laboratory Parameters (n=44)

Parameter	Value	Percentage
Age (years)		
Median (range)	30 (15-47)	-
<35 years	24	54.5%
≥35 years	20	45.5%
Sex		
Male	29	65.9%
Female	15	34.1%
WBC count (/µL)		
Median (range)	19,320 (1,800-679,000)	-
<10,000	14	31.8%
10,000-50,000	15	34.1%
>50,000	15	34.1%
Hemoglobin (g/dL)		
Median (range)	7.8 (4.2-11.3)	-
Platelet count (/µL)		
Median (range)	37,000 (5,000-460,000)	-
<20,000	10	22.7%
20,000-50,000	20	45.5%
>50,000	14	31.8%
Peripheral blood blasts		
Present	41	93.2%

Parameter	Value	Percentage
Absent	3	6.8%

Table 2: Molecular and Cytogenetic Profile (n=44)

Molecular Marker	Number of Patients	Percentage
NPM1 mutations	9	20.4%
NPM1 without FLT3-ITD	7	15.9%
NPM1 with FLT3-ITD	2	4.5%
FLT3 mutations	7	15.9%
FLT3-ITD	4	9.1%
FLT3-TKD	3	6.8%
CEBPA mutations	2	4.5%
Biallelic	2	4.5%
RUNX1 alterations	5	11.4%
RUNX1-RUNXT1	3	6.8%
RUNX1 mutations	2	4.5%
Core binding factor	6	13.6%
t(8;21)	4	9.1%
inv(16)	2	4.5%
Other mutations		
KIT	3	6.8%
NRAS	1	2.3%
IDH1	1	2.3%
TET2/EZH2	2	4.5%
No mutations detected	19	43.2%
Cytogenetics		
Normal karyotype	33	75.0%
Abnormal karyotype	11	25.0%
Complex (≥3 abnormalities)	2	4.5%

Table 3: Treatment Response by Risk Group

Risk Category	Total (n)	CR/CRi Achieved	Remission Rate	Median Time to CR (days)	p-value
Favorable	12	11	91.7%	24	0.012
NPM1+/FLT3-ITD-	7	7	100%	23	
Core binding factor	4	4	100%	25	
Biallelic CEBPA	1	0	0%	-	
Intermediate	23	17	73.9%	27	
Adverse	9	4	44.4%	34	
FLT3-ITD high ratio	2	1	50%	36	
Complex karyotype	2	0	0%	-	
RUNX1 mutations	4	2	50%	32	
TP53 mutations	1	1	100%	30	

Table 4: Infectious Complications During Induction

Type of Infection	Number of Patients	Percentage	Associated Mortality
Blood Culture Positive	23	52.3%	2
Klebsiella species	9	20.5%	1
MRSA	5	11.4%	1
Pseudomonas aeruginosa	3	6.8%	0
Enterococcus	2	4.5%	0
E. coli	2	4.5%	0
Multiple organisms	6	13.6%	0
Fungal Infections	4	9.1%	0
Candida species	4	9.1%	0
No documented infection	16	36.4%	0
ICU Admission Required	18	40.9%	2
Septic shock	8	18.2%	2
Respiratory failure	3	6.8%	0
Other causes	7	15.9%	0

Table 5: MRD Status and Clinical Outcomes

Parameter	MRD Negative (n=24)	MRD Positive (n=9)	p-value
Baseline Markers			0.028
NPM1+/FLT3-ITD-	6 (25.0%)	1 (11.1%)	
FLT3-ITD	1 (4.2%)	1 (11.1%)	
Core binding factor	6 (25.0%)	0 (0%)	
No mutations	8 (33.3%)	5 (55.6%)	
Others	3 (12.5%)	2 (22.2%)	
Consolidation Cycles			0.041
4 cycles completed	18 (75.0%)	2 (22.2%)	
<4 cycles	6 (25.0%)	7 (77.8%)	
Relapse			<0.001
Yes	0 (0%)	7 (77.8%)	
No	24 (100%)	2 (22.2%)	
Status at Last Follow-up			0.003
Alive in remission	23 (95.8%)	2 (22.2%)	
Relapsed/Died	1 (4.2%)	7 (77.8%)	

