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ARTICLE

Prognostic significance of PAK family kinases in acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) is a clonal and heterogeneous disease characterized by a myriad of genetic defects. Genetic abnormalities are powerful prognostic factors. P21-activated kinases (PAKs) are a kind of serine/threonine protein kinases, which is regulator of plenty of oncogenic signaling pathways. The clinical and prognostic value of PAKs in AML is unclear. A total of 155 AML patients with PAK expression data from The Cancer Genome Atlas database were enrolled in this study. Eighty-four patients underwent chemotherapy only, 71 also underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT). In the chemotherapy-only group, high *PAK3* and *PAK7* expression were both bound up with poor EFS and OS (all $P < 0.05$). However, high *PAK2* expressers had better EFS and OS (all $P < 0.05$). Multivariate analysis demonstrated that high *PAK7* expression was an adverse independent prognostic factor in patients who received chemotherapy only. PAKs have no influence in EFS and OS in patients who underwent allo-HSCT. In conclusion, high *PAK2* expression is a favorable prognostic factor, as to the high expression of *PAK3* and *PAK7*, they are poor prognostic factors, and *PAK7* has better prognostic value, but their prognostic effects can be offset by allo-HSCT.

Introduction

Acute myeloid leukemia (AML) is a clonal and heterogeneous disease characterized by a myriad of genetic defects [1]. AML is characterized by complex and dynamic, coexisting competing clones, and disease evolution over time [2]. Genetic abnormalities are powerful prognostic factors in AML. Previous studies point several factors bound up with positive or negative outcomes. High expression of *CPNE3*, *MAP7*, *ETS2*, *FHL2*, and *iASPP*, and mutations of *FLT3-ITD* and *DNMT3A* are belong to the latter group [3–7]. As for the former group, it includes mutations in *NPM1* and double *CEBPA* [8]. Furthermore, gene–gene interactions should be taken into consideration, for example, only in the absence of *FLT3-ITD* can *NPM1* mutation have a "favorable" prognosis [8–11], whereas mutations in *ASXL1* and *RUNX1* cause a particularly poor prognosis in AML [12]. Due to the complexity of pathogenesis and clinical manifestations of AML, more comprehensive studies are needed to stratify risks and determine treatment directions.

P21-activated kinases (PAKs) are a kind of serine/threonine protein kinases. They can regulate plenty of oncogenic signaling pathways. There are six members in PAK family including PAK1–6 (PAK5 is known as PAK7). Based on the structure, it can be divided into group I (PAK1–3) and group II (PAK4–6). They mainly exist at the intersection of plenty of signaling pathways required for oncogenesis. PAKs play a central role in carcinogenic signals and promote several

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processes, which are markers of cancer initiation, growth, and spread [13]. Cell proliferation, migration, evasion of apoptosis, promotion of invasion, and metastasis caused by increased PAK expression or activation of mutations are mechanism of tumorigenesis [14]. They are regulated by different self-restraining mechanisms. At the same time, there are overlaps and differences in their functions. In many human tumors, the activity of PAKs increase significantly, and also relate to advanced grade and lower survival [13–17]. PAK kinases are often overexpressed in various human tumors and are targets for cancer treatment. Previous study shows that *PAK1* and *PAK4* are upregulated and over-activated in pancreatic cancer, and promote the movement and invasion of pancreatic cancer cells. Furthermore, *PAK3* knockout, rather than *PAK1* or *PAK2*, can inhibit the proliferation of pancreatic cancer cells in vitro and the growth of tumors in vivo [18].

However, the clinical and prognostic values of PAK family kinases in AML remain unclear. Allogeneic hematopoietic stem cell transplantation (allo-HSCT), a curative treatment for AML, may reduce relapse of AML and prolong survival through significant reduction of leukemia residual disease [18]. Here we performed a biomarker study to investigate the effects of PAK family kinases on the survival of AML patients, and whether allo-HSCT can overcome their prognostic effects.

