

## Prognostic Factor Analysis in Core-Binding Factor-positive Acute Myeloid Leukemia

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**Abstract.** *Background: Acute myeloid leukemia (AML) cases with t(8;21) or inv(16) have a favorable outcome, but the associated prognoses are heterogeneous and complicated by additional molecular aberrations. Patients and Methods: Between January 2000 to December 2010, 67 patients were diagnosed with t(8;21) or inv(16) AML. We collected cytogenetic variables and analyzed treatment outcomes. Results: Among 67 patients, 51 (7.8%) had t(8;21) AML and 16 (2.4%) had inv(16) AML. Thrombocytopenia, and a high percentage of blasts in the peripheral blood and bone marrow were associated with poor overall survival. Twenty-five (49.0%) patients with t(8;21) had an additional chromosomal abnormality, while only six (37.5%) patients with inv(16) AML had a secondary chromosomal abnormality. The most common chromosomal abnormalities were deletion of the Y or X sex chromosomes. Conclusion: Deletion of the Y chromosome may be a favorable prognostic factor in patients with core binding factor-positive AML.*

Core-binding factor (CBF) is a heterodimeric transcription factor complex composed of alpha and beta subunits that plays a pivotal role in normal hematopoiesis (1). CBF-positive acute myeloid leukemia (AML) results from the disruption of the  $\alpha$  and  $\beta$  subunits of CBF genes, and is characterized by the presence of t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) (2). The CBF $\alpha$  subunit is

encoded by one of the three homologous genes belonging to the *Runt-related transcription factor (RUNX)* family, the *RUNX1 (AML1; CBFA2; PEBP2aB)* gene, whereas the CBF $\beta$  subunit is encoded by the *CBFB (PEBP2B)* gene. The CBF $\alpha$  subunit binds directly to the DNA promoter sequences of target genes involved in hematopoiesis, whereas the beta subunit does not bind to DNA but enhances the DNA affinity of the CBF complex and protects it from proteolysis. t(8;21) targets the *RUNX1* gene on chromosome 21q22 and the *Eight twenty One (ETO)* gene on chromosome 8q22.

RUNX proteins bind DNA as heterodimers through CBF $\beta$ , and both DNA-binding and heterodimerization are mediated through a central RUNX domain that has a high degree of homology with the *Drosophila* protein Runt. As described above, CBF $\beta$  does not directly contact DNA, but instead increases the stability and DNA-binding affinity of RUNX proteins. CBFs play a critical role in the development of several different cellular lineages. For example, RUNX1 is a master regulatory protein that controls the formation of definitive hematopoietic stem cells (HSCs) (3). Expression of RUNX1 and CBFB genes appears to be essential for the development of normal hematopoiesis. In murine knockout models, homozygous loss of *Runx1* or *Cbfb* results in embryonic death due to lack of definitive hematopoiesis (4). At the molecular level, chromosomal aberrations form *RUNX1-RUNXIT1* and *BCFB-Myosin, heavy chain 11, smooth muscle (MYH11)* fusion genes, which disrupt subunits  $\alpha$  and  $\beta$  of CBF. *RUNX1-RUNXIT1* can be generated not only by t(8;21), but also by its infrequent variants, including complex translocations involving one or two additional chromosomes (5-8) and insertions [e.g. ins(21;8)(q22q22;q22) and ins(8;21)(q22;q22q22)] (5-7, 9), although these are more rare. CBF-AML represents one of the most common cytogenetic abnormalities in acute myeloid leukemia (10), comprising of 4-12% of newly-diagnosed cases of AML (11). Patients with CBF-AML have a relatively favorable outcome, with a significantly lower median age and better prognosis than

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patients with AML associated with a normal karyotype or chromosomal aberrations. The favorable outcome, with higher complete remission (CR) rate and lower incidence of relapse, especially for patients who receive high-dose cytarabine in post-remission treatment (12-14), has led to the formation of several collaborations aimed at discussing the indications for allogeneic stem cell transplantation (SCT) in CBF-AML (15-17).

Unlike the cytogenetic definition of CBF-AML, patient outcome does not appear to be as homogeneous as its cytogenetic definition. Indeed, 30-50% of patients with CBF-AML experience relapse and the 5-year survival is only 50% (18). The prognosis of patients with CBF-AML is heterogeneous, and is frequently complicated by additional chromosomal or molecular aberrations. Specifically, additional chromosomal aberrations are detected at diagnosis in 40% of patients with inv(16)/t(16;16) and in 70% of t(8;21) patients (2, 19). Several studies have suggested that secondary chromosome aberrations may have clinical significance, and that there is also a difference between patients with inv(16)/t(16;16) and those with t(8;21). Schlenk *et al.* reported that secondary aberrations such as +8, +21, and +22 are more common in those with inv(16)/t(16;16), while -Y, -X, and del(9q) are more common in those with t(8;21) (17). However, most of these additional chromosomal aberrations fail to exhibit clinical significance. For example, male patients with t(8;21) and -Y have a short overall survival and duration of first CR (17). On the contrary, a study from China showed that male patients with t(8;21) and -Y have a good prognosis (11). Several acquired genetic mutations and changes in both gene and microRNA expression that occur in addition to t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22), the cytogenetic hallmarks of CBF-AML, have been recently reported. The most common mutations are receptor tyrosine kinase (RTK) *c-KIT* mutations and *Fms-like tyrosine kinase 3 (FLT3)* mutations, which are present in 12-47% of patients with t(8;21), and in 22-38% of those with inv(16)/t(16;16). Haploinsufficiency of the putative tumor suppressor genes *Transducer-like enhancer of split-1 (TLE1)* and *Transducer-like enhancer of split-1 (TLE4)* in patients with t(8;21) with del(9q), *Meningioma 1 (MNI)* overexpression in patients with inv(16), and epigenetic and post-transcriptional silencing of *CCAAT/enhancer-binding protein alpha (CEBPA)* have also been reported (2). Rat sarcoma (*RAS*) mutations have been identified in 36% of inv(16)/t(16;16) and 5% of t(8;21) cases. In a model proposed by Gilland *et al.*, AML was suggested to result from class-I mutations conferring a proliferative advantage to AML and class-II mutations leading to impaired hematopoietic differentiation. With respect to CBF-AML, the *c-KIT*, *FLT3*, and *RAS* genes may be sites of class I mutations, while *RUNX1-ETO* and *CBFβ-MYH11* gene fusions represent class-II mutations. Several other studies have shown that *c-KIT* mutations are associated with shorter event-free survival and overall survival. Especially for t(8;21) AML, *KIT*

