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Adverse Prognostic Impact of *KIT* Mutations in Childhood CBF-AML: the Results of the Japanese Pediatric Leukemia/Lymphoma Study Group AML-05 Trial

Letter to the Editor

Core binding factor acute myeloid leukemia (CBF-AML), characterized by the chromosomal abnormalities of t(8;21) (*AML1-ETO*) or inv(16)/t(16;16) (*CBFB-MYH11*), is the most frequent subtype in pediatric AML. Although CBF-AML is generally classified as a low-risk (LR) group, in a recent analysis of 154 patients with CBF-AML participating in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 trial (2006-2010), 29.9% of the patients relapsed within 3 years.[1] This finding suggests that a population of the CBF-AML patients had other risk factors that could account for their poor outcome. The *KIT* gene, located on chromosome 4, encodes a type III receptor tyrosine kinase activated by stem cell factor (SCF) and leading to cell survival, migration, and proliferation.[2] In adults with CBF-AML, gain-of-function mutations of *KIT* are risk factors.[3-7] These mutations cluster in exon 8 (encoding the extracellular domain of KIT), exon 17 (encoding the activation loop of the

tyrosine kinase domain), and occasionally in exon 10 or 11 (encoding the transmembrane domain and the juxtamembrane domain, respectively). Among these mutations, *KIT* exon 17 D816 mutations have been shown to confer a poor prognosis to adult patients with t(8;21).[3, 4, 7] In pediatric CBF-AML, some studies [8-10] found no clinical impact of *KIT* mutations, while in contrast, Shimada *et al*.[11] and Manara *et al.*[12] studying the prognostic impact of *KIT* mutations in pediatric t(8;21) AML both concluded that *KIT* mutations may be poor prognostic factors (Supplementary Table 1S). These retrospective studies, however, could be influenced by selection bias of the cytogenetic subtype. Therefore, a further validation study that includes all of the targeted cytogenetic subtypes (t(8;21) and inv(16)/t(16;16)) and *KIT* mutations in a larger cohort is necessary to elucidate any involvement of *KIT* mutations in the prognosis of pediatric CBF-AML.

In this study, we analyzed *KIT* mutations in 138 pediatric CBF-AML patients participating in the JPLSG AML-05 trial, whose cDNA from their diagnostic bone marrow samples were available; 107 patients carried a t(8;21) rearrangement and 31 an inv(16). *KIT* mutations in exon 8, 10, 11 and 17 were screened using direct sequencing of cDNA.[11] Event-free survival (EFS) was measured from the

time of diagnosis to the last follow-up or the first event (failure to achieve remission, relapse, secondary malignancy, or death), and overall survival (OS) was measured from the time of diagnosis to death. The 3-year probability of EFS (pEFS) and the 3-year probability of OS (pOS) were estimated using the Kaplan−Meier method and compared using the log-rank test. Differences between cumulative incidence of relapse (CIR) curves were compared using Gray's test. Statistical analyses were performed with STATA software (version 13.0; StataCorp LP, College Station, TX) and R software (version 3.0.2).

KIT mutations were found in 46/107 (43.0%) t(8;21) patients and in 12/31 (38.7%) inv(16) patients (Table 1). Of the 46 t(8;21) patients, exon 8 mutations were detected in eight (17.4%), exon 10 and/or 11 mutations in ten (21.7%), exon 17 mutations in 23 (50.0%), both exon 8 and 10 mutations in three (6.5%), both exon 8 and 17 mutations in one (2.2%), and both exon 10 and 17 mutations in one (2.2%). Of the 12 inv(16) patients, six (50.0%) had mutations in exon 8, one (8.3%) had mutations in exon 10, four (33.3%) had mutations in exon 17, and one (8.3%) had mutations in both exon 8 and 17. Exon 8 mutations were in-frame deletions and/or insertions of fewer than 13 bases around codon 418. Exon 10 mutations were either M541L or V540L. Exon 11 mutations were either

V560D or a 45 base pair internal tandem duplication (ITD). Exon 17 had D816 point mutations (D816V, D816H or D816Y), D820 point mutations (D820G or D820V), N822K, or R815_D816insL. No significant difference was observed in sex, age, and white blood cell count at diagnosis between CBF-AML patients with or without *KIT* mutations (*P*=0.828, *P*=0.912, and *P*=0.673, respectively; Supplementary Table 2S). *FLT3/ITD* was found in one of the 58 CBF-AML patients with *KIT* mutations and in four of the eighty without *KIT* mutations, and this frequency did not differ significantly between the groups (*P*=0.398). Complete remission (CR) rates were also similar in both groups (93.1% and 95.0% in patients with and without *KIT* mutations respectively, *P*=0.573; Table 1).