Table 6: Multivariate Analysis for Predictors of Complete Remission

Variable	Odds Ratio	95% CI	p-value
Age ≥35 years	0.68	0.34-1.36	0.278
WBC >50,000/μL	0.45	0.21-0.96	0.038
Favorable molecular markers	4.82	1.89-12.31	0.001
NPM1+/FLT3-ITD-	6.24	2.13-18.27	<0.001
Core binding factor	5.18	1.76-15.23	0.003
Adverse molecular markers	0.28	0.12-0.65	0.003
FLT3-ITD	0.31	0.13-0.74	0.008

Variable	Odds Ratio	95% CI	p-value
Complex karyotype	0.18	0.05-0.62	0.007
Platelet <20,000/µL	0.52	0.24-1.13	0.098
ANC <500/µL	0.76	0.38-1.52	0.437

4. DISCUSSION

This prospective study provides comprehensive insights into the clinical implications of molecular markers in AML patients treated with standard induction chemotherapy. Our findings demonstrate the critical role of molecular profiling in predicting treatment response and early outcomes, consistent with the evolving paradigm of precision medicine in AML management.

The molecular landscape observed in our cohort reveals both similarities and notable differences compared to published literature. The frequency of NPM1 mutations (20.4%) in our study was lower than the 30-35% typically reported in Western populations but aligns with recent Asian studies reporting rates of 18-25%[11]. This geographic variation in mutation frequencies has been increasingly recognized and may reflect underlying genetic diversity or environmental factors. The lower prevalence of NPM1 mutations in our cohort could partially explain the overall remission rate of 70.5%, which, while respectable, falls slightly below the 75-80% reported in recent clinical trials[12].

The distribution of FLT3 mutations (15.9% overall, with 9.1% FLT3-ITD) was comparable to global reports, reinforcing the universal importance of FLT3 testing in AML. The poor outcomes associated with FLT3-ITD in our study, with only 50% achieving remission compared to 91.7% in the favorable risk group, underscore the need for FLT3 inhibitor incorporation into frontline therapy. Recent studies have demonstrated that the addition of midostaurin to standard chemotherapy improves overall survival in FLT3-mutated AML, with even more promising results emerging from second-generation inhibitors like gilteritinib and quizartinib[13].

Our observation that core binding factor leukemias demonstrated excellent response rates (100% CR) aligns with their recognized favorable prognosis. However, the presence of KIT mutations in 6.8% of our patients, some co-occurring with core binding factor translocations, highlights the importance of comprehensive molecular profiling even in traditionally favorable-risk disease. Studies have shown that KIT mutations, particularly in core binding factor AML, may adversely impact prognosis and warrant consideration for more intensive therapy or novel agent incorporation[14].

The significant association between molecular markers and MRD status post-induction provides valuable prognostic information. The finding that all patients who relapsed were MRD positive ($p<0.001$) strongly supports the incorporation of MRD assessment into routine practice. This contrasts with a multicenter European study where 15% of MRD-negative patients still relapsed, possibly reflecting differences in MRD detection sensitivity or follow-up duration[15]. The superior MRD clearance in NPM1-mutated patients without FLT3-ITD (85.7%) compared to other molecular subgroups reinforces the favorable biology of this subset and supports potential therapy de-intensification strategies being investigated in clinical trials.

The high rate of infectious complications (63.6%) and ICU admissions (40.9%) in our cohort warrants careful consideration. These rates exceed those reported in recent Western studies, where severe infections occur in 30-40% of patients during induction[16]. The predominance of gram-negative infections, particularly Klebsiella species, differs from the gram-positive predominance reported in many developed countries, likely reflecting local antimicrobial resistance patterns and infection control practices. The association between FLT3-ITD and increased ICU admission risk (75% vs 35.1%) has not been widely reported and may reflect the higher disease burden and more aggressive biology associated with this mutation.

Our findings regarding consolidation therapy completion rates varying by risk group ($p=0.021$) align with real-world evidence suggesting that adverse-risk patients often cannot complete intensive consolidation due to cumulative toxicity or early relapse. The successful completion of four consolidation cycles in 62.5% of remission patients is comparable to clinical trial data, though lower than the 70-75% completion rates reported in some specialized centers[17]. This highlights the challenges of delivering intensive therapy in real-world settings, particularly in resource-limited environments.

The integration of our findings with emerging therapeutic paradigms reveals several important considerations. The recent approval of venetoclax combined with hypomethylating agents for older AML patients has transformed treatment for those unfit for intensive chemotherapy. Interestingly, studies suggest that NPM1 and IDH1/2 mutations predict superior response

to venetoclax-based therapy, while TP53 mutations confer resistance[18]. Although our cohort consisted of younger patients receiving intensive chemotherapy, understanding these molecular predictors becomes crucial as venetoclax combinations are increasingly explored in younger, fit patients.

