


MECOM gene overexpression in pediatric patients with acute myeloid leukemia

Mariam Elsherif^a, Mahmoud Hammad^{a,b} , Hanafy Hafez^{a,b}, Dina Yassin^{c,d}, Mohamed Ashraf^c, Nouran Yasser^e, Leslie Lehmann^f and Alaa Elhaddad^{a,b}

^aDepartment of Pediatric Oncology, Children's Cancer Hospital Egypt (CCHE), Cairo, Egypt; ^bDepartment of Pediatric Oncology, National Cancer Institute, Cairo University, Cairo, Egypt; ^cDepartment of Clinical Pathology, Children's Cancer Hospital Egypt (CCHE), Cairo, Egypt; ^dDepartment of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt; ^eDepartment of Research, Children's Cancer Hospital (CCHE), Cairo, Egypt; ^fPediatric Stem Cell Transplantation Unit, Dana Farber Cancer Institute, Boston, MA, USA

ABSTRACT

Background: Acute myeloid leukemia (AML) is characterized by blocked or aberrant differentiation of hematopoietic stem cells. The *MECOM* gene overexpression in hematopoietic progenitors induces myeloid differentiation block, resulting in increased self-renewal and survival of these transformed progenitors. However, its exact role in AML remains unclear. We aimed to estimate the prevalence of *MECOM* overexpression among pediatric AML patients, and assess its impact on clinical outcome.

Patients and Methods: Real-time quantitative polymerase chain reaction and Livak method ($2^{-\Delta\Delta Ct}$) were used to determine relative *MECOM* expression level among 243 pediatric patients with AML. *MECOM* overexpression was considered if the cumulative relative expression was above 1 ($2^{-\Delta\Delta Ct}$) and was designated as *MECOM*^{pos}.

Results: Of 243 AML patients tested 57(23.5%) demonstrated *MECOM*^{pos}. Patients with *MECOM*^{pos} had significantly lower median age. The frequency of *MECOM*^{pos} was significantly higher among AML patients with 11q23 abnormalities, complex karyotypes and among high- and intermediate-risk groups compared to low-risk group ($p = .014$). *MECOM*^{pos} patients had significantly lower overall survival (OS) (38.7 vs. 78.9%, $p < .001$), event-free survival (EFS) (37.3% vs. 68.4%, $p < .001$), and had higher cumulative incidence of relapse (49.5% vs. 23.5%, $p = .002$) at 36 months compared to *MECOM*^{neg} patients. Multivariate analysis revealed that *MECOM*^{pos} was an adverse prognostic factor for OS (hazards ratio (HR) = 2.11, 95% confidence interval (CI) 1.24–3.60, $p = .006$) and EFS (HR = 1.71, 95% CI 1.07–2.75, $p = .025$). The logistic regression model showed that *MECOM*^{pos} was an independent prognostic factor regardless of minimal residual disease status post first induction therapy in the intermediate-risk group (odds ratio 2.89; 95% CI 1.19–6.57, $p = .018$).

Conclusion: The aberrant *MECOM* gene expression is an adverse prognostic factor, especially in patients without previously known cytogenetic risk factors. Our results suggest the potential benefit from pretreatment screening for *MECOM* gene overexpression in newly diagnosed AML patients for better risk stratification and treatment adjustment.

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Introduction

Acute myeloid leukemia (AML) is characterized by blocked or aberrant differentiation of hematopoietic stem cells. AML accounts for 20% of childhood leukemia with 5-year overall survival rate of 68% for children younger than 15 years [1,2]. Overexpression of certain hematopoietic-associated genes is commonly found in AML and can define clinically relevant biological subsets that lack other previously identified prognostic markers [3].

The *MDS1* and *EV11* complex locus protein (*MECOM*) gene is located on chromosome 3q26 and encodes a zinc-finger protein that functions as a transcriptional regulator of hematopoietic stem cell self-renewal and long-term activity [3]. The gene was first identified as a common site of viral integration in retrovirus-induced murine leukemia, suggesting its

role in the transformation of hematopoietic cells [4]. Overexpression of the *MECOM* gene in hematopoietic progenitors induces a myeloid differentiation block, resulting in increased self-renewal and survival of these transformed progenitors [5].

