Pharmacogenomics and Personalized Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Impact of *HOXB4* and *PRDM16* Gene Expressions on Prognosis and Treatment Response in Acute Myeloid Leukemia Patients

Yomna M El-Meligui[1], Naglaa M Hassan[1], Amira B Kassem[2], Nora A Gouda[3], Marwa Mohanad[4], Manal A Hamouda[5], Ahmad Salahuddin[6]

[1]Clinical Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt; [2]Clinical Pharmacy and Pharmacy Practice Department, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt; [3]Cancer Epidemiology and Biostatistics Department, National Cancer Institute, Cairo University, Cairo, Egypt; [4]Biochemistry Department, College of Pharmaceutical Sciences and Drug Manufacturing, Misr University for Science and Technology, Giza, Egypt; [5]Clinical Pharmacy Department, Faculty of Pharmacy, Menoufia University, Shibin El Kom, Egypt; [6]Biochemistry Department, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt

Correspondence: Amira B Kassem, Email amira.kassem@pharm.dmu.edu.eg

Introduction: Acute myeloid leukemia (AML) is the most common type of leukemia among adults and is characterized by various genetic abnormalities. *HOXB4* and *PRDM16* are promising markers of AML. Our objective is to assess the potential roles of *HOXB4* and *PRDM16* as prognostic and predictive markers in newly diagnosed AML patients and determine the correlation between their expressions and other prognostic markers as *FLT3-ITD, NPM1 exon 12* mutations, response to treatment, and patient's survival.
Methods: This study included 83 de novo AML adult patients. All patients were subjected to clinical, morphological, cytochemical, and molecular analysis to detect *HOXB4* and *PRDM16* gene expressions and *FLT3-ITD, NPM1 exon 12* mutations.
Results: The results showed that a low expression of *HOXB4* was found in 31.3% of AML patients, whereas a high expression of *PRDM16* was evident in 33.8% of AML patients. *FLT3-ITD* mutations were detected in 6 patients (7.2%), while *NPM1 exon 12* mutations were detected in 7 patients (19.4%) out of 36 patients with intermediate genetic risk. Out of the 50 patients who achieved complete remission (CR), relapse occurred in 16% of the cases. Low expression of *HOXB4* and high expression of *PRDM16* were associated with CR of 32% and 28%, respectively, and a short overall survival (OS) and disease-free survival (DFS).
Conclusion: Further larger study should be conducted to verify that high *PRDM16* and low *HOXB4* gene expressions could be used as a poor prognostic predictor for AML. The correlation between *PRDM16* and *HOXB4* gene expressions and *FLT3-ITD* and *NPM1 exon 12* mutations might have a role on CR, relapse, OS, and, however, this should be clarified in analysis with a larger number of samples.
Keywords: AML, HOXB4, PRDM16, NPM1 exon 12, FLT3-ITD

Introduction

Acute myeloid leukemia (AML), the most prevalent myeloid malignancy in adults, is characterized by genetic and epigenetic abnormalities in hematopoietic progenitors that result in dysregulation of critical processes, including proliferation self-renewal, and terminal differentiation.[1,2] It is responsible for 15–20% of acute childhood leukemia.[3] AML is diverse both across individuals and within the same patient, with varying clinical symptoms, molecular abnormalities, cytogenetics, and responsiveness to therapy.[4] AML has been divided into eight French-American-British (FAB) subgroups based on morphologic-genetic heterogeneity.[5]

The use of the newly approved treatment approaches to personalize therapy and improve outcomes in AML patients has progressed since 2017.[6] Advances in genome-wide molecular profiling and immunophenotyping (IPT) have identified mutations in genes associated with apoptosis (p53, nucleoplasmin, etc.) and regulation of cell proliferation (RAS, Fms-like tyrosine kinase 3, c-KIT, etc.) as possible prognostic biomarkers in AML. However, targeting these factors has failed to tackle the increasing disease heterogeneity and outcome, limiting personalized approaches for AML

Received: 30 March 2022
Accepted: 14 June 2022
Published: 25 June 2022

© 2022 El-Meligui et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

patients. The average 2-yr and 5-yr overall survival (OS) rates of patients diagnosed with AML are approximately 32% and 27%, respectively.[7,8] Thus, there is an urgent need for novel prognostic and predictive genetic biomarkers to guide patient-tailored treatment and improve survival outcomes.

The homeobox (HOX) family of transcription factors is required for normal anatomical development. *HOXB4* is a positive regulator of hematopoietic stem cell self-renewal that has been classified as a tumor suppressor or oncogene depending on the kind of cancer. *HOXB4* overexpression is required for the development and progression of several forms of cancer, including lung,[9] ovarian,[10] bladder, renal,[11] mesothelioma,[12] and leukemia.[13,14] Additionally, elevated *HOXB4* expression is related to a poor prognosis for malignant mesothelioma.[12] Other investigations have shown downregulation of the *HOXB4* gene in cancer tissues.[15,16]

PRDM16 is a member of the PRDI-BF1 and RIZ domain-containing protein families. It is structurally distinct from the others by having a conserved N-terminal PR domain and a variable number of zinc fingers.[17,18] *PRDM16* has intrinsic histone methyltransferase activity, allowing it to catalyze histone-3 lysine methylation (H3K9me1).[19] As a result, *PRDM16* may also participate as a transcriptional regulator, either directly or indirectly, via complex formation with histone-modifying enzymes.[20] *PRDM16* is required to maintain hematopoietic stem cells,[21] which makes it an attractive potential gene for leukemogenesis induction.[22] While new research suggests that *PRDM16* may contribute to the prediction of poor outcomes in juvenile AML patients,[23] the prognostic importance of *PRDM16* remains uncertain.

