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LETTER TO THE EDITOR

High *DNA-methyltransferase 3B* expression predicts poor outcome in acute myeloid leukemia, especially among patients with co-occurring *NPM1* and *FLT3* mutations

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DNA methyltransferases (DNMTs) are epigenetic regulators targeted to the treatment of hematological malignancies.[1–4] Mutations in the DNA methyltransferase *DNMT3A* and high expression of its paralogue *DNMT3B* have been associated with inferior outcome in acute myeloid leukemia (AML) and other hematological malignancies.[5–8] Using a publicly available gene expression data set,[9] we studied whether DNMT3B expression correlates with outcome in genetically well-defined AML subgroups. We first validated the expression data from the microarray by quantitative PCR, using 39 patient samples (Supplementary Figure S1A). *DNMT3B* micro-array analyses showed that the expression was not normally distributed among AML patients; in the quartile with highest expression, a larger variation in expression was observed compared to the three quartiles with relatively lower expression (Supplementary Figure S1B). The median *DNMT3B* expression in AML samples was significantly lower compared with that observed in normal bone marrow (NBM)-derived $CD34^+$ cells ($P < 0.0001$), while it was higher compared to NBM cells, but the latter difference was not statistically significant (Supplementary Figure S1B). Subsequently, we investigated the correlation between *DNMT3B* expression and overall survival (OS) and event-free survival (EFS). In univariate Cox regression analyses, continuous *DNMT3B* expression was significantly associated with poor survival ($P < 0.001$ for both OS and EFS, data not shown). To visualize the prognostic impact, we performed Kaplan–Meier analyses on the four quartiles based on expression levels. The quartile including the patients with the highest *DNMT3B* expression, exhibiting the largest variation in expression, showed a significantly reduced OS and EFS compared to the other quartiles (Supplementary Fig. S2). As the survival between the lower three quartiles did not differ significantly, we grouped these patients together as having lower *DNMT3B* expression, whereas the remaining patients were ranked as having higher *DNMT3B* expression. Using these criteria, the 5-year OS and EFS were 17.2% ± 3.3% and 13.6% ± 3.0% for patients with higher *DNMT3B* expression compared to 43.8% ± 2.5% and 34.4% ± 2.4% for patients with lower *DNMT3B* levels ($P < 0.001$, Figure 1a). We next performed a multivariate Cox regression analysis including known prognostic factors (including age >60 years; white blood cell counts $>100 \times 10^9$/l; transplantation status; karyotypes t(8;21), t(15;17) and inv(16); nucleophosmin 1 (*NPM1*), *FLT3-ITD*, *DNMT3A* and double *CEBPA* mutations, and ecotropic viral integration site 1 (*EVI1*) overexpression), which revealed that higher *DNMT3B* expression carried an independent prognostic risk for both OS and EFS (hazard ratio (HR): 1.768, 95% confidence interval (CI): 1.384–2.260; $P < 0.001$ and HR: 1.706, 95% CI: 1.342–2.168; $P < 0.001$, respectively, Table 1), in line with a recently published study.[7] In fact, higher *DNMT3B* expression showed a higher hazard ratio for OS than that of well-known adverse prognostic factors such as internal tandem duplications of the fms-related tyrosine kinase 3 (*FLT3-ITD*, HR: 1.675, 95% CI: 1.287–2.179; $P < 0.001$) and overexpression of the *EVI1* gene (HR: 1.430, 95% CI: 0.999–2.047; $P = 0.051$).