MECOM gene overexpression reportedly occurs in approximately 8–15% of pediatric patients with AML and has been associated with an adverse prognosis in both adult and pediatric myeloproliferative neoplasms [6–8]. Therefore, assessment of *MECOM* gene expression, in addition to other known molecular and cytogenetic abnormalities, could provide a wider biological landscape and further clinical information that will help in the application of a better risk stratified therapy, especially in patients with AML that lack other known genetic abnormalities [9]. Previous studies of *MECOM* expression in pediatric AML from European

cooperative groups and from the Children Oncology Group (COG) study AAML03P1 reported that its overexpression adversely impacted event-free survival (EFS) and overall survival (OS) in univariate analyses, but not in multivariate analyses [8,10].

The aim of the current study was to estimate the prevalence of *MECOM* gene overexpression in pediatric patients with AML at a single large pediatric oncology center treating children with the modified COG protocol (AAML-1031) and to assess the prognostic impact of *MECOM* gene overexpression on survival and disease outcome.

Patients and methods

This was a retrospective study that included 248 pediatric patients diagnosed with AML who have been treated at the Children's Cancer Hospital Egypt (CCH-57357) from January 2015 to October 2017, with a minimum one-year follow-up from the end of treatment. The institutional review board approved the study, and informed consent was obtained before diagnostic assessment or therapy was initiated.

Patients with acute promyelocytic leukemia (APML), Down syndrome myeloid neoplasm, myelodysplastic syndrome (MDS), therapy-related AML (t-AML), relapsed AML, and myeloid sarcoma were excluded. Patients who died before response assessment during induction I were also excluded from the analysis.

The following data were collected: age at presentation, initial total leukocyte count (TLC), French-American-British (FAB) classification, cytogenetics, and molecular abnormalities.

Patients were initially classified according to modified COG protocol AAML 1031 into:

- Low Risk (LR): Favorable cytogenetics - (t8;21), inversion 16, nucleophosmin (*NPM1*), *CEBPA*
- Intermediate Risk (IR): Neither favorable nor unfavorable criteria
- High risk (HR): Unfavorable cytogenetics, monosomy 7, monosomy 5, complex karyotype (more than three chromosomal aberrations), *FLT3* internal tandem duplications (*FLT3/ITD*) with high allelic ratio defined as > 0.4 (*FLT3/ITD positive*)
- Using flow cytometry after induction I, patients in the IR group were further classified into HR and LR according to the minimal residual disease (MRD) response, with LR defined as MRD $< 0.1\%$.

Treatment protocols

Patients were registered onto the CCH 57357 AML protocol adopted from the modified COG protocol AAML 1031. The details of the chemotherapy regimens and doses are provided in 'Supplementary Table 1.'

Definitions

Complete remission (CR) was defined as bone marrow (BM) blasts $< 5\%$. Refractory disease was defined as the persistence of BM blasts $\geq 5\%$ at the end of induction II.

Allogenic hematopoietic stem cell transplantation (Allo-HSCT) from a matched sibling donor was offered to high-risk patients in complete remission 1 (CR1), refractory disease, and relapsing cases if they reached a second complete remission (CR2). Busulfan and cyclophosphamide were used as conditioning regimens.

Identification of *MECOM* expression using real-time quantitative PCR

Total RNA was extracted from the BM samples using the RNeasy Mini Kit (Qiagen, Germany).

