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# Clinical features and prognostic impact of *PRDM16* expression in adult acute myeloid leukemia

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## Abstract

High *PRDM16* (also known as *MEL1*) expression is a representative marker of acute myeloid leukemia (AML) with *NUP98-NSD1* and is a significant predictive marker for poor prognosis in pediatric AML. However, the clinical features of adult AML with *PRDM16* expression remain unclear. *PRDM16* is highly homologous to *MDS1/EVI1*, which is an alternatively spliced transcript of *MECOM* (also known as *EVI1*). We investigated *PRDM16* expression in 151 AML patients, with 47 (31%) exhibiting high *PRDM16* expression (*PRDM16/ABL1* ratio $\geq 0.010$). High *PRDM16* expression significantly correlated with *DNMT3A* (43% vs. 15%, $P < 0.001$) and *NPM1* (43% vs. 21%, $P = 0.010$) mutations and partial tandem duplication of *KMT2A* (22% vs. 1%, $P < 0.001$). Remarkably, high-*PRDM16*-expression patients were frequent in the noncomplete remission group (48% vs. 21%, $P = 0.002$). Overall survival (OS) was significantly worse in high-*PRDM16*-expression patients than in low-*PRDM16*-expression patients (5-year OS, 18% vs. 34%; $P = 0.002$). This trend was observed more clearly among patients aged <65 years (5-year OS, 21% vs. 50%; $P = 0.001$), particularly in *FLT3*-ITD-negative patients in the intermediate cytogenetic risk group (5-year OS, 25% vs. 59%; $P = 0.009$). These results suggest that high *PRDM16* expression is a significant predictive marker for poor prognosis in adult AML patients, similar to pediatric AML patients.

  

## 1 | INTRODUCTION

Acute myeloid leukemia (AML) is a complex disease caused by various genetic alterations. Several prognosis-associated cytogenetic aberrations or gene mutations, such as *t*(8;21)(q22;q22)/*RUNX1-RUNX1T1*, inv(16)(p13q22)/*CBFB-MYH11*, *t*(16;21)(p11;q22)/*FUS-ERG*, *KMT2A* rearrangements, *KIT*, *NPM1*, *CEBPA*, and *FLT3*-internal tandem duplication (ITD), are used to stratify the risk.[1–3] Furthermore, some gene mutations have been implicated in AML pathogenesis, including *DNMT3A*, *IDH1/2*, and *TET2*, by recently developed massively parallel sequencing technologies.[2–11] However, even after incorporating these molecular markers, there are many patients whose prognosis remains uncertain.

Recently, *NUP98-NSD1* was identified as a poor prognostic factor for both adult and pediatric AML.[12,13] We have reported that all pediatric AML patients with *NUP98-NSD1* showed high expression of the PR domain containing 16 (*PRDM16*; also known as *MEL1*),[13] which is a zinc finger transcription factor located at 1p36.3 identified from the breakpoint of *t*(1;3)(p36;q21)/*RPM1-PRDM16*.[14] Interestingly, *PRDM16* is highly homologous to *MDS1/EVI1*, which is an alternatively spliced transcript of *MECOM* (also known as *EVI1*).[14] Furthermore, *PRDM16* is essential for the maintenance of hematopoietic stem cells[15]; hence, it is a remarkable candidate gene to induce leukemogenesis.[16] Although recent reports revealed that high *PRDM16* expression was a significant predictive marker for poor prognosis in pediatric AML patients,[17,18] the significance of *PRDM16* expression in adult AML patients is unclear. Thus, we investigated *PRDM16* and *MECOM* expression and its correlation with other gene aberrations to verify the prognostic impact of *PRDM16* expression.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients and samples

A total of 151 patients with *de novo* AML referred to our institutions between 1996 and 2015 were included in this study. The characteristics of patients are described in Table 1. The Chromosomal Classification according to the 2013 NCCN guidelines classified these patients into those with favorable cytogenetic risk, intermediate cytogenetic risk, and adverse cytogenetic risk. Patients diagnosed with acute promyelocytic leukemia or Down syndrome-associated AML were excluded from this study. The protocols were approved by the institutional review boards of Gunma University Hospital and Nippon Medical School Hospital. The present study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

### 2.2 | Quantitative RT-PCR analysis

All leukemic samples were obtained from either bone marrow or peripheral blood at diagnosis, with DNA and RNA prepared using the ALLPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). Quantitative RT-PCR analysis was performed using the 7900HT Fast Real Time PCR System, TaqMan Gene Expression Master Mix, and TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA).[18] *ABL1* was also evaluated as a control gene.[19] TaqMan Gene Expression Assays Hs00922674_ml, Hs00602795_ml, and Hs01104728_m1 were used for *PRDM16*, *MECOM*, and *ABL1*, respectively. cDNA was prepared using 0.8–1.0 μg of total RNA and Ready-To-Go RT-PCR Beads (GE Healthcare, Buckinghamshire, UK); 1/200 dilution of the prepared cDNA was used as a template for each PCR reaction. We defined *PRDM16* and *MECOM* expression cutoff point as *PRDM16/ABL1* ratio $\geq$0.010 and *MECOM/ABL1* ratio $\geq$0.10, according to the results of pediatric AML, which were previously reported.[17,18]

