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

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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
patients. The average 2-yr and 5-yr overall survival (OS) rates of patients diagnosed with AML are approximately 32% and 27%, respectively. 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, ovarian, bladder, renal, mesothelioma, and leukemia. Additionally, elevated HOXB4 expression is related to a poor prognosis for malignant mesothelioma. Other investigations have shown downregulation of the HOX gene in cancer tissues.

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. PRDM16 has intrinsic histone methyltransferase activity, allowing it to catalyze histone-3 lysine methylation (H3K9me1). As a result, PRDM16 may also participate as a transcriptional regulator, either directly or indirectly, via complex formation with histone-modifying enzymes. PRDM16 is required to maintain hematopoietic stem cells, which makes it an attractive potential gene for leukemogenesis induction. While new research suggests that PRDM16 may contribute to the prediction of poor outcomes in juvenile AML patients, 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. 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.

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 (e.g., 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. All patients were diagnosed with AML using the FAB and WHO criteria.

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/m², Day 1–3) and cytarabine for seven days (100 mg/m², 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^-ΔΔCt 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’-CAATTTAGGATGAAAGCC-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 MgCl₂, 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/CTGATGTCTATGAAAGTGTTGGTTTCC (sense) and NPMex12R/CTCTGCATTATAAAAAGGACAGCCAG (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 MgCl2, 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,
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).

### 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*                  |       |       |
| Hb (gm/dl) *                     | 30.0 (0.5–616.0) |       |
| Platelets x10^9 /mm^3*           |       |       |
| 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).*
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 |       |
| Age: (years)*           | N | % | N | % |
|                         | 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)
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. 

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.

**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. 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.
### 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 x10⁹ /mm³*           | 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 x10⁹ /mm³*     | 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)
The human HOX gene family consists of 39 members clustered on four distinct chromosomes. Although overexpression of the HOX family has been seen in AML with normal karyotypes, the prognostic relevance of each HOX gene differs.

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.

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, 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 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, 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 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 and Umeda et al, who observed that high PRDM16 and low HOXB4 expressions are significant predictive markers for poor prognosis in AML patients.

### 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 | 26 | 41 | 2.401 | 0.200 | NS | 16 | 3 | 26.908 | 0.646 | NS |
| High Expression | 57 | 18 | 5.395 | 0.280 |          | 33 | 5 |         |          |          |
| PRDM16    |        |                |                   |                  |          |        |                |                   |                  |          |
| Low Expression | 55 | 39 | 4.211 | 0.245 | NS | 36 | 6 | 26.91 | 0.660 | NS |
| High Expression | 28 | 20 | 1.678 | 0.214 |          | 13 | 2 |         |          |          |

Note: *NR (median not reached).
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. 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.

**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.

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