Subjects and methods

Patients

The study included 84 patients who received chemotherapy only and 71 patients who also received allo-HSCT. The data were obtained from The Cancer Genome Atlas database. The end points of this study were event-free survival (EFS) and overall survival (OS). OS referred to the time from diagnosis to death for any reason or the last follow-up time. EFS refers to the time from diagnosis to the first event, such as relapse, death, etc. Plenty of clinical and molecular characteristics were obtained, including peripheral blood (PB), white blood cell (WBC) counts, PB blasts, bone marrow (BM) blasts, French-American-British (FAB) subtypes, and the frequencies of known recurrent genetic mutations. The informed consent of patients was obtained, and the study protocol was approved by the Washington University Human Studies Committee.

Statistical analysis

The clinical and molecular characteristics of patients were summarized using descriptive statistics. Data sets were described with median and/or range. Numerical data were compared using the Mann–Whitney *U* test, and categorical data were compared using the chi-square test. The Kaplan–Meier methods and log-rank test were used for survival analysis. Multivariate Cox proportional hazard models were constructed for EFS and OS, using a limited backward elimination procedure. The confidence interval is 95%. All statistical analyses were performed by SPSS software 24.0 and GraphPad Prism software 5.0.

Results

Clinical and molecular characteristics of the patients

Clinical and molecular characteristics of the patients are shown in Table 2. Median age was 66.5 (range 22–88) years, with 58 cases older than 60 years. Thirty-nine cases were women. The median WBC count was 14.7×10^9/L (range $0.7–297.4 \times 10^9$/L), and in 41 cases it was $>15 \times 10^9$/L. Forty-six patients had a BM blast percentage of >70%, and 46 had PB blasts > 20%. The primary FAB subtypes were M1, M2, and M4 (72.6%). Forty-four patients had abnormal karyotypes. The proportion of good, intermediate, and poor-risk patients were 14.6, 56.1, and 29.3%, respectively. *NPM1* had the highest mutation frequency ($n = 27$, 32.1%), followed by *DNMT3A* ($n = 23$, 27.4%), *FLT3* ($n = 22$, 26.2%), *IDH1/2* ($n = 15$, 17.9%), *NRAS/KRAS* ($n = 12$, 14.3%), *TET2* ($n = 11$, 13.1%), *TP53* ($n = 11$, 13.1%), and *RUNX1* ($n = 8$, 9.5%).

Evaluation of the prognostic significance of PAK family kinases in AML

In order to assess the prognostic value of PAK family in AML, this study divided the data into two groups by the median expression levels of each PAK member. The comparison results are shown in Table 1. In the chemotherapy-only group, high expression of *PAK3* and *PAK7* had adverse effects on EFS and OS (all $P < 0.05$, Fig. 1c–f), whereas high *PAK2* expression had favorable effects on EFS and OS (all $P < 0.01$, Fig. 1c, b). In the allo-HSCT group, the expression levels of all PAK family kinases were independent of survival.

Association of *PAK2/3/7* expressions with clinical and molecular characteristics in the chemotherapy-only group

The clinical and molecular characteristics of high and low *PAK2*, *PAK3*, and *PAK7* expression groups were compared, respectively (Tables 2 and 3). The $PAK2^{high}$ group had

Table 1 Comparison of EFS and OS between different expression levels of *PAK1–7*

Variables	EFS		OS	
	χ^2	*P*-value	χ^2	*P*-value
Chemotherapy-only group				
PAK1 (high vs. low)	1.461	0.227	2.195	0.138
PAK2 (high vs. low)	7.947	0.005	7.203	0.007
PAK3 (high vs. low)	5.632	0.018	6.599	0.010
PAK4 (high vs. low)	0.000	0.996	0.036	0.849
PAK6 (high vs. low)	0.025	0.875	0.000	0.986
PAK7 (high vs. low)	72.399	0.000	85.211	0.000
Allo-HSCT group				
PAK1 (high vs. low)	0.776	0.378	0.934	0.334
PAK2 (high vs. low)	0.611	0.434	0.648	0.421
PAK3 (high vs. low)	0.137	0.712	0.066	0.798
PAK4 (high vs. low)	0.012	0.912	1.345	0.246
PAK6 (high vs. low)	0.808	0.369	0.07	0.792
PAK7 (high vs. low)	0.015	0.902	0.041	0.840