### 2.3 | Chromosomal and genetic analyses

Chromosomal abnormalities were screened using conventional G-banding. Gene rearrangement of *NUP98-NSD1* in all 151 patients was analyzed with RT-PCR.[12] Mutational analyses of genes located in known hot spots (*FLT3*-ITD and *NPM1*), genes for which probe design for emulsion sequencing was difficult (*CEBPA*), and those for which analysis with Ion torrent personal genome machine (Thermo Fisher Scientific, Waltham, MA) was difficult [partial tandem duplication of *KMT2A* (*KMT2A-PTD*)] were performed in 112 patients at the Nippon Medical School Hospital using previously reported methods.[20] An oligonucleotide library was generated with emulsion PCR using order-made probes designed against all *DNMT3A* exons.[21] The library was analyzed using the next-generation sequencer the Ion torrent personal genome machine (Thermo Fisher Scientific). A satisfactory depth of coverage was obtained for all these exons in each sample (59–2000). As for the 39 patients at the Gunma University Hospital, mutational analysis of *FLT3*-ITD, *NPM1*, *CEBPA*, and *DNMT3A* were performed with Sanger sequencing using previously reported methods.[20]

### 2.4 | Statistical analysis

All analyses were performed using EZR (version 1.32. Saitama Medical Centre, Jichi Medical University, Saitama, Japan).[22] Continuous variables are presented as means $\pm$ standard deviations (SD) and/or medians with ranges. Categorical variables are presented as frequencies and percentages. For all analyses, *P* values were two-tailed and a *P* value of $<$0.05 was considered statistically significant. Fisher's exact test, $\chi^2$ analysis, and Mann–Whitney test were used as appropriate for comparisons between groups. Moreover, the Kaplan–Meier method was used to analyze overall survival (OS). Differences in survival were assessed using the log-rank test. OS was defined according to the European Leukemia Net (ELN) recommendations of 2017.[23] The median length of the follow-up for censored patients was 34.0 months (1.4–60.0 months).

With respect to prognostic factors, multivariate analysis was conducted with the Cox proportional hazards model. Initially, we included all genetic variables, age ($\geq$65 years), stem cell transplantation (SCT) status at first complete remission (CR), and *PRDM16* and *MECOM* expression patterns in the first model and then sequentially removed

**TABLE 1** Characteristics of all AML patients

| Characteristics | Total AML $n = 151$ | Favorable $n = 21$ | Cytogenetic classification Intermediate $n = 105$ | Adverse $n = 25$ |
|---|---|---|---|---|
| | | *t*(8;21): 14 | Normal karyotype: 68 | Complex karyotype: 11[a] |
| | | inv(16), *t*(16;16): 7 | Trisomy 8: 5 | *t*(11q23) excluding *t*(9;11): 9 |
| | | | Others: 25 | Monosomy 7: 5[a] |
| | | | Not determined: 7[b] | *t*(3q21): 1 |
| | | | | *t*(6;9): 2 |
| Male/female | 87/64 | 15/6 | 62/43 | 10/15 |
| Age at diagnosis median (range) | 61 (17–88) | 53 (30–74) | 64 (17–83) | 55 (21–83) |
| 15–24 | 6 | 0 | 5 | 1 |
| 25–34 | 11 | 2 | 7 | 2 |
| 35–44 | 20 | 5 | 11 | 4 |
| 45–54 | 19 | 4 | 11 | 4 |
| 55–64 | 25 | 3 | 18 | 4 |
| 65–74 | 46 | 7 | 31 | 8 |
| ≥75 | 24 | 0 | 22 | 2 |
| FAB subtype | | | | |
| M0 | 3 | 0 | 2 | 1 |
| M1 | 40 | 2 | 34 | 4 |
| M2 | 45 | 13 | 24 | 8 |
| M4 | 22 | 6 | 14 | 2 |
| M5 | 22 | 0 | 17 | 5 |
| M6 | 3 | 0 | 3 | 0 |
| M7 | 1 | 0 | 0 | 1 |
| Not determined | 15 | 0 | 11 | 4 |
| Induction therapy[c] | 134 | 21 | 92 | 21 |
| IDA/AraC or DNR/AraC | 55 | 10 | 34 | 11 |
| Others | 79 | 11 | 58 | 10 |
| HSCT on 1st CR | 8 | 2 | 5 | 1 |

IDR, idarubicin; AraC, cytarabine; DNR, daunorubicin; HSCT, hematopoietic stem cell transplantation.
[a]Three patients had both complex karyotype and monosomy 7.
[b]At G-band analysis, dividing cells could not be identified in seven patients.
[c]One hundred and thirty four patients who received induction therapy were analyzed. BHAC-DM-like regimen, high dose AraC-containing regimen or CAG regimen were chosen for initial induction therapy other than IDA/AraC or DNR/AraC.

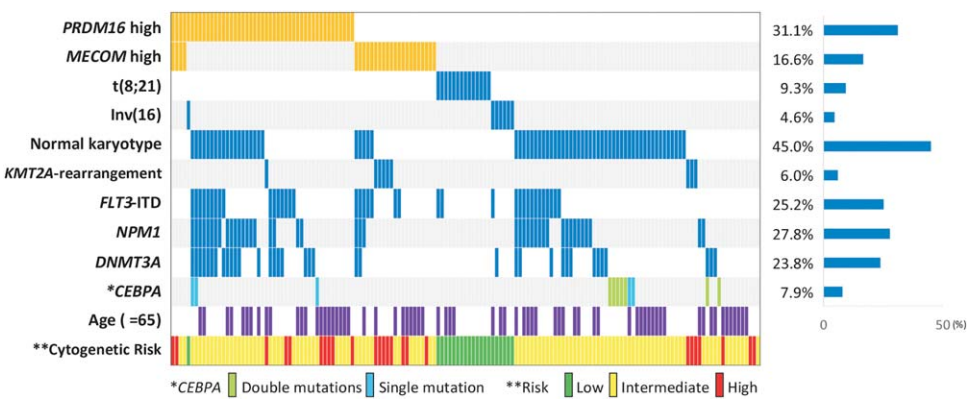


**FIGURE 1** Associations between *PRDM16* expression and cytogenetic features and mutations in AML patients. Relationships between *PRDM16* expression levels and cytogenetics and mutations in 151 patients with AML. Orange and blue indicate the presence of high *PRDM16* expression and the presence of the specified mutation in the designated patient, respectively. Blanks indicate the absence of mutations. Cytogenetic risks are shown by three colors. Red, yellow, and green indicate adverse risk, intermediate risk, and favorable risk, respectively [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** Clinical characteristics of 151 AML patients with or without high *PRDM16* expression