mutations occur mostly in exon 17 and confer an adverse prognosis (20-25), whereas the prognostic significance of *KIT* mutations (both in exons 8 and 17) in inv(16) AML is not well-established. *KIT* mutations can be targeted by tyrosine kinase inhibitors, which are selectively active against specific *KIT* mutations. For instance, imatinib is active against various exon 8 mutations and the exon 17 mutation involving codon N822, but not against mutations involving codon D816, which can be successfully targeted with dasatinib and midostaurin (26).

The mutation status of *RAS* is not associated with patient prognosis, and thus the clinical relevance of *RAS* mutations remains controversial. Among patients with inv(16)/t(16;16) and t(8;21) AML, clinical outcomes are heterogeneous, which is contrary to the cytogenetic definition. This observation may be related to various gene aberrations, additional chromosomal abnormalities, and specific clinical features. For these reasons, it is important to consider clear standards on the basis not only of chromosomal differences, but also of comprehensive prognostic factor analysis including gene aberration and clinical features. Thus, the aims of the present study were to analyze genetic and clinical markers in patients with CBF-AML patients and to identify patients who require intensive treatment.

## Patients and Methods

Between January 2000 to December 2010, 657 patients were diagnosed with AML. We identified the patients with t(8;21) AML and the patients with inv(16)/t(16;16) AML through successful cytogenetic analysis of bone marrow (BM). The following clinicopathological variables and treatment outcomes were retrospectively collected: Patient demographics, complete blood count, liver function test, renal function test, and C-reactive protein levels. We also collected information regarding additional chromosomal abnormalities and gene mutations *via* analysis of bone marrow or peripheral blood blast samples. The presence of *FLT3-Internal-tandem duplications (ITD)* was analyzed by *Polymerase chain reaction (PCR)* on genomic DNA using primer pair 11F and 12R. Detection of *c-KIT* mutations was performed using genomic DNA with PCR fragment analysis and direct sequencing. We only screened exon 17 D816 and N822 mutations, which were assayed together. To analyze the mutation status of exon 8 in *c-KIT*, we performed direct sequencing.

**Treatment.** Patients received induction therapy according to the following doses: cytarabine of 200 mg/m<sup>2</sup> for seven days in combination with idarubicin of 12 mg/m<sup>2</sup> for three days. As part of consolidation, patients received three to four cycles of high-dose cytarabine and idarubicin as follows: cytarabine of 3 g/m<sup>2</sup> every 12 h on days 1, 3, and 5 in combination with idarubicin of 12 mg/m<sup>2</sup> on days 2 and 4.

**Statistical analysis.** We analyzed prognostic factors related to overall survival, progression-free survival (PFS), and leukemia-free survival (LFS). For overall survival, events were defined as death from any cause. Overall survival was measured from the first date of chemotherapy to the date of death or the date of the last follow-up

Table I. *Clinical and laboratory findings before treatment.*

Characteristic	t(8;21) (n=51)	Inv(16) (n=16)	p-Value
Median age (range),years	40 (18-70)	47 (18-75)	0.84
Age >60 years	7 (13.7)	1 (6.3)	0.67
WBC count ( $10^3/\mu\text{l}$ )	10.5 (0.3-46.2)	11.6 (3.5-113)	1.00
Hemoglobin (g/dl)	7.7 (2.4-13.9)	8.8 (3.7-10.8)	0.02
Platelets ( $10^3/\mu\text{l}$ )	30 (5-117)	33 (8-92)	0.59
Percentage of PB blasts (%)	28 (0-81)	41 (10-88)	0.51
Percentage of BM blasts (%)	41.4 (20-83.2)	64 (22.5-90.0)	0.23
Total bilirubin	0.5 (0.2-3.1)	0.7 (0.3-1.3)	0.36
AST (U/l)	24 (8-108)	27 (11-95)	0.43
ALT (U/l)	19 (7-361)	30 (15-66)	0.75
ALP (U/l)	63 (31-501)	65 (35-336)	0.78
Creatinine (>1.1 mg/dl);	0.8 (0.4-1.5)	0.8 (0.6-2.1)	0.77
LDH (IU/l)	845 (3-2382)	443 (1110-3494)	0.31
Ferritin (ng/dl)	742 (480-3101)	256 (1228-4176)	0.39
C-Reactive protein (mg/dL)	2.17 (0.03-23.7)	2.3 (0.23-21.2)	0.86

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; Creatinine: serum creatinine; LDH: lactate dehydrogenase; BM: bone marrow; PB: peripheral blood; WBC: white blood cell.

visit. CR was defined as recovery of morphologically-normal BM and normal blood counts (neutrophils  $\geq 1500/\mu\text{l}$  and platelets  $\geq 100,000/\mu\text{l}$ ) and no circulating leukemic blasts or evidence of extramedullary leukemia. Relapse was defined by  $\geq 5\%$  BM blasts, circulating leukemic blasts, or development of extramedullary leukemia. Relapse-free survival was measured from the date of the first administration of chemotherapy to the date of relapse, death, or last follow-up visit.