Assessment for RNA quantity used Nanodrop 2000 (ThermoScientific, US), quality was tested by gel electrophoresis. A total of 1 μg of RNA was transcribed into cDNA using high-capacity reverse transcriptase (Life Technologies, Merelbeke, Belgium). Real-time quantitative PCR amplification was performed using QuantStudio 5 (Applied Biosystems, Thermo Scientific) with 20 μL mix containing: 12.5 μL TaqMan™ Universal Master Mix II, with UNG (Applied Biosystems Thermo Scientific), 7.5 pmol of forward and reverse primers each (Eurofins Genomics), 10 nM dual-labeled probe HPLC grade (Eurofins Genomics), labeled at the 5' end with the reporter dye molecule FAM (6-carboxy-fluorescein) and at the 3' end with the quencher dye molecule TAMRA (6-carboxy-tetramethylrhodamine; Eurofins Genomics) for *EV11*, *EV11/MDS*, and *ABL* genes), and 50 ng of cDNA. The thermal cycling conditions included 10 min at 95 °C, followed by 40 cycles of denaturation for 15 s at 95 °C and annealing/extension at 60 °C for 60 s. To determine the expression levels in AML, all samples were tested in duplicates and the average values were used for quantification, and all the results were subjected to the Livak method for relative gene expression determination [11]. In addition to 15 healthy BM samples, the relative expression of *EV11* and *EV11/MDS* transcripts was calculated using the comparative cycle time (ΔCt) method, with the *ABL* gene as the housekeeping gene. We used the $\Delta\Delta\text{Ct}$ value to calculate the cumulative relative expression ($2^{-\Delta\Delta\text{Ct}}$). The sample was considered to have *MECOM* overexpression if the cumulative relative expression was above 1 [12]. *MECOM* overexpression was designated as *MECOM*^{pos}, with the remainder of the samples designated as *MECOM*^{neg}. Five samples had low RNA concentrations after extraction and were therefore excluded. The analysis cohort comprised 243 patients.

The oligonucleotide sequences of the primers and probes used for *EV11*, *EV11/MDS*, and *ABL* are shown in 'Supplementary Table 2.'

Statistical analysis

The characteristics of patients who were *MECOM*^{pos} and *MECOM*^{neg} were compared using the chi-square test with

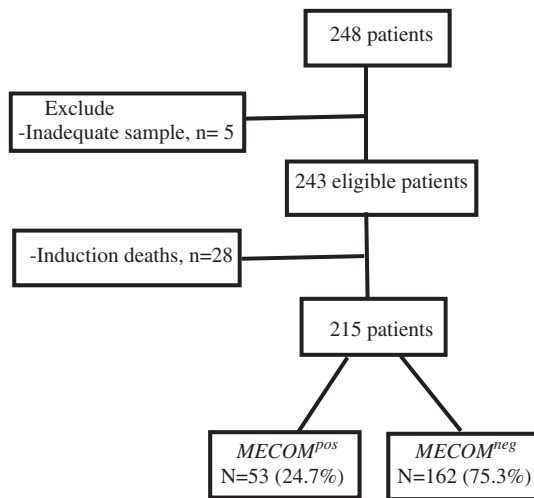


Figure 1. Prevalence of *MECOM* overexpression gene among pediatric patients with AML.

Bonferroni multiple hypothesis *P*-value adjustment. Logistic regression was performed to evaluate the impact of *MECOM* gene overexpression and MRD on relapse in the IR group. Overall and event-free survival probabilities were calculated using Kaplan-Meier analysis, and comparisons between patients who were *MECOM*^{pos} and *MECOM*^{neg} were performed using the log-rank test. OS was defined as the duration from the date of registration to the date of death or last contact date. EFS was defined as the duration from the registration date until the event date where the event was either relapse, refractory (induction failure), or death. The effects of different risk factors on death and events were tested using univariate Cox regression, and the statistically significant variables were reevaluated using a multivariate Cox regression model. The cumulative incidence of relapse (CIR) was estimated for *MECOM*^{pos} and *MECOM*^{neg} groups and compared using the Gray test.

Results

Prevalence of *MECOM* gene overexpression among pediatric patients with AML

Of the 215 eligible patients, 53 (24.7%) had *MECOM* overexpression (Figure 1).

Patient characteristics and patterns of *MECOM* gene overexpression

As shown in Table 1, the median age for the entire cohort was 7 years (range, 0.2–17.9 years). Patients who were *MECOM*^{pos} had a lower median age (range, 0.2–16.3 years) at diagnosis than those who were *MECOM*^{neg} (range, 0.4–17.9 years) (66% of *MECOM*^{pos} <7 vs. 44% of *MECOM*^{neg} <7 years; *p* = .042).