| | *PRDM16* high expression ($n = 47$) | *PRDM16* low expression ($n = 104$) | *P* value |
|---|---|---|---|
| Age at diagnosis median (range) | 60 (21 to 85) | 61 (17 to 88) | 0.797 |
| Gender male, *n* (%) | 24 (51) | 63 (61) | 0.291 |
| White-cell count at diagnosis; median (range) ($\times 10^9$/L) | 42.2 (0.5 to 677.0) | 27.5 (0.9 to 396.6) | 0.467 |
| Blast (%) | 60 (1.0 to 98.5) | 61 (3 to 99.5) | 0.816 |
| LDH | 817 (157 to 3915) | 723 (165 to 4803) | 0.790 |
| Cytogenetic risk, *n* (%) | | | |
| Favorable risk | 1 (2) | 20 (19) | 0.004 |
| Intermediate risk | 36 (77) | 69 (66) | 0.253 |
| Adverse risk | 10 (21) | 15 (14) | 0.346 |
| No complete remission, *n*/total (%)[a] | 20/42 (48) | 19/92 (21) | 0.002 |
| AML subtype, *n* (%) | | | |
| M0 | 2 (4) | 1 (1) | 0.229 |
| M1 | 13 (28) | 27 (26) | 0.884 |
| M2 | 9 (19) | 36 (35) | 0.058 |
| M4 | 7 (15) | 15 (14) | 1.000 |
| M5 | 7 (15) | 15 (14) | 1.000 |
| M6 | 1 (2) | 2 (2) | 1.000 |
| M7 | 1 (2) | 0 (0) | 0.311 |
| Other | 7 (15) | 8 (8) | |
| Chromosomal abnormality | | | |
| Normal karyotype, *n* (%) | 19 (40) | 49 (47) | 0.483 |
| Complex karyotype, *n* (%) | 6 (13) | 6 (6) | 0.192 |
| *t*(8;21) | 0 (0) | 14 (13) | 0.005 |
| inv(16) | 1 (2) | 6 (6) | 0.436 |
| *KMT2A* rearrangement | 1 (2) | 8 (8) | 0.275 |
| Monosomy7 | 3 (6) | 3 (3) | 0.376 |
| Genetic mutation, *n*/total (%) | | | |
| *FLT3*-ITD | 16 (34) | 22 (21) | 0.110 |
| *NPM1* | 20 (43) | 22 (21) | 0.010 |
| *DNMT3A* | 20 (43) | 16 (15) | $< 0.001$ |
| *CEBPA* | 3 (6) | 9 (9) | 0.755 |
| *CEBPA (biallelic mutation)* | 0 (0) | 7 (7) | 0.099 |
| *KMT2A*-PTD[b] | 8/36 (22) | 1/76 (1) | $< 0.001$ |
| Gene expression, *n*/total (%) | | | |
| *MECOM* (*EVI1*) high expression | 4 (9) | 21 (28) | 0.098 |
| Induction therapy, *n*/total (%)[a] | | | |
| IDA/AraC or DNR/AraC | 19 | 36 | 0.572 |
| Others[c] | 23 | 56 | |

IDR, idarubicin; AraC, cytarabine; DNR, daunorubicin.
[a]One hundred and thirty four patients who received induction therapy were analyzed.
[b]One hundred and Twelve patients were analyzed because of lack of samples from the remaining patients.
[c]BHAC-DM-like regimen, high dose AraC-containing regimen or CAG regimen were chosen for initial induction therapy other than IDA/AraC or DNR/AraC.

the nonsignificant variables ($P \geq 0.050$). A stepwise backward procedure selection model was used to extract independent events.

## 3 | RESULTS

### 3.1 | Clinical and molecular features of AML with *PRDM16* and *MECOM* expression

Among 151 AML patients, high *PRDM16* and *MECOM* expression was identified in 47 (31%) and 25 (17%), respectively (Figure 1). *PRDM16* and *MECOM* were expressed in a nearly mutually exclusive manner. We compared the clinical and molecular features between high-*PRDM16*-expression and low-*PRDM16*-expression patients (Table 2). There were no significant differences in age at diagnosis, sex distributions, white blood cell counts at diagnosis, blast ratio in bone marrow, and selection of induction therapy. Mutations of *DNMT3A* (43% vs. 15%, $P < 0.001$), *NPM1* (43% vs. 21%, $P = 0.010$), and *KMT2A*-PTD (22% vs. 1%, $P < 0.001$) were frequently observed in high-*PRDM16*-expression patients. In addition, *FLT3*-ITD (34% vs. 21%, $P = 0.110$) tended to be more frequently observed in high-*PRDM16*-expression patients. Conversely, high-*PRDM16*-expression patients had a significantly lower coincidence of *t*(8;21) (0% vs. 13%, $P = 0.005$) and