Around 30% of AML patients display the *FLT3-ITD* mutation. Patients with this mutation have a poor prognosis. Early detection of *FLT3-ITD* may allow for more sustained and permanent remissions.[24] Additionally, previous articles revealed that mutations in Nucleophosmin 1 (*NPM1*) *exon 12* may have prognostic importance in Egyptian AML patients, providing vital new prognostic information and potentially significantly affecting therapy choices.[25,26]

The current study evaluated the potential prognostic and predictive roles of *HOXB4* and *PRDM16* in newly diagnosed AML patients and established a correlation between their expression and other prognostic factors such as cytogenetic abnormalities, *FLT3-ITD, NPM1 exon 12* mutations, response to treatment, and patient survival.

Methods

Subjects and Samples

This study was conducted at the National Cancer Institute (NCI), Cairo University, Egypt, and included 83 newly diagnosed AML adult patients (median 40 years) referred to Medical Oncology Department between January 2018 and June 2021.

The inclusion criteria were that the patient had to be newly diagnosed with AML and have no prior treatment history. Exclusion criteria included being a secondary AML patient, having significantly compromised hepatic or renal function, having concomitant severe or uncontrolled medical problems (eg, uncontrolled diabetes, infection, or hypertension), or having a family history of hematological malignancies.

Ten apparently healthy persons (age and sex-matched) who had bone marrow (BM) aspiration for reasons other than malignancy served as normal controls.

All participants provided written informed consent. The research was approved by the institutional review board of the National Cancer Institute, Cairo University, Cairo, Egypt, following the Helsinki Declaration and its recent amendments.

Clinical, cytomorphological, cytogenetic, and molecular analyses of BM samples were used to diagnose all patients. The European LeukemiaNet-2017 (ELN-2017) genetic risk categorization system was used to classify AML patients.[27] All patients were diagnosed with AML using the FAB and WHO criteria.[28]

Treatment Protocol and Follow-Up

In general, all patients were given the standard front-line (3+7) IA/DA-like induction regimens consisting of idarubicin/ daunorubicin for three days (10/45 mg/m^2, Day 1–3) and cytarabine for seven days (100 mg/m^2, Day 1–7).

Complete remission (CR) was described as when less than 5% of leukemia blasts remain in the BM; extramedullary disorders were absent, neutrophil counts >1 x 10^9/L, and counting of platelets >100 x 10^9/L in the peripheral blood. Following CR, the consolidation was achieved by four cycles of high-dose cytarabine (2 g/m^2).

All patients were followed until June 2021. The OS was calculated from the date of AML diagnosis to the date of death and was censored at the time of the final follow-up. Patients who underwent hematopoietic stem cell transplantation (HSCT) were censored at the start of the procedure. DFS was estimated from the date of initial diagnosis to the date of relapse or death from any cause, whichever occurred first.

Sampling and Laboratory Work-Up

All patients had the following laboratory evaluations: peripheral blood examination (CBC: hemoglobin (Hb) level, total leukocyte count (TLC), platelet count, and blast cell percentage), bone marrow examination, IPT, and cytogenetic analysis.

Two drops of BM aspirate specimens were collected from all patients. The first was collected on K-EDTA for IPT and molecular analyses, and the second was collected on sodium heparin for conventional karyotyping and fluorescence in situ hybridization (FISH). Two BM aspirates were withdrawn to perform smear slides for morphology and cytochemistry.

Quantitative Real-Time PCR (qRT-PCR) of *PRDM16* and *HOXB4* mRNA

Total RNA was extracted from BM cells according to the manufacturer's recommendations using a QIAamp RNA extraction blood micro kit (QIAGEN® Austin, TX, USA, catalog no. 52304). The purity and concentration of extracted RNA were determined using a spectrophotometer NanoDrop (Quawell, Q-500, Scribner, USA) and the samples were kept at −80 °C until further evaluation.

Complementary DNA (cDNA) was produced according to the manufacturer's instructions using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA; catalog no. 4368814). The purity and concentration of complementary DNA were determined and then kept at −20 °C until qRT-PCR was performed.

The expression of *PRDM16* and *HOXB4* mRNA in enrolled samples was evaluated using TaqMan Universal PCR Master Mix II (Applied Biosystems, USA; Thermo Fisher Scientific, Cat. no. 4440040) and the *PRDM16* and *HOXB4* TaqMan Gene Expression Assay (Applied biosystems, USA, Thermo Fisher Scientific, Cat no 4453320, Hs 00223161-m1, Hs 00256884-m1). The expression of *PRDM16* and *HOXB4* was normalized to the endogenous control β-actin. qRT-PCR was performed using cDNA with the concentration adjusted depending on the abundance of mRNA. The thermal reaction conditions were as follows: 95 °C for 10 minutes (polymerase activation), followed by 40 cycles of 95 °C for 30 seconds (denaturation) and 60 °C for 60 seconds (annealing and extension), in which fluorescence was acquired and detected by StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

The relative expression of *HOXB4* and *PRDM16* gene expression was assessed relative to the housekeeping gene using the $2^{-\Delta\Delta Ct}$ method.[29] The data were expressed as the fold change in *HOXB4* and *PRDM16* gene expression in patients relative to healthy controls and normalized to the expression levels of the endogenous control.