EFS event-free survival, *OS* overall survival, *allo-HSCT* allogeneic hematopoietic stem cell transplantation

fewer old patients (≥60 years) ($P = 0.009$), higher WBC count ($P = 0.024$), fewer patients with complex karyotype ($P = 0.007$), more good-risk ($P = 0.026$) and fewer poor-risk patients ($P = 0.028$), and fewer patients with *TP53* mutations ($P = 0.007$) than the $PAK2^{low}$ group. No significant differences were found BM blasts, PB blasts, FAB subtypes, and frequency of other recurrent genetic mutations (*FLT3*, *NPM1*, *DNMT3A*, *IDH1/IDH2*, *RUNX1*, *NRAS/KRAS*, and *TET2*).

Compared with $PAK3^{low}$ group, $PAK3^{high}$ group had lower WBC count ($P = 0.006$), more patients with complex karyotype ($P = 0.007$) and fewer *CBFβ-MYH11* ($P = 0.026$), more poor-risk patients ($P = 0.029$), and more *TP53* mutation ($P = 0.048$). No significant differences were found in age, gender distribution, BM blasts, PB blasts, FAB subtypes, and frequency of other recurrent genetic mutations (*FLT3*, *NPM1*, *DNMT3A*, *IDH1/IDH2*, *RUNX1*, *NRAS/KRAS*, and *TET2*) between the two groups.

Compared with $PAK7^{low}$ group, $PAK7^{high}$ group had more old patients (≥60 years) ($P = 0.009$), more patients with complex karyotype ($P = 0.048$) and fewer *RUNX1-RUNX1T1* ($P = 0.026$), and fewer good-risk ($P = 0.003$) and more poor-risk ($P = 0.003$) patients. No significant differences were found in gender distribution, WBC count, BM blasts, PB blasts, FAB subtypes, and frequency of other recurrent genetic mutations (*FLT3*, *NPM1*, *DNMT3A*, *IDH1/IDH2*, *RUNX1*, *NRAS/KRAS*, *TET2*, and *TP53*) between the two groups.

Multivariate analysis of possible prognostic factors in the chemotherapy-only group

To further assess the prognostic significance of *PAK2/3/7*, expression levels of *PAK2/3/7* (high vs. low), age (≥60 vs. <60 years), WBC count (≥15 vs. $<15 \times 10^9$/L), *FLT3-ITD* (positive vs. negative), and other common genetic mutations (*NPM1*, *DNMT3A*, *RUNX1*, and *TP53*; mutated vs. wild) were selected for multivariate analysis (Table 4). Several independent risk factors for EFS and OS were identified, including high *PAK7* expression, age ≥60 years, and mutations in *NPM1*, *DNMT3A*, *RUNX1*, and *TP53* (all $P < 0.05$). Besides, high *PAK3* expression was an independent risk factor for EFS ($P = 0.029$).

Discussion

In our study, we found that high expressions of *PAK3* and *PAK7* were poor prognostic factors, and high *PAK2* expression was a good prognostic factor in AML patients who received chemotherapy only. However, the prognostic effects of *PAK2/3/7* on survival were not significant in patients who underwent allo-HSCT. This suggests that allo-HSCT may offset their prognostic impact.