**TABLE 3** Clinical characteristics of 151 AML patients with or without high *MECOM* expression

| | *MECOM* high expression ($n = 25$) | *MECOM* low expression ($n = 126$) | *P* value |
|---|---|---|---|
| Age at diagnosis Median (range) | 54 (21 to 87) | 63 (17 to 88) | 0.527 |
| Gender male, *n* (%) | 11 (44) | 76 (60) | 0.183 |
| White-cell count at diagnosis; Median (range) ($\times 10^9$/L) | 56.3 (1.1 to 396.6) | 28.0 (0.5 to 677.0) | 0.231 |
| Blast (%) | 59 (2.5 to 98.5) | 61 (1.0 to 99.5) | 0.633 |
| LDH | 744 (249 to 2471) | 737 (157 to 4803) | 0.698 |
| Cytogenetic risk, *n* (%) | | | |
| Favorable risk | 0 (0) | 21 (17) | 0.025 |
| Intermediate risk | 15 (60) | 90 (71) | 0.341 |
| Adverse risk | 10 (40) | 15 (12) | 0.002 |
| No complete remission, *n*/total (%)[a] | 9/21 (43) | 30/113 (27) | 0.189 |
| AML subtype, *n* (%) | | | |
| M0 | 2 (8) | 1 (1) | 0.071 |
| M1 | 8 (32) | 32 (25) | 0.470 |
| M2 | 5 (20) | 40 (32) | 0.339 |
| M4 | 1 (4) | 21 (17) | 0.127 |
| M5 | 3 (12) | 19 (15) | 1.000 |
| M6 | 0 (0) | 3 (2) | 1.000 |
| M7 | 0 (0) | 1 (1) | 1.000 |
| other | 6 (24) | 9 (7) | |
| Chromosomal abnormality | | | |
| Normal karyotype, *n* (%) | 5 (20) | 63 (50) | 0.008 |
| Complex karyotype, *n* (%) | 4 (16) | 8 (6) | 0.114 |
| *t*(8;21) | 0 (0) | 14 (11) | 0.128 |
| inv(16) | 0 (0) | 7 (6) | 0.601 |
| *KMT2A* rearrangement | 5 (20) | 4 (3) | 0.007 |
| Monosomy7 | 3 (12) | 3 (3) | 0.058 |
| 3q26 abnormalities | 2 (8) | 0 (0) | 0.027 |
| Genetic mutation, n/total (%) | | | |
| *FLT3*-ITD | 7 (28) | 31 (25) | 0.801 |
| *NPM1* | 3 (12) | 39 (31) | 0.085 |
| *DNMT3A* | 2 (8) | 34 (27) | 0.043 |
| *CEBPA* | 0 (0) | 12 (10) | 0.218 |
| *CEBPA (biallelic mutation)* | 0 (0) | 7 (6) | 0.601 |
| *KMT2A*-PTD[b] | 0/16 (0) | 9/96 (9) | 0.354 |
| Induction Therapy, *n*/total (%)[a] | | | |
| IDA/AraC or DNR/AraC | 11 | 44 | 0.334 |
| Others[c] | 10 | 69 | |

IDR, idarubicin; AraC, cytarabine; DNR, daunorubicin.
[a]One hundred and thirty four patients who received induction therapy were analyzed.
[b]One hundred and twelve patients were analyzed because of lack of sample from the remaining patients.
[c]BHAC-DM-like regimen, high dose AraC-containing regimen or CAG regimen were chosen for initial induction therapy other than IDA/AraC or DNR/AraC.

favorable risk group (2% vs. 19%, $P = 0.004$; Table 2). In addition, high *PRDM16* expression was mutually exclusive with *CEBPA* double mutations (0% vs. 7%, $P = 0.099$). Remarkably, high-*PRDM16*-expression patients were more frequently observed in the noncomplete remission (non-CR) group (48% vs. 21%, $P = 0.002$). When all patients were divided into four groups according to risk category of the ELN 2010,[24] high-*PRDM16*-expression patients were more frequently observed in the intermediate II group (Supporting Information Table 1).

Regarding *MECOM* expression, there were no significant differences in age at diagnosis, sex distributions, and white blood cell counts at diagnosis between high-*MECOM*-expression and low-*MECOM*-expression patients (Table 3). Cases with high *MECOM* expression had a higher incidence of *KMT2A* rearrangement (20% vs. 3%, $P = 0.007$) and 3q26 abnormalities (8% vs. 0%, $P = 0.027$) and a significantly lower incidence of normal karyotype (20% vs. 50%, $P = 0.008$) and *DNMT3A* mutation (8% vs. 27%, $P = 0.043$). High-*MECOM*-expression patients were frequently observed in the adverse risk ($P = 0.002$), but not in the favorable risk group ($P = 0.025$). Moreover, high *MECOM* expression was mutually exclusive with t(8;21), inv(16), *CEBPA* mutation, and *KMT2A*-PTD (Figure 1, Table 3).