Analysis of FLT3 Gene Mutations

According to the manufacturer's protocol, high molecular weight DNA was extracted from BM/EDTA samples using QIAamp DNA Blood Mini Kit (QIAGEN). PCR amplification was carried out using *FLT3-ITD* master mix containing dNTPs, the forward (5′-CAATTTAGGTATGAAAGCC-3′) and reverse (5′-GTACCTTTCAGCATTTTGAC-3′) primers (Invivoscribe Technologies, Inc., USA). Positive and negative control DNAs (Invivoscribe Technologies, Inc., USA), AmpliTaq DNA polymerase (Applied Biosystems, Life Technologies, USA), and 100 bp DNA ladder (Invitrogen, Life Technologies, USA) were used.

In brief, 1 µL DNA was amplified in a volume of 25 µL containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 Mm $MgCl_2$, 200 mM dNTPs, 0.5 µM of each primer, and 1 U Taq DNA polymerase (QIAGEN). The PCR consisted of an initial incubation step at 94 °C for 150 seconds followed by 35 cycles at 94 °C for 30 seconds, 57 °C for 60 seconds, 72 ° C for 120 seconds, and a final elongation step at 94 °C for 30 seconds, and 60 °C for 10 minutes. The PCR product was

analyzed on standard 3% agarose gel stained with ethidium bromide. A fragment of 328 base pairs (bp) was produced from wild-type (WT) alleles. All patients with an additional higher molecular weight band were considered *FLT3-ITD*+.

Analysis of *NPM1 Exon 12* Mutations

Patients with intermediate genetic risk (normal cytogenetic results) were selected for molecular analysis of *NPM1 exon 12* mutations.

Genomic DNA was extracted from BM/EDTA samples using QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's protocol. For *NPM1* mutation analysis, *NPM1 exon 12* was amplified by genomic PCR using primers NPMex12F/ CTGATGTCTATGAAGTGTTGTGGTTCC (sense) and NPMex12R/ CTCTGCATTATAAAAAGG ACAGCCAG (antisense). The reaction mixture was made up of 50 µL of the following constituents: 100 ng of genomic DNA, 0.5 U Taq DNA polymerase, 1X Taq polymerase buffer, 1.75 mM $MgCl_2$, 0.4 µM *NPM1* primers, and 0.4 mM dNTP. The samples were amplified by initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 53 °C for 1 minute, 72 °C for 2 minutes, and final extension at 72 °C for 10 minutes. They were checked on 2% agarose gel electrophoresis using a DNA marker.

PCR products were mixed with ten volumes of loading buffer, denatured at 96 °C for 5 minutes, quenched on ice immediately, and applied to 10% polyacrylamide gel electrophoresis. Normal *NPM1* exhibits a specific conformational pattern. A mutant gene displays a pattern with different electrophoretic mobility (mobility shift)

Statistical Analysis

The statistical analyses were carried out using the IBM SPSS (SPSS for Windows release, version 22.0, SPSS, Chicago, IL, USA). Continuous variables were expressed as mean and standard deviation or median and range, whichever is appropriate. To represent categorical variables, the frequency and percentage were used. Chi-square and Fisher's exact test investigated the correlation between qualitative variables. The Kruskal–Wallis test (non-parametric ANOVA) compared three groups, followed by post-hoc pair-wise comparisons. Mann–Whitney test was used to compare two groups. The Kaplan-Meier method was used for survival analysis, and the Log rank test was used to compare the two survival curves. The tests were two-tailed, and a p-value <0.05 was deemed significant.

Results

Patient's Characteristics

Table 1 shows the baseline patient characteristics. The median age at diagnosis for the entire study cohort was 40 (range, 18–57) years, with 46 males (55.4%) and 37 females (44.6%) present. BM hypercellularity was found in 62 patients (74.7%). AML-M2 was the most frequent FAB subtype representing 39.8% of the patients, followed by M4 and M1 subtypes. Sixteen patients (19.3%) were classified as high-risk, 36 (43.4%) were intermediate-risk, and 31 (37.3%) were low-risk. Mutational analysis has shown that six patients (7.2%) had *FLT3-ITD* mutations while seven patients (19.4%) had *NPM1 exon 12* mutations.

Expression of *HOXB4* and *PRDM16* in AML Patients

Figure 1 shows that the mean fold change of *HOXB4* and *PRDM16* gene expressions were significantly higher in AML (23.49 and 17.36, respectively) compared to the control (0.94 and 1.16, respectively; p <0.001). The *HOXB4* and *PRDM16*mRNA expression was classified into two categories (low vs high) according to the median of the *HOXB4* and *PRDM16* gene expression (3.21 and 0.67, respectively). High expression of *HOXB4* was found in 68.67% (57/83) of AML patients, whereas high expression of *PRDM16* was evident in 33.73% (28/83) of AML patients, as shown in Table 2.