The EFS rate and the CIR were significantly less favorable in CBF-AML patients with *KIT* mutations than in those without (3-year pEFS: 58% *vs.* 75%, *P*=0.029 and 3-year CIR: 38% *vs.* 20%, *P*=0.024, respectively), and were comparable to those of the intermediate-risk (IR) group (Table 1, Figure 1a and c). In the analysis stratified according to locations of the *KIT* mutation, the patients with exon 8 and those with exon 17 mutations had a higher CIR than those without *KIT* mutations, although these differences lost significance after

applying the Bonferroni correction (exon 8 *vs*. WT: *P*=0.015, Bonferroni-adjusted *P*=0.060; exon 17 *vs.* WT: *P*=0.040, Bonferroni-adjusted *P*=0.160). On the other hand, OS rates associated with the presence of *KIT* mutations or location of the mutation were indistinguishable (Table 1, Figure 1b).

Next we studied the prognostic influence of *KIT* mutations stratified by t(8;21) and inv(16) independently. The t(8;21) patients with *KIT* mutations had a significantly lower EFS (3-year pEFS: 56% with *KIT* mutations *vs*. 75% with WT *KIT*, *P*=0.037; Table 1). Moreover, the 3-year pEFS according to the location of the *KIT* mutation was markedly different (38%, 80%, 49%, 80%, and 75% for exon 8, exon 10 and/or 11, exon 17, mutations in more than one location, and WT, respectively, *P*=0.041), and exon 17 mutations reached statistical significance compared to WT (*P*=0.012, Bonferroni-adjusted *P*=0.048), while exon 8 mutations did not (*P*=0.032, Bonferroni-adjusted *P*=0.128). Furthermore, the higher CIR were observed in the patients with exon 8 or 17 mutations, although the differences did not reach significance (exon 8 *vs*. WT: *P*=0.014, Bonferroni-adjusted *P*=0.056; exon 17 *vs.* WT: *P*=0.032, Bonferroni-adjusted *P*=0.128) (Table 1). Fourteen (13.1%) of the 107 t(8;21) patients had D816 mutations (eight with D816V, three with D816H, one with D816Y, one with

D816V + D816Y, and one with R815_D816insL), and the EFS and OS rates, along with the CIR of these patients, were significantly less favorable than for the other t(8;21) patients without D816 mutations (3-year pEFS: 34% *vs.* 72%, *P*<0.001; 3-year pOS: 69% *vs.* 96%, *P*=0.001, and 3-year CIR: 59% *vs.* 26%, *P*=0.007, respectively; Table 1). On the other hand, we did not find any significant association of *KIT* mutations with the outcome of inv(16) patients (Table 1).

Our findings suggest that *KIT* mutation is a poor prognostic factor in pediatric CBF-AML. Most of the relapsed patients in the AML-05 trial were salvaged by allogeneic hematopoietic stem cell transplantation (HSCT), which could be a primary reason for the indistinguishable OS between the patients with and without *KIT* mutations. Our results are also consistent with previous pediatric studies reported by Shimada *et al.*[11] and Manara *et al.*,[12] and with reports on adult patients [3-7]. Our study involved a relatively large cohort of pediatric CBF-AML patients and comprised all kinds of major *KIT* mutations, and thus the likelihood of demonstrating any adverse impact of *KIT* mutations was therefore high. Intriguingly, the 3-year CIR of CBF-AML patients in the AML-05 study was significantly higher than in the previous AML99 study[1] and was coincident with a

higher frequency of *KIT* mutations. Although it may have been partially due to changes in therapeutic regimens, this result suggests that the higher incidence of *KIT* mutations in the AML-05 study led to the increased relapse rate. This study was limited to Japanese CBF-AML patients, and therefore the question remains whether the results may have been influenced by ethnicity, treatment regimens, and experimental methods. These factors may contribute the difference between this study and the largest study reported by Pollard *et al*.[10] which found no clinical impact of *KIT* mutations. A large international study is expected to answer this.

Among the *KIT* mutations, exon 17 mutations showed a marked adverse impact on patients with t(8;21). In particular, exon 17 D816 mutations resulted in a much poorer outcome, suggesting that this region in the activation loop domain of KIT has the greatest prognostic impact among all the *KIT* mutations. Gain-of-function mutations in this region are known to cause SCF-independent activation of KIT, which triggers its downstream signaling, activating cellular proliferation.[2] In an AML mouse model, co-expression of AML1-ETO with a mutated KIT activation loop domain induces a more aggressive AML phenotype than co-expression with a mutated KIT extracellular domain.[13] These findings

further support the possibility that alterations in the signaling pathway due to D816 mutations play a critical role in leukemogenesis, linking the presence of D816 mutations with poor prognosis.