We then studied the association of higher *DNMT3B* expression with specific AML subcategories. Higher *DNMT3B* expression was under-represented in FAB-M4 and mutually exclusive with the favorable karyotypes t(8;21) and inv(16), as shown in Supplementary Table S1. In contrast, higher *DNMT3B* expression was over-represented in the FAB-M1 subcategory (Supplementary Table S1), and predicted poor OS and a trend toward poor EFS in this group (data not shown). A significant association between higher *DNMT3B* expression and *EVI1* overexpression and *IDH2* mutations was also observed (Supplementary Table S1), but not with *IDH1* mutations. *DNMT3B* expression did not predict clinical outcome in these subgroups (data not shown). We also observed that higher *DNMT3B* expression was associated with a normal karyotype (NK, $P = 0.017$), and strongly associated with mutations in *NPM1* (*NPM1*$^+$, $P < 0.001$) and *FLT3-ITD* (*FLT3-ITD*$^+$, $P < 0.001$, Supplementary Table S1). Patients with NK can be classified based on *NPM1* and *FLT3-ITD* mutational status. *NPM1* mutations, particularly in the absence of *FLT3-ITD*, display a favorable disease outcome, whereas the presence of a *FLT3-ITD* mutation is generally considered as an adverse prognostic factor.[10] Remarkably, among patients with NK, higher *DNMT3B* expression did not significantly associate with *NPM1*$^-$/*FLT3-ITD*$^-$ status and was under-represented in the *NPM1*$^+$/*FLT3-ITD*$^-$ and *NPM1*$^-$/*FLT3-ITD*$^+$ groups, but was significantly over-represented in the *NPM1*$^+$/*FLT3-ITD*$^+$ group (Supplementary Table S2). Within the latter group, patients with higher *DNMT3B* expression showed a significantly worse outcome than patients with lower *DNMT3B* expression (Figure 1b). The 5-year OS and EFS of patients with higher *DNMT3B* expression were 16.7% ± 6.2% ($P = 0.001$) and 16.7% ± 6.2% ($P = 0.005$) compared to 47.6% ± 8.8% and 39.0% ± 8.6% in patients with lower *DNMT3B* expression. The survival of patients with co-occurring *NPM1* and *FLT3-ITD* mutations is negatively influenced by high *FLT3-ITD* allelic burden.[11–13] Higher *DNMT3B* expression was observed in both patients with low and those with high *FLT3-ITD* allelic burden (Supplementary Table S2). We next analyzed whether *DNMT3B* expression had an effect on survival among these subgroups. Higher *DNMT3B* expression did not exhibit a significant effect on OS and EFS among patients with low *FLT3-ITD* allelic burden (data not shown). However, patients with higher *DNMT3B* expression showed an extremely poor OS and EFS (5-year OS: 0.0% ± 0.0%, 5-year EFS: 0.0% ± 0.0%, $P < 0.001$), compared to patients with lower *DNMT3B* expression (5-year OS: 38.9% ± 12.9%, 5-year EFS: 32.0% ± 12.4%) within the subgroup with high *FLT3-ITD* allelic burden (Figure 1c). In multivariate analysis, higher *DNMT3B* expression showed an independent prognostic value for OS and EFS, with a high hazard ratio both in the *NPM1*$^+$/*FLT3-ITD*$^+$ subgroup (HR: 4.850, 95% CI: 1.980–11.880) and among those patients with high *FLT3-ITD* allelic burden, indicating that the latter subgroup can be separated into two groups, one with an intermediate and the other with an extremely poor survival, based on *DNMT3B* expression (Table 1).

In conclusion, these data show that higher *DNMT3B* expression is a strong independent predictive factor for poor disease