Relations Between *HOXB4* and *PRDM16* Expression and Patient's Characteristics

No significant associations were found between *HOXB4* and *PRDM16* expression and patient characteristics except for the significant-high *HOXB4* expression in the male group (36/46, 78.3%) as compared to the female group (21/37,

Table 1 Demographic Data of All Studied Patients

Variables		**N= 83**	**%**
Age: (years)*		40.0 (18–57)	
Gender	Male	46	55.4
	Female	37	44.6
TLC x10^9 /mm^3*		30.0 (0.5–616.0)	
Hb (gm/dl) *		7.7 (3.7–13.2)	
Platelets x10^9 /mm^3*		36.0 (5.0–826.0)	
Peripheral blood blasts %*		42.0 (0.0–96.0)	
BM blasts %*		60.0 (20.0–97.0)	
BM cellularity	Normocellular	17	20.5
	Hypercellular	62	74.7
	Hypocellular	4	4.8
FAB	M0	2	2.4
	M1	12	14.5
	M2	33	39.8
	M3	2	2.4
	M4	25	30.1
	M5	6	7.2
	M7	3	3.6
IPT	Myeloid	49	59.0
	Monocytic	6	7.2
	Myelomonocytic	25	30.1
	Megakaryoblastic	3	3.6
Genetic risk	Low	31	37.3
	Intermediate	36	43.4
	High	16	19.3
FLT3-ITD	Wild	77	92.8
	Mutant	6	7.2
NPM1 exon 12 (N=36)	Wild	29	80.6
	Mutant	7	19.4

Note: *Median (Min-Max).

56.8%) (p=0.036) and high *PRDM16* among patients with hypocellular BM (4/4, 100%) as compared to those with hypercellular BM (2/17, 11.8%) and normocellular BM (22/62, 35.5%) (p=0.002). Out of the 50 (50/83, 60.24%) patients who achieved CR, relapse occurred in 16% of the cases (8/50). Expressions of *HOXB4* and *PRDM16* were not significantly associated with CR or relapse. (Tables 3 and 4).

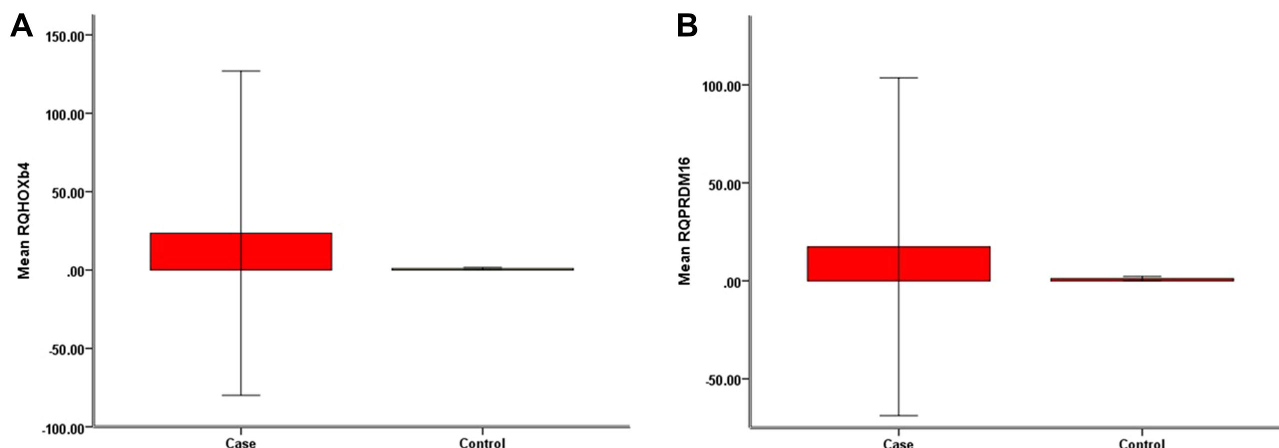

Figure 1 The mean fold change of *HOXB4* (**A**) and *PRDM16* (**B**) gene expressions.

Survival Analysis and Response to Treatment

The median follow-up period was 29.3 months. The survival analyses revealed no significant differences in the OS and DFS outcomes between *HOXB4* or *PRDM16* high and low expressers in AML patients (Table 5, Figure 2).

Table 2 Expression of *HOXB4* and *PRDM16*

Variables		AML	
		N=83	%
HOXB4	Low Expression	26	31.3
	High Expression	57	68.7
PRDM16	Low Expression	55	66.2
	High Expression	28	33.8

Table 3 Relation Between *HOXB4* and All Other Variables

Variables		*HOXB4*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
Age: (years)*		38.50 (18–56)		40.00 (18–57)		−0.692	NS
TLC x10^9 /mm^3*		25.00 (1.3–358)		40.00 (0.50–616)		−1.164	NS
Hb (gm/dl)*		8.00 (4.0–12.0)		7.60 (3.7–13.2)		−0.692	NS
Platelets x10^9 /mm^3*		33.50 (10.0–225.0)		36.00 (5.0–826.0)		−0.191	NS
Peripheral blood blasts %*		38.50 (10.0–85.0)		47.00 (0.0–96.0)		−0.285	NS
BM blasts %*		49.50 (25.0–97.0)		63.00 (20–0–96.0)		−1.287	NS
Sex	Male	10	21.7	36	78.3	4.408	0.036 #
	Female	16	43.2	21	56.8		

(*Continued*)

Table 3 (Continued).

Variables		*HOXB4*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
BM cellularity	Normocellular	6	35.3	11	64.7	1.974	NS
	Hypercellular	20	32.3	42	67.7		
	Hypocellular	0	0.0	4	100.0		
FAB	M0	0	0.0	2	100.0	9.405	NS
	M1	3	25.0	9	75.0		
	M2	13	39.4	20	60.6		
	M3	2	100.0	0	0.0		
	M4	4	16.0	21	84.0		
	M5	3	50.0	3	50.0		
	M7	1	33.3	2	66.7		
IPT	Myeloid	18	36.7	31	63.3	4.479	NS
	Monocytic	3	50.0	3	50.0		
	Myelomonocytic	4	16.0	21	84.0		
	Megakaryoblastic	1	33.3	2	66.7		
Genetic risk	LR	13	41.9	18	58.1	4.268	NS
	IR	7	19.4	29	80.6		
	HR	6	37.5	10	62.5		
FLT3-ITD	Wild	22	28.5	55	71.5	0.012	NS
	Mutant	4	66.7	2	33.3		
NPM1 exon 12	Wild	7	24.1	22	75.2	2.419	NS
	Mutant	0	0.0	7	100.0		
CR	No	10	30.3	23	69.7	1.850	NS
	Yes	16	32.0	34	68.0		
Relapse	No	13	31.0	29	69.0	0.132	NS
	Yes	3	37.5	5	62.5		

Note: *Median (Min-Max), NS: non-significant, p value set significant at ≤0.05, # significant difference between high expression and low expression groups.