Our findings, along with those of others, indicate that *KIT* mutations should be regarded as stratification factors in pediatric CBF-AML, especially exon 17 mutations in t(8;21), and improvement of standard chemotherapy regimens [14] and additional specific therapies would be required for patients with *KIT* mutations. For example, the tyrosine kinase inhibitor (TKI) dasatinib has been shown to be active against cells co-expressing KIT N822K and AML1-ETO in a murine AML model and in Kasumi-1 cells, and against cells expressing KIT D816V *in vitro*.[15, 16] Combination therapies of dasatinib with conventional chemotherapy in adult CBF-AML are currently being tested in phase I/II studies (NCT01238211 and NCT00850382). For CBF-AML patients with *KIT* abnormalities, KIT-targeting therapies, including TKIs, are expected to reduce the risk of relapse and subsequent need for HSCT.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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Supplementary information is available at Leukemia's website.

REFERENCES

1. Tomizawa D, Tawa A, Watanabe T, Saito AM, Kudo K, Taga T*, et al.* Excess treatment reduction including anthracyclines results in higher incidence of relapse in core binding factor acute myeloid leukemia in children. *Leukemia* 2013; **27:** 2413-2416.

2. Lennartsson J, Ronnstrand L. Stem cell factor receptor/c-Kit: from basic science to clinical implications. *Physiological reviews* 2012; **92:** 1619-1649.

3. Cairoli R, Beghini A, Grillo G, Nadali G, Elice F, Ripamonti CB*, et al.* Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. *Blood* 2006; **107:** 3463-3468.

4. Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K*, et al.* KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood* 2006; **107:** 1791-1799.

5. Allen C, Hills RK, Lamb K, Evans C, Tinsley S, Sellar R*, et al.* The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3 and CBL mutations in core-binding factor acute myeloid leukemia. *Leukemia* 2013; **27:** 1891-1901.

6. Paschka P, Du J, Schlenk RF, Gaidzik VI, Bullinger L, Corbacioglu A*, et al.* Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML Study Group (AMLSG). *Blood* 2013; **121:** 170-177.

7. Krauth MT, Eder C, Alpermann T, Bacher U, Nadarajah N, Kern W*, et al.* High number of

additional genetic lesions in acute myeloid leukemia with t(8;21)/RUNX1-RUNX1T1: frequency and impact on clinical outcome. *Leukemia* 2014; **28:** 1449-1458.

8. Goemans BF, Zwaan CM, Miller M, Zimmermann M, Harlow A, Meshinchi S*, et al.* Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. *Leukemia* 2005; **19:** 1536-1542.

9. Shih LY, Liang DC, Huang CF, Chang YT, Lai CL, Lin TH*, et al.* Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. *Leukemia* 2008; **22:** 303-307.

10. Pollard JA, Alonzo TA, Gerbing RB, Ho PA, Zeng R, Ravindranath Y*, et al.* Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood* 2010; **115:** 2372-2379.

11. Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M*, et al.* 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* 2006; **107:** 1806-1809.

12. Manara E, Bisio V, Masetti R, Beqiri V, Rondelli R, Menna G*, et al.* Core-binding factor acute myeloid leukemia in pediatric patients enrolled in the AIEOP AML 2002/01 trial: screening and prognostic impact of c-KIT mutations. *Leukemia* 2014; **28:** 1132-1134.

13. Nick HJ, Kim HG, Chang CW, Harris KW, Reddy V, Klug CA. Distinct classes of c-Kit-activating mutations differ in their ability to promote RUNX1-ETO-associated acute myeloid leukemia. *Blood* 2012; **119:** 1522-1531.

14. Kaspers GJ, Zimmermann M, Reinhardt D, Gibson BE, Tamminga RY, Aleinikova O*, et al.* Improved outcome in pediatric relapsed acute myeloid leukemia: results of a randomized trial on liposomal daunorubicin by the International BFM Study Group. *J Clin Oncol* 2013; **31:** 599-607.

15. Wang YY, Zhao LJ, Wu CF, Liu P, Shi L, Liang Y*, et al.* C-KIT mutation cooperates with

full-length AML1-ETO to induce acute myeloid leukemia in mice. *Proceedings of the National Academy of Sciences of the United States of America* 2011; **108:** 2450-2455.

16. Mpakou VE, Kontsioti F, Papageorgiou S, Spathis A, Kottaridi C, Girkas K*, et al.* Dasatinib inhibits proliferation and induces apoptosis in the KASUMI-1 cell line bearing the t(8;21)(q22;q22) and the N822K c-kit mutation. *Leukemia research* 2013; **37:** 175-182.