A study shows that the protein stability of β-catenin is regulated by *PAK3* via AKT/GSK-3β signaling pathway in pancreatic cancer cells. In *PAK3* knockout cells, the formation of mammary gland globules, aldehyde dehydrogenase activity, and cancer stem cell-related markers are downregulated. These findings suggest that *PAK3*, as a major regulator of Akt/GSK-3β/β-catenin signaling, can promote tumor growth [18]. There are increased proliferation and migration of tumor cells and inhibition of apoptosis, which caused by higher *PAK7* expression in colorectal cancer, ovarian cancer, neuroglioma, and breast cancer [19–25]. Increased *PAK7* expression positively bound up with pathological differentiation and tumor node metastasis stage of breast cancer. By activating the Wnt/β-catenin signaling pathway, *PAK7* is involved in the progression of breast cancer [26]. Furthermore, *PAK7* plays an important role in tumorigenesis, invasion, and metastasis [27]. *PAK7* is upregulated in different gastric cancer cell tissues and lines, particularly in SGC-7901 and MGC-803 cells. By downregulating *PAK7* expression via lentivirus-mediated small interfering RNA and knocking out *PAK7*, it is confirmed that *PAK7* may promote tumor growth by affecting the expression of cell cycle regulator [28]. Consistent with these previous studies, we found that high expressions of *PAK3* and *PAK7* were more likely to occur in poor-risk patients, and co-exist with complex