Discussion

AML is a malignant illness of the bone marrow defined by the arrest of hematopoietic precursors at an early stage of development. It is the most prevalent form of leukemia in adults and is associated with a poor prognosis.[30]

Additionally, genetic anomalies affect the progression and recurrence of AML, which may aid in targeting treatment and improving prognosis. AML is a clonal illness characterized by various genetic defects, but little is known about the molecular processes behind clinical variability within the same cytogenetic risk group.[31,32]

Table 4 Relation Between *PRDM16* and All Other Variables

Variables		*PRDM16*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
Age: (years)*		39.00 (18–56)		45.50 (18–57)		−1.123	NS
TLC x10^9 /mm^3*		30.00 (0.5–616.0)		27.50 (1.90–242.0)		−0.356	NS
Hb (gm/dl)*		7.60 (3.8–13.2)		7.90 (3.7–12.0)		−1.243	NS
Platelets x10^9 /mm^3*		40.00 (5.0–283.0)		29.00 (9.0–826.0)		−1.146	NS
Peripheral blood blasts %*		42.00 (0.0–96.0)		43.50 (5.0–90.0)		−0.058	NS
BM blasts %*		57.00 (25.0–97.0)		64.50 (20.0–88.0)		−0.092	NS
Sex	Male	27	58.7	19	41.3	2.645	NS
	Female	28	75.7	9	24.3		
BM Cellularity	Normocellular	15	88.2	2	11.8	10.771	0.002 #
	Hypercellular	40	64.5	22	35.5		
	Hypocellular	0	0.0	4	100.0		
FAB	M0	1	50.0	1	50.0	8.276	NS
	M1	7	58.3	5	41.7		
	M2	26	78.8	7	21.2		
	M3	2	100.0	0	0.0		
	M4	12	48.0	13	52.0		
	M5	5	83.3	1	16.7		
	M7	2	66.7	1	33.3		
IPT	Myeloid	36	73.5	13	26.5	5.453	NS
	Monocytic	5	83.3	1	16.7		
	Myelomonocytic	12	48.0	13	52.0		
	Megakaryoblastic	2	66.7	1	33.3		
Genetic risk	LR	23	74.2	8	25.8	3.265	NS
	IR	20	55.6	16	44.4		
	HR	12	75.0	4	25.0		
FLT3-ITD	Wild	53	68.8	24	31.2	0.000	NS
	Mutant	2	33.3	4	66.7		
NPM1 exon 12	Wild	15	51.7	14	48.3	0.042	NS
	Mutant	5	71.4	2	28.6		
CR	No	19	57.6	14	42.4	0.132	NS
	Yes	36	72.0	14	28.0		

(*Continued*)

Table 4 (Continued).

Variables		*PRDM16*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
Relapse	No	30	71.4	12	28.6	0.043	NS
	Yes	6	75.0	2	25.0		

Notes: *Median (Min-Max), p-value set significant at ≤0.05, # significant difference between high expression and low expression groups.
Abbreviation: NS, non-significant.

Table 5 Relation Between *HOXB4* and *PRDM16* Expressions and Survivals

Variables		OS					DFS				
		No=83	No. of Events	Median Survival Time	24-Month Survival Estimate	P-value	No=49	No. of Events	Median Survival Time	24-Month Survival Estimate	P-value
HOXB4	Low Expression High Expression	26 57	41 18	2.401 5.395	0.200 0.280	NS	16 33	3 5	NR 26.908	0.606 0.646	NS
PRDM16	Low Expression High Expression	55 28	39 20	4.211 1.678	0.245 0.214	NS	36 13	6 2	26.91 NR	0.660 0.700	NS

Note: *NR (median not reached).

The human *HOX* gene family consists of 39 members clustered on four distinct chromosomes.[33] Although overexpression of the *HOX* family has been seen in AML with normal karyotypes, the prognostic relevance of each *HOX* gene differs.[34] Additionally, *PRDM16* is a transcription factor required for the maintenance of hematopoietic stem cells. *PRDM16* has been documented to be mutated, translocated, or expressed abnormally in several subgroups of AML.[21,35–37]

We reported that the mean fold change of *HOXB4* and *PRDM16* expression was significantly higher in AML compared to the control. These results agree with Shiba et al,[23] who discovered overexpression of the *PRDM16* gene in 23% (84/369) of juvenile de novo AML patients after establishing an optimum *PRDM16* gene expression cutoff threshold. Further, Yamato et al[38] studied *PRDM16* expression in 151 AML patients and found that 47 (31%) individuals had elevated *PRDM16* expression. Our results are consistent with Umeda et al,[34] who examined the expression of newly defined hematopoietic stem cell factors including *HOXB4* in BM from de novo AML patients, and found that *HOXB4* was substantially more abundant in AML than in normal controls. In cell culture and murine BM transplantation assays, Bansal et al[39] reported that *HOX* genes also were dysregulated in leukemic BM with up-regulation of *HOXB4* mainly.