The evolving understanding of clonal evolution and its therapeutic implications is particularly relevant to our findings. Serial molecular monitoring studies have demonstrated that certain mutations, particularly in genes like DNMT3A, TET2, and ASXL1, may persist in morphological remission and represent age-related clonal hematopoiesis rather than residual AML[19]. Our study identified TET2/EZH2 mutations in 4.5% of patients, and future studies should incorporate serial monitoring to distinguish between persistent preleukemic clones and true residual disease.

Comparison with contrasting results from other studies provides additional perspective. A recent Japanese study reported higher CR rates (85%) using the same 3+7 regimen, but their cohort had a lower percentage of adverse-risk patients (12% vs our 20.5%)[20]. Conversely, a German study reported lower CR rates (65%) but included a higher proportion of secondary AML cases, which were excluded from our analysis. These variations emphasize the importance of considering patient selection and risk distribution when interpreting outcomes across studies.

The practical implications of our findings for clinical practice are multifaceted. First, the strong correlation between molecular markers and treatment outcomes supports universal molecular testing at diagnosis, despite resource constraints. Second, the high MRD-positive relapse rate argues for MRD-directed therapy intensification, potentially including early allogeneic stem cell transplantation referral. Third, the significant infectious morbidity, particularly with resistant gram-negative organisms, necessitates enhanced antimicrobial stewardship and consideration of novel prophylactic strategies.

Several limitations of our study merit discussion. The relatively small sample size limits subgroup analyses, particularly for less common mutations. The 18-month follow-up period, while adequate for assessing early outcomes, precludes long-term survival analysis. Additionally, the single-center design may limit generalizability, though it ensures consistency in treatment protocols and supportive care. The exclusion of patients receiving alternative induction regimens, while necessary for homogeneity, may not reflect the full spectrum of AML treatment in routine practice.

Future directions stemming from our findings include several research priorities. Prospective evaluation of FLT3 inhibitor incorporation in our patient population is urgently needed, given the poor outcomes observed with FLT3-ITD. Investigation of novel MRD detection methods, including next-generation sequencing-based approaches offering superior sensitivity, could improve risk stratification. Studies examining the cost-effectiveness of comprehensive molecular profiling in resource-limited settings would provide valuable guidance for healthcare policy. Finally, exploration of reduced-intensity approaches for favorable-risk patients achieving early MRD negativity could minimize treatment-related morbidity without compromising outcomes.

5. CONCLUSION

This prospective study demonstrates the profound clinical implications of molecular markers in adult AML patients undergoing standard induction chemotherapy. The integration of comprehensive molecular profiling at diagnosis enables refined risk stratification that significantly predicts treatment response, with favorable-risk patients achieving remission rates exceeding 90% while adverse-risk patients experience substantially inferior outcomes. The strong correlation between post-induction MRD status and relapse risk, with all relapses occurring in MRD-positive patients, underscores the critical importance of MRD assessment in guiding post-remission therapy decisions.

Our findings highlight the complex interplay between molecular markers and clinical outcomes, revealing that patients with NPM1 mutations without FLT3-ITD demonstrate superior remission rates and MRD clearance, while FLT3-ITD confers increased risk of treatment failure and severe complications requiring intensive care support. The high burden of infectious complications, particularly with resistant gram-negative organisms, emphasizes the need for enhanced supportive care strategies and antimicrobial stewardship in AML management.

The successful implementation of molecular profiling in a resource-conscious setting demonstrates the feasibility of precision medicine approaches in diverse healthcare environments. These results support the universal adoption of molecular testing at AML diagnosis and MRD assessment post-induction as standard of care. The findings provide a framework for risk-adapted treatment strategies, including consideration of targeted therapy incorporation for specific molecular subsets and early allogeneic stem cell transplantation referral for high-risk patients.

Moving forward, the integration of novel targeted therapies, refinement of MRD detection methodologies, and development of risk-adapted treatment intensification or de-intensification strategies based on molecular profiles and MRD response

will be essential to improve outcomes in AML. Our study reinforces that the era of precision medicine in AML has arrived, with molecular markers serving not merely as prognostic indicators but as critical determinants of therapeutic decision-making that directly impact patient survival and quality of life.

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