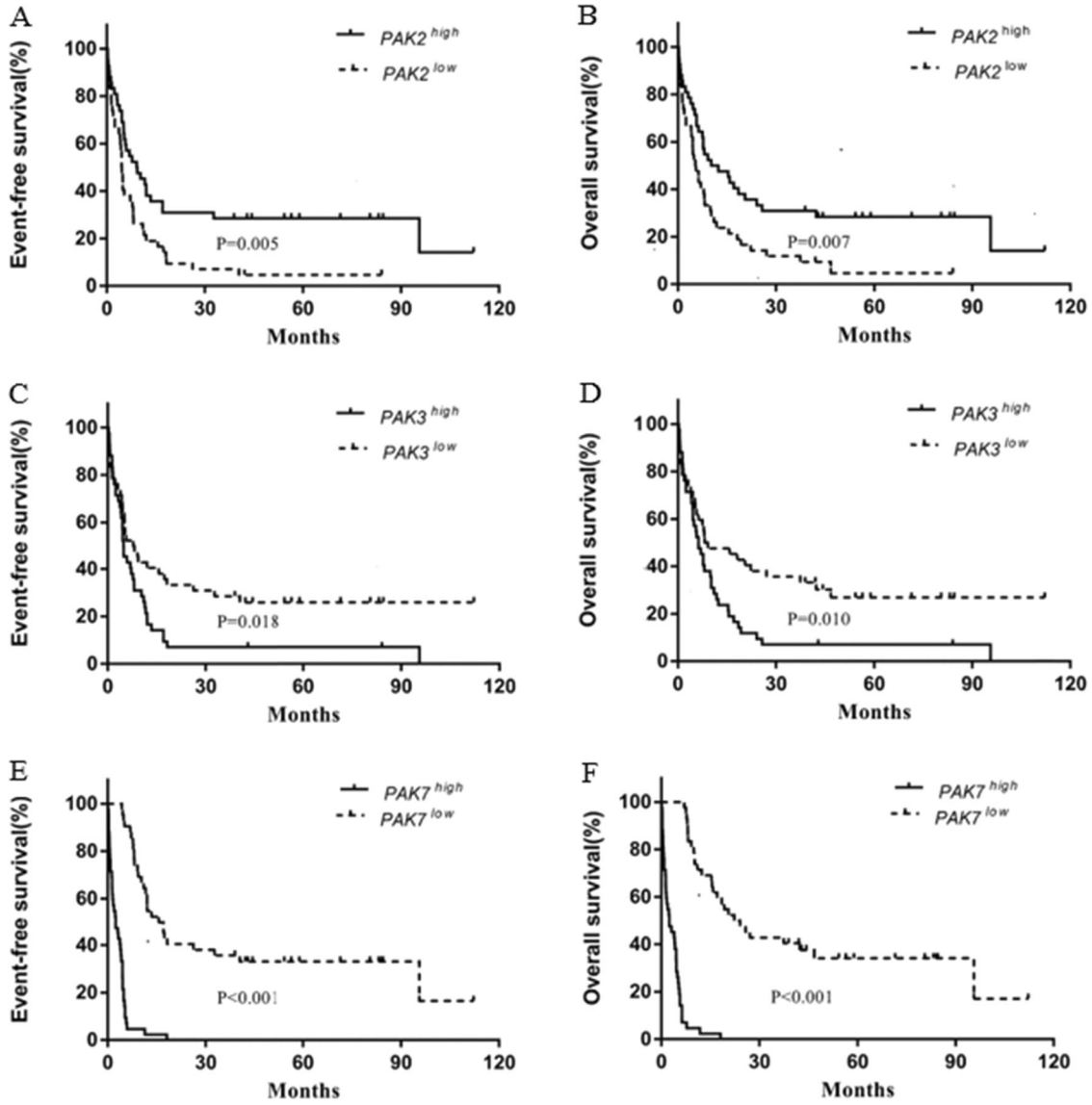

Fig. 1 Kaplan–Meier curves of event-free survival (EFS) and overall survival (OS) in patients who received chemotherapy only. **a**, **b** High *PAK2* expressers had longer EFS and OS than the low expressers. **c**, **d** High *PAK3* expressers had shorter EFS and OS than the low expressers. **e**, **f** High *PAK7* expressers had shorter EFS and OS than the low expressers

karyotypes. High *PAK3* and *PAK7* expression had adverse effects on EFS and OS, indicating that they may be involved in leukemogenesis.

Overexpression of *PAK2* is observed in ovarian cancer, and cell migration and invasion are decreased due to the knockdown of *PAK2* in ovarian cancer cell lines, suggesting that the phosphorylation of *PAK2* plays a vital role in ovarian carcinogenesis [29]. AP-1 lead to the activation of multiple tumor-related genes and promotion of tumorigenesis and malignant transformation, but activity of AP-1 is downregulated due to the knockdown of *PAK2* in JB6 Cl41 (P+) cells and sk-mel-5 melanoma cells, suggesting that PAK2 might act as the target of cancer chemoprevention [30]. Unlike the above findings, we found that high *PAK2* expression had favorable effect on EFS and OS in AML. It indicates that *PAK2* may play different roles in different diseases. Furthermore, multivariate analysis indicated that compared with *PAK2* and *PAK3*, *PAK7* has better prognostic value.

In multivariate analysis, we also found that age ≥60 years independently contribute to poor EFS and OS. This is consistent with previous finding that older AML patients along with poorer performance status, lower CR rate, and shorter survival because poorly tolerate intensive chemotherapy [31, 32]. Mutations in *DNMT3A*, *RUNX1*, and *TP53* were independent risk factors for survival in AML,

Table 2 Comparison of clinical and molecular characteristics in different *PAK2* expression groups among chemotherapy group