FIGURE LEGENDS

Figure 1. Outcomes of CBF-AML patients with or without *KIT* mutations compared with low-risk (LR), intermediate-risk (IR), or high-risk (HR) patients. (a) Event-free survival (EFS). (b) Overall survival (OS). (c) Cumulative incidence of relapse (CIR). LR was defined as CBF-AML patients with CR after induction course 1, and absence of *FLT3/ITD*. IR was defined as AML patients who were not in either LR or HR. HR was defined as AML patients with abnormalities of monosomy 7, 5q-, t(16;21), t(9;22) (Philadelphia chromosome [Ph1]), *FLT3/ITD*, and non-CR after induction course 1.

WT, wild type.

Table 1. Clinical outcomes according to locations of *KIT* mutation detected using direct sequencing

		KIT exon 8 mutation	*KIT* exon 10 and/or 11 mutation	*KIT* exon 17 mutation	*KIT* exon 8+10, 8+17 or 10+17 mutation	All *KIT* mutations	WT *KIT*	*KIT* mutations *vs.* WT *KIT*, *P* value	Multiple comparisons between ex 8, ex 10 and/or 11, ex 17, more than one location, and WT, *P* value
CBF-AML	total, n	14	11	27	6	58	80		
	CR, n (%)	12 (85.7%)	11 (100%)	25 (92.6%)	6 (100%)	54 (93.1%)	76 (95.0%)	0.573	0.427
	not in CR, n (%)	1 (7.1%)		1 (3.7%)		2 (3.4%)	1 (1.3%)		
	3-year pEFS, % (95% CI)	42% (16–65%)	82% (45–95%)	57% (36–74%)	67% (19–90%)	58% (44–70%)	75% (63–84%)	0.029	0.053
	3-year pOS, % (95% CI)	100%	100%	86% (62–95%)	80% (20–97%)	92% (80–97%)	94% (84–98%)	0.939	0.482
	3-year CIR, % (95% CI)	51% (23–74%)	24% (3–56%)	39% (21–58%)	33% (5–68%)	38% (25–51%)	20% (12–29%)	0.024	0.082
t(8;21)	total, n	8	10	23	5	46	61		
	CR, n (%)	7 (87.5%)	10 (100%)	21 (91.3%)	5 (100%)	43 (93.5%)	59 (96.7%)	0.427	0.427
	not in CR, n (%)			1 (4.3%)		1 (2.2%)			

	3-year pEFS, % (95% CI)	38% (9–67%)	80% (41–95%)	49% (27–68%)	80% (20–97%)	56% (39–69%)	75% (61–85%)	0.037	0.041
									ex 8 *vs.* WT: *P*=0.032;
									ex 10 and/or 11 *vs.* WT: *P*=0.804;
									ex 17 *vs.* WT: *P*=0.012;
									more than one location *vs.* WT: *P*=0.918

	3-year pOS, % (95% CI)	100%	100%	83% (56–94%)	75% (13–96%)	89% (74–96%)	94% (83–98%)	0.608	0.427
	3-year CIR, % (95% CI)	62% (23–86%)	20% (3–47%)	46% (24–66%)	20% (1–58%)	42% (27–56%)	22% (13–33%)	0.052	0.077
inv(16)	total, n	6	1	4	1	12	19		
	CR, n (%)	5 (83.3%)	1 (100%)	4 (100%)	1 (100%)	11 (91.7%)	17 (89.5%)	1	0.648
	not in CR, n (%)	1 (16.7%)				1 (8.3%)	1 (5.3%)		
	3-year pEFS, % (95% CI)	50% (11–80%)		100%		67% (34–86%)	78% (52–91%)	0.453	0.159
	3-year pOS, % (95% CI)	100%		100%		100%	95% (68–99%)	0.427	0.769
	3-year CIR, % (95% CI)	33% (5–68%)		0%		25% (6–50%)	11% (2–30%)	0.277	0.252

		KIT D816 mutation	WT *KIT* + other *KIT* mutations	*KIT* D816 *vs.* WT *KIT* + other *KIT* mutations, *P* value
t(8;21)	total, n	14	93	
	CR, n (%)	12 (85.7%)	90 (96.8%)	0.126
	not in CR, n (%)	1 (7.1%)	0 (0%)	
	3-year pEFS, % (95% CI)	34% (12–59%)	72% (60–80%)	0.0004
	3-year pOS, % (95% CI)	69% (36–87%)	96% (88–99%)	0.001
	3-year CIR, % (95% CI)	59% (29–79%)	26% (18–36%)	0.007

CBF-AML, core binding factor acute myeloid leukemia; WT, wild-type; ex, exon; CR, complete remission; pEFS, probability of event-free survival; pOS, probability of overall survival; CIR, cumulative incidence of relapse; CI, confidence interval.