Leukemia-free survival was measured from the date of the first CR to the date of relapse, death, or last follow-up visit. Survival analysis was estimated using the Kaplan-Meier method and compared by the log-rank test. Multivariate survival analysis was carried out using a Cox regression model. Results that reached a level of  $p < 0.05$  were considered statistically significant. Estimates for hazard ratios and corresponding 95% confidence interval (CI) were obtained for each significant prognostic factor.

## Results

**Clinical features and treatment outcomes.** Between January 2000 to December 2010, approximately 67 (10.2%) out of the 657 patients with newly-diagnosed of AML were found to have CBF-positive AML. We identified 51 (7.8%) patients with t(8;21) AML and 16 (2.4%) patients with inv(16)/t(16;16) AML through successful cytogenetic analysis of BM. The median age of patients was 44 (range: 18-75) years, and 31 (46.3%) patients were male. Clinical and laboratory findings obtained before starting treatment are summarized in Table I. Only hemoglobin levels were significantly different between patients with t(8;21) and patients with inv(16)/t(16;16). A total of seven patients were enrolled in the t(8;21) AML group, while only one patient was enrolled in the inv(16) AML group; the age of patients

in both groups was >60 years. The overall CR rate was 92.3% (62/67), the median overall survival was 80.6 months and median relapse-free survival was 68.4 months.

We analyzed the relationship among pre-treatment clinical and laboratory findings and treatment outcomes including overall survival and leukemia-free survival. Univariate analysis indicated that thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{l}$ ), a high percentage of blasts in peripheral blood (>50%), and a high percentage of blasts in BM (>50%) were all significantly associated with poor overall survival (Table II, Figure 1). However, sex, old age (>60 years), leukocytosis or leucopenia, and low hemoglobin levels were not significantly associated with survival. In leukemia-free survival, thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{l}$ ) and high percentage of blasts in BM (>50%) were significantly associated with a shorter duration of leukemia-free survival, while patients with Y chromosome deletions exhibited longer leukemia-free survival (Table II). With respect to relapse-free survival, thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{l}$ ) and high percentage of blasts in BM (>50%) were significantly associated with poor progression-free survival, while Y chromosome deletions were favorable factors for relapse-free survival. A total of 11 (21.6%) patients with t(8;21) AML and 5(31.3%) patients with inv(16)/t(16;16) AML received BM transplantations. Most patients who received BM transplantation had additional chromosomal abnormalities or *c-KIT* mutations. In our study two patients with multi-lineage dysplasia identified during an initial bone marrow study also experienced a relapse after 24 months and had a poor overall survival of less than 36 months. Ultimately, these two patients died from uncontrolled infections.

Table II. *Clinical features and treatment outcomes.*

Characteristic	No. of patients (%)	Overall survival			Leukemia-free survival		
		HR	95% CI	p-Value	HR	95% CI	p-Value
Male	32 (47.8)	0.76	0.37-1.54	0.45	0.84	0.41-1.72	0.63
Age >60 years	8 (11.9)	1.90	0.72-5.00	0.19	2.07	0.79-5.45	0.14
WBC ( $10^3/\mu\text{L}$ )							
≤3,800	12 (17.9)	0.92	0.41-2.08	0.84	0.75	0.27-2.54	0.11
3,800-10,580	20 (29.9)	1.00					
>10,580	35 (52.2)	1.02	0.40-2.62	0.96	0.92	0.39-2.85	0.31
Hemoglobin (≤8.0 g/dl)	29 (43.3)	1.37	0.60-3.10	0.46	0.83	0.37-1.87	0.66
Platelets (≤ $20 \times 10^3/\mu\text{L}$ )	25 (37.3)	2.29	1.12-4.66	0.02	1.03	1.00-1.04	0.008
PB Blasts >50%	19 (28.4)	2.25	1.02-4.96	0.04	2.02	0.90-4.54	0.08
BM Blasts >50%	34 (50.7)	2.25	1.02-4.96	0.03	2.97	1.36-6.51	0.006
Additional chromosome	12 (17.9)	1.03	0.40-2.69	0.95	2.98	0.70-12.67	0.14
Y Deletion	18 (58.1)	0.39	1.15-9.14	0.02	0.37	0.14-1.03	0.03
X Deletion	5 (7.5)	1.16	0.26-5.18	1.16	1.05	0.23-4.69	0.95
9 Deletion	2 (2.9)	1.19	0.16-8.76	0.87	0.94	0.22-3.95	0.93
Trisomy 22	3 (4.8)	1.30	0.15-11.80	0.81	2.41	0.56-10.30	0.24
c-KIT mutation	5 (7.5)	2.15	0.27-16.90	0.47	3.16	0.41-24.49	0.27
Multi-lineage dysplasia	2 (5.4)	3.00	0.71-12.78	0.14	2.98	0.70-12.67	0.14

WBC: White blood cell; PB: peripheral blood; BM: bone marrow; HR: hazard ratio; CI: confidence interval.

**Additional chromosomal abnormalities and gene mutations.** A total of 25 (49.0%) patients with t(8;21) had additional chromosomal abnormalities, the most common of which were deletions of the sex chromosomes X or Y. Specifically, 15 of the 25 male patients with t(8;21) AML and one of the 6 male patients with inv(16) were found to have a Y chromosome deletion. In addition, two patients with a Y chromosome deletion also had a deletion of chromosome 9. The remaining chromosomal abnormalities were deletion of chromosome 9 or 7, complex chromosomal abnormality, and abnormal 19q 13. In patients with inv(16)/t(16;16) AML, only 6 (37.5%) patients had a secondary chromosomal abnormality, of which trisomy 21 was the most common (Table III).

There were two patients with multi-lineage dysplasia observed at the initial BM examination, both of whom had a poor prognosis. Conversely, male patients with Y chromosome deletions had a good prognosis with a hazard ratio of 0.39 (95% CI=1.15-9.14) and *p*-value of 0.02. Y chromosome deletions were also associated with short relapse-free survival (*p*-value=0.04). The statistical power of the Y chromosome deletion was maintained not only for cases of t(8;21) AML, but also for cases of total CBF-AML.