Regarding FAB classification, M7 was frequently encountered with *MECOM* overexpression (15/53, 28.3% vs. 7/162,

Table 1. Distribution of *MECOM* gene overexpression depending on patient's characteristics with multiple hypothesis adjustment.

	<i>MECOM</i> gene overexpression		<i>p</i> Value
	Yes (total = 53 cases) N(%)	No (total = 162 cases) N(%)	
Age (median)			.042^a
<7.2 years	35 (66%)	71 (43.8%)	
≥7.2 years	18 (33.9%)	91 (56.1%)	
Initial TLC (median)			1
<20.6	24 (45.2%)	82 (50.6%)	
≥20.6	20 (37.7%)	89 (54.9%)	
FAB Classification			.014^a
0	8 (13.2%)	9 (5.6%)	
1	3 (5.7%)	25 (15.4%)	
2	6 (11.3%)	83 (51.2%)	
4	8 (15.1%)	28 (17.3%)	
5	9 (17.0%)	10 (6.2%)	
6	4 (7.5%)	0 (0.0%)	
7	15 (28.3%)	7 (4.3%)	
MLL-rearrangement			.014^a
Negative	41 (77.4%)	152 (93.8%)	
Positive	12 (22.6%)	10 (6.2%)	
Trisomy 8			.154
No	41 (77.4%)	147 (90.7%)	
Yes	12 (22.6%)	15 (9.3%)	
Trisomy 21			.168
No	46 (86.8%)	156 (96.3%)	
Yes	7 (13.2%)	6 (3.7%)	
Monosomy 7			.154
No	48 (90.6%)	159 (98.1%)	
Yes	5 (9.4%)	3 (1.9%)	
Complex Karyotype			.014^a
No	43 (81.1%)	158 (97.5%)	
Yes	10 (18.9%)	4 (2.5%)	
FLT3/ITDs			1
Mutant (< 0.4 AR)	0 (0.0%)	1 (.6%)	
Mutant (≥ 0.4 AR)	1 (1.9%)	12 (7.4%)	
Wild type	52 (98.2%)	149 (92%)	
Initial risk			.014^a
High Risk	16 (30.2%)	20 (12.3%)	
Intermediate risk	35 (66%)	78 (48.1%)	
Low risk	2 (3.8%)	64 (39.5%)	

^aStatistically significant.

TLC: total leukocyte count (TLC); FAB: French-American-British classification; MLL: mixed Lineage leukemia; ITDs: internal tandem duplications; AR: Allelic Ratio.

4.3%; *p* = .014). The rate of mixed lineage leukemia (*MLL*) gene rearrangement was significantly higher in patients who were *MECOM*^{pos} (12/53, 22.6%) versus those who were *MECOM*^{neg} (10/162, 6%) (*p* = .014), as well as those with complex karyotypes (10/53 18.8% vs. 4/162 2%, *p* = .014 in *MECOM*^{pos} vs. *MECOM*^{neg}, respectively). No significant association was found among monosomy 7, trisomy 8, trisomy 21, or *FLT3/ITD* and *MECOM* overexpression.

Using the COG risk classification, patients with *MECOM* overexpression were more often in the IR (35/53 66% vs. to 78/162 48%) and HR groups (16/53 30% vs. 20/162 12%) (*p* = .014). Only two patients with *MECOM* overexpression were classified as LR.

MECOM gene overexpression and survival

Patients with *MECOM* gene overexpression had significantly lower 3-year OS (38.7% vs. 78.9%, *p* < .001) and EFS (37.3% vs. 68.4%, *p* < .001), and significantly higher cumulative