In addition, we reported that *FLT3-ITD* mutations were detected in 6 patients (7.2%), while *NPM1 exon 12* mutations were detected in 7 patients (19.4%) out of 36 patients with intermediate genetic risk. We found that all patients with *NPM1 exon 12* mutations and 33.3% of patients with *FLT3-ITD* mutations had high expression of *HOXB4*. On the other hand, our results revealed that 71.4% of patients with *NPM1 exon 12* mutations and 33.3% of patients with *FLT3-ITD* mutations had low expression of *PRDM16*. The relation between these mutations and the expressions of *HOXB4* and *PRDM16* may be illustrated by their impact on good CR and relapse rate.

Furthermore, we observed that the expression of *HOXB4* and *PRDM16* was not significantly associated with CR or relapse. Low expression of *HOXB4* and high expression of *PRDM16* were associated with CR of 32% and 28%, respectively. These results disagree with Yamato et al[38] and Umeda et al,[34] who observed that high *PRDM16* and low *HOXB4* expressions are significant predictive markers for poor prognosis in AML patients.

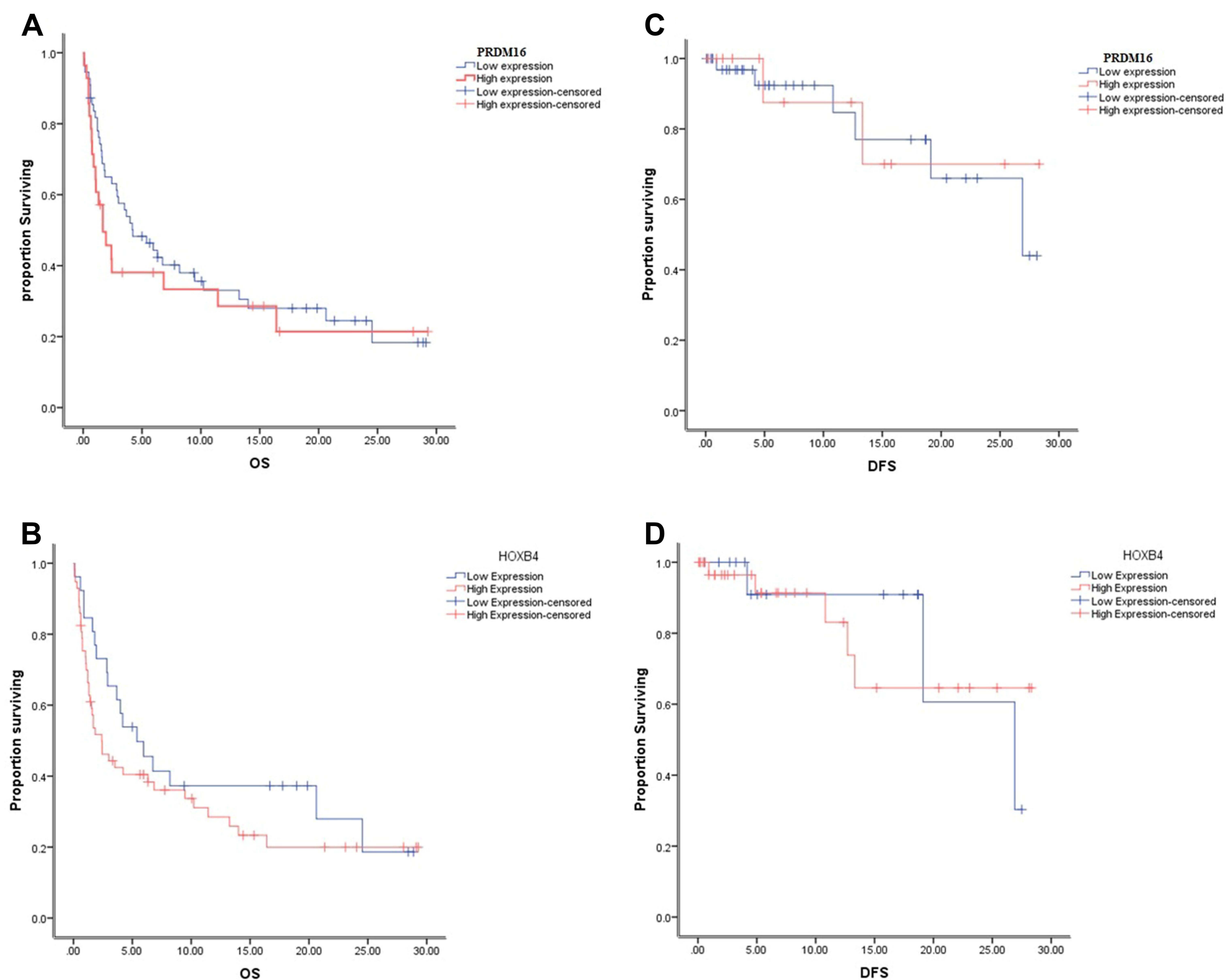

Figure 2 (**A** and **B**) Kaplan–Meier curves with log-rank statistics describe OS according to *PRDM16* and *HOXB4* expression level. (**C** and **D**) Kaplan–Meier curves with log-rank statistics describe DFS according to *PRDM16* and *HOXB4* expression levels.