## 3.2 | High expression of *PRDM16* or *MECOM* was associated with poor survival

Among the 151 AML patients, 134 patients were analyzed for survival; 17 patients were excluded because 11 died before induction therapy

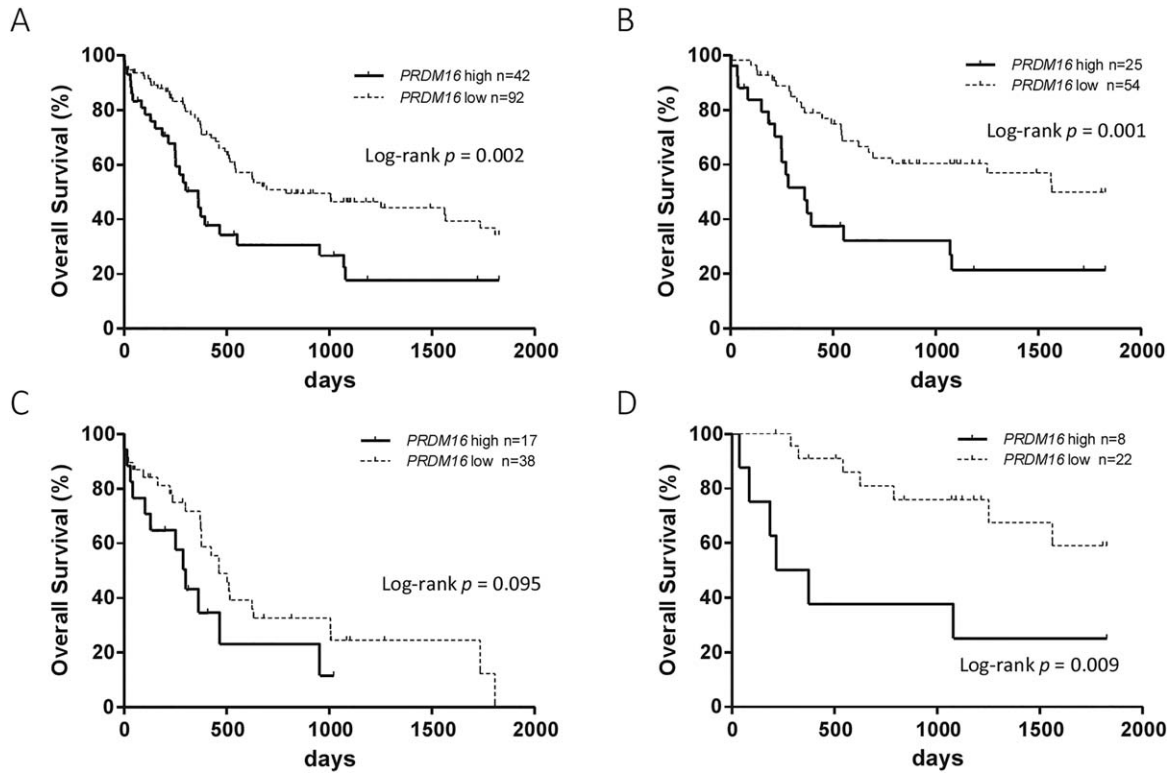


**FIGURE 2** Prognostic impact of high *PRDM16* expression in AML patients. (A) Kaplan–Meier curves of overall survival (OS) according to high or low *PRDM16* expression (all patients). (B) Kaplan–Meier curves of OS according to high or low *PRDM16* expression (patients aged <65 years). (C) Kaplan–Meier curves of OS according to high or low *PRDM16* expression (patients aged ≥65 years). (D) Kaplan–Meier curves of OS according to high or low *PRDM16* expression (patients aged <65 years, *FLT3*-ITD negative, and intermediate cytogenetic risk)

and six were lost to follow-up. The OS and median survival time (MST) of patients with high *PRDM16* expression were significantly worse than in those with low expression (5-year OS, 18% vs. 34%; MST, 361 days vs. 788 days; $P = 0.002$; Figure 2A). This trend was observed more clearly among patients aged <65 years (5-year OS, 21% vs. 50%; MST, 361 days vs. 1565 days; $P = 0.001$; Figure 2B). On the other hand, there was no significant difference among patients aged ≥65 years (5-year OS, 12% vs. 0%; MST, 299 days vs. 462 days; $P = 0.095$; Figure 2C) because of their worse prognosis and limited patient number. Remarkably, high *PRDM16* expression was a significant prognostic factor for *FLT3*-ITD-negative patients aged <65 years in the intermediate cytogenetic risk group (5-year OS, 25% vs. 59%; MST, 294 days vs. undefined; $P = 0.009$; Figure 2D). There was no significant difference in choice of induction therapy between the patients with or without high *PRDM16* expression under all of four conditions (all patients, patients aged <65 years, patients aged ≥65 years, and *FLT3*-ITD-negative patients aged <65 years in the intermediate cytogenetic risk group) (Supporting Information Table 2). Moreover, there was no association between the choice of induction therapy and OS under all of four conditions (Supporting Information Figure 1A–D). High *PRDM16* expression was also associated with a high rate of cumulative incidence of relapse (CIR) in all patients (5-year CIR, 85% vs. 76%, $P = 0.001$) (Supporting Information Figure 2). These results suggested that high *PRDM16* expression was a significant predictive marker for poor prognosis in adult AML patients, which is similar to the finding in pediatric AML patients.[17,18] Although the number of *FLT3*-ITD-negative patients with intermediate cytogenetic risk was limited, high *PRDM16* expression showed significant poor prognostic impact in patients whose prognosis is unclear.

High *MECOM* expression was also associated with poor survival among all patients (5-year OS, 14% vs. 32%; MST, 298 days vs. 631 days; $P = 0.039$; Figure 3A) or patients aged <65 years (5-year OS, 19% vs. 46%; MST 286 days vs. 1250 days; $P = 0.040$; Figure 3B). However, there was no significant difference among patients aged ≥65 years because of their limited patient number (5-year OS, 0% vs. 0%; MST, 298 days vs. 462 days; $P = 0.092$; Figure 3C).