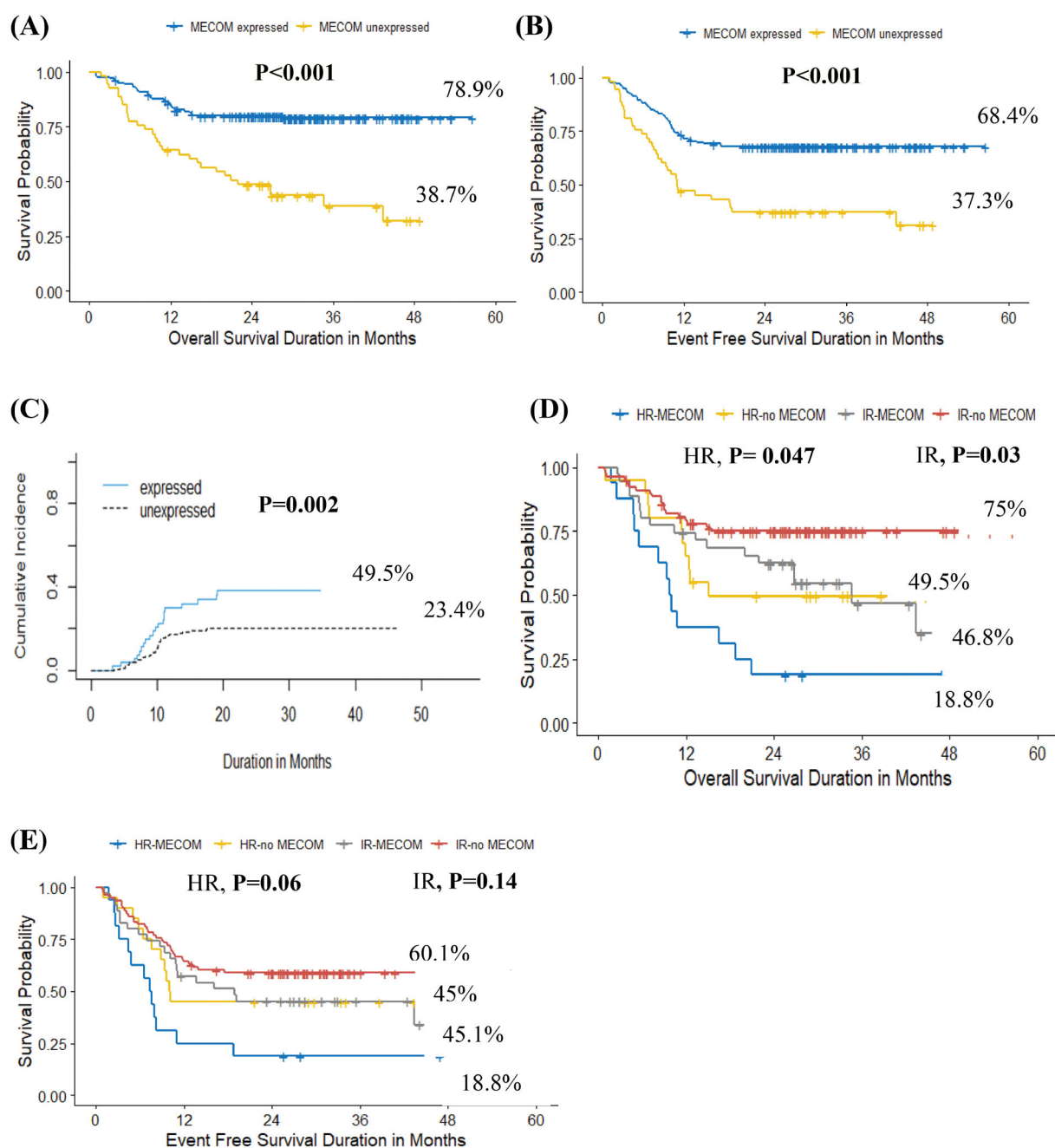


Figure 2. Impact of MECOM gene overexpression on survival. (A) Kaplan–Meier curves of overall survival of patients with MECOM^{pos} vs MECOM^{neg} among the whole cohort, (B) Kaplan–Meier curves of event-free survival of patients with MECOM^{pos} vs MECOM^{neg} among the whole cohort, (C) Cumulative incidence of relapse among patients with MECOM^{pos} vs MECOM^{neg} among the whole cohort, (D) Kaplan–Meier curves of overall survival of patients with MECOM^{pos} vs MECOM^{neg} within disease risk group, (E) Kaplan–Meier curves of event-free survival of patients with MECOM^{pos} vs MECOM^{neg} within disease risk group.

incidence of relapse (49.5% vs. 23.5%, $p = .002$) than patients who were MECOM^{neg} (Figure 2(A–C) respectively, Supplementary Tables 3(A–C)).