Interestingly, concerning the OS, our analyses revealed a short OS and DFS in high *PRDM16* and low *HOXB4* expressions AML patients with no significant differences. In accordance with our finding, Shiba et al[23] reported that the OS among *PRDM16*-overexpressing patients was significantly worse than in patients with low *PRDM16* expression (51% vs 81%, $P < 0.001$). Further, the 5yr OS was significantly worse in high-*PRDM16*-expression patients than in low-*PRDM16*-expression patients (18% vs 34%; $P=0.002$), as reported by Yamato et al.[38]

Conclusion

High *PRDM16* and low *HOXB4* gene expressions may be used as poor prognostic and predictive markers in newly diagnosed AML adult patients, but larger studies are needed to prove these results. The correlation between *PRDM16* and *HOXB4* gene expressions and *FLT3-ITD* and *NPM1 exon 12* mutations might have a role in CR, relapse, OS, and DFS, however, this should be clarified in analysis with a larger number of samples.

Funding

This research received no external funding.

Disclosure

The authors declare no conflicts of interest in relation to this work.

References

1. Jordan: CT. Unique molecular and cellular features of acute myelogenous leukemia stem cells. *Leukemia*. 2002;16(4):559–562. doi:10.1038/sj.leu.2402446
2. Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer*. 2006;107(9):2099–2107. doi:10.1002/cncr.22233
3. de Rooij JD, Zwaan CM, van den Heuvel-Eibrink M. Pediatric AML: from biology to clinical management. *J Clin Med*. 2015;4(1):127–149. doi:10.3390/jcm4010127
4. Potter N, Miraki-Moud F, Ermini L, et al. Single cell analysis of clonal architecture in acute myeloid. *leukaemia*. 2019;33(5):1113–1123. doi:10.1038/s41375-018-0319-2
5. Acute myeloid leukemia early detection, diagnosis, and types. In: American Cancer Society. Available from: http://www.cancer.org/cancer/leukemia-acutemyeloidaml/detailedguide/leukemia-acute-myeloid-myelogenous-classified. Accessed August 21, 2018.
6. Illangeswaran RSS, Das S, Paul DZ, Mathews V, Balasubramanian P. A personalized approach to acute myeloid leukemia therapy: current options. *Pharmgenomics Pers Med*. 2019;12:167–179. doi:10.2147/pgpm.s168267
7. Gbadamosi B, Ezekwudo D, Bastola S, Jaiyesimi: I. Predictive and prognostic markers in adults with acute myeloid leukemia: a single-institution experience. *Clin Lymphoma Myeloma Leuk*. 2018;18(7):e287–e294. doi:10.1016/j.clml.2018.05.005
8. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan: AM. Epidemiology of acute myeloid leukemia: recent progress and enduring challenges. *Blood Rev*. 2019;36:70–87. doi:10.1016/j.blre.2019.04.005
9. Bodey B, Bodey B Jr., Gröger AM, Siegel SE, Kaiser: HE. Immunocytochemical detection of homeobox B3, B4, and C6 gene product expression in lung carcinomas. *Anticancer Res*. 2000;20(4):2711–2716.
10. Li N, Gou J-H, Xiong J, You -J-J, Li Z-Y. *HOXB4* promotes the malignant progression of ovarian cancer via DHDDS. *BMC Cancer*. 2020;20(1):222. doi:10.1186/s12885-020-06725-4
11. Shears L, Plowright L, Harrington K, Pandha HS, Morgan R. Disrupting the interaction between HOX and PBX causes necrotic and apoptotic cell death in the renal cancer lines CaKi-2 and 769-P. *J Urol*. 2008;180(5):2196–2201. doi:10.1016/j.juro.2008.07.018
12. Morgan R, Simpson G, Gray S, et al. HOX transcription factors are potential targets and markers in malignant mesothelioma. *BMC Cancer*. 2016;16(1). doi:10.1186/s12885-016-2106-7
13. Wang H, Jia XH, Chen JR, et al. *HOXB4* knockdown reverses multidrug resistance of human myelogenous leukemia K562/ADM cells by downregulating P-gp, MRP1 and BCRP expression via PI3K/Akt signaling pathway. *Int J Oncol*. 2016;49(6):2529–2537. doi:10.3892/ijo.2016.3738
14. Dumas PY, Mansier O, Prouzet-Mauleon V, et al. MiR-10a and *HOXB4* are overexpressed in atypical myeloproliferative neoplasms. *BMC Cancer*. 2018;18(1):1098. doi:10.1186/s12885-018-4993-2
15. Zhou G, Liu X, Xiong B, Sun: Y. Homeobox B4 inhibits breast cancer cell migration by directly binding to StAR-related lipid transfer domain protein 13. *Oncol Lett*. 2017;14(4):4625–4632. doi:10.3892/ol.2017.6825
16. Benezeder T, Tiran V, Treitler AAN, et al. Multigene methylation analysis of enriched circulating tumor cells associates with poor progression-free survival in metastatic breast cancer patients. *Oncotarget*. 2017;8(54):92483–92496. doi:10.18632/oncotarget.21426
17. Hohenauer T, Moore: AW. The Prdm family: expanding roles in stem cells and development. *Development*. 2012;139(13):2267–2282. doi:10.1242/dev.070110
18. Di Zazzo E, De Rosa C, Abbondanza C, Moncharmont: B. PRDM proteins: molecular mechanisms in signal transduction and transcriptional regulation. *Biology*. 2013;2(1):107–141. doi:10.3390/biology2010107
19. Pinheiro I, Margueron R, Shukeir N, et al. Prdm3 and *PRDM16* are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. *Cell*. 2012;150(5):948–960. doi:10.1016/j.cell.2012.06.048
20. Chi J, Cohen: P. The multifaceted roles of *PRDM16*: adipose biology and beyond. *Trends Endocrinol Metab*. 2016;27(1):11–23. doi:10.1016/j.tem.2015.11.005
21. Aguilo F, Avagyan S, Labar A, et al. *PRDM16* is a physiologic regulator of hematopoietic stem cells. *Blood, J Am Soci Hematol*. 2011;117(19):5057–5066.
22. Morishita: K. Leukemogenesis of the EVI1/MEL1 gene family. *Int J Hematol*. 2007;85(4):279–286. doi:10.1532/IJH97.06174
23. Shiba N, Ohki K, Kobayashi T, et al. High PRDM 16 expression identifies a prognostic subgroup of pediatric acute myeloid leukaemia correlated to FLT 3-ITD, KMT 2A-PTD, and NUP 98-NSD 1: the results of the Japanese Paediatric Leukaemia/Lymphoma Study Group AML-05 trial. *Br J Haematol*. 2016;172(4):581–591. doi:10.1111/bjh.13869
24. El-Meligui YM, Abd Elrhman HE, Salahuddin A, Hamouda MA, Kassem: AB. Correlation Study on HLA-DR and CD117 (c-Kit) expressions: its prognosis and treatment response in acute myeloid leukemia patients. *Pharmgenomics Pers Med*. 2021;14(381):381–393. doi:10.2147/PGPM.S268986
25. Zidan M, Shaaban H, El Ghannam: D. Prognostic impact of nucleophosmin 1 (*NPM1*) gene mutations in Egyptian acute myeloid leukemia patients. *Turk J Hematol*. 2013;30(2):129. doi:10.4274/Tjh.2012.0048
26. Kassem N, Hamid AA, Attia T, et al. Novel mutations of the nucleophosmin (NPM-1) gene in Egyptian patients with acute myeloid leukemia: a pilot study. *J Egypt Natl Canc Inst*. 2011;23(2):73–78. doi:10.1016/j.jnci.2011.09.003
27. Herold T, Rothenberg-Thurley M, Grunwald VV, et al. Validation and refinement of the revised 2017 European LeukemiaNet genetic risk stratification of acute myeloid leukemia. *Leukemia*. 2020;34(12):3161–3172. doi:10.1038/s41375-020-0806-0
28. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood, J Am Soci Hematol*. 2016;127(20):2391–2405.
29. Rao X, Huang X, Zhou Z, Lin: X. An improvement of the 2ˆ(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath*. 2013;3(3):71–85.