The results of univariate and multivariate Cox regression analyses are presented in Table 4. In the univariate analysis, factors significantly associated with OS included high *PRDM16* expression (HR = 2.101, 95% CI: 1.310–3.370), high *MECOM* expression (HR = 1.814, 95% CI: 1.028–3.201), favorable cytogenetic risk (HR = 0.368, 95% CI: 0.169–0.801), adverse cytogenetic risk (HR = 2.047, 95% CI: 1.408–3.537), *FLT3*-ITD (HR = 2.702, 95% CI: 1.006–2.702), *DNMT3A* (HR = 1.662, 95% CI: 1.010–2.737), and age (≥65 years) (HR = 2.231, 95% CI: 1.408–3.537). The multivariate analysis revealed that high *PRDM16* and *MECOM* expression was an independent poor prognostic factor associated with OS (*PRDM16*, HR = 2.127, 95% CI: 1.244–3.637; *MECOM*, HR = 2.248, 95% CI: 1.172–4.313). Supporting Information Table 3 shows results of univariate and multivariate Cox regression analyses using another

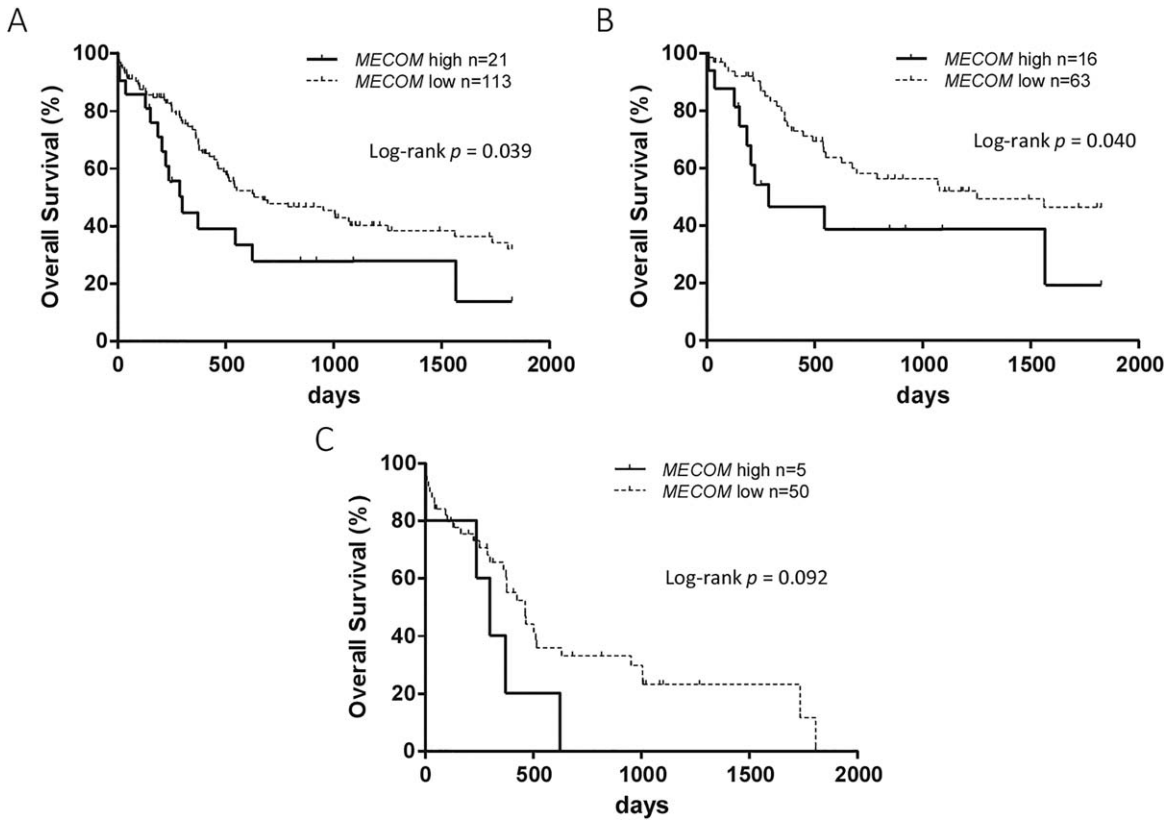


**FIGURE 3** Prognostic impact of high *MECOM* expression in AML patients. (A) Kaplan–Meier curves of overall survival (OS) according to high or low *MECOM* expression (all patients). (B) Kaplan–Meier curves of OS according to high or low *MECOM* expression (patients aged <65 years). (C) Kaplan–Meier curves of OS according to high or low *MECOM* expression (patients aged ≥65 years)

approach that included further distinct cytogenetic groups such as inv(16), *t*(8;21), *KMT2A* rearrangement, *MECOM* rearrangement, and complex karyotype. Consequently, both high *PRDM16* and high *MECOM* expressions were independent poor prognostic factors associated with OS in both approaches.