As expected, in the entire cohort, patients in the HR group had lower OS and EFS (35.4% and 33.3%, respectively) than patients in the IR and LR groups (OS 64.4% and 91.9%, respectively; EFS 55.4% and 84.8%, respectively) ($p < .001$) (Supplementary Figures 1(A,B)).

MECOM gene overexpression influences overall survival in high risk and intermediate risk groups

Within the risk groups, MECOM^{pos} conferred a negative impact on OS (HR: 18.8% vs. 49.5%, $p = .047$; IR: 46.8% vs. 75%, $p = .03$). However, the differences in EFS among the groups were not statistically significant (Figure 2(D,E), Supplementary Table 4).

Table 2. Cox regression for univariate and multivariate analyses of *MECOM* overexpression, FAB, *MLL-r*, complex karyotype, and initial risk.

OS	Univariate			Multivariate		
	Hazards Ratio	95%CI for HR Lower– Upper	<i>p</i> Value	Hazards Ratio	95%CI for HR Lower –Upper	<i>p</i> Value
<i>MECOM</i> gene overexpression	3.48	2.13–5.68	<.001	2.11	1.24–3.60	.006
FAB (M7 vs. others)	1.57	0.78–3.19	.208			
<i>MLL</i> gene	1.99	1.04–3.81	.038	1.33	0.67–2.63	.419
Complex Karyotype	3.78	1.92–7.45	<.001	1.02	0.43–2.39	.970
Initial Risk			<.001			<.001
Low	1(–)	1(–)		1(–)	1(–)	
Intermediate	5.38	2.11–13.81		3.89	1.47–10.31	
High	13.17	4.98–34.81		9.41	3.29–26.9	

EFS	Univariate			Multivariate		
	Hazards Ratio	95%CI for HR Lower – Upper	<i>p</i> Value	Hazards Ratio	95%CI for HR Lower –Upper	<i>p</i> Value
<i>MECOM</i> gene overexpression	2.58	1.67–3.99	<.001	1.72	1.07–2.76	.025
FAB (M7 vs. others)	1.39	0.74–2.61	.314			
<i>MLL</i> gene	1.52	0.83–2.80	.177	1.05	0.55–1.99	.878
Complex Karyotype	2.84	1.46–5.51	.002	1.01	0.436–2.33	.983
Initial Risk			<.001			<.001
Low	1(–)	1(–)		1(–)	1(–)	
Intermediate	3.80	1.93–7.49		3.16	1.56–6.42	
High	7.23	3.45–15.18		5.82	2.55–13.27	

EFS: event-free survival; OS: overall survival; CI: confidence interval; HR: hazards ratio.

Univariate and multivariate cox regression analyses

MECOM gene overexpression, *MLL* gene-r, complex karyotype, and initial disease risk were significant predictors of survival rates in the univariate analysis (Table 2). However, only two factors were associated with lower OS and EFS in the multivariate analysis: *MECOM* gene overexpression for OS (hazard ratio (HR) = 2.11, 95% confidence interval [CI], 1.24–3.60, $p = .006$) and for EFS (HR = 1.71, 95% CI 1.07–2.75, $p = .025$), and initial risk stratification for OS ($p < .001$) and EFS ($p < .001$) (Table 2).

MECOM gene overexpression as a poor prognostic factor in the intermediate risk group regardless of minimal residual disease response

In the current study, *MECOM* gene overexpression did not affect the MRD response after induction I in the IR group (Supplementary Table 5). However, 19 out of 57 patients had no identifiable marker to follow post-induction I; thus, MRD could not be assessed, and according to the modified COG protocol AAML1031, these patients were considered MRD-negative (Figure 3).

We analyzed the impact of *MECOM* gene overexpression and MRD on relapse using a logistic regression model in the IR group. *MECOM*^{pos} had a significant impact on relapse regardless of MRD status post induction I (odds ratio 2.79, 95% CI 1.19–6.56, $p = .018$) (Supplementary Table 6).