We next checked the *c-KIT* mutation status in 27 patients, and found that *c-KIT* mutations were present in 5 (18.5%) patients with CBF-AML. A total of two patients had exon 17 t(8;21) AML, one patient had a mutation in exon 8, and three patients had mutations in exons 10 and 11. However, in our study, *c-KIT* mutations were not clinically significant

because of the small number of patients. We also analyzed the status of *FLT3*-ITD in 26 patients with CBF-AML, but did not identify any mutations.

We next performed a multivariate analysis of all of the variables that showed a significant effect on overall survival in univariate analysis including thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{L}$ ), high percentage of blasts in the peripheral blood (>50%), high percentage of blasts in BM (>50%), and Y chromosome deletions. In male patients, thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{L}$ ), high percentage of blasts in peripheral blood (>50%), high percentage of blasts in BM (>50%), and Y chromosome deletions retained their significance in multivariate analysis (Table IV). In female patients however, only thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{L}$ ) and a high percentage of blasts in the peripheral blood (>50%) were associated with poor overall survival in multivariate analysis (Table IV).

## Discussion

CBF-AML is defined by the presence of either t(8;21)(q22;q22) or inv(16)(p13;q22)/t(16;16)(p13;q22) chromosomal re-arrangements that result in the disruption of *CBFA* and *CBFB* genes encoding CBF  $\alpha$  and  $\beta$  subunits, respectively. CBF-AML accounts for approximately 4-12% of all cases of AML. CBF-AML is associated with a significantly lower median age of patients, as well as improved prognosis compared to patients with AML with a normal karyotype or other chromosomal aberrations. The favorable outcome associated with CBF-AML is also

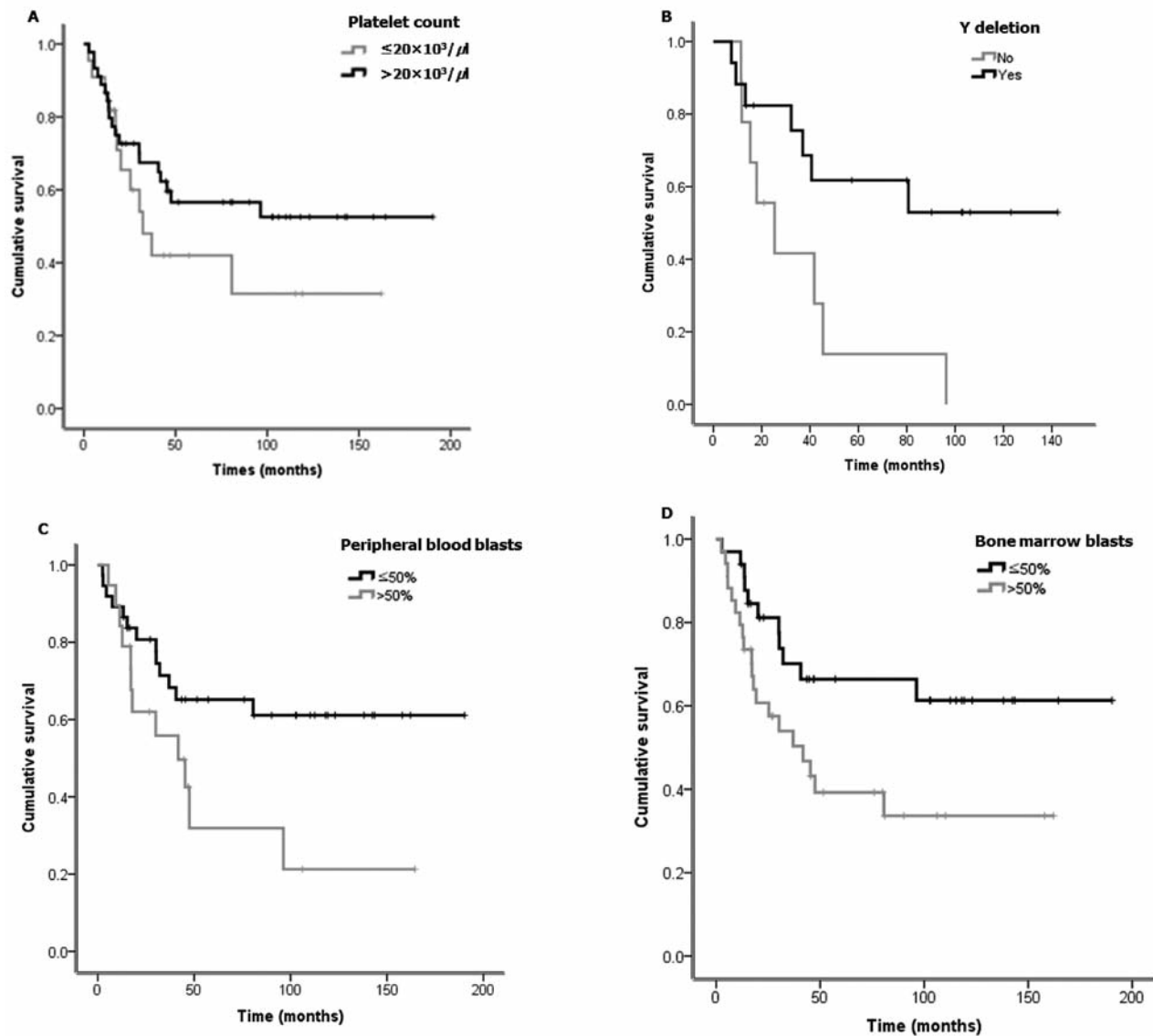


Figure 1. Survival curves based on the factors: platelet count (A), Y chromosome deletion (B), peripheral blood blasts (C), and bone marrow blasts (D).