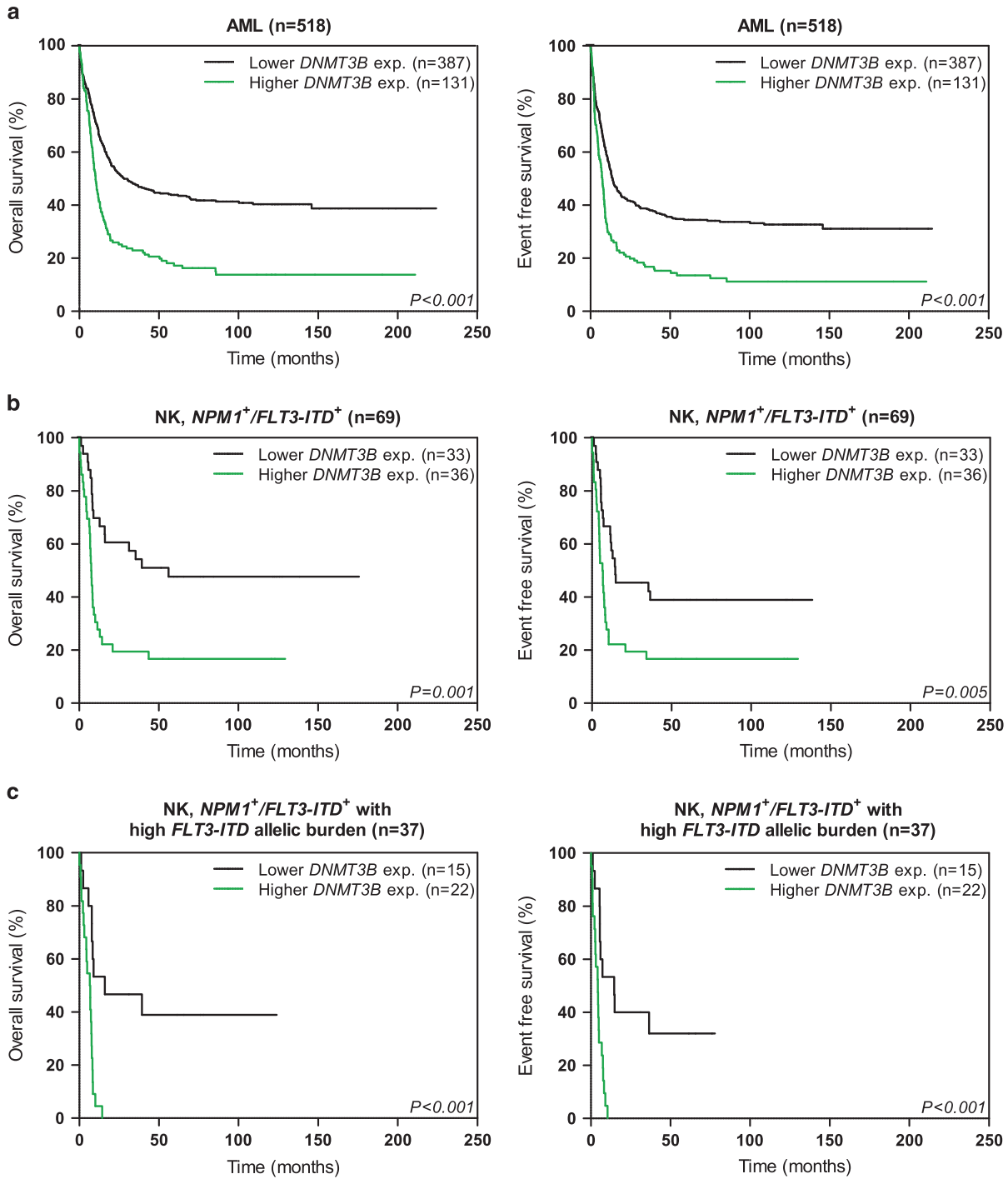

Figure 1. Higher *DNMT3B* expression correlates with inferior OS and EFS in AML. (**a**) Kaplan–Meier plots for OS and EFS showed that higher *DNMT3B* expression correlated significantly with a poor OS and EFS among AML patients (5-year OS: 17.2% ± 3.3% vs 43.8% ± 2.5% and 5-year EFS: 13.6% ± 3.0% vs 34.4% ± 2.4% for patients with higher and lower *DNMT3B* expression, respectively). (**b**) Higher *DNMT3B* expression predicted a very poor OS and EFS among patients with normal karyotype carrying *NPM1* and *FLT3-ITD* mutations compared to patients with lower *DNMT3B* expression (5-year OS: 16.7 ± 6.2% vs 47.6 ± 8.8%, $P = 0.001$ and 5-year EFS: 16.7 ± 6.2% vs 39.0 ± 8.6%, $P = 0.005$ for patients with higher and lower *DNMT3B* expression, respectively). (**c**) Within the group of patients with normal karyotype that carries *NPM1* mutations with high *FLT3-ITD* allelic burden, higher *DNMT3B* expression correlated with an extremely poor OS and EFS compared to patients with lower *DNMT3B* expression (5-year OS: 0% ± 0.0% vs 38.9% ± 12.9%, $P < 0.001$ and 5-year EFS: 0% ± 0.0% vs 32.0% ± 12.4%, $P < 0.001$ for patients with higher and lower *DNMT3B* expression, respectively). *P* values were determined with the log-rank test. In agreement with de Jonge *et al.*,[13] high *FLT3-ITD* allelic burden was defined as an allelic *FLT3-ITD/FLT3* ratio > 1.