30. Abdellateif MS, Kassem AB, El-Meligui: YM. Combined expression of CD34 and FLT3-internal tandem duplication mutation predicts poor response to treatment in acute myeloid leukemia. *Int J Gen Med*. 2020;13(867):867–879. doi:10.2147/IJGM.S276138
31. Moarii M, Papaemmanuil: E. Classification and risk assessment in AML: integrating cytogenetics and molecular profiling. *Hematology Am Soc Hematol Educ Program*. 2017;2017(1):37–44. doi:10.1182/asheducation-2017.1.37
32. Kumar: CC. Genetic abnormalities and challenges in the treatment of acute myeloid leukemia. *Genes Cancer*. 2011;(2)(2):95–107. doi:10.1177/1947601911408076
33. Gonçalves CS, Le Boiteux E, Arnaud P, Costa: BM. HOX gene cluster (de)regulation in brain: from neurodevelopment to malignant glial tumours. *Cell Mol Life Sci*. 2020;77(19):3797–3821. doi:10.1007/s00018-020-03508-9
34. Umeda S, Yamamoto K, Murayama T, et al. Prognostic significance of *HOXB4* in de novo acute myeloid leukemia. *Hematology*. 2012;17(3):125–131. doi:10.1179/102453312x13376952196250
35. Hu T, Morita K, Hill MC, et al. *PRDM16*s transforms megakaryocyte-erythroid progenitors into myeloid leukemia-initiating cells. *Blood*. 2019;134(7):614–625. doi:10.1182/blood.2018888255
36. Gudmundsson K, Nguyen N, Oakley K, et al. *PRDM16* is a critical regulator of adult long-term hematopoietic stem cell quiescence. *Proc Natl Acad Sci*. 2020;117:202017626. doi:10.1073/pnas.2017626117
37. Corrigan D, Luchsinger L, Snoeck: H. The role of *PRDM16* and its isoforms in acute myeloid leukemia. *Exp Hematol*. 2016;44(9):S65. doi:10.1016/j.exphem.2016.06.111
38. Yamato G, Yamaguchi H, Handa H, et al. Clinical features and prognostic impact of *PRDM16* expression in adult acute myeloid leukemia. *Genes Chromosomes Cancer*. 2017;56(11):800–809. doi:10.1002/gcc.22483
39. Bansal D, Scholl C, Fröhling S, et al. Cdx4 dysregulates Hox gene expression and generates acute myeloid leukemia alone and in cooperation with Meis1a in a murine model. *Proc Natl Acad Sci USA*. 2006;103(45):16924–16929. doi:10.1073/pnas.0604579103

Pharmacogenomics and Personalized Medicine Dovepress

Publish your work in this journal

Pharmacogenomics and Personalized Medicine is an international, peer-reviewed, open access journal characterizing the influence of genotype on pharmacology leading to the development of personalized treatment programs and individualized drug selection for improved safety, efficacy and sustainability. This journal is indexed on the American Chemical Society's Chemical Abstracts Service (CAS). The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/pharmacogenomics-and-personalized-medicine-journal