Characteristics	Total	*PAK2*		*P*
		High ($n = 42$)	Low ($n = 42$)	
Age (years), median (range)	66.5 (22–88)	62.5 (22–82)	70 (40–88)	0.003[a]
Age group, *n* (%)				0.009[b]
<60 years	26 (31.0)	19 (45.2)	7 (16.7)	
≥60 years	58 (69.0)	23 (54.8)	35 (83.3)	
Gender, *n* (%)				0.382[b]
Male	45 (53.6)	20 (47.6)	25 (59.5)	
Female	39 (46.4)	22 (52.4)	17 (40.5)	
WBC ($\times 10^9$/L), median (range)	14.7 (0.7–297.4)	31.5 (1–297.4)	8.6 (0.7–171.9)	0.024[a]
BM blasts (%), median (range)	72 (30–99)	72 (32–99)	71 (30–98)	0.463[a]
PB blasts (%), median (range)	23.5 (0–98)	32 (0–98)	22 (0–91)	0.248[a]
FAB subtypes, *n* (%)				
M0	7 (8.3)	2 (4.8)	5 (11.9)	0.433[b]
M1	20 (23.8)	9 (21.4)	11 (26.2)	0.798[b]
M2	21 (25)	11 (26.2)	10 (23.8)	1.000[b]
M4	20 (23.8)	13 (31)	7 (16.7)	0.200[b]
M5	12 (14.3)	7 (16.7)	5 (11.9)	0.756[b]
M6	1 (1.2)	0 (0.0)	1 (2.4)	1.000[b]
M7	3 (3.6)	0 (0.0)	3 (7.1)	0.241[b]
Cytogenetics, *n* (%)				
Normal	40 (47.6)	22 (52.4)	18 (42.9)	0.512[b]
Complex	11 (13.1)	1 (2.4)	10 (23.8)	0.007[b]
inv(16)/CBFβ-MYH11	6 (7.1)	5 (11.9)	1 (2.4)	0.202[b]
t(8;21)/RUNX1-RUNX1T1	6 (7.1)	5 (11.9)	1 (2.4)	0.202[b]
11q23/MLL	3 (3.6)	3 (7.1)	0 (0.0)	0.241[b]
−7/7q−	3 (3.6)	0 (0.0)	3 (7.1)	0.241[b]
t(9;22)/BCR-ABL1	1 (1.2)	0 (0.0)	1 (2.4)	1.000[b]
Others	14 (16.7)	6 (14.3)	8 (19)	0.771[b]
Risk, *n* (%)				
Good	12 (14.6)	10 (24.4)	2 (4.9)	0.026[b]
Intermediate	46 (56.1)	24 (58.5)	22 (53.7)	0.824[b]
Poor	24 (29.3)	7 (17.1)	17 (41.5)	0.028[b]
FLT3, *n* (%)				0.872[b]
FLT3-ITD	15 (17.9)	8 (19)	7 (16.7)	
FLT3-TKD	7 (8.3)	4 (9.5)	3 (7.1)	
Wild type	62 (73.8)	30 (71.4)	32 (76.2)	
NPM1, *n* (%)				0.641[b]
Mutation	27 (32.1)	15 (35.7)	12 (28.6)	
Wild type	57 (67.9)	27 (64.3)	30 (71.4)	
DNMT3A, *n* (%)				1.000[b]
Mutation	23 (27.4)	12 (28.6)	11 (26.2)	
Wild type	61 (72.6)	30 (71.4)	31 (73.8)	
IDH1/IDH2, *n* (%)				1.000[b]
Mutation	15 (17.9)	7 (16.7)	8 (19)	
Wild type	69 (82.1)	35 (83.3)	34 (81)	
RUNX1, *n* (%)				1.000[b]
Mutation	8 (9.5)	4 (9.5)	4 (9.5)	
Wild type	76 (90.5)	38 (90.5)	38 (90.5)	
NRAS/KRAS, *n* (%)				0.756[b]
Mutation	12 (14.3)	7 (17.5)	5 (11.9)	
Wild type	72 (90.5)	35 (83.3)	37 (88.1)	
TET2, *n* (%)				1.000[b]
Mutation	11 (13.1)	5 (11.9)	6 (14.3)	
Wild type	73 (86.9)	37 (88.1)	36 (85.7)	
TP53, *n* (%)				0.007[b]
Mutation	11 (13.1)	1 (2.4)	10 (23.8)	
Wild type	73 (86.9)	41 (97.6)	32 (76.2)	

WBC white blood cell, *BM* bone marrow, *PB* peripheral blood, *FAB* French American British

[a]Mann–Whitney *U*-test

[b]Chi-square test

which supported by the fact that *DNMT3A* mutation is highly recurrent in patients with de novo AML with an intermediate-risk cytogenetic profile and is independently associated with a poor outcome [33], *TP53* mutation is associated with complex karyotype, monosomal karyotype, and specific chromosomal aneuploidies, and predicts for very poor outcome [34, 35], and that patients with *RUNX1* mutation have shorter OS and EFS compared to *RUNX1* wild-type cases [36]. As to *NPM1* mutation, it is associated with a significantly better prognosis in AML. However, *NPM1* mutation has adverse prognostic effect in survival in our study. This may be caused by multifactor interaction and small sample size. In summary, our results can be used for risk stratification and treatment of AML.

In conclusion, our findings confirm that high expression of *PAK3* and *PAK7* indicate poor prognosis, and high *PAK2* expression predicts good prognosis in AML, but their prognostic effects on survival can be overcome by allo-HSCT. Overall, *PAK7* is an independent prognostic indicator in AML. Due to the small sample size, larger prospective researches are needed for further validation. As for the mechanism behind PAKs, more precise experiments are needed to explore.