associated with a higher rate of CR and a lower incidence of relapse, especially for patients receiving high-dose cytarabine in post-remission treatment, which has led to several collaborative efforts to discuss the indications of allogeneic SCT in CBF-AML. The clinical heterogeneity of CBF-AML is well known, with 30-50% of patients with CBF-AML experiencing relapse and a 5-year survival of only 50%. The heterogeneity of CBF-AML prognosis is likely complicated by additional chromosomal or molecular aberrations, and thus several attempts have been made to identify prognostic factors to identify aspects of pre-treatment clinical features and genetic findings (27). Previous studies on prognostic factor analysis can be divided into pre-treatment clinical

features including ethnicity, sex, and laboratory findings; additional chromosomal abnormalities including +8, +21, +22, and deletion of sex chromosomes; estimation of minimal residual disease; and mutations of *c-KIT*, *FLT3*, and *RAS*. However, it has not yet been possible to build a risk stratification strategy based on existing data.

The present study was designed to analyze all factors that might be related to treatment outcomes, including clinical, laboratory, and genetic factors in CBF-AML. Importantly, prognostic analysis in our study showed that deletion of the Y sex chromosome in male patients with CBF-AML was an independent indicator of favorable disease outcome, and may thus be considered as a favorable prognostic biomarker.

Table III. Additional chromosomal abnormalities.

Additional abnormalities	t(8;21) AML		inv(16) AML		p-Value
	No. of patients	%	No. of patients	%	
Y Deletion	15/25	60.0%	1/6	16.7%	0.03
X Deletion	4/26	15.4%	0/10	0%	0.68
9 Deletion	2	3.9%	0	0%	0.42
7 Deletion	1	1.9%	0	0%	0.43
Complex ( $\geq 3$ )	2	3.9%	1	6.3%	0.57
Abn19q13	1	0%	0	0%	0.12
Trisomy 22	0	0%	3	18.8%	0.57
Other	0	0%	2	11.1%	

Table IV. Multivariate analysis.

Characteristic	Overall survival			Leukemia-free survival		
	HR	95% CI	p-Value	HR	95% CI	p-Value
Male						
Platelets ( $\leq 20 \times 10^3/\mu\text{l}$ )	5.03	1.28-9.65	0.02	1.00	0.95-1.06	0.90
PB Blasts ( $>50\%$ )	3.32	0.55-20.16	0.19	1.69	0.45-6.29	0.44
BM Blasts ( $>50\%$ )	2.27	0.54-9.57	0.02	3.00	0.29-30.4	0.35
Y Deletion	3.88	0.97-4.12	$<0.001$	0.61	0.15-2.47	0.49
Female						
Platelets ( $\leq 20 \times 10^3/\mu\text{l}$ )	1.04	0.97-1.11	0.22	1.00	0.94-1.07	0.81
PB Blasts ( $>50\%$ )	1.91	0.58-6.33	0.29	1.60	0.51-5.07	0.42
BM Blasts ( $>50\%$ )	1.64	0.05-8.28	0.73	2.91	0.25-33.9	0.39

PB: Peripheral blood; BM: bone marrow; CI: confidence interval; HR: hazard ratio.

According to multivariate analysis, thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{l}$ ) and a high percentage of blasts in BM ( $>50\%$ ) were also associated with a poor prognosis.

Several reports have discussed the clinical significance of additional sex chromosome abnormalities (28). Deletion of the Y chromosome is more common in children than in adults (29), and thus it is possible that there is an age-related heterogeneity of chromosomal aberrations and different genetic mechanisms between children and adults (30). In a previous study on additional sex chromosomal abnormalities in CBF-AML, the clinical significance of the results proved controversial. Specifically, Schelink *et al.* reported that male patients with a Y chromosome deletion had significantly reduced overall survival and duration of first CR (17). However, in a study performed in children, additional chromosomal abnormalities, including sex chromosomes, were found to reflect relatively late stages of leukemia and their presence before treatment accurately predicted poor prognosis (31-33). Several other reports showed that the

prognostic impacts of these additional changes were negligible (34-38). Consistent with the results of our study, several other reports have shown that male patients with Y chromosomal deletions have a good prognosis (39); however, most of these studies consisted of sub-group analyses of a small number of patients, and thus the clinical significance of Y deletions in CBF-AML has not yet been clearly defined.

In our study, two patients with multi-lineage dysplasia during an initial BM study experienced a relapse after 24 months and had a poor survival of less than 36 months. These patients died due to uncontrolled infection. According to a study by Ma *et al.*, *de novo* AML involving multi-lineage dysplasia is associated with poor response to therapy, while dysplasia in t(8;21) AML has not been shown to influence prognosis (40). However, they analyzed dysplasia in hematopoietic lineages rather than multilineage dysplasia, and thus their results suggest that patients with multilineage dysplasia may exhibit increased vulnerability to infections, although this will require evaluation in a large-scale.

In the present study, we analyzed 67 patients with CBF-AML, taking into account the incidence of secondary genetic aberrations (*c-KIT*, *FLT3*) (2). However, the patient sample size was small, and thus our results did not appear clinically significant. *c-KIT* is a proto-oncogene that encodes a type-III trans-membrane tyrosine kinase that functions as a receptor for the cytokine stem cell factor, also known as mast cell growth factor. Mutations of *c-KIT* are common in CBF-AML, and are present in 12.7% to 48.1% of all cases of AML1-ETO leukemia (20). Mutations at Asp816 and Arg822 in the tyrosine kinase domain are the two most common mutation in t(8;21) AML (24), and several reports have shown that various *c-KIT* mutations adversely affect the frequency of relapse and overall survival of patients with CBF-AML (41, 42). Several *c-KIT* mutations are associated with poor treatment outcome, including mutations at position Asp816 in exon 17 and several mutations appearing in exon 8. In particular, the Asp816 mutation is found in 10.5% of all cases of t(8;21) AML and is associated with poor overall survival and a high rate of relapse.