MECOM gene overexpression in patients transplanted to the HR group

Twenty-nine patients underwent Allo-HSCT, 9 of whom were *MECOM*^{pos} (4 in CR1 and 5 in CR2), while the remaining 20 were *MECOM*^{neg} (13 in CR1 and 7 in CR2). Seven patients relapsed after Allo- HSCT (three of whom were *MECOM*^{pos} and four *MECOM*^{neg}). Due to the small sample size, definitive

conclusions regarding the impact of *MECOM* gene overexpression on relapse after Allo- HSCT could not be made.

Discussion

Risk stratification in AML continues to evolve as new information emerges about both the role of biological factors identified at diagnosis and the response to up-front therapy. The ELN guidelines, updated in 2017, classify patients into 'favorable,' 'intermediate,' and 'adverse' groups based on cytogenetics and molecular mutations [13]. Research on molecular testing has attempted to further refine the prognosis of intermediate cases by identifying new mutations to explain the heterogeneity of outcomes in this group [14].

When fused with *MDS1*, the ecotropic viral integration site 1 (*EV11*) creates *MECOM*, a unique protein that acts as a proto-oncogene controlling transcription in early hematopoietic development. Aberrant *EV11* expression has been reported in 8–10% of adult AML cases and up to 27% of pediatric mixed-lineage leukemia (*MLL*). The prevalence of *MECOM* gene overexpression in childhood AML reportedly ranges from 9% to 28%, which was similar to our results (23.5%) [8,10]. We found that *MECOM* gene overexpression was strongly associated with the FAB M7 morphologic subtype of AML (28.3% of *MECOM*^{pos} cases were M7), similar to what was found in some previous pediatric studies [10,15]. This result contrasts with that of other pediatric studies where *MECOM* gene expression is associated with FAB M4 and M5 [16].

Cytogenetic aberrations in relation to *MECOM* overexpression have also been observed. *MLL* gene rearrangements on 11q23 were found in 15–20% of de novo pediatric AML cases. Several studies have shown a high incidence of *MECOM* expression and *MLL-r*, ranging from 28% to 40% [8,10,17]. In the current study, over half of *MLL* rearranged cases were *MECOM*^{pos}, and the complex karyotype where almost three-quarters of patients had *MECOM* gene overexpression.

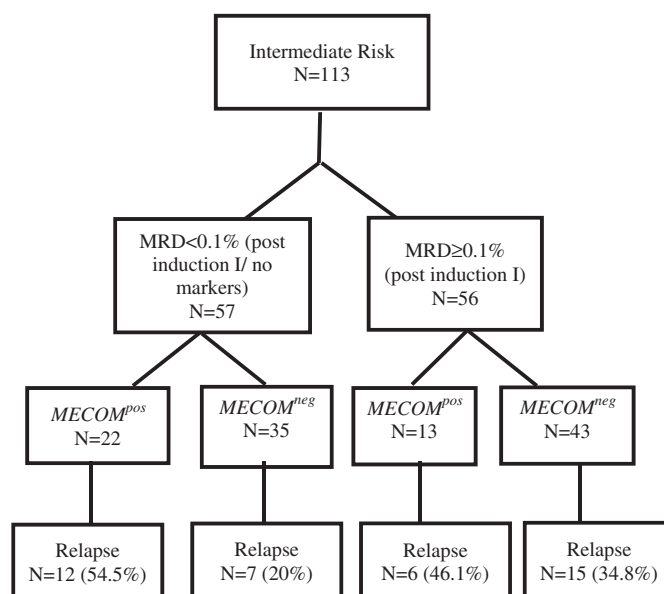


Figure 3. MECOM overexpression among patients in the intermediate risk group.

Monosomy 7 is a common cytogenetic abnormality frequently found in myeloid malignancies, and its association with MECOM overexpression has been reported in adult and pediatric AML, which may be explained by the presence of MECOM on 3q26, a region commonly involved in rearrangements in monosomy 7 [8,10,18]. In our study, although small numbers precluded a detailed analysis, five out of eight patients with monosomy 7 were MECOM^{pos}.