Table 3 Comparison of clinical and molecular characteristics in different *PAK3/7* expression groups among chemotherapy group

Characteristics	*PAK3*		*P*	*PAK7*		*P*
	High ($n = 42$)	Low ($n = 42$)		High ($n = 42$)	Low ($n = 42$)	
Age (years), median (range)	68.5 (33–88)	63.5 (22–81)	0.085[a]	68.5 (41–88)	62 (22–81)	0.011[a]
Age group, *n* (%)			0.098[b]			0.009[b]
<60 years	9 (21.4)	17 (40.5)		7 (16.7)	19 (45.2)	
≥60 years	33 (78.6)	25 (59.5)		35 (83.3)	23 (54.8)	
Gender, *n* (%)			0.662[b]			0.662[b]
Male	24 (57.1)	21 (50)		24 (57.1)	21 (50)	
Female	18 (42.9)	21 (50)		18 (42.9)	21 (50)	
WBC ($\times 10^9$/L), median (range)	5.9 (0.7–171.9)	35 (2.3–297.4)	0.006[a]	15.6 (0.7–297.4)	13.7 (1–131.5)	0.658[a]
BM blasts (%), median (range)	67 (32–97)	73.5 (30–99)	0.388[a]	75.5 (30–99)	67 (33–98)	0.415[a]
PB blasts (%), median (range)	17.5 (0–91)	48 (0–98)	0.128[a]	27 (0–98)	22.5 (0–97)	0.774[a]
FAB subtypes, *n* (%)						
M0	6 (14.3)	1 (2.4)	0.109[b]	4 (9.5)	3 (7.1)	1.000[b]
M1	8 (19)	12 (28.6)	0.443[b]	14 (33.3)	6 (14.3)	0.071[b]
M2	12 (28.6)	9 (21.4)	0.615[b]	8 (19)	13 (31)	0.314[b]
M4	8 (19)	12 (28.6)	0.443[b]	6 (14.3)	14 (33.3)	0.071[b]
M5	5 (11.9)	7 (16.7)	0.756[b]	7 (16.7)	5 (11.9)	0.756[b]
M6	1 (2.4)	0 (0.0)	1.000[b]	1 (2.4)	0 (0.0)	1.000[b]
M7	2 (4.8)	1 (2.4)	1.000[b]	2 (4.8)	1 (2.4)	1.000[b]
Cytogenetics, *n* (%)						
Normal	16 (38.1)	24 (57.1)	0.126[b]	16 (38.1)	24 (57.1)	0.126[b]
Complex	10 (23.8)	1 (2.4)	0.007[b]	9 (21.4)	2 (4.8)	0.048[b]
inv(16)/CBFβ-MYH11	0 (0.0)	6 (14.3)	0.026[b]	1 (2.4)	5 (11.9)	0.202[b]
t(8;21)/RUNX1-RUNX1T1	3 (7.1)	3 (7.1)	1.000[b]	0 (0.0)	6 (14.3)	0.026[b]
11q23/MLL	2 (4.8)	1 (2.4)	1.000[b]	2 (4.8)	1 (2.4)	1.000[b]
−7/7q−	3 (7.1)	0 (0.0)	0.241[b]	2 (4.8)	1 (2.4)	1.000[b]
t(9;22)/BCR-ABL1	0 (0.0)	1 (2.4)	1.000[b]	1 (2.4)	0 (0.0)	1.000[b]
Others	8 (19)	6 (14.3)	0.771[b]	11 (26.2)	3 (7.1)	0.038[b]
Risk, *n* (%)						
Good	3 (7.1)	9 (22.5)	0.064[b]	1 (2.5)	11 (26.2)	0.003[b]
Intermediate	22 (52.4)	24 (60)	0.513[b]	21 (52.5)	25 (59.5)	0.657[b]
Poor	17 (40.5)	7 (17.5)	0.029[b]	18 (45)	6 (14.3)	0.003[b]
FLT3, *n* (%)			0.390[b]			0.872[b]
FLT3-ITD	6 (14.3)	9 (21.4)		7 (16.7)	8 (19)	
FLT3-TKD	5 (11.9)	2 (4.8)		3 (7.1)	4 (9.5)	
Wild type	31 (73.8)	31 (73.8)		32 (76.2)	30 (71.4)	
NPM1, *n* (%)			0.160[b]			1.000[b]
Mutation	10 (23.8)	17 (40.5)		13 (31)	14 (33.3)	
Wild type	32 (76.2)	25 (59.5)		29 (69)	28 (66.7)	
DNMT3A, *n* (%)			0.328[b]			1.000[b]
Mutation	9 (21.4)	14 (33.3)		12 (28.6)	11 (26.1)	
Wild type	33 (78.6)	28 (66.7)		30 (71.4)	31 (73.8)	