outcome in AML in general, and especially among *NPM1*$^+$/*FLT3-ITD*$^+$ patients with normal karyotype. Drugs that inhibit DNA methylation are tested for clinical efficacy in AML.[1–4] Because *DNMT3B* catalyzes DNA methylation, its expression level may affect the therapeutic sensitivity to these drugs. Thus, it will be interesting to investigate whether *DNMT3B* expression predicts therapy responses to treatments affecting DNA methylation.

Table 1. Data proving that *DNMT3B* expression is a prognostic factor in AML, particularly in *NPM1*$^+$/ *FLT3-ITD*$^+$ patients with normal karyotype

	OS		*EFS*	
	HR (95% CI)	P	*HR (95% CI)*	P
AML				
Higher *DNMT3B* exp.	1.768 (1.384–2.260)	< **0.001**	1.706 (1.342–2.168)	< **0.001**
Age > 60 years	1.523 (1.111–2.087)	**0.009**	1.339 (0.985–1.821)	0.063
WBC count > 100×10^9/l	1.448 (1.098–1.909)	**0.009**	1.531 (1.173–1.997)	**0.002**
FLT3-ITD mutations	1.675 (1.287–2.179)	< **0.001**	1.649 (1.274–2.136)	< **0.001**
NPM1 mutations	0.397 (0.291–0.541)	< **0.001**	0.381 (0.280–0.518)	< **0.001**
Favorable karyotype	0.388 (0.266–0.565)	< **0.001**	0.457 (0.323–0.646)	< **0.001**
CEBPA double mutation	0.338 (0.177–0.645)	**0.001**	0.361 (0.199–0.653)	**0.001**
DNMT3A mutations	1.631 (1.204–2.210)	**0.002**	1.531 (1.136–2.063)	**0.005**
EVI1 overexpression	1.430 (0.999–2.047)	0.051	1.711 (1.208–2.423)	**0.002**
Transplantation status	0.700 (0.610–0.806)	< **0.001**	0.758 (0.665–0.865)	< **0.001**
Normal karyotype				
NPM1$^+$/*FLT3-ITD*$^+$				
Higher *DNMT3B* expression	3.090 (1.623–5.883)	**0.001**	2.414 (1.309–4.451)	**0.005**
Age > 60 years	2.703 (1.134–6.438)	**0.025**	1.951 (0.831–4.583	0.125
WBC count > 100×10^9/l	2.365 (1.270–4.404)	**0.007**	1.763 (0.958–3.246)	0.069
High *FLT3-ITD* AB	3.791 (1.971–7.294)	< **0.001**	3.130 (1.685–5.813)	< **0.001**
NPM1$^+$/*FLT3-ITD*$^+$ *with high FLT3-ITD AB*				
Higher *DNMT3B* expression	4.850 (1.980–11.880)	**0.001**	4.428 (1.824–10.749)	**0.001**
Age > 60 years	2.007 (0.660–6.101)	0.219	1.478 (0.489–4.464)	0.489
WBC count > 100×10^9/l	1.356 (0.634–2.901)	0.433	0.954 (0.446–2.043)	0.904

Abbreviations: AB, allelic burden; 95% CI, 95% confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival; WBC, white blood cell. Multivariate Cox regression model for probability of OS and EFS. Favorable karyotype includes inv(16), t(8;21) and t(15;17). Transplantation status includes no transplantation, autologous transplantation or allogeneic transplantation. Age and WBC count are dichotomized. *DNMT3B* expression is dichotomized in multivariate analysis. *P*-values (bold) indicate whether differences are significant at the level of 0.05.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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