Laboratory findings have implicated both leukocytosis and thrombocytopenia in influencing CBF-AML outcome. Several additional reports have shown that low platelet counts may reflect poor treatment outcomes (18, 37). Similarly, Billstrom *et al.* showed that the presence of leukocytosis at the initial diagnosis of CBF-AML is associated with a less favorable prognosis (18, 38, 40, 43). In the present study, however, neither leukocytosis nor leucopenia appeared to be related to treatment outcome.

A limitation of the present study was the gene mutation test, which was performed for only a few patients and thus had very limited statistical power. However, according to a previous study (44), of a total of 116 patients, *mutKIT17* and *mutKIT8* were identified in 36 (31%) and seven (6%) patients, respectively. In patients with t(8;21), prognosis was significantly worse in patients with *mutKIT17* compared to those without the mutation. Interestingly, this difference was limited to adults. In patients with inv(16), there was no prognostic impact of *c-KIT* mutations, and therefore an analysis of *mutKIT17* in adult CBF-AML patients with t(8;21) is recommended for a predictive prognosis (44). The incidence of aberrant cluster of differentiation-56 (CD56) expression was significantly higher in patients with a mutation of *c-KIT* in exon 17 compared to those without a mutation (20-21).

*FLT3* is a class-III receptor tyrosine kinase in a family that also includes KIT. *FLT3* is expressed in both early hematopoietic stem cells and in a subset of dendritic cell progenitors. FLT3 signaling activates intracellular pathways [e.g. *RAS*- *Rapidly Accelerated Fibrosarcoma* (*Raf*)-mitogen-activated protein kinase/extracellular signal-regulated kinase (Mek), phosphatidylinositol 3-kinase (PI3K)]- v-akt murine thymoma viral oncogene homolog 1 provided (*AKT*)

that promote proliferation and inhibition of apoptosis. The most commonly described *FLT3* mutation in AML is the ITD mutation of the juxtamembrane segment. The *FLT3*-ITD mutation leads to loss of the autoinhibition exerted by the juxtamembrane domain over the tyrosine kinase domain, generating a constitutively active FLT3 molecule. *FLT3*-ITD mutations are found in 20% to 30% of patients with AML and are more common in normal karyotype AML, acute promyelocytic leukemia, and AML with a t(6;9)(p23;q34) translocation (9-14). Patients who have *FLT3*-ITD-positive normal-karyotype AML have a higher leukocyte count and exhibit a rate of complete response similar to that of *FLT3*-ITD-negative patients, but have shorter disease-free survival and overall survival, mainly because of frequent relapses (45-50). We did not observe any *FLT3*-ITD mutations in the patients analyzed in this study.

The results of our study are meaningful because of our decision to collect and analyze all factors that might be related with treatment outcomes including clinical, laboratory and genetic factors in CBF-AML in South Korea. Indeed, previous studies have only analyzed a few factors. Additionally, the heterogeneity of CBF-AML may be related to ethnicity and age, and this is the first such prognostic factor analysis of CMF-AML in Korea.

## Conclusion

The results of the present study suggest that deletion of the Y chromosome may be a favorable prognostic factor for patients with CBF-AML, while thrombocytopenia ( $\leq 20 \times 10^3/\mu\text{l}$ ) and a high percentage of blasts in BM ( $>50\%$ ) were associated with a poor prognosis. Further studies are required to confirm the feasibility of risk stratification for use in risk-adapted therapy.

## References

- 1 Speck NA and Gilliland DG: Core-binding factors in haematopoiesis and leukaemia. *Nat Rev Cancer* 2: 502-513, 2002.
- 2 Mrozek K, Marcucci G, Paschka P and Bloomfield CD: Advances in molecular genetics and treatment of core-binding factor acute myeloid leukemia. *Curr Opin Oncol* 20: 711-718, 2008.
- 3 Okuda T, van Deursen J, Hiebert SW, Grosveld G and Downing JR: AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 84: 321-330, 1996.
- 4 Downing JR: The core-binding factor leukemias: Lessons learned from murine models. *Curr Opin Genet Dev* 13: 48-54, 2003.
- 5 Huang L, Abruzzo LV, Valbuena JR, Medeiros LJ and Lin P: Acute myeloid leukemia associated with variant t(8;21) detected by conventional cytogenetic and molecular studies: A report of four cases and review of the literature. *Am J Clin Pathol* 125: 267-272, 2006.