### 3.3 | Combination of high *PRDM16* and *MECOM* expressions is a convincing poor prognostic marker

As *PRDM16* and *MECOM* were expressed in a nearly mutually exclusive manner (Figure 1), a combination of high *PRDM16* and *MECOM*

**TABLE 4** Univariate and multivariate Cox regression gene analyses of overall survival

| | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | | 95% CI | | | | 95% CI | | |
| | HR | Inferior | Superior | *P* value | HR | Inferior | Superior | *P* value |
| Age (≥65-year-old) | 2.231 | 1.408 | 3.537 | < 0.001 | 2.761 | 1.692 | 4.507 | < 0.001 |
| Favorable cytogenetic risk[a] | 0.368 | 0.169 | 0.801 | 0.012 | 0.587 | 0.254 | 1.354 | 0.211 |
| Adverse cytogenetic risk[a] | 2.047 | 1.158 | 3.621 | 0.014 | 2.834 | 1.418 | 5.662 | 0.003 |
| SCT at 1st CR | 0.149 | 0.021 | 1.071 | 0.059 | 0.095 | 0.013 | 0.708 | 0.022 |
| High *PRDM16* expression | 2.101 | 1.310 | 3.370 | 0.002 | 2.127 | 1.244 | 3.637 | 0.006 |
| High *MECOM* expression | 1.814 | 1.028 | 3.201 | 0.040 | 2.248 | 1.172 | 4.313 | 0.015 |
| *CEBPA* double mutations | 0.309 | 0.076 | 1.260 | 0.101 | | | | |
| *NPM1* | 1.217 | 0.740 | 2.000 | 0.439 | | | | |
| *FLT3*-ITD | 1.648 | 1.006 | 2.702 | 0.048 | 1.663 | 0.930 | 2.975 | 0.090 |
| *DNMT3A* | 1.662 | 1.010 | 2.737 | 0.046 | 2.419 | 1.342 | 4.358 | 0.003 |

CI, confidence interval; HR, hazard ratio.
[a]The chromosomal classification was classified according to the 2013 NCCN guidelines.

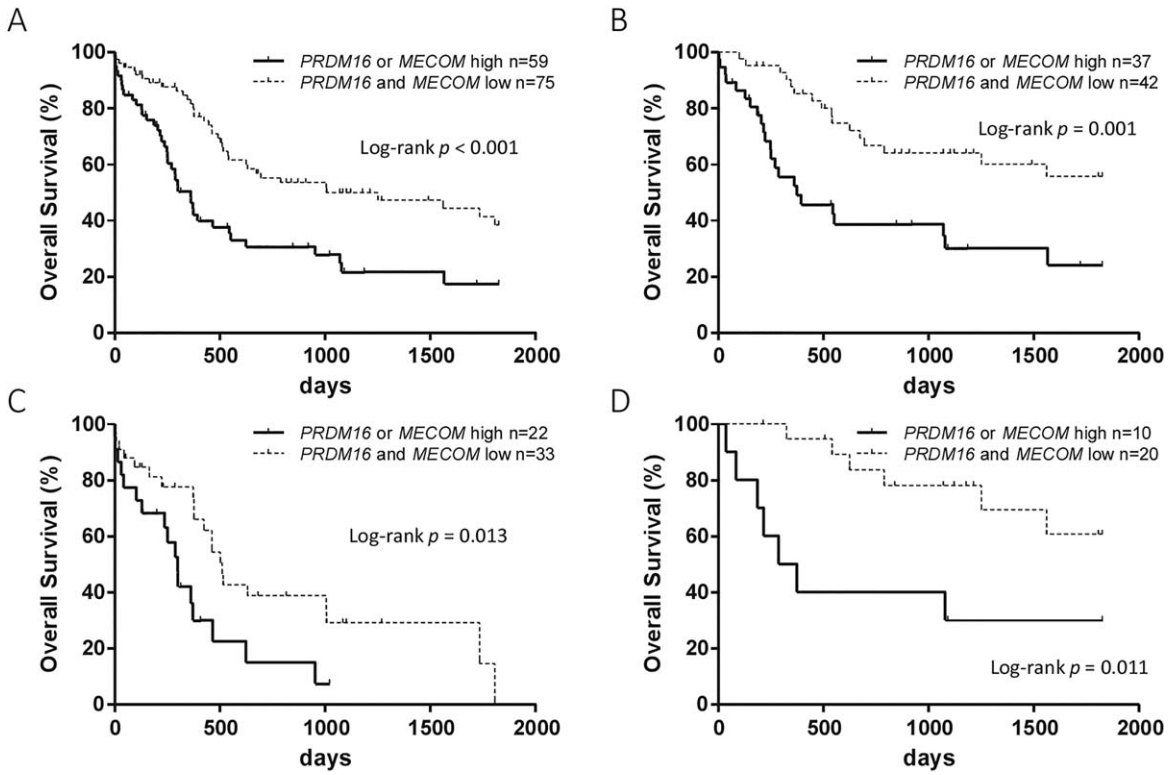


**FIGURE 4** A combination of high *PRDM16* and *MECOM* expressions is expected to be a poor prognostic marker in AML patients. (A) Kaplan–Meier curves of overall survival (OS) between high *PRDM16* or *MECOM* expression and both low *PRDM16* and *MECOM* expression (all patients). (B) and (C) Kaplan–Meier curves of OS between high *PRDM16* or *MECOM* expression and both low *PRDM16* and *MECOM* expression (patients aged <65 years or aged ≥65 years). (D) Kaplan–Meier curves of OS between high *PRDM16* or *MECOM* expression and both low *PRDM16* and *MECOM* expression (patients aged <65 years, *FLT3*-ITD negative, and intermediate cytogenetic risk)

expressions is expected to become a poor prognostic marker. When 59 patients with high *PRDM16* or *MECOM* expression were selected, including four patients with both *PRDM16* and *MECOM* overexpression, their prognosis was extremely poor (5-year OS, 17% vs. 38%; MST, 361 days vs. 1006 days; $P < 0.001$; Figure 4A). The same trend was also observed among patients aged <65 years (5-year OS, 24% vs. 56%; MST, 373 days vs. undefined; $P = 0.001$; Figure 4B) and patients aged ≥65 years (5-year OS, 7% vs. 0%; MST, 298 days vs. 510; $P = 0.013$; Figure 4C). This trend was observed more clearly among *FLT3*-ITD-negative patients aged <65 years in the intermediate cytogenetic risk group (5-year OS, 30% vs. 61%; MST, 330 days vs. undefined; $P = 0.011$; Figure 4D).