Importantly, this study found that patients with MECOM^{pos} had significantly lower OS and EFS than those who were MECOM^{neg}, mostly due to a higher cumulative incidence of relapse among patients who were MECOM^{pos}. Ho *et al.* reported that high *EV11* gene expression adversely influenced OS ($51 \pm 14\%$ vs. $68 \pm 8\%$, $p = .015$) and EFS ($40 \pm 13\%$ vs. $52 \pm 8\%$, $p = .042$) [10]. While Balgobind showed that patients with MECOM gene overexpression had significantly worse EFS (28% vs. 44%, $p = .04$), this did not extend to OS (56% vs. 64%, $p = .34$), as confirmed by multivariate analysis, including favorable karyotype, age, and WBC count [8].

Many factors contribute to AML outcomes and survival, including initial risk stratification and response assessment after induction therapy by flow cytometry. In our cohort, high MECOM gene expression remained a significant predictor of inferior survival after adjustment for other variables, including initial risk stratification.

We also found that the majority of patients with MECOM overexpression were classified as IR (35/53 patients), whereas only two patients were considered as LR. Ho *et al.* reported similar results, wherein 81% of patients with *EV11* belonged to the intermediate-risk group, lacking other cytogenetic or molecular prognostic markers, whereas patients in the favorable-risk group accounted for only 4% of the patients with *EV11* [10].

One of the main goals of this study was to identify a subgroup of patients within the IR group with an increased risk of relapse and poor outcomes, thus setting the stage for

different approaches to upfront therapy for this group. We noticed that MECOM gene overexpression adversely affected the survival in patients from the IR group, resulting in outcomes similar to those in the HR group (OS: 46.8% vs. 49.5%, EFS 45.1% vs. 45%). Within the limits of our ability to identify markers for MRD, MECOM^{pos} remained an independent predictor of poor outcome regardless of MRD status post-induction I. Among the HR group, patients with MECOM overexpression had significantly worse OS (18.8% vs. 49.5%, $p = .047$) and EFS (18% vs. 45%, $p = .06$) than those without MECOM expression.

Conclusion

Despite advances in the treatment of AML, relapse remains a major problem. While some children can be risk-stratified based on cytogenetic or molecular findings, a large percentage of children lacks these markers and are thus categorized into the IR group. Our study shows that MECOM overexpression is an adverse prognostic marker in pediatric patients with AML and can identify patients in the IR group that have a poorer prognosis. More studies are still needed to prove the need of more intensified treatment for this group of patients including Allo-HSCT in CR1. Opportunities for targeted therapy also exist; in 2011, Saito showed that overexpression of MECOM gene was linked to aberrant overexpression of *CD52*, a target of the monoclonal antibody alemtuzumab, and a study by De Weer showed the *MIR4494* was a crucial target of *EV11* involved in the pathogenesis of leukemia. Another study by Heller *et al* found 56 unique genes responsive to *EV11* in the human myeloid cell line U937T using gene expression microarray analysis in which 27 genes were up-regulated, and 29 genes down-regulated. The most strongly (~16-fold) repressed gene was *MS4A3*. In addition support of the regulatory relationship between *EV11*

and *MS4A3*, analysis of gene expression omnibus (GEO) microarray data sets GSE35159, GSE6891, GSE14471, and GSE35784 revealed that human myeloid cell lines and primary AML patient samples with high *EV11* mRNA levels exhibited low expression of *MS4A3* and vice versa [19–21]. Our results suggest screening for *MECOM* gene overexpression in pediatric patients newly diagnosed with AML may be of use and serve as a potential prognostic variable for risk stratification and treatment adjustment.

Institutional review board statement

This Study was approved by the Scientific Advisory Committee (SMAC) and the institutional review board (IRB) approved the study, informed consent was obtained before diagnostic assessment or therapy was initiated.

Disclosure statement

The authors declare no conflict of interest.

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ORCID

Mahmoud Hammad  <http://orcid.org/0000-0003-1677-0360>

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