- 6 Udayakumar AM, Alkindi S, Pathare AV and Raeburn JA: Complex t(8;13;21)(q22;q14;q22)—a novel variant of t(8;21) in a patient with acute myeloid leukemia (AML-M2). *Arch Med Res* 39: 252-256, 2008.
- 7 Ahmad F, Kokate P, Chhedha P, Dalvi R, Das BR and Mandava S: Molecular cytogenetic findings in a three-way novel variant of t(1;8;21)(p35;q22;q22): A unique relocation of the *AML1/ETO* fusion gene 1p35 in AML-M2. *Cancer Genet Cytogenet* 180: 153-157, 2008.
- 8 Mrozek K, Prior TW, Edwards C, Marcucci G, Carroll AJ, Snyder PJ, Koduru PR, Theil KS, Pettenati MJ, Archer KJ, Caligiuri MA, Vardiman JW, Kolitz JE, Larson RA and Bloomfield CD: Comparison of cytogenetic and molecular genetic detection of t(8;21) and inv(16) in a prospective series of adults with *de novo* acute myeloid leukemia: a Cancer and Leukemia Group B Study. *J Clin Oncol* 19: 2482-2492, 2001.
- 9 Gamberdinger U, Teigler-Schlegel A, Pils S, Bruch J, Viehmann S, Keller M, Jauch A and Harbott J: Cryptic chromosomal aberrations leading to an *AML1/ETO* rearrangement are frequently caused by small insertions. *Genes Chromosomes Cancer* 36: 261-272, 2003.
- 10 Rowley JD: Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. *Ann Genet* 16: 109-112, 1973.
- 11 Liu XP, Xue YP, Liu SH, Mi YC, Han MZ, Xiao ZJ, Bian SG and Wang JX: An analysis of cytogenetic characteristics and prognosis of 189 t (8; 21) acute myeloid leukemia patients. *Zhonghua Nei Ke Za Zhi* 45: 918-921, 2006 (in Chinese).
- 12 Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R, Patil SR, Davey FR, Berg DT, Schiffer CA, Arthur DC and Mayer RJ: Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res* 58: 4173-4179, 1998.
- 13 Marcucci G, Caligiuri MA and Bloomfield CD: Molecular and clinical advances in core binding factor primary acute myeloid leukemia: A paradigm for translational research in malignant hematology. *Cancer Invest* 18: 768-780, 2000.
- 14 Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA and Bloomfield CD: Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with *de novo* acute myeloid leukemia: Results from Cancer and Leukemia Group B (CALGB 8461). *Blood* 100: 4325-4336, 2002.
- 15 Nguyen S, Leblanc T, Fenaux P, Witz F, Blaise D, Pignaux A, Thomas X, Rigal-Huguet F, Liouze B, Auvrignon A, Fiere D, Reiffers J, Castaigne S, Leverger G, Harousseau JL, Socie G and Dombret H: A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia (AML): A survey of 161 cases from the French AML Intergroup. *Blood* 99: 3517-3523, 2002.
- 16 Delaunay J, Vey N, Leblanc T, Fenaux P, Rigal-Huguet F, Witz F, Lamy T, Auvrignon A, Blaise D, Pignaux A, Mugneret F, Bastard C, Dastugue N, Van den Akker J, Fiere D, Reiffers J, Castaigne S, Leverger G, Harousseau JL and Dombret H: Prognosis of inv(16)/t(16;16) acute myeloid leukemia (AML): A survey of 110 cases from the French AML Intergroup. *Blood* 102: 462-469, 2003.
- 17 Schlenk RF, Benner A, Krauter J, Buchner T, Sauerland C, Ehninger G, Schaich M, Mohr B, Niederwieser D, Krahl R, Pasold R, Dohner K, Ganser A, Dohner H and Heil G: Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: A survey of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol* 22: 3741-3750, 2004.
- 18 Chen CC, Gau JP, Yu YB, Lu CH, Lee KD and You JY: Prognosis and treatment outcome in patients with acute myeloid leukemia with t(8;21)(q22;q22). *Adv Ther* 24: 907-920, 2007.
- 19 Lai YY, Qiu JY, Jiang B, Lu XJ, Huang XJ, Zhang Y, Liu YR, Shi HL and Lu DP: Characteristics and prognostic factors of acute myeloid leukemia with t (8; 21) (q22; q22). *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 13: 733-740, 2005 (in Chinese).
- 20 Boissel N, Leroy H, Brethon B, Philippe N, de Botton S, Auvrignon A, Raffoux E, Leblanc T, Thomas X, Hermine O, Quesnel B, Baruchel A, Leverger G, Dombret H and Preudhomme C: Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). *Leukemia* 20: 965-970, 2006.
- 21 Cairoli R, Beghini A, Grillo G, Nadali G, Elice F, Ripamonti CB, Colapietro P, Nichelatti M, Pezzetti L, Lunghi M, Cuneo A, Viola A, Ferrara F, Lazzarino M, Rodeghiero F, Pizzolo G, Larizza L and Morra E: Prognostic impact of *c-KIT* mutations in core binding factor leukemias: An Italian retrospective study. *Blood* 107: 3463-3468, 2006.
- 22 Paschka P, Marcucci G, Ruppert AS, Mrozek K, Chen H, Kittles RA, Vukosavljevic T, Perrotti D, Vardiman JW, Carroll AJ, Kolitz JE, Larson RA and Bloomfield CD: Adverse prognostic significance of *KIT* mutations in adult acute myeloid leukemia with inv(16) and t(8;21): A Cancer and Leukemia Group B Study. *J Clin Oncol* 24: 3904-3911, 2006.
- 23 Bacher U, Haferlach T, Schoch C, Kern W and Schnittger S: Implications of NRAS mutations in AML: A study of 2502 patients. *Blood* 107: 3847-3853, 2006.
- 24 Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K and Schoch C: *KIT*-D816 mutations in *AML1-ETO*-positive AML are associated with impaired event-free and overall survival. *Blood* 107: 1791-1799, 2006.
- 25 Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M, Hanada R, Tsukimoto I and Hayashi Y: *KIT* mutations, and not *FLT3* internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): A study of the Japanese Childhood AML Cooperative Study Group. *Blood* 107: 1806-1809, 2006.
- 26 Paschka P: Core binding factor acute myeloid leukemia. *Semin Oncol* 35: 410-417, 2008.
- 27 Hagihara M, Kobayashi H, Miyachi H and Ogawa T: Clinical heterogeneity in acute myelogenous leukemia with the 8;21 translocation. *Keio J Med* 40: 90-93, 1991.
- 28 Rege K, Swansbury GJ, Atr AA, Horton C, Min T, Dainton MG, Matutes E, Durosini M, Treleaven JG, Powles RL and Catovsky D: Disease features in acute myeloid leukemia with t(8;21)(q22;q22). Influence of age, secondary karyotype abnormalities, CD19 status, and extramedullary leukemia on survival. *Leuk Lymphoma* 40: 67-77, 2000.
- 29 Mertens F, Johansson B and Mitelman F: Age- and gender-related heterogeneity of cancer chromosome aberrations. *Cancer Genet Cytogenet* 70: 6-11, 1993.