Table 3 (continued)

Characteristics	*PAK3*		*P*	*PAK7*		*P*
	High ($n = 42$)	Low ($n = 42$)		High ($n = 42$)	Low ($n = 42$)	
IDH1/IDH2, *n* (%)			0.570[b]			1.000[b]
Mutation	6 (14.3)	9 (21.4)		8 (19)	7 (16.7)	
Wild type	36 (85.7)	33 (78.6)		34 (81)	35 (83.3)	
RUNX1, *n* (%)			0.265[b]			0.713[b]
Mutation	6 (14.3)	2 (4.8)		5 (11.9)	3 (7.1)	
Wild type	36 (85.7)	40 (95.2)		37 (88.1)	39 (92.9)	
NRAS/KRAS, *n* (%)			0.756[b]			1.000[b]
Mutation	5 (11.9)	7 (17.1)		6 (14.3)	6 (14.3)	
Wild type	37 (88.1)	35 (83.3)		36 (85.7)	36 (85.7)	
TET2, *n* (%)			1.000[b]			0.194[b]
Mutation	6 (14.3)	5 (11.9)		3 (7.1)	8 (19)	
Wild type	36 (85.7)	37 (88.1)		39 (92.9)	34 ((81)	
TP53, *n* (%)			0.048[b]			0.194[b]
Mutation	9 (21.4)	2 (4.8)		8 (19)	3 (7.1)	
Wild type	33 (78.6)	40 (95.2)		34 (81)	39 (92.9)	

WBC white blood cell, *BM* bone marrow, *PB* peripheral blood, *FAB* French American British

[a]Mann–Whitney *U*-test

[b]Chi-square test

Table 4 Multivariate analysis of EFS and OS in chemotherapy group

Variables	EFS		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
PAK2 (high vs. low)	1.019 (0.589–1.762)	0.947	1.075 (0.612–1.889)	0.801
PAK3 (high vs. low)	1.909 (1.069–3.409)	0.029	1.701 (0.947–3.054)	0.075
PAK7 (high vs. low)	12.182 (6.089–24.372)	0.000	21.517 (9.691–47.774)	0.000
Age (≥60 vs. <60 years)	2.461 (1.295–4.679)	0.006	2.131 (1.123–4.045)	0.021
WBC (≥15 vs. $<15 \times 10^9$/L)	1.131 (0.650–1.966)	0.663	1.106 (0.637–1.920)	0.720
FLT3-ITD (positive vs. negative)	1.667 (0.841–3.302)	0.143	1.728 (0.849–3.516)	0.131
NPM1 (mutated vs. wild)	2.386 (1.277–4.459)	0.006	2.059 (1.080–3.928)	0.028
DNMT3A (mutated vs. wild)	2.041 (1.084–3.845)	0.027	1.967 (1.057–3.659)	0.033
RUNX1 (mutated vs. wild)	2.516 (1.057–5.988)	0.037	3.187 (1.327–7.656)	0.010
TP53 (mutated vs. wild)	3.632 (1.556–8.477)	0.003	4.178 (1.749–9.981)	0.001

EFS event-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidential interval, *WBC* white blood cell, *BM* bone marrow, *PB* peripheral blood

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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