## 4 | DISCUSSION

We here provide evidence that high *PRDM16* expression is a recurrent event characterizing clinically relevant features in adult AML. The results are consistent with the previous pediatric AML report showing that high expression of *PRDM16* correlated with higher coincidence of non-CR and *KMT2A*-PTD, and a lower incidence of t(8;21).[18] Notably, high *PRDM16* expression was significantly associated with *DNMT3A* mutations in adult AML. This is the first report showing a correlation between *DNMT3A* and high *PRDM16* expression in leukemia, because *DNMT3A* mutations are extremely rare in pediatric AML.[25] Hence, the pathogenesis and clinical impact of *DNMT3A* mutation has a similar pattern to *NUP98-NSD1* from the viewpoint of gene expression pattern because high *PRDM16* expression was the representative factor of pediatric AML patients with *NUP98-NSD1*.[12] The *NUP98-NSD1* fusion was not identified in this study. *NUP98-NSD1* methyltransferase activity gives rise to abnormally high levels of methylation at lysine 36 on histone 3, enforcing oncogene activation by activated *HOX* expression,[26] whereas *DNMT3A* mutations confer a global hypomethylation pattern that specifically targets *HOX*.[27] However, the molecular mechanisms through which *PRDM16* expression plays an important role for leukemogenesis in both adult and pediatric AML patients still require clarification.

High *MECOM* expression was recurrent in AML patients with 3q26 abnormalities and *KMT2A* rearrangements and was associated with a poor prognosis. In addition, *KMT2A*-rearranged patients with high *MECOM* expression tended to have poor prognosis (5-year OS, 0% vs. 67%; MST, 421 days vs. undefined; $P = 0.311$). These results are consistent with those of previous reports.[28–32] Remarkably, we identified that high *MECOM* expression correlated with a lower incidence of *DNMT3A* mutations.

Interestingly, *PRDM16* is highly homologous to *MECOM*, and both *PRDM16* and *MECOM* encode histone H3 lysine 9 monomethyltransferases that function in the maintenance of heterochromatin integrity.[33] In this study, *PRDM16* and *MECOM* presented some similarities as follows. High *PRDM16* and *MECOM* expressions were

nearly mutually exclusive with CBF-AML and *CEBPA* double mutations and were associated with poor survival. On the other hand, their high expressions presented their own features. In short, although high *PRDM16* expression correlated with *DNMT3A*, *NPM1*, and *KMT2A*-PTD, high *MECOM* expression was nearly mutually exclusive with these mutations but was associated with 3q26 abnormalities and *KMT2A* rearrangements. Because of their mutual exclusiveness, the combination of high *PRDM16* and *MECOM* expressions was expected to be effective in the detection of higher-risk patients (Figure 4). *SETBP1*, which is located downstream of both *PRDM16* and *MECOM* in different pathways, is considered to play a key role in the mutual exclusiveness of high *PRDM16* and high *MECOM* expression because overexpression of *SETBP1* was reported to promote leukemogenesis by increasing *SET* expression.[34,35] As a result, either high *PRDM16* or high *MECOM* expression may be sufficient for leukemogenesis to activate the expression of *SETBP1*. Therefore, high *PRDM16* and high *MECOM* expressions may be mutually exclusive in most AML patients.

With respect to 30 *FLT3*-ITD-negative patients aged <65 years in the intermediate cytogenetic risk group, high *PRDM16* or *MECOM* expression was observed in 10 (33%) of those patients (Supporting Information Table 4). Notably, all two non-CR and all five relapsed patients with high *PRDM16* or *MECOM* expression died. This indicates that high-*PRDM16*-expression or high-*MECOM*-expression patients may need an SCT at the first CR. On the other hand, all *CEBPA* double mutations were observed in patients with low *PRDM16* and *MECOM* expression (7/20, 35%). Remarkably, there was no significant difference in mortality between the low-*PRDM16*-expression and low-*MECOM*-expression patients with or without *CEBPA* double mutations (42% vs. 38%, $P = 1.000$). These findings suggest that low *PRDM16* and *MECOM* expression might be a useful marker for identifying favorable-risk patients.

With respect to the risk classification of the ELN recommendations of 2017, we could not classify our patients based on it because our genetic analyses were started before the ELN recommendations were updated. Consequently, gene mutations in *RUNX1*, *ASXL1*, and *TP53* used for risk classification in the ELN recommendations of 2017 were not analyzed in several patients. Therefore, we adopted the ELN recommendations of 2010 in Supporting Information Table 1. We will analyze these genes in the next study, which targets more patients.

In conclusion, high *PRDM16* expression was independently associated with non-CR, *KTM2A*-PTD, and adverse outcome, and mutually exclusive with *t*(8;21) in both adult and pediatric AML patients.[18] Our findings indicate that the same pathogenesis might exist in both adult and pediatric AML patients through *PRDM16* expression. Measuring *PRDM16* expression could be a powerful predictive tool for prognostication of adult AML patients. Moreover, the combination of *PRDM16* and *MECOM* expression might be effective in clarifying the genetic backgrounds and risks of AML. Further studies will be required to use *PRDM16* expression in AML treatment decisions.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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