- 30 Gritsaev SV, Martynkevich IS, Martynenko LS, Ivanova MP, Aksenova V, Moskalenko MV, Zapreeva IM and Abdulkadyrov KM: Age-specific characteristics of acute myeloid leukemia karyotype. *Ter Arkh* 83: 51-55, 2011 (in Russian).
- 31 Fleishman EV, Sokova OI, Popa AV, Shneider MM, Kirichenko OP, Konstantinova LN and Metel'kova NF: Chromosomal translocation t(8;21) in acute myeloid leukemia of children: Prognostic value of additional karyotype abnormalities. *Vestn Ross Akad Med Nauk*: 9-16, 2009 (in Russian).
- 32 Betts DR, Ammann RA, Hirt A, Hengartner H, Beck-Popovic M, Kuhne T, Nobile L, Caflisch U, Wacker P and Niggli FK: The prognostic significance of cytogenetic aberrations in childhood acute myeloid leukaemia. A study of the Swiss Paediatric Oncology Group (SPOG). *Eur J Haematol* 78: 468-476, 2007.
- 33 Rowley JD: General report on the Second International Workshop on Chromosomes in Leukemia. *Int J Cancer* 26: 531-533, 1980.
- 34 Second MIC Cooperative Study Group: Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukaemias. *Br J Haematol* 68: 487-494, 1988.
- 35 Groupe Français de Cytogénétique Hématologique: Acute myelogenous leukemia with an 8;21 translocation. A report on 148 cases from the Groupe Français de Cytogenetique Hematologique. *Cancer Genet Cytogenet* 44: 169-179, 1990.
- 36 Keating MJ, Cork A, Broach Y, Smith T, Walters RS, McCredie KB, Trujillo J and Freireich EJ: Toward a clinically relevant cytogenetic classification of acute myelogenous leukemia. *Leuk Res* 11: 119-133, 1987.
- 37 Fenaux P, Preudhomme C, Lai JL, Morel P, Beuscart R and Bauters F: Cytogenetics and their prognostic value in *de novo* acute myeloid leukaemia: A report on 283 cases. *Br J Haematol* 73: 61-67, 1989.
- 38 Billstrom R, Johansson B, Fioretos T, Garwicz S, Malm C, Zettervall O and Mitelman F: Poor survival in t(8;21) (q22;q22)-associated acute myeloid leukaemia with leukocytosis. *Eur J Haematol* 59: 47-52, 1997.
- 39 Haferlach T, Bennett JM, Loffler H, Gassmann W, Andersen JW, Tuzuner N, Cassileth PA, Fonatsch C, Schoch C, Schlegelberger B, Becher R, Thiel E, Ludwig WD, Sauerland MC, Heinecke A and Buchner T: Acute myeloid leukemia with translocation (8;21). Cytomorphology, dysplasia and prognostic factors in 41 cases. AML Cooperative Group and ECOG. *Leuk Lymphoma* 23: 227-234, 1996.
- 40 Dzeletovic N, McGuire J, Daujat M, Tholander J, Ema M, Fujii-Kuriyama Y, Bergman J, Maurel P and Poellinger L: Regulation of dioxin receptor function by omeprazole. *J Biol Chem* 272: 12705-12713, 1997.
- 41 Jiao B, Wu CF, Liang Y, Chen HM, Xiong SM, Chen B, Shi JY, Wang YY, Wang JH, Chen Y, Li JM, Gu LJ, Tang JY, Shen ZX, Gu BW, Zhao WL, Chen Z and Chen SJ: *AML1-ETO9A* is correlated with *C-KIT* overexpression/mutations and indicates poor disease outcome in t(8;21) acute myeloid leukemia-M2. *Leukemia* 23: 1598-1604, 2009.
- 42 De J, Zanjani R, Hibbard M and Davis BH: Immunophenotypic profile predictive of *KIT* activating mutations in *AML1-ETO* leukemia. *Am J Clin Pathol* 128: 550-557, 2007.
- 43 O'Brien S, Kantarjian HM, Keating M, Gagnon G, Cork A, Trujillo J and McCredie KB: Association of granulocytosis with poor prognosis in patients with acute myelogenous leukemia and translocation of chromosomes 8 and 21. *J Clin Oncol* 7: 1081-1086, 1989.
- 44 Park SH, Chi HS, Min SK, Park BG, Jang S and Park CJ: Prognostic impact of c-KIT mutations in core binding factor acute myeloid leukemia. *Leuk Res* 35: 1376-1383, 2011.
- 45 Santos FP, Jones D, Qiao W, Cortes JE, Ravandi F, Estey EE, Verma D, Kantarjian H and Borthakur G: Prognostic value of *FLT3* mutations among different cytogenetic subgroups in acute myeloid leukemia. *Cancer* 117: 2145-2155, 2011.
- 46 Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, Goldstone AH and Linch DC: The presence of a *FLT3* internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: Analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* 98: 1752-1759, 2001.
- 47 Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Loffler H, Sauerland CM, Serve H, Buchner T, Haferlach T and Hiddemann W: Analysis of *FLT3* length mutations in 1003 patients with acute myeloid leukemia: Correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood* 100: 59-66, 2002.
- 48 Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, Wermke M, Bornhauser M, Ritter M, Neubauer A, Ehninger G and Illmer T: Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* 99: 4326-4335, 2002.
- 49 Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR and Reilly JT: Identification of novel *FLT3* Asp835 mutations in adult acute myeloid leukaemia. *Br J Haematol* 113: 983-988, 2001.
- 50 Whitman SP, Ruppert AS, Radmacher MD, Mrozek K, Paschka P, Langer C, Baldus CD, Wen J, Racke F, Powell BL, Kolitz JE, Larson RA, Caligiuri MA, Marcucci G and Bloomfield CD: *FLT3* D835/I836 mutations are associated with poor disease-free survival and a distinct gene-expression signature among younger adults with *de novo* cytogenetically normal acute myeloid leukemia lacking *FLT3* internal tandem duplications. *Blood* 111: 1552-1